

## Final Project Evaluation Report

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Your Details	
Full Name	Roberto-Emiliano Trejo-Salazar
Project Title	<i>Leptonycteris yerbabuenae</i> in a Natural Experiment for Conservation: Contribution with Bat Friendly Tequila Production
Application ID	24085-1
Grant Amount	£5000
Email Address	<a href="mailto:retrejo@gmail.com">retrejo@gmail.com</a>
Date of this Report	19-02-2019

**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
Samples tissues collection of Long-nosed bats				Sampling work was successfully conducted. I visited seven roosting caves (Fig. 1) of <i>Leptonycteris</i> bats and I took samples of tissues (n: <i>L. yerbabuenae</i> =165; <i>L. nivalis</i> = 109) for genetic and genomic analysis. I was not be able to visit all nectar-feeding bats roosting caves historically registered, however, I obtained samples from other collaborators who donated the tissue samples (Table 1).
Recording the presence of <i>Leptonycteris</i> bats in tequila or mezcal crops				I collected small wing membrane tissue samples (1mm diameter) out of <i>Leptonycteris</i> bats inhabiting near of The Tequila Route in three different sites associated with tequila plantations. We were able to detect the presence of nectar feeding bats in this new area with agave plants.
To increase preservation of Agave blooming plants inside plantations in order to feed nectar-feeding bats.				This goal was partially completed just because I am expecting for the implementation of the programme in other sites outside and other agave species. Three local trademarks participated actively in this conservation initiative. They allowed blooming of <i>Agave tequilana</i> inflorescences in a 5% per ha. Moreover, there are some mezcal producers interested in this programme and we still have permanent communication with them to begin good bat friendly practices in their crops.
Building a model of potential distribution of <i>Leptonycteris</i> bats				I developed a potential distribution model for three species of the Genus <i>Leptonycteris yerbabuenae</i> (Fig. 4). This tool is very useful to define priority sites for conservation because potential distribution of <i>Leptonycteris</i>

				bats and agave plants in Mexico overlap to each other, which means that every conserve inflorescence of tequila, mezcal or pulque works as a food source for nectar feeding bats.
Analysis of genetic diversity of Agave tequilana				This is a long-term goal, I have made first step. I germinated first seeds produced by natural pollination by bats (n=27). Also, I have tissues from three different localities of tequila route (n=90) and other from an outside place (Queretaro, Mexico) which will be used as sister group.
Phylogeographic analysis of <i>Leptonycteris</i> bats				This goal is currently building. I have obtained statistic resume of genetic diversity (Table 2; Fig. 2)
Science Communication				I had the chance to share my experience with local people, crops workers who are directly involved in conservation of agaves. In addition, I offered a speech in Mexico City with guides in Natural History Museum. Lastly, this proposal was published by Oikos, a magazine of the Instituto de Ecología, UNAM.

**2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.**

I faced different difficulties during the development of the project. The record of the presence of nectar feeding bats during blooming of agaves depends on different factors. For instance, If weather is suitable, bats can be captured while they are foraging but with the presence of rain or full moon, bats may be absent. Besides, when bats do not follow the season of migration one must wait for their arrival. In this study, we had to wait for the arrival of bats because they did not migrate according to the season.

Another pending objective is the quest of new roosting caves, this was not completed because of little information from local people. Also many people in Mexico are distrustful due to the lack of social safety conditions at some locations. When we asked for information about caves with bats, people suggested that we did not visit lonely zones and it was impossible to convince them the go with us. Field guides always thought that visit new places was not a good idea.

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

- a) I obtained a significant amount of samples from long-nosed bats in the different caves visited. These samples will allow to explain how they move during migration seasons, and if there have been historical demographic

fluctuations. In the future, it will be possible to know how climate change can affect this species. This information is necessary to improve management plans and, help to persuade that the conservation can be an economical support for more agave and mezcal producers and communities.

- b) The record of presence of *Leptonycteris* bats is an important outcome of this project because now we can prove that nectar feeding bats are able to pollinate flowers of *Agave tequilana* and that they can move the genetic material long distances between crops. Tequila and mezcal producers are interested in this area since they want to increase the possibilities that *A. tequilana* can cope with climate change or plagues and diseases.
- c) I germinated several seed produced by natural pollination of *A. tequilana*. It is important because one of the most popular ideas about these plants is that they are not viable to be fertilised due to their clonal origin. Subsequently, I will the plan to extract DNA and make genetic diversity analysis in these plants to compare with plant cultivated by clonal methods.

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

People who lives around tequila and mezcal routes are directly concerned by the conservation of their economic and social sources. Nowadays, they are aware of the risk of climate change or apparition of plagues in their clonal crops. They also realised the main role nectar-feeding bats play in pollination of Agave.

Tequila producers have begun a new strategy to cultivate *A. tequilana* var. *blue*, particularly, in Arandas town, the manager of "Tequila Ocho" installed a greenhouse to germinate agave from seed and not depend on clonal methods. We hope that other producers take the same way in the future.

I established a network with people who also use agave to produce food complements and mezcal in Tehuacán, Puebla. I will give them a course about the pollination by nectar-feeding bats and the advantages of permit that a little percentage of actives inflorescences can helps to keep or increase genetic diversity of agaves.

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

Yes, there are a lots of sites along Mexico where agaves are the most important economic and social sources. I also have a particular interest in conservation of Agave and nectar-feeding bats because they can be used as umbrella species. More than 60% of Mexican territory is occupied by dry vegetation, and it is possible that we find at least one species of agave in every part of the country. In addition, I consider that is very important to study the rest of nectar-feeding bats species in

Mexico and their evolutionary and ecological relationship with different groups' of plants they visit.

I have finished laboratory work but with tissue samples, I was able to send 95 DNA samples to obtain genomic information (SNPs) which work to explain other important biological, ecological and evolutionary aspects of *Leptonycteris* bats and their capability of fly long distances, digest sugars, pollinate different groups of plants and their potential to survive in climate change scenarios.

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

I have published beside my committee tutor thesis our proposal in a scientific journal and a magazine for general public. But, I will published three scientific papers where I mention this project and my PhD thesis. On the other hand, I will present this project in the next Mexican Congress of Ecology and if it is possible, I will participate in an international congress related with this topic.

I also showed partial results in Natural History Museum of Mexico City and I collaborated with other colleagues giving talks about the ecosystems services bats gave. I have two invitations to participate in workshops focus on "jimadores" and mezcal producers in Oaxaca and Puebla. On March (2019) I will present the bat friendly project in Botany Garden on Zapotitlán Salinas, Puebla.

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

The grant was used for 2018 field work and some supplies of laboratory support as in the original time plan of my proposal with some changes to the schedule. All activities for which the funding was used are embedded in my last year of my PhD programme which mostly includes last field and laboratory work. I still needed more time to complete the overall objective of my PhD thesis.

The first step of the project and present laboratory work both have been supported by Dr Luis Eguiarte with a financed project of CONACyT (government agency of Mexican science) about the *Leptonycteris yerbabuenae* genome.

#### 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
Field work				

Field fees	313	422	+109	This amount was calculated for 3 persons, but it increase because I had to travel with one person more. The amount also increased because of the sampling period was extended due to the late migrate season. So I had to do other trips.
Airfare to monitoring site (roundtrip)	1,130	1241		The addition of one person more and the other trips not contemplated at the initial budget.
Gas for in site Transportation	965	1139	+559	The extended time in fieldwork waiting for migrate bats caused an increase in this topic because of the movement between sites monitoring.
Lodging	1304	1415	+111	The same reason above.
Field Guides	174	196	+76	The increase in fieldwork trips required the help of field guides more time than the expected.
Vehicle maintenance	17	21	+4	It was needed a car revision because there were problems with it in the second trip.
<b>Capture equipment</b>				
Mist nets	164	164		According to the original plan
Sample collection kits (tubes 1.5ml)	86	87	+1	There was a different counting taxes
Wing punch	126	120	-6	I had to buy more wing punches because of the use during field work and trips.
Gloves	4	5	+1	I used more gloves during extraction DNA procedure
Head lamp	17	17		According to the original plan
Poles	64	64		According to the original plan
Bag for mist net poles	20	20		According to the original plan
Hard disk (1TB)	50	50		According to the original plan
<b>Laboratory equipment</b>				
Tubes Eppendorf 0.6 ml	80	80		According to the original plan
Taq DNA Polymerase (250 U)	284	295	+11	I used another tube of Taq to complete the total of amplification
PCR primers	157	157		According to the original plan
<b>TOTAL</b>	<b>5000</b>	<b>5492</b>	<b>+492</b>	The difference was paid by Dr. Luis Eguiarte with a Conacyt financed project.

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

First, I have to persuade new mezcal, pulque and tequila producers about the key role that nectar-feeding bats play on pollination along the mezcal and tequila route. If we protect more hectares of wild and cultivated agaves it will be probably that the nectar corridor works helping the migratory routes of *Leptonycteris* females. In the short term, I will finish phylogeographic analysis to infer diversity hotspots, migratory routes and centres of origin of the species to give major priority. Finally, I will propose conservation plans in those sites and the implement of bat friendly programme.

**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?**

I used the Rufford Foundation logo on my oral presentations with local people and in the workshops I participated. I advised Rufford Foundation as a financial option for supporting students and colleagues' projects. I also plan to use the logo in my PhD presentation and congress in the future. Also, the logo will be listed on my PhD thesis and in the papers I will published to communicate my results.

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

**Dr. Luis Eguiarte.** He is part of my Ph.D. committee, he has given their guidance and expertise to the project, increasing the success for all steps of this project.

**Dr. Rodrigo Medellin.** He is my Ph.D. advisor, he supported me planning travels for sampling and monitoring of bats and agaves.

**Ing. Carlos Camarena.** He spent time with me in his crops, he allowed me in the conservation of inflorescences and gave material, transport and people during our stay in Arandas, Jalisco.

**Dr. Ana Ibarra** and student **Marco Antonio Reyes** helped me during travels, monitoring and sampling of bats and agave tissues.

**12. Any other comments?**

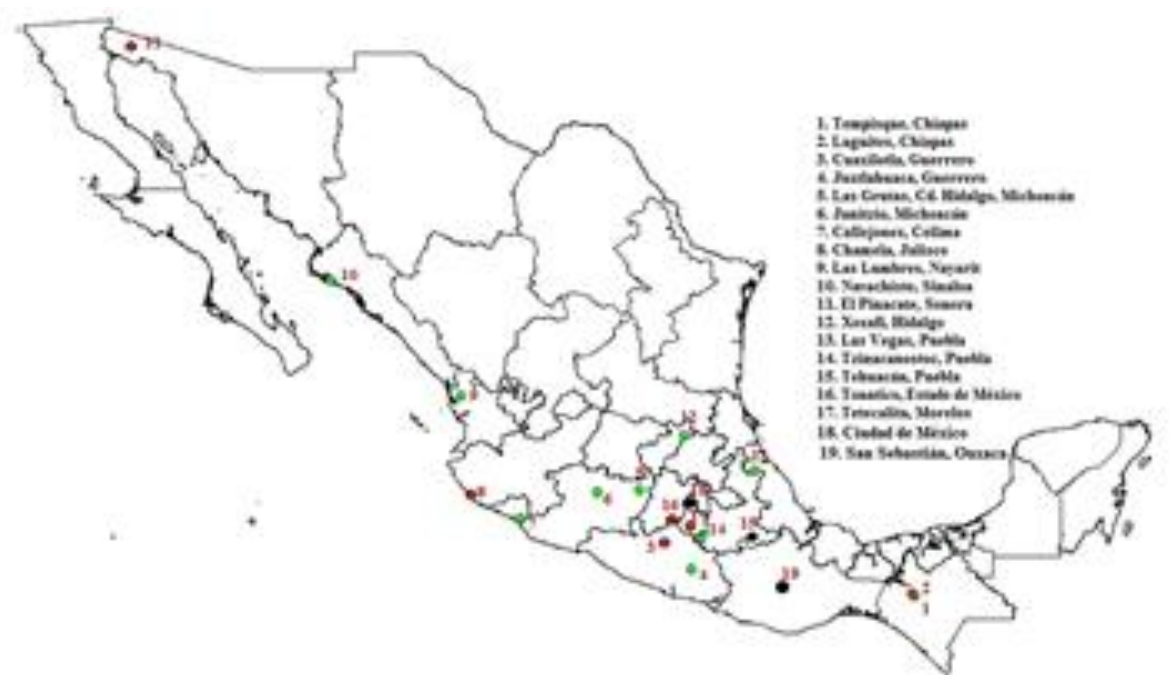
The development of this project has been a great challenge for me, mainly because it is very difficult to apply theoretical knowledge in harmony with life style of local people. Every place in Mexico can be totally different one from the other in both cultural and social interactions, so one must adjust the original plan according to the external factors. With, sometimes, little variants.

As a biologist, I learned not just about science and bats, but also how to communicate my ideas with people who is not immersed in this topics. I think people can be opened to implement conservation actions, but we need to explain better the importance and benefits it carry out in local communities but in global level too.



**Table 1. Total of tissue samples in each Mexican state.**

Mexican States	No. Samples
Chiapas	25
Ciudad de México	11
Colima	19
Guerrero	25
Hidalgo	20
Jalisco	30
Michoacán	25
Morelos	30
Nayarit	16
Puebla	27
Sinaloa	15
Sonora	40
Estado de México	5
Oaxaca	20



**Figure 1. Sampling sites of *Leptoncyteris yerbabuenae***



Table 2. Haplotipic and Nucleotide Diversity for three mitochondrial and one associated-chromosomal Y gene in *Leptonycteris yerbabuenae*.

	Sample Size	No. of Haplotypes	Haplotypic diversity (Hd)	Nucleotide Diversity (Pi)
<b>NADH</b>	<b>125</b>	<b>58</b>	<b>0.831</b>	<b>0.345</b>
<b>Cyt-b</b>	<b>31</b>	<b>32</b>	<b>0.995</b>	<b>0.439</b>
<b>D-loop</b>	<b>128</b>	<b>36</b>	<b>0.528</b>	<b>0.105</b>
<b>DBY</b>	<b>93</b>	<b>55</b>	<b>0.978</b>	<b>0.298</b>

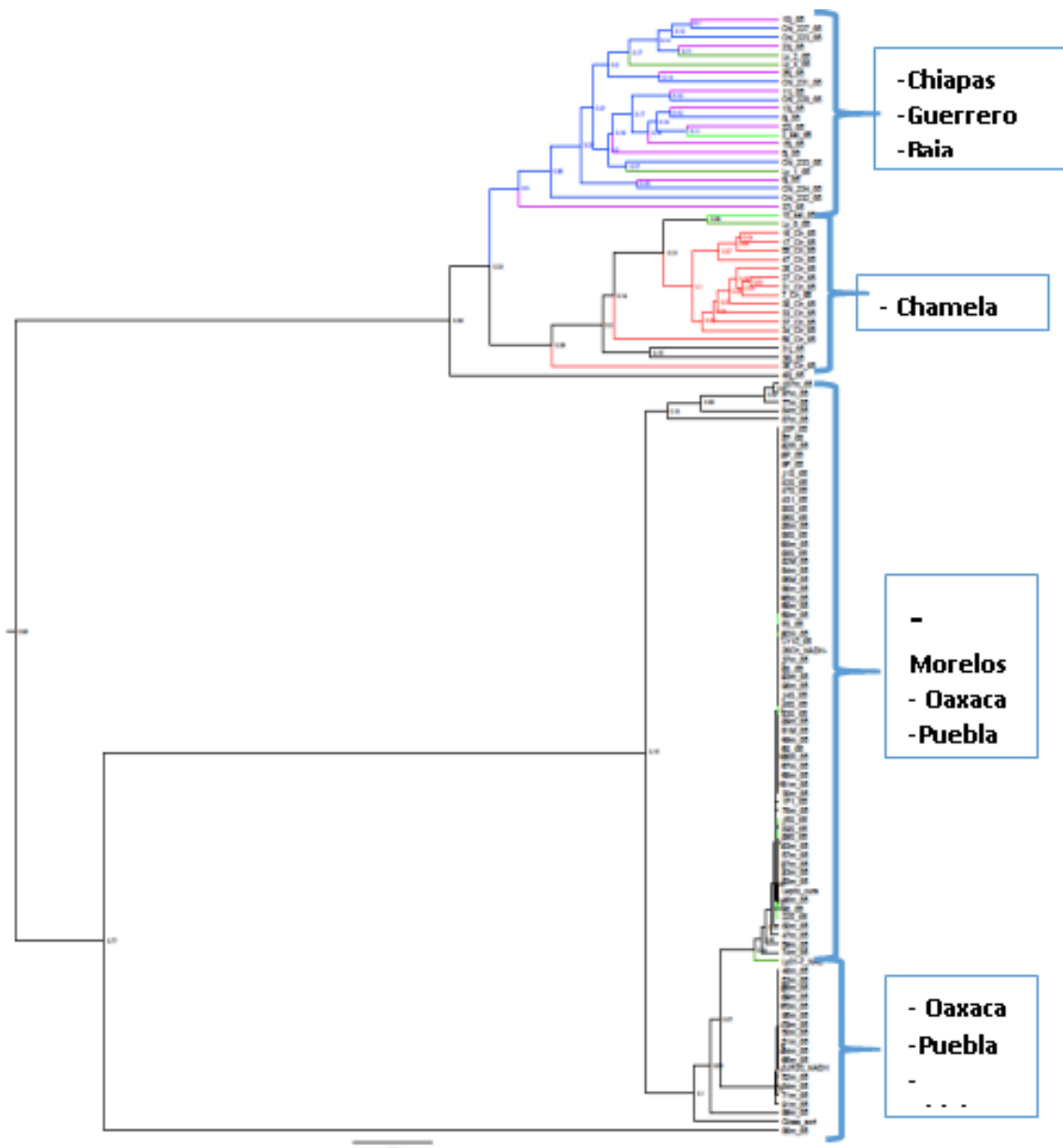


Figure 2. UPGMA tree of *Leptonycteris yerbabuenae*, built with NADH (mitochondrial gene).

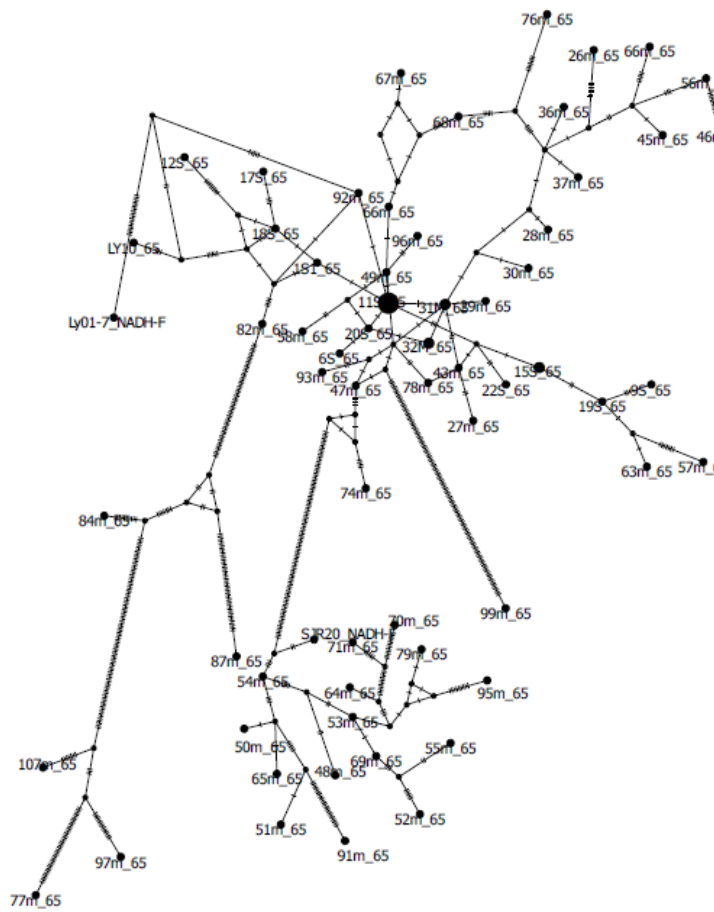


Figure 3. Haplotypic net (Medianjoining) based on NADH mitochondrial gene of *L. yerbabuena*.

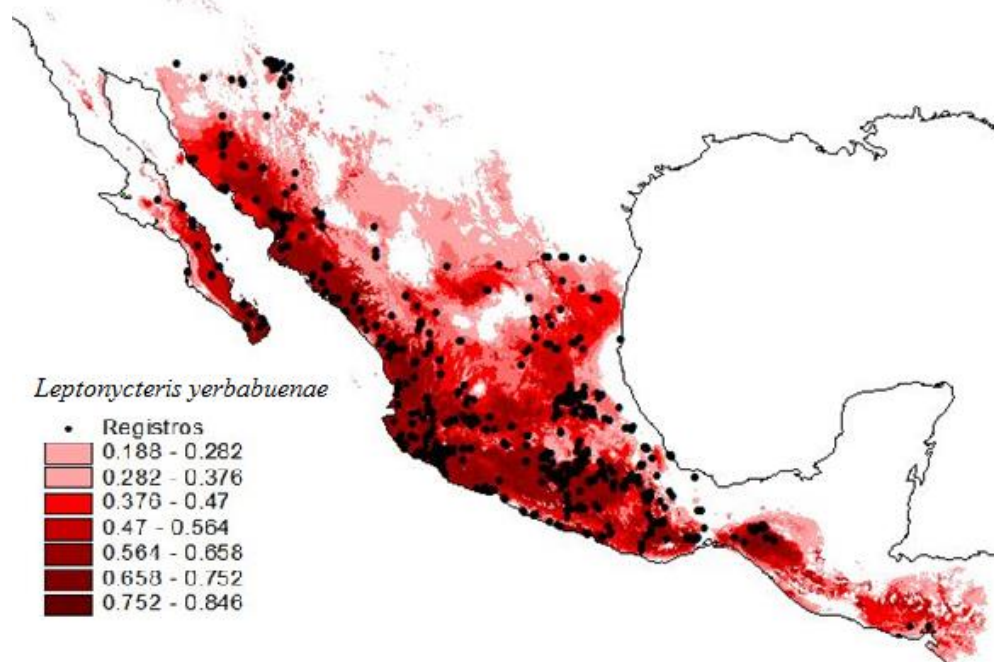


Figure 4. Present potential distribution model of *L. yerbabuena* (MaxEnt)



Left & Middle: Sampling in Tequila Route. Left: counting flowers and cutting a piece of leaf. Middle: wrapping and storing the biological material. Right: Landscape of Tequila plantations with inflorescences conserved.



Far left: Taking tissue sample of Agave tequilana. Middle left: Ing. Carlos Camarena is reviewing his plantations and mature inflorescences he conserved. Middle right: Mature fruit collected to obtain seed produced by natural bats pollination. Far right: Greenhouse built in "Tequila Ocho" lands (Photograph taken from Ing. Carlos Camarena's Facebook site).



Left: The fruit collected in tequila crops were dried in Institute of Ecology Greenhouse (UNAM). Middle & Right: First germinated plants in greenhouse.





Preparing mist nets to capture *Leptonycteris* bats in order to take tissue samples.



There was a meeting with local people in Hidalgo and I play with children didactic games showing the ecological role of nectar feeding bats.



Workshop organized by Natural History Museum Mexico City where I showed the Bat friendly project and partial results.



I gave oral presentation in communities visited, this picture show a chat with general public.