

## The Rufford Small Grants Foundation

### Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. 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. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to [jane@rufford.org](mailto:jane@rufford.org).

Thank you for your help.

Josh Cole

Grants Director

Grant Recipient Details	
<b>Your name</b>	Aly Veronica Valderrama Villarroel
<b>Project title</b>	Conservation biology of <i>Tilia mexicana</i> , an endangered medicinal tree
<b>RSG reference</b>	13765-1
<b>Reporting period</b>	January 2014 – March 2015
<b>Amount of grant</b>	5985
<b>Your email address</b>	avalderrama@cieco.unam.mx
<b>Date of this report</b>	March 31 <sup>th</sup> 2015

**1. Please 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
Evaluate the current conservation status of <i>T. mexicana</i> and will provide updated information on its distribution and localisation, as well as on the degree of threat imposed to the species by anthropogenic pressures like flower harvest, logging, deforestation, etc.			X	The development of the fieldwork on this project was useful to update the current distribution and population size of <i>Tilia mexicana</i> . The more recent herbarium information was from 1985, when the degree of disturbance to the populations was very different than at present. For this reason, the field work was hard, since in the CONABIO database there were many recorded localities in which we could not find the species. In total, we sampled 16 populations and 198 individuals from 10 states of Mexico.
The genetic data will allow understanding the geographic distribution of the genetic variability, and the level of gene flow among populations.			X	We successfully analysed the collected individuals with five universal chloroplast microsatellites (cpSSR) and eight specific nuclear microsatellites (nSSR).
The scientific data obtained in this research will be published in at least two scientific articles in high quality international journals.		X		The articles are in preparation. We are currently writing two manuscripts. The first one focuses on the demography and ecology of the 16 populations sampled. The second manuscript will describe the conservation genetics of the species in Mexico.
Additionally, the information will be transferred to the National Commission for Use and Knowledge of Biodiversity (CONABIO) of Mexico, so it can be utilised for designing conservation and management strategies for the species.		X		The transference of the new knowledge will be made when the final version of all the results are ready. The conservation management information will be transferred to the CONABIO (National Commission for Knowledge and Use of Biodiversity) and CONAFOR (National Forestry Commission). Additionally, we will present the results to wider audiences and local people through talks, social networks, newspaper articles and printed materials.

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

The main unforeseen difficulty was the frequent out-of-date records in the database information, both from CONABIO and from different herbariums in the country. This means that several populations of the species have recently disappeared, given the rapid rate of transformation of the *Tilia* habitat. For example, during the first two field work trips we did not find any *Tilia* trees. After that, we decided to visit localities in which other species of the mountain cloud forest were reported. This decision was fundamental for a successful field work.

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

The three most important outcomes of the project relate to: 1. a field evaluation of the current conservation status and threat degree of *Tilia mexicana* in Mexico on the basis of ecological data and interviews with local people, 2. the assessment of genetic variation, genetic structure and gene flow in these populations with nuclear and chloroplast microsatellites, and 3. the prioritisation of populations for conservation purposes, on the basis of the ecological, genetic and local management information. Each of these topics is presented with more detail below:

**1. Conservation status of *T. mexicana* in Mexico**

The field work was conducted from March to June 2014. We sampled 16 populations (Figure 1) which were found on different vegetation types, i.e. cloud forest, *Quercus* forest and *Pinus* forest. We collected 198 individuals. During the field work it was important to observe that considerable land-use changes have occurred in many of the localities listed in the database that we compiled from the records of CONABIO and INECOL. Consequently, several populations of *Tilia* have probably disappeared or reduced considerably in size.

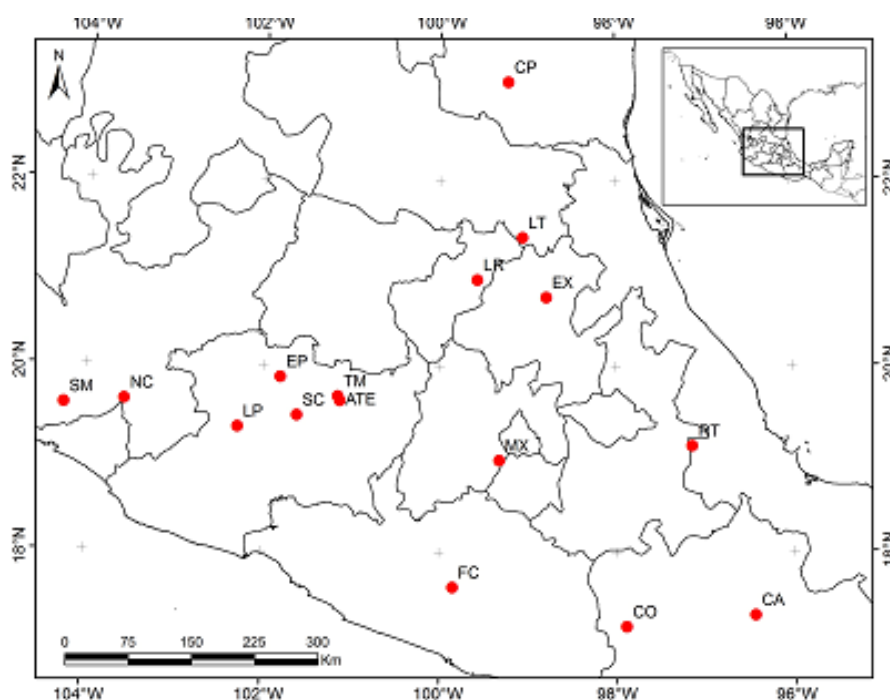


Figure 1. Map of localities of *Tilia mexicana* visited on this research. The locality codes are shown in Table 1.

Table 1. Localities of *Tilia mexicana* visited and collected during this research

Code	Locality	State	Longitude	Latitude
CA	Capulalpan	Oaxaca	96° 26' 53.42" W	17° 19' 5.70" N
CO	Camino a Oaxaca	Oaxaca	97° 53' 17.55" W	17° 11' 59.34" N
CP	Casa de Piedra	Tamaulipas	99° 13' 15.03" W	23° 3' 41.45" N
EP	El Pinal	Michoacán	101° 48' 11.72" W	19° 52' 35.84" N
EX	Eloxochitlan	Hidalgo	98° 47' 13.48" W	20° 44' 47.87" N
FC	Filo de Caballos	Guerrero	99° 50' 46.57" W	17° 37' 21.59" N
LP	La Peña	Michoacán	102° 16' 57.01" W	19° 20' 11.72" N
LR	Las Ranas	Querétaro	99° 34' 18.40" W	20° 55' 59.40" N
LT	La Trinidad	San Luis Potosí	99° 3' 25.64" W	21° 23' 19.02" N
MX	Mexicapan	México	99° 19' 13.91" W	18° 59' 21.07" N
NC	Nevado Colima	Jalisco	103° 33' 59.95" W	19° 37' 10.04" N
RT	Río Tlaxcoa	Veracruz	97° 8' 23.44" W	19° 8' 35.50" N
SC	Santa Clara del Cobre	Michoacán	101° 36' 50.06" W	19° 28' 1.98" N
SM	Sierra Manantlan	Jalisco	104° 14' 49.38" W	19° 33' 55.00" N
TM	Los Filtros	Michoacán	101° 9' 1.60" W	19° 40' 31.15" N
ATE	Atecuaro	Michoacán	101° 7' 34.95" W	19° 37' 37.84" N

Additionally, the population ecology data and the information provided by local inhabitants permitted us to rank populations according to their conservation status. The distribution of the values of diameter at breast height (DBH) indicated that the site with the lowest DBH was Santa Clara, Michoacán State with a mean of 2.1 cm, while in populations La Trinidad (San Luis Potosí), Las Ranas (Querétaro) and Eloxochitlán (Hidalgo) the mean DBH values were 6.1, 6.0 and 8.0 cm, respectively. These data were included into a data matrix along with a record of the following activities: logging, flower harvest, cattle grazing, farming inside the forest, knowledge of the species by the inhabitants and general conservation degree of the locality.

The localities with the highest perturbation degree according to the variables evaluated in the survey were: Casa Piedra (Tamaulipas), Mexicapan (México), Río Tlaxco (Veracruz) and Santa Clara (Michoacán) due to damage by logging; Atécuaro, El Pinal, La Peña, Los Filtros, Santa Clara (Michoacán), Capulalpan, Camino a Oaxaca (Oaxaca), Eloxochitlán (Hidalgo), Filo de Caballos (Guerrero), and Sierra de Manantlán (Jalisco) due to flower harvest; El Pinal, Santa Clara (Michoacán), Las Ranas (Querétaro), and Mexicapan (Mexico) due to damage by cattle grazing; La Peña (Michoacán) and Mexicapan (Mexico) due to farming inside the forest. Overall, the localities with the highest conservation status were Capulalpan (Oaxaca), Eloxochitlán (Hidalgo), Filo de Caballos (Guerrero), Las Ranas (Querétaro), La Trinidad (San Luis Potosí), Nevado de Colima and Sierra de Manantlán (Jalisco) (table 2).

Table 2. Mean diameter at breast height (DBH), mean number of branches and qualitative assessment of anthropogenic perturbation at each sampled *Tilia mexicana* site.

Code	State	DBH <sup>1</sup>	Nº branches <sup>1</sup>	Logging	Flowers Harv.	Cattle grazing	Farming	Knowledge by inhabitants	Conservation status
Ca	Oaxaca	11.6 ± 10.0	4 ± 2	0	1	0	0	0	1
Co	Oaxaca	63.1 ± 53.5	2 ± 1	0	1	0	0	0	0
Cp	Tamaulipas	12.3 ± 13.7	4 ± 3	1	0	0	0	0	0
Ep	Michoacan	24.8 ± 14.4	2 ± 1	0	1	1	0	0	0
Ex	Hidalgo	8.0 ± 10.1	5 ± 3	0	1	0	0	0	1
Fc	Guerrero	11.2 ± 16.6	6 ± 4	0	1	0	0	0	1
Lp	Michoacan	39.8 ± 28.6	1 ± 0	0	1	0	1	0	0
Lr	Queretaro	6.0 ± 5.6	7 ± 3	0	0	1	0	0	1
Lt	San Luis Potosi	6.1 ± 7.1	5 ± 4	0	0	0	0	1	1
Mx	Mexico	14.3 ± 22.2	3 ± 2	1	0	1	1	0	0
Nc	Jalisco	16.0 ± 15.6	4 ± 3	0	0	0	0	0	1
Rt	Veracruz	12.3 ± 13.5	4 ± 3	1	0	0	0	0	0
Sc	Michoacan	2.1 ± 5.4	1 ± 0	1	1	1	0	0	0
Sm	Jalisco	26.5 ± 23.1	3 ± 2	0	1	0	0	0	1

<sup>1</sup> Values showed as mean ± standard deviation.

## 2. Genetic diversity and structure

The genetic analysis was conducted using nuclear and chloroplast DNA microsatellite markers (nSSRs and cpSSRs, respectively). The level of genetic diversity determined with the cpSSRs was not very high (13 haplotypes in total) and the geographic distribution of haplotypes suggested high levels of historical gene flow through seed dispersal. The coefficient of genetic differentiation was low ( $G_{ST} = 0.093$ ).

The nSSRs also revealed a scenario of low genetic diversity within the Mexican populations of *Tilia*, with an average expected heterozygosity ( $H_E$ ) between 0.167 and 0.407. The level of genetic differentiation for the nuclear microsatellites was moderate ( $F_{ST} = 0.15$ ).

Combined, these results indicate that at present, the Mexican populations of *Tilia* maintain relatively low levels of genetic variation, which could be due to their small size and high degree of fragmentation. During historical times (probably during the cold periods of the Pleistocene) the level of genetic connectivity among *Tilia* populations was probably high (as indicated by genetic differentiation for cpSSRs), but at present is probably lower (as indicated by nSSRs).

## 3. Proposed conservation measures

We found that Mexican *Tilia* populations are threatened due to land-use change and direct logging. Activities such as cattle grazing are also harmful because of their impact on regeneration stages of the species. Interestingly, it would seem that flower extraction has a relatively low impact if performed moderately, and has the potential to provide a sustainable resource for local inhabitants.

## 4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

The execution of the fieldwork involved an informal participation of some members of the local communities. For example sometimes local inhabitants participated as field guides and also provided information during the course of the interviews. This information was extremely valuable for the project.

Interestingly, we found that a very different species *Ternstroemia sylvatica* is also consumed as an infusion in many of the localities and the fruit of this species is also named “Flor de Tilia (Tilia flower)”. However, there are health risks in the use of *T. sylvatica* since it causes hypoxia in blood. During the course of our field work we explained to the local inhabitants the differences between the two species and warned them about the use of *T. sylvatica*.

Finally, we expect that in the long-term the local inhabitants will benefit from the results of this research because it will provide information for the conservation and sustainable use of this valuable resource.

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

Yes. The results obtained with the support of this grant are very promissory scientifically and in terms of their potential applications to the management and conservation of *Tilia* populations in Mexico. It would be very important to perform exhaustive field surveys to determine accurately the

current distribution range of *Tilia mexicana* and to establish the number of populations still present and their census sizes. The analysis of further samples with other molecular markers (for example chloroplast and nuclear DNA sequences) would provide more detail about the population history of the species in Mexico. On the other hand, Pigott (2012) proposed the existence of three subspecies of *Tilia* in Mexico and our present and future work will contribute to clarify this taxonomic issue.

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

Our intention is to publish two scientific articles, with the first of them focusing on the ecology of the species and the second on the phylogeography and conservation genetics of *Tilia* in Mexico. We have made significant progress in the preparation of both manuscripts and we expect to send them for review within the next 3 months.

In addition, we will write two or three articles for the general public and we are planning to use social networks to share some of the results. Finally, we will produce printed material and will conduct workshops for the local inhabitants in nearby communities as a part of an ecological education program.

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

We started the activities for our Rufford Small Grant on March 2014 because *Tilia* starts producing new leaves in the spring and during the previous January and February it was not possible to collect the samples. As explained above, the field work was more difficult and time consuming than anticipated. The laboratory work also offered some challenges since, for example, the DNA extraction technique required a lot of optimisation to obtain DNA with enough quality to perform the analyses. For this reasons we request three extra months to finalise the products of the project (two scientific manuscript and the dissemination articles and workshops).

#### 8. Budget: Please 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.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Field work (£ 105/day) Fuel, foods, accommodation and road taxes for 4 people in 25 days	2625	3166	-541	Due to the trouble to found the <i>Tilia</i> localities, the field work was longer than estimated.
Storage material for plant samples (bags, tubes, cooler, etc) (300 units)	100	38	+62	
Liquid nitrogen to grind samples (20 L)	210	210	0	
Forest diametric steel tape	120	90	+30	The cost was higher than estimated.
Nuclear Microsatellite synthesis (12 primers) with fluorescent labels (FAM and VIC and NED and PET)	1250	0	+1250	These were paid with money from other project



Laboratory material (mortar, electrophoresis reagents, ladder, etc.)	500	500	0	
PCR reagents	350	687	-337	The cost was lower than estimated
Sequencer reagents (LIZ, formamide, etc.)	830	912	-82	The cost was higher than estimated.
<b>TOTAL</b>	5985	<b>5603</b>	+382	

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

The first important next step would be to continue with the scientific research of *Tilia mexicana* to elucidate the historical colonization process of the species. Another important aspect would be to understand the ecological associations and interactions of *Tilia* with other species at a community level, to gain a better understanding on which would be the best strategies for the conservation of these endangered ecosystems.

In addition, it would be very important to contribute further to elaborate local programs of environmental education directed to young local inhabitants emphasizing the relevance preserving cloud forests and their constituent species, such as *Tilia*.

### 10. Did you use the RSGF logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

The RSGF logo was used in all the results of the Project. Specifically, in the Latinoamerican Botanical Congress in Brazil, we showed a poster with preliminary results of the Project and we used the RSGF logo in the head section.

