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Evolution, history and conservation of two species of sea turtle in the coast of Oaxaca, Mexico

Progress Report II

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1.- Advances

The field work for this project started the 27 of July 2021, we collected tissue samples of 30 olive ridley turtles In Oaxaca, Mexico. Of those samples, 15 were taken in La Escobilla beach that is a sanctuary to sea turtles' conservation. The other 15 samples were taken in Morro Ayuta in the community of Río Seco, that beach is another important place for these sea turtle's nesting process. The importance of both beaches to this species is that these are places were the phenomenon of "arribada" occurs. In here thousands of females emerge from the sea to the beach at the same time to lay its eggs and thus minimize the risk of mortality due to predation. With this nesting strategy the olive ridley increase the probability of survivor for the hatchlings.

Figure 1. An olive Ridley turtle emerging from the sea to nests in the Morro Ayuta beach.

First, we did nocturnal surveys together with the staff of the turtle camps, whom oversee monitoring and caring for the nesting sea turtles that come to lay its eggs on the beaches. They work for the CONANP that is a governmental institute in charge of administrating the natural protected areas in Mexico. In the nocturnal surveys we first located the nesting females and then we proceeded to approach to

the turtle and if it was already nesting, we began to take the sample. The collected tissue was taken with a 6 mm biopsy punch. First, we wait until the female start to lay its eggs to proceed with the sample, this mainly to not scare the turtle before it began its nesting process. At the time of sampling, the skin of the sea turtle was cleaned with an antibenzyl solution to avoid possible infections. Subsequently, a piece of skin was extracted with the biopsy punch and finally the skin was cleaned with antibenzyl once again.

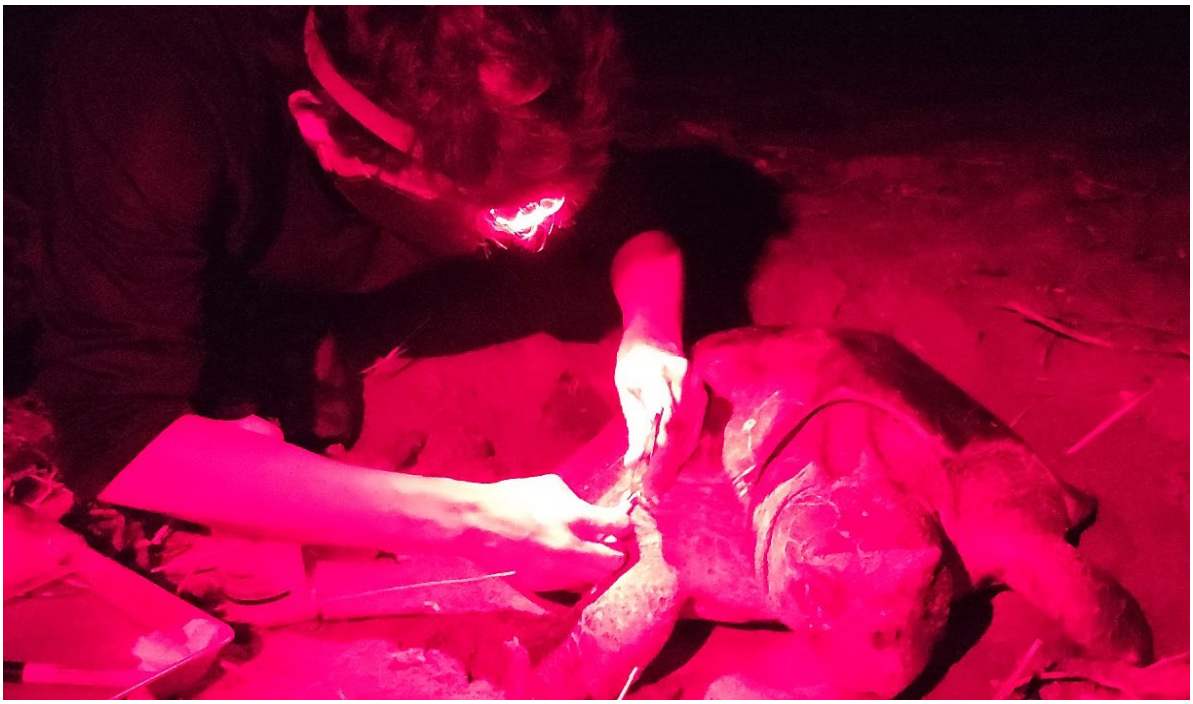

Figure 2. The process of collect the tissue samples in an olive ridley turtle.

After collecting the samples, we take them to the ECOSUR genetics laboratory and start the processing for the genetic analyses. First the DNA isolation was performed using the Phenol-Chloroform-Isoamyl Alcohol method. After extracting the DNA from the 30 samples, we use the PCR technique to amplify the DNA regions of interest. One of these regions is the mitochondrial DNA (mtDNA) control region or d-loop. The study of this region is important because in animals it is only maternally inherited, also lacks genetic recombination, it has a rapid mutation rate and has polymorphic nature. The control region is the most variable region of the mitochondrial genome, and this help us to study the molecular evolution, the population structure and even help us in conservation and migration studies. For mitochondrial control region in the olive ridley we used the H950 and LTEi9 primers designed by Abreu-Grobois et al 2006.

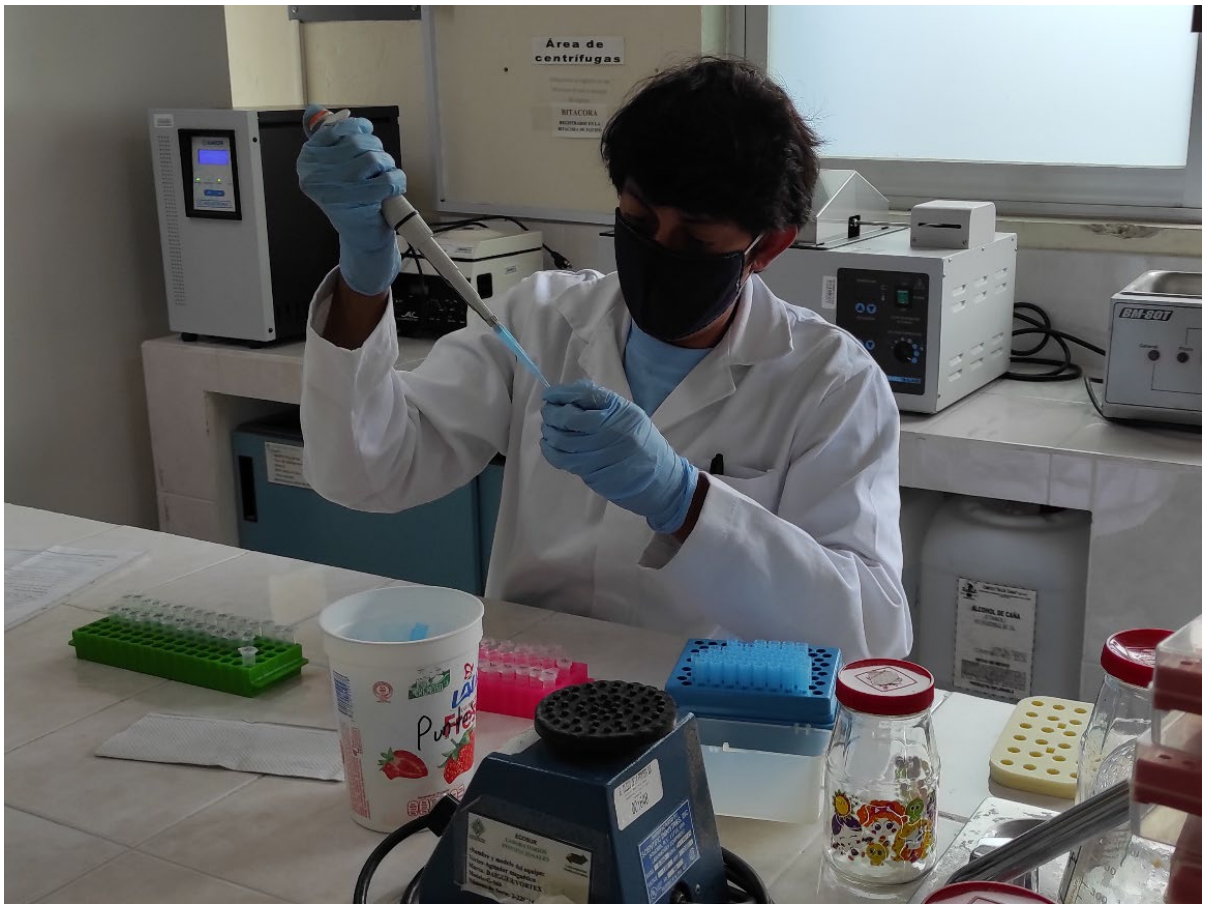

Figure 3. Part of the DNA extraction process.

At the same time, we are using 15 microsatellites to study this species at genetic level. The microsatellites could help us to understand the population structure, the kinship between individuals, the genetic diversity and the recent population's history. From these 15 microsatellites (Table 1) six (all with Or-n) were original designed for the olive ridley, seven (all the LB and the 14_15) were original designed for the leatherback and the other two (Cm84 and Ei8) for green turtle (*Chelonia mydas*) and the hawksbill sea turtle (*Eretmochelys imbricata*) respectively.

Nuclear DNA Microsatellites		
Or-1	**Or-2**	**Or-4**
Or-7	**Or-9**	**Or-14**
Cm84	**Ei8**	**14_15**
LB99	**LB106**	**LB128**
LB141	**LB142**	**LB133**

Table 1. The 15 microsatellites used in this study.

On November 22 of 2021 we started our field season to collect leatherback's tissue. We collected 30 samples in two beaches of Oaxaca. For this species we

visited Cahuitán and Barra de la Cruz communities. Both are considered “playas índice”- index beaches- a category used in Mexico for the main beaches where sea turtles’ nests and have been monitored since the 1990s. These places are two of the main nesting areas in the country, where some of the biggest nesting processes for the leatherback in Mexico have been monitored for almost 3 decades by CONANP. With this species the process for collect the tissue was like the one described for the Olive Ridley Turtle. We did the night survey accompanied by the official rangers protecting these turtles. As for the olive ridley we wait until the female start to lay the eggs to approach it. In this case when we found a leatherback, we proceed to put a metallic tag in their rear flipper and a PIT (Passive Integrated Transponders) in the front shoulder for future identifications of the turtle.

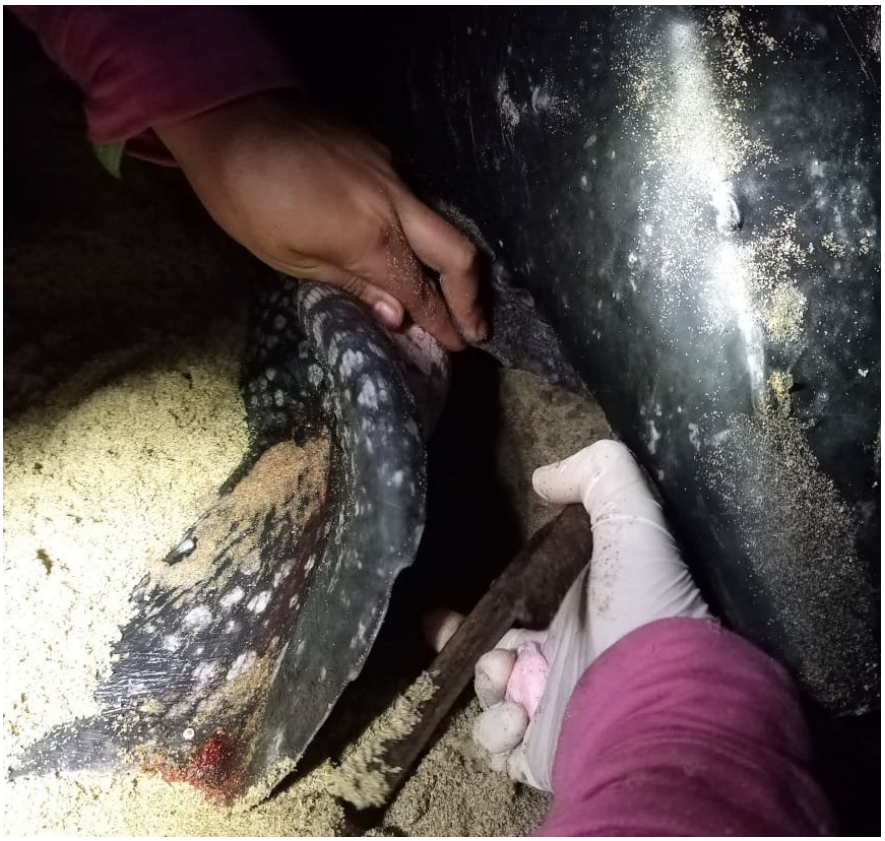

Figure 4. CONANP’s staff placing a metallic tag in the rear flipper of a leatherback female.

When we conclude the collection of leatherback tissue, we take the samples to ECOSUR’s genetic lab, where we started with the DNA isolation using the same method as the one used for the olive ridley. At the moment that I’m writing this we have finished the DNA isolation with success for both species, and we start to do the PCR for the mtDNA control region amplification. For the olive ridley we have finished the mtDNA amplification and we have a progress about 50% in

microsatellites amplification. For leatherback, once we finish with the mtDNA control region, we will start to amplify with the microsatellites at the nuclear DNA.

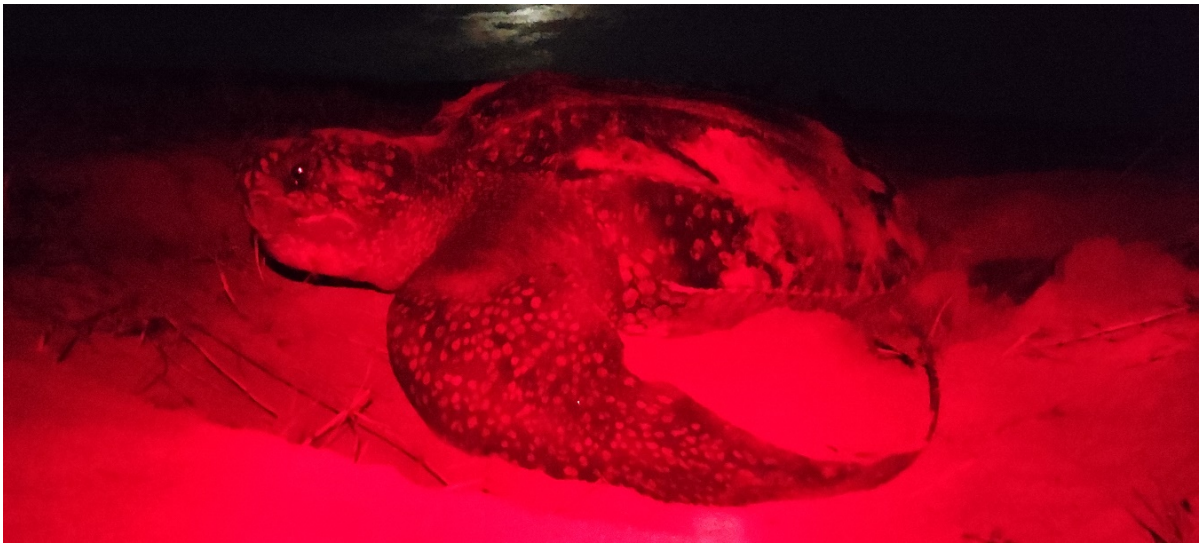

Figure 5. A leatherback turtle finishing the nest.

We also conducted two interviews in Barra de la Cruz with older people to learn about the abundance, size and the human exploitation of the nesting sea turtles of the area in the past. Through these interviews we learned that the population's numbers have decreased over time by orders of magnitude which is not properly assessed with the current monitoring. We also learned that in the past the exploitation was higher than in the present and that patterns of beach fidelity might have change over time with different species. In the next months we will make some other interviews and insight will be duly presented in the future.

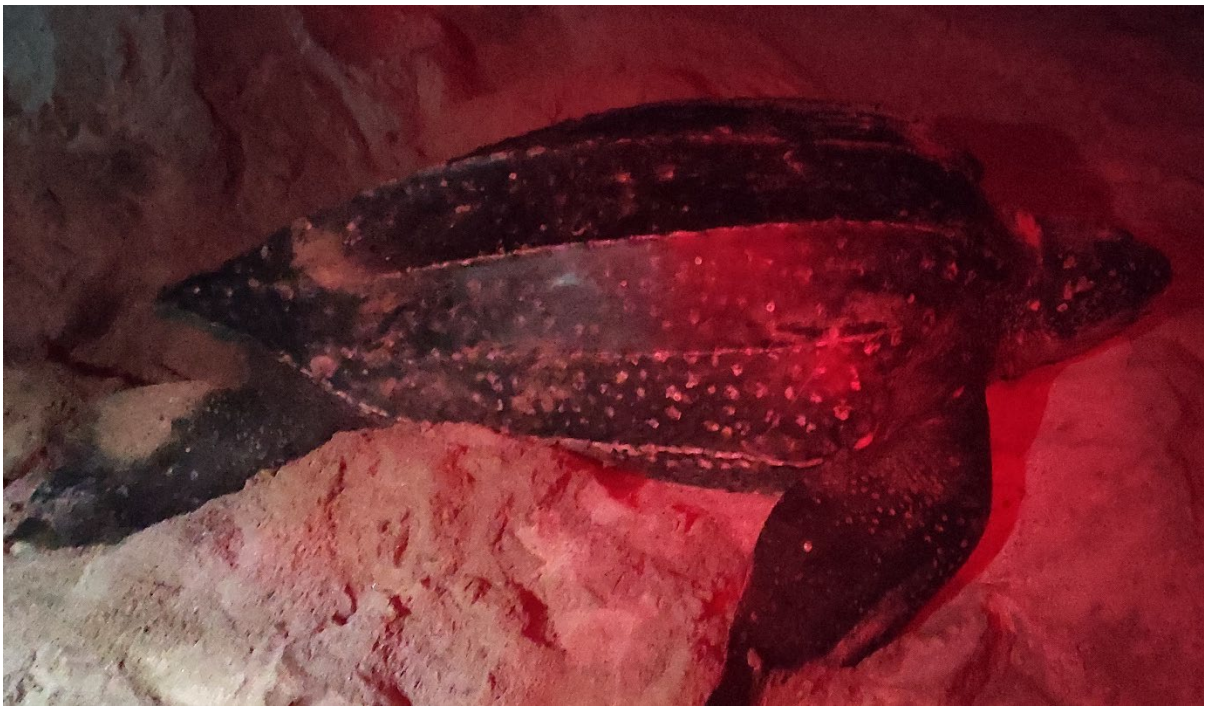

Figure 6. A leatherback sea turtle that has just finished its nest.

2.- Obstacles to the original Plan

Some obstacles have been founded in this project since we presented the idea to the foundation. Due to the COVID pandemic many things have changed. First the collection permit was delayed and, because we are working with endangered species, it is impossible (rather illegal) to take the tissue sample or even manipulate these animals without a permit. So, for this reason we lost the opportunity of collect the tissue sample for the leatherback in the 2020-2021 season. We had to wait until the 2021-2022 nesting season for leatherback start, to begin with the work field for this species. For this reason, our project has been delayed, anyway we fully trust that the project will be concluded successfully this year.

3.- Plans for the future

We are planning to submit my thesis first, and then to organize the workshop with experts. For the workshop, beyond the results of my thesis we will like to synthetize some of the observations provided by local people, so we can discuss this in the workshop with scientist and managers. We think we will have the results of the workshops by summer and submit an article with the results of my thesis and the insight provide by all stakeholders during the workshop

At the end of this project, I will continue working in the conservation of sea turtles, I would like to continue researching about the genetic diversity of the leatherback

turtle in my country and even in other countries in Latin America. At the present I'm applying for a PhD at El Colegio de La Frontera Sur in Mexico. In this part of my career, I will continue researching about sea turtles' conservation that is an important species for the coastal and marine ecosystems. I would like to continue gathering genetic information and local ecological knowledge, which in combination might bring fresh insight to help preserving these species.