Project Update: April 2019

Achievements to date:

To collect new samples of Galapagos shark (*Carcharhinus galapagensis*)

- Partially achieved - After a careful consideration of high genetic connectivity between San Cristóbal and Santa Cruz, we decided to sample in Isabela and San Cristóbal only. We have successfully sampled San Cristóbal, and waited until preliminary analyses will reveal the technique was appropriate before sampling the next location in Isabela. The following trip will take place during May and will comprise one location in Isabela Island (Roca Unión).

To extract genomic DNA and perform PCR

- Partially achieved - DNA was successfully extracted from all samples at the Galapagos Science Center facility. PCRs are in progress

mtDNA sequencing

- Partially achieved - A first batch of samples was sent for mtDNA sequencing to check on the quality of the PCR product. Once the results are back from the sequencing facility and the PCRs are completed in the lab, we will send a second batch with all the remaining samples.

SNP sequencing

- Not achieved - Waiting for permits to be renewed to be able to export the samples for SNP sequencing.

Data analyses

- Partially achieved - Preliminary analyses were required to identify the SNPs with the potential to detect origin of the samples. Unfortunately, we found that only the Galapagos population possess the strong unique genetic signature required to inform about provenience. And even for the Galapagos population, the validation of the markers (SNPs position and function within the genome) requires a reference genome. We have used all shark genomes available to try to accomplish these goals. However, we have not been successful.
  All these preliminary analyses, however, brought a very important finding, that can lead to the development of a different, but equally useful forensic tool.

Results communication

- Not achieved - Results have not been communicated yet

Difficulties so far:

We have had four major difficulties during the development of the project:

1) Some of the laboratory reagents (including the DNA extraction Kit – QIAGEN DNeasy tissue and blood extraction kit) used for the lab work came from international providers, and took 4 months to arrive to the Galapagos, delaying the very first Laboratory procedure.
2) The permits used for the collection of the samples (permits granted by the Ministry of Environment of Ecuador) are currently under a renovation process,

and as a result of this, all export activities (needed to send samples for sequencing at international facilities) are delayed until the process is completed.

3) Preliminary analyses showed the only population in the Eastern Pacific with a genetic signature capable to show provenience is the Galápagos population. Meaning we can only tell (with intermediate confidence) if a sample was caught in the Galápagos or not, but not determine the origin from other locations unless we are capable to increase the resolution of the data and potentially target specific regions of the genome, and for that we need a much larger investment.

Outcomes so far:

I describe the major finding on this ongoing project bellow:
During our effort to identify provenience markers, we were able to find high resolution markers capable to tell *Carcharhinus galapagensis* and *C. obscururs* apart. While for many other shark species mitochondrial DNA provides enough resolution to define taxonomy from a simple fin clip sample, this is NOT the case for these two species. The divergence between these closely related species is so recent, and the morphological similarities so high, that mitochondrial DNA is not capable to distinguish one from the other. We have investigated further the potential of SNPs for this purpose and are currently developing a PCR-based essay that can be used for forensics purposes in the future to distinguish these two species from carcases or body parts. This tool will be useful to enhance conservation efforts and to avoid erroneous data on landings, which will also be useful to update the vulnerability status of both species.

Continuation/ Next Steps:

Yes, now that we have identified the lack of resolution to identify provenience from places other than the Galápagos Islands, we aim at increasing our sampling in other Eastern Pacific areas as well as increasing the genomic resources from this locations in order to improve the resolution of our data, and sequencing a draft reference genome for a *C.galapagensis* individual. This to achieve two main goals:

1. To expand our knowledge on evolutionary and local adaptation processes of the species within the Eastern Tropical Pacific.
2. To validate the markers currently identified in order to confidently define provenience from Galápagos by identifying the function and position of the SNPs within the genome. Even if restricted to the Galápagos Islands, this is still a valuable research as we are still finding new cases of illegal shark fishing in waters of the Galápagos Marine Reserve.

Important next steps for the development of the tool to differentiate these two species is to ensure proper communication and training for those end users of such tool. Next steps to accomplish our original objective include the sequence of an entire reference genome (or at least a draft) to confidently validate the origin traceability markers for the Galapagos population. This requires a larger investment but is well justified due to the multiple applications of having an entire genome for a Carcharhinid species (e.g. evolutionary and local adaptation studies, development of applied conservation tools, etc.).

Communities and Sharing:

The potential of this applied tool goes beyond the local community, as these species are widely distributed and caught in fisheries around the world. Once completed, this tool can be used for landing assessments of fisheries in areas where the two species are caught.

Strategies to communicate the results include:

1. Results will be presented at the Galapagos Science Centre Symposium to be held in 2020, and discussed with governmental institutions involved in fisheries and conservation in the Galapagos (e.g. Galapagos National Park Authority).
2. Additionally, we will publish a bilingual case study on Galapagos Conservation Trust's (GCT) educational website, Discovering Galapagos (DG) (average monthly usership of 3,500 in the UK and 9,000 in Ecuador).
3. Finally, we will work in the promotion of resources via partners' social media (twitter/Facebook/ Instagram) and Galápagos Science Centre email communications with supporting media (video clips/images/infographics).

Timescale:

I have requested a 6-month extension for the project due to the challenges described in section 2.