[Anonymized Student Name]

YSEA Undergraduate Grant Proposal

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EXTINCTION IS NOT FOREVER: LOOKING FOR A LOST LINEAGE OF GIANT GALAPAGOS TORTOISES

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Home to the iconic Galapagos tortoise, the Galapagos Archipelago offers evolutionary biologists an ideal location to study evolutionary history and diversification within species. The Galapagos Archipelago consists of 13 main islands located in the Pacific Ocean about 900 km off the coast of South America. Throughout the Archipelago's history, the chain has experienced major geographical changes with islands connecting, splitting, and drifting apart (Ali and Aitchison 2014). These changes significantly impacted the colonization of native species, such as the Galapagos Giant Tortoise.

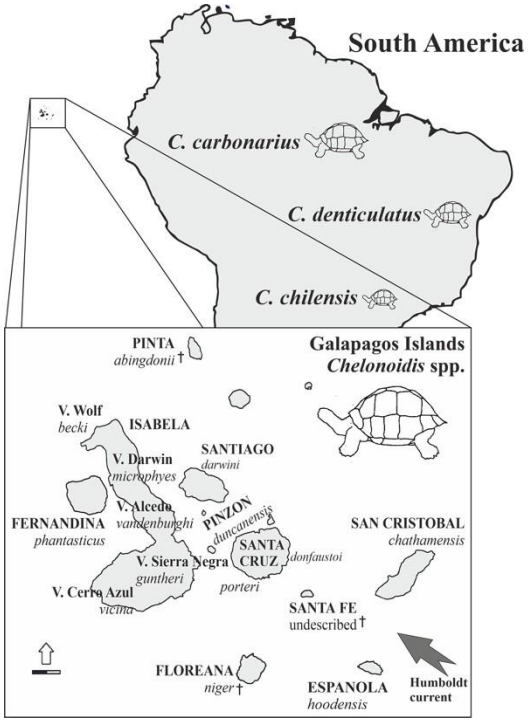

Figure 1: Map of South America and the Galapagos Archipelago with Galapagos Tortoise species in the respective islands. Extinct Species appear with a black cross beside their name. The dark gray arrow represents the Humboldt current that brought tortoises to the island. Figure Courtesy of N. Poulakakis (Caccone 2021)

Giant Galapagos tortoises include a recently diverged group of 15 species, all belonging to the genus *Chelonoidis*. These unique animals are the largest extant cold-blooded terrestrial vertebrate, and they can weigh up to 400 pounds and live for more than 120 years. The 15 species vary in morphology, with the most striking variation being in the carapace shape. The shapes range from completely round in some species to a curved, saddle-like shape in others (Galapagos means saddle in Spanish). These two forms are called domed and saddleback. Some species also have intermediate carapace shapes. Typically, only one species of tortoise lives on an island. However, the islands of Isabela and Santa Cruz have 4 and 2 species respectively. Three of the 15 species are now extinct, while several others have experienced dramatic declines due to anthropogenic effects, such as human harvesting for food, invasive species eating eggs or young, and competition for food resources. Current population estimates report that the population of tortoises in the Galapagos is now 10% of its historical size (Cayot 2008). Giant tortoises first arrived on the islands via the Humboldt ocean current from continental South America. Their colonization of the archipelago likely started from San Cristobal, as it is one of the oldest emerged islands in the Galapagos chain (Caccone 2021). Because of this, understanding the genetic history of tortoises on the island of San Cristobal is vital in constructing a clear picture of the colonization and diversification of the tortoises in the archipelago. Modern tortoises on San Cristobal—*Chelonoidis chatamenis*—are described as having an intermediate and varied carapace morphology. In the 1970s the population of tortoises on the island bottlenecked at around 500-700 individuals, but, after successful countermeasures such as a breeding program, San Cristobal's population has managed to bounce back to about 6,700 tortoises (Cayot 2008: Tapia et al. 2020).

Recently, a study analyzing the mitochondrial DNA of tortoises on San Cristobal found that bone remains from a cave on the island carried a different mitochondrial DNA (mtDNA) sequences than the extant tortoises living on San Cristobal, which all have only one mtDNA haplotype. This lack of mtDNA haplotype diversity in the extant tortoises is most likely due to the previous bottleneck (Caccone 2021). The five cave samples' mtDNA haplotypes were distantly related to the haplotype of the contemporary population. This raises the possibility of two distinct groups of Galapagos tortoises existing on San Cristobal in the past, with the cave samples belonging to a different, now extinct, species (Caccone 2021).

Figure 2: Tortoise on a Volcano Wolf Slope Photo by P. Gibbons (Caccone 2021)

This preliminary data prompted the Galapagos National Park to organize an expedition to verify if the putative second species is truly extinct. Since an island-wide survey had not been completed before, the expedition took blood samples from tortoises across San Cristobal. These samples included tortoises in impervious locations that could be descendants of the possibly extinct taxon. The survey resulted in ~200 tortoises blood samples, which recently arrived in the Laboratory of Dr. Caccone and are ready for analyses.

My project will focus on screening contemporary tortoises from San Cristobal to look for individuals with the extinct mitochondrial haplotype found in the cave samples. Using the new blood samples received from San Cristobal, I will carry out mitochondrial DNA sequencing of the same DNA fragment analyzed in the previous study. This data will then be analyzed together with the homologous DNA fragment from the cave and extant samples already available, allowing to test the hypothesis. This research will be carried out in the Caccone laboratory, where protocols for doing this have been already developed, and I will have the support to learn how to collect the genetic data, carry out the statistical analyses, and write a scientific report of my findings.

As a first-year, this research will provide me with valuable lab experience and will help prepare me for continued research throughout my time at Yale and beyond. Through the project, I will be able to build basic lab skills such as pipetting, DNA extraction, gel electrophoresis, PCR amplification, DNA sequencing, and population genetic analyses. These skills will provide me with a starting tool box to explore future projects in evolutionary biology and conservation, a field that deeply interests me.

References

Ali, J., & Aitchison, J. (2014). Exploring the combined role of eustasy and oceanic island thermal subsidence in shaping biodiversity on the Galápagos. *Journal of Biogeography, 41*(7), 1227-1241. Retrieved January 29, 2021, from http://www.jstor.org/stable/24035276

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Cayot L (2008) The restoration of giant tortoise and land iguana populations in Galápagos. Galápagos Res 65:39-43.

Chiari Y (2020) Morphology. In: Gibbs JP, Cayot L, Tapia A W, editors. Galapagos Giant Tortoises. New York: Elsevier. p.

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Total Budget for 200 samples: $ 1, 200

Itemized Budget:

DNA Extraction and quality control: $200 ($1/individual)

PCR Amplification of one mtDNA fragment and quality control: $400 ($2/individual)

DNA Sequencing of one PCR fragment in both directions: $600 ($3/individual)