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Crocodile Specialist Group IUCN-SSC Student Research Assistance Scheme (SRAS)

PHYLOGEOGRAPHY AND TESTING PARALLEL EVOLUTION AND ADAPTATION IN NEOTROPICAL CROCODILES

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CONCEPTUAL FRAMEWORK AND MAJOR RESEARCH QUESTION | Speciation studies have been a major topic of study in the last three decades increasing in an exponential way since the 80's[1] and research in new non-model systems continue to understand the process of speciation[2]. In a strict sense, speciation occurs when groups of populations that interbreed become reproductively isolated by geographical, ecological, and/or genetic barriers[3]. From this, an interesting question arises: How do species as discrete entities evolve from a population with limited or continuous variation? It can be seen as a process of gradual addition of genetic changes and reproductive isolation[4]. A helpful analytical way to measure this phenomenon is the "speciation continuum" - a model that considers successive stages of differentiation between populations to quantitatively examine the speciation process[5]. In the special case of ecological speciation[6], divergent selection caused by various ecological factors strongly influences the populations (i.e., future species) along the speciation continuum. An approach for studying speciation is the four states of the speciation continuum[4].

The proposed project investigates different states of the speciation continuum in Neotropical crocodile populations integrating climate, geography, and genetics to detect evolutionary patterns and ecological processes. The Neotropical *Crocodylus* genus ranges from Florida to Venezuela on the Atlantic coasts, from Baja to Ecuador on the Pacific coasts, and several inland regions in between. One species that ranges over much of Mexico and Pacific central America is *Crocodylus acutus*. My research focuses on answering three questions with this species.

1. Does the wide distribution of Crocodylus correspond to local adaptation across the wide variety of climatic conditions over its range? To understand the process and patterns of gene flow and local adaptations, detailed knowledge of how landscape characteristics structure populations is required[7]. Crocodile populations in the Neotropics are present in a variety of latitudes, altitudes, water conditions (salt, brackish, soft), and from inland to coastal locations[8]. This geographic range provides great potential to explore how landscape characteristics relate to population genetics and evolutionary patterns of these species.

2. Is genetic divergence associated to adaptation to different environmental conditions? Ecological speciation and local adaptation can be tested by an ecological, reproductive isolation, and genetic distance approach (ERG)[5]. I will test if crocodile populations have strongly discontinuous variation with strong but reversible reproductive isolation and local adaptation. The research will use Restriction site associated DNA (RAD) sequencing and SNP's, and will be prioritized by the bioclimatic and geographic analysis.

3. Do these populations exhibit parallel evolution? The broad geographical range of *C. acutus* is spread across multiple Pacific and Atlantic localities with similar environmental conditions. The species colonized the Pacific regions via the former isthmus of Panama. Thus, this radiation can be used to test if migrations to similar environmental conditions support a model of parallel evolution of these populations. The RAD sequencing results will be used to establish high resolution genetic differences and similarities between Pacific and Atlantic populations in similar environments. The bioclimatic data will be used to determine which Pacific-Atlantic comparison pairs live in similar climate regimes.

RESEARCH PLAN AND METHODS | I used Single Nucleotide Polymorphism's to understand how environment drives genetic local adaptation. I focused on three main objectives to begin this research. Each are centered on the Yucatan Peninsula populations to test the sequencing strategies and degree of data resolution.

Objective 1: Analyze and model the distribution of Neotropical crocodiles. To analyze and model the distribution of crocodiles a Geographical Information System (GIS), R[9] and the environmental layers from WORLDCLIM[10] will be used to obtain data about all the known localities. Potential distributions will be generated using different prediction algorithms. The data will be analyzed to construct climatic zones throughout crocodile's distribution.

Objective 2: Prepare, sequence and analyze C. acutus RAD libraries to detect variable nucleotide sites associated to local adaptation. I will use RAD seq methods[11]. With this method hundred to thousands of SNP's can be obtained at a moderate cost and applied to non-model organisms[12]. The information will be mapped back to the available C. *porosus* reference genome[13].

Objective 3: Analyze the genetic structure of C. acutus populations in the Yucatan Penisnsula. These will be measured based on the RADseq data retrieved.

Objective 4: Detect parental and pure populations of C. acutus in the Yucatan Peninsula. Test for genomic divergence of coastal versus insular *C. acutus* populations using RADseq data.

PRELIMINARY RESULTS| I analyzed the distribution of the species based on the 439 localities reported by Thobjarnarson *et al*.[14]. Most of the variance in the distribution is explained by the precipitation (Fig.1) And the bioclimatic profile of the species for 16 climatic variables (Table 1).

I analyzed 48 samples from five populations in the Yucatan Peninsula (Fig. 2). More than 25,000 alleles were retrieved for each population and all of them are mapped to the *C. porosus* genome (Fig. 3). Only 691 alleles contribute most to the diversity within populations (Fig. 5). Using Bayesian inference methods, I detected 4 genetic clusters (Fig. 6). The island populations (Cozumel and Banco Chinchorro Islands) are well differentiated from the coastal populations (Fig. 7). The population of Banco Chinchorro Island is the most differentiated populations and has the longest branch length (Figs. 8 and 9).

FIGURES

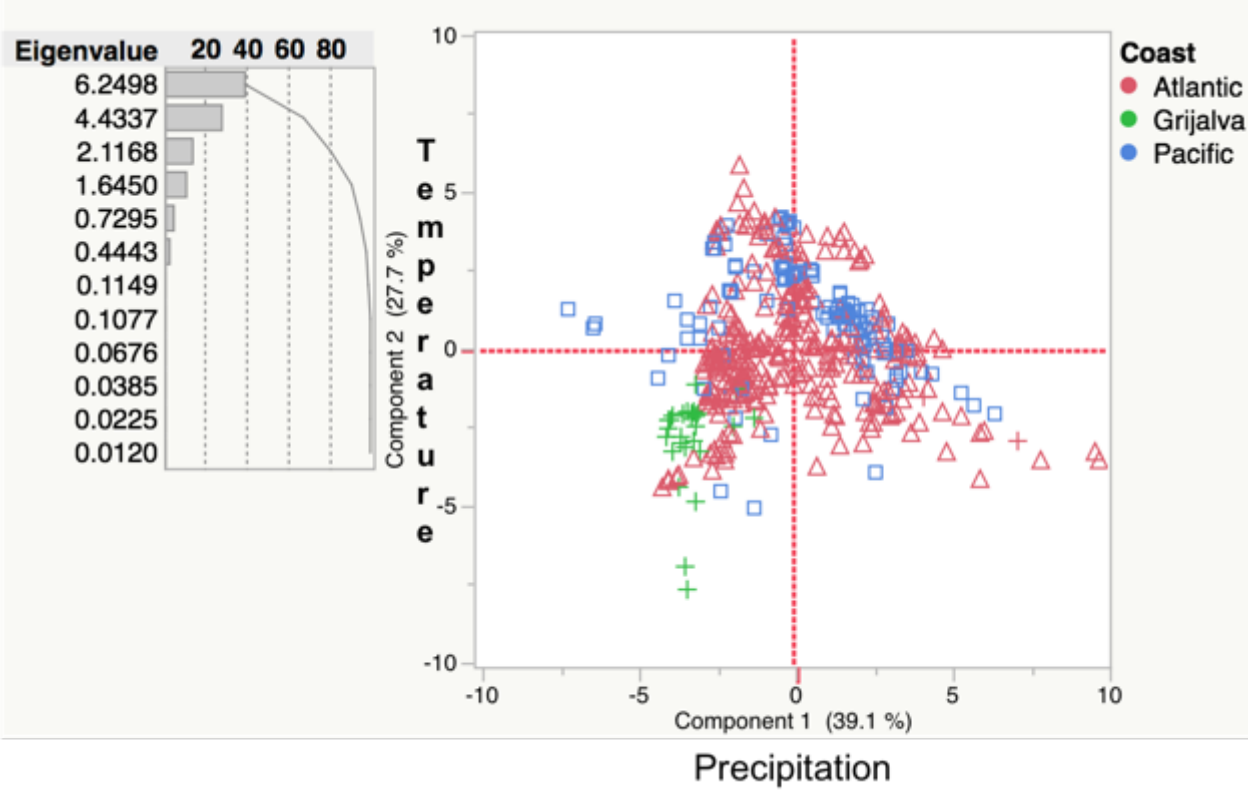


Figure 1. Principal Component Analysis of the bioclimatic conditions of 16 climatic variables of the distribution of *C. acutus*

Table 1. Bioclimatic profile of *C. acutus*

TEMPERATURE	Annual Mean Temperature	Mean Diurnal Range	Max Temperature of Warmest Month	Min Temperature of Coldest Month	Temperature Annual Range	Mean Temperature of Wettest Quarter	Mean Temperature of Driest Quarter	Mean Temperature of Warmest Quarter	Mean Temperature of Coldest Quarter
Average	26.09	9.76	32.98	19.06	13.91	26.67	25.23	27.51	24.37
Max	28.90	15.00	37.40	23.40	25.00	31.20	28.50	31.20	27.80
Min	21.20	5.90	29.10	11.10	6.90	22.30	18.80	22.90	17.80
PRECIPITATION	Annual Precipitation	Precipitation of Wettest Month	Precipitation of Driest Month	Precipitation of Wettest Quarter	Precipitation of Driest Quarter	Precipitation of Warmest Quarter	Precipitation of Coldest Quarter		
Average	1789.179954	301.0273349	29.21184501	777.4259681	114.0227709	434.1457859	379.3895216		
Max	5760	783	186	2058	735	1525	1981		
Min	173	57	0	128	0	22	0		



Figure 2. Sampled. *C.acutus* populations: Cozumel, Reserva de la Biosfera Sian ka'an (RBSK), Xcalak, Huach, and Banco Chinchorro (BCH).

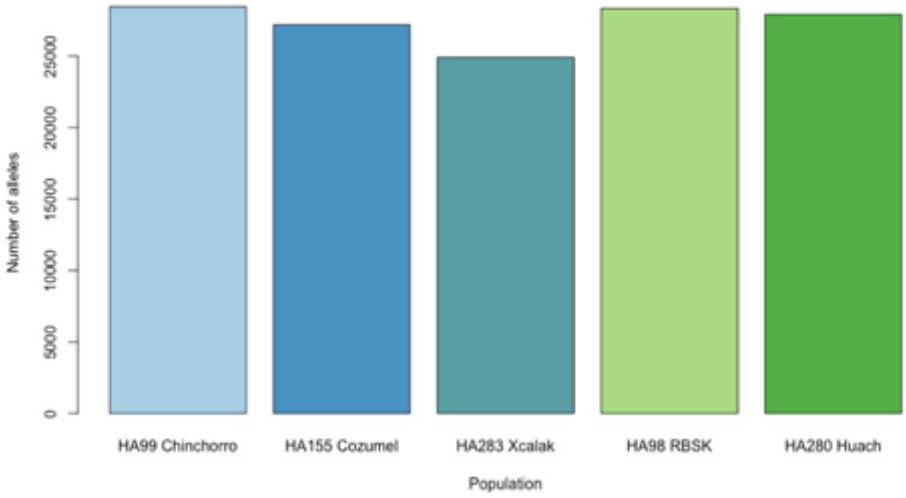


Figure 3. Number of alleles per population.

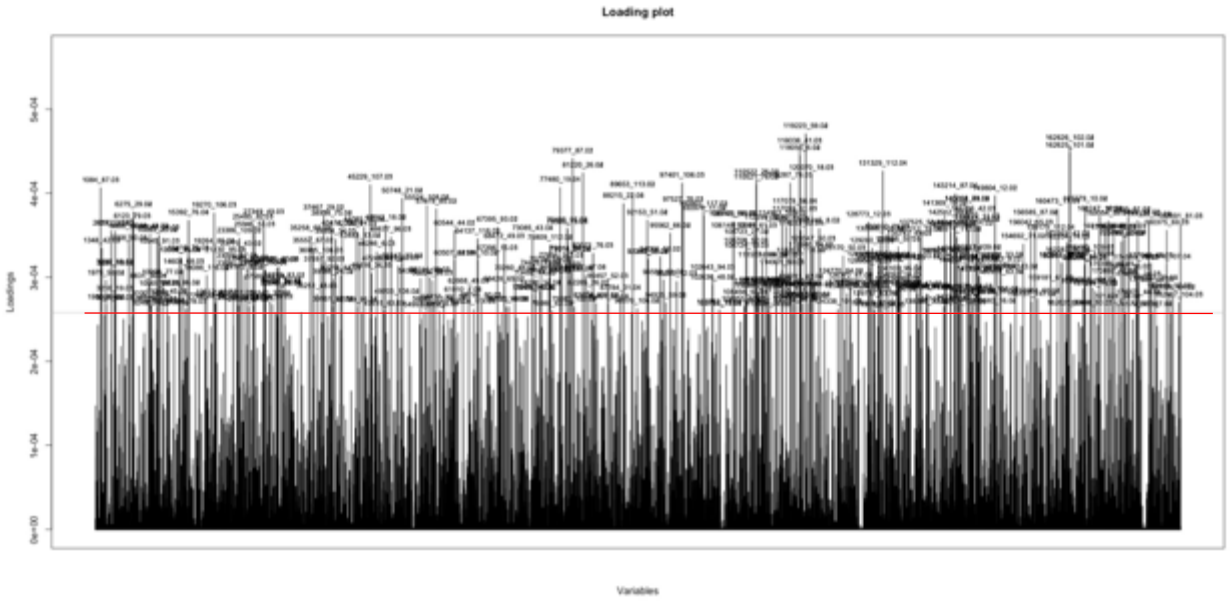


Figure 4. The 2% of alleles contributing most to explain the diversity within populations are above the red line.

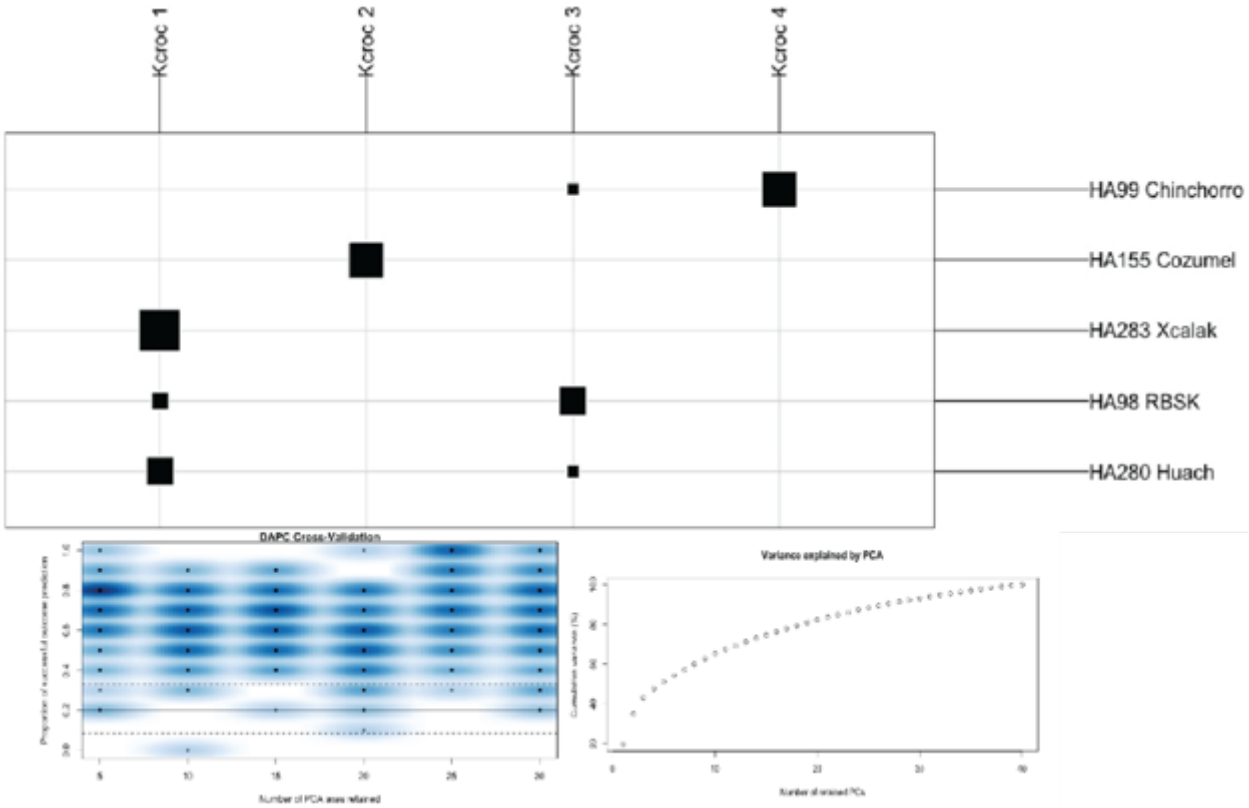


Figure 5. Genetic clusters found using Bayesian inference.

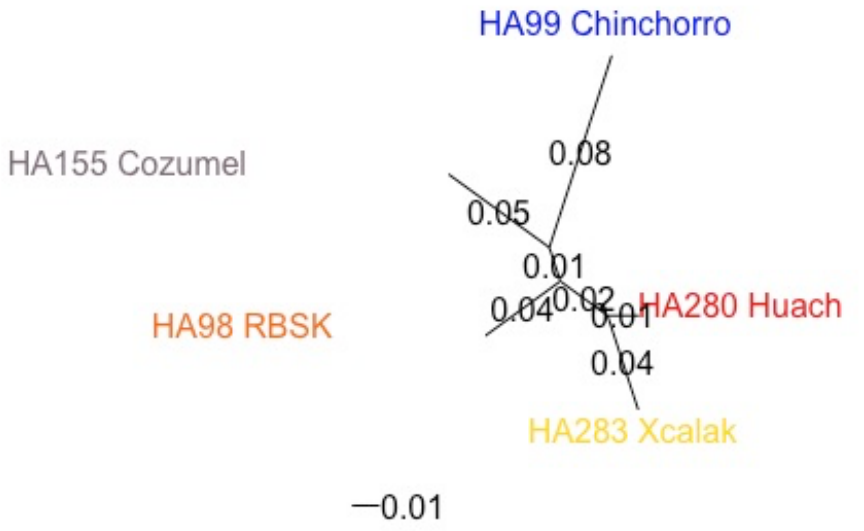


Figure 6. Neighbour joining tree based on paired Fst.

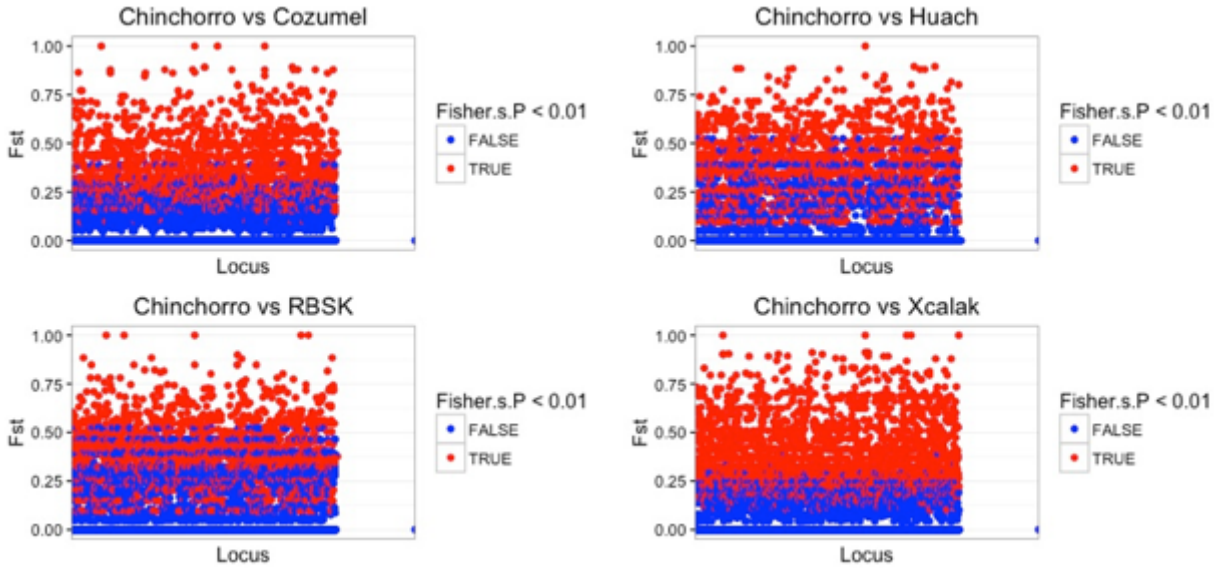


Figure 7. Fst comparison of all loci of Banco Chinchorro against the other populations. The red dots represent significant alleles (P<0.01).

EXPECTED OUTCOME AND SIGNIFICANCE | This is the first high-resolution genomic study of any crocodile species looking for ecological evolution using next generation sequencing, geography, and environment. The main goal of this research is to use genomic data to understand the evolution, introgression and hybridization of the genome of Neotropical crocodiles. The results will have significant inputs towards predicting future trends within the species and propositions for conservation strategies.

FUTURE DIRECTIONS| Hundreds of samples of the Yucatan peninsula and the Pacific Coast are currently being analyzed to retrieve more loci and a higher resolution of the evolutionary and ecological processes of *C. acutus* populations.

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