**A Final Report on**

# "POPULATION GENETICS OF THE AMERICAN CROCODILE IN COIBA NATIONAL PARK, PANAMA"

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ABSTRACT

Population genetic studies provide information on the various aspects of genetics of a species and therefore have a wide spread application in conservation research. This study assesses the genetic diversity, population subdivision, and genetic structure within populations of the American crocodile (*Crocodylus acutus*) in three sampling regions (mainland, northern and southern Coiba Island) in south -western Panama. A total of 143 individuals were characterized genetically by amplifying polymorphic microsatellite loci developed for *Crocodylus.* Microsatellite loci utilized in this study showed considerable allelic variation with a total of 61 alleles reported with an average of 6.8 alleles per locus. A model based clustering analysis revealed the presence of three spatially overlapping genetic clusters among the sampled populations. Both estimates of population subdivision ($F_{ST}$ and $R_{ST}$) are consistent with the finding that the fine scale analysis of sampled populations revealed an overall presence of genetic subdivisions among and within the sampled regions. The Panamanian government should consider this information when designing conservation programs for the American crocodile in Coiba National Park.

INTRODUCTION

The Crocodile Specialist Group (CSG) promotes world conservation strategies for crocodilians and emphasizes the need for investigation on population genetics of several species of crocodilians, including the American crocodile (*Crocodylus acutus)*, to assess their genetic diversity and to develop conservation programs (Ross 1998). Population genetic studies of *C. acutus* can provide basic information on their phylogeography, genetic population structure, migration patterns and gene flow, as well as introgression and hybridization events (Dever *et al.* 2002; Ray *et al.* 2004). This information will be critical in designing and implementing conservation programs for *C. acutus* in a particular local habitat as well as throughout their range.

In Panama, American crocodiles are distributed in and around the Coiba National Park (CNP). The CNP comprises an archipelago of nine main islands and about 30 islets. These islands, including the Coiba, were part of the continental mainland (Gulf of Chiriqui and Gulf of Montijo) until they were separated at the end of the last glacial period during the Late Pleistocene, between 18,000 and 12,000 years before present (Castroviejo and Ibanez 2001). Due to this separation event, we hypothesize that the populations of *C. acutus* on these Islands also were separated from their mainland conspecifics. After this separation, either the populations of *C. acutus* on the Island of Coiba and the mainland were completely isolated from each other with no gene flow between them and the sea has been acting as a barrier to gene flow; or there has been some level of exchange of migrants between these populations, in which case panmixia would be expected. American crocodiles are salt tolerant in nature and are known to disperse and/or migrate large distances (Kushlan and Mazzotti 1989a; Neill 1971). This ability of American crocodiles can potentially increase the amount of gene flow that could occur between

neighboring or even distant populations. Therefore, we expected to find no significant population subdivision and genetic structure within the sampled populations from mainland and Coiba Island.

The main purpose of this study was to estimate genetic diversity, and to assess the population subdivision and genetic structure within the populations of American crocodile from Coiba Island and the continental mainland. The specific objectives were to: 1) estimate the genetic diversity of populations of the American crocodile from Coiba Island and the mainland by calculating levels of heterozygosity, 2) assess the population subdivision within populations of the American crocodile from Coiba Island and the mainland by using different estimates of population differentiation or subdivision, and 3) assess the genetic structure within the sampled populations using a model based clustering analysis. Highly polymorphic microsatellite loci developed for *Crocodylus* were employed to accomplish these specific objectives.

## MATERIALS AND METHODS

This study was conducted in CNP and the adjacent continental mainland to the CNP (Gulf of Chiriqui and Gulf of Montijo) in Panama (Figure 1). CNP lies off the coast in the south-west Pacific of Panama in the south-eastern border of Gulf of Chiriqui. CNP was declared as a protected area in 1991, and was included in Pacific Biological Corridors in 2002; which is comprised of Islands of Malpelo and Gorgona (Colombia), Galapagos (Ecuador), and Cocos (Costa Rica) (Guzman *et al.* 2004).

CNP covers a total of 2,700 square kilometer, more than 80% of which is marine (Cardiel *et al.* 1997). The islands inside CNP are mostly covered by dense tropical rainforest and are characterized by several rivers of variable flows, depending upon the season and hydrographic

basin. Coiba is the largest island within the park system with a total area of about 50,314 ha. A unique geological feature of this island is the central fault which crosses the island diagonally from northwest to southeast and is conditioned by topography, low permeability soil, and rainfall (Cardiel *et al.* 1997; Kolarsky and Mann 1995). This central fault divides Coiba into two watersheds, herein referred to as northern and southern Coiba. Thorbjarnarson *et al*. (2006) classified Coiba Island and the Gulf of Montijo as an important Crocodile Conservation Unit for *C. acutus* in Panama, characterized by high quality habitat with high connectivity and low habitat destruction with an estimated population size of 500-1000 in Coiba and 100-500 in Gulf of Montijo.

Field work was conducted in two successive years from May to August in 2009 and from May to December in 2010; and 143 individuals were collected across all the sampling localities. Night-time spotlight surveys were conducted in most of the major rivers, estuaries, and mangroves in all the localities. Crocodiles were located utilizing their night eye shine (Magnusson 1982). Identified crocodiles were captured by non-lethal harpoon technique (Venegas-Anaya 1992) and by noose. Blood was collected via the caudal or dorsal sinus and stored in cell lysis buffer (Bayliss 1987; Gorzula *et al.* 1976). Whereas, skin clips were collected from tail scutes and stored in 95% ethanol. Each crocodile was uniquely marked by a numerical scute clipping system and released at the site of capture.

Total genomic DNA was isolated using the CTAB/phenol/chloroform technique (Palumbi *et al.* 1996; Sambrook *et al.* 1989), electrophoresed on a 2% agarose gel, and visualized with ethidium bromide under ultra violet light. Nine polymorphic microsatellite loci described for crocodilians (Dever and Densmore 2001; FitzSimmons *et al.* 2001; Miles *et al.* 2009) were amplified for each of the samples collected. These microsatellite loci were amplified

using the Polymerase Chain Reaction (PCR) based on a modified three primer competition protocol as described by Schuelke (2002). Various phylogenetic softwares were employed for subsequent data analyses to achieve the aforementioned objectives.

## RESTULTS

All the microsatellite loci showed considerable allelic variation with a total of 61 alleles reported in this study. The number of alleles per locus (A) ranged from two to 15 with an average of 6.8 alleles per locus. Among sampling regions, the highest allelic diversity was found in southern Coiba. Out of a total of 61 alleles reported, 53 were found in southern Coiba, 38 on the mainland, and 37 in northern Coiba. The number of private alleles ($A_P$) were highest in southern Coiba ($A_P$ = 13) compared to the mainland ($A_P$ =5), and northern Coiba ($A_P$ =2), although the frequency of these private alleles in each of the regions were relatively low.

The results from BOTTLENECK showed that two loci had observed heterozygosity ($H_e$) greater than expected heterozygosity at equilibrium ($H_{eq}$) under both the Step wise Mutation and Two Phase models of microsatellite mutation. A Wilcoxon's sign test did not detect a significant excess of heterozygosity ($P$ >0.05) under either of the mutation models showing no evidence of a recent genetic bottleneck for the sampled populations. When the Mode-Shift indicator was calculated to distinguish between the populations that have undergone recent bottleneck and those with the stable populations, a normal L-shaped distribution was obtained further indicating the absence of recent bottleneck.

Among regions, all the pair-wise $F_{ST}$ values were statistically significant ($P$ <0.01) suggesting significant subdivision among three populations. The $F_{ST}$ value between mainland and northern Coiba (0.057) and between mainland and southern Coiba (0.048) was slightly

higher than the $F_{ST}$ value between northern and southern Coiba (0.03). Similarly, pair-wise $R_{ST}$ value between mainland and northern Coiba (0.044) was slightly higher than the $R_{ST}$ value between mainland and southern Coiba (0.027). The AMOVA results revealed minor partitioning between sampled regions ($\Phi_{PT}$ = 0.06, $P$ <0.01) and a high degree of within-region variation (that is, over 94% of the variation detected). A model- based clustering analysis revealed the presence of three spatially overlapping genetic clusters ($\Delta K$ being the highest for $K$=3).

## DISCUSSION

All nine microsatellites utilized in this study were informative in describing genetic diversity, population subdivision, and assessing population structure of the American crocodile in and around CNP. The mean observed heterozygosity for the populations of American crocodiles in this study was 0.40. This value is comparable although slightly lower than the mean heterozygosity ($H$) estimated for different populations of American crocodiles in various parts of their range. American crocodiles have undergone a significant decline in their population size throughout their range (Ross 1998). Evidence of a recent bottleneck in the population of American crocodiles in Costa Rica has been reported by Cotroneo (2010). Taking this historic population reduction into account, it is interesting that the populations of American crocodiles on Coiba Island and the mainland did not show any analogous indication of a recent bottleneck. This absence of a bottleneck could be attributed to the fact that Coiba Island served as a penal colony since 1950s until recently. This establishment as a penal colony and its relative isolation from human habitat might have restricted the illegal hunting of American crocodiles in this region.

Our *a priori* assumption of an absence of genetic structure in the sampled populations was based on the fact that the American crocodile is a large, salt- tolerant species with higher mobility (Kushlan and Mazzotti 1989a), and seemingly unrestricted migration pathway between the populations from the mainland and Coiba Island. In contrast, model based clustering analysis revealed the presence of three genetic clusters among the sampled populations. However, these three genetic clusters do not strictly correspond to the three sampling regions in this study (mainland, northern and southern Coiba). Both the estimates of population subdivision are consistent with the finding that the fine scale analysis of sampled populations revealed an overall presence of genetic subdivision among and within the sampled populations. Pair-wise $F_{ST}$ and $R_{ST}$ values suggested lower level of population differentiation between the populations from northern and southern Coiba Island. In contrast, population differentiation was higher between populations from the mainland and northern Coiba Island, and between the mainland and southern Coiba Island. This implies that there is more gene flow between two populations on Coiba Island than between the island populations and the mainland, likely due to their geographic proximity.

## CONSERVATION IMPLICATIONS

This study was carried out with the aim of collecting baseline information on the genetic aspects of populations of the American crocodile in and around CNP. The information gathered from this study will be provided to the Panamanian government in hopes of helping them design a conservation program targeting the populations of American crocodile in this region. The Panamanian Government should consider the presence of three distinct genetic clusters and significant population differentiation among the sampled populations while designing conservation programs for the American crocodile in and around CNP. The knowledge about

which sub-populations are in migratory contact with each other can highlight important dispersal corridors as well as identify isolated areas, revealing the areas of priority for conservation (Bergl and Vigilant 2007). Considering these findings would be beneficial to help preserve the genetic diversity of each cluster, which is actually desirable in any conservation program. Any management strategies such as translocation of an individual, setting up a harvest quota, commercial farming and sustainable use programs should also be designed individually for each of these three genetic clusters rather than treating them as a one panmictic population.

The Crocodile Specialist Group (CSG) emphasizes the need of population genetic information for the American crocodile throughout their range in order to optimize the conservation strategy as designed by them. Given their wide distribution, local population genetic studies on the population of American crocodiles are necessary because it provides information on various aspects of genetics for this species. This particular study aims to fill this gap that exists for populations of American crocodile in Panama. The findings of this study in combination with other population genetic studies like Cotroneo (2010) in Costa Rica, Rodriguez *et al* (2011) in Florida, Cedeno-Vazquez *et al* (2008) in Mexico and other such studies will provide the information on the genetic diversity of American crocodiles throughout their range. This would help the CSG to create conservation strategies for the American crocodile. This will also broaden our understanding of American crocodiles in various aspects such as genetic diversity and genetic structure.

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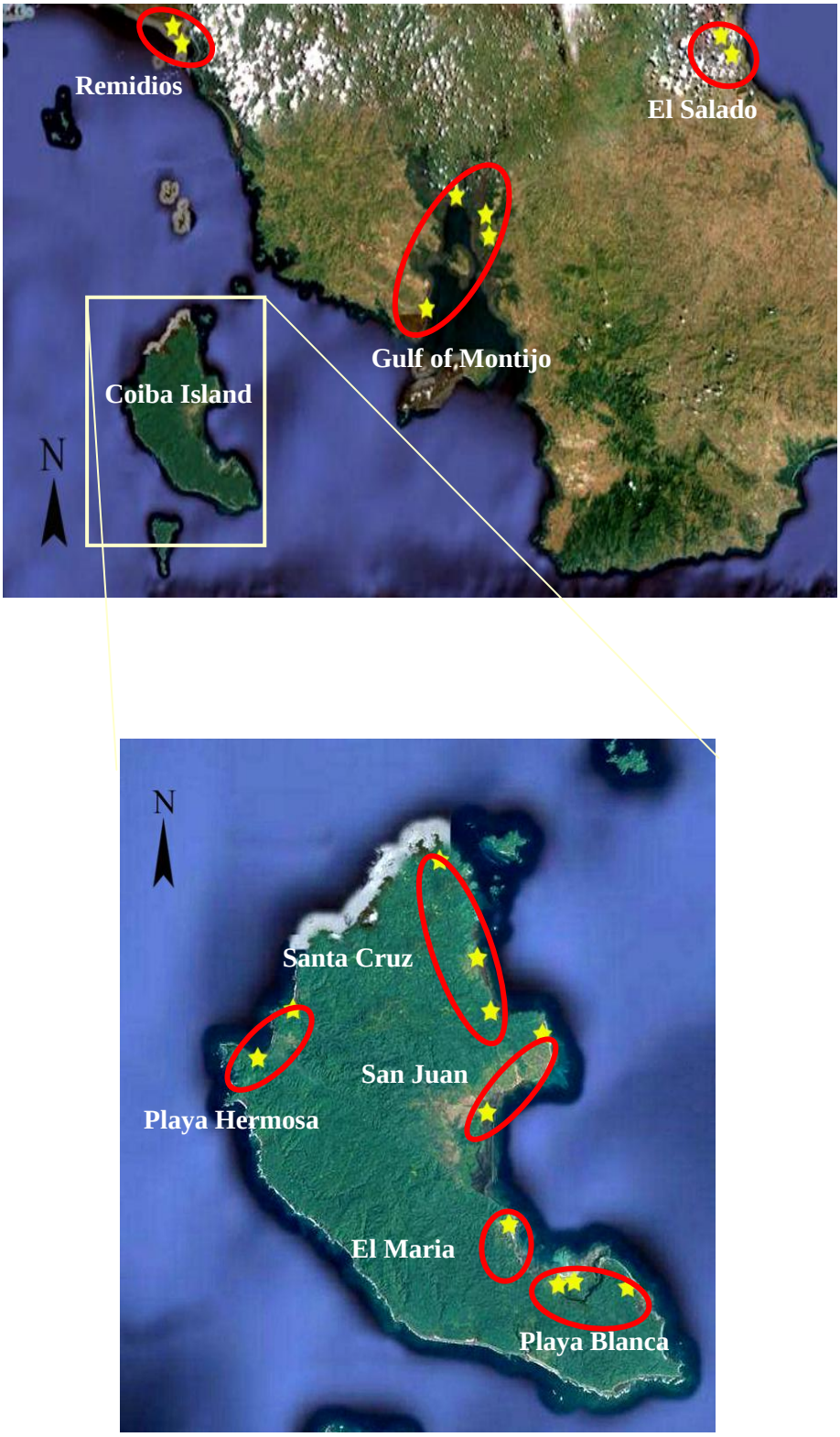



Figure 1. Map of Coiba National Park and mainland showing the sampling localities in red ovals.

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