Population genetic structure of Morelet's and American crocodiles in Belize: hybridization, connectivity and conservation

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Summary Report

Hybridization can influence the evolutionary potential of wild species and can be especially detrimental where one species is abundant, and the other much rarer. In Belize, the Morelet's crocodile (*Crocodylus moreletii*) primarily inhabits inland freshwater lagoons, lakes and rivers, whereas the less abundant American crocodile (*Crocodylus acutus*) is more prevalent among the offshore cayes and atolls. Both species are historically sympatric along the brackish coastline (Cissell and Steinberg 2020). Evidence of hybridization between the two species has previously been identified in coastal zones of both Belize and Mexico using mitochondrial DNA sequences, microsatellite allele frequencies and morphological characters (Ray et al. 2004; Cedeño-Vázquez et al. 2008; Rodriguez et al. 2008; González-Trujillo et al. 2012; Hekkala et al. 2015; Pacheco-Sierra et al. 2016). However, it remains unclear whether admixture between the two is affecting genetic integrity of the species. Key aims of this study were to identify geographic locations where hybridization is currently ongoing and to identify purebred populations that may benefit from conservation measures.

Sampling of wild *C. moreletii* and *C. acutus* was carried out in Belize by the Crocodile Research Coalition as part of a national population survey between 2016 to 2018. Double digest restriction-site associated DNA libraries were prepared and sequenced at the University of Bristol Genomics Facility, UK following the established protocol (Peterson et al. 2012). A total of 80 wild crocodilian samples from Belize samples were used and three additional reference samples from captive crocodiles including one captive *C. moreletii*, one wild *C. acutus* from Mexico, and one captive *C. cf. rhombifer*. Raw sequence reads were demultiplexed and aligned to a *Crocodylus porosus* reference genome GCA_001723895.1 (Ghosh et al. 2020). Filtering resulted in a total of 1092 biallelic SNPs for further analyses. We used Principal Component and Admixture to categorise individuals as either pure *C. moreletii*, pure *C. acutus*, or admixed. To explore population genetic structure, we used Discriminant Analysis of Principal Components (DAPC) with population groups predetermined based on their sampling localities and admixture status. FST values and individual co-ancestry coefficients of the 80 wild individuals were calculated to further investigate shared ancestry and relatedness.

All individuals sampled inland (Chiquibul National Park and Spanish Lookout) were categorised as pure *C. moreletii*, presumably reflecting the low tolerance to salinity of the species (Hekkala et al. 2015). We placed purebred *C. moreletii* individuals into five groupings based on collection localities. The greatest geneflow was present between the northern groups, across a lowland area where seasonal floods may enable dispersal. A unique *C. moreletii* population located in Chiquibul National Park was genetically distinct from other pure groups of that species, potentially representing a key refuge for the preservation of purebred individuals. Purebred *C. acutus* individuals were placed into two groups, one on the mainland (southeast coast) and the other on the offshore islands (Ambergris Caye and Caye Caulker). Despite being identified as pure, the mainland individuals had relatively high shared co-ancestry coefficients with hybrids, suggesting these individuals are likely to be part of a broader hybrid population, rather than a distinct group of purebreds. This would imply that offshore islands are the only

remaining stronghold for *C. acutus* in Belize whereby oceanic waters between the mainland and offshore act as a protective barrier against hybridization.

Hybrids were only identified along the central-southern coastline and were in close geographic proximity to coastal purebreds. This is similar to a previous study investigating hybridization in Belize using samples collected between 1996 – 1997 (Hekkala et al. 2015). The pattern of admixture we observed may be reflective of natural hybridization that has taken place for hundreds of generations (Pacheco-Sierra et al. 2016 and Pacheco-Sierra and Amavet 2021). Additionally, or alternatively, introgression may have resulted from changing behaviours or habitat use by one or both species, due to coastal development. It is possible the coastal regions of Belize represent a stable hybrid zone where the preferred habitats of the two species meet.

This work has validated and extended previous studies of hybridization between *C. moreletii* and *C. acutus* in Belize by using >1000 SNPs across the genome. We identified locations containing pure groups of *C. moreletii* and *C. acutus* that may benefit form continued monitoring and enhanced protection. Future research expanding the spatial scale of this work and enhancing the number of genetic variants may highlight previously unknown genetic reservoirs, potentially informing new conservation and management action plans, including the creation of new protected areas.

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