SRAS Final Report: Phylogeography of the American crocodile, *Crocodylus acutus* (Crocodylia: Crocodylidae) in Colombia

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Abstract

The American crocodile (*Crocodylus acutus*) is the most widely distributed crocodilian species in the Neotropics and plays a vital role in the ecosystems it inhabits. However, population declines caused by unsustainable exploitation and habitat degradation have been observed throughout its range. In Colombia, the species is classified as Endangered by local authorities, leading to the establishment of protective legislation, including hunting bans and captive breeding programs. The success of these initiatives relies on the availability of foundational genetic data, which remains limited for this species. This study examines the genetic diversity, phylogeography, and demographic patterns of *C. acutus* in Colombia using three mitochondrial markers (*cox1*, *cytb*, and *trnP/trnF/D-Loop*) and proposes strategies for its conservation and management. We analyzed 33 samples of American crocodiles from wild and captive populations, performing genetic variability assessments, phylogenetic and phylogeographic analyses, and neutrality tests, incorporating previously reported sequences. Our findings reveal high genetic diversity in Colombia, the presence of distinct mitochondrial phylogenetic clades, and potential human-driven or stochastic demographic fluctuations. Based on these results, we recommend guidelines for the translocation of American crocodiles and provide evidence of possible dispersal events in the Caribbean and Magdalena regions. Lastly, we advocate for the establishment of management units and highlight key research priorities for future efforts

Introduction

Like other crocodilian species, the American crocodile (*Crocodylus acutus* Cuvier, 1807) plays a crucial role in ecosystem stability as a predator in both aquatic and terrestrial environments (Somaweera et al., 2020). However, overexploitation for its skin and human activities have caused significant population declines across its range (Thorbjarnarson et al., 2006). Currently, the species is classified as 'Vulnerable' on the International Union for Conservation of Nature (IUCN) Red List (Rainwater et al., 2021) and listed as 'Endangered' or 'Critically Endangered' in some countries (Carvajal et al., 2005; Rivas et al., 2012; Morales-Betancourt et al., 2015). To prevent further population loss, the American crocodile has been included in Appendices I and II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), depending on the region, such as Appendix I for most of its range and Appendix II for Cuba, Mexico, and Cispatá Bay in Colombia (CITES, 2022).

In Colombia, the management and conservation of the American crocodile have undergone several stages, transitioning from unrestricted exploitation to regulated trade through captive farming systems (Medem, 1981; Balaguera-Reina & Densmore, 2014). The unsustainable use of the species during the 19th century led to hunting bans, regulatory policies, and the establishment of captive breeding programs with defined criteria (Ardila-Robayo et al., 2009; Balaguera-Reina & Densmore, 2014). These policies allowed local communities to continue using crocodilian resources while promoting the recovery of natural populations through retributive measures, such as releasing 5% of hatchlings from captive farms into the wild (Ardila-Robayo et al., 2009). However, the lack of ecological and genetic studies nationwide has hindered the effective implementation of these measures.

Genetic diversity, a fundamental level of biodiversity (Fenster et al., 2018), is essential for maintaining adaptive potential, avoiding declines in biological fitness, and preventing the accumulation of harmful alleles (Frankham, 2003; Spielman et al., 2004). Understanding genetic diversity enables researchers and policymakers to assess the status of threatened species, define evolutionarily significant units (ESUs) and management units (MUs; Moritz, 1994, 2002), and develop effective conservation strategies (Rocha & Gasca, 2007; Godoy, 2009) to promote both

population recovery and sustainable use (Amavet et al., 2017; Zucoloto et al., 2021). Mitochondrial markers are among the most widely used tools in conservation genetics due to their variability and sensitivity. They provide insights into phylogeographic histories by linking phylogenetic relationships with genetic diversity across geographic regions (Moore, 1995; Arif et al., 2011).

Over the past two decades, genetic studies of American crocodile populations in Central America and the Greater Antilles have expanded our understanding of the species' phylogenetics, lineage distribution, and relationships with other crocodilian species (e.g., Ray et al., 2004; Weaver et al., 2008; Milián-García et al., 2018; Pacheco-Sierra & Amavet, 2021). These studies have provided valuable insights for population-level inferences and conservation priorities in regions such as the Greater Antilles and Mexico (Milián-García et al., 2018; Pacheco-Sierra et al., 2018; Rossi et al., 2020). In Colombia, limited genetic studies have revealed unexpectedly high molecular diversity in wild and captive populations (Bloor et al., 2015; Viloria-Lagares, 2017) and facilitated the relocation of an American crocodile based on genetic data (Balaguera-Reina et al., 2020). However, gaps remain in understanding the genetic relationships among populations nationwide, particularly in unsampled regions, which restricts our knowledge of the species' phylogeography across its distribution.

Effective management and conservation strategies require a foundational understanding of the evolutionary history, ecology, genetic diversity, and population dynamics of the species (Alonso Roldán et al., 2011; Hekkala et al., 2011). Studies addressing these aspects and the genetic relationships between wild and captive populations are critical as such information informs translocation efforts and ensures the preservation of genetic diversity and integrity in wild populations (Loeschcke et al., 1994; Crandall et al., 2000).

In this study, we analyzed the genetic diversity, phylogeography, and demographic patterns of the American crocodile in Colombia, integrating ex situ and in situ data using three mitochondrial markers [cytochrome oxidase 1 (*cox1*), cytochrome b (*cytb*), and the tRNA-Pro, tRNA-Phe, *D-Loop* region (*trnP/trnF/D-Loop*)]. Our sampling focused on previously unsampled areas (e.g., Huila and Chocó departments) and areas with limited prior sampling (e.g., Tayrona National Natural Park). We also examined the genetic diversity of captive populations (zoos and farms) and their relationships to wild populations to propose guidelines for translocation and reintroduction. This work aims to address knowledge gaps in the demographic and molecular structure of American crocodile populations in Colombia and provide robust genetic data to support management (e.g., retributive farming policies) and conservation (e.g., wild population recovery) efforts.

Methods

Sampling

We analyzed a total of 33 scute samples from American crocodiles collected across Colombia's Caribbean region and inter-Andean valleys. Of these, 19 samples were obtained from wild populations, including specimens from Paramillo National Natural Park (NNP; 5), Salamanca Island Road (VIPIS) NNP (5), Tayrona NNP (2), Acandí Wildlife Sanctuary (WS; 1), Ciénaga Grande de Santa Marta (CGSM) WS (2), Cabrera River in Doche Municipality (2), Santa Marta (1), and Sardinata River in the Catatumbo region (1). Additionally, 11 samples were taken from captive individuals housed at Barranquilla Zoo (2), Vivarium del Caribe Zoo (4), and Reptibol Farm (5). Three samples were obtained from animals unintentionally released into the wild (escapees) and subsequently captured between 2014 and 2021 (Fig. 1). All samples were collected and preserved in 96% ethanol by park rangers or environmental agency staff and donated for this research. The samples were then transported to the Molecular Biology Laboratory at the University of Ibagué for storage at −20 °C and further processing.

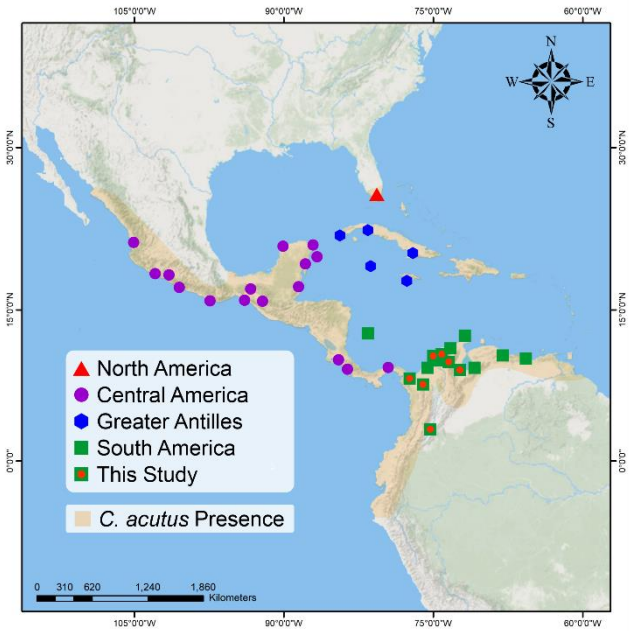


Fig. 1 American crocodile distribution (cream color), including both locations of samples retrieved from databases (North, Central, and South America including the Greater Antilles) and samples processed in the present study (green square with red dot).

Mitochondrial extraction, amplification, and sequencing

Mitochondrial DNA was extracted using the 'DNeasy Blood & Tissue' commercial kit (QIAGEN), and its concentration and purity were evaluated with QUBIT4 and Nanodrop (Picó Sirvent & Sánchez Busó, 2013). We amplified the full sequences of the *cox1* and *cytb* genes, as well as the *trnP/trnF/D-Loop* region, through PCR using a Veriti thermocycler (Applied Biosystems). The primers used were those described by Bloor et al. (2015) and Weaver et al. (2008).

Each PCR reaction contained 22 µL of water, 25 µL of 2× PCR Taq Master Mix (ABM), 1 µL of each primer, and 1 µL of template DNA (20–100 ng/µL). For the *cox1* and *cytb* genes, amplification conditions included an initial denaturation at 94 °C for 2 minutes, followed by 34 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 2 minutes, ending with a final extension at 72 °C for 10 minutes. For the *trnP/trnF/D-Loop* region, amplification involved an initial denaturation at 94 °C for 2 minutes, followed by 33 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 60 seconds, and extension at 72 °C for 45 seconds, concluding with a final extension at 72 °C for 10 minutes.

The PCR products were quantified with QUBIT4 and visualized through electrophoresis on a 1.5% agarose gel. Amplicons were purified using the ammonium acetate and ethanol method (Fregel et al., 2010) and sent to the SSiGMol Molecular Laboratory at the Genetic Institute of the National University of Colombia for sequencing via the Sanger method (Sanger & Coulson, 1975).

Data Analysis

Chromatograms were inspected for errors in base calls and gaps to ensure high-quality sequences. Forward and reverse contigs were aligned to produce consensus sequences for all genes from each sample using Geneious Prime v.2.9 (Kearse et al., 2012). Sequences from this study (33 individuals) were aligned with those from Bloor et al. (2015; 40 individuals) and Viloria-Lagares (2017; 75 individuals), for individual markers (*cox1*, *cytb*, and *trnP/trnF/D-Loop*) and concatenated *cox1* and *cytb* sequences for Colombian samples, using the MUSCLE algorithm (Edgar, 2004) within Geneious Prime v.2.9.

For each marker and concatenated sequences, we calculated genetic diversity metrics including the number of polymorphic sites (S), number of haplotypes (H), nucleotide diversity (π), haplotype diversity (h), and the number of indels (Indels) using DnaSP v.6.12 (Rozas et al., 2017). Sequences were matched to existing haplotypes or assigned new ones following Bloor et al. (2015) nomenclature (Cac01–Cac20).

Phylogeographic and Phylogenetic Analyses

We conducted phylogeographic analyses on two alignments: (A) concatenated *cox1–cytb* sequences and (B) the *trnP/trnF/D-Loop* region, incorporating sequences from GenBank (Ray et al., 2004; Cedeño-Vázquez et al., 2008; and others). Sequences from Villegas et al. (2022) were excluded due to incomplete *cox1* data (641 bp fragment). A third alignment (C) included all available Colombian data to study phylogenetic relationships within the country. Outgroups included sequences from *C. intermedius*, *C. moreletii*, *C. rhombifer*, and *C. niloticus*.

Evolutionary models and nucleotide partition schemes were identified using PartitionFinder v.2.1.1 (Lanfear et al., 2017) with the corrected Akaike Information Criterion (AICc). Bayesian Inference (BI) reconstructions were performed using MrBayes v.3.2 (Ronquist et al., 2012), with 10 million Markov chain Monte Carlo iterations (four chains in parallel, tree sampling every 100 generations, and the first 25,000 trees discarded as burn-in). Phylogenetic trees were visualized and edited using ITOL (Letunic & Bork, 2021).

For Colombian sequences, haplotype networks were generated with the TCS algorithm (Clement et al., 2000) in PopArt v.1.7 (Leigh & Bryant, 2015). Haplogroups were defined based on phylogroups following Lanteri & Confalonieri (2003), and their spatial distributions were described across wild and captive populations.

Nei's genetic distances (Tamura & Nei, 1993) between haplotypes and haplogroups were calculated using the ape package (Paradis & Schliep, 2019) in R. AMOVA analyses were conducted in Arlequin v.3.5 (Excoffier & Lischer, 2010) with 20,000 permutations using the Tamura-Nei method. Neutrality tests (Fu's D and F, Tajima's D, and Fu's F*) were carried out for haplogroups and all Colombian sequences with DnaSP v.6. Mismatch distributions and the raggedness (r) statistic were analyzed to detect demographic changes.

Finally, the relationship between pairwise FST values (from wild populations) and geographic distances (calculated in Google Earth) was assessed with the Mantel test (10,000 replicates, significance level of 0.05) using the ade4 package (Chessel et al., 2004) in R.

Results

Genetic diversity

We successfully sequenced the full-length *cox1* (1557 bp) and *cytb* (1200 bp) genes, along with a 675 bp fragment of the *trnP/trnF/D-Loop* region, from 33 individuals sampled in Colombia. Gene-specific alignments, including sequences from all 148 Colombian individuals, revealed that the *cox1* gene had the highest number of haplotypes (H = 13) and haplotype diversity (h = 0.78), while the *cytb* gene exhibited the highest nucleotide diversity (π = 0.0039; Table 1). Both genes contained the same number of polymorphic sites (16), while the *trnP/trnF/D-Loop* region had the only indel observed among all three markers. From the concatenated *cox1–cytb* alignment (2757 bp; N = 148; Table 1), we identified 19 haplotypes. However, upon analyzing the variation in the *trnP/trnF/D-Loop* marker, we found that two individuals (Cac13 and Cac14) were identical in the *cox1* and *cytb* genes but differed in the *trnP/trnF/D-Loop* region. The most common haplotypes were Cac01, with 47 individuals across nine localities [VIPIS NNP, Ciénaga Grande de Santa Marta WS, and Reptibol Farm from our study; Santa Marta, Cispatá Bay, Canal del Dique, San Onofre, Buritaca reported by Viloria-Lagares (2017); and farms from Bloor et al. (2015)]; Cac08, with 36 individuals from three localities [Paramillo NNP from our study; Cispatá Bay reported by Viloria-Lagares (2017); and farms from Bloor et al. (2015)]; and Cac18, with 24 individuals from four localities [Tayrona NNP and the Catatumbo region from our study; and Portete Bay and Michiragua reported by Viloria-Lagares (2017)] (Table 2).

Table 1. Genetic variability values for different mitochondrial markers in Colombian American crocodiles. *N* = Number of individuals, *bp* = Sequence length in base pairs, *S* = Polymorphic sites, *Indels*= Number of insertions and deletions, *H* = Number of haplotypes, *h* = Haplotype diversity, and π = Nucleotide diversity.

Dataset	**Marker**	**N**	**bp**	**S**	**Indels**	**H**	**h**	**π**
Colombia	*cox1*	148	1557	16	0	13	0.7850	0.00291
	cytb	148	1200	16	0	10	0.7440	0.00389
	trnP/trnF/D-Loop	33	675	3	1	5	0.6761	0.00130
	cox1-cytb	148	2757	32	0	19	0.8103	0.00334

Table 2. Number of American crocodiles sampled in Colombia per locality, haplotype and haplogroup including samples collected in the present study and those reported in the literature (Bloor et al. 2015, Viloria-Lagares 2017). *Bloor farms refers to the crocodile farms sampled by Bloor et al. (2015) due to the lack of specific information (farm names or location) on the paper.

Haplogroup	Haplotype	Localities and number of individuals	Total Individuals
Northwest/ Paramillo	Cac07	Cispata Bay (4); Bloor Farms (2)	6
	Cac08	Cispata Bay (12); Paramillo NNP (15); Bloor Farms (9)	36
	Cac13	Barranquilla Zoo (1)	1
	Cac14	Barranquilla Zoo (1)	1
	Cac16	Paramillo NNP (1)	1
Magdalena Basin	Cac01	VIPIS NNP (5); Santa Marta (2); Cispata Bay (2); Canal del Dique (10); San Onofre (2); Buritaca (2); Reptibol Farm (1); Cienaga Grande de Santa Marta WS (2); Bloor Farms (21)	47
	Cac02	Cispata Bay (3); Bloor Farms (1)	4
	Cac03	Cabrera River - Doche (2); Bloor Farms (1)	3
	Cac04	Bloor Farms (2)	2
	Cac05	Puerto Colombia (1); Puerto Mocho (1); Vivarium del Caribe Zoo (1); Tayrona NNP (1); Reptibol Farm (3); Bloor Farms (3)	10
	Cac06	Bloor Farms (1)	1
	Cac09	Vivarium del Caribe Zoo (2); San Andres Island (1)	3
	Cac10	Tayrona NNP (1)	1
	Cac12	Vivarium del Caribe Zoo (1)	1
	Cac20	Reptibol Farm (1); Santa Marta (2)	3
Northeast/ Catatumbo	Cac15	Sardinata River - Catatumbo (1)	1
	Cac17	Tayrona NNP (1)	1
	Cac18	Sardinata River - Catatumbo (5); Portete Bay (9); Michiragua (7); Tayrona NNP (3)	24
	Cac19	Michiragua (1)	1
Acandí	Cac11	Acandí WS (1)	1
Total	20 haplotypes	21 Localities (148)	148

Phylogenetic analyses

Bayesian inference reconstructions, which included individuals from the entire *C. acutus* distribution (Fig. 2), revealed that the American crocodile is a non-monophyletic group comprising three distinct clades: (1) American crocodiles from Mexico, which are closely related to *C. moreletii*; (2) American crocodiles from the USA, Mexico, Jamaica, the Cayman Islands, and Cuba, which are closely related to *C. rhombifer*; and (3) American crocodiles from the USA, Mexico, Belize, Costa Rica, Cuba, Colombia, and Venezuela, which are closely related to *C. intermedius*.

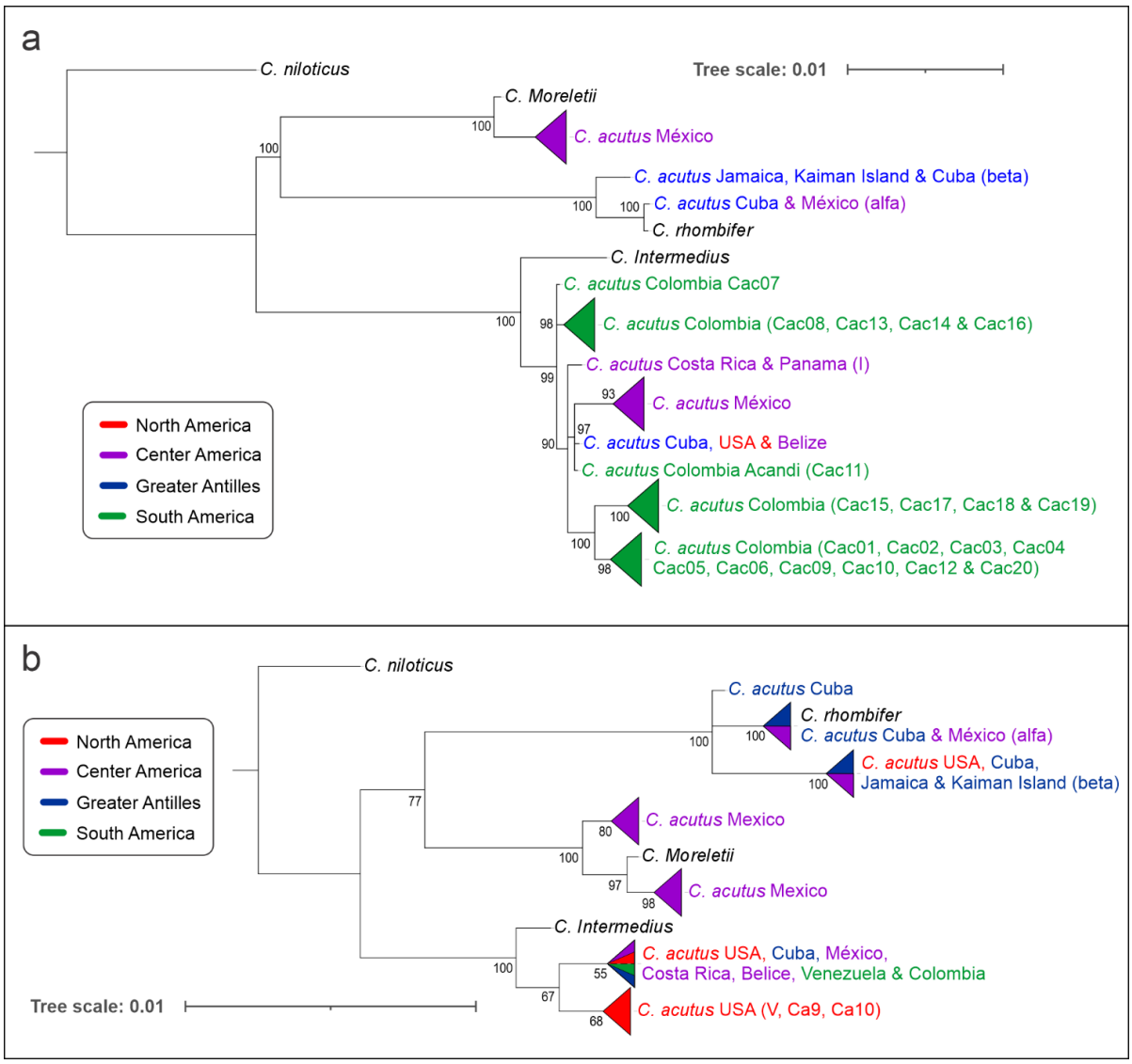


Fig. 2 Bayesian Inference (BI) reconstructions for American crocodiles based on **A** *cox1* and *cytb* genes and **B** *TrnP/trnF/D-Loop* sequenced region. Colors correspond to its distribution, North America countries (red), Center America countries (purple), Greater antilles (blue), and South America countries (green).

In the Bayesian inference reconstruction for Colombia and the TCS network (Fig. 3), we identified four well-defined haplogroups, listed from west to east: Acandí (Cac11), north-west/Paramillo (Cac07, Cac08, Cac13, Cac14, and Cac16), Magdalena Basin (Cac01, Cac02, Cac03, Cac04, Cac05, Cac06, Cac09, Cac10, Cac12, and Cac20), and north-east/Catatumbo (Cac15, Cac17, Cac18, and Cac19). Haplotypes from these haplogroups were also found in captive populations (north-west/Paramillo and Magdalena Basin; Table 2; Fig. 3B), as well as in individuals that had escaped from farms and were later recaptured in the wild (American crocodiles captured in Puerto Colombia, Puerto Mocho, and San Andres Island; Table 2; Fig. 3B). Notably, the two escaped American crocodiles identified in this study (captured in Puerto Colombia and Puerto Mocho; haplotype Cac05) were from farms located in the lower Magdalena River Basin (Bolivar and Atlántico), and one of these crocodiles shared the same origin as the American crocodile captured in San Andres Island (Zambrano, Bolivar; Cac09; Balaguera-Reina et al., 2020).

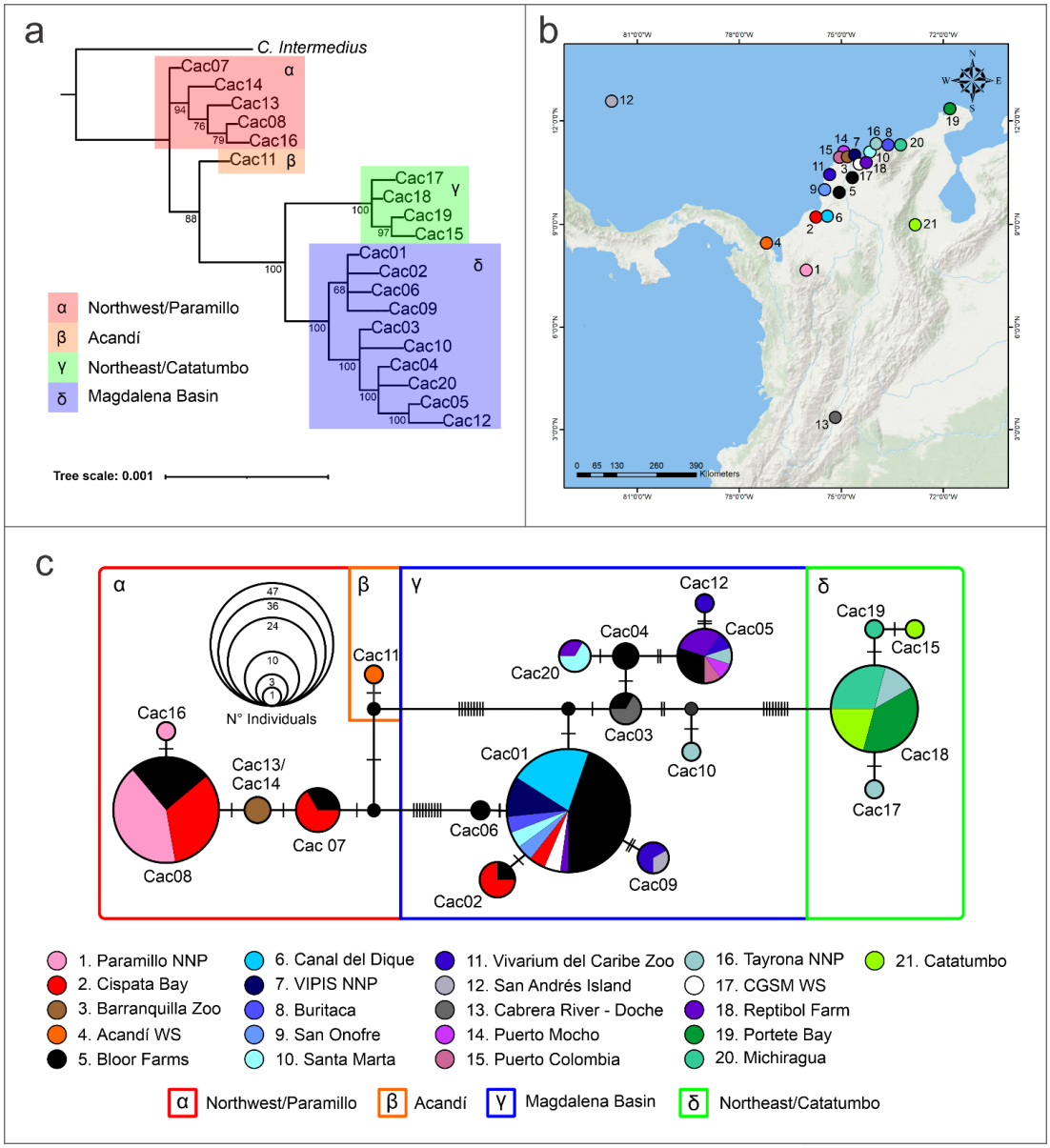


Fig.3 Colombian haplogroups of American crocodile using *coi* and *cytb* genes, and *TrnP/trnF/D-Loop* marker. **A** bayesian Inference (BI) reconstruction for Colombian American crocodile haplotypes, including the four identified haplogroups, Northwest/Paramillo (red, α), Acandí (Orange, β), Northeast/Catatumbo (green, γ), and Magdalena Basin (blue, δ). **B** geographical distribution of the 21 included Colombian localities in this study. **C** TCS haplotype network for Colombian American crocodile using *coi* and *cytb* genes, together with the 21 included localities, and the four identified haplogroups.

Nei's genetic distances revealed the greatest differentiation between haplogroups was between Acandí and north-east/Catatumbo (0.63 ± 0.06), followed by northwest/Paramillo and Magdalena Basin (0.49 ± 0.10), Magdalena Basin and north-east/Catatumbo (0.48 ± 0.08), and Magdalena Basin and Acandí (0.43 ± 0.04). The phylogenetic relationships of the American crocodile in Colombia remained consistent whether the analysis used three markers (*cox1*, *cytb*, and *trnP/trnF/D-Loop*) or two markers (*cox1* and *cytb*), always showing the same topology and proximity to *C. intermedius*.

Population structure and demographic analyses

The AMOVA by localities (21) using the *cox1–cytb* concatenated alignment resulted in an FST index value of 0.65 ($P < 0.05$), which was lower than the FST index calculated when grouping by haplogroups (0.90; $P < 0.05$). Sequences from American crocodiles collected in Colombia (148 individuals) showed no significant deviations from the standard neutral model when analyzed either together or by haplogroup ($P > 0.10$), based on Fu and Li's D and Fu's F* tests. However, weak deviations from neutrality were observed for Colombia when using Fu and Li's F and Tajima's D tests (1.56 and 1.77, respectively; P-values between 0.05 and 0.10), which may be related to demographic contractions or bottlenecks. Mismatch distribution patterns aligned with the neutrality tests, showing a multimodal pattern across Colombian sequences, in contrast to the unimodal tendency observed for each haplogroup (Fig. 4). Lastly, the Mantel test revealed a positive correlation between the pairwise FST matrix and the geographic distribution of the sampled localities (0.49, $P = 0.00029$).

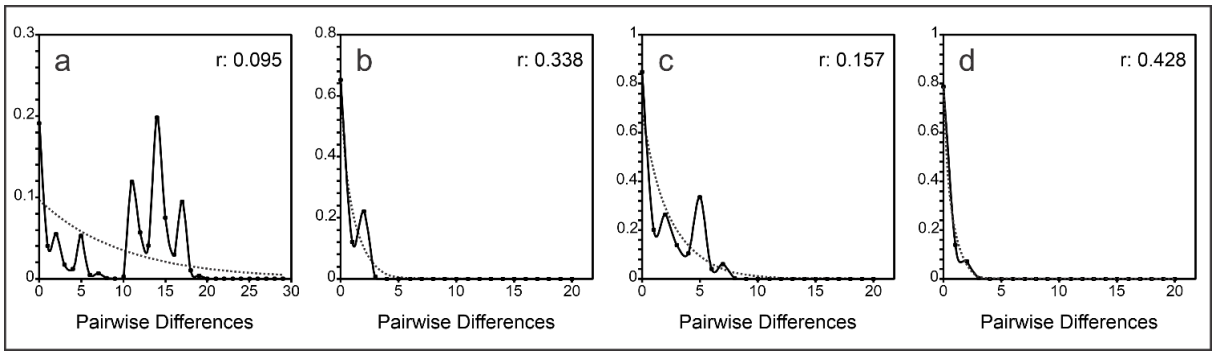


Fig.4 Mismatch distributions for Colombian American crocodile using *coi* and *cytb* genes, including the raggedness statistic (r), observed frequency in solid line and expected frequency in dotted line. **A** dataset of all Colombian crocodiles. **B** Northwest/Paramillo haplogroup. **C** Magdalena basin haplogroup. **D** Northeast/Catatumbo haplogroup.

Discussion

Phylogenetic analyses

As previously reported, our Bayesian Inference (BI) reconstructions revealed that the American crocodile is a nonmonophyletic group based on mitochondrial markers (species complex; Fig. 1), comprising at least three major clades related to sister species (*C. moreletii*, *C. rhombifer*, and *C. intermedius*; Milián-García et al., 2011, 2015; Pacheco-Sierra et al., 2016, 2018). We found that all South American individuals clustered with a sister clade of *C. intermedius*, while individuals from Central America were distributed in clades associated with *C. intermedius*, *C. rhombifer*, and *C. moreletii*. Individuals from the Greater Antilles were grouped with *C. intermedius* and *C. rhombifer* (Figs. 1, 2). This distribution may be the result of an introgression process through hybridization between American crocodiles and other Crocodylus species where they coexist (Central America and

the Greater Antilles), or due to long-range movements of the species (from South to Central America; Balaguera-Reina et al., 2020).

This finding highlights the geographic variation in the genetic diversity of the American crocodile. In our study, we did not detect evidence of hybridization or introgression, either in the individuals sampled or in the South American crocodiles included in the analysis, suggesting the absence of migration from Central and North America to South America. However, it remains unclear how much of this genetic introgression may be the result of translocated or unintentionally released individuals (e.g., USA populations; Rodriguez et al., 2011) versus natural dispersal (e.g., the individual from San Andrés Island; Balaguera-Reina et al., 2020). This is a crucial topic to explore, as it has significant implications for management and conservation efforts. As a precaution, all releases, whether from in situ (e.g., translocations) or ex situ (e.g., restocking programs) projects, must ensure a thorough understanding of the phylogeographical relationships between individuals and wild populations before reintroducing animals into the wild. This will help prevent the unintended increase in introgression or the introduction of hybrids in areas where pure populations exist. Therefore, it is important to consider both the geographical origin and the specific genetic characteristics of the population when implementing conservation strategies for the species (Loeschcke et al., 1994; Crandall et al., 2000).

Genetic diversity

The nucleotide and haplotype diversity values observed in this study were comparable to those reported for American crocodile populations from Belize, Cuba, the USA, Mexico, and Venezuela (Milián-García et al., 2018; Rossi et al., 2020), as well as those for populations of *C. moreletii* in Mexico (Ray et al., 2004; González-Trujillo et al., 2012), indicating a high level of diversity within Colombian populations.

Population structure and demographic of American crocodiles in Colombia

In Colombia, we identified four haplogroups (Acandí, north-west/Paramillo, Magdalena Basin, and north-east/Catatumbo; Fig. 3), with the north-west/Paramillo and Magdalena Basin haplogroups previously documented by Bloor et al. (2015) and Viloria-Lagares (2017). Our Bayesian Inference (BI) reconstruction did not recover the north-west/Paramillo haplogroup as a monophyletic clade, excluding haplotype Cac07, which was still considered part of the haplogroup due to its genetic similarity to other haplotypes in the network. The Acandí and north-east/Catatumbo haplogroups were represented by only one individual each, likely due to the absence of farms in these regions.

The Magdalena Basin haplogroup was the most numerous, with the highest number of haplotypes and localities sampled, ranging from the upper Magdalena River Basin to the Caribbean Sea. An American crocodile that traveled from the Magdalena River to San Andrés Island (~700 km; Balaguera-Reina et al., 2020) was part of this haplogroup, which could explain its broad distribution. The genetic distances between haplogroups were relatively high, but under 1%, and FST values were higher when grouped by haplogroups (0.91) compared to localities (0.64), indicating that haplogroups better represent genetic variation in Colombia. The Mantel test confirmed a positive correlation between genetic and geographical distances, supporting the geographic distribution of the haplogroups.

Neutrality tests and mismatch distribution analyses (Fig. 4) suggested weak evidence of a demographic contraction in Colombian populations, which might relate to the initial separation of haplogroups followed by demographic expansion. This pattern was also observed in the haplotype network, where the north-west/Paramillo, Magdalena Basin, and north-east/Catatumbo haplogroups displayed star-like patterns, typical of populations undergoing expansion (Slatkin & Hudson, 1991; Lanteri & Confalonieri, 2003). The absence of intermediate haplotypes and several mutational steps might indicate demographic contractions or bottleneck events (Ramos-Onsins & Rozas, 2002). One explanation for this pattern could be historical climatic events causing local extinctions, as seen in other species (Zigouris et al., 2013; Arias-Alzate et al., 2017). The unsustainable exploitation of crocodilians in Colombia might have exacerbated this pattern. Similar contraction and expansion events have been observed in other crocodilian species, such as the American crocodile in Florida (Rodriguez et al., 2011), saltwater crocodiles in Australia (Luck et al., 2012), and South American

caimans (Vasconcelos et al., 2006). Larger sample sizes are needed to improve the detection of demographic variation signals (Fu, 1997; Ramos-Onsins & Rozas, 2002).

Based on our BI reconstructions, we propose that these haplogroups represent Evolutionarily Significant Units (ESUs; Fig. 3). However, further analyses with nuclear markers are needed to validate gene flow between haplogroups (Moritz, 1994). We recommend designating Management Units (MUs) for areas where all sampled individuals belong to a single haplogroup based on mitochondrial genetic distinctiveness (Moritz, 2002), considering the impact on the genetic diversity and conservation of the species (Palsbøll et al., 2007). These areas include: Paramillo NNP for the north-west/Paramillo haplogroup, VIPIS NNP for the Magdalena Basin haplogroup, Portete Bay and Catatumbo for the north-east/Catatumbo haplogroup, and Acandí WS for the Acandí haplogroup. Populations in certain areas (e.g., Tayrona NNP and Cispatá Bay) and in captivity (e.g., farms sampled by Bloor et al., 2015) already exhibit a mixture of individuals from different haplogroups (Table 2).

Finally, we strongly recommend that future releases of American crocodiles be based on the haplogroups identified in this study to maintain the genetic integrity of populations (Newton et al., 1999; Fraser & Bernatchez, 2001). This type of management has already been implemented in Colombia, with the first documented release of a crocodile from San Andrés Island (Balaguera-Reina et al., 2020). Translocations should occur in areas with minimal human-crocodile conflict to reduce potential risks for both the animals and local human populations (Nyhus, 2016; Balaguera-Reina et al., 2019). We recommend using nuclear markers to further characterize allelic frequencies and genetic diversity in natural and ex situ populations in Colombia to improve understanding of the genetic status and structure of the species, aiding compliance with national legislation for crocodile conservation (Balaguera-Reina & Densmore, 2014).

Acknowledgements

I express my gratitude to my tutors and coauthors who worked with me on this project: Felipe Hernandez-Gonzalez, Carlos F. Prada-Quiroga, Lina M. García-Calderon, Yessy García, Elkin Hernandez, Aristides Lopez, Arnold Argel, Juan Manuel Polo, Nidia Farfan-Ardila, and Sergio A. Balaguera-Reina

Furthermore, I express my gratitude to the International Union for Conservation of Nature (IUCN) Crocodile Specialist Group, the University of Tolima, the University of Ibagué, and NaturalSIG Corporation for their financial and logistical support. We also appreciate the regional environmental agencies [Corporación para el Desarrollo Sostenible del Archipiélago de San Andrés, Providencia y Santa Catalina (CORALINA), Corporación Autónoma Regional del Alto Magdalena (CAM), Corporación Autónoma Regional de la Frontera Nororiental (CORPONOR), and Corporación Autónoma Regional del Atlántico (CRA)] and local agencies (Environmental Public Establishment Barranquilla Verde), as well as the National Natural Parks (NNP; Tayrona NNP, Salamanca Island Road NNP, Paramillo NNP, Acandí Playón and Playona Wildlife Sanctuary, and Ciénaga Grande de Santa Marta Wildlife Sanctuary), farms (Reptibol Farm), and zoos (Barranquilla and Vivarium del Caribe Zoos) for their time and efforts in collecting the samples for this study. All samples were gathered by agencies and park researchers/rangers and donated to the study under agreement 20202000005803, dated October 7, 2020. Lastly, we thank the reviewers of this manuscript for their valuable contributions.

This work was funded by the Student Research Assistance Scheme grant from the Crocodile Specialist Group (CSG) of the International Union for Conservation of Nature (IUCN) and supported by University of Ibague Molecular Biology Lab resources.

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