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**Evaluación genética preliminar de
poblaciones *in situ* del Caimán Llanero
(*Crocodylus intermedius*) en la Orinoquía
colombiana**

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*A mis padres,
a la memoria de mis abuelas*

Se van sucediendo animales de los tipos más distintos. «Es como el paraíso», decía nuestro timonel. Y en realidad todo recuerda aquí el estado original del mundo. En ese terreno intermedio se ven los cocodrilos, a menudo ocho o diez, tendidos sobre la arena, inmóviles, abiertas las quijadas en ángulo recto, reposando unos al lado de otros, sin brindarse ninguna de esas señales de afecto que se observan en otros animales que viven en sociedad

Alexander von Humboldt

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Fecha 30/01/2023

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Resumen

Evaluación genética preliminar de poblaciones *in situ* del Caimán Llanero (*Crocodylus intermedius*) en la Orinoquía colombiana

El Caimán Llanero o Cocodrilo del Orinoco (*Crocodylus intermedius*) se encuentra críticamente amenazado debido a la caza indiscriminada que sufrió durante el siglo pasado con el fin de satisfacer la empresa curtiembre estadounidense y europea. Por lo que hoy, la especie es representada por individuos aislados, pocas agrupaciones remanentes, y en gran medida, poblaciones *ex situ* establecidas con fines de conservación en Colombia y Venezuela. Se han desarrollado legislaciones y planes de conservación estatales, que, en el caso de Colombia, incluyen la importancia y urgente necesidad de evaluar su estado genético en vida silvestre, buscando conservar su potencial evolutivo. La presente investigación se desarrolló con el fin de empezar a llenar este vacío de información y proponer acciones concretas y efectivas para la conservación de la especie. En el primer capítulo, reevaluamos el planteamiento efectuado a partir de fragmentos de ADN mitocondrial que sugería el manejo de la especie como una única unidad genética. Para ello, usamos marcadores moleculares variables (microsatélites y la región control de la mitocondria). Como resultado, identificamos tres agrupaciones genéticas con correspondencia geográfica en la Orinoquía Colombiana: i) Cuenca Oriental del Río Meta, ii) Cuenca Occidental del Río Meta y Cuenca del Río Vichada, y iii) Cuenca del Río Guaviare. Estimamos aspectos sobre su flujo genético y planteamos hipótesis que puedan explicar esta estructuración. Así mismo, evaluamos la asignación de individuos decomisados y cuyo origen era desconocido. En el segundo capítulo, efectuamos la caracterización genética de la población que habita el sistema de ríos Cravo Norte-Ele-Lipa y del programa de rancheo de huevos que allí se desarrolla, con el objetivo de aportar herramientas para seguir con su conservación y manejo. Identificamos a la población como un valioso recurso para la conservación de la especie, e identificamos aspectos demográficos históricos y actuales, entre los que destaca su bajo tamaño efectivo poblacional. Finalmente, en cada capítulo se proponen acciones concretas para la conservación y manejo de esta especie en Colombia.

Palabras clave: Conservación genética, Neotrópico, microsatélites, región control de la mitocondria.

Abstract

Preliminary genetic evaluation of *in situ* populations of the Orinoco Crocodile (*Crocodylus intermedius*) in the Colombian Orinoquía

The Orinoco Crocodile (*Crocodylus intermedius*) is critically endangered due to the indiscriminate hunting it suffered during the last century to satisfy the American and European leather demand. Therefore, today it is represented by isolated individuals, few remaining groups, and to a large extent, *ex situ* populations established for conservation purposes in Colombia and Venezuela. Likewise, legislation and state conservation plans have been developed, which, in the case of Colombia, have suggested the evaluation of the *in situ* genetic status of the species, seeking to preserve its evolutionary potential. The present investigation was developed aiming baseline information to propose concrete and effective actions towards the species conservation. In the first chapter, we re-evaluated the approach made using mitochondrial DNA fragments that suggested managing the species as a single genetic unit. To do this, we used variable molecular markers (microsatellites and the control region of mitochondria). As a result, we identified three genetic groups with geographic correspondence in the Colombian Orinoquía: i) Eastern Meta River Basin, ii) Western Meta and Vichada River Basins, and iii) Guaviare River Basin. Furthermore, we estimated aspects such as gene flow, propose hypotheses that may explain its structure, and performed the assignment of seized individuals whose origin was unknown. In the second chapter, we achieved the genetic characterization of the population that inhabits the Cravo Norte-Ele-Lipa River System and the egg ranching program for conservation purposes that is being locally developed. We identified the population as a valuable resource for the conservation of the species and evaluated historical and present demographic aspects, among which its low effective population size stands out. Finally, in each chapter we propose concrete actions for the conservation and management of the species in Colombia.

Keywords: Conservation genetics, Neotropics, microsatellites, mitochondrial control region.

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Introduction

With the development of this dissertation, we aim to advance in the conservation program of the Orinoco Crocodile (*Crocodylus intermedius*, Graves 1819), by preliminary assessing the genetic diversity of *in situ* populations that inhabits the Colombian Orinoquía. For this purpose, we used variable molecular markers; microsatellites (SSRs) and the Domain III of the control region of the mitochondria. In the first chapter, we reevaluate the population structure of *C. intermedius* and assessed the provenance of confiscated individuals that are part of the founders of the Estación de Biología Tropical Roberto Franco (EBTRF) *ex situ* population. In the second chapter, we provide a genetic characterization of the remanent population that inhabits the Cravo Norte-Ele-Lipa Rivers System and explore its demographic history. We aim at proposing management actions based on the analyzed information to contribute to the conservation of this critically endangered crocodile.

Genetic inferences in endangered species and populations

Conservation genetics makes use of tools from molecular biology, population genetics, phylogenetic systematics, and phylogeography to understand, from an evolutionary context, the dynamics that affect processes within and between species in order to provide solid concepts for taking conservation actions (Allendorf et al., 2012; Frankham, 2015). By employing the theoretical concepts of these disciplines and molecular genetics tools, it is possible to infer population-level parameters, including evidence for bottlenecks, inbreeding, and effective population size (N_e). In this context, the main object of study in population genetics is the fitness and frequency of genotypes within and between populations of the same species or of closely related by ancestry (Gillespie, 2004; Hartl & Clark, 1997). These parameters serve as an indicator of conservation status; for instance, threatened species according to the IUCN (International Union for Conservation of Nature) categories show reduced heterozygosity and allelic richness indexes (Willoughby et al., 2015).

In the case of threatened species, they are typically characterized by reduced and fragmented populations, which increases the negative effects of genetic drift and inbreeding by reducing their genetic diversity (Frankham, 1996; Willoughby et al., 2015). Genetic drift, the change in allele frequencies in a population as a result of chance, could quickly lead to the loss of genetic variation due to fixation and consequent loss of alleles in the population, which decreases the evolutionary adaptive potential of the species, i.e., their ability to react to new environmental challenges

(Allendorf et al., 2012; Markert et al., 2010). Inbreeding, or the reproduction between closely related individuals, causes a rapid increase in homozygosity and exposure of rare deleterious alleles, reducing reproductive capacity and survival (Frankham, 2015). Populations with a small N_e , which is referred to the size of an idealized population that loses genetic diversity at the same rate as the focal population (Willi et al., 2022; Wright, 1939), are more sensitive to these effects (Allendorf et al., 2012).

Therefore, conservation genetics provides critical information in deciding what management actions are necessary (Willi et al., 2022). It provides tools for individual identification and origin identification, detects and quantifies the effect of events such as habitat loss, fragmentation, and reproductive isolation, informs about the reintroduction viability of individuals from *ex situ* populations (populations that are kept outside their natural habitat, i.e., within zoos or biological stations), and serves to identify evolutionary significant units (ESUs) that should be the focus of management actions (Allendorf et al., 2012; Moritz, 1994; Willi et al., 2022). By doing so, it aims at reducing the effects of the stochastic events associated with small population size (demographic, environmental and genetic), that together with systematic human-associated threats (habitat loss, pollution, and climate change) drive a species to extinction (Frankham et al., 2014; Gilpin & Soulé, 1986; Shaffer, 1981).

***Crocodylus intermedius* as a model species**

In this dissertation, the model species correspond to the Orinoco Crocodile (Figure **I-1**). It is one of the largest crocodile species in the world, reaching a size of up to seven meters (Medem, 1981). When young, its diet consists of invertebrates and small fishes, while as it grows, the Orinoco Crocodile includes larger preys such as larger fishes, reptiles, birds, and mammals (Antelo et al., 2008; Medem, 1981; Seijas et al., 2015). Likewise, it has been observed scavenging and having cannibalistic behaviors (Antelo et al., 2008; Medem, 1981; Seijas et al., 2015). Sexual maturity is reached after approximately 2 m of total length, and reproduction occurs in the dry season, when the availability of suitable places for its postures, such as river beaches, increase (Morales-Betancourt et al., 2015; Thorbjarnarson & Hernández, 1993). Its clutches have an average of 40 eggs, and multiple paternity had been reported in the reintroduced population of El Frío Biological Station, Venezuela (Castro Casal, 2012; Rossi Lafferriere et al., 2016). Historically, *C. intermedius* inhabited the Orinoco River basin, in almost all large rivers in a wide diversity of aquatic habitats in the

Orinoquía (Medem, 1981). Explorers of the XIX century described abundant populations easily observed on sandy river beaches (Antelo et al., 2008; von Humboldt, 1958). That situation changed as a consequence of the unsustainable hunt that the species suffered between 1930 and 1970, with an estimate of 90,000 skins exported *per annum* for the American and European leather industry (Antelo et al., 2008; Medem, 1981). Consequently, the Orinoco Crocodile population diminished dramatically along all its distributional range, further influenced by eggs recollection, juveniles trade and habitat loss (Casal et al., 2013; Medem, 1981; Morales-Betancourt et al., 2015). In 1975 it was estimated that the population in Colombia was less than 1000 individuals (Medem 1981), while currently a remnant of 250 adult individuals is expected (Morales-Betancourt et al., 2015).

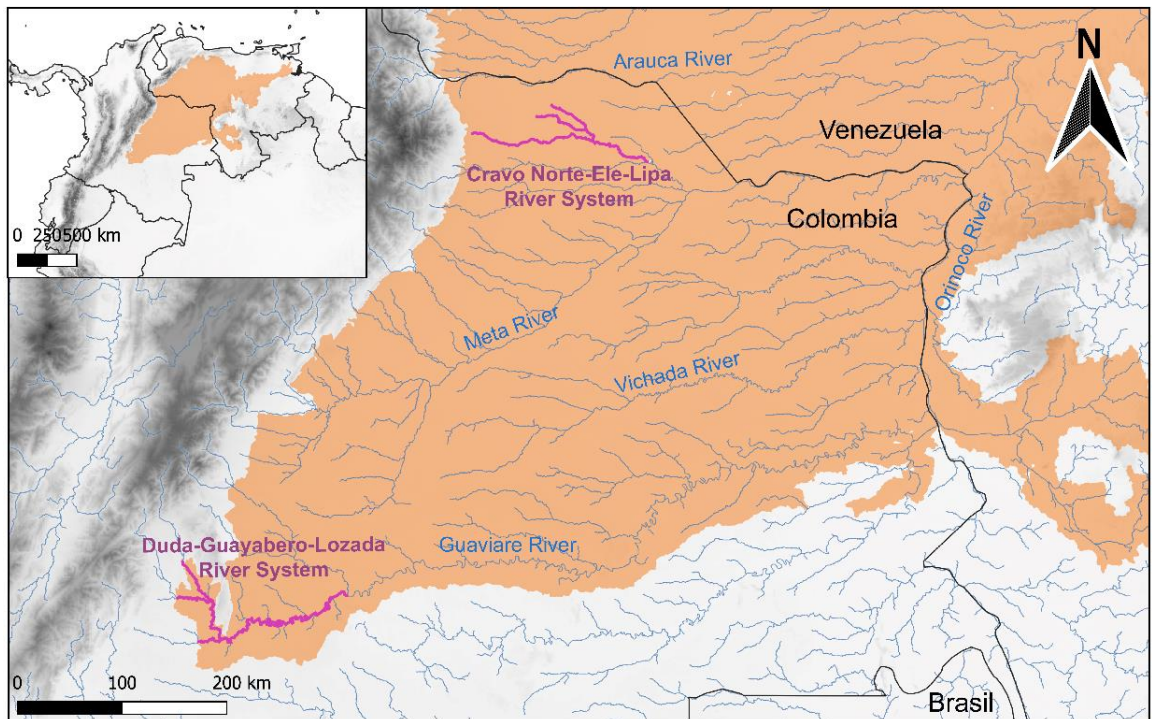
Figure I-1: Adult *C. intermedius* individual from the EBTRF *ex situ* strategy.



Currently, the Orinoco Crocodile is one of the most endangered crocodylian species in the Neotropics; it is included in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2017), is listed as critically endangered by the IUCN (Balaguera-Reina et al., 2008) and the Colombian Red List (Morales-Betancourt et al., 2015), and endangered in the Venezuela Red List (Seijas et al., 2015). Since its *in situ* presence corresponds mainly to isolated individuals and few population relics, Balaguera-Reina et al. (2017) identified areas where it is necessary and urgent to carry out management and conservation actions, defined as

Regional Habitat Priority/Crocodile Conservation Units (RHP/CCUs). In this research, the better-suited areas for the long-term preservation of the species correspond to the Cojedes System and some localities in the Apure State, in Venezuela, and the Duda-Guayabero-Lozada, and Cravo Norte-Ele-Lipa River Systems in Colombia (Figure I-2).

Figure I-2: River systems described as Regional Habitat Priority/Crocodile Conservation Units for *C. intermedius* in Colombia by Balaguera-Reina et al. (2017) are shown in purple. Historical distribution of *C. intermedius* is shown in orange (Adapted from Balaguera-Reina et al., 2018).



To face this situation, the Colombian government led by the Ministry of the Environment, the Universidad Nacional de Colombia (UNAL) represented by the Estación de Biología Tropical Roberto Franco (EBTRF), and the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH), established in 1998 the Program for the Conservation of the Orinoco Caiman (PROCAIMAN for its acronym in Spanish). It was updated in the year 2002, and defined as main objectives of the program to prevent the extinction of the species in Colombia and to promote the recovery of populations in its natural range of distribution (MMA, 2002). One of the biggest achievements for the conservation of the species in Colombia, has been the establishment of an *ex situ* population in the Estación de Biología Tropical Roberto Franco (EBTRF). Efforts for its

constitution began in the 70's counting with crocodiles from the Meta basin and confiscated individuals of unknown origin. Currently it counts with more than 593 individuals (Saldarriaga-Gómez, 2021). Likewise, an interinstitutional action plan for the Orinoco Crocodile conservation has been recently agreed between Corporinoquia, Cormacarena, Parques Nacionales Naturales de Colombia, EBTRF, Fundación Palmarito Casanare, Gobernación de Casanare and Wildlife Conservation Society (WCS-Colombia) (Antelo et al., 2022). It aims to establish three wild populations in protected areas within the historical distribution of the species, with at least five reproductive females in a period of 15 years. The accomplishment of this objective includes wild population assessments, captive breeding, newborn rescue, reintroductions, genetic management, and environmental education activities.

Advances in the genetic knowledge of the Orinoco Crocodile

Management actions, including reintroductions, had been limited since it was recognized the need for genetic evaluations in order to avoid genetic diversity loss (Antelo et al., 2022; MMA, 2002). Regarding *ex situ* population, Saldarriaga-Gómez (2021) used a set of 16 nuclear DNA (nDNA) microsatellites or simple sequence repeats (SSRs) to characterize the genetic variation of the *ex situ* EBTRF population. She identified that the living crocodiles maintain much of the founder diversity, high levels of heterozygosity, and low inbreeding. Consequently, it is a valuable resource for reintroduction and the conservation of the species.

The only approach that has been made for *in situ* population, was the population structure assessment using mitochondrial DNA (mtDNA) markers; the cytochrome b (Cyt B) and cytochrome c oxidase subunit I (COI) made by Posso-Peláez et al. (2018). They identified four haplotypes separated by no more than two mutational steps and concluded that the Orinoco Crocodile can be managed as a single genetic unit in Colombia. However, they did not include samples from the Duda-Guayabero-Lozada River System, which, as mentioned before, represents one of the two most important populations relicts of the species in the country. In addition, it is in one of the limits of the species distribution, and its likely to have its own genetic identity. Furthermore, other molecular markers that have been well documented and are common in crocodylian conservation activities may be more accurate to assess population structure parameters than the used by Posso-Peláez et al. (2018). These are SSRs (Hekkala et al., 2010; Hinlo et al., 2014; Rossi Lafferriere et al., 2020; Russello et al., 2007; van Asch et al., 2019; Vashistha et al., 2020; Velo-Antón et al., 2014), and mtDNA represented by the

D-loop or control region (CR) (Ray & Densmore, 2002, 2003; Rossi Lafferriere et al., 2020; van Asch et al., 2019).

1. Chapter 1

Population genetic structure evaluation of *Crocodylus intermedius* in Colombia: a change of perspective to improve conservation actions

Abstract

Conservation actions of threatened species and populations aim to preserve their genetic diversity and avoid processes such as inbreeding or outbreeding depressions. This perspective has been included in the Colombian management plans proposed for the critically endangered Orinoco Crocodile (*Crocodylus intermedius*). An approximation of the population structure of the species performed with the mitochondrial cytochrome b and cytochrome c oxidase subunit I identified a low diversity and proposed that the Orinoco Crocodile *in situ* population management does not present genetic restrictions. In this research, we re-evaluated this proposal by including other molecular markers that have successfully identified population-level parameters in other crocodylians (microsatellites and the control region of the mitochondria) and a wider temporal and geographic sampling. We provide evidence for a population structure pattern comprising three genetic clusters with geographic correspondence in the Colombian Orinoquía that might be a historical consequence of the ecological and geographical features of the region. We assessed the genotypic and haplotypic diversity of each of the inferred genetic clusters, evidenced gene flow between them, and managed to assign most of the confiscated individuals with unknown origin that constituted part of the F0 population in the Colombian *ex situ* population of the species. We propose that the described genetically distinctive clusters must be considered as independent management units.

Keywords: *In situ* conservation, microsatellites, mitochondrial control region, assignment analysis, population genetics.

Resumen

Las acciones de conservación de especies y poblaciones amenazadas tienen como objetivo preservar su diversidad genética y evitar procesos como depresiones por endogamia o exogamia. Esta perspectiva ha sido incluida en los planes de manejo propuestos en Colombia para el críticamente amenazado Caimán Llanero (*Crocodylus intermedius*). Una aproximación a la estructura poblacional de la especie realizada a partir de la evaluación del citocromo B y de la subunidad I del complejo citocromo C oxidasa de la mitocondria, identificó una baja diversidad y propuso que el manejo poblacional *in situ* del Caimán Llanero no presenta restricciones genéticas. En esta investigación reevaluamos ese planteamiento al incluir otros marcadores moleculares que han identificado con éxito parámetros a nivel de población en otros crocodilios (microsatélites y la región de control de la mitocondria) y un muestreo temporal y geográfico más amplio. Aportamos evidencia a favor de un patrón de estructura poblacional de tres grupos genéticos con correspondencia geográfica en la Orinoquía colombiana, como posible consecuencia histórica de las características ecológicas y geográficas de la región. Evaluamos la diversidad genotípica y haplotípica de cada uno de los grupos genéticos inferidos, evidenciamos la presencia de flujo de genes entre ellos, y logramos asignar la mayor parte de los individuos decomisados de origen desconocido que formaban parte de la población F0 en la población *ex situ* de la especie establecida en el país. Proponemos que los grupos genéticos descritos deben ser considerados como unidades de manejo independientes para la especie.

Palabras clave: Conservación *in situ*, microsatélites, región control de la mitocondria, análisis de asignación, genética de poblaciones.

1.1 Introduction

The Orinoco Crocodile (*Crocodylus intermedius*) is one of the most threatened crocodylian species in the Neotropics; it is listed as critically endangered (CR) by the International Union for the Conservation of Nature (IUCN; Balaguera-Reina et al., 2008) and included in the Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2017). This species used to be abundant in several different aquatic ecosystems along the Orinoco Basin, including rivers in tropical forests and piedmont streams in the foothills of the Andean mountains (Antelo et al., 2008; Medem, 1981). Nevertheless, the unsustainable hunt of the species during the last century, together with eggs consumption, juveniles trade, and habitat loss, caused a huge population decline along all its distributional range (Casal et al., 2013; Medem, 1981; Morales-Betancourt et al., 2015).

In Colombia, as few as 250 adult individuals are expected to remain in the wild (Morales-Betancourt et al., 2015), mainly grouped in two remanent populations that have been proposed as regional habitat priorities/crocodile conservation units: the river systems Duda-Guayabero-Lozada in the Meta department, and Cravo Norte-Ele-Lipa in the Arauca department (Balaguera-Reina et al., 2017). The last one corresponds to the better-studied *in situ* population of the species in Colombia, regarding aspects of its ecology (Anzola, 2017; Anzola & Antelo, 2015; Ardila-Robayo, Barahona-Buitrago, Bonilla-Centeno, et al., 2002; Barahona-Buitrago & Bonilla-Centeno, 1999; Lugo-Rugeles & Ardila-Robayo, 1998), anthropogenic interactions (Antelo et al., 2022; Preciado-Salas, 2018), and genetic status (this research Chapter 2). Furthermore, crocodiles in the Duda-Guayabero-Lozada River System were registered during the years 1994-1997, 2001, and 2010 (Ardila-Robayo et al., 2010; Ardila-Robayo, Barahona-Buitrago, & Bonilla-Centeno, 2002; Morales-Betancourt et al., 2015), without any recent assessments. This is a consequence of the historical context of the zone that currently presents one of the highest deforestation levels in the country and where the armed conflict has had an enormous incidence (IDEAM, 2020; Ministerio de Ambiente, 2016).

In Colombia, governmental established conservation actions include reinforcements of remanent populations as well as reintroductions in localities where the Orinoco Crocodile was once distributed but is currently extinct or just represented by isolated individuals (Antelo et al., 2022; MMA, 2002). Notwithstanding, these actions had been limited since there are many pending genetic assessments to avoid genetic diversity loss, inbreeding or outbreeding depression. In this context, Saldarriaga-

Gómez (2021) performed a genetic characterization of the *ex situ* population harbored by the Estación de Biología Tropical Roberto Franco (EBTRF) using a set of 16 polymorphic nuclear DNA (nDNA) microsatellites or simple sequence repeats (SSRs), which was recognized as a valuable source for reintroductions of individuals of the species. Regarding *in situ* populations characteristics, Posso-Peláez et al. (2018) used 27 wild caught and confiscated individuals to evaluate the species' genetic population structure using the mitochondrial (mtDNA) cytochrome b (Cyt B) and cytochrome c oxidase subunit I (COI) markers. They found minor differences and suggested that at least in Colombia, population management of this species can operate without genetic restrictions. Nonetheless, this conclusion could be in discussion since i) other molecular markers had been useful in revealing crocodylians' genetic variability at a population level (Vashistha et al., 2020), and ii) the lack of inclusion of samples from the Duda-Guayabero-Lozada River System, which, located in one of the geographic limits of the species distribution may have a distinctive genetic identity.

Consequently, the goal of this study is to reevaluate *C. intermedius* genetic population structure using a wide temporal and spatial sampling of the Colombian Orinoquía. For this purpose, we used molecular markers that have successfully identified population structure in other crocodylians and have been useful to propose robust conservation actions: SSRs (Hekkala et al., 2010; Hinlo et al., 2014; Rossi Lafferriere et al., 2020; Russello et al., 2007; van Asch et al., 2019; Vashistha et al., 2020; Velo-Antón et al., 2014), and mtDNA represented by the D-loop or control region (CR) (Ray & Densmore, 2002, 2003; Rossi Lafferriere et al., 2020; van Asch et al., 2019). By doing so, we reassess the definition of independent management units (Moritz, 1994) of the Orinoco Crocodile in Colombia. Likewise, with the results, we aim at assigning the possible origin of individuals that have been confiscated and are part of the EBTRF conservation program.

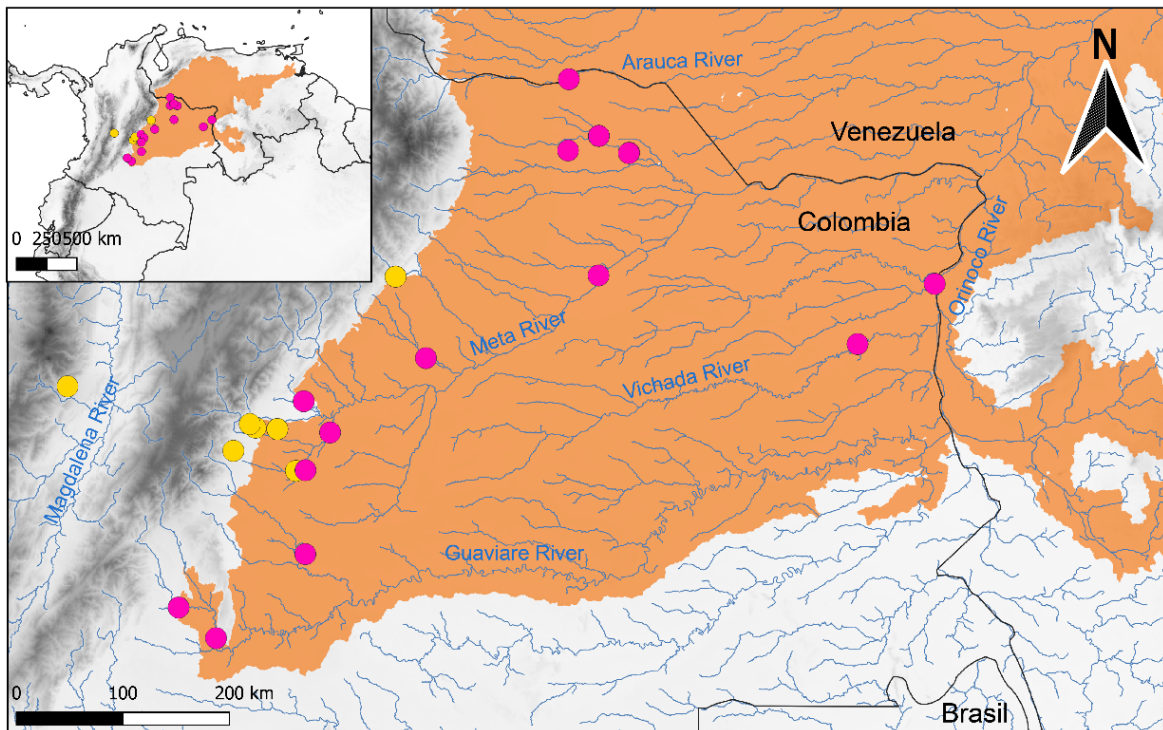
1.2 Methods

1.2.1 Sample collection

We used tissue from caudal scale or bone samples extracted from skulls located in the reptile collections from the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH-R) and the Instituto de Ciencias Naturales-UNAL (ICN). These samples came from 25 confiscated individuals of unknown origin and 18 individuals that were captured between 1956 and 2019 in the following rivers of the Colombian Orinoquía: Arauca (one sample), Ariari (one sample),

Casanare (one sample), Cravo Norte (two samples), Cusiana (one sample), Guayabero (two samples), Humea (one sample), Ele (three samples), Meta (two sample), Metica (one sample), Orinoco (one sample), and Vichada (two samples) (Figure 1-1; Table S6). Because of the difficulty of access and sampling, as well as the low number of individuals in the natural environment, no more than three individuals came from the same sample locality. All the confiscated individuals, as well as seven of the ones with known origin, were part of the F0 generation of the EBTRF *ex situ* population. All samples are preserved in 96% ethanol and stored at -20°C in the Collection Banco de ADN y Tejidos de la Biodiversidad Colombiana (BTBC) of the Instituto de Genética, Universidad Nacional de Colombia (IGUN).

Figure 1-1: Sampling sites (pink dots) in the Colombian Orinoquía. Locations of confiscated individuals are illustrated in yellow dots. The historical distribution of *C. intermedius* is shown in orange (Adapted from Balaguera-Reina et al., 2018).



1.2.2 Laboratory procedures, genotyping, and sequencing

Laboratory procedures were conducted in the Molecular Ecology Laboratory of the IGUN. We extracted genomic DNA from tissue using the NucleoSpin® Tissue Kit (Machery-Nagel, Germany) following manufacturer protocol. We used the same set of SSRs previously used for cross-amplification with *C. intermedius* by Rossi Lafferriere et al. (2016) and for the *ex situ* population evaluation of the EBTRF performed by Saldarriaga-Gómez (2021), comprising 17 microsatellite loci (Table S1) developed for the genus *Crocodylus* (C391, Cj16, Cj18, Cj101, Cj122, Cj127, CUJ131, and Cu5123; Fitzsimmons et al., 2001), *C. moreletii* (Cj109; Dever & Densmore, 2001), and *C. porosus* (CpDi13, CpP302, CpP305, CpP314, CpP801, CpP1409, CpP1610, and CpP3216; Miles et al., 2009). Polymerase chain reactions (PCR) were performed following Saldarriaga Gómez (2021) in four PCRs multiplex (Table S1) prepared in a final volume of 10 µL including 5 µL of MyTaq™ HS Mix (Bioline, USA), 0.2 µL of 10X each primer (except for Cj122 and Cj109 for which 0.4 µL were added), a final concentration of 4ng/µL of DNA and the excess of ultra-pure water to complete. The thermocycle was: an initial denaturation stage at 95 °C for 4 minutes was followed by 30 denaturation cycles at 95 °C for 30 seconds, annealing at 57°C (except for the multiplex composed by primers Cj18, CUJ131, Cj109, and C391, for which a temperature of 60°C was used) for 45 seconds, and elongation at 72 °C for 30 seconds; finally, an ending with a temperature of 72 °C for 5 minutes was settled. Subsequently, 1 µl of a dilution made with 1 µl of the PCR product and 99 µl ultra-pure water was mixed with 8.5 µl of Hi-Di Formamide (Applied Biosystems, USA), 0.25 µl of pure water, and 0.25 µl of GeneScan-600 LIZ Size Standard (Applied Biosystems, USA), for fragment length determination using an ABI 3500 Genetic Analyzer. Genotypes were identified with GENE-MAPPER 3.7 (Applied Biosystems, USA) and OSIRIS 2.13.1 (NCBI, USA) software, using as a reference the reported alleles by Saldarriaga-Gómez (2021).

A fragment of 453 base pairs (bp) of the Domain III of the CR was amplified with primers CR2H and 12SH1 (Ray & Densmore, 2002). PCR reactions were performed at a final volume of 30 µL including 3 µl of 10X PCR buffer, 3 µl of MgCl₂ solution 25 mM, 0.5 µl of dNTP solution 10mM, 0.7 µl of each primer at 10µM, 0.90 µl of BSA at 10mg/ml, 0.3 µl of Taq polymerase at 5 U/µl, 3 µl of DNA at a 5-20 µg/µl concentration, and 17.9 of ultra-pure water. PCR cycle was initiated with three cycles of denaturation at 95°C for 30 seconds, annealing at 56° C for 1 minute, and extension at 72° C for 1 minute 30 seconds; it was followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing at 58° C for 1 minute, and extension at 72° C for 1 minute. An ending temperature of 72°C for 8 minutes was used. PCR products were purified with an ammonium acetate protocol (Bensch et

al., 2000). Fragment lengths and sequences were obtained using an ABI 3130XL Genetic Analyzer automatic sequencer (Applied Biosystems, USA) in the Servicio de Secuenciación y Análisis Molecular (SSIGMOL)-IGUN.

1.2.3 Genotypic and haplotypic variation

- Genotypic variation

Genotyping inconsistencies such as null allele frequencies at each locus and allele dropout were assessed with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). GENEPOP 4.7.5 (Rousset, 2008) was used to evaluate the tendency to Hardy Weinberg (HW) equilibrium for all loci using the implemented exact test, and genotypic linkage disequilibrium (LD) between each pair of loci using the log likelihood ratio statistic. Significance levels were estimated using a Markov chain Monte Carlo (MCMC) algorithm with 10,000 dememorization steps, 1,000 batches, and 10,000 iterations per batch. Bonferroni corrections were applied to both, HW and LD calculations. Observed (H_o) and expected heterozygosities (H_e) were calculated using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010), while observed allelic diversity (A_{ob}), allelic richness (A_R) and private allelic richness (PA) per population were estimated in HP-RARE 1.0 (Kalinowski, 2005), which integrates rarefaction to cope effects of the sample size disparity between populations.

- Haplotypic variation

Sequences obtained from the CR fragment were edited and aligned with CHROMAS 2.6.6 (<http://www.technely-sium.com.au/chromas.html>) and BIOEDIT (Hall, 2005), using the Clustal W algorithm. We used ARLEQUIN and DNASP 6 (Rozas et al., 2017) to calculate haplotype diversity (H_d), nucleotide sequence diversity (π), and mean number of pairwise differences among sequences (k). In order to infer genealogical relationships between haplotypes, a parsimony haplotype network was drawn using TCS algorithm (Clement et al., 2000) implemented in POPART 1.7 (Leigh, Jessica, Bryant, 2015).

1.2.4 Population structure

- Microsatellite data

To assess population structure, we used two clustering Bayesian methods that search for partitions corresponding to populations in HW and linkage equilibrium proportions. The first one, implemented on STRUCTURE 2.3.4 (Hubisz et al., 2009; Pritchard et al., 2000), was used without sampling location priors. Posterior probabilities were calculated for genetic clusters (K) from 1 to 10, through 20 independent runs, 1'500,000 iterations, and a burn-in period of 500,000. We assumed an admixture model and correlated allele frequencies as it considers that individuals can have mixed ancestry. The optimal value of K was estimated using STRUCTURESELECTOR (Li & Liu, 2018) as it implements both the Puechmaille method (Puechmaille, 2016) and the Evanno method (Evanno et al., 2005). The resulting cluster plots were obtained with CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) and STRUCTURE PLOT 2.0 (Ramasamy et al., 2014).

The second method was implemented in the package GENELAND 4.9.2 (Guillot et al., 2005) in R 4.2.2 (R Development Core Team, 2022). This approximation has an algorithm based on a spatial model that assesses genotypes and geographical coordinates to assign individuals to a K number. By doing so, it does not assume all clustering solutions as equally possible but weights individual's location information to define the most likely K. The number of iterations was set to 1'500,000 with a burn-in of 500,000, and possible Ks as implemented in STRUCTURE. As suggested by Guillot et al. (2005), a correlated allele model was used with a maximum rate of Poisson process equal to the number of individuals, and a maximum number of nuclei in the Poisson-Voronoi tessellation of three times the number of individuals. An uncertainty of 177 km for spatial coordinates was set since it is the largest recorded distance for Orinoco Crocodile seasonal movements (Moreno-Arias & Ardila-Robayo, 2020). The optimal value of K was defined by the highest posterior probability between ten independent runs.

In addition, population structure was evaluated using a Principal Component Analysis (PCA) in ADEGENET 2.1.8 (Jombart, 2008). To estimate the degree of differentiation between and within possibly genetic groups an Analysis of Molecular Variance (AMOVA) was performed in ARLEQUIN (Jombart, 2008). Likewise, pairwise F_{ST} comparisons were assessed using the same software.

- Mitochondrial sequence data

For mitochondrial data, we explored *in situ* population structure by performing a PCA in ADEGENET 2.1.8 (Jombart, 2008). We also used ARLEQUIN 2.1.8 (Jombart, 2008) to perform an AMOVA and pairwise comparisons based on haplotype frequencies (F_{ST}). The significance of values was tested with 10,000 permutations. And finally, we tested for significant differences in haplotype frequency distributions among groups with a Chi-square analysis in DNASP 6 (Rozas et al., 2017).

1.2.5 Assignment analyses

To establish the putative provenience of individuals from the EBTR *ex situ* population that had been confiscated, we used the microsatellite data set to perform three genetic assignment/exclusion approaches in GENECLASS 2.0 (Piry et al., 2004). In the first method, we used a Bayesian test based on Rannala & Mountain (1997) that estimates the population's allele frequencies and individual assignment's statistical significance. In the second method, we used a frequencies-based method proposed by Paetkau et al., (1995), which assigned individuals to genetic groups where each of their genotypic frequencies is expected to be the highest. Finally, we used a genetic distance approach between the individual to be assigned and candidate groups; for it, we implemented the Nei's DA genetic distance (Nei et al., 1983). For each method, we applied the computation probability with the simulation algorithm proposed by Paetkau et al. (2004) with a MCMC resampling of 1'000,000 steps. Likewise, we explored the assignment of the seized individuals by performing a STRUCTURE analyses (via Q value), as previously described for population structure analyses, and including the USEPOPINFO to use the identified clusters as priors. Furthermore, a PCA was performed in ADEGENET 2.1.8 (Jombart, 2008) to explore the spatial correspondence of each assigned individual to a cluster. Additionally, with the purpose of assessing CR mtDNA haplotypes of the assigned individuals to each genetic cluster, we developed a parsimony haplotype network using TCS algorithm (Clement et al., 2000) in POPART 1.7 (Leigh, Jessica, Bryant, 2015). Finally, after including the individuals that were successfully assigned, we assessed the genotypic and haplotypic variation indexes of each cluster and performed an AMOVA and F_{ST} pairwise comparison between them.

1.2.6 Migration

Migration across genetic clusters was assessed using the Bayesian multilocus procedure implemented in BAYESASS (Wilson & Rannala, 2003). Values for mixing parameters of allele frequencies, inbreeding coefficients, and migration rates were adjusted so that acceptance rates for proposed changes between chains at the end of the run ranged between 20 and 60% of the total chain length, as optimal for chain mixing proposed by Wilson & Rannala (2003) based on empirical analyses. To test convergence, we carried out several independent runs initiated with different randomly set seeds. A MCMC was performed with 10'000,000 iterations with the first 1'000,000 set as burn-in, and samples were collected every 100 iterations to infer posterior probability distributions of the migration proportions. The migration rate is defined as m_{ij} or the fraction of individuals in the population i that are migrants derived from population j per generation. In addition, we inferred individual immigrant ancestries estimated by a population assignment test (Rannala & Mountain, 1997). For this analysis, we included the individuals that were successfully assigned to any of the inferred clusters since increasing the sample size rise the accuracy of the estimates (Meirmans, 2014; Wilson & Rannala, 2003).

1.3 Results

1.3.1 Genotypic and haplotypic variation

- Genotypic variation

We amplified all loci for the 18 wild captured individuals, except for locus Cj122 that was amplified for 15 of them. Significant values of LD were not detected between any pairs of loci, while the locus Cp305 differed significantly from HW equilibrium. Likewise, null alleles were detected for that locus. Consequently, the locus Cp305 was excluded from the following analyses. Additionally, we did not include locus CP1610 since, as defined for the *ex situ* population in the EBTRF (Saldarriaga-Gómez, 2021) it resulted monomorphic. In the Table **1-1** we present the genotypic variation of our Orinoco Crocodile *in situ* sampling for 15 loci. H_O and H_E were 0.499 and 0.611, respectively, A_{Ob} ranged from two (CpP3216) to 13 (Cj391) with a mean of 4.667 alleles per locus, and A_R from 1.833 (Cj127) to 9.251 (Cj391) with a mean of 3.857.

Table 1-1: Genetic diversity of the microsatellite data set for our sampling of *C. intermedius* from the Colombian Orinoquía. All loci were amplified for 18 individuals except for locus Cj122 which was amplified for 15 individuals. A_{Ob} observed allelic diversity; A_R allelic richness; H_O observed heterozygosity; H_E expected heterozygosity.

Locus	A_{Ob}	A_R	H_O	H_E
CpP3216	2	2.000	0.389	0.475
CpP1409	3	2.416	0.278	0.494
CpP302	5	4.548	0.556	0.754
CpP314	3	2.997	0.556	0.667
Cj16	7	5.698	0.611	0.757
CU5123	4	3.389	0.611	0.600
Cj122	5	4.780	0.600	0.752
Cj18	4	3.933	0.667	0.729
CUJ131	3	2.389	0.167	0.332
Cj109	6	4.722	0.556	0.765
Cj391	13	9.251	0.889	0.922
CCj101	3	2.897	0.556	0.552
CpDi13	3	2.417	0.444	0.538
Cj127	3	1.833	0.111	0.110
CpP801	6	4.592	0.500	0.717
Mean	4.667	3.857	0.499	0.611
s.d.	2.717	1.906	0.199	0.204

- Haplotypic variation

We obtained a 453 bp long fragment of the mitochondrial CR of the 18 wild-captured individuals included in this analysis. We identified 11 haplotypes differing in seven polymorphic sites (one transition and six transversions). Haplotype Ci5 was identified as the most frequent, found in four samples from the rivers Cusiana, Humea and Vichada. Four haplotypes are represented by two individuals each: Ci2 (Ariari and Guayabero rivers), Ci4 (Meta and Metica rivers), Ci8 (Arauca and Cravo Norte rivers, and Ci9 (Casanare and Ele rivers). While six haplotypes are represented by one individual each: Ci1 (Guayabero River), Ci6 (Orinoco River), Ci7 (Ele River), Ci10 (Ele River), Ci11 (Cravo Norte River). We grouped the sampling sites according to the proximity of river systems in the Orinoquía as follow: Arauca-Casanare-Cravo Norte-Ele, Ariari-Guayabero, Cusiana-Humea, Meta-Metica, and Vichada-Orinoco. Furthermore, we identified that the CR haplotypes do not form

a phylogeographic structure pattern (Figure 1-2). Haplotypes were distinct by one to 12 mutational steps. Overall diversity indexes were $H_d = 0.935$, $\pi = 0.007$, and $k = 2.124$ (Table 1-2).

Figure 1-2: Haplotype network of the mtDNA CR for *C. intermedius* in the Colombian Orinoquía. Circle size corresponds to haplotype frequency, and hatch mark to a mutation step.

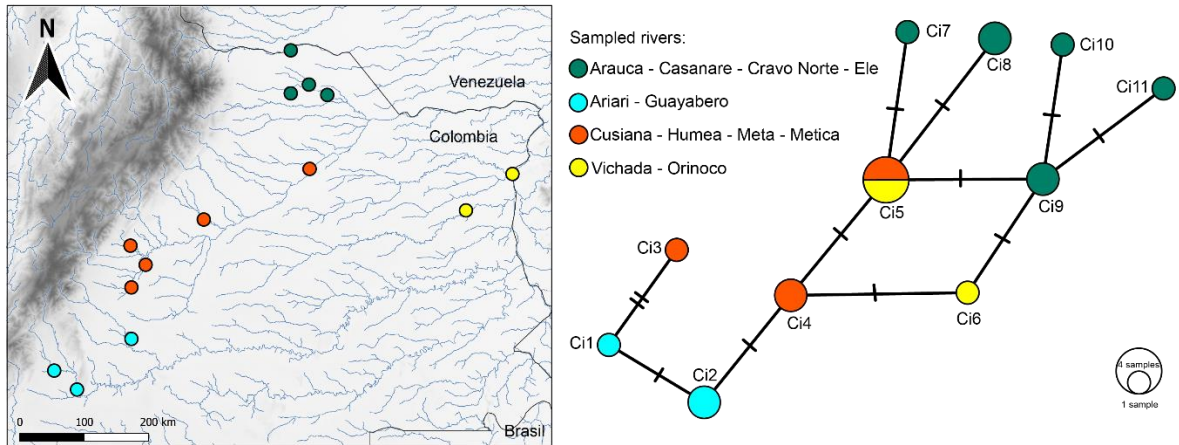


Table 1-2: Genetic diversity of the mitochondrial data for the sampling of 18 individuals of *C. intermedius* from the Colombian Orinoquía. H_d haplotype diversity; π nucleotide sequence diversity; k mean number of pairwise differences among sequences. Standard deviations in brackets.

Control Region mtDNA			
H_d	π	k	Number of haplotypes
0.935	0.007	2.124	11
(0.038)	(0.007)	(0.410)	

1.3.2 Population structure

We identified three distinct genetic units with a geographical correspondence by using both implemented Bayesian algorithms. The spatially explicit method of GENELAND was consistent in all 10 independent runs by recovering a geographic correspondence with some of the Colombian Orinoquía river basins: i) the Guaviare, ii) the Western Meta and Vichada, and iii) the Eastern Meta. In Figure 1-3 we present the correspondence posterior probability of each sampling site to each of these three inferred clusters. With the non-spatial clustering method applied in STRUCTURE, we

estimated an optimal $K=3$ with the Evanno method (Delta $K = 55.585$, Table S2), while the Puechmaille method was not able to detect any subdivision (Table S3). Nevertheless, combined with the GENELAND result, and the visual inspection of barplot for $K = 3$ that reflects individuals with $\sim 0.9\%$ probability of belonging to each of the three clusters (Figure 1-4), we proposed the Evanno method result to be the most accurate for our study case.

Figure 1-3: A) Colombian Orinoquía River Basins that correspond to the genetic clusters inferred for *C. intermedius*. B) Maps of posterior probabilities for each cluster, coordinates in planar CTM12 format. A darker color reflects a higher posterior probability.

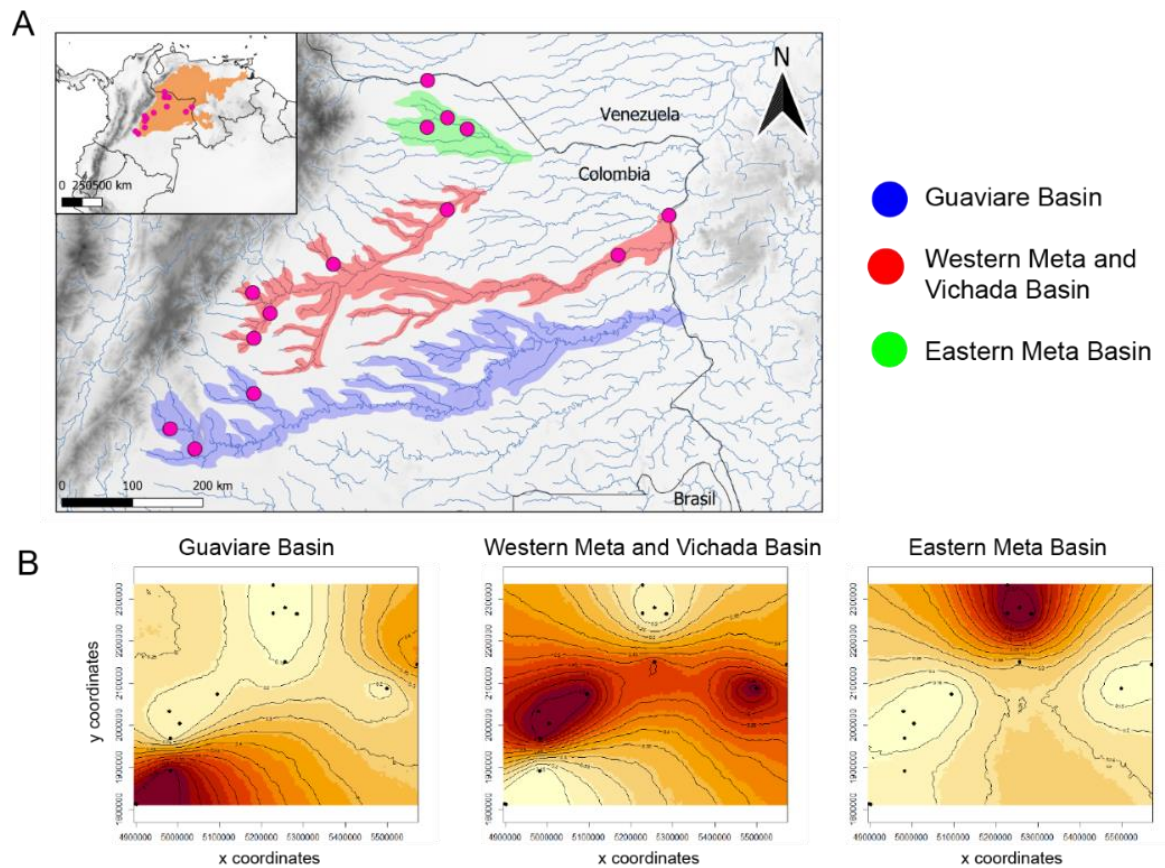
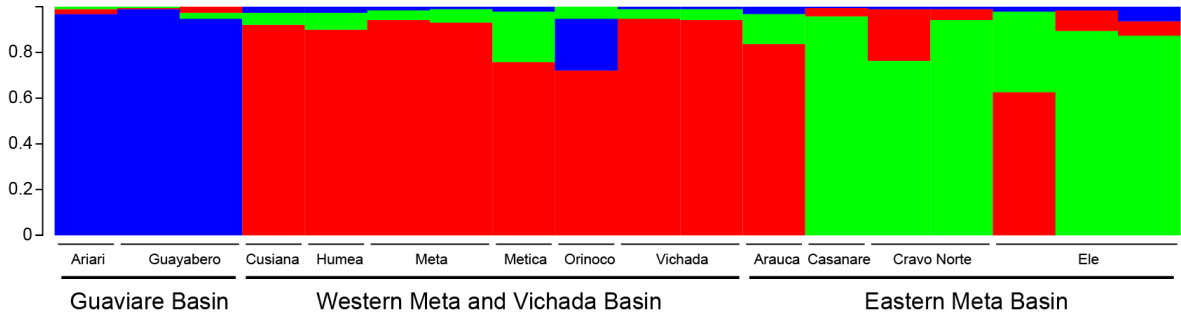


Figure 1-4: STRUCTURE barplot for the sampling of 18 *C. intermedius* individuals from the Colombian Orinoquía based on 15 microsatellite loci with K = 3.



The resulting PCA using microsatellites data indicates the spatial differentiation between these genetic clusters with the first principal component explaining 18.86 % of the variance in allele frequencies among samples, and the second principal component explaining a 17.37 % (Figure 1-5, A). The AMOVA identified that 15.98 % of the total variation occurred among these three clusters, while 84.02% occurred within them. We identified the among-groups component as significant with an $F_{ST} = 0.160$ ($P < 0.0001$). Likewise, every pairwise F_{ST} comparison was significant (Table 1-3). This evidences a greater differentiation between the clusters from the Guaviare basin and the Eastern Meta basin, and less differentiation between both clusters from the Meta basin. Consequently, three genetically distinctive units of the Orinoco Crocodile in Colombia are supported with the SSRs data set.

Figure 1-5: A) Scatter plot of PCA with PC1 and PC2 across 15 microsatellite loci. B) Scatter plot of PCA with PC1 and PC2 performed with mtDNA CR data.

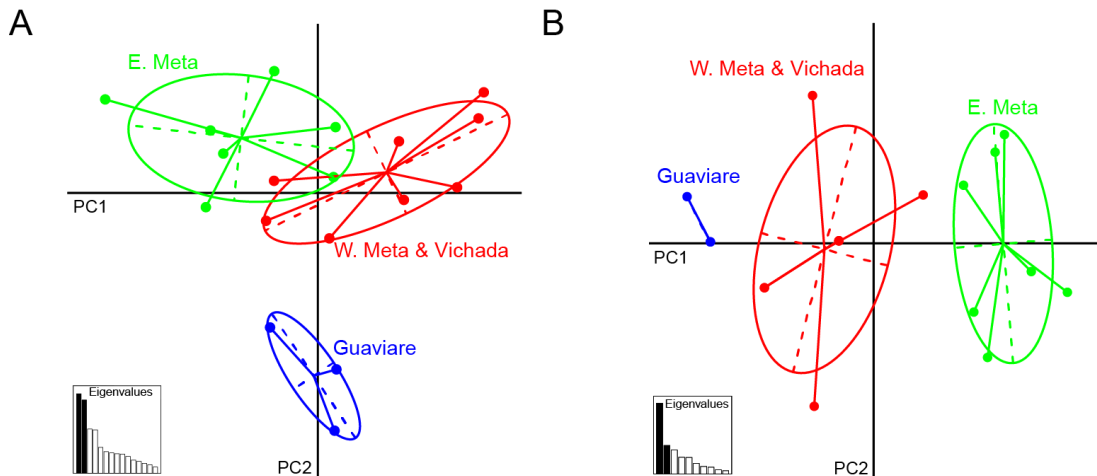


Table 1-3: Pairwise estimates of F_{ST} between inferred clusters of *C. intermedius* in the Colombian Orinoquía. Values below the diagonal correspond to comparisons based on microsatellite data, and above the diagonal based on mtDNA. Values bearing an asterisk are statistically significant: $P < 0.05$.

	Guaviare	W. Meta and Vichada	Eastern Meta
Guaviare	-	0.144	0.929*
W. Meta and Vichada	0.200*	-	0.477*
Eastern Meta	0.263*	0.099*	-

The results obtained with the CR fragment agree with the previously described microsatellite results. We performed a PCA with the mitochondrial data; the first principal component explains 36.65% of the variance and the second principal component explains 14.85% (Figure 1-5, B). We identified a spatial differentiation between the genetic clusters described for the Colombian Orinoquía with the microsatellite data set. Likewise, the conducted AMOVA identified 54.92 % of the total variation among genetic clusters and 45.08 % to occur within them. We recognized the among-groups component as significant with an $F_{ST} = 0.549$ ($P < 0.004$). In addition, F_{ST} values were significant in two pairwise comparisons; between the clusters from the Eastern Meta Basin and the Western Meta and Vichada Basins, and between the Eastern Meta Basin and the Guaviare Basin (Table 1-3). Regarding the Chi-square analysis, we obtained a significant value that supports the genetic differentiation hypothesis between the inferred clusters ($X^2 = 36$; $P < 0.015$; $df = 20$).

For each of these distinctive clusters, we assessed its genotypic and haplotypic variation (Table 1-4). The Western Meta and Vichada cluster presents the highest values for the microsatellite indexes, while the Eastern Meta cluster for the mtDNA indexes. It is remarkable that, even if the Guaviare basin cluster has the lowest sampling size (three samples), number of polymorphic loci, and genotypic variation indexes values, we were able to detect private alleles (4.125). In addition, even if we do not identify a phylogeographic structure with the CR haplotypes (Figure 1-2), we identified two private haplotypes in the Guaviare basin (Ci1 and Ci2), four in the Western Meta and Vichada basins (Ci3, Ci4, Ci5, and Ci6), and five in the Eastern Meta basin (Ci7, Ci8, Ci9, Ci10, and Ci11).

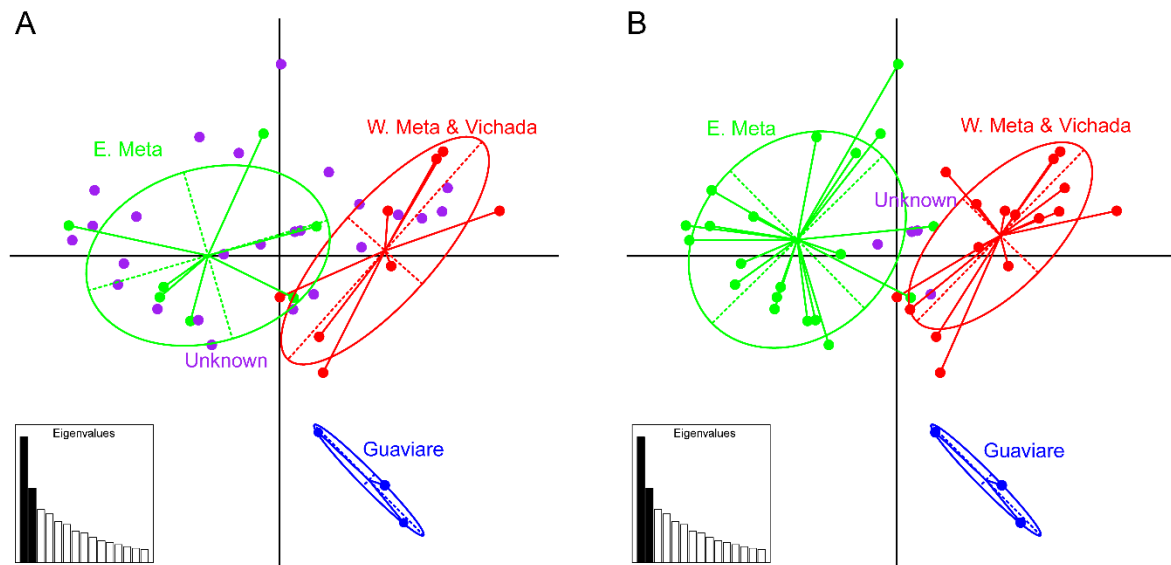
Table 1-4: Genetic diversity indexes for the proposed genetic clusters of *C. intermedius* from the Colombian Orinoquía. N sampling size; A_{Ob} observed allelic diversity; A_R allelic richness; H_O observed heterozygosity; H_E expected heterozygosity; PA private allelic richness; Hd haplotype diversity; π nucleotide sequence diversity; k mean number of pairwise differences among sequences. Private haplotypes per cluster in bold.

Basin	N	Microsatellites (nDNA)						Control Region (mtDNA)			
		Polymorphic loci	A_{Ob}	A_R	H_O	H_E	PA	Hd	π	k	Haplotypes
Guaviare	3	9	2.444	2.444	0.444	0.556	4.125	0.667	0.002	0.667	Ci1, Ci2
Western Meta and Vichada	8	15	3.933	3.858	0.581	0.626	17.313	0.750	0.005	1.536	Ci3, Ci4, Ci5, Ci6
Eastern Meta	7	13	3.385	2.933	0.571	0.604	5.250	0.905	0.006	2.476	Ci7, Ci8, Ci9, Ci10, Ci11
Total/mean	18	15	4.667	3.857	0.499	0.611	–	0.935	0.007	2.124	–

1.3.3 Assignment analysis

We defined the genetic clusters herein identified as priors to assign sized individuals from the EBTRF *ex situ* population whose providence was unknown. The methods implemented in GENECLASS successfully identified 32% of individuals with a correspondence probability higher than 70% to correspond to the Western Meta and Vichada basins, and the Eastern Meta basin clusters, 24% with a correspondence probability higher than 80%, and just one sample (4%) with a correspondence probability higher than 90% (Table S4). Nevertheless, none of individuals with unknown providence were related to the Guaviare basin cluster. This is also evident when performing a PCA, since none of the individuals to be assigned was spatially close to the Guaviare Basin samples (Figure 1-6, A). Regarding the analyses performed in STRUCTURE, it successfully assigned 84% of individuals with a membership coefficient (Q) higher than 70% to either the Western Meta and Vichada basins or the Eastern Meta basin clusters, 68% with a Q higher than 80%, and 52% with a Q higher than 90% (Table S4). In agreement with the GENECLASS methods, no individual was associated to the Guaviare basin cluster.

Figure 1-6: Scatter plot of a PCA performed with known and unknown origin individuals of *C. intermedius* across 15 microsatellite loci A) previous assignment test evaluation, and B) after assignment test evaluation. PC1 explains 19.1% of the variance and PC2 11.3%.

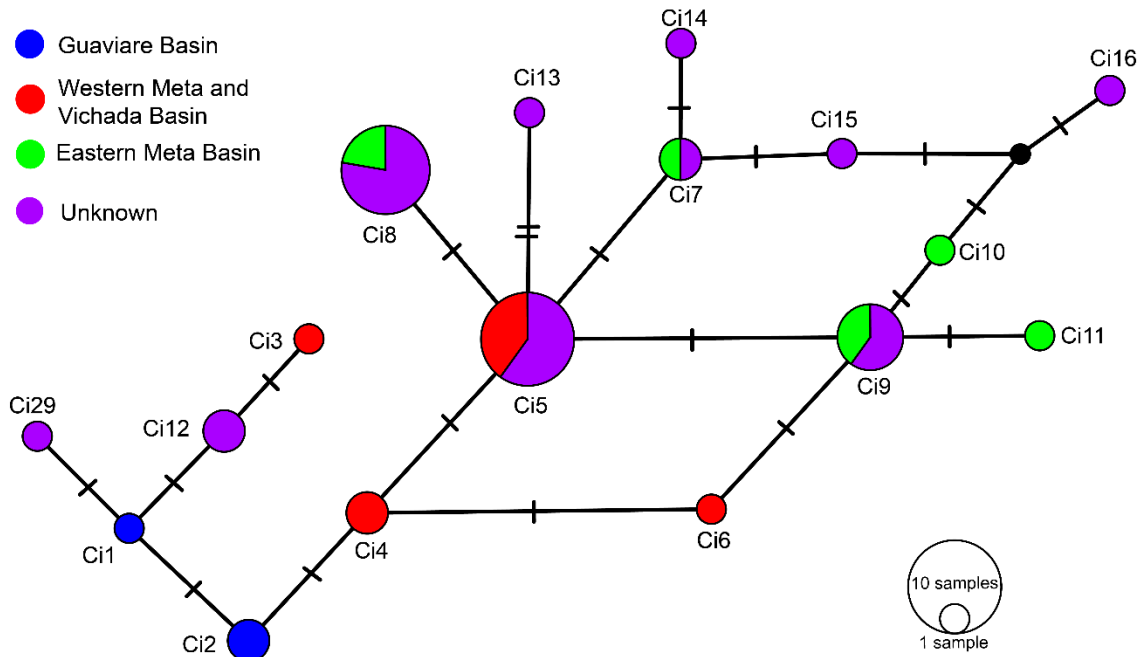


Since neither of the evaluated methods contradicted the other result, we managed to assign 84% of the individuals to one of these clusters if at least one of the microsatellite data approaches exposed a correspondence probability or a Q value higher than 70% (Table S4). We used this threshold since in study cases of endangered or invasive species it corresponds to: i) minimum assignment values obtained for positive controls (Rivera-Ortíz et al., 2021), ii) minimum assignment values of individuals defined as residents (Rollins et al., 2009), and iii) a defined threshold based on the compromise of maximizing correct and reducing incorrect assignments (Dominguez et al., 2019). In Figure 1-6, B we present in a PCA the spatial distributions of these assigned individuals in agreement with the previous defined clusters.

After the performed assignments, we identify a higher haplotypic diversity of the CR by identifying six new haplotypes (Ci12-Ci16, Ci29; Figure 1-7). Five of these (Ci13, Ci14, Ci15, Ci16, and Ci29) were detected in one individual each, all of them assigned to the Eastern Meta Basin cluster. Haplotype Ci12 was found in two individuals, one unassigned and another one assigned to the Western Meta and Vichada Basins cluster. In regard to previously described haplotypes, we identified that Ci7 and Ci9, which were formerly identified for the Eastern Meta Basin, were detected in individuals assigned to this cluster. While haplotypes Ci5 -previously described for the Western

Meta and Vichada Basins cluster- and Ci8 -previously described for the Easter Meta Basin cluster- were identified in seized individuals assigned to both clusters (Figure 1-7; Table S4).

Figure 1-7: Haplotype network of the mtDNA CR for the sampling of *C. intermedius* in the Colombian Orinoquía and confiscated individuals. Circle size corresponds to haplotype frequency, and hatch mark to a mutation step.



In addition, we evaluate the genotypic and haplotypic variation of each of the inferred genetic cluster after including assigned individuals (Table 1-5). The Western Meta and Vichada cluster and the Eastern Meta continue to present the highest values for genotypic and haplotypic diversity indexes, respectively. Even if none of the confiscated individuals were assigned to the Guaviare Basin cluster, it still counted with private microsatellites alleles (4.810), and haplotypes (Ci1 and Ci2). Furthermore, we described two new private haplotypes in the Western Meta and Vichada basins (Ci12 and Ci29), and four in the Eastern Meta basin (Ci13, Ci14, Ci15, and Ci16), while identified that haplotypes Ci5 and Ci8 are shared between these two clusters.

Table 1-5: Genetic diversity indexes of the microsatellite and mitochondrial data set for the proposed genetic clusters of *C. intermedius* from the Colombian Orinoquía with the inclusion of seized individuals that were successfully assigned to any of them. N sampling size; A_{Ob} observed allelic diversity; A_R allelic richness; H_O observed heterozygosity; H_E expected heterozygosity; PA private allelic richness; Hd haplotype diversity; π nucleotide sequence diversity; k mean number of pairwise differences among sequences. Private haplotypes per cluster in bold.

Basin	N	Microsatellites (nDNA)						Control Region (mtDNA)			
		Polymorphic loci	A_{Ob}	A_R	H_O	H_E	PA	Hd	π	k	Haplotypes
Guaviare	3	9	2.444	2.444	0.444	0.556	4.810	0.667	0.002	0.667	Ci1, Ci2
Western Meta and Vichada	16	15	4.400	3.691	0.620	0.615	15.360	0.750	0.005	1.658	Ci3, Ci4, Ci5, Ci6, Ci8, Ci12, Ci29
Eastern Meta	20	14	3.571	3.005	0.536	0.516	4.515	0.884	0.007	2.879	Ci5, Ci7, Ci8, Ci9, Ci10, Ci11, Ci13, Ci14, Ci15, Ci16
Total/mean	39	15	5.000	3.590	0.534	0.591	–	0.890	0.006	2.132	–

Moreover, we performed an AMOVA and F_{ST} pairwise comparison between the inferred clusters after including assigned individuals. For the SSRs data set, we identified with the performed AMOVA that 17.39 % of the total variation occurred among clusters, while 82.61 % occurred within them. The among-groups component was recognized as significant with an $F_{ST} = 0.174$ ($P < 0.0001$). Likewise, every F_{ST} pairwise comparison was identified as significant (Table 1-6). For the mtDNA data set, the performed AMOVA exposed a 34.66 % of the total variation among clusters and 65.34 % within them. In this case, F_{ST} values were significant in the pairwise comparisons between the Eastern Meta Basin and the Western Meta and Vichada Basins, and between the Eastern Meta Basin and the Guaviare Basin Clusters (Table 1-6). These results confirm the degree of differentiation previously described between clusters without the addition of assigned individuals.

Table 1-6: Pairwise estimates of F_{ST} between inferred clusters of *C. intermedius* in the Colombian Orinoquía with the inclusion of seized individuals that were successfully assigned to any of them. Values below the diagonal correspond to comparisons based on microsatellite data, and above the diagonal based on mtDNA. Values bearing an asterisk are statistically significant: $P < 0.05$.

	Guaviare	W. Meta and Vichada	Eastern Meta
Guaviare	-	0.209	0.677*
W. Meta and Vichada	0.204*	-	0.267*
Eastern Meta	0.318*	0.138*	-

1.3.4 Migration

For this analysis, we recognize the limitations of our results since it did not count with the sampling sizes that some authors had suggested as accurate to identify the magnitudes of the gene flow process (Faubet et al., 2007; Meirmans, 2014). Nevertheless, it is important to approximate to identifying this process rather than its magnitude, especially in endangered species (Gottelli et al., 2013; Neuwald, 2010; Yang & Jiang, 2011). Likewise, even if sampling sizes are not as expected, threatened populations, as the herein study case, are likely to present a high population structure ($F_{ST} > 0.1$), which had been detected as an important factor for accurately approximating gene flow detection (Faubet et al., 2007; Meirmans, 2014).

Migration rates are considered to be high when $m \geq 0.1$, and very low when $m = 0.01$ (Faubet et al., 2007). We assessed migration rates including the 21 confiscated individuals that were successfully assigned to the Western Meta and Vichada Basin and the Eastern Meta Basin clusters to increase our sampling size. We detected a gene flow process with estimated migration rates (Figure 1-8; Table 1-7) that suggest asymmetric migration. We identified a higher influx from the cluster in the Guaviare basin to the proposed one for the Western Meta and Vichada basins. Likewise, this was also the case between the Eastern Meta cluster and the Western Meta and Vichada cluster, where a higher influx from the first to the second was revealed.

Figure 1-8: Gene flow pattern among *C. intermedius* populations. Gene flow direction is represented by arrows and its relative amount by the arrow's thickness. Each value indicates its mean migration rate m .

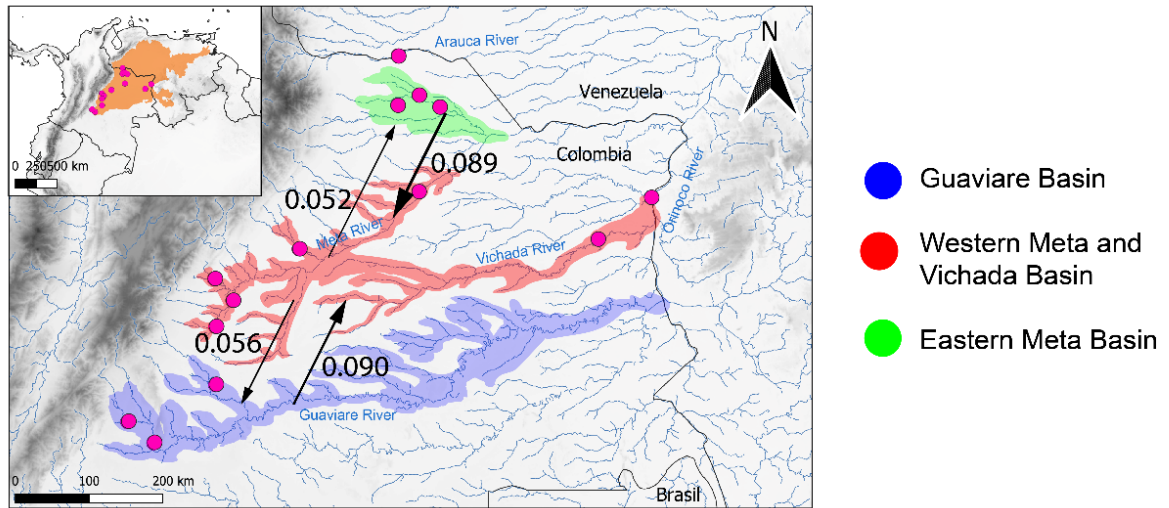


Table 1-7: Gene flow estimates for each genetic cluster of *C. intermedius* in the Colombian Orinoquia. Estimates of gene flow represent the mean migration rate m and 95% confidence interval in brackets. $m = m_{ij}$ or the fraction of individuals in the population i that are migrants derived from population j per generation.

Cluster pair	Gene flow (m) B
Guaviare – W. Meta and Vichada	0.056 (0-0.150)
Guaviare – E. Meta	0.056 (0-0.149)
W. Meta and Vichada – Guaviare	0.090 (0.014-0.166)
W. Meta and Vichada – E. Meta	0.089 (0.017-0.160)
E. Meta – Guaviare	0.018 (0-0.050)
E. Meta – W. Meta and Vichada	0.052 (0-0.118)

Additionally, we evidence the isolated status of the Guaviare Basin cluster, since the three individuals assigned to it were identified as residents or no migrants with a high percentage (99.4; Table 1-8). In concern to the Western Meta and Vichada Basins and the Eastern Meta basin clusters, we identified that these are composed mainly of residents (51.4% and 86.8%, respectively), but second-generation migrants or migrants' offspring are an important component of them. For the Western Meta and Vichada cluster, these are derived from both, Guaviare (14.5%), and Eastern Meta

basins clusters (27.4%). In the case of the Eastern Meta cluster, second-generation migrants are derived mainly from the Western Meta and Vichada cluster (11.9%)

Table 1-8: Percentage of assignment to no migrants, migrants, and migrant's offspring per genetic cluster evaluated with BAYESASS.

Assigned from	1			2			3		
	Guaviare Basin Cluster			Western Meta and Vichada Basins Cluster			Eastern Meta Basin Cluster		
	No migrant	Migrant	Migrant's offspring	No migrant	Migrant	Migrant's offspring	No migrant	Migrant	Migrant's offspring
1	99.4	–	–	–	6.6	14.5	–	0.0	1.2
2	–	0.0	0.5	51.4	–	–	–	0.1	11.9
3	–	0.0	0.1	–	0.0	27.4	86.8	–	–

1.4 Discussion

1.4.1 Evidence of *in situ* population structure

We detected that the Orinoco Crocodile presents a genetic population structure with geographic correspondence in the Colombian Orinoquía. This means a change of perspective in our understanding and management actions for the species since Posso-Peláez et al. (2018) suggested that at least in the country, the species could be managed as a single genetic unit based on four closely related CytB and COI mtDNA haplotypes. Since that research was also conducted with crocodiles from the EBTRF, we share nine sampled individuals in our analyses (Table S6), identifying that the haplotypes that the authors described for the CytB and COI do not correspond to the ones herein described for the CR. We identified that individuals with haplotype Cin1 (CytB and COI) present CR haplotypes Ci16, Ci4, Ci5, Ci8, and Ci9; while individuals with haplotype Cin2 (CytB and COI) present CR haplotypes Ci3, Ci5, and Ci9.

It illustrates how genetic diversity, as well as population structure inferences, vary depending on which molecular markers are used, as a consequence of mutation rate differences (Amavet et al., 2021). In this research, we evidence how the use of SSRs and the mitochondrial CR present a genetic pattern for *C. intermedius* that is not observed with the Cyt B and COI fragments evaluation.

Contrasting results had been obtained with regard to this aspect for different crocodylians species: consistent population structure evidence had been recovered for *Paleosuchus trigonatus* between the Brazilian Madeira and Xingu river populations with both Cyt B (Bittencourt et al., 2019) and ten microsatellites loci analyses (Muniz et al., 2019). Likewise, *Melanosuchus niger* Ecuadorian population was identified as genetically distinct from the remaining ones in the Amazon basin with both Cyt B (Vasconcelos et al., 2008) and analyses of eight microsatellites loci (de Thoisy et al., 2006). Further, differentiation between coastal (Davis et al., 2002) and inland (Ryberg et al., 2002) populations of *Alligator mississippiensis* had been found through microsatellites assessments, while mtDNA was unable to detect differentiation among populations (Glenn et al., 2002). Similarly, an increase in microsatellite variability was reported in *Caiman latirostris* Argentinian populations, while the Cyt B counted with a low genetic diversity (Amavet et al., 2017). In regard to the genus *Crocodylus*, in the case of *C. acutus*, individuals from Cuba and Jamaica are included in the same cluster according to Cyt B and COI analyses (Balaguera-Reina et al., 2021; Milián-García et al., 2018; Pacheco-Sierra et al., 2018), while a microsatellite assessment counting on 11 loci, identified a distinctive genetic identity between individuals from those islands (Rossi Lafferriere et al., 2020).

The genetic clusters that we identified correspond to i) the Guaviare, ii) the Western Meta and Vichada, and iii) the Eastern Meta basins and are supported by Bayesian (Figures 1-3 and 1-4) and statistical approaches (Figure 1-5), as well as by the presence of private alleles and haplotypes (Table 1-4). The resulting PCAs particularly illustrate the wide separation of the samples from the rivers Guayabero and Ariari (i.e., the Guaviare Basin). Even if the variance explained by the first two components of the microsatellites PCA corresponds to the 36.23 % of the total, it is between the reported values for recent PCA analyses performed for different vertebrates using microsatellites (see Cohen & Ruane, 2022; Jablonski et al., 2021; Rossi Lafferriere et al., 2020; Turba et al., 2022) and also with Single Nucleotide Polymorphisms (see Gustafson et al., 2022; Weaver et al., 2022). We evidenced the differentiation of these populations expressed by significant pairwise F_{ST} comparisons (except for the Western Meta and Vichada-Guaviare comparison of mtDNA data; Tables 1-3 and 1-6), whose higher value correspond to the comparison between the Guaviare Basin cluster, especially with the Eastern Meta Basin cluster for both, microsatellite ($F_{ST} = 0.263$, $P < 0.05$) and mitochondrial data ($F_{ST} = 0.929$, $P < 0.05$). It is remarkable since these two basins contain the remanent populations of the species located in the Duda-Guayabero-Lozada and the Cravo Norte-Ele-Lipa River Systems, respectively (Morales-Betancourt et al., 2015). On the other side, the Western Meta and Vichada basin and the Eastern Meta basin clusters presented a higher connection degree. This is expressed by a lower pairwise F_{ST} comparison value of the microsatellite data ($F_{ST} =$

0.099, $P < 0.05$) and the magnitude of second-generation migrants present between both clusters (Table 1-8); both areas are influenced by the Meta River and are likely connected through the floodable ecosystems of the region.

Even if we did not identify a phylogeographic structure expressed in the CR mtDNA haplotype network (Figure 1-2), the presence of some unique haplotypes per cluster, and the spatial separation illustrated in the obtained PCA performed with mtDNA evidence (Figure 1-5, B), suggest that the population structure pattern revealed by the microsatellite data analyses has been present for a relatively long time and may have originated due to historical processes and maintained since then by biogeographical and anthropic factors. Recently, it has been proposed that a reduction in the sea level of more than 60 m during the Last Glacial Maximum, limited the gene flow between the *Crocodylus acutus* Pacific and Caribbean populations in Panama (Avila-cervantes & Larsson, 2023). *C. acutus* and *C. intermedius* exhibit a high dependency on aquatic systems, but while *C. acutus* is the Neotropical crocodile most widely distributed, inhabiting hypersaline and riverine ecosystems (Martin, 2008; Oaks, 2011; Thorbjarnarson, 1989), *C. intermedius* is restricted to the water system of the Orinoco (Martin, 2008). During the Late Pleistocene, the Orinoco River discharge was reduced and presented larger differences in its maximum and minimum (Iriando, 1999). This process might have produced a parallel phenomenon to one proposed for *C. acutus*, where a reduction in the level of the Orinoquía rivers acted in favor of structuring the Orinoco Crocodile populations.

In addition, some geographical accidents may constitute a natural barrier for the species. Medem (1981) proposed that the Maipures and Atures streams, located approximately 120 km North of the Guaviare River mouth in the Orinoco River, may limit the movement of *C. intermedius* individuals. Our results support Medem's hypothesis since the only individual –apart from the individuals from the Guayabero and Ariari Rivers– with an important $Q = 22.6\%$ inferred by STRUCTURE to belong to the Guaviare cluster is the one collected in the Guahibo stream, Orinoco River, which neighbors the Maipures stream (Figure 1-4). Furthermore, the Angosturas and the Mapiripana streams, respectively located in the Guayabero and the middle Guaviare river -the West to East direction of its course-, are likely to increase the isolation degree of the species populations in that basin.

Furthermore, we recognize the importance that ecological factors may have in this process since each of the inhabiting areas of identified clusters do present important ecological differences: the sampled areas in the Eastern Meta Basin are characterized by lowland floodplains and aeolian savannas (Bustamante, 2019; Mora-Fernández et al., 2015); the Western and Meta Vichada Basins

mainly correspond to high plains and no floodable savannas southern the Meta River (Bustamante, 2019); and, the Guaviare Basin that is characterized by riparian and floodable forests, as a consequence of its Amazonian influence (Bustamante, 2019; Hernández-Camacho et al., 1992).

Currently, the isolation between the inferred clusters might have increased as a consequence of habitat fragmentation across the Orinoquía (Bustamante, 2019) and the population decline (Morales-Betancourt et al., 2015). Furthermore, the high degree of isolation identified for the genetic cluster described for the Guaviare Basin might be a consequence of the reasons exposed, as well as its location as the southern and western distributional limit of the species (Balaguera-Reina et al., 2018; Morales-Betancourt et al., 2015). Nevertheless, since our sampling for this cluster consisted of three samples from the Guayabero and Ariari Rivers, we highlight the importance to assess more individuals from the Duda-Guayabero-Lozada River System as well as to evaluate other locations in the Guaviare basin where some individuals may subsist. Even if there are no recent records of the species in other localities of the basin (Balaguera-Reina et al., 2017; Morales-Betancourt et al., 2015), it is probable that some remanent individuals may persist. This had been proposed for other areas of the Colombian Orinoquia (Antelo et al., 2022) and may occur along the Guaviare River, particularly in the Iteviare and Siare rivers mouths, since it is still an area with a low human population and good habitat quality (Balaguera-Reina et al., 2017; Morales-Betancourt et al., 2019).

1.4.2 Genetic diversity status

As a result of our wide temporal framework, our analysis does not reflect the current genetic diversity status of the species, but it aimed to serve as a point of reference for future studies of its populations. By analyzing our complete data set with known sampling locality, we identified that the Orinoco Crocodile presented a relatively high genetic diversity expressed by both genotypic and haplotypic diversity indexes, in comparison to other wild populations of *Crocodylus* including species listed as least concerned according to the IUCN categories (Table S5). Besides, we inferred that some of the *in situ* diversity has not been recovered by any *ex situ* initiative, since it presents higher microsatellite diversity indexes than the *ex situ* populations of the species, except for the El Frío Biological Station, that presented a higher A_{ob} index of 5.294 (Rossi Lafferriere et al., 2016). This might be expected since this *ex situ* population was established by introducing individuals from different wild populations, such as the Capanaparo and the Cojedes rivers, and from unknown origin crocodiles without considering their genetic identity (Antelo et al., 2008).

Regarding the genetic clusters proposed, we identified that the Western Meta and Vichada basins have the highest H_E , A_{Ob} , and A_R (Table 1-4). Nevertheless, this result does not reflect the current status of the population since most of the sampling localities of the individuals included in our analyses may be places where the species is locally extinct or characterized by few remanent individuals, i.e., the Meta and Vichada rivers, and most of their tributaries (Castro et al., 2011). It is important to evaluate new localities in the area where the species had been recently observed, particularly in the Manacacías and Yucao rivers (Antelo et al., 2022), in order to identify the current *in situ* status of this cluster.

With respect to the clusters from the Eastern Meta and the Guaviare basins, our evaluation with the microsatellites data set revealed that the first one presents moderate to high genotypic variation with a relatively high H_E and moderate A_{Ob} and A_R . Regarding its haplotypic variation, it showed high value indexes, especially on its Hd . In the Chapter 2 of this thesis dissertation, we assessed the genetic status of the current remanent of this genetic cluster, i.e., the Lipa-Ele-Cravo Norte River System (Eastern Meta Basin). On the other hand, even if we defined the Guaviare basin cluster based on solely three samples, they consistently differentiated from the rest of our sampling and showed higher H_O and H_E values (0.444 and 0.556, respectively) than the reported for other *Crocodylus* species listed as critically endangered (*C. mindorensis* [H_O from 0.408 to 0.457; H_E from 0.423 to 0.446] and *C. rhombifer* [$H_O = 0.490$; $H_E = 0.540$]), or even as least concern (*C. moreletii* [H_O from 0.350 to 0.579; H_E from 0.300 to 0.552], *C. niloticus* [H_O from 0.400 to 0.717; H_E from 0.250 to 0.745] and *C. porosus* [H_O from 0.371 to 0.633; H_E from 0.456 to 0.622]). Nevertheless, its A_{Ob} and A_R are some of the lowest values ever reported for wild populations of *Crocodylus*. This is also the case for its haplotypic variation indexes; it presents a relatively high Hd (0.667), while moderate π (0.002), and k (0.667), in comparison of the vulnerable *C. acutus* (Hd from 0 to 0.610; π from 0 to 0.15; k from 0 to 2.820), and other *Crocodylus* listed as least concerned (*C. moreletii* [Hd from 0 to 0.610; π from 0 to 0.15; k from 0 to 2.820], *C. niloticus* [Hd from 0 to 0.861; π from 0 to 0.15; k from 0 to 8.144] and *C. porosus* [$Hd = \pi = k = 0$]). Nowadays, this genetic cluster is represented by the crocodiles inhabiting the Duda-Guayabero-Lozada River System. It is a priority to assess its status to evaluate changes in its genetic diversity indexes values, and to evaluate ecological, demographical, and human-related attributes.

1.4.3 Conservation implications

We were able to assign unknown providence individuals to one of the genetically inferred clusters by means of the proposed genetic data analyses. This is relevant for the conservation of the species since i) Ascertain that the F0 generation of the EBTRF *ex situ* population is composed of individuals from the Western Meta and Vichada basins and the Eastern Meta basin clusters and do not count with representatives of the Guaviare basin cluster, ii) provide a reference tool for identifying a likely origin of new confiscated Orinoco Crocodiles, and iii) add new information for the selection of individuals to be reintroduced.

Based on the evidence in favor of the population structure of *C. intermedius*, we propose that the identified genetically distinctive clusters for the Colombian Orinoquía must be treated as three different independent management units *sensu* Moritz (1994). This may lead to an improvement in the conservation actions of the species in the wild since provide a better understanding of its biology and by doing so, the chance of taking the most accurate decisions for the conservation of its genetic diversity and structure, translated in the avoiding of local adaptations dilutions by outbreeding depression. Even if the risk of this phenomenon is lower than what previously thought (Frankham et al., 2011; Weeks et al., 2017), it increases if populations inhabit different environments or had no gene flow in the last 500 years (Frankham et al., 2011). This may be the case due to the environmental heterogeneity that characterizes the Orinoquía, and the isolation that may have historically suffered by the population inhabiting the Guaviare Basin.

Currently, the crocodiles that inhabit the Western Meta and Vichada Basin correspond mainly to isolated individuals without any sign of population recovery (Castro et al., 2011), and consequently, the institutions that conform PROCAIMÁN (MMA, 2002) and the interinstitutional action plan for the Orinoco Crocodile conservation in Colombia (Antelo et al., 2022) are leading reinforcements and establishments of *de novo* population in those areas. Nevertheless, we encourage the individuals to release represent the genetic identity of the genetic cluster that we identified for the basin. This is possible since some of the individuals that conform the F0 generation of the EBTRF *ex situ* population are known to come from those areas or, in the case of the ones that were confiscated, are assigned to it with a high probability (Table S4, Table S6). Furthermore, we argue for the evaluation of measures for increasing the connectivity between remanent populations, since, as we detected a

low but existing gene flow between the inferred clusters (Figure 1-8; Tables 1-7 and 1-8) it is critical to maintaining it functional in order to not affect the subsistence of the populations.

On the other hand, we put forward attention on the need for genetic assessments for both remaining *in situ* concentrations of Orinoco Crocodiles that persist in the Colombian Orinoquía. We accomplished this objective in Chapter 2 of this thesis dissertation for the one that inhabits the Lipa-Ele-Cravo Norte River System (Eastern Meta basin), but it remains a pending action in the case of the Duda-Guayabero-Lozada River System (Guaviare basin) individuals. Since none of the individuals that we assessed recovered the Guayabero and Ariari Rivers genetic identity, it is likely to be restricted to that area and represent a valuable resource for the species' long-term survival. Therefore, we strongly recommend avoiding any management action that could affect its genetic identity or structure. This means, that at least until an evaluation of the genetic status of the crocodiles in the area is attained, reintroductions from the *ex situ* population must be avoided in favor of the demographic increase with local individuals by hatchlings or egg ranching. These activities are currently taking place in the Cravo Norte River vicinities as autonomous conservation initiatives (Antelo et al., 2022) and are an example of what might be supported in the Duda-Guayabero-Lozada River System area.

2. Chapter 2

First genetic evaluation of a *Crocodylus intermedius* wild population: new insights for the recovery of a critically endangered species

Abstract

During the last century, the Orinoco Crocodile (*Crocodylus intermedius*) suffered a process of hunting driven by the American and European leather industry demand. This situation led to a sharp decline of its populations. Nowadays, there persist only two remanent populations in the Colombian Orinoquía: in the Guayabero-Duda-Lozada and the Cravo Norte-Ele-Lipa Rivers Systems. The last has been the most studied, counting recent surveys and local initiatives of conservation such as egg ranching. Furthermore, population recovery has been evidenced based on the increase of observed clutches in the area. Nevertheless, information about its genetic status was pending to assess. With this research, we aim to provide the genetic characterization of this remanent population and evaluate the diversity that a period of the egg ranching program recovered. For this purpose, we used variable molecular markers represented by microsatellites and the control region of the mitochondria. We described a high genetic diversity expressed on its genotypic and haplotypic variation relative to other *Crocodylus* and *C. intermedius in situ* populations inferred in Chapter 1. Nevertheless, allele richness estimates might indicate a process of genetic diversity loss. We identify an effective population size of 11.5-17, which is far below minimum values proposed as required for short-term subsistence. We did not find evidence of inbreeding but recognize it as a likely risk. In addition, we detected an historical bottleneck possibly influenced by the arid periods that affected the region since the Pleistocene, and a process of population expansion that suggests its subsequent recovery. We conclude that the evaluated population presents a unique opportunity for *C. intermedius* conservation, and that the main action to be taken is the support of the egg ranching program, which successfully recovered most of the genetic diversity present in the population.

Keywords: Neotropics, bottlenecks, population expansion, effective population size, *in situ* conservation.

Resumen

Durante el siglo pasado, el Caimán Llanero o Cocodrilo del Orinoco (*Crocodylus intermedius*) sufrió un proceso de caza impulsado para satisfacer la demanda de la industria de cuero estadounidense y europea. Esta situación provocó una fuerte disminución de sus poblaciones. En la actualidad, persisten sólo dos poblaciones remanentes en la Orinoquía colombiana en los sistemas de ríos Guayabero-Duda-Lozada y Cravo Norte-Ele-Lipa. Este último ha sido el más estudiado, contando con censos recientes e iniciativas locales de conservación como la cría de huevos. Además, se ha evidenciado una recuperación poblacional a partir del aumento de nidadas observadas en la zona. Sin embargo, aspectos referentes de su estado genético permanecían sin evaluar. Con esta investigación pretendemos brindar la caracterización genética de esta población remanente y evaluar la diversidad que fue recuperada durante un período del programa de ranqueo de huevos. Para ello, utilizamos marcadores moleculares variables representados por microsatélites y la región de control de las mitocondrias. Describimos una diversidad genética alta expresada en términos de su variación genotípica y haplotípica, en comparación de otras poblaciones *in situ* de *Crocodylus* y de *C. intermedius* evaluadas en el Capítulo 1. No obstante, las estimaciones de riqueza alélica podrían indicar un proceso de pérdida de diversidad genética. Así mismo, identificamos un tamaño poblacional efectivo de 11.5-17, que está muy por debajo de los valores mínimos propuestos como necesarios para la subsistencia a corto plazo. No encontramos evidencia de endogamia, pero la reconocemos como un riesgo probable. Adicionalmente, detectamos un cuello de botella histórico posiblemente influenciado por los periodos áridos que afectaron a la región desde el Pleistoceno, así como un proceso de expansión poblacional que sugiere su posterior recuperación. Concluimos que la población evaluada presenta una oportunidad única para la conservación de la especie y que la principal acción a tomar es el apoyo al programa de crianza de huevos, el cual recuperó exitosamente la mayor parte de la diversidad genética presente en la población.

Palabras clave: Neotrópico, cuellos de botella, expansión poblacional, tamaño efectivo población, conservación *in situ*.

2.1 Introduction

Crocodylus intermedius, or the Orinoco Crocodile, is one of the most endangered species in the Neotropics (Balaguera-Reina et al., 2017; Seijas et al., 2010). In the past, the species was widely distributed along the Orinoco River basin in Colombia and Venezuela, in several different aquatic ecosystems, including rivers in tropical forests and piedmont streams in the foothills of the Andean mountains (Antelo et al., 2008; Medem, 1981). Nonetheless between 1928 to 1969 *C. intermedius* suffered an enormous population decline caused by an unsustainable hunt process to supply the American and European leather industry demand (Medem, 1981). This situation, in addition to habitat loss and eggs recollection for local consumption, has led the Orinoco Crocodile to be recognized as critically endangered by the IUCN red list (Balaguera-Reina et al., 2018), and listed in Appendix I of CITES (CITES, 2017). To face this critical situation the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH), the Universidad Nacional de Colombia (UNAL) represented by the Estación de Biología Tropical Roberto Franco (EBTRF), and the Colombian Environment Ministry (MMA) established in 1998 the National Program for the Conservation of the Plain Caiman (PROCAIMAN for its acronym in Spanish), a governmental program to avoid the extinction of this species in the country that recognized the importance of including genetic assessments of the species and its populations (MMA, 2002).

The inclusion of genetic evaluations is a critical aspect of integrative management plans for threatened populations and species since enables access to population-level parameters such as diversity indexes and provides evidence of events such as bottlenecks or inbreeding (Willi et al., 2022). By doing so, is possible to assess population status and decide which management actions are needed, with the aim of increasing population size while preserving genetic diversity (Jamieson et al., 2008; Willi et al., 2022). This is translated into the conservation of population evolutionary potential, or, its ability to adapt to a changing environment (Reed & Frankham, 2003; Vandewoestijne et al., 2008). In the case of the Orinoco Crocodile, new efforts have been made to evaluate its genetic status: A characterization of the *ex situ* Colombian population, established by the EBTRF as a conservation strategy, was performed with a set of 16 microsatellites or SSRs nDNA. This research found high levels of heterozygosity and low inbreeding, which makes these individuals suitable for reintroductions in the wild (Saldarriaga-Gómez, 2021). In regard of the *in situ* populations of the species, Posso-Peláez et al. (2018), based on the mitochondrial cytochrome b (Cyt B) and cytochrome c oxidase subunit I (COI), proposed that population management in Colombia

can operate without genetic restrictions. Nevertheless, the inclusion of more variable genetic data such as SSRs, and the Domain III of the mitochondrial control region (CR), revealed that the Orinoco Crocodile in the wild cannot be considered as a unique management unit, but three: the Eastern Meta Basin, the Western Meta and Vichada Basins and the Guaviare Basin (Chapter 1 of this thesis).

Nowadays, the presence of *C. intermedius* in the wild consists mainly of isolated individuals (e.g. in the Meta and Vichada Rivers; Castro et al., 2011) and few population relicts identified as regional habitat priorities/crocodile conservation units: the Cojedes System and some localities in the Apure State, in Venezuela, and the Duda-Guayabero-Lozada / Cravo Norte-Ele-Lipa River Systems in Colombia (Balaguera-Reina et al., 2017). This last relict population is the most studied, with surveys in 1994-1995, 2000-2001, 2012, and 2014-2015 (Anzola, 2017; Ardila-Robayo, Barahona-Buitrago, Bonilla-Centeno, et al., 2002; Barahona-Buitrago & Bonilla-Centeno, 1999; Lugo-Rugeles & Ardila-Robayo, 1998). It was first estimated a population of 50 to 54 adults (Ardila-Robayo, Barahona-Buitrago, Bonilla-Centeno, et al., 2002; Lugo-Rugeles & Ardila-Robayo, 1998), and in the latest survey, 102 adults were sighted (Anzola, 2017). Likewise, an increase in the number of observed nests had been recognized: from 1994 to 2012 reported nests ranged from seven to 11 (Ardila-Robayo, Barahona-Buitrago, Bonilla-Centeno, et al., 2002; Barahona-Buitrago & Bonilla-Centeno, 1999; Castro et al., 2012), while Anzola & Antelo (2015) reported 24 from December 2014 to April 2015. These results may suggest a process of population recovery (Anzola, 2017; Anzola & Antelo, 2015). In addition, it is noteworthy the development of local initiatives that are taking place and that are currently supported by the Wild Conservation Society (WCS), which include egg ranching for conservation purposes (Antelo et al., 2022). Nevertheless, neither the genetic status of this population nor how much of its diversity is being recovered by the egg ranching program has been evaluated.

Therefore, the goal of this research project is to perform the first genetic characterization of one *in situ* remanent population of *C. intermedius* by evaluating individuals belonging to the crocodile conservation units of Cravo Norte-Ele-Lipa Rivers System. To accomplish this objective, we include the analyses of variable molecular markers represented by 17 microsatellite loci and the Domain III of the CR to evaluate its genetic diversity and insights into its demographic history. Besides, we aimed at using the same set of microsatellite loci to evaluate the diversity that was recovered during a period of the egg ranching program, in comparison to the identified for the population. This research is a contribution to the conservation and surveillance of this emblematic and endangered crocodile.

2.2 Methods

2.2.1 Sample collection

In this research we used two data sets; one for performing the genetic characterization of the population, and another for evaluating the diversity recovered during a period of egg ranching. For the first one we used 38 caudal scale samples taken between 2009 and 2017 from individuals coming from the Cravo Norte (24 individuals) and Ele Rivers (14 individuals), in the Arauca department, Colombia (Figure 2-1; Table S7). Of these, 30 crocodiles are part of the captive breeding program coordinated by the EBTRF, and 8 are part of the local initiative of egg ranching for conservation intents that take place in the municipality of Cravo Norte, Arauca. When nest information was available, and to avoid overrepresentation, no more than two individuals hatched from the same nest were included. Regarding our second data set, we included a sample of 81 of a total of 139 individuals that were born from the egg ranching initiative in 2015 (Table S8). Its clutches were initially collected in the sector of Playa Campo Abierto, which corresponds to a sand beach of the Cravo Norte River. All samples were preserved in 96% ethanol and stored at -20°C in the Collection Banco de ADN y Tejidos de la Biodiversidad Colombiana (BTBC) from the Instituto de Genética of the Universidad Nacional de Colombia (IGUN). In addition, we included in some of the performed analyses, one representative of each haplotype described for the mitochondrial CR in the Chapter 1 of this thesis dissertation (Table S7).

Figure 2-1: Sampling sites (pink dots) in the Cravo Norte and Ele rivers, in the Arauca department, Colombia. The historical distribution of *C. intermedius* is shown in the upper-right corner map in orange (Adapted from Balaguera-Reina et al., 2018).



2.2.2 Laboratory procedures, sequencing, and genotyping

All laboratory procedures were conducted in the Molecular Ecology Laboratory of the IGUN. We extracted genomic DNA from tissue using the NucleoSpin® Tissue Kit (Machery-Nagel, Germany) following manufacturer protocol. We amplified the same set of 17 microsatellites loci previously used for cross-amplification with *C. intermedius* by Rossi Lafferriere et al. (2016), for the *ex situ* EBTRF population by Saldarriaga-Gómez (2021), and for the *in situ* assessment in Chapter 1 (Table S1). These molecular markers were developed for the genus *Crocodylus* (Fitzsimmons et al., 2001), *C. moreletii* (Dever & Densmore, 2001), and *C. porosus* (Miles et al., 2009). Polymerase chain reactions (PCR) were performed as proposed by Saldarriaga-Gómez (2021) in four PCRs multiplex prepared in a final volume of 10 μ L including 5 μ L of MyTaq™ HS Mix (Bioline, USA), 0.2 μ L of 10X each primer (except for Cj122 and Cj109 for which 0.4 μ L were added), a final concentration

of 4ng/ μ L of DNA and the excess of ultra-pure water to complete. The PCR thermocycle was performed as follows: An initial denaturation stage at 95 °C for 4 minutes was followed by 30 denaturation cycles at 95 °C for 30 seconds, annealing at 57°C (except for the multiplex composed by primers Cj18, CUJ131, Cj109, and C391, for which a temperature of 60 °C was used) for 45 seconds, and elongation at 72 °C for 30 seconds, and finally an ending at 72 °C for 5 minutes was settled. Subsequently, 1 μ l of a dilution made with 1 μ l of the PCR product and 99 μ l ultra-pure water was mixed with 8.5 μ l of Hi-Di Formamide (Applied Biosystems, USA), 0.25 μ l of pure-water, and 0.25 μ l of GeneScan-600 LIZ Size Standard (Applied Biosystems, USA). For fragment length determination, an ABI 3500 Genetic Analyzer was used. Genotypes were identified with GENE-MAPPER 3.7 (Applied Biosystems, USA) and OSIRIS 2.13.1 (NCBI, USA) software, using as a reference the reported alleles by Saldarriaga-Gómez (2021).

A Fragment of 450 base pairs (bp) of the Domain III of the CR was amplified with primers CR2H and 12SH1 (Ray & Densmore, 2002) for the *in situ* population samples. PCR reactions were performed at a final volume of 30 μ l including 3 μ l of 10X PCR buffer, 3 μ l of MgCl₂ solution 25 mM, 0.5 μ l of dNTP solution 10mM, 0.7 μ l of each primer at 10 μ M, 0.90 μ l of BSA at 10mg/ml, 0.3 μ l of Taq polymerase at 5 U/ μ l, 3 μ l of DNA at a 5-20 μ g/ μ l concentration, and 17.9 μ l of ultra-pure water. PCR cycle was initiated with three cycles of denaturation at 95°C for 30 seconds, annealing at 56° C for 1 minute, and extension at 72 °C for 1 minute 30 seconds; it was followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing at 58° C for 1 minute, and extension at 72° C for 1 minute. An ending temperature of 72 °C for 8 minutes was used. PCR products were purified with an ammonium acetate protocol (Bensch et al., 2000) and sequences were obtained with an ABI 3130XL Genetic Analyzer automatic sequencer (Applied Biosystems, USA). Both fragment length analysis and sequences obtention were done by the Servicio de Secuenciación y Análisis Molecular (SSIGMOL)-IGUN.

2.2.3 Microsatellites data analysis

- Population genotypic variation

For the population data set, genotyping inconsistencies such as null alleles frequencies at each locus and allele dropout were assessed with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). GENEPOP 4.7.5 (Rousset, 2008) was used to evaluate the tendency to Hardy Weinberg (HW)

equilibrium for all loci using the implemented exact test, and genotypic linkage disequilibrium (LD) between each pair of loci using the log-likelihood ratio statistic. Significance levels were estimated using a Markov chain (MC) algorithm with 10,000 dememorization steps, 1,000 batches, and 10,000 iterations per batch. Bonferroni corrections were applied to HW equilibrium and LD calculations. Observed allelic diversity (A_{ob}) and allelic richness (A_R), were calculated using HP-RARE 1.0 (Kalinowski, 2005), which integrates rarefaction to cope effects of sample size disparity between populations (Goudet, 2003). Observed (H_O), and expected heterozygosities (H_E) were calculated using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010).

- Inbreeding and effective population size

Inbreeding coefficient (F_{IS} ; Weir & Cockerham, 1984) was assessed in FSTAT 2.9.4 (Goudet, 2003). Its significance for excess or deficiency of heterozygotes (Rousset & Raymond, 1995) was evaluated in GENEPOP applying Bonferroni corrections.

Effective population size (N_e) was assessed using two methods for comparative purposes: The first one correspond to the sibship assignment method proposed by Wang (2009) and implemented in COLONY (Jones & Wang, 2010). It assumes that in small populations the probability that two randomly taken individuals results are siblings increases, N_e , might be inferred from sibship frequencies in a sibship assignment analysis. A 95% confidence interval (C.I.) was calculated by assuming a t-student distribution. The second evaluation is the bias-corrected method based on LD (Hill, 1981; Waples, 2006; Waples & Do, 2010) and implemented in NEESTIMATOR 2.1 (Do et al., 2014). It presumes that N_e is correlated to the degree of LD between two neutral markers in isolated populations of constant size and stable structure. To exclude low frequency and singletons alleles bias in the estimation, we did not use alleles that occur at a frequency less than 0.02 as recommended by Waples & Do (2010). We implemented the jackknife-across-samples method for the 95% C.I. estimation. Random mating was assumed for both methods. Additionally, the ratio between N_e and the adult census population size (N_c) was assessed for each N_e estimate by assuming a $N_c = 102$, as it is the more recent number of adult specimens detected on the evaluated geographic area (Anzola, 2017).

- Bottlenecks

We used three methods to detect sharp population decline signatures: The first one, implemented in BOTTLENECK 1.2.02 (Piry et al., 1999), identifies a significant H_O excess compared to H_E for the number of observed loci (Cornuet & Luikart, 1996). We chose the two-phase mutation model (TPM) since it fits most microsatellite data sets compared to infinite allele or stepwise mutation models (Di Rienzo et al., 1994). We set a 95% of single-step mutation, 5% of multiple step mutation, and the variance among multiple steps to 12. For statistical significance determination, a Wilcoxon sign-rank test was used (Luikart & Cornuet, 1998). The second method, that is also implemented in BOTTLENECK, is qualitative and indicates if the allele frequency distribution is approximately L-shaped, as a mutation-drift equilibrium expectation, or is shifted, as a recent bottleneck may cause (Piry et al., 1999). The third method is the M -ratio test (Garza & Williamson, 2001). It assesses the relation between the number of alleles (k) and the overall range in fragment sizes (r), with the statistic $M = k/r + 1$, as corrected by Excoffier & Lischer (2010). Since k is expected to decrease faster than r because of the loss of rare alleles by genetic drift, declining populations may have a smaller M -ratio than non-declining ones. This statistic was calculated in ARLEQUIN and established as significant if it was lower than a critical value (M_c) obtained in simulations performed in CRITICAL_M (Garza & Williamson, 2001). We implemented the TPM with an average repetition frequency of multi-step mutations $\Delta_g = 3.1$, a proportion of multi-step mutations $p_g = 0.22$, as recommended by Peery et al. (2012) to reduce I type error rates, and a θ value defined as $4 N_e \mu$ (where μ = mutation rate) ranging from 0.1 to 10. By the inclusive application of these assessments, we may discern either a recent or a historical population decline. Since the M -ratio test recovers from its signal much more slowly than the heterozygosity excess and the allele frequency distribution methods (Garza & Williamson, 2001), a historical process might be detected if only the M -ratio is conclusive in favor of a population decline.

- Genotypic diversity recovered by the egg ranching program

We inferred the same diversity indexes for the egg ranching program sample as for the *in situ* population and then compared their values. Observed allelic diversity (A_{Ob}) and allelic richness (A_R), were calculated using HP-RARE 1.0 (Kalinowski, 2005). Observed (H_O), and expected heterozygosities (H_E) were calculated using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010).

2.2.4 Mitochondrial sequence data

- Haplotypic variation

The obtained sequences from the CR fragment were edited and aligned with CHROMAS 2.6.6 (<http://www.technely-sium.com.au/chromas.html>) and BIOEDIT (Hall, 2005), using the Clustal W algorithm. We used ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010) and DNASP 6 (Rozas et al., 2017) to calculate haplotype diversity (H_d), nucleotide sequence diversity (π), and mean number of pairwise differences among sequences (k). A parsimony haplotype network was drawn using the TCS algorithm (Clement et al., 2000) in POPART 1.7 (Leigh, Jessica, Bryant, 2015), in order to infer genealogical relationships between haplotypes. For comparison purposes, we included one representative of each haplotype described in Chapter 1 of this thesis dissertation.

- Demographic history

We assessed population expansion in DNASP 6 (Rozas et al., 2017) through a mismatch distribution test and the expansion parameters τ , Θ_1 and Θ_0 (Rogers & Harpending, 1992). The fit of the observed distribution to the demographic expansion model was evaluated with the Harpending's raggedness index (r) with 10,000 bootstrap replicates. A non-significant index suggests that the data does not deviate from the expectation of population expansion. A pairwise mismatch distribution graph was generated in DNASP. Additionally, Tajima's D and Fu's FS tests of selective neutrality were performed in DNASP, since, its negative value suggests an historical population growth (Fu, 1997; Tajima, 1989).

2.3 Results

2.3.1 Microsatellites

- Population genotypic variation

We excluded the loci Cj127 and CP1610 of our analyses since they were monomorphic for the evaluated sampling, the last was also reported as monomorphic for the *ex situ* population in the EBTRF (Saldarriaga-Gómez, 2021) and for the *in situ* evaluation in Chapter 1. No significant LD

was detected between any pairs of loci, while, as detected in Chapter 1, locus CpP305 differed significantly from HW equilibrium. Consequently, the following analyses were conducted with the 14 markers and 38 individuals (Table 2-1). A_{Ob} ranged from two (CpP3216 and CpP1409) to eight (Cj391) alleles with a mean of 3.857 alleles per locus, while A_R presented values from 1.981 (CpP1409) to 5.575 (Cj391) with a mean of 3.109. H_O and H_E values were 0.592 and 0.573, respectively. We did not detect null alleles for any of the analyzed loci.

Table 2-1: Genetic diversity and inbreeding coefficient (F_{IS}) of the microsatellite data for the *C. intermedius* population in the Cravo Norte-Ele-Lipa River System with a sample size of 38 individuals. A_{Ob} observed allelic diversity; A_R allelic richness; H_O observed heterozygosity; H_E expected heterozygosity.

Loci	A_{Ob}	A_R	H_O	H_E	F_{IS}
CpP3216	2.000	2.000	0.421	0.501	0.161
CpP1409	2.000	1.981	0.368	0.337	-0.095
CpP302	4.000	3.429	0.763	0.635	-0.206
CpP314	3.000	2.800	0.553	0.586	0.058
Cj16	4.000	3.422	0.526	0.501	-0.052
CU5123	4.000	3.201	0.632	0.602	-0.050
Cj122	5.000	4.688	0.684	0.775	0.118
Cj18	4.000	3.932	0.842	0.739	-0.142
CUJ131	3.000	2.104	0.211	0.195	-0.080
Cj109	3.000	2.980	0.684	0.640	-0.070
Cj391	8.000	5.575	0.816	0.804	-0.015
CCj101	4.000	2.555	0.658	0.543	-0.216
CpDi13	3.000	2.197	0.526	0.488	-0.080
CpP801	5.000	3.802	0.605	0.681	0.113
Mean	3.857	3.190	0.592	0.573	-0.040
s.d.	1.512	1.060	0.175	0.166	0.116

- Inbreeding and effective population size

The estimated mean F_{IS} has a value of -0.040 (Table 2-1) and did not show any significant value for neither excess nor deficiency of heterozygotes evaluated per loci and population. Consequently, there is no evidence of current inbreeding. However, both N_e estimation methods (Table 2-2) indicate

a concerning value for this parameter: 17 (95% C.I. = 10.0-34.0) by sibship assignment and 11.5 (95% C.I. = 5.7-21.7) by linkage disequilibrium. N_e / N_c ratio for each estimation was 0.167 and 0.113, respectively.

Table 2-2: Estimates of effective population size (N_e) for the *C. intermedius* population in the Cravo Norte-Ele-Lipa River System and its ratio with adult census estimated size (N_c). 95% confidence intervals are indicated in brackets.

Method	N_e estimation	N_e / N_c
Sibship assignment	17 (10.0-34.0)	0.167
Linkage disequilibrium	11.5 (5.7-21.7)	0.113

- Bottlenecks

Population decline evidence was detected with the M -ratio test with consistent results by defining the M_c threshold under different θ values (Table 2-3). Nonetheless, it was not detected either with the heterozygosity excess or with the allele frequency distribution approximations, which may indicate that we are detecting a historical bottleneck but not the population decline that the species suffered during the last century. However, it is noticed that the Wilcoxon sig-rank test employed for the heterozygosity excess method presented a $P = 0.059$, which is very close to the statistical significance limit.

Table 2-3: Tests assessments for past bottlenecks in the *C. intermedius* population inhabiting the Cravo Norte-Ele-Lipa River System. Values bearing an asterisk are statistically significant: $P < 0.05$ for heterozygosity excess, and below a critical value (M_c) for the M -ratio test.

Wilcoxon sign-rank test (one-tailed P value for heterozygosity excess)	Allele frequency distribution mode shift test	M -ratio test	
		M -ratio	M_c
TPM			
0.059	Normal L-shaped	0.365*	0.679 - 0.802

- Genotypic diversity recovered by the egg ranching program

As for the *in situ* population data set, we identified both loci Cj127 and CP1610 as monomorphic and therefore were not included in the analyses. In Table 2-4 we report the diversity indexes found for the 81 samples of the egg ranching program that were evaluated. We identified a very slight reduction of the mean value for every index in comparison to the identified for the population (Table 2-1). A_{Ob} ranged from two (CpP3216, CpP1409, and CpDi13) to eight (Cj391) alleles with a mean of 3.714 alleles per locus, which corresponds to a reduction of 3.7% of the value reported for the population. A_R ranged from 1.860 (CpP1409) to 5.602 (Cj391) with a mean of 3.165, which corresponds to a reduction of 0.8% of the value reported for the population. It is remarkable that for the locus Cj109 we identified one allele more than for the population, as a possible consequence of increasing the sampling size. It corresponds to allele 382 and was previously identified for the clusters in the Western Meta and Vichada basins and the Eastern Meta basin that were inferred in Chapter 1. Regarding the heterozygosity measures, H_O has a value of 0.574, which is a reduction of 3% of the reported for the population. And H_E value is 0.569, which indicates a reduction of 0.7% of the reported for the population.

Table 2-4: Genotypic variation for the sample of 81 *C. intermedius* individuals that were part of the egg ranching program in the Cravo Norte River during the year 2015. Reduction corresponds to a percentage that each index reduced in comparison to the values identified for the population (Table 2-1). A_{Ob} observed allelic diversity; A_R allelic richness; H_O observed heterozygosity; H_E expected heterozygosity.

Locus	A_{Ob}	A_R	H_O	H_E
CpP3216	2	1.989	0.469	0.374
CpP1409	2	1.860	0.235	0.208
CpP302	4	3.483	0.654	0.590
CpP314	3	2.920	0.704	0.619
Cj16	4	3.191	0.519	0.489
CU5123	3	2.858	0.556	0.574
Cj122	5	4.347	0.642	0.735
Cj18	4	3.856	0.815	0.707
CUJ131	3	2.656	0.370	0.462
Cj109	4	3.064	0.556	0.647
Cj391	8	5.602	0.877	0.808
CCj101	4	2.566	0.482	0.544
CpDi13	2	2.000	0.407	0.478
CpP801	4	3.914	0.753	0.737
Mean	3.714	3.165	0.574	0.569
s.d.	1.541	1.027	0.179	0.161
Reduction	3.7%	0.8%	3%	0.7%

2.3.2 Mitochondrial sequence data

- Haplotypic variation

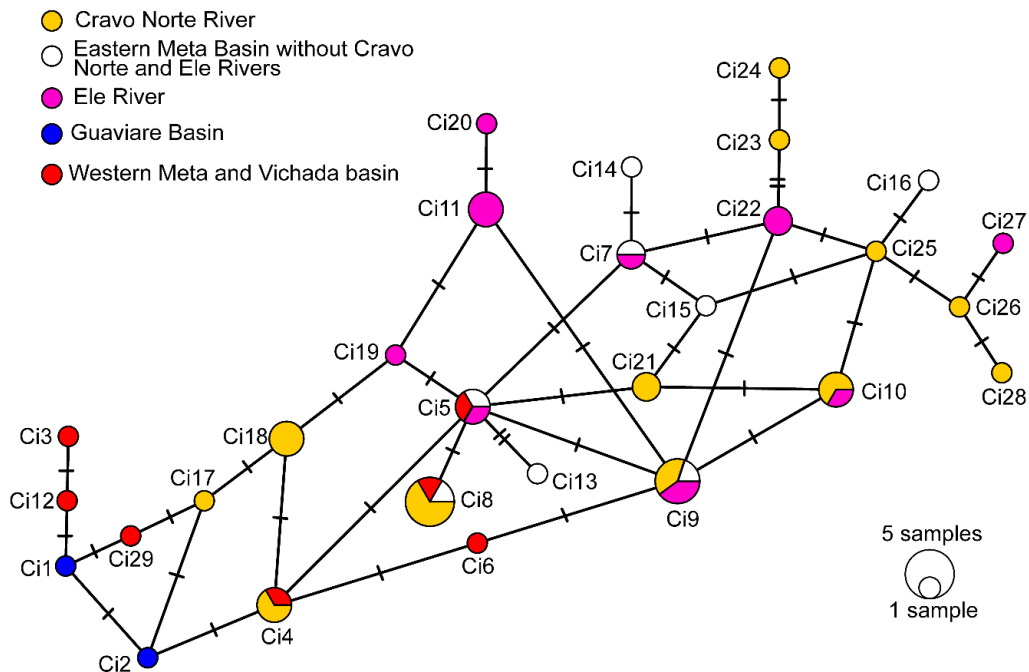
A fragment of 453 bp long of the mitochondrial CR was obtained for 21 individuals from the Cravo Norte River and 13 from the Ele River, for a total of 34. We identified 19 haplotypes that differed in 20 segregation sites, counting with three transitions and 17 transversions. We detected haplotypes Ci8 and Ci9 in four samples; Ci10, Ci11, and Ci18 in three samples; Ci4, Ci21, and Ci22 in two

samples; and 11 haplotypes (Ci5, Ci7, Ci17, Ci19, Ci20, Ci23, Ci24, Ci25, Ci26, Ci27 and Ci28) in one sample each. 12 of these haplotypes were not detected during the analyses performed in the Chapter 1 of this thesis dissertation. In the Figure 2-2 we present a haplotype network of the assessed individuals inhabiting the Cravo Norte-Ele-Lipa River System and one representative of the haplotypes previously described for the CR. We identify a high diversity of haplotypes but not a phylogeographic correspondence of them. Haplotypes in the evaluated river system were distinct by one to 14 mutational steps. Overall diversity indexes were: $Hd = 0.977$, $\pi = 0.011$, and $k = 4.041$ (Table 2-5).

Table 2-5: Genetic diversity of the mitochondrial data for the *C. intermedius* population in the Cravo Norte-Ele-Lipa River System with a sample size of 34 individuals. H_d haplotype diversity; π nucleotide sequence diversity; k mean number of pairwise differences among sequences.

Control Region mtDNA			
H_d	π	k	Number of haplotypes
0.977 (0.013)	0.011 (0.001)	4.041 (0.501)	19

Figure 2-2: Haplotype network of the mtDNA CR for *C. intermedius* in the Cravo Norte-Ele-Lipa River System, with the addition of the described haplotypes for the Colombian Orinoquía. Circle size corresponds to haplotype frequency, and hatch mark to a mutation step.



- Demographic history

We identified that the distribution of pairwise nucleotide difference did not differ significantly from the expected under population expansion (Figure 2-3; Table 2-6). Likewise, Tajima's D and Fu's FS values were negative, which may support historical population growth. Nevertheless, we only identified the FS as statistically significant, in favor of this demographic process.

Figure 2-3: Pairwise mismatch distributions performed with mtDNA CR data for the *C. intermedius* population in the Cravo Norte-Ele-Lipa River System.

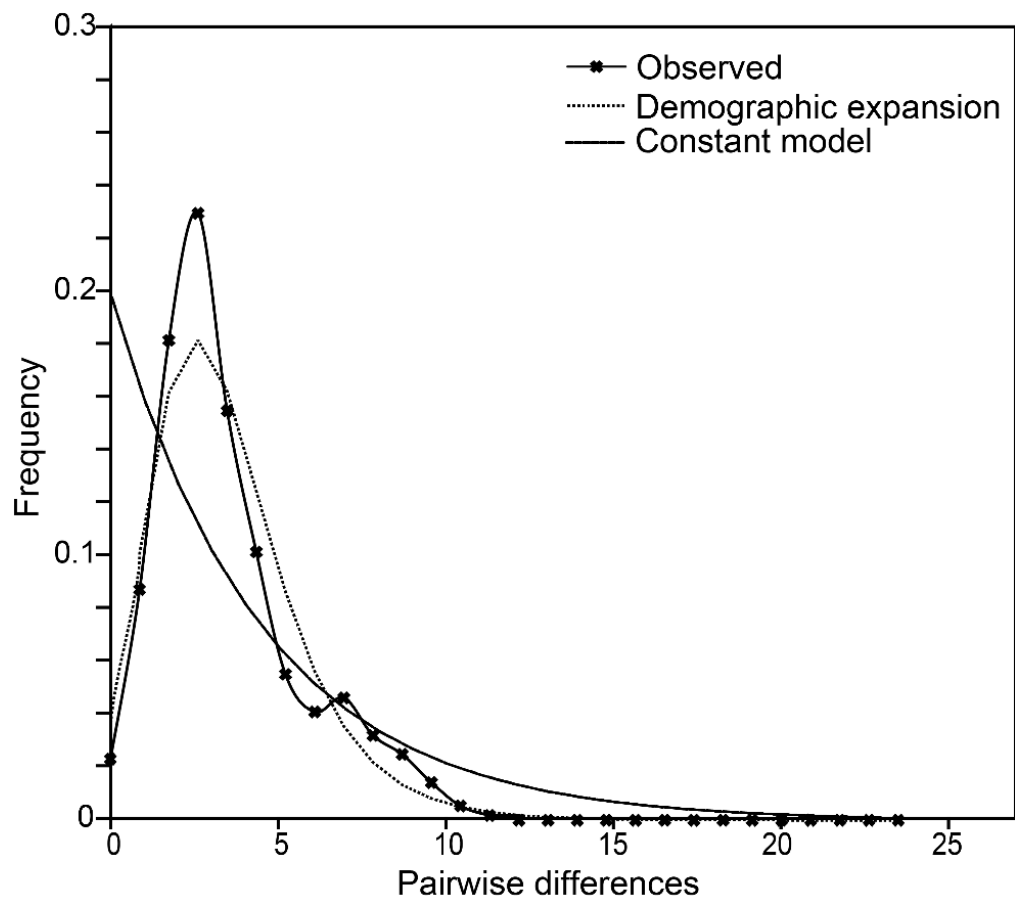


Table 2-6: Population expansion tests for the *C. intermedius* population in the Cravo Norte-Ele-Lipa River System. N sampling size; S segregation sites; τ age of expansion; θ_0 population size before expansion; θ_1 population size after expansion. Values bearing an asterisk are statistically significant: $P < 0.05$.

N	34
S	18
τ	2.491
θ_0	1.550
θ_1	1000
Raggedness R	0.027
P	0.097
Tajima's D	-0.276
P	0.435
Fu's Fs	-17.813
P	0.001*

2.4 Discussion

2.4.1 Population genetic status

Genetic diversity parameters indicated that the Orinoco Crocodile population inhabiting the Cravo Norte-Ele-Lipa River System has moderate to high genetic diversity, in comparison with other *Crocodylus* wild populations (Table S5). Concerning the microsatellite indexes, and in the matter of IUCN categories (Cedeño-Vázquez et al., 2012; Choudhury & de Silva, 2013; Isberg et al., 2019; Rainwater et al., 2021; Targarona et al., 2008; van Weerd et al., 2016; Webb et al., 2021), the herein reported $H_O = 0.592$ and $H_E = 0.573$ are the highest for any critically endangered *Crocodylus* species (*C. mindorensis* [H_O from 0.408 to 0.457; H_E from 0.423 to 0.446] and *C. rhombifer* [$H_O = 0.490$; $H_E = 0.540$]), and are even higher than most of the registered values for species categorized as vulnerable (*C. acutus* [H_O from 0.455 to 0.620; H_E from 0.503 to 0.640] and *C. palustris* [$H_O = 0.350$; $H_E = 0.430$]), or even as least concerned (*C. moreletii* [H_O from 0.350 to 0.579; H_E from 0.300 to 0.552], *C. niloticus* [H_O from 0.400 to 0.717; H_E from 0.250 to 0.745] and *C. porosus* [H_O from 0.371 to 0.633; H_E from 0.456 to 0.622]). In respect of the mean number of alleles per locus, $A_{Ob} = 3.857$,

is quite lower than the reported median. This parameter ranges from 1.720 (*C. niloticus* population of West Africa; Hekkala et al., 2010) to 7.273 (*C. acutus* population of Black River Lower Morass, Jamaica; Rossi Lafferriere et al., 2020). On the other hand, mtDNA diversity analyses are not as frequently implemented as microsatellite evaluations in *Crocodylus*. For instance, *C. intermedius* is the only critically endangered species that now count with these estimates. However, the evaluated population exposes very high values of these indexes; an $Hd = 0.977$ represents the highest value of this index reported in the gender (Table S5), while both $\pi = 0.011$, and $k = 4.041$, are the second higher values reported in the gender, followed by the *C. niloticus* assessed population in the Lower Shire River, Malawi ($\pi = 0.015$; $k = 8.144$; van Asch et al., 2019).

The evaluated *in situ* population presents microsatellite genetic diversity indexes lower to the *ex situ* populations of the species that had been assessed (Table S5). Its H_E is lower than the assessed for the EBTRF populations (Table S5; Saldarriaga-Gómez, 2021); while its A_{Ob} and A_R is lower than the reported for any of the *ex situ* populations of the species (Table S5; Rossi Lafferriere et al., 2016; Saldarriaga-Gómez, 2021). Since Orinoco Crocodile *ex situ* assessments do not represent wild populations, they do not serve as a proper point of comparison to explore the dynamics of the Cravo Norte-Ele-Lipa River System population. In comparison to the described genetic clusters of the Orinoco Crocodile for the Colombian Orinoquía, we identify lower H_E , A_{Ob} , and A_R estimations than the Western Meta and Vichada basins, but higher than the estimations for the Eastern Meta, and the Guaviare basins clusters (Chapter 1; Table S5). Consequently, we argue that the herein evaluated population is a valuable resource for the conservation of the species since, relative to other *Crocodylus* and *C. intermedius in situ* populations it presents a high genetic diversity expressed on its genotypic and haplotypic variation. Nevertheless, the moderate to low A_{Ob} , and A_R estimates may indicate a process of genetic diversity loss, since population declines -such as the suffered by the species during the last century driven by anthropical actions (Medem, 1981; Morales-Betancourt et al., 2015)-, alleles are lost faster than heterozygosity (Luikart & Cornuet, 1998).

With respect to contemporary N_e estimations, the obtained results (Table 2-2) were conclusive in identifying an extremely low value of this parameter for the evaluated Orinoco Crocodile population. When N_e began to be used as a useful tool in conservation management, the 50-500 rule was proposed: a population must have at least a $N_e = 50$ to reduce the likelihood for short-term extinction due to inbreeding depression, and a minimum of $N_e = 500$ to maintain a balance between genetic drift and mutation, i.e., to retain enough genetic variation for long-term adaptation (Franklin, 1980). More recent approaches suggest those minimum values as $N_e \geq 100$, and $N_e \geq 1000$, respectively for

each case (Frankham et al., 2014). Nevertheless, it is still in discussion which N_e value, 50 or 100, is the minimum for the short-term conservation (García-Dorado, 2015). In any case, the upper N_e estimated bound estimated for the *C. intermedius* inhabiting the Cravo Norte-Ele-Lipa River System has a value of 34.0, detected by the sibship assignment method, is lower than any of the proposed value for short-term subsistence. This critical panorama likely reflects the population's recent demographic history of intensive hunting and evidences its huge affectation even on the biggest Orinoco Crocodile wild population in Colombia.

The absence of evidence for current inbreeding may imply that this population is not in the extinction vortex (Gilpin & Soulé, 1986), but the inferred N_e reflects the risk of beginning an inbreeding depression process, which may reduce its genetic diversity and fitness (Blomqvist et al., 2010). Recent bottlenecks may result in F_{IS} values that underestimate the true level of inbreeding, since this demographic process temporarily increases H_O , relative to H_E , because of low-frequency alleles loss (Rhode et al., 2014; van Asch et al., 2019). Consequently, we are not able to dismiss that the evaluated population is very likely to present inbreeding. Hinlo et al. (2014) reported a similar case for wild populations of critically endangered *C. mindorensis*, with even lower N_e estimates but without inbreeding evidence, but unlike *C. intermedius*, this species presented low genetic diversity measured in terms of H_E (0.423-0.446; Table S5). In addition, a process that may reduce the risk of inbreeding may be multiple paternity, which may maintain high levels of genetic diversity and has been reported for the species (Rossi Lafferriere et al., 2016).

In relation to N_e/N_c ratio estimates, a value of 0.14 had been described as the median, nevertheless, it changes tremendously between species depending on their natural history (Jamieson & Allendorf, 2012; Palstra & Ruzzante, 2008). Compared with other *Crocodylus in situ* populations, our evaluation reflects a higher value (ranging from 0.113 to 0.167; Table 2-2) than the reported for the also critically endangered *C. mindorensis* (0.03-0.06; Hinlo et al., 2014) and the non-endangered *C. niloticus* (0.04-0.10; Bishop et al., 2009). Consequently, the Orinoco Crocodile might have a lower risk of suffering immediate deleterious effects of a population decline than those species, as it increases in scenarios of reduced N_e relative to N_c (Bishop et al., 2009; Frankham, 1995).

2.4.2 Demographic history

Crocodylus intermedius is a species with a long longevity whose populations were known to be abundant, for instance, Alexander von Humboldt mentioned numerous encounters with this crocodile during its exploration in the Orinoco during the early XIX century, and actually, how the species was part of the daily life of the human communities therein inhabiting (von Humboldt, 1958). Even though the sparse information, Medem (1981), estimated that an average of 90,000 skins per year were exported between 1930 and 1970, while other authors suggest an estimate of a minimum of two or three million during that period (Casal et al., 2013; Thorbjarnarson, 1987); which caused a huge population decline of this species (Antelo et al., 2008; Casal et al., 2013; Medem, 1981; Thorbjarnarson, 1987). Nevertheless, our results suggest that we are not detecting that anthropical caused population decline (Table 2-3) but a historical one. A mode-shifted distribution remains for a few dozen generations, but, for instance, it may take five to ten generations to occur when counting 20 breeders (Luikart & Cornuet, 1998). In the case of the heterozygosity excess method, theoretical models suggest that this process is detectable during a period of 0.2–2.5 N_e after the population reduction (Cornuet & Luikart, 1996). While, the M -ratio test maintains that signal for more than 100 generations (Garza & Williamson, 2001). Consequently, and considering the species' longevity, the sharp population decline that the Orinoco Crocodile suffered during the last century might be too recent to be detected with heterozygosity excess or mode shift distribution approximations. The same scenario was detected for the critically endangered gharial (*Gavialis gangeticus*) in India (Sharma et al., 2021), where even if the species suffered a dramatic population decline during the last century, it was not detected by the heterozygosity excess method.

A process of biogeographic importance than might explain the population decline herein detected are the historical changes that had affected the hydrological and habitat conditions in the Orinoquía. According to the pollen record, this region presented an arid period of lower precipitation and a longer dry seasons between the Last Glacial Maximum (20,000 to 18,000 years ago) and the late Pleistocene (13,000 to 10,000 years ago) (Behling & Hooghiemstra, 2001; Wijmstra & van der Hammen, 1966). Furthermore, it has been suggested that the aeolian savannas that characterize the area where the Cravo Norte-Ele-Lipa River System is located, derived from a desertic climate dated in the Late Pleistocene (Iriondo, 1999; Roa, 1979). Consequently, during that period, the hydrology of the region exhibited a reduced discharge of the Orinoco River (Iriondo, 1999). In Chapter 1 we discussed that this factor may influence the degree of population structure described for the species. But, since *C. intermedius* exhibits a high degree of dependence on aquatic environments –it is a riverine species restricted to the water systems in the Orinoco (Martin, 2008) that prefers distant areas from the shore (Villamarín et al., 2021)–, it is very likely that this process not only influence

the structure of the species population, but also the population decline that we are detecting by reducing the habitat quality required for the species. Moreover, there had been arid periods during the early and middle Holocene that might cause similar scenarios (Behling & Hooghiemstra, 2001; Iriondo, 1999; Wijmstra & van der Hammen, 1966).

Nevertheless, we suggest that after the historical decline that it suffered, the population did demographically recover. This hypothesis is supported by the mismatch distribution analyses and a negative and significant Fu's F_S value, despite a non-significant Tajima's D result (Figure 2-3; Table 2-6), since the Fu's F_S present a higher powerful of detecting expansions (Ramos-Onsins & Rozas, 2002).

2.4.3 Conservation implications

This research represents a step forward in the conservation of *C. intermedius* and accomplishes one of the pending aspects in the current initiatives aimed at preventing the extinction of the species in Colombia, i.e. PROCAIMÁN (MMA, 2002) and the interinstitutional action plan for the Orinoco Crocodile conservation (Antelo et al., 2022), as it constitutes an advance in the genetic evaluation of the wild populations. The crocodiles inhabiting the Cravo Norte-Ele-Lipa River System correspond to the better studied wild population of the species, containing in addition to this genetic evaluation, available data about its abundance and ecology (Anzola, 2017; Anzola & Antelo, 2015; Ardila-Robayo, Barahona-Buitrago, Bonilla-Centeno, et al., 2002; Barahona-Buitrago & Bonilla-Centeno, 1999; Lugo-Rugeles & Ardila-Robayo, 1998), as well as promising approaches with the coexisting human community. Even if the killing of adults and harvest of some nests have been reported (Castro et al., 2012), the community has shown its interest in being part of the conservation of this representative species (Antelo et al., 2022; Preciado-Salas, 2018). These aspects, together with the apparent process of population recovery (Anzola, 2017; Anzola & Antelo, 2015), represent a unique opportunity for *C. intermedius* long-term survival.

Nonetheless, the human pressures that the species suffered during the last century led to a genetic signature on this population, evidenced by the detection of recent population decline and whose effect is exemplified on a reduced N_e . In any event, this Orinoco Crocodile population still counts with a significant genetic diversity reservoir, even in comparison with other wild populations of not threatened crocodiles. Likewise, we identified that during 2015, the egg ranching program

successfully recovered most of the population diversity (Table 2-4), even if it was restricted to the clutches located in one single sand beach of the Cravo Norte River. In order to increase the diversity recovered by this initiative, we encourage for the collection of clutches in other areas of the herein evaluated River System, which is possible due to the recent increase in reported nest locations (Anzola & Antelo, 2015).

Consequently, and considering the encouraging insights about other aspects of the Orinoco Crocodiles in the area, we argue that the conservation upon this population must be focused on two actions: First, on the demographic increase with local individuals, and its strength with hatchlings or egg ranching, which we evidenced as successfully in terms of genotypic diversity. And second, to evaluate the viability of population reinforcement (considering the risks that it may lead in terms of diseases, and factors as learned behaviors) with specimens whose genetic identity mainly correspond to the Eastern Meta Basin Cluster proposed in Chapter 1, since the evaluated population is part of it. In addition, individuals with a genetic identity of the Western Meta and Vichada Basins Cluster might simulate a process of migration that likely used to occur (Chapter 1). It is important since population fragmentation is a serious threat to the genetic diversity subsistence of a population (Amavet et al., 2021; Frankham et al., 2014). Likewise, if gene flow is re-established between isolated populations, N_e can substantially increase (Frankham et al., 2014). On the other hand, even if the risk of outbreeding depression is lower than was previously thought (Frankham et al., 2011; Weeks et al., 2017), it might be elevated if populations had been isolated for more than 500 years or inhabit different environments (Frankham et al., 2011). As a consequence, reinforcements with individuals of the Guaviare Basin population must be avoid since it is located in one of the distribution limits of the species, inhabits an area characterized by riparian and floodable forests, while the Cravo Norte-Ele-Lipa River System is characterized by floodplains ecosystems (Bustamante, 2019), and presents a distinctive genetic identity (Chapter 1) and most probably an adaptive separated group.

In addition, it is necessary to continuously survey the genetic status of the population in the search of i) maintain its genetic diversity, ii) revise for changes in its N_e , and iii) opportunely identify processes of inbreeding. As Jamieson & Allendorf (2012) discussed, conservation programs goal should emphasize for the maintenance of genetic diversity during the recovery stage, and not solely reach a minimum recovery size, implying that an N_e might guarantee the viability of the population must be a long-term aspirational purpose. Finally, we emphasize the importance of continuing and strengthening the Orinoco Crocodile management from the integration of genetic, ecological, and

anthropological perspectives. This is fundamental in the aim of involving not just a scientific point of view to improve our knowledge of the species and its habitat, but the co-existing human community, whose role is directly responsible for the real success of any conservation program.

3. Conclusions

With the selection of microsatellites and the control region of the mitochondria as molecular markers, we inferred a genetic pattern previously undetected for *C. intermedius* and described three genetic clusters with an hydrogeographic correspondence with the river basins of the Colombian Orinoquía: i) the Eastern Meta River Basin, ii) the Western Meta and Vichada River Basins, and iii) the Guaviare River Basin, which must be considered as different independent management units in consideration of its distinctive genetic identity. It constitutes an important change of perspective in our understanding and management actions of this species and evidence how genetic diversity and population structure inferences vary depending on the molecular marker selection.

We identified crucial aspects of the remanent population that inhabits the Cravo Norte-Lipa and Ele basin. It presents a high genetic diversity expressed on most of its genotypic and haplotypic variation indexes. Nevertheless, allele richness estimates were relatively low and might indicate a process of genetic diversity loss. Likewise, we determine important demographic factors to considering in the management actions: a low effective population size and the apparent absence of inbreeding. Which indicates that even if the risk is high, the population is not in the extinction vortex. However, it may be possible that inbreeding is still not detectable due to the proximity of the sharp population decline.

In addition, we identify the importance that historical ecological and hydrogeographic processes may have in the populational history of the species. Reductions in the Orinoquía Rivers level during the Late Pleistocene might had influence the described high degree of structure by reducing the available habitat of *C. intermedius*, and furthermore, inducing a population decline that we detect in the Cravo Norte-Ele-Lipa River System population.

The results presented in this research aim at informing direct conservation actions for the species: To encourage that the reinforcements and *de novo* populations that are been established in the Eastern Meta and Vichada Basin should be developed with individuals that present the genetic identity matching the genetic cluster of that areas, which is possible since some of the individuals that conform the F0 generation of the EBTRF *ex situ* population present that identity. To avoid any population reinforcement in the isolated remanent population of the Guayabero-Duda-Lozada River System assigned to the Guaviare Basin cluster, in order to preserve its genetic identity. And, to support demographic increase with local individuals as the main conservation action for the Orinoco Crocodiles remanent populations: the Guayabero-Duda-Lozada and the Cravo Norte-Ele-Lipa River

Systems. Consequently, we argue in favor of the egg ranching initiative that successful in terms of recovered genetic diversity.

In addition, we urge for the crucial need to assess the population aspects of the Guayabero-Duda-Lozada River System population, since even if our sampling of that area was low, we identify a completely unique genetic identity. Likewise, we argue in favor of the survey of areas where unidentified remanent groups of the species might persist.

Finally, we recognize the importance that the Orinoco Crocodile management plan integrates genetic and ecological information sources, but to include the local perspectives of the co-existing human community, whose role is critical for the success of any conservation long-term program.

4. Supplementary information

Table S1: Set of primers used to *in situ* population assessments.

Locus	Primer sequences (5'-3')	Annealing temperature	Multiplex Mix
CpP1409	F: GTTTATGCCCTACTGGTTATCTATC	57°C	1
	R: CAGTCGGGCGTCATCAGGGAAGGGGATTTAATAAT		
CpP1610	F: CAGTCGGGCGTCATCATAGAGGGATTTGACTGT	57°C	1
	R: GTTTGATTATTTTGTCTGGGTTCTT		
CpP302	F: GTTTGGAAACCCAAGAACTTACAAC	57°C	1
	R: CAGTCGGGCGTCATCATTGGGTTTAGTCAGCACATA		
CpP305	F: GTTTGTAGCTGGAACCTGATAGTG	57°C	1
	R: CAGTCGGGCGTCATCAGGTTAACACGTGGTAACTACA		
CpP314	F: GTTTGAAATGCCACTAATACACACA	57°C	1
	R: CAGTCGGGCGTCATCACCAATTCTTCAGGTCCTTAT		
CpP3216	F: CAGTCGGGCGTCATCAGATTAATTCATTGGCTCTC	57°C	1
	R: GTTTATGCCTTTGCCTTTAG		
C391	F: ATGAGTCAGGTGGCAGGTTC	57°C	2
	R: CATAAATACACTTTTGAGCAGCAG		
CUJ131	F: GTCCCTTCCAGCCCAAATG	57°C	2
	R: CGTCTGGCCAGAAAACCTGT		
Cj122	F: GTTTCATGCTGACTGTTTCTAATCACC	57°C	2
	R: GGAActACAATTGGTCAACCTCAC		
Cj16	F: CATGCAGATTGTTATTCCTGATG	57°C	2
	R: TGTCATGGTGTCAATTAACCTC		
Cj109	F: GTATTGTCAACCCACCGTGC	60°C	3
	R: GTTCCCCTCCACAGATTTACTTGC		
Cj18	F: ATCCAAATCCCATGAACCTGAGAG	60°C	3
	R: CCGAGTGCTTACAAGAGGCTGG		
Cu5123	F: GGGAAGATGACTGGAAT	60°C	3
	R: AAGTGATTAActAAGCGAGAC		
Cj101	F: ACAGGAGGAATGTGCGATAATTG	57°C	4
	R: GTTTATACCGTGCCATCCAAGTTAG		
Cj127	F: CCCATAGTTTCCTGTTACCTG	57°C	4
	R: GTTCCCTCTCTGACTTCAGTGTTG		
CpDi13	F: GTTTGTGTCAGCCTATACATGTT	57°C	4
	R: CAGTCGGGCGTCATCAGTCTCAGAGTATGCCTAGAA		
CpP801	F: CAGTCGGGCGTCATCATTGGCATTAGATTGGTAGAC	57°C	4
	R: GTTCTATGCCAAAGCTACAAC		

Table S2: Mean LnP(D) values for different Ks, their standard deviations and ΔK values obtained for *in situ* *C. intermedius* analyses in the Colombian Orinoquía. Maximum values in bold.

K	Mean LnP(D)	LnP(D) s.d.	ΔK
1	-645.290	0.445	–
2	-662.300	69.053	1.325
3	-587.840	1.473	55.585
4	-595.260	2.607	1.412
5	-606.360	4.171	0.776
6	-614.225	5.240	0.926
7	-617.240	4.831	0.095
8	-620.715	5.843	0.517
9	-627.210	5.528	0.514
10	-630.865	5.642	–

Table S3: MedMed (median of medians), MedMean (median of means), MaxMed (maximum of medians), and MaxMean (maximum of means) for different Ks obtained for *in situ* *C. intermedius* analyses in the Colombian Orinoquía.

K	MedMed	MedMean	MaxMed	MaxMean
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
	MedMedK	MedMeaK	MaxMedK	MaxMeaK
All	1	1	1	1

Table S4: Assignment tests performed for seized *C. intermedius* individuals that are part of the F0 *ex situ* population of the EBTRF based on 15 microsatellites (nDNA) loci and the proposed genetic clusters for the species *in situ* population. 1 = probability to correspond to the Guaviare basin cluster, 2 = probability to correspond to the Western Meta and Vichada basins cluster, and 3 = probability to correspond to the Eastern Meta basin cluster. CR indicates the control region mtDNA haplotype.

Collection number	Chip EBTRF	Origin location	Assignment method												Putative cluster > 70%	Putative cluster > 80%	Putative cluster > 90%	CR
			Bayesian test			Frequencies-based method			Genetic distance method			STRUCTURE Q value						
			1	2	3	1	2	3	1	2	3	1	2	3				
UNAL:BTBC:10510	95931063	Seized by INDERENA	0.000	0.233	0.194	0.000	0.200	0.123	0.000	0.146	0.187	0.016	0.184	0.800	3	3	-	Ci9
UNAL:BTBC:10519	95929742	Seized in Villavicencio, Meta	0.000	0.069	0.411	0.000	0.098	0.300	0.001	0.089	0.421	0.105	0.178	0.717	3	-	-	Ci16
UNAL:BTBC:10524	95910725	Seized in unknown locality	0.000	0.004	0.655	0.000	0.010	0.674	0.000	0.006	0.570	0.014	0.010	0.977	3	3	3	Ci9
UNAL:BTBC:10564	95920569	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.147	0.108	0.000	0.204	0.126	0.003	0.450	0.250	0.099	0.668	0.233	-	-	-	Ci8
UNAL:BTBC:10565	9594922	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.093	0.094	0.001	0.147	0.111	0.019	0.476	0.377	0.093	0.810	0.098	2	2	-	Ci8
UNAL:BTBC:10581	95926814	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.020	0.921	0.000	0.038	0.926	0.000	0.038	0.895	0.008	0.010	0.982	3	3	3	Ci7
UNAL:BTBC:10582	95926429	Seized by INDERENA to "Zoocriadero Zootechal" in San Carlos de Guaroa, Meta	0.000	0.005	0.354	0.000	0.005	0.317	0.000	0.004	0.150	0.011	0.016	0.973	3	3	3	Ci5
UNAL:BTBC:10586	95919858	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.109	0.615	0.001	0.103	0.641	0.003	0.258	0.772	0.041	0.108	0.851	3	3	-	Ci13
UNAL:BTBC:10587	95928876	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.389	0.277	0.000	0.445	0.301	0.001	0.570	0.357	0.080	0.688	0.231	-	-	-	Ci8
UNAL:BTBC:10605	95916660	Seized by the Agriculture Secretary in Yopal, Casanare	0.000	0.848	0.002	0.001	0.774	0.003	0.002	0.720	0.004	0.030	0.955	0.015	2	2	2	Ci5
UNAL:BTBC:10613	959275506	Seized by Cormacarena in Guamal, Meta	0.000	0.033	0.190	0.000	0.038	0.145	0.000	0.108	0.362	0.036	0.454	0.510	-	-	-	-

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Collection number	Chip EBTRF	Origin location	Assignment method												Putative cluster > 70%	Putative cluster > 80%	Putative cluster > 90%	CR
			Bayesian test			Frequencies-based method			Genetic distance method			STRUCTURE Q value						
			1	2	3	1	2	3	1	2	3	1	2	3				
UNAL:BTBC:10615 EBTRF-C-104	95926121	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.028	0.654	0.000	0.037	0.663	0.000	0.058	0.598	0.018	0.042	0.940	3	3	3	Ci8
UNAL:BTBC:10616	95926695 97725521968	Unknown	0.001	0.076	0.477	0.009	0.085	0.476	0.033	0.059	0.362	0.179	0.042	0.780	3	-	-	Ci5
UNAL:BTBC:10621	95922139	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.077	0.880	0.000	0.064	0.871	0.000	0.086	0.815	0.011	0.014	0.975	3	3	3	Ci9
UNAL:BTBC:10622	95924718	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.118	0.334	0.000	0.146	0.363	0.000	0.340	0.768	0.027	0.076	0.897	3	3	-	Ci14
UNAL:BTBC:10672 EBTRF-C-107	95918077	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.221	0.131	0.000	0.237	0.131	0.001	0.262	0.154	0.041	0.749	0.210	2	-	-	Ci8
UNAL:BTBC:10673 EBTRF-C-109	95927678	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.036	0.846	0.000	0.034	0.839	0.000	0.047	0.756	0.012	0.014	0.975	3	3	3	Ci8
UNAL:BTBC:10674 EBTRF-C-108	95927333	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.024	0.805	0.000	0.016	0.791	0.000	0.029	0.595	0.012	0.013	0.975	3	3	3	Ci8
UNAL:BTBC:10675 EBTRF-C-110	95931154	Unknown	0.000	0.656	0.152	0.000	0.602	0.181	0.001	0.604	0.198	0.036	0.767	0.198	2	-	-	Ci5
UNAL:BTBC:10842 EBTRF-C-72	95923925	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	0.000	0.063	0.551	0.000	0.075	0.547	0.000	0.071	0.514	0.022	0.050	0.928	3	3	3	Ci15
UNAL:BTBC:10843 EBTRF-C-335	95913410	Unknown	0.000	0.811	0.000	0.000	0.757	0.001	0.000	0.695	0.001	0.016	0.972	0.012	2	2	2	Ci5
UNAL:BTBC:10844 EBTRF-C-334	95919779	Unknown	0.000	0.637	0.003	0.000	0.565	0.005	0.000	0.466	0.006	0.019	0.956	0.025	2	2	2	Ci5
UNAL:BTBC:10846 EBTRF-C-389	95922103	Seized in Villavicencio, Meta	0.000	0.174	0.235	0.001	0.237	0.265	0.007	0.510	0.353	0.130	0.625	0.245	-	-	-	Ci12
UNAL:BTBC:10849 EBTRF-C-71	95910922	Seized by the Agriculture Secretary in Yopal, Casanare	0.000	0.525	0.001	0.000	0.447	0.002	0.000	0.608	0.009	0.026	0.960	0.014	2	2	2	Ci12
UNAL:BTBC:13121		Seized by CORTOLIMA in Ibagué, Tolima	0.000	0.068	0.000	0.000	0.082	0.001	0.000	0.139	0.003	0.015	0.952	0.033	2	2	2	Ci29

Table S5: Genetic diversity parameters inferred from microsatellite and mtDNA data of *in situ* *Crocodylus intermedius* assessed in this thesis dissertation (in bold) with other wild populations of the genus and previous *C. intermedius* evaluations for *ex situ* populations (indicated with asterisk). N sample size; H_O observed heterozygosity; H_E expected heterozygosity; A_{Ob} observed allelic diversity; A_R allelic richness; H_d haplotype diversity; π nucleotide sequence diversity; k mean number of pairwise differences among sequences. IUCN categories as follow: NE not evaluated; LC least concerned; VU vulnerable; CE critically endangered.

Taxon	IUCN category	Region	Marker											Reference
			Microsatellite nDNA						mtDNA					
			N	Loci	H_O	H_E	A_{Ob}	A_R	N	Fragment	H_d	π	k	
<i>C. acutus</i>	VU	Black River Lower Morass, Jamaica	17	11	0.455	0.547	7.273	–	17	CR	0.000	0.000	0.000	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Venezuela	—	–	–	–	–	–	11	CR	0.550	0.000	1.640	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Costa Rica	–	–	–	–	–	–	5	CR	0.000	0.000	0.000	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Everglades National Park, United States	35	11	0.562	0.629	6.091	–	35	CR	0.150	0.010	2.360	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Portland Bight Protected Area, Jamaica	82	11	0.455	0.545	3.909	–	82	CR	0.000	0.000	0.000	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Quintana Roo, Mexico	–	–	–	–	–	–	19	CR	0.610	0.000	0.730	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Turneffe Atoll, Belize	31	11	0.497	0.503	6.364	–	31	CR	0.460	0.000	0.460	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Wildlife Refuge Monte Cabaniguan, Cuba	60	11	0.494	0.534	7.000	–	60	CR	0.000	0.000	0.000	Rossi Lafferriere et al., 2020
<i>C. acutus</i>	VU	Zapata Swamp National Park, Cuba	14	9	0.620	0.640	5.100	5.100	5	CR	0.400	0.010	2.820	Milián-García et al., 2015; Rossi Lafferriere et al., 2020
<i>C. intermedius</i>	CE	Eastern Meta Basin, Colombia	7	13	0.571	0.604	3.385	2.933	7	CR	0.905	0.006	2.476	Chapter 1
<i>C. intermedius</i>	CE	Eastern Meta Basin, Colombia	20	14	0.536	0.516	3.571	3.005	20	CR	0.884	0.007	2.879	Chapter 1
<i>C. intermedius</i>	CE	EBTRF Alive population, Colombia*	64	16	0.617	0.574	4.000	3.369	–	–	–	–	–	Saldarriaga-Gómez, 2021
<i>C. intermedius</i>	CE	EBTRF F0, Colombia*	37	16	0.573	0.587	4.190	3.852	–	–	–	–	–	Saldarriaga-Gómez, 2021
<i>C. intermedius</i>	CE	El Frío Biological Station, Venezuela*	20	17	0.524	0.544	5.294	–	–	–	–	–	–	Rossi Lafferriere et al., 2016
<i>C. intermedius</i>	CE	Guaviare Basin, Colombia	3	9	0.444	0.556	2.444	2.444	3	CR	0.667	0.002	0.667	Chapter 1
<i>C. intermedius</i>	CE	Lipa-Ele-Cravo Norte River System, Eastern Meta Basin, Colombia	38	14	0.592	0.573	3.857	3.190	34	CR	0.977	0.011	4.041	Chapter 2
<i>C. intermedius</i>	CE	Colombian Orinoquía, Colombia	18	15	0.499	0.611	4.667	3.857	18	CR	0.935	0.007	2.124	Chapter 1

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Taxon	IUCN category	Region	Marker											Reference
			Microsatellite nDNA						mtDNA					
			<i>N</i>	Loci	<i>H_O</i>	<i>H_E</i>	<i>A_{Ob}</i>	<i>A_R</i>	<i>N</i>	Fragment	<i>H_d</i>	π	<i>k</i>	
<i>C. intermedius</i>	CE	Colombian Orinoquía, Colombia	39	15	0.534	0.591	5.000	3.590	39	CR	0.890	0.006	2.132	Chapter 1
<i>C. intermedius</i>	CE	Western Meta and Vichada Basins, Colombia	8	15	0.581	0.626	3.933	3.858	8	CR	0.750	0.005	1.536	Chapter 1
<i>C. intermedius</i>	CE	Western Meta and Vichada Basins, Colombia	16	15	0.620	0.615	4.400	3.691	16	CR	0.750	0.005	1.658	Chapter 1
<i>C. mindorensis</i>	CE	Isabela, Philippines	84	11	0.408	0.423	3.900	2.159	–	–	–	–	–	Hinlo et al., 2014
<i>C. mindorensis</i>	CE	Liguasan, Philippines	14	11	0.457	0.446	3.700	2.317	–	–	–	–	–	Hinlo et al., 2014
<i>C. moreletii</i>	LC	Gold Button Lagoon, Belize	9	42	0.460	0.410	3.556	–	–	–	–	–	–	Dever et al., 2002
<i>C. moreletii</i>	LC	Habanero, Belize	11	9	0.350	0.300	2.111	–	11	CR	0.346	0.001	–	Dever et al., 2002; Ray et al., 2004
<i>C. moreletii</i>	LC	Macal River, Belize	–	–	–	–	–	–	11	CR	0.182	0.000	–	Ray et al., 2004
<i>C. moreletii</i>	LC	New River and Gold Button Lagoons, Belize	52	5	0.579	0.552	4.600	–	–	–	–	–	–	Mcvay et al., 2008
<i>C. niloticus</i>	LC	Kenya	17	12	0.520	0.420	3.250	2.400	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	KwaZulu-Natal, South Africa	10	11	0.612	0.745	4.318	4.919	9	CR	0.861	0.006	3.000	Hinlo et al., 2014
<i>C. niloticus</i>	LC	Limpopo River Basin, South Africa	13	11	0.480	0.480	3.450	2.320	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	Limpopo River, South Africa	12	11	0.717	0.634	3.818	3.737	12	CR	0.773	0.005	2.591	van Asch et al., 2019
<i>C. niloticus</i>	LC	Lower Kunene River, Namibia	12	11	0.495	0.583	4.182	3.307	12	CR	0.000	0.000	0.000	van Asch et al., 2019
<i>C. niloticus</i>	LC	Lower Shire River, Malawi	52	11	0.621	0.674	6.909	5.529	47	CR	0.332	0.015	8.144	van Asch et al., 2019
<i>C. niloticus</i>	LC	Northern Madagascar	15	8	0.430	0.470	3.250	2.190	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	Northwest Madagascar	11	12	0.490	0.440	2.580	2.490	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	Okavango River, Botswana and Namibia	62	11	0.596	0.610	5.182	3.650	52	CR	0.000	0.000	0.000	van Asch et al., 2019
<i>C. niloticus</i>	LC	Okavango River, Botswana and Namibia	142-153	7	0.510	0.720	6.700	–	–	–	–	–	–	Bishop et al., 2009
<i>C. niloticus</i>	LC	Southeast Madagascar	13	10	0.470	0.410	2.700	2.180	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	Tanzania	12	6	0.480	0.610	3.000	2.780	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	West Africa	6	10	0.400	0.250	1.720	–	–	–	–	–	–	Hekkala et al., 2010
<i>C. niloticus</i>	LC	Zimbabwe	11	12	0.570	0.540	3.330	2.450	–	–	–	–	–	Hekkala et al., 2010
<i>C. palustris</i>	VU	Sarbaz-Bahukalat basin, Iran	10	12	0.350	0.430	2.750	–	–	–	–	–	–	Campos et al., 2018
<i>C. porosus</i>	LC	Australia	5	6	0.633	0.622	3.830	–	–	–	–	–	–	Russello et al., 2007

Taxon	IUCN category	Region	Marker											Reference
			Microsatellite nDNA						mtDNA					
			N	Loci	H_O	H_E	A_{Ob}	A_R	N	Fragment	H_d	π	k	
<i>C. porosus</i>	LC	New Britain, Papua New Guinea	21	6	0.444	0.536	4.330	–	–	–	–	–	–	Russello et al., 2007
<i>C. porosus</i>	LC	North Solomons Province, Solomon Islands	12	6	0.458	0.530	3.170	–	–	–	–	–	–	Russello et al., 2007
<i>C. porosus</i>	LC	Northeast Papua New Guinea	32	6	0.551	0.569	4.330	–	–	–	–	–	–	Russello et al., 2007
<i>C. porosus</i>	LC	Northwest Papua New Guinea	7	6	0.371	0.456	3.670	–	–	–	–	–	–	Russello et al., 2007
<i>C. porosus</i>	LC	Palau	39	6	0.570	0.575	4.330	–	39	CR	0.000	0.000	0.000	Russello et al., 2007
<i>C. porosus</i>	LC	Southern Papua New Guinea	31	6	0.483	0.529	5.170	–	–	–	–	–	–	Russello et al., 2007
<i>C. porosus</i>	LC	Sulawesi	11	6	0.561	0.561	3.170	–	–	–	–	–	–	Russello et al., 2007
<i>C. porosus</i>	LC	Sunda Shelf	19	6	0.490	0.600	4.330	–	–	–	–	–	–	Russello et al., 2007
<i>C. rhombifer</i>	CE	Zapata Swamp National Park, Cuba	27	9	0.490	0.540	4.100	3.600	27	tRNAPro-tRNAPhe-CR	0.090	–	–	Milián-García et al., 2015
<i>C. suchus</i>	NE	Gabbou, Mauritania	8	12	0.470	0.560	3.330	2.370	–	–	–	–	–	Velo-Antón et al., 2014
<i>C. suchus</i>	NE	Gorgod el Abiod, Mauritania	6	12	0.540	0.610	3.580	2.590	–	–	–	–	–	Velo-Antón et al., 2014
<i>C. suchus</i>	NE	Gorgol el Akhdar-Garfa, Mauritania	4	12	0.620	0.740	3.580	2.870	–	–	–	–	–	Velo-Antón et al., 2014
<i>C. suchus</i>	NE	Karakoro-Kolimbiné, Mauritania	16	12	0.540	0.610	4.000	2.460	–	–	–	–	–	Velo-Antón et al., 2014

Table S6: Sampled individuals used for the analyses performed in the Chapter 1 of this thesis dissertation. All sampling location are in Colombia.

Collection number	Chip EBTRF	Tissue sample	Locality	Approximate coordinates	Collection date	mtDNA CR Haplotype	mtDNA CytB-Coi Haplotype (Posso-Peláez et al., 2018)
UNAL:BTBC:7845		Caudal scale	Río Guayabero, La Macarena, Meta	2.300756, -73.915899	15/04/2019	Ci1	
UNAL:BTBC:10510	95931063	Caudal scale	Seized by INDERENA		Before 1999	Ci9	Cin1
UNAL:BTBC:10519	95929742	Caudal scale	Seized in Villavicencio, Meta	4.137105, -73.633623	04(mes)-1996	Ci16	Cin1
UNAL:BTBC:10522	95919774	Caudal scale	Santa Rita, Vichada River, Vichada	4.779854, -68.501235	12/04/1995	Ci5	Cin2
UNAL:BTBC:10524	95910725	Caudal scale	Seized in unknown locality		1993	Ci9	
UNAL:BTBC:10538	95929345	Caudal scale	Santa Rita, Vichada River, Vichada	4.779854, -68.501235	12/04/1995	Ci5	Cin2
UNAL:BTBC:10564	95920569	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	Cin1
UNAL:BTBC:10565	9594922	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	Cin1
UNAL:BTBC:10567	95928277	Caudal scale	Río Cusiana, Casanare	4.6617950, -72.1447016	4/02/1975	Ci5	Cin1
UNAL:BTBC:10569	95922435	Caudal scale	Charco Gaitán, Río Humea, Meta	4.2985145, -73.1761907	1/03/1976	Ci5	
UNAL:BTBC:10570	95918827	Caudal scale	Puerto López, Río Meta, Meta	4.034414, -72.953808	17/01/1970	Ci3	Cin2
UNAL:BTBC:10571	95930354	Caudal scale	Caño Yatea, Bocas Guachiría, Río Meta, Casanare	5.3586111, -70.6880556	1986	Ci4	Cin1
UNAL:BTBC:10581	95926814	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci7	
UNAL:BTBC:10582	95926429	Caudal scale	Seized by INDERENA to "Zoocriadero Zootecal" in San Carlos de Guaroa, Meta	3.711138, -73.242014	22/08/1995	Ci5	
UNAL:BTBC:10586	95919858	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci13	
UNAL:BTBC:10587	95928876	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	
UNAL:BTBC:10605	95916660	Caudal scale	Seized by the Agriculture Secretary in Yopal, Casanare	5.348598, -72.400680	10/07/1992	Ci5	
UNAL:BTBC:10613	959275506	Caudal scale	Seized by Cormacarena in Guamal, Meta	3.880475, -73.769877	Before 1999	-	
UNAL:BTBC:10615 EBTRF-C-104	95926121	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	
UNAL:BTBC:10616	95926695 97725521968	Caudal scale	Unknown		Before 1999	Ci5	
UNAL:BTBC:10618	95924517		San Carlos de Guaroa, Metica River, Meta, Colombia	3.716205, -73.161037	4/02/1975	Ci4	
UNAL:BTBC:10621	95922139	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci9	

Collection number	Chip EBTRF	Tissue sample	Locality	Approximate coordinates	Collection date	mtDNA CR Haplotype	mtDNA CytB-Coi Haplotype (Posso-Peláez et al., 2018)
UNAL:BTBC:10622	95924718	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci14	
UNAL:BTBC:10672 EBTRF-C-107	95918077	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	
UNAL:BTBC:10673 EBTRF-C-109	95927678	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	
UNAL:BTBC:10674 EBTRF-C-108	95927333	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci8	
UNAL:BTBC:10675 EBTRF-C-110	95931154	Caudal scale	Unknown		1995	Ci5	
UNAL:BTBC:10842 EBTRF-C-72	95923925	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci15	
UNAL:BTBC:10843 EBTRF-C-335	95913410	Caudal scale	Unknown		Before 1999	Ci5	
UNAL:BTBC:10844 EBTRF-C-334	95919779	Caudal scale	Unknown		Before 1999	Ci5	
UNAL:BTBC:10846 EBTRF-C-389	95922103	Caudal scale	Seized in Villavicencio, Meta	4.105368, -73.630791	Before 1999	Ci12	
UNAL:BTBC:10849 EBTRF-C-71	95910922	Caudal scale	Seized by the Agriculture Secretary in Yopal, Casanare	5.348598, -72.400680	10/07/1992	Ci12	
UNAL:BTBC:12301		Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8	
UNAL:BTBC:13096		Caudal scale	Río Ele, Cravo Norte, Arauca	6.534468, -70.685535	10/07/2017	Ci7	
UNAL:BTBC:13099		Caudal scale	Río Ele, Cravo Norte, Arauca	6.534733, -70.685381	10/07/2017	Ci9	
UNAL:BTBC:13103		Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci11	
UNAL:BTBC:13110		Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci10	
UNAL:BTBC:13116 ICN-1851		Skull	Angosturas, Alto Río Guayabero, La Macarena, Meta	2.558574, -74.232140	24/01/1959	Ci2	
UNAL:BTBC:13117 ICN-1852		Skull	Bajo Río Ariari, Meta	3.008173, -73.162617	20/01/1956	Ci2	
UNAL:BTBC:13118 ICN-12409		Skull	Río Casanare, Arauca	6.412223, -70.945066	17/06/1995	Ci9	
UNAL:BTBC:13119		Caudal scale	Río Arauca, Arauca	7.012940, -70.938537	2017	Ci8	
UNAL:BTBC:13120 IAvH 1620		Skull	Raudal Guahibo, Río Orinoco, Vichada	5.287906, -67.850306	1/07/1974	Ci6	
UNAL:BTBC:13121		Caudal scale	Seized by CORTOLIMA in Ibagué, Tolima	4.423723, -75.170028	17/02/2023		

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Table S7: Sampled individuals used for the analyses performed in the Chapter 2 of this thesis dissertation. All sampling location are in Colombia.

Collection number	Chip EBTRF	Tissue sample	Location	Approximate coordinates	Collection date	mtDNA CR Haplotype
UNAL:BTBC:7845	-	Caudal scale	Río Guayabero, La Macarena, Meta	2.300756, -73.915899	15/04/2019	Ci1
UNAL:BTBC:10519	95929742	Caudal scale	Seized in Villavicencio, Meta	4.137105, -73.633623	1996	Ci16
UNAL:BTBC:10522	95919774	Caudal scale	Santa Rita, Vichada River, Vichada	4.779854, -68.501235	12/04/1995	Ci5
UNAL:BTBC:10570	95918827	Caudal scale	Puerto López, Río Meta, Meta	4.034414, -72.953808	17/01/1970	Ci3
UNAL:BTBC:10571	95930354	Caudal scale	Caño Yatea, Bocas Guachiría, Río Meta, Casanare	5.3586111, -70.6880556	1986	Ci4
UNAL:BTBC:10581	95926814	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci7
UNAL:BTBC:10586	95919858	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci13
UNAL:BTBC:10622	95924718	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci14
UNAL:BTBC:10842 EBTRF-C-72	95923925	Caudal scale	Seized by INDERENA to "Zoocriadero Arango Ruds" in Pompeya, Meta	4.063703, -73.398333	7/07/1994	Ci15
UNAL:BTBC:10849 EBTRF-C-71	95910922	Caudal scale	Seized by the Agriculture Secretary in Yopal, Casanare	5.348598, -72.400680	10/07/1992	Ci12
UNAL:BTBC:10984 EBTRF-C-291	972752300	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	25/04/2009	-
UNAL:BTBC:10988 EBTRF-C-313	972753397	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	25/04/2009	Ci18
UNAL:BTBC:10990 EBTRF-C-321	972754014	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	23/04/2009	Ci18
UNAL:BTBC:10993 EBTRF-C-292	972759045	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	25/04/2009	Ci9
UNAL:BTBC:11001 EBTRF-C-322	972755840	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	23/04/2009	-
UNAL:BTBC:11002 EBTRF-C-312	972744397	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	23/04/2009	Ci4
UNAL:BTBC:11172 EBTRF-C-314	972759002	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	23/04/2009	Ci17
UNAL:BTBC:11196 EBTRF-C-324	972752318	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	25/04/2009	Ci18
UNAL:BTBC:11323 EBTRF-C-320	972760517	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.530272, -70.816362	25/04/2009	Ci4
UNAL:BTBC:12258	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8
UNAL:BTBC:12259	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci21
UNAL:BTBC:12296	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci27
UNAL:BTBC:12301	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8

Collection number	Chip EBTRF	Tissue sample	Location	Approximate coordinates	Collection date	mtDNA CR Haplotype
UNAL:BTBC:12332	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8
UNAL:BTBC:12333	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8
UNAL:BTBC:12367	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci24
UNAL:BTBC:12371	-	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci21
UNAL:BTBC:13096	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.534468, -70.685535	10/07/2017	Ci7
UNAL:BTBC:13098	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.532556, -70.674422	10/07/2017	Ci11
UNAL:BTBC:13099	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.534733, -70.685381	10/07/2017	Ci9
UNAL:BTBC:13100	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.532556, -70.674422	10/07/2017	Ci19
UNAL:BTBC:13101	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.534733, -70.685381	10/07/2017	Ci20
UNAL:BTBC:13102	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.534733, -70.685381	10/07/2017	Ci11
UNAL:BTBC:13103	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci11
UNAL:BTBC:13104	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci22
UNAL:BTBC:13105	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci10
UNAL:BTBC:13106	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci9
UNAL:BTBC:13107	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci5
UNAL:BTBC:13108	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci9
UNAL:BTBC:13109	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	-
UNAL:BTBC:13110	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci10
UNAL:BTBC:13111	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci23
UNAL:BTBC:13112	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci26
UNAL:BTBC:13113	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci10
UNAL:BTBC:13114	-	Caudal scale	Río Cravo Norte, Cravo Norte, Arauca	6.390450, -70.432211	10/07/2017	Ci25
UNAL:BTBC:13115	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.532556, -70.674422	10/07/2017	-
UNAL:BTBC:13116 ICN-1851	-	Skull	Angosturas, Alto Río Guayabero, La Macarena, Meta	2.558574, -74.232140	24/01/1959	Ci2
UNAL:BTBC:13118 ICN-12409	-	Skull	Río Casanare, Arauca	6.412223, -70.945066	17/06/1995	Ci9
UNAL:BTBC:13119	-	Caudal scale	Río Arauca, Arauca	7.012940, -70.938537	2017	Ci8

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Collection number	Chip EBTRF	Tissue sample	Location	Approximate coordinates	Collection date	mtDNA CR Haplotype
UNAL:BTBC:13120 IAvH 1620	-	Skull	Raudal Guahibo, Río Orinoco, Vichada	5.287906, -67.850306	1/07/1974	Ci6
UNAL:BTBC:13122	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.535203, -70.684808	10/07/2017	Ci28
UNAL:BTBC:13123	-	Caudal scale	Río Ele, Cravo Norte, Arauca	6.534394, -70.685561	10/07/2017	Ci22

Table S8: Sampled individuals from the egg ranching program in the Cravo Norte municipality, Arauca, Colombia that were used for the analyses performed in the Chapter 2 of this thesis dissertation.

Collection number	Tissue sample	Location	Coordinates	Collection date	mtDNA CR Haplotype
UNAL:BTBC:12258	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8
UNAL:BTBC:12259	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci21
UNAL:BTBC:12262	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12263	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12264	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12265	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12266	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12267	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12268	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12269	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12270	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12271	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12272	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12273	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12274	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12275	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12276	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12277	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12278	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12296	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci27
UNAL:BTBC:12297	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12298	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12299	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12300	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12301	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	Ci8
UNAL:BTBC:12302	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12303	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12304	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12305	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12306	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12307	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12308	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12309	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	

Collection number	Tissue sample	Location	Coordinates	Collection date	mtDNA CR Haplotype
UNAL:BTBC:12379	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12380	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12381	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12382	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12384	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12385	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12386	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12387	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	
UNAL:BTBC:12388	Caudal scale	Playa Campo Abierto, Río Cravo Norte, Cravo Norte, Arauca	6.3934861, -70.428436111	2015	

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