SRAS Final Report
Phylogenetic position, divergence time and climatic niche differentiation of *Crocodylus acutus* in Ecuador–Peru

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Abstract

The American crocodile (*Crocodylus acutus*) is widely distributed throughout the Neotropics, but populations at the southern limit of its Pacific distribution in Ecuador and Peru remain poorly represented in genetic, biogeographic and climatic studies. This project evaluated the phylogenetic position, mitochondrial divergence time and climatic niche differentiation of *C. acutus* from southern Ecuador and northern Peru relative to *C. acutus* from the Caribbean and northern mainland range and to *C. intermedius*. Mitochondrial COI and D-loop sequences generated from local samples were analyzed together with comparative GenBank records. Maximum-likelihood phylogenies were inferred in IQ-TREE, genetic distances were estimated under the Kimura 2-parameter model in MEGA12, and a mitochondrial chronogram was generated in BEAST2 using secondary TimeTree calibrations. Climatic comparisons used nine bioclimatic variables and 177 filtered occurrence records, analyzed through PCA, PCA-env, Schoener's D, Warren's I, and niche equivalency and similarity tests. The concatenated COI + D-loop phylogeny placed the Ecuador–Peru samples within *C. acutus* and recovered a recognizable mitochondrial grouping. K2P distances between Ecuador–Peru and Caribbean–northern mainland *C. acutus* were 0.869% for COI and 1.031% for D-loop. The local sequence IKIAM 076 diverged from the clade formed by the reference *C. acutus* and *C. intermedius* sequences at a mean age of 1.98 Ma (95% HPD: 1.16–3.12 Ma). Climatic overlap was low between the two *C. acutus* regional groups (D = 0.050; I = 0.219) and null relative to *C. intermedius*. These results support a regional mitochondrial signal and climatic differentiation in the analyzed occurrences, while emphasizing the need for broader sampling and nuclear or genomic data before defining independent conservation units.

Introduction

Crocodylus acutus occupies coastal, estuarine, lagoon, mangrove and riverine environments from southern Florida through Mexico, Central America and the Caribbean to northern South America, reaching Ecuador and Peru along the Pacific coast (Rainwater et al., 2022). Genetic studies have shown that variation is not homogeneous across this broad distribution. Regional mitochondrial lineages, island–mainland dispersal, population structure and complex relationships involving introgression and mito-nuclear discordance have been documented in different parts of the range (Bloor et al., 2015; Milián-García et al., 2018, 2020; Pacheco-Sierra et al., 2018).

The southern Ecuador–northern Peru region represents the southern limit of the species' Pacific distribution, yet it remains underrepresented in comparative genetic and climatic analyses. Local occurrence information and molecular material have become available through field research in the Puyango–Tumbes region, creating an opportunity to place these populations in a broader evolutionary context. Climatic differentiation is also relevant because the species spans regions with contrasting thermal and precipitation regimes, and previous distribution studies have identified temperature and precipitation variables as important correlates of crocodilian occurrence.

The objective of this project was therefore to estimate the phylogenetic position, evolutionary divergence time and climatic niche differences of *C. acutus* populations from southern Ecuador and northern Peru, comparing them with *C. acutus* from the Caribbean and northern mainland range and with *C. intermedius*. The project integrated mitochondrial phylogenetics, genetic distances, temporal inference and climatic niche comparisons to provide a first regional assessment and identify priorities for subsequent conservation-oriented research.

Methods

Biological samples were obtained under Ecuadorian genetic-resource access authorization MAATE-DBI-CM-2023-0307 within the institutional cooperation framework between Universidad Regional Amazónica Ikiam and Escuela Politécnica Nacional. Tissue was obtained from caudal scales using sterile material; blood samples were also collected from some individuals. Samples were processed at Ikiam's Integrative Biology Laboratory. Comparative sequences of C. acutus, C. intermedius and other Crocodylus species were obtained from GenBank/NCBI (Sayers et al., 2022), with Mecistops cataphractus used as the outgroup.

Mitochondrial COI sequences were generated by PCR and Sanger sequencing. D-loop sequences were complemented with data generated in a related study and processed from long-read data using NanoPack tools and NGSpeciesID (De Coster et al., 2018; Sahlin et al., 2021). Sequences were curated in Geneious Prime (Kearse et al., 2012), aligned separately by marker with MAFFT v7 (Katoh & Standley, 2013), trimmed to homologous regions and organized into COI and D-loop matrices. A concatenated COI + D-loop matrix was prepared for the principal phylogenetic analysis, retaining both markers as separate partitions.

Maximum-likelihood inference was performed in IQ-TREE with ModelFinder model selection (Kalyaanamoorthy et al., 2017; Minh et al., 2020). Node support was assessed using SH-aLRT and UFBoot2 with 1,000 replicates (Anisimova et al., 2011; Hoang et al., 2018). Genetic distances were calculated separately for COI and D-loop in MEGA12 (Kumar et al., 2024) using the Kimura 2-parameter model (Kimura, 1980). Sequences were grouped as C. acutus Ecuador–Peru, C. acutus Caribbean–northern mainland and C. intermedius.

Divergence time was estimated in BEAST2 v2.7.7 (Bouckaert et al., 2019) using a concatenated COI + D-loop subset containing representative Crocodylus taxa and M. cataphractus. Only one local sequence, IKIAM 076, had coverage for both markers and was included in the temporal analysis. HKY + Γ models, an uncorrelated lognormal relaxed clock and a Yule tree prior were used. Secondary calibrations from TimeTree (Kumar et al., 2022) were applied to the C. acutus + C. intermedius and C. rhombifer + C. moreletii nodes. The MCMC ran for 20 million generations, with 10% burn-in, and convergence was evaluated in Tracer.

For the climatic component, wild occurrence records from project data, published sources and GBIF were filtered for incomplete or doubtful coordinates, duplicates and cells without climate data. One occurrence per WorldClim 2.1 cell (2.5 arc-min resolution; Fick & Hijmans, 2017) was retained within each group, yielding 177 records: eight C. acutus Ecuador–Peru, 104 C. acutus Caribbean–northern mainland and 65 C. intermedius. Nine bioclimatic variables were retained after literature-based preselection and Pearson correlation screening ($|r| \geq 0.80$). Climatic structure was evaluated using PCA and PCA-env following Broennimann et al. (2012), implemented with ecospat (Di Cola et al., 2017). Niche overlap was quantified with Schoener's D and Warren's I (Schoener, 1970; Warren et al., 2008), and equivalency and bidirectional similarity tests were performed with 1,000 randomizations.

Results

The concatenated COI + D-loop phylogeny placed all Ecuador–Peru samples within *C. acutus*. The newly generated sequences formed a recognizable mitochondrial grouping with high support among themselves (SH-aLRT/UFBoot = 92.1/99). In the sampled mitochondrial dataset, this group was recovered as sister to the *C. intermedius* clade with lower support (49.1/79), while both were related to the comparative *C. acutus* clade with high support. Given the mitochondrial nature of both markers, this topology is interpreted as a regional mitochondrial signal rather than evidence of species-level separation.

Mean K2P distances were below 1.3% in all comparisons. Between Ecuador–Peru and Caribbean–northern mainland *C. acutus*, distances were 0.869% for COI and 1.031% for D-loop. Distances

between Ecuador–Peru *C. acutus* and *C. intermedius* were 0.874% for COI and 0.901% for D-loop. The largest distances were between Caribbean–northern mainland *C. acutus* and *C. intermedius* (1.112% for COI and 1.247% for D-loop).

In the mitochondrial chronogram, IKIAM 076 was sister to the clade formed by *C. acutus* NC_015647.1 and *C. intermedius* NC_015648.1. The node separating IKIAM 076 from that clade had a mean age of 1.98 Ma (95% HPD: 1.16–3.12 Ma), while the node between the two reference sequences was estimated at 1.29 Ma (95% HPD: 1.01–1.58 Ma) (Figure 1). These estimates describe the mitochondrial lineages included in the analysis and should not be interpreted as population-wide divergence dates.

The climatic PCA showed that PC1 explained 44.35% and PC2 19.09% of total variation, together accounting for 63.45%. The eight Ecuador–Peru occurrences occupied a relatively compact portion of the represented climatic space, partially overlapping with the broader Caribbean–northern mainland group (Figures 2–3). Niche overlap between the two *C. acutus* regional groups was low (D = 0.050; I = 0.219), while overlap between Ecuador–Peru *C. acutus* and *C. intermedius* was zero for both indices. Equivalency tests were not significant. Similarity tests were also non-significant in most directions; the only significant result was Caribbean–northern mainland *C. acutus* → Ecuador–Peru *C. acutus* (D: p = 0.014; I: p = 0.011).

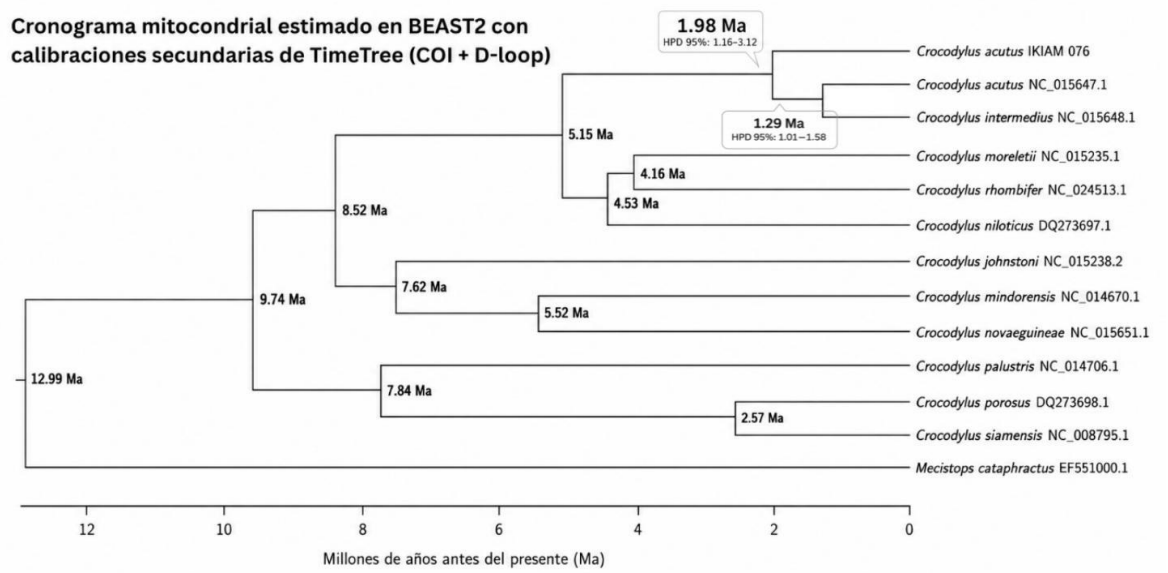


Figure 1. Mitochondrial chronogram estimated in BEAST2 for the concatenated COI + D-loop matrix using secondary TimeTree calibrations. IKIAM 076 is the only Ecuador–Peru sequence included in the temporal analysis. The focal node was estimated at 1.98 Ma (95% HPD: 1.16–3.12 Ma).

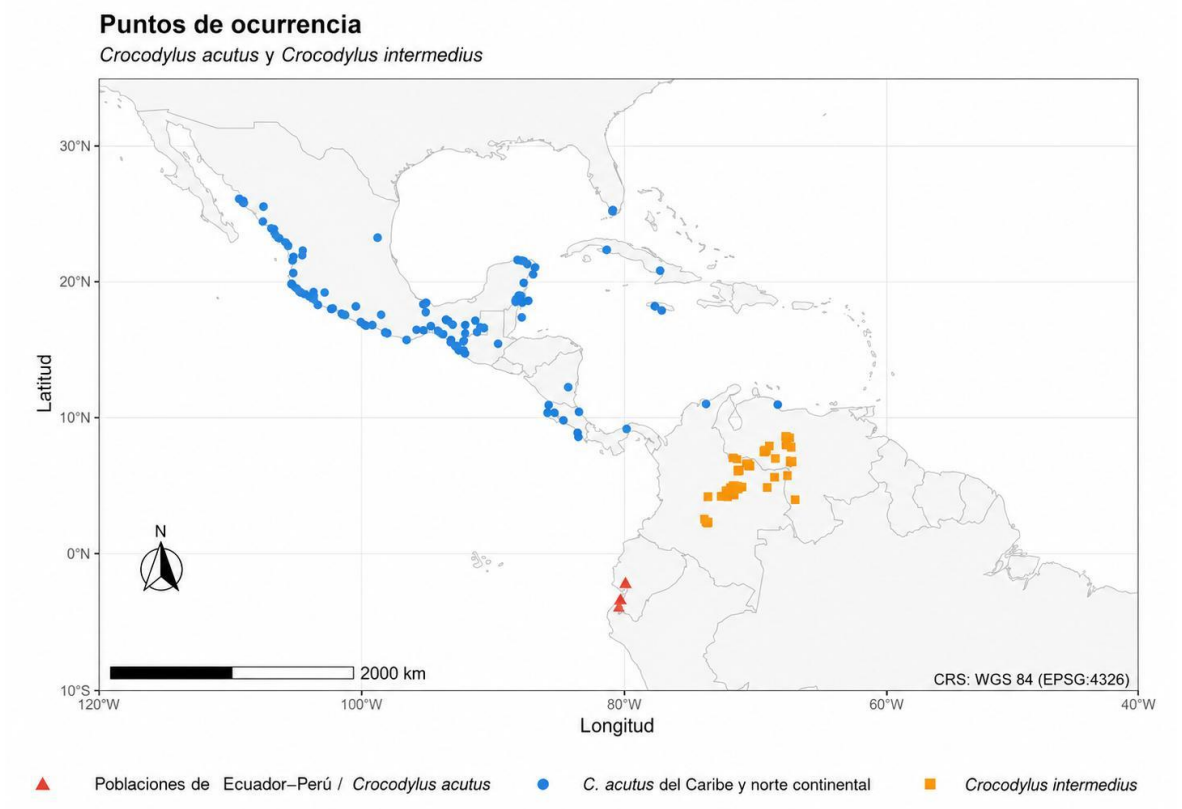


Figure 2. Geographic distribution of occurrence records used in the climatic analysis: eight C. acutus records from Ecuador–Peru, 104 C. acutus records from the Caribbean and northern mainland range, and 65 C. intermedius records. Coordinates are in WGS84 (EPSG:4326).

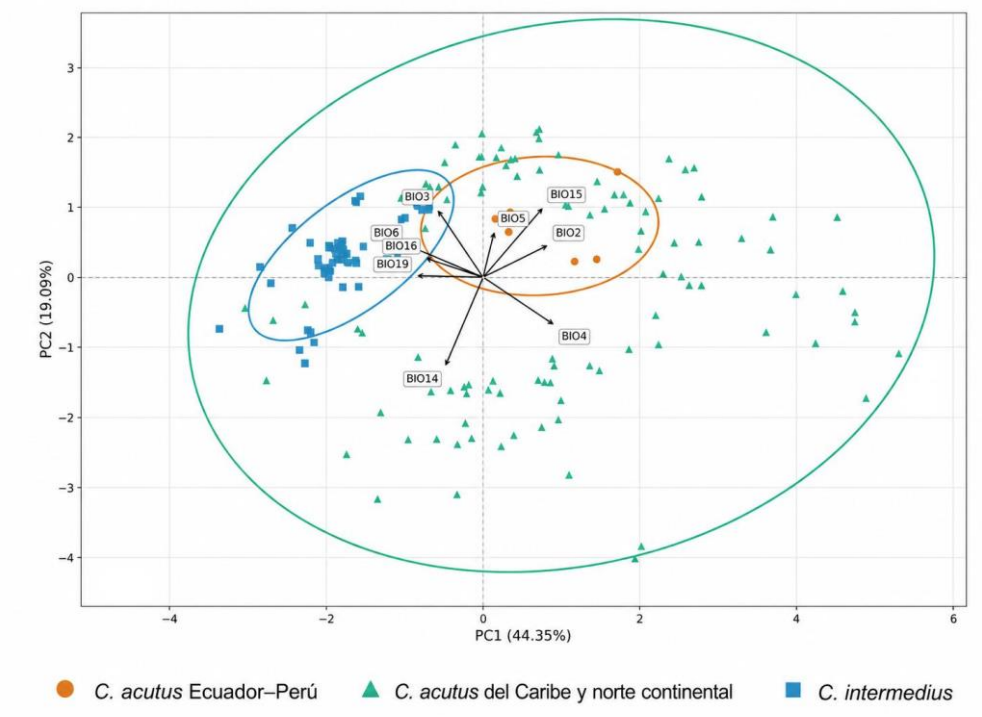


Figure 3. Principal component analysis of climatic conditions associated with the occurrence records. PC1 explained 44.35% and PC2 19.09% of variation (63.45% cumulative). Arrows indicate the direction and relative magnitude of loadings for the nine retained bioclimatic variables.

Discussion

The principal result is the recovery of a recognizable mitochondrial signal for C. acutus from southern Ecuador and northern Peru. The local sequences clustered within C. acutus, while their grouping and the small but non-zero K2P distances indicate regional differentiation in the mitochondrial markers analyzed. Similar geographic structure has been reported elsewhere in the species' range, including Colombia, the Greater Antilles and Pacific Costa Rica (Bloor et al., 2015; Mauger et al., 2017; Milián-García et al., 2018). However, COI and D-loop belong to the same mitochondrial genome and therefore do not represent independent hereditary lines (Hurst & Jiggins, 2005). The present results should consequently be tested with nuclear or genomic markers before drawing conclusions about population structure or conservation-unit status.

The temporal analysis provides a preliminary evolutionary timescale for one local mitochondrial sequence. The mean age of 1.98 Ma places the focal node in the early Pleistocene, a period during which sea-level changes and changing connectivity among coastal and riverine habitats could have influenced dispersal and isolation. Nevertheless, the estimate is based on IKIAM 076 as the only local sequence with both markers and on secondary calibrations. It should be treated as an estimate for the sampled mitochondrial genealogy, not as the origin or divergence time of the Ecuador–Peru population as a whole.

The climatic analyses likewise indicate differentiation while requiring cautious interpretation. The Ecuador–Peru records occupied a compact part of the climatic space and showed low overlap with Caribbean–northern mainland *C. acutus* and no detectable overlap with *C. intermedius* under the variables, resolution, backgrounds and occurrences used. The significant similarity test in only one direction reflects the asymmetry of the test and suggests that the climatic space represented by Ecuador–Peru resembles part of the broader Caribbean–northern mainland environmental space more than expected under randomization. It does not demonstrate niche equivalency or complete inclusion of one niche within another.

Sampling remains the main limitation of the climatic component: only eight Ecuador–Peru occurrences were available after filtering, compared with 104 and 65 records in the two comparative groups. The analysis was also restricted to WorldClim bioclimatic variables and did not include salinity, hydrology, mangrove cover, habitat connectivity or human pressure. The results therefore describe climatic space rather than the complete ecological niche.

Taken together, the genetic, temporal and climatic evidence identifies southern Ecuador and northern Peru as a region that merits more intensive study within the Pacific distribution of *C. acutus*. The current dataset does not justify designation of an independent evolutionary unit, but it provides a baseline for expanding geographic sampling, incorporating nuclear or genomic markers and integrating hydrological and habitat variables. These additions would allow stronger evaluation of connectivity, genetic diversity and potential implications for regional monitoring and conservation management.

Conclusions and Future Work

The project achieved its revised objectives by integrating mitochondrial phylogenetics, genetic distances, divergence-time estimation and climatic niche comparisons. Ecuador–Peru samples were placed within *C. acutus* and showed a recognizable regional mitochondrial grouping, while climatic analyses indicated low overlap with comparative regional datasets. The results are best viewed as a baseline rather than definitive evidence of an independent conservation unit. Future work should prioritize additional sampling across Ecuador and Peru, nuclear or genome-wide markers, and environmental variables that capture aquatic habitat, connectivity and anthropogenic pressures. These data will be necessary to determine whether the regional mitochondrial and climatic patterns observed here correspond to broader population-level differentiation relevant to conservation management.

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