Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

ORIGINAL RESEARCH

Rivers and spatial distance are drivers of genetic diversity in the South American dwarf caiman (*Paleosuchus palpebrosus*)

B. D. Vasconcelos[1], F. Camurugi[1], J. R. Mudrek[2], R. A. Brandão[3] & D. J. Santana[1]

[1]Laboratório de Sistemática e Biogeografia de Anfíbios e Répteis–Mapinguari, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande, Brazil
[2]Universidade Federal de Mato Grosso – UFMT, Cuiabá, Brazil
[3]Laboratório de Fauna e Unidades de Conservação, Departamento de Engenharia Florestal, Universidade de Brasília, Brasília, Brazil

Keywords
barrier; crocodilians; landscape elements; topography; *Paleosuchus palpebrosus*; genetic diversity; gene flow; South America.

Correspondence
Beatriz Diogo Vasconcelos, Laboratório de Sistemática e Biogeografia de Anfíbios e Répteis, Mapinguari, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande-MS, CEP 79.070-900, Brazil.
Email: beatrizdiogov@gmail.com

Editor: Anthony Herrel
Associate Editor: Kevin Arbuckle

Received 22 January 2024; revised 10 September 2024; accepted 13 September 2024

doi:10.1111/jzo.13226

Abstract

The distribution of species and the way that lineages are structured are the result of intrinsic historical processes of the species and their relationships with landscape features. *Paleosuchus palpebrosus* is one of the smallest crocodilians in the world and has a wide geographic distribution in South America, occurring in different habitats. Here, we analyzed mitochondrial cytochrome b sequences of 227 individuals, investigated how elements of landscape heterogeneity impact genetic differentiation, and evaluated the evolutionary and phylogeographic history of the species. Thus, we hypothesized that landscape structures, such as aridity and slope, should act as resistance surfaces to population connectivity, while rivers could mediate the dispersion of the species as a conductor of the gene flow. The analyses recovered three main lineages of *P. palpebrosus*. While most of the observed genetic variation was explained by geographic resistance distance, river connectivity had a smaller contribution for the observed variation. We also found a recent history with limited genetic divergence throughout the wide distribution of the species. Our findings highlight the main drivers for the evolutionary history of the species and how landscape features can shape the diversification, especially if we consider rivers as a facilitator of gene flow.

Introduction

Historical process, landscape features, and demographic history of the organisms shaped their distribution and genetic structure (Camurugi et al., 2020; He et al., 2013). In the Neotropical region, phylogeographic patterns and diversification processes are usually explained by different coupled events, such as geomorphological changes and climatic fluctuations (Hoorn et al., 2010; Turchetto-Zolet et al., 2013). Such geomorphological events promoted greater availability and diversity of habitats, niches, and reconfiguration of riverine habitats (Hoorn et al., 2010; Rull, 2011).

Among the Neotropical aquatic and semi-aquatic fauna, crocodilians are long-lived, large-bodied animals that inhabit aquatic environments, such as rivers, small streams, lakes, estuaries, swamps, and marshes (Amavet & Zucoloto, 2021). In these environments, they occupy the top of the food chain and play a key role in the structure and function of ecosystems and the density, behavior, and foraging of their prey (Layman et al., 2015; Nifong & Silliman, 2013). Crocodilians are an ancient group that has undergone relatively little morphological and genetic diversification compared to other archosaurs, such as birds (Grigg & Kirshner, 2015). Their diversification is congruent with the diversification of other herpetofauna groups in South America, in which tectonic and palaeogeographic reorganizations during the Neogene (~23–2.6 Mya) or environmental shifts caused by the glacial–interglacial periods in the Quaternary (~2.58 Mya to today) drove lineage diversification (Bittencourt et al., 2019; Turchetto-Zolet et al., 2013). In addition, landscape features have potential to represent important forces that drove the pattern of lineage structuring of these organisms, such as the opening of the Panamanian Isthmus during extreme sea-level lows that restricted gene flow between Pacific and Caribbean *Crocodylus acutus* in Panama (Avila-Cervantes & Larsson, 2023).

Paleosuchus palpebrosus is one of the smallest extant crocodilians (Campos et al., 2010), has a wide geographic distribution in South America, and occurs in different habitats throughout its distribution. In the Amazon, it occurs in seasonally flooded forests near large rivers and lakes (Campos et al., 2010). While in the Pantanal it occurs in headwaters of rivers and streams of rapids with rocky substrate (Campos & Mourão, 2006) and in the floodplain (Silva-Alves et al., 2022). Recently, Muniz et al. (2018), using mitochondrial data (Cytochrome b), demonstrated that *P. palpebrosus* comprises at least three intraspecific lineages with restricted gene flow. Since *P. palpebrosus* prefers cold waters and specific nesting environments (Rueda-Almonacid et al., 2007), habitat modifications can alter lineage characteristics (Ross, 1998). Thus, we hypothesized that aridity and terrain slope should act as resistance surfaces to population connectivity, while rivers could mediate the dispersion of the species. Whether aridity is an important driver of genetic differentiation, we expect that more arid regions could reduce connectivity between populations since the species is associated with aquatic environments. Conversely, rivers can shape the patterns of gene flow and genetic diversity, facilitating connections between lineages (Fonseca et al., 2021). In addition, whether species presents philopatry and/or limited dispersion ability, we expected to find a high correlation between geographic and genetic distances (IBD). In this way, the present study has three main goals: (1) Evaluate the evolutionary history and phylogeography of *P. palpebrosus* along its distribution; (2) verify possible lineage signatures and the time of divergence between the lineages; and (3) investigate how the landscape features impacted the species' genetic differentiation testing for IBD and IBR models.

Materials and methods

Sampling and DNA extraction, amplification, and sequencing

Paleosuchus palpebrosus tissue samples were obtained through scientific collection donations (Appendix S1). We complemented our dataset by downloading available sequences of *P. palpebrosus* from GenBank (http://www.ncbi.nlm.nih.gov/genbank), totaling 227 samples from 40 localities (Fig. 1; Appendix S1). We extracted DNA from the tissues of 21 individuals of *P. palpebrosus* using the phenol-chloroform extraction protocol (Sambrook et al., 1989). We amplified the partial sequences of the mitochondrial cytochrome b (Cytb) (mtDNA) loci using the standard Polymerase Chain Reaction (PCR) technique (Mullis et al., 1986) in a reaction volume of 25 μL, using primers 5′-3′ described by Hrbek et al. (2008) (CytbCRf.3 CCATACATYGGAGACACCAT and CytbCRr.2 AAGATYAGGTGGGTKATGAG). For all reactions, the PCR cycling program used was 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 72°C for 1 min, and concluding with a 1 min extension at 72°C. PCR conditions for amplification consisted of 1× buffer, dNTP at 0.2 mM, each primer at 0.2 μM, MgCl at 2 mM, 1 U Taq polymerase, and 2 μL of template DNA, in a total reaction volume of 25 μL. We ran the agarose gel and quantified the PCR product using a nanodrop. Center for Biological Resources and Genomic Biology (CREBIO) conducted the sequencing protocol. We visually checked all subsequent nucleotide sequences to ensure and aligned them using the Muscle algorithm (Edgar, 2004) in the Geneious v.7.1.3 (https://www.geneious.com). The final alignment checked in Geneious v.7.1.3 comprised 634 bp and we found no stop codons in the electropherograms. We used the species *Paleosuchus trigonatus* as an outgroup. All sequences used here are listed in Appendix S1.

Phylogenetic analyses

We estimated the best substitution model and partition schemes based on the Bayesian Information Criterion using PartitionFinder 2.1.1 (Lanfear et al., 2012), with a linked branch lengths model and a 'greedy' search. The best models were HKY + G for Cytb 1st position, TrN + G for Cytb 2nd position, and HKY + I for Cytb 3rd position. We then estimated a calibrated Bayesian gene tree using the most appropriate substitution models and assumed a Yule Process as tree prior. For clock prior calibration, we used a strict clock as priors and time-calibrated the tree by using a Crocodylidae-specific substitution rate of complete mtDNA genomes of 0.01 site/million years (SD = 0.004) (Eo & DeWoody, 2010). We implemented three independent runs each consisting of 5×10^7 generations sampled at every 5×10^3 generations in BEAST v2.6.4 (Bouckaert et al., 2019). We assessed the convergence of MCMC runs based on effective sample sizes (ESS ≥ 200) using Tracer v1.7 (Rambaut et al., 2018). We discarded the initial 10% generations of each run as burn-in and combined the results with LogCombiner v2.6.4 (Bouckaert et al., 2019). A maximum clade credibility tree was generated with TreeAnnotator v2.6.4 (Bouckaert et al., 2019). Finally, we visualized the tree in Figtree v1.4.3 (Rambaut, 2016).

Lineage delimitation and genetic statistics

We implemented a Bayesian Analysis of Population Structure (BAPS) to assess the presence and nature of spatial genetic structure of *P. palpebrosus* using BAPS v6.0 (Corander et al., 2008), with a population mixture model and k ranging from 1 to 10. Then, we conducted population admixture analyses with the number of iterations to estimate the admixture coefficients set to 100, 200 reference individuals from each lineage, and 20 iterations per individual. BAPS infers the lineage structure and determines the number of genetic clusters, testing 'mixture and admixture' through the geographic locations of each sample (Corander et al., 2013). To delimit lineages, we used the Generalized Mixed Yule Coalescent (GMYC; Pons et al., 2006) implemented in the R package splits (Fujisawa & Barraclough, 2013). The GMYC uses an ultrametric phylogenetic tree to estimate the transition between interspecific (i.e., speciation) and intraspecific events (modeled as Yule and coalescent processes, respectively; Pons et al., 2006). Since the assessment of model adequacy is fundamental for robust phylogeographic inferences (Carstens et al., 2022), we assessed the fit of the GMYC model to our empirical dataset using the P2C2M.GMYC R package (Fonseca et al., 2020). P2C2M.GMYC implements a parametric bootstrap

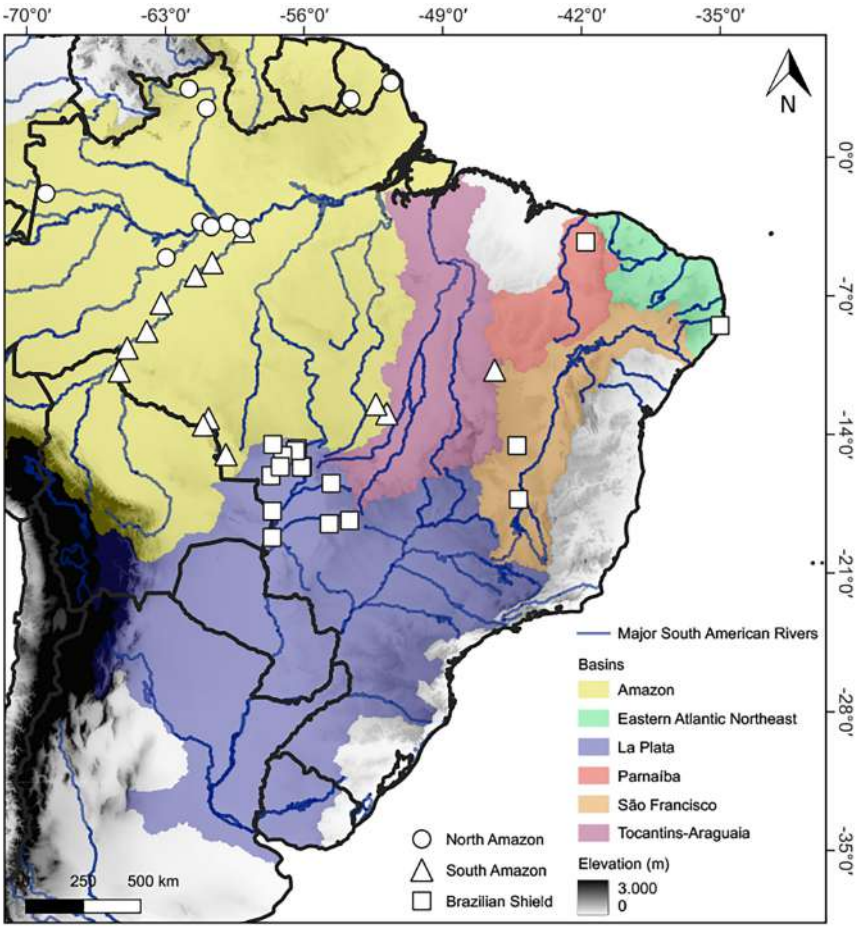

Figure 1 Distribution of sampled localities for *Paleosuchus palpebrosus* based on the Cytb mitochondrial dataset, including samples sent by scientific collections and available on GenBank. Colors represent the basins sampled in the study. The polygons represent each of the three recovered lineages: Circle represents the North Amazon lineage; Triangle represents the South Amazon lineage and; Square represents the Brazilian Shield lineage.

strategy where simulated datasets are built conditional to the same number of species recovered in the empirical dataset by the GMYC analysis. We simulated a total of 100 independent datasets and found no evidence of model violation using pseudo-*P*-values ($P < 0.05$).

To assess the patterns of genetic diversity for *P. palpebrosus* along the different lineages (see Results), we calculated the number of segregation sites (S), nucleotide diversity (π), number of haplotypes (H), and the haplotype diversity (Hd) in DnaSP (Rozas, 2009). We also used the program Arlequin 3.5 (Excoffier & Lischer, 2010) to perform Tajima's D neutrality tests (Tajima, 1989) and Fu's F_s (Fu, 1997). Results of Tajima's D can be used as a statistic test to assess whether the data are consistent with the lineage being at a neutral mutation-drift equilibrium following two cases: (1) when negative and significant, it suggests an excess of low-frequency polymorphisms relative to expectation, indicating lineage expansion; (2) when positive and significant, means low levels of both low and high-frequency polymorphisms, indicating a decrease in lineage size and/or balancing selection. Meanwhile, Fu's F_s is a statistic based on the infinite sites model of mutation. A negative and significant value of F_s is evidence for an excess number of alleles, as would be expected from a recent lineage size expansion or from genetic hitchhiking. A positive and significant value of F_s is evidence of a deficiency of alleles, as would be expected from a recent bottleneck (Holsinger, 2006). Also, with Arlequin v.3.5.2 (Excoffier & Lischer, 2010), we used a Hierarchical Analysis of Molecular Variance (AMOVA) to assess the departure of lineage genetic structure from panmictic expectations, using the BAPS haplogroups, GMYC delimitation groups, and clustering of individuals by river basin. We calculated average genetic distances (uncorrected *p*-distance) among haplogroups in MEGA X v10.0.5 (Kumar et al., 2018). Finally, we built a haplotype network using the median-joining method with PopArt (Leigh & Bryant, 2015), with the epsilon parameter set to 0, to describe the genealogical relationships between the localities and to investigate the genealogical relationships of haplotypes.

Spatiotemporal phylogeographic reconstruction and demographic history

We reconstructed the spatiotemporal history of *P. palpebrosus* using a Bayesian approach implemented in BEAST v1.8.4 (Drummond et al., 2012). To reconstruct lineage dispersal across the distribution of the species, we applied a lognormal relaxed random walk (RRW) model. This method is a time heterogeneous approach and generates a graphical image with branches that represent the tree and polygons that represent the 80% high posterior density of the geographical coordinates (Bielejec et al., 2011; Gehara et al., 2013). Thereby, we can visualize the reconstruction of the dispersal of lineages continuously in space and time. We implemented a coalescent prior with constant population size and applied the following substitution models for each codon position: F81 + *F* for 1st position, TrN + *F* for 2nd position, and HKY + FI for 3rd position. Because some sample coordinates were duplicated, we used a jitter option of 0.05, adding a random noise to identical coordinates. We ran two independent MCMC chains of 5×10^7 generations sampled at every 5×10^3 generations and assessed the stability of the analysis in Tracer v1.7 ensuring that all ESS values were >200 (Rambaut et al., 2018). To time-calibrate the tree, we used a Crocodylidae-specific substitution rate of complete mtDNA genomes of 0.01 site/million years (Eo & DeWoody, 2010) (SD = 0.004). The resulting tree was summarized with TreeAnnotator v1.8.2, the first 10% of samples were discarded as burn-in. After, we used SPREAD v1.0.4 to generate a kml file of the calibrated tree (Bielejec et al., 2011) which was plotted on Google Earth (http://earth.google.com).

To evaluate the population size changes of lineages through time, we used the single locus Bayesian Skyline Plot method (BSP) in BEAST v2.6.4 (Bouckaert et al., 2019) applied separately for each lineage identified by delimitation analyses (see Results). To time-calibrate the BSP, we used the same strategy implemented in the BEAST analysis (see section above), but using a Coalescent Bayesian Skyline tree prior and running MCMC simulations of 2×10^7 generations sampled at every 2×10^3 generations discarding the first 10% generations as burn-in. Parameter convergence of runs (ESS ≥ 200) and skyline graphs were visualized in Tracer v1.7 (Rambaut et al., 2018).

Identifying determinants of genetic differentiation

To test if landscape features (aridity, slope, and rivers) and geographic distance could mediate dispersal and genetic differentiation, we tested the effects of these predictors through the relative contributions of Isolation by Distance (IBD) and Isolation by Resistance (IBR).

For testing our IBD and IBR hypotheses we used only locations with more than two samples, that is the minimum number to calculate the genetic distance for a given deme. Our hypothesis of IBD posits a positive and significant correlation between spatial distance and genetic distance. To estimate the pairwise distance between localities, we used Geographic Distance Matrix Generator 1.2.3 (Ersts, 2011) and obtained a matrix of geographic distances. We tested a simple IBD null model by regressing both pairwise matrices distances (pairwise genetic distances vs. Euclidean geographic distance) ($R^2 = 0.35$, $P < 0.001$). For testing the IBR scenario, in which landscape connectivity/resistance can influence gene flow of lineages, we used three predictors that can lead to genetic differentiation. We used aridity and slope as resistant surfaces and rivers as conductance surfaces (IBR). Our river network raster contains the presence of main rivers from South America (stream lines at 1:10 m scale; https://www.naturalearthdata.com/). Because our study system depends on aquatic environments as habitat requirements for reproduction and feeding behavior, we used the global aridity index (Trabucco & Zomer, 2019; retrieved from https://csidotinfo.wordpress.com/data/global-aridity-and-pet-database/) as a measure of aridity gradient in the landscape, representing the contrasting mesic/dry conditions for the organisms. We obtained the elevation raster from NASA Jet Propulsion Laboratory (https://landscape.jpl.nasa.gov/). We used the elevation map to derive a slope raster in Arcmap v.10.3 (ESRI). We assumed that steeper slopes meant a higher cost for gene flow. To calculate pairwise resistance, we built environmental resistance surfaces with Circuitscape 5.0 package (Anantharaman et al., 2019) in Julia v. 5.0.0 (Hall et al., 2021). All rasters were at a spatial resolution of 2.5 arc-minutes. Circuitscape reads zeros as hard barriers and because of that we changed all 0 by 0.0001. Predictor matrices were standardized with the *scale* function in R. Then, we assessed the multicollinearity among them with Pearson's correlation tests and variance inflation factors (VIF) (Prunier et al., 2015) and found that geographic distance and slope resistance distance were highly correlated (Pearson correlation > 0.90, $P = 0.0001$; VIF > 5). So, in our isolation by distance null model, we integrated slope as an indicator of the landscape's topographic complexity (Vasconcellos et al., 2019; Zellmer & Knowles, 2009). Thus, our slope distance matrix represents now on our predictor of geographic resistance distance (IBD), instead of the pure Euclidean distances, to be tested in a multivariate approach (see below).

A pairwise matrix of genetic differentiation among localities (φst) was obtained with an analysis of molecular variance (AMOVA) in Arlequin 3.5.2 (Excoffier & Lischer, 2010). The significance of pairwise comparisons was assessed by 10 000 permutations. We replaced negative φst values ($n = 11$) with 0 because negative values of φst are stated as 0.

To test whether different landscape features promoted genetic differentiation in *P. palpebrosus*, we conducted a multiple regression on distance matrices (MRDM; Legendre et al., 1994; Wang, 2013) and a commonality analysis (CA; Prunier et al., 2015; Ray-Mukherjee et al., 2014) implemented in the *ecodist* (Goslee & Urban, 2007) and that packages (Nimon et al., 2013) in R, respectively. MRDM is useful for relating phylogenetic to spatial and environmental distance, examining the effect of a group of explanatory matrices on the response matrix (Lichstein, 2007; Swenson, 2014). CA analysis is a detailed variance-partitioning procedure that decomposes common and shared contributions of variables into non-overlapping components of variance to determine the variance of each predictor in the regression effect (Prunier et al., 2015). The predictor variables were the matrices of

geographic distance topographically corrected, resistance distances due to aridity, and conductance distances due to rivers, while the pairwise genetic distance matrix was used as the response variable. The MRDM model was run with 10 000 permutations to assess significance. With CA, we estimated the unique (U) and common (C) contributions of predictor variables, in which U represents the variance attributed solely to that single predictor and C represents the variance explained by that predictor along with one or more others. The total effect ($U + C$) accounts for the overall contribution of a predictor, regardless of collinearity with other predictors.

Results

Phylogenetic analysis and demographic history

The topology of the mitochondrial cytb tree recovered three strongly supported lineages for *P. palpebrosus* (PP = 1.00) (Fig. 2): (1) from the north of the Amazon basin, (2) from the south of the Amazon basin, including the samples from the Tocantins-Araguaia River basin, and (3), called Brazilian Shield, which encompasses samples from the La Plata River, São Francisco River, Parnaíba River and, Eastern Atlantic Northeast basins. The divergence among lineages of *P. palpebrosus* started almost simultaneously, with a first split of the North Amazon lineage at 440 Kya (90% HPD; 640–270 Kya), followed by a split between South Amazon and Brazilian Shield lineages at 370 Kya (95% HPD; 490–200 Kya). However, the support among lineages was low supported (PP = 0.66) and thus the divergence time considering any lineage split should be interpreted cautiously.

Lineages delimitation and genetic statistics

The BAPS and GMYC analyses recovered three mtDNA lineages for *P. palpebrosus*: (1) from the north of the Amazon basin, (2) from the south of the Amazon basin, including the samples from the Tocantins-Araguaia River basin, and (3), called Brazilian Shield, which encompasses samples from the La Plata River, São Francisco River, Parnaíba River and, Eastern Atlantic Northeast basins. We found low levels of genetic diversity for *P. palpebrosus*, with 26 polymorphic sites, 13 haplotypes, and a nucleotide diversity of 0.69 (Fig. 3). Tajima's D and Fu's F statistics showed negative values but not significant (Table 1). All comparisons between the lineages

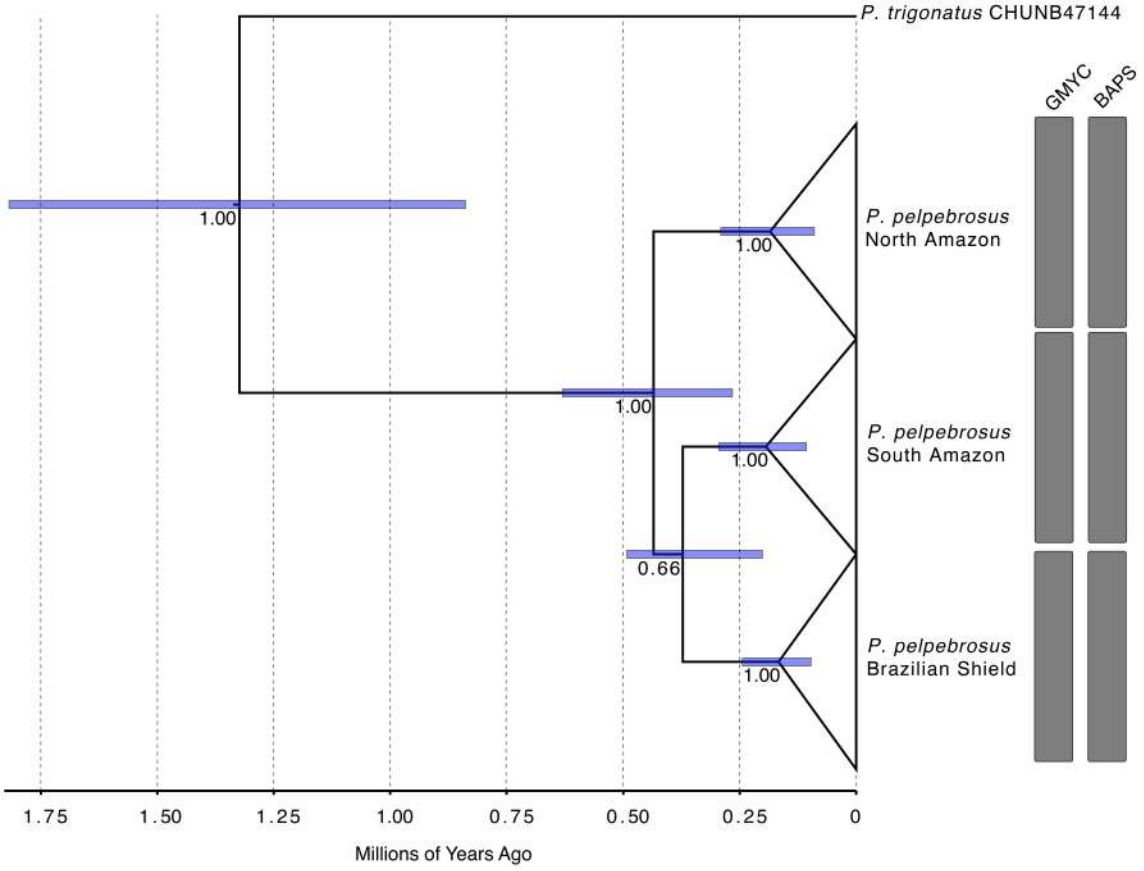

Figure 2 Tree topology generated using BEAST based on the Cytb mitochondrial dataset composed of 227 *Paleosuchus palpebrosus* sequences and 1 sequence of *Paleosuchus trigonatus*, used as outgroup (634 bp). BAPS and GMYC analysis indicate the three recovered lineages.

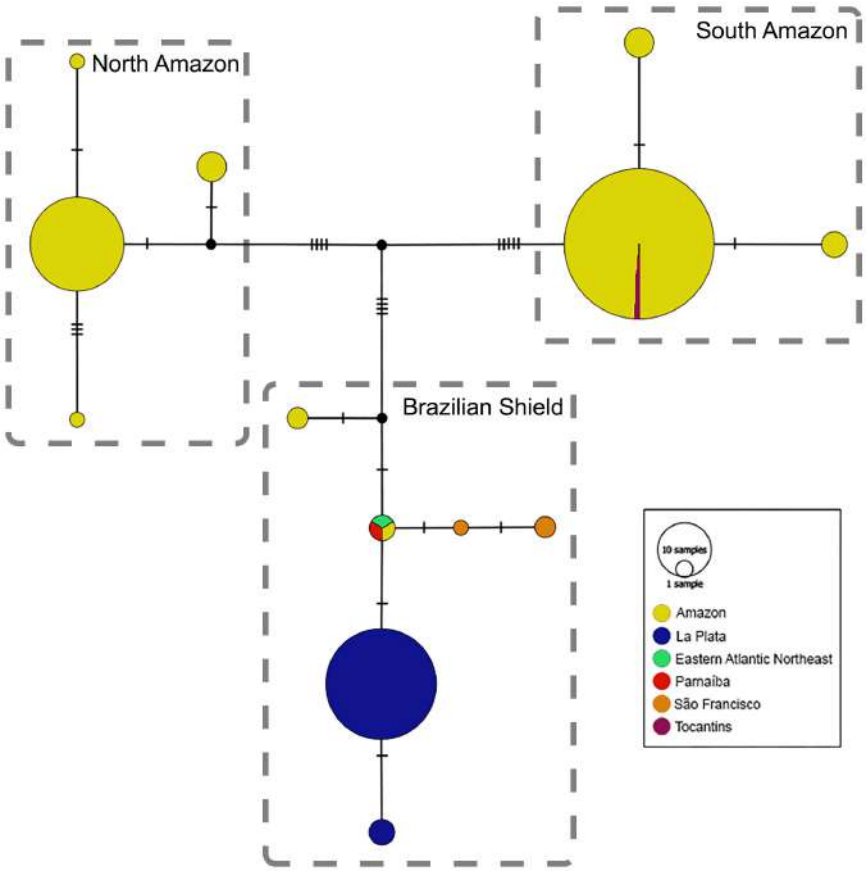

Figure 3 Haplotype network showing the low genetic diversity among *Paleosuchus palpebrosus* individuals throughout their distribution based on the Cytb mitochondrial dataset. Each colored circle represents a haplotype. Black circles represent unsampled haplotypes and the dashes represent mutational steps.

Table 1 General genetic parameters of *Paleosuchus palpebrosus* clusters, where N = number of samples; S = number of segregation sites; π = nucleotide diversity; H = number of haplotypes; Hd = haplotype diversity; Tajima's D with simulated P-value (alpha = 0.05, 10.000 simulations); Fu's F_s with simulated P-value (alpha < 0.02, 10.000 simulations)

Groups	N	S	π	H	Hd	Tajima's D	Fu's F_s (P)
North Amazon	47	6	0.48844	4	0.2359	−1.65 ($P \leq 0.05$)	−0.93 ($P > 0.05$)
South Amazon	112	2	0.12210	3	0.1202	−1.02 ($P > 0.05$)	−1.93 ($P > 0.05$)
Brazilian Shield	68	6	0.38876	6	0.2959	−1.34 ($P > 0.05$)	−2.58 ($P > 0.05$)

show φst values greater than 0.9 (Table 2). The uncorrected *p*-distance among lineages showed genetic differences of 1.74% between South Amazon and Brazilian Shield, 1.59% between North Amazon and South Amazon, and 1.74% between North Amazon and Brazilian Shield groups.

Spatiotemporal phylogeographic reconstruction and demographic history

The phylogeographic analysis suggested an Amazonian origin for *P. palpebrosus* (~2 Mya) during the early Pleistocene (Fig. 4), with subsequent dispersal to Tocantins-Araguaia River basin then to La Plata River basin (~500 Kya). Finally, the species dispersed to the São Francisco River basin, Parnaíba River, and Eastern Atlantic Northeast basins, while it increases dispersion to other regions of the Amazon basin (~200Kya). The Bayesian Skyline Plot analyses for all lineages showed that effective population sizes were relatively constant through time (Appendix S2).

Identifying determinants of genetic differentiation

The MRDM model retrieved a significant effect of IBD (including slope) and other two predictors of landscape resistance (aridity and rivers) as important predictors of genetic

Table 2 Results of the analyses of molecular variance (AMOVAs) considering different structure hypotheses

	Source of variation		
Grouping	Among populations	Within populations	F_{ST}
GMYC/ BAPS	96.95	3.05	0.9695
River basins	70.98	29.02	0.7098

The variance components (%) are presented accounting for GMYC and BAPS delimitation results and considering the clustering of samples by river basins. All *P*-values <0.0001.

differentiation in *P. palpebrosus*. The full model explained 37% of the total observed genetic variation ($R^2 = 0.37$, R^2 adjusted = 0.36, $F = 67.47$, $P < 0.0001$), considering all three variables. From all the explained variables, two were significant: geographic resistance distance ($\beta = 0.56$, $P < 0.0001$) and river connectivity ($\beta = 0.09$, $P = 0.03$). Only resistance distance due to aridity had no significant contribution for the observed genetic differentiation ($\beta = 0.03$, $P = 0.57$). The variation partitioning analysis indicated that geographic distance (including slope) accounted for most of the unique contribution of variables (61.38% of the explained variation), while rivers and aridity retrieved solely 2.24% and 0.16%, respectively. The shared contributions of geographic resistance distance and river connectivity represented 27.7% of the explained variation (Table 3).

Discussion

Resonances of the past: Historical events and the structuring of lineages

We found that *P. palpebrosus* is spatially structured in three lineages: North Amazon, South Amazon, and Brazilian Shield (see Fig. 1). Muniz et al. (2018) explored data from the Amazon and La Plata basins, and we complement it with more data from the La Plata basin and with sampling from the other basins where the species occurs: São Francisco River, Tocantins-Araguaia River, Parnaíba River and, Eastern Atlantic Northeast basins. Related to the diversification periods, Bittencourt et al. (2019) and Roberto et al. (2020) estimated the divergence of the Brazilian Shield lineage from the remaining lineages at approximately 4.28 Mya (95% HPD = 7.1–2.2 Mya), while the North Amazon and South Amazon diverged at 3.32 Mya (95% HPD = 5.6–1.5 Mya), using fossil calibrations to date their phylogenetic trees. In contrast, our study employed a Crocodylidae-specific substitution rate of complete

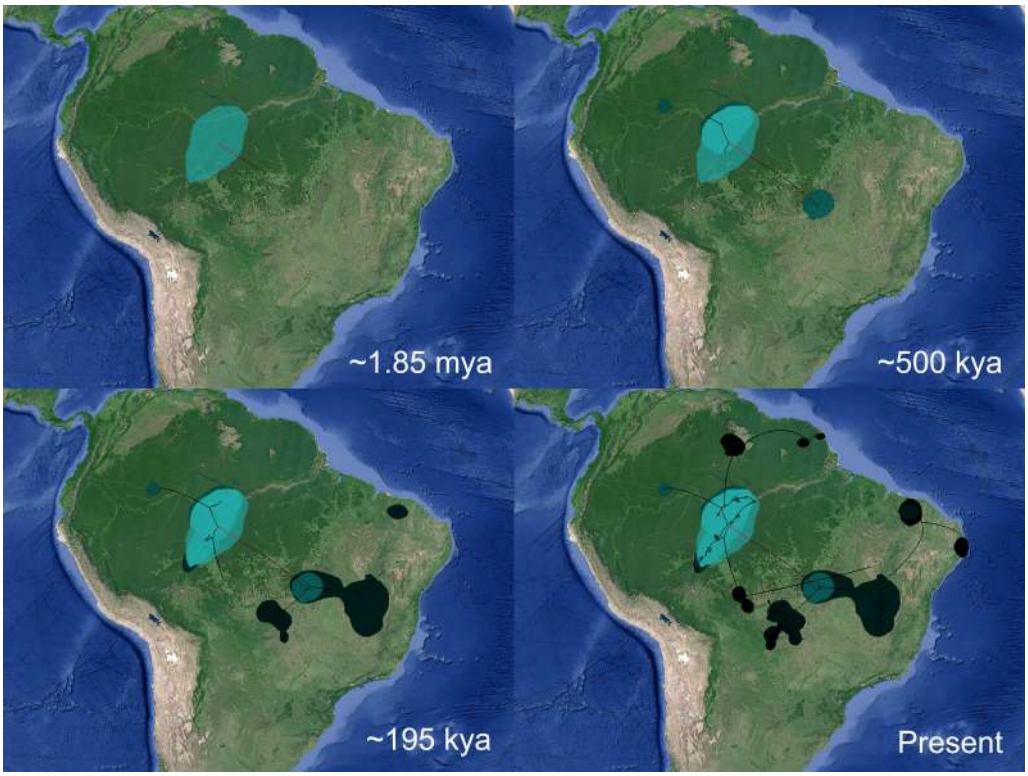

Figure 4 Phylogeographic reconstruction of *Paleosuchus palpebrosus*. Phylogeographical analysis of the species based on the Cytb mitochondrial dataset using a Relaxed Random Walk model for continuous trait reconstruction in Beast software. In the upper left corner, center of origin of *P. palpebrosus*. In the upper right corner, the dispersal to Tocantins-Araguaia River basin and La Plata basin. In the lower left corner, the dispersal to northeast of Brazil, while it increases dispersion to other regions of the Amazon basin. In the lower right corner, recent dispersals. Green polygons and red branches indicate relatively older events. Maps were generated using google earth (earth.google.com).

Table 3 Results of MRDM and commonality analysis

Predictor	β	P	U	C	T
Geographic distance	0.56	<0.0001	0.226	0.133	0.359
Rivers	0.09	0.03	0.008	0.046	0.054
Aridity	0.03	0.57	0.001	0.111	0.112

The contributions of predictor variables in explaining the genetic differentiation of populations are presented as predictor unique effect (U), the common contribution of predictors as the sum of shared effects of one predictor with other ones (C), and total contributions to the variance of the response variable ($T = U + C$).

mtDNA genomes, which led to divergence estimates that, although still occurring within the Pleistocene, yielded different time frames. Specifically, our divergence estimates are more recent, highlighting the sensitivity of molecular dating methods to both the choice of calibration points and substitution rates. The Pleistocene is recognized as a period of significant geological and climatic shifts, such as the Andean orogeny and glacial cycles, which have been implicated in shaping Neotropical biodiversity (Antonelli & Sanmartín, 2011; Ledo et al., 2020). However, the discrepancies between our dates and those of previous studies may reflect differences in the scale and impact of these historical processes across taxa. For example, other groups like the frog *Boana albopunctata* (1.7–2.6 Mya; Prado et al., 2012) and the snake *Bothrops neuwiedi* (3.06–1.71 Mya; Machado et al., 2014) also show divergence in the Pleistocene, but with varying timing and diversification drivers, such as the uplift of the Chapada dos Guimarães plateau and the Central Brazilian Plateau. These differences suggest that the Pleistocene's impact on species diversification may not have been uniform, further justifying the variance in our molecular dating results compared to earlier studies.

The phylogeographic relationship among lineages of *P. palpebrosus* indicates that they diversified recently, possibly during the Pleistocene. At this time, there was further Andean uplift (Hoorn et al., 2010) near western Amazon and neotectonic processes that caused development of river systems and patterns that led to the landscape we know currently (Toivonen et al., 2007). Amazonian lowlands, known for their dynamic headwaters of the Amazon River, have experienced geological events that significantly altered their water courses throughout history (Toivonen et al., 2007), also influencing other river basins (Rosa et al., 2003). Such historical processes may have influenced the diversification of *P. palpebrosus* into two Amazonian lineages and a lineage in the Brazilian shield area.

Isolation and connection: Dynamics in lineage delimitation

Three lineages were recovered for the species, which can be ratified by the high level of genetic differentiation among lineages shown by AMOVA (Table 2). The species has a pattern of low haplotype diversity and shared haplotypes (Fig. 3) along its wide distribution through the hydrographic basins (Amazon, La Plata River, São Francisco River, Tocantins-Araguaia River, Parnaíba River, and Eastern Atlantic Northeast). Also, the occurrence of populations in different hydrographic basins suggests connections between the basins and a similar hydrological history (Finston et al., 2006). The Amazon basin serves as a center of origin (Fig. 4) and harbors most haplotypes, including those shared with other basins, suggesting its ancient nature. The compartmentalisation of river basins can represent barriers for dispersal of aquatic species (Abell et al., 2008). Nevertheless, in this study this pattern is widespread in each lineage for each basin because the individuals may be able to move between basins, as the species can locomote long distances searching for available pond habitats (Marioni et al., 2022; Roberto & Albano, 2014). Hernández-Rangel et al. (2023) recently discovered evidence of possible female migration between different clusters of *P. palpebrosus* in the Amazon basin, potentially linking nearby regions. A similar pattern is also found in the pink dolphin (*Inia geoffrensis*), where some gene flow is maintained between two lineages (Hollatz et al., 2011). A potentially similar pattern is seen in the lineages of Morelet's crocodile (*Crocodylus moreletii*), where the lineages of one river function as a 'mainland,' while the surrounding smaller lineages appear as 'islands' (Dever et al., 2002).

Research carried out with Amazonian organisms usually finds that how historic processes have promoted barriers and corridors for dispersal (Pirani et al., 2020; Silva et al., 2019). In our study, samples from Amazon basin recovered seven unique haplotypes, in addition to two shared with other basins. Research on aquatic organisms has shown a connection between the Amazon and Parnaíba River basins (Albert & Reis, 2011; Garda & Cannatella, 2007), with records indicating that endemic species from the Parnaíba and the Eastern Atlantic Northeast basins share affinities with Amazonian species (Vari, 1989). This overlap is likely due to the Parnaíba River's historical role as a tributary of the Amazon River (Agassiz & Agassiz, 1938). Presumably, the evolutionary history of landscape features promotes isolation between lineages that, even when isolated, share haplotypes due to incomplete lineage sorting, given that the diversification of the species is very recent.

Mapping the past: Phylogeographic reconstruction and demographic dynamics

Despite its broad distribution, the species exhibits low genetic diversity. Historical demographic analysis reveals a recent evolutionary history with stable effective lineage sizes. This stability may be attributed to the long lifespan of crocodilians and the lower genetic diversity of long-lived vertebrates compared to shorter-lived organisms (Cao et al., 2020; Velo-Antón et al., 2014). The generalist ecology (Campos et al., 1995; Magnusson et al., 1987; Vasconcelos et al., 2020) and demographic stability despite environmental changes in the Pleistocene (Rossetti et al., 2017; Werneck et al., 2012) likely explain why *P. palpebrosus* lineages are continuously distributed with limited genetic divergence throughout its wide distribution. Species with wide distributions can be affected by various historical processes across their range limits due to abiotic factors and responses of individuals and populations to habitat quality, availability, and interactions (Mott, 2010), but even so, *P. palpebrosus* showed notable demographic stability during evolutionary time, which leads us to infer that lineages have

sufficient geographical stability to preserve the phylogenetic characteristics (Zhang et al., 2020). The species occupies different aquatic environments throughout its distribution (Campos & Mourão, 2006; Magnusson, 1985; Vasconcelos et al., 2020), including primary and secondary streams (*igarapés*), that were not greatly affected by historical processes, and its flexibility in the use of different kinds of water courses may have contributed to this scenario.

Drivers of genetic differentiation

We found that IBD and IBR significantly explain the genetic divergence of *P. palpebrosus*, where the topographically corrected geographic distances and aridity act as landscape features that reduce the migration of individuals, and rivers act as conductance between populations. Aridity, however, had no significant contribution for the IBR model. These findings agree with previous studies on faunal diversification in the Neotropics, in which IBR explained part of the genetic differentiation found in different lineages. Specifically, IBR was found to explain most of the genetic differentiation in *Podocnemis erythrocephala* and *P. sextuberculata*, two Amazonian riverine turtle species where the Amazon River acts as a barrier and river color acts as a resistance factor for connectivity, respectively (Oliveira et al., 2019).

The Madeira River, a major tributary of the Amazon basin, serves as a crucial zone of contact between the two recovered Amazonian lineages of *P. palpebrosus*. Supporting this, Roberto et al. (2020) identified a distinct lineage of the species within the Madeira River basin. This river has the largest water volume and sediment flow among all Amazon River tributaries (Li et al., 2020). The presence of high concentrations of chemicals and sediment load (Leite et al., 2011), and the difficulty that large species have to overcome their waterfalls during the floods (Farias et al., 2010) may be an obstacle for *P. palpebrosus* to cross it. In addition, the Madeira River represents a transitional zone between the Ombrophilous dense forest and Ombrophilous open forest (Ortiz et al., 2018), which can be important for isolation by resistance since rivers have an effect on the structuring of *P. palpebrosus*. Further south of the Madeira River, the Parecis plateau likely separates the Amazonian lineages of *P. palpebrosus* from the Brazilian Shield lineage. For *P. palpebrosus*, the Parecis mountains may represent one of the landscape elements that reflects the influence of slope in the genetic structuring of the species (IBR).

Recently, the Riverine Thruway Hypothesis (RTH) has been proposed, suggesting that rivers act as facilitators of gene flow, allowing population connectivity (Fonseca et al., 2021). The pattern observed in *P. palpebrosus* corroborates the RTH since rivers may act to structure the observed lineages herein. The rivers acting as dispersal facilitators have been reported in previous studies with aquatic and semi-aquatic species (Lawson, 2013; Oliveira et al., 2019). Possibly *P. palpebrosus* dispersed from the Amazon basin to the Tocantins-Araguaia River basin, where there is haplotype sharing, and then toward the La Plata River basin and other basins of the Brazilian Shield lineage. Despite being strongly associated with aquatic environments, *P. palpebrosus* likely can disperse by land routes, in order to reach ephemeral wetlands (Paolilla & Gorzula, 1985). In this way, events after the Last Glacial Maximum, when the Amazon delta extended further east and regions were connected to the Amazon basin, may be attributed to their dispersal and colonization of new areas, now outside the Amazon basin (Vasconcelos et al., 2006).

Conclusion

We demonstrated that *P. palpebrosus* corresponds to a single and widespread species, composed of three structured lineages that diverged in the Pleistocene and experienced a stable demographic history. We shed light on the main drivers of the evolutionary history of the species, finding that mainly isolation by distance taking the terrain topography into account, and the river connectivity at a lesser proportion, shaped the genetic structure of this species.

Acknowledgments

We are grateful to the curators of scientific collections for sending tissues under their care. BDV thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Funding code 001. FC thanks FUNDECT (Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul) and CNPq (Conselho Nacional de Desenvolvimento Cientıfico e Tecnológico) for financial support and his post doc fellowship (processes #71/700.083/2020). RAB thanks CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for his research fellowships and for financial support (processes 302162/2020-8; 306644/2023-7; 309420/2020-2; 402519/2016-7; 311284/2023-0). DJS thanks CNPq for his research fellowships and for financial support (processes 311284/2023-0; 402519/2016-7).

Author contributions

BDV, FC and DJS conceived the ideas, designed methodology, and analyzed the data; BDV and FC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Our study was conducted with the assistance of each author in the area in which they are experts. Just as the study species is widely distributed, so was the contribution to this publication. In addition to presenting gender diversity, the geographical distribution of the authors is also wide, with emphasis on the first two authors who are from the Northeast of Brazil, a region that is often overlooked for research collaboration but which has brilliant professionals.

References

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., . . . Petry, P. (2008). Freshwater ecoregions of the

world: A new Mmap of biogeographic units for biodiversity conservation. *Bioscience*, **58**, 403–414.

Agassiz, L., & Agassiz, E. C. (1938). *Viagem ao Brasil: 1865–1866*. São Paulo: Itatiaia.

Albert, J. S., & Reis, R. E. (2011). *Historical biogeography of neotropical freshwater fishes*. University of California Press.

Amavet, P. S., & Zucoloto, R. B. (2021). Perspectives and final considerations about the molecular ecology of new-world crocodilians. In R. B. Zucoloto, P. S. Amavet, L. M. Verdade, & I. P. Farias (Eds.), *Conservation genetics of New World crocodilians: 215–222*. Springer.

Anantharaman, R., Hall, K., Shah, V., & Edelman, A. (2019). Circuitscape in Julia: High performance connectivity modelling to support conservation decisions. *ArXiv* Preprint. http://arxiv.org/abs/1906.03542

Antonelli, A., & Sanmartín, I. (2011). Why are there so many plant species in the Neotropics? *Taxon*, **60**, 403–414.

Avila-Cervantes, J., & Larsson, H. C. E. (2023). Ice age effects on genetic divergence of the American crocodile (*Crocodylus acutus*) in Panama: Reconstructing limits of gene flow and environmental ranges: A reply to O'Dea et al. *Evolution*, **77**, 329–334.

Bielejec, F., Rambaut, A., Suchard, M. A., & Lemey, P. (2011). SPREAD: Spatial phylogenetic reconstruction of evolutionary dynamics. *Bioinformatics*, **27**, 2910–2912.

Bittencourt, P. S., Campos, Z., Muniz, F. D. L., Marioni, B., Souza, B. C., Da Silveira, R., de Thoisy, B., Hrbek, T., & Farias, I. P. (2019). Evidence of cryptic lineages within a small south American crocodilian: The Schneider's dwarf caiman *Paleosuchus trigonatus* (Alligatoridae: Caimaninae). *PeerJ*, **7**, e6580.

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., . . . Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **15**, e1006650.

Campos, Z., Coutinho, M., & Abercrombie, C. (1995). Size structure and sex ratio of dwarf caiman in the Serra Amolar, Pantanal, Brazil. *Herpetological Journal*, **5**, 321–322.

Campos, Z., & Mourão, G. (2006). Conservation status of the dwarf caiman, *Paleosuchus palpebrosus*, in the region surrounding Pantanal. *Crocodile Specialist Group Newsletter*, **25**, 9–10.

Campos, Z., Sanaiotti, T., & Magnusson, W. (2010). Maximum size of dwarf caiman, *Paleosuchus palpebrosus* (Cuvier, 1807), in the Amazon and habitats surrounding the Pantanal, Brazil. *Amphibians and Reptiles*, **31**, 439–442.

Camurugi, F., Gehara, M., Fonseca, E. M., Zamudio, K. R., Haddad, C. F. B., Colli, G. R., Thomé, M. T. C., Prado, C. P. A., Napoli, M. F., & Garda, A. A. (2020). Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed Neotropical treefrog. *Journal of Biogeography*, **2020**, 1–13.

Cao, R., Somaweera, R., Brittain, K., FitzSimmons, N. N., Georges, A., & Gongora, J. (2020). Genetic structure and diversity of Australian freshwater crocodiles (*Crocodylus johnstoni*) from the Kimberley, Western Australia. *Conservation Genetics*, **21**, 421–429.

Carstens, B. C., Smith, M. L., Duckett, D. J., Fonseca, E. M., & Thomé, M. T. C. (2022). Assessing model adequacy leads to more robust phylogeographic inference. *Trends in Ecology & Evolution*, **37**, 402–410.

Corander, J., Cheng, L., Marttinen, P., Sirén, J., & Tang, J. (2013). *BAPS: Bayesian analysis of population structure*. University of Helsinki.

Corander, J., Marttinen, P., Sirén, J., & Tang, J. (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics*, **16**(9), 539. https://doi.org/10.1186/1471-2105-9-539

Dever, J. A., Strauss, R. E., Rainwater, T. R., McMurry, S. T., & Densmore, L. D., III. (2002). Genetic diversity, population subdivision, and gene flow in Morelet's crocodile (*Crocodylus moreletii*) from Belize, Central America. *Copeia*, **4**, 1078–1091.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797.

Eo, S. H., & DeWoody, J. A. (2010). Evolutionary rates of mitochondrial genomes correspond to diversification rates and to contemporary species richness in birds and reptiles. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 3587–3592.

Ersts, P. J. (2011). *Geographic distance matrix generator (version 1.2.3)*. American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh.org/open_source/gdmg

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and windows. *Molecular Ecology Resources*, **10**, 564567.

Farias, I. P., Torrico, J. P., García-Dávila, C., Santos, M. C. F., Hrbek, T., & Renno, J. (2010). Are rapids a barrier for floodplain fishes of the Amazon Basin? A demographic study of the keystone floodplain species *Colossoma macropomum* (Teleostei: Characiformes). *Molecular Phylogenetics and Evolution*, **56**, 1129–1135.

Finston, T. L., Johnson, M. S., Humphreys, W. F., Eberhard, S. M., & Halse, S. A. (2006). Cryptic speciation in two widespread subterranean amphipod genera reflects historical drainage patterns in an ancient landscape. *Molecular Ecology*, **16**, 355–365.

Fonseca, E. M., Duckett, D. J., & Carstens, B. C. (2020). P2C2M.GMYC: An R package for assessing the utility of the generalized mixed yule coalescent model. *Methods in Ecology and Evolution*, **12**, 487–493.

Fonseca, E. M., Garda, A. A., Oliveira, E. F., Camurugi, F., Magalhães, F. M., Lanna, F. M., Zurano, J. P., Marques, R., Vences, M., & Gehara, M. (2021). The riverine thruway hypothesis: Rivers as a key mediator of gene flow for the aquatic paradoxical frog *Pseudis tocantins* (Anura, Hylidae). *Landscape Ecology*, **36**, 3049–3060.

Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Fujisawa, T. T., & Barraclough, T. G. (2013). Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology*, **62**, 707–724.

Garda, A. A., & Cannatella, D. C. (2007). Phylogeny and biogeography of paradoxical frogs (Anura, Hylidae, Pseudae) inferred from 12S and 16S mitochondrial DNA. *Molecular Phylogenetics and Evolution*, **44**, 104–114.

Gehara, M., Summers, K., & Brown, J. L. (2013). Population expansion, isolation and selection: Novel insights on the evolution of color diversity in the strawberry poison frog. *Evolutionary Ecology*, **27**, 797–824.

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, **22**, 1–19.

Grigg, G., & Kirshner, D. (2015). *Biology and evolution of crocodylians* (1st ed.). Csiro Publishing.

Hall, K. R., Anantharaman, R., Landau, V. A., Clark, M., Dickson, B. G., Jones, A., Platt, J., Edelman, A., & Shah, V. B. (2021). Circuitscape in julia: Empowering dynamic approaches to connectivity assessment. *Landscape*, **10**, 301.

He, Q., Edwards, D. L., & Knowles, L. L. (2013). Integrative testing of how environments from the past to the present shape genetics structure across landscapes. *Evolution*, **67**, 3386–3402.

Hernández-Rangel, S. M., Morales-Betancourt, M. A., Muniz, F. L., Vargas-Ramírez, M., Rojas-Runjaic, F. J. M., Lasso, C. A., & Caballero, S. (2023). Phylogenetic identity and population structure of the dwarf caimans *Paleosuchus* spp. in The Orinoco basin of Colombia and Venezuela: Filling gaps. *Biological Journal of the Linnean Society*, **142**, 1–13.

Hollatz, C., Vilaça, S. T., Redondo, R. A. F., Marmontel, M., Baker, C. S., & Santos, F. R. (2011). The Amazon River system as an ecological barrier driving genetic differentiation of the pink dolphin (*Inia geoffrensis*). *Biological Journal of the Linnean Society*, **102**, 812–827.

Holsinger, K. E. (2006). *Lecture notes in population genetics* (1st ed.). University of Connecticut.

Hoorn, C., Wesselingh, F. P., Steege, H. T., Bermudez, M. A., Mora, A., Sevinik, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C. L., Figueiredo, J. P., Jaramillo, C., Riff, D., Negri, F. R., Hooghiemstra, H., Lundberg, J., Stadler, T., Sarkinen, T., & Antonelli, A. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, **330**, 927–931.

Hrbek, T., Vasconcelos, W. R., Rebelo, G., & Farias, I. P. (2008). Phylogenetic relationships of south American Alligatorids and the caiman of Madeira River. *The Journal of Experimental Zoology*, **309A**, 588–599.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**, 1547–1549.

Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695–1701.

Lawson, L. P. (2013). Diversification in a biodiversity hot spot: Landscape correlates of phylogeographic patterns in the African spotted reed frog. *Molecular Ecology*, **22**, 1947–1960.

Layman, C. A., Giery, S. T., Buhler, S., Rossi, R., Penland, T., Henson, M. N., Bogdanoff, A. K., Cove, M. V., Irizarry, A. D., Schalk, C. M., & Archer, S. K. (2015). A primer on the history of food web ecology: Fundamental contributions of fourteen researchers. *Food Webs*, **4**, 14–24.

Ledo, R. M. D., Domingos, F. M. C. B., Giugliano, L. G., Sites, J. W., Jr., Werneck, F. P., & Colli, G. R. (2020). Pleistocene expansion and connectivity of mesic forests inside the south American dry diagonal supported by the phylogeography of a small lizard. *Evolution*, **74**, 1988–2004.

Legendre, P., Lapointe, F. J., & Casgrain, P. (1994). Modeling brain evolution from behavior: A permutational regression approach. *Evolution*, **48**, 1487–1499.

Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, **6**, 1110–1116.

Leite, N. K., Krusche, A. V., Ballester, M. V. R., Victoria, R. L., Richey, J. E., & Gomes, B. M. (2011). Intra and interannual variability in the Madeira River water chemistry and sediment load. *Biogeochemistry*, **105**, 37–51.

Li, D., Lu, D., Moran, E., & da Silva, R. F. B. (2020). Examining water area changes accompanying dam construction in the Madeira River in the Brazilian Amazon. *Water*, **12**, 1921.

Lichstein, J. W. (2007). Multiple regression on distance matrices: A multivariate spatial analysis tool. *Plant Ecology*, **188**, 117–131.

Machado, T., Silva, V. X., & Silva, M. J. J. (2014). Phylogenetic relationships within *Bothrops neuwiedi* group (Serpentes, Squamata): Geographically highly-structured lineages, evidence of introgressive hybridization and Neogene/quaternary diversification. *Molecular Phylogenetics and Evolution*, **71**, 1–14.

Magnusson, W. (1985). Habitat selection, parasites and injuries in amazonian crocodilians. *Amazoniana*, **9**, 193–204.

Magnusson, W. E., Da Silva, E. V., & Lima, A. P. (1987). Diets of amazonian crocodilians. *Journal of Herpetology*, **21**, 85–95.

Marioni, B., Magnusson, W. E., Vogt, R. C., & Villamarín, F. (2022). Home range and movement patterns of male dwarf caimans (*Paleosuchus palpebrosus* and *Paleosuchus trigonatus)* living in sympatry in Amazonian floodplain streams. *Neotropical Biodiversity*, **8**, 156–166.

Mott, C. L. (2010). Environmental constraints to the geographic expansion of plant and animal species. *Nature Education Knowledge*, **3**, 72.

Mullis, K., Faloona, F., Scharf, S., Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology*, **25**, 263–273.

Muniz, F. L., Campos, Z., Hernandez Rangel, S. M., Martínez, J. G., Souza, B. C., de Thoisy, B., Botero-Arias, R., Hrbek, T., & Farias, I. (2018). Delimitation of evolutionary units in Cuvier's dwarf caiman, *Paleosuchus palpebrosus* (Cuvier, 1807): Insights from conservation of a broadly distributed species. *Conservation Genetics*, **19**, 599–610.

Nifong, J., & Silliman, B. R. (2013). Impacts of a large-bodied, apex predator (*Alligator mississippiensis* Daudin 1801) on salt marsh food webs. *Journal of Experimental Marine Biology and Ecology*, **440**, 185–191.

Nimon, K., Oswald, F. L., & Roberts, J. K. (2013). *Yhat: Interpreting regression effects*. R package version 2.0.0. https://cran.r-project.org/web/packages/yhat/index.html

Oliveira, J. A., Farias, I. P., Costa, G. C., & Werneck, F. P. (2019). Model-based riverscape genetics: Disentangling the roles of local and connectivity factors in shaping spatial genetic patterns of two Amazonian turtles with different dispersal abilities. *Evolutionary Ecology*, **33**, 273–298.

Ortiz, D. A., Lima, A. P., & Werneck, F. P. (2018). Environmental transition zone and rivers shape intraspecific population structure and genetic diversity of an Amazonian rain forest tree frog. *Evolutionary Ecology*, **32**, 359–378.

Paolilla, A., & Gorzula, S. (1985). Life history notes: *Paleosuchus palpebrosus* (Dwarf caiman) migration. SSAR. *Herpetological Review*, **16**, 27.

Pirani, R. M., Peloso, P. L. V., Prado, J. R., Polo, E. M., Knowles, L. L., Ron, S. R., Rodrigues, M. T., Sturaro, M. J., & Werneck, F. P. (2020). Diversification history of clown tree frogs in Neotropical rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group). *Molecular Phylogenetics and Evolution*, **150**, 106877.

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D., & Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, **55**, 595–609.

Prado, C. P. A., Haddad, C. F. B., & Zamudio, K. R. (2012). Cryptic lineages and Pleistocene population expansion in a Brazilian Cerrado frog. *Molecular Ecology*, **21**, 921–941.

Prunier, J. G., Colyn, M., Legendre, X., Nimon, K. F., & Flamand, M. C. (2015). Multicollinearity in spatial genetics: Separating the wheat from the chaff using commonality analyses. *Molecular Ecology*, **24**, 263–283.

Rambaut, A. (2016). *FigTree v1.4.3. Molecular evolution, phylogenetics and epidemiology*. http://tree.bio.ed.ac.uk/software/figtree

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in bayesian phylogenetics using tracer 1.7. *Systematic Biology*, **67**, 901–904.

Ray-Mukherjee, J., Nimon, K., Mukherjee, S., Morris, D. W., Slotow, R., & Hamer, M. (2014). Using commonality analysis in multiple regressions: A tool to decompose regression effects in the face of multicollinearity. *Methods in Ecology and Evolution*, **5**, 320–328.

Roberto, I. J., & Albano, C. G. (2014). *Paleosuchus palpebrosus* (Cuvier's smooth-fronted caiman) habitat use. *Herpetological Review*, **45**, 121–122.

Roberto, I. J., Bittencourt, P. S., Muniz, F. L., Hernández-Rangel, S. M., Nóbrega, Y. C., Ávila, R. W., Souza, B. C., Alvarez, G., Miranda-Chumacero, G., Campos, Z., Farias, I. P., & Hrbek, T. (2020). Unexpected but unsurprising lineage diversity within the most widespread Neotropical crocodilian genus caiman (Crocodylia, Alligatoridae). *Systematics and Biodiversity*, **18**, 1–19.

Rosa, R. S., Menezes, N. A., Britski, H. A., Costa, W. J. E. M., & Groth, F. (2003). Diversidade, padrões de distribuição e conservação dos peixes da Caatinga. In I. R. Leal, M. Tabarelli, & J. M. C. Silva (Eds.), *Ecologia e conservação da Caatinga* (pp. 135–180). Universitária da UFPE.

Ross, J. P. (1998). *Crocodiles: Status survey and conservation action plan* (1st ed.). IUCN/SSC Crocodile Specialist Group.

Rossetti, D. F., Cohen, M. C. L., & Pessenda, L. C. R. (2017). Vegetation change in southwestern Amazonia (Brazil) and relationship to the late Pleistocene and Holocene climate. *Radiocarbon*, **59**, 68–89.

Rozas, J. (2009). DNA sequence polymorphism analysis using DnaSP. In D. Posada (Ed.), *Bioinformatics for DNA sequence analysis* (pp. 337–350). Springer Protocols.

Rueda-Almonacid, J. V., Carr, J. L., Mittermeier, R. A., & Rodriguez-Mahecha, J. V. (2007). *Las tortugas y los cocodrilianos de los países andinos del trópico* (1st ed.). Conservación Internacional Série de Guías Tropicales de Campo.

Rull, V. (2011). Neotropical biodiversity: Timing and potential drivers. *Trends in Ecology & Evolution*, **26**, 508–513.

Sambrook, J., Fritsch, E. F., & Maniatis, R. (1989). *Molecular cloning: A laboratory manual* (1st ed.). Cold Spring Harbor Laboratory Press.

Silva, S. M., Peterson, A. T., Carneiro, L., Burlamaqui, T. C. T., Ribas, C. C., Sousa-Neves, T., Miranda, L. S., Fernandes, A. M., D'horta, F. M., Araújo-Silva, L. E., Batista, R., Bandeira, C. H. M. M., Dantas, S. M., Ferreira, M., Martins, D. M., Oliveira, J., Rocha, T. C., Sardelli, C. H., Thom, G., … Aleixo, A. (2019). A dynamic continental moisture gradient drove Amazonian bird diversification. *Science Advances*, **5**, eaat5752.

Silva-Alves, V. D., Mudrek, J. R., Silva-Diogo, O., Canale, G. R., Santos-Filho, M., Muniz, C. C., & Silva, D. J. (2022). A first approximation for the Herpetofauna species composition of the Taiamã Ecological Station, Pantanal of Mato Grosso, Brazil. *Brazilian Journal of Biology*, **82**, e264737.

Swenson, N. G. (2014). *Functional and phylogenetic ecology in R* (1st ed.). Springer.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Toivonen, T., Mäki, S., & Kalliola, R. (2007). The riverscape of Western Amazonia – A quantitative approach to the fluvial biogeography of the region. *Journal of Biogeography*, **34**, 1374–1387.

Trabucco, A., & Zomer, R. (2019). *Global aridity index and potential evapotranspiration (ET0) climate database v2*. https://doi.org/10.6084/m9.figshare.7504448.v3

Turchetto-Zolet, A. C., Pinheiro, F., Salgueiro, F., & Palma-Silva, C. (2013). Phylogeographical patterns shed light on evolutionary processes in South America. *Molecular Ecology*, **22**, 1193–1213.

Vari, R. P. (1989). Systematics of the neotropical characiform genus *Psectrogaster* Eigenmann and Eigenmann (Pisces, Characiformes). *Smithsonian Contributions to Zoology*, **481**, 1–41.

Vasconcellos, M. M., Colli, G. R., Weber, J. N., Ortiz, E. M., Rodrigues, M. T., & Cannatella, D. C. (2019). Isolation by instability: Historical climate change shapes population structure and genomic divergence of treefrogs in the Neotropical Cerrado savanna. *Molecular Ecology*, **28**, 1748–1764.

Vasconcelos, B. D., Abreu, J. M. N. S., & Brandão, R. A. (2020). História natural do jacaré-paguá (*Paleosuchus palpebrosus*), o pequeno notável das veredas dos sertões da Trijunção entre Bahia, Goiás e Minas Gerais. In R. Brandão, R. D. Françoso, T. H. Machado, & N. J. Santos (Eds.), *História Natural do Sertão da Trijunção do Nordeste, Centro-Oeste e Sudeste do Brasil* (pp. 149–164). PerSe.

Vasconcelos, W. R., Hrbek, T., Silveira, R., Thoisy, B., Marioni, B., & Farias, I.P. (2006). Population genetic analysis of Caiman crocodilus (Linnaeus, 1758) from South America. *Genetics and Molecular Biology*, **29**, 220–230.

Velo-Antón, G., Godinho, R., Campos, J. C., & Brito, J. C. (2014). Should I stay or should I go? Dispersal and population structure in small, isolated desert populations of West African crocodiles. *PLoS One*, **9**, e94626.

Wang, I. J. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution*, **67**, 3403–3411.

Werneck, F. P., Gamble, T., Colli, G. R., Rodrigues, M. T., & Sites, J. W., Jr. (2012). Deep diversification and long-term persistence in the south American "dry diagonal": Integrating continent-wide phylogeography and distribution modeling of geckos. *Evolution*, **66**, 3014–3034.

Zellmer, A. J., & Knowles, L. L. (2009). Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. *Molecular Ecology*, **18**, 3593–3602.

Zhang, D., Hui, H., & Yu, G. (2020). Shared response to changes in drainage basin: Phylogeography of the yunnan small narrow-mouthed frog, *Glyphoglossus yunnanensis* (Anura: Microhylidae). *Ecology and Evolution*, **10**, 1567–1580.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Voucher information and GenBank numbers. Specimens used for the molecular analyses, including GenBank numbers for mitochondrial Cytb sequences.

Appendix S2. Bayesian skyline plots for each mtDNA (Cytb) lineage of *Paleosuchus palpebrosus*, where A is North-Amazon lineage, B is South-Amazon lineage, and C is Brazilian Shield lineage. The *x*-axis represents years from present to past, while *y*-axis represents the effective lineage size (Ne). The blue line in the middle indicates the median of Ne, while the blue lines at the ends indicate a 95% higher posterior probability.