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Programa de Pós-Graduação em Biologia Animal

**The genetic diversity in a South American Dwarf Caiman
(*Paleosuchus palpebrosus*) is structured by landscape features**

Beatriz Diogo Vasconcelos

Dissertação apresentada à Fundação Universidade Federal de Mato Grosso do Sul, como requisito à obtenção do título de Mestre em Biologia Animal. Área de concentração: Zoologia.

Orientador: Prof. Dr. Diego José Santana

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“Do or do not. There is no try.”

(Yoda em “Star Wars Episódio V: O Império Contra-Ataca”, 1980)

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Resumo

A distribuição das espécies e a forma como as linhagens se estruturam é resultado de processos históricos intrínsecos das espécies e suas relações com as características da paisagem. Estudos com diferentes organismos aquáticos e semi-aquáticos mostram como sua distribuição e estrutura genética são moldadas por características da paisagem e adequação climática. *Paleosuchus palpebrosus* é um dos menores crocodilianos do mundo e possui ampla distribuição com diferentes condições de habitat. Neste trabalho, investigamos como elementos de heterogeneidade da paisagem impactam a diferenciação genética e avaliamos a história evolutiva e filogeográfica da espécie. Nós hipotetizamos que elementos das estruturas da paisagem poderiam mediar a dispersão das espécies, onde aridez e declividade atuam como superfícies de resistência e rios como facilitadores de conectividade. As três linhagens recuperadas trouxeram informações únicas sobre a estrutura genética da espécie para outras áreas onde reportamos dados genéticos pela primeira vez. Parte da diferenciação genética resultou do isolamento por resistência. Além disso, nossa análise de demografia histórica encontrou uma história recente e nenhuma mudança nos tamanhos efetivos das linhagens durante todo o tempo, diferentemente do que aconteceu com a grande maioria da fauna Neotropical durante o Plioceno e o Pleistoceno. Nossas descobertas destacam os principais impulsionadores da história evolutiva da espécie e como as características da paisagem podem ter moldado sua diversificação, especialmente se considerarmos os rios como facilitadores do fluxo gênico.

Palavras-chave: Jacaré, Isolamento por resistência, Rio como facilitador, Topografia

Abstract

The distribution of species and the way that lineages are structured is the result of intrinsic historical processes of the species and their relationships with landscape features. Studies with different aquatic and semi-aquatic organisms show how their distribution and genetic structure are shaped by landscape features and climatic suitability. *Paleosuchus palpebrosus* is one of the smallest crocodylians in the world and has a wide distribution with different habitat conditions. Here, we investigate how elements of landscape heterogeneity impact genetic differentiation and evaluate the evolutionary and phylogeographic history of the species. We hypothesized that elements of landscape structures, such as aridity and slope as resistance surfaces and river as conductance surface could mediate the dispersion of the species. The three lineages recovered brought unique information about the genetic structure of the species for other areas where we reported genetic data for the first time. Part of the genetic differentiation resulted from isolation by resistance. Furthermore, our historical demography analysis found a recent history and no changes on effective lineages sizes through time, differently from what happened with the vast majority of the Neotropical fauna during the Pliocene and Pleistocene. 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.

Keywords: Alligator, Isolation by resistance, River as facilitator, Topography

Introdução geral

A distribuição das espécies e, conseqüentemente, a maneira como as linhagens são estruturadas hoje é o resultado de processos históricos intrínsecos das espécies e suas relações com outros organismos (Manel et al 2003). Através da distribuição espacial dessas linhagens, conhecemos a origem, diversificação e processos históricos responsáveis pela estruturação e distribuição geográfica de variações genéticas dentro das espécies (Beheregaray 2008; Hickerson et al 2009). A filogeografia avalia essa distribuição histórico-geográfica de linhagens genealógicas baseado em informações genéticas (Avice 2000), permitindo testar características da paisagem na determinação da diversidade genética (Adams et al 2016; Jaisuk et al 2018). Estudos filogeográficos com diferentes organismos testaram o papel de barreiras geográficas (e.g. rios) na estruturação das linhagens e o histórico evolutivo deles (Hrbek et al 2008; Oliveira et al 2019; Santos et al 2016). Tais elementos podem atuar como barreiras (Silva et al 2019) ou como facilitadores do fluxo gênico (Fonseca et al 2021).

A biodiversidade Neotropical está relacionada a uma história complexa e contínua de eventos geológicos e dinâmicas climáticas (Rull 2008). Nesse contexto, a história evolutiva de diferentes linhagens de espécies neotropicais com traços de história de vida distintos deve gerar diferentes respostas demográficas aos processos históricos. (De Oliveira et al 2021). As rãs de grande porte do complexo de espécies *Leptodactylus latrans*, que são morfologicamente crípticas, exibem um forte padrão de estrutura geográfica relacionado a altas distâncias genéticas intraespecíficas (Magalhães et al 2022). Contudo, é importante buscar outros fatores que expliquem a demografia histórica, como elementos da paisagem, que podem influenciar as linhagens ao longo do tempo (Fenker et al 2021).

Na região Neotropical, a paisagem apresenta grande variação, o que se reflete na distribuição e nos padrões genéticos das espécies (Lawson 2013). Por exemplo, aspectos físicos da paisagem podem influenciar a conectividade espacial e as taxas de fluxo gênico entre linhagens do lagarto *Ameivula ocellifera*, uma vez que os rios desempenharam um papel importante na diferenciação genética da espécie (Oliveira et al 2018). Apesar da ampla distribuição de *Physalaemus cuvieri*, o soerguimento do Planalto Central Brasileiro e os rios estruturaram a diversidade genética da espécie (Miranda et al 2019). Da mesma forma, o isolamento das linhagens do sudoeste da lagartixa *Phyllopezus pollicaris* ocorreu devido a um intenso soerguimento dos Andes e do Escudo Central Brasileiro (Werneck et al 2012). Embora alguns estudos busquem explicar os padrões filogeográficos neotropicais como vemos hoje (Soley-Guardia et al 2019; Rull 2020), um passo fundamental é encontrar e, de forma robusta, delimitar a estrutura populacional dos organismos (Hosegood et al 2020) e, como próximo passo, testar a influência de fatores geográficos e históricos que contribuíram para a distribuição da variabilidade genética de uma espécie (Eizirik 2001).

Dentre os organismos que ocorrem na região Neotropical, estão os crocodilianos. Nos últimos anos, estudos científicos aumentaram o conhecimento da biologia do grupo (Marioni et al 2020), permitindo a descoberta de várias linhagens (i.e. Godshalk 2006; Eaton et al 2009; Franke et al 2013; Shirley et al 2014; Bloor et al 2015; Pacheco-Sierra et al 2018) e validação de espécies (Hekkala et al 2011; Shirley et al 2018). *Paleosuchus palpebrosus* é um dos menores crocodilianos do mundo (Campos et al. 2010) e está associado a diferentes tipos de ambientes (e.g. florestas alagadas, áreas antropizadas e ambientes aquáticos com solo arenoso e lagoas) (Campos e Magnusson et al 2013; Magnusson e Campos, 2010). A espécie possui ampla distribuição geográfica na América do Sul e ocorre em várias bacias hidrográficas, desde a Bacia do Alto Paraguai até a Bacia Amazônica (Magnusson e Campos 2010; Muniz et al 2018; Muniz et al 2021). Recentemente, Muniz et al. (2018) e Bittencourt et al. (2019) demonstraram que *P. palpebrosus* compreende pelo menos três linhagens intraespecíficas com fluxo gênico altamente restrito, o que leva a especulações de que pode representar um complexo de espécies crípticas escondidas sob o mesmo epíteto específico (Muniz et al., 2018). A descoberta dessas linhagens permite compreender melhor a diversidade do grupo, seus modos de dispersão, tempos de diversificação e outros processos em nível de espécie e linhagens (Diniz-Filho et al 2008).

Referências Bibliográficas

Adams, R.V.; Lazerte, S.E.; Otter, K.A.; Burg, T.M. (2016). Influence of landscape features on the microgeographic genetic structure of a resident songbird. *Heredity*, 117:63-72.

Beheregaray, L. (2008). Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology*, 17:3754-3774.

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 crocodylian: The Schneider's dwarf Caiman *Paleosuchus trigonatus* (Alligatoridae: Caimaninae). *PeerJ*, 7:e6580

Bloor P, Ibáñez C, Vilorio-Lagares TA (2015) Mitochondrial DNA analysis reveals hidden genetic diversity in captive populations of the threatened American crocodile (*Crocodylus acutus*) in Colombia. *Ecology and Evolution* 5:130–140

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. *Amphibia-Reptilia*, 31:439-442.

Campos, Z.; Magnusson, W. (2013). Thermal relations of dwarf caiman, *Paleosuchus palpebrosus*, in a hillside stream: Evidence for an unusual thermal niche among crocodylians. *Journal of Thermal Biology*, 38:20-23.

de Oliveira FFR, Gehara M, Solé M et al (2021) Quaternary climatic fluctuations influence the demographic history of two species of sky-island endemic amphibians in the Neotropics. *Molecular Phylogenetics and Evolution* 160:107113

Diniz-Filho, J.A.F.; de Campos Telles, M.P.; Bonatto, S.L.; Eizirik, E.; Freitas, T.R.O.; Marco Jr, P.; Santos, F.R.; Sole-Cava, A.; Soares, T.N. (2008). Mapping the evolutionary twilight zone: molecular markers, populations and geography. *Journal of Biogeography*, 35(5):753-763.

Eaton MJ, Martin A, Thorbjarnarson J et al (2009) Species-level diversification of African dwarf crocodiles (Genus *Osteolaemus*): A geographic and phylogenetic perspective. *Molecular Phylogenetics and Evolution* 50:496–506

Eizirik E, Kim JH, Menotti-Raymond M et al (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). *Molecular Ecology* 10:65–79

Fenker J, Tedeschi LG, Melville J et al (2021) Predictors of phylogeographic structure among codistributed taxa across the complex Australian monsoonal tropics. *Molecular Ecology* 2021:1–16

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 the gene flow for the aquatic paradoxical frog *Pseudis tocantins* (Anura, Hylidae). *Landscape Ecology*, <https://doi.org/10.1007/s10980-021-01257-z>.

Franke FA, Schmidt F, Borgwardt C (2013) Genetic differentiation of the African dwarf crocodile *Osteolaemus tetraspis* Cope, 1861 (Crocodylia: Crocodylidae) and consequences for European zoos. *Organisms Diversity and Evolution* 13:255–266

Godshalk R (2006) Phylogeography and conservation genetics of the yacare caiman (*Caiman yacare*) of South America. Thesis, University of Florida

Hekkala E, Shirley MH, Amato G et al (2011) An ancient icon reveals new mysteries: Mummy DNA resurrects a cryptic species within the Nile crocodile. *Molecular Ecology* 20:4199–4215

Hickerson, M. J.; Carstens, B.C.; Cavender-Bares, J.; Crandall, K.A.; Graham, C.H.; Johnson, J.B.; Rissler, L.; Victoriano, P.F.; Yoder, A.D. (2009). Phylogeography's past, present, and future? 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution*, 54(1):291-301.

Hosegood J, Humble E, Ogden R et al (2020) Phylogenomics and species delimitation for effective conservation of manta and devil rays. *Molecular Ecology* 29:4783–4796

Hrbek, T.; Vasconcelos, W.R.; Rebelo, G.; Farias, I.P. (2008). Phylogenetic relationships of South American alligatorids and the Caiman of Madeira River. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309A(10):588–599

Jaisuk, C.; Senanan, W. (2018). Effects of landscape features on population genetic variation of a tropical stream fish, Stone lapping minnow, *Garra cambodgiensis*, in the upper Nam river drainage basin, northern Thailand. *PeerJ*, 6:e4487.

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

Magalhães FM, Camurugi F, Lyra ML et al (2022) Ecological divergence and synchronous Pleistocene diversification in the widespread South American butter frog complex. *Molecular Phylogenetics and Evolution* 169:107398

Magnusson, W.; Campos, Z. (2010). Cuvier's smooth-fronted Caiman *Paleosuchus palpebrosus*. In: Manolis, S.C.; Stevenson, C. *Crocodiles. Status Survey and Conservation Action Plan*. 3a ed. Crocodile Specialist Group: Darwin. pp 10-42.

Manel. S.; Schwartz, M.K.; Luikart, G.; Taberlet, P. (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, 18(4):189–197.

Marioni B, Barão-Nóbrega JAL, Botero-Arias R et al (2020) Science and conservation of Amazonian crocodylians: a historical review. *Aquatic Conservation: Marine and Freshwater Ecosystems* 31:1056–1067

Miranda EO, Maciel NM, Lima-Ribeiro MS et al (2019) Diversification of the widespread neotropical frog *Physalaemus cuvieri* in response to Neogene-Quaternary geological events and climate dynamics. *Molecular Phylogenetics and Evolution* 132:67–80

Muniz, F.; Bittencourt, P.S.; Hernández-Rangel, S.M.; Roberto, I.J.; Farias, I.P.; Hrbek, T. (2021). Biogeography and Comparative Phylogeography of New-World Crocodylians. In: Zucoloto, R.B.; Amavet, P.S.; Verdade, L.M.; Farias, I.P. (eds). *Conservation Genetics of New World Crocodylians*. Springer Nature Switzerland AG. pp 95-122.

Muniz, F. L.; Campos, Z.; Hernandez Rangel, S.M.; Martinez, J.G.; Souza, B.C.; De Thoisy, B.; Botero-Arias, R.; Hrbek, T.; Farias, I.P. (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(3):599–610.

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.

Pacheco-Sierra G, Domínguez-Laso J, Pérez-Alquicira J et al (2018) Ancestral hybridization yields evolutionary distinct hybrids lineages and species boundaries in Crocodiles, posing unique conservation conundrums. *Frontiers in Ecology and Evolution* 6:1–15

Rull V (2008) Speciation timing and Neotropical biodiversity: The Tertiary-Quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology* 17:2722–2729

Rull V (2020) Neotropical Diversification: Historical overview and conceptual insights. In: Rull V, Carnaval AC (ed) *Neotropical diversification: Patterns and processes*, 1st edn. Springer Nature, Switzerland, pp 13–49

Santos, R.C.; Viana, M.N.S.; Monjeló, L.A.S.; Andrade, P.C.M.; Pantoja-Lima, J.; Oliveira, P.H.G.; Vogt, R.C.; Pezzuti, J.C.B.; Sites Jr, J.W.; Hrbek, T.; Farias, I.P. (2016). Testing the Effects of Barriers on the Genetic Connectivity in *Podocnemis erythrocephala* (Red-Headed Amazon River Turtle): Implications for Management and Conservation. *Chelonian Conservation and Biology*, 15(1):12-22.

Silva, S.M.; Peterson, A.T.; Carneiro, L.; Burlamaqui, T.C.T.; Ribas, C.C.; SousaNeves, T. et al (2019). A dynamic continental moisture gradient drove Amazonian bird diversification. *Science Advances*, 5(7):eaat5752.

Soley-Guardia M, Carnaval AC, Anderson RP (2019) Sufficient versus optimal climatic stability during the Late Quaternary: using environmental quality to guide phylogeographic inferences in a Neotropical montane system. *Journal of Mammalogy* 100:1783–1807

Werneck FP, Gamble T, Colli GR et al (2012) Deep diversification and long-term persistence in the South American “Dry Diagonal”: Integrating continent-wide phylogeography and distribution modeling of geckos. *Evolution* 66:3014–303

The genetic diversity in a South American Dwarf Caiman (*Paleosuchus palpebrosus*)
is structured by landscape features

Abstract

The distribution of species and the way that lineages are structured is the result of intrinsic historical processes of the species and their relationships with landscape features. Studies with different aquatic and semi-aquatic organisms show how their distribution and genetic structure are shaped by landscape features and climatic suitability. *Paleosuchus palpebrosus* is one of the smallest crocodylians in the world and has a wide distribution with different habitat conditions. Here, we investigate how elements of landscape heterogeneity impact genetic differentiation and evaluate the evolutionary and phylogeographic history of the species. We hypothesized that elements of landscape structures, such as aridity and slope as resistance surfaces and river as conductance surface could mediate the dispersion of the species. The three lineages recovered brought unique information about the genetic structure of the species for other areas where we reported genetic data for the first time. Part of the genetic differentiation resulted from isolation by resistance. Furthermore, our historical demography analysis found a recent history and no changes on effective lineages sizes through time, differently from what happened with the vast majority of the Neotropical fauna during the Pliocene and Pleistocene. 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.

Keywords: Alligator, Isolation by resistance, River as facilitator, Topography

Introduction

The historical process, landscape features, and demographic history of the organisms shaped their distribution and genetic structure (Gehara et al 2017; Vasconcellos et al 2019; Camurugi et al 2020). Phylogeographic studies with different aquatic and semi-aquatic organisms (Hrbek et al 2008; Santos et al 2016; Oliveira et al 2019; Pirani et al 2019) tested the role of geomorphological elements (i.e. rivers) structuring lineages and the evolutionary history of species. These landscape features can structure lineages acting as barriers (Silva et al 2019), but also as dispersion facilitators (Fonseca et al 2021). One case is the allopatric speciation of the clown tree

frog (*Dendropsophus ebraccatus*), caused by the final uplift of northern Andes, that separated the species from the others species of *D. leucophyllatus* group (Pirani et al 2020). Differently from what happened with the African spotted reed frog (*Hyperolius substriatus*), where the rivers act as facilitators of gene flow (Lawson 2013).

In the South America Neotropical region, the phylogeographic patterns and diversification processes are usually explained by different coupled events, such as geomorphological features and climatic fluctuations (Hoorn et al 2010; Turchetto-Zolet et al 2013). There are usually large-scale reconfigurations of the landscape that explain the observed phylogeographic structures nowadays (Smith et al 2014). The Andean mountain, for example, generated many changes, including the formation of the Amazon river system, which limits species distributions and isolates sister species (Ribas et al 2012). Even though for some regions these patterns provide demographic synchronism (Gehara et al 2017), in other regions the lineage sizes of species may vary in different ways (Phillipsen et al 2015), since each species has their own traits, biotic interactions and habits a heterogeneous environment that influences isolation and/or persistence of lineages (Harvey et al 2017; Myers et al 2019).

Spatial isolation, environmental heterogeneity, geographic barriers and the dispersal abilities of organisms are considered drivers of species divergence and speciation (Manel et al 2003). Lineages that are geographically restricted may experience isolation by distance (IBD) promoted by landscape features and geographical distances (Wright 1943). The Amazonas lineage of the fish *Arapaima gigas*, for example, is further structured by isolation by distance with the Amapá locality, possibly a result of demographic and evolutionary processes mediated by current landscape structure (Farias et al 2019). Heterogeneous environmental conditions can reduce gene flow on lineages experiencing contrasting ecological conditions and isolation by environment (IBE) (Thomassen et al 2010). When lineages are isolated, each can respond to local interactions with the environment, resulting in local variation in selective pressures (Funk et al 2006). *Boana lundii* experienced the role of climatic fluctuations structuring their populations, which is relevant since the species occurs in the heterogeneous Cerrado landscape, with different environmental requirements (Vasconcellos et al 2019). Also, genetic divergence can be influenced by physical restrictions to dispersal and connectivity among lineages, promoting isolation by resistance (IBR) (McRae 2006). For example, vicariance was the most likely process responsible for the divergence of the continent-wide frog *Dermatonotus*

muelleri lineages in a scenario where each lineage is on opposite sides of the Brazilian Plateau with no gene flow (Oliveira et al 2018).

Among the Neotropical aquatic and semi-aquatic fauna, crocodylians are long-lived, large-sized animals that inhabit aquatic environments, such as rivers, small streams, lakes, estuaries, swamps, and marshes (Amavet and 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, as the impact on the density, behavior and foraging of their prey (Layman et al 2015; Nifong and Sillman 2013), including hosts of diseases of high importance in human medicine and veterinary (Coutinho et al 2013; Rueda-Almonacid et al 2007). Crocodylians are an ancient group that had diversified morphologically and genetically little compared to other archosaurs, such as birds (Grigg and Kirshner 2015). Their diversification is congruent with the diversification of the herpetofauna in South America (Turchetto-Zolet et al 2013; Bittencourt et al 2019), during tectonic and palaeogeographical reorganizations (Neogene), or environmental shifts caused by the glacial-interglacial periods (Quaternary) (Rull 2011). Such geomorphological events promoted greater availability and diversity of habitats, niches and reconfiguration of riverine habitats (Hoorn et al 2010; Rull 2011; Waddell et al 2018). In addition, landscape features has potential to represent important elements in the pattern of lineage structuring of these organisms.

Paleosuchus palpebrosus is one of the smallest extant crocodylians (Campos et al 2010), has a wide geographic distribution in South America and occurs in different types of environment 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 and Mourão 2006). Recently, Muniz et al (2018), using mitochondrial data (Cytochrome b gene), demonstrated that *P. palpebrosus* comprises at least three intraspecific lineages with highly restricted gene flow. Since *Paleosuchus palpebrosus* has a close relationship with the environment (Rueda-Almonacid et al 2007) and the habitat modifications can alter lineage characteristics (Ross 1998). We hypothesized that elements of landscape structures, such as aridity and slope as resistance surfaces and river as conductance surface could mediate the dispersion of the species. If rivers facilitate the connection between lineages, it can promote gene flow (Fonseca et al 2021). In addition, our hypothesis of IBD posits that geographic distance influences genetic distance, since it happens when gene flow is reduced among lineages at

greater distances from each other (Wright 1943). For IBE, our hypothesis postulates that climate differences along the distribution of *P. palpebrosus* can shape patterns of genetic differentiation in the species. In this way, the present study has three main goals: (1) Evaluate the evolutionary history and phylogeography of *Paleosuchus palpebrosus* along its distribution; (2) verify possible lineage signatures and the time of divergence between the lineages; and (3) investigate how the isolation by distance (IBD), isolation by environment (IBE) and isolation by resistance (IBR) influences the lineages structure.

Materials and Methods

Sampling and DNA extraction, amplification, and sequencing

The tissue samples were obtained through scientific collections (Appendix 1). We complemented our dataset by downloading available sequences of *Paleosuchus palpebrosus* from GenBank (<http://www.ncbi.nlm.nih.gov/genbank>), totalling 227 specimens from 40 localities (Figure 1). We extracted DNA from 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) gene using the standard Polymerase Chain Reaction (PCR) technique (Mullis et al, 1986) and in reaction volume at 25 μ L, using primers described by Hrbek et al (2008). We performed PCR with the following conditions: 34 cycles of 45s denaturation at 94 $^{\circ}$ C, 45s annealing at 52 $^{\circ}$ C, and 60s extension at 72 $^{\circ}$ C. Center for Biological Resources and Genomic Biology (CREBIO) conducted sequencing. We visually checked all nucleotide sequences (619 pb) using the Muscle algorithm (Edgar 2004) in the Geneious v.7.1.3 software (<https://www.geneious.com>). We used the species *Paleosuchus trigonatus*, *Caiman yacare*, *Melanosuchus niger*, *Alligator mississippiensis* and *A. sinensis* as outgroups. We deposited the sequences generated in this study into GenBank (Appendix 1).

Phylogenetic Analysis

We estimated the best substitution model and partition schemes based on the Bayesian Information Criterion using PartitionFinder2.1.1 (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, partitioned gene tree using the best substitution models, a Yule tree model, and a strict clock as priors. To calibrate the tree based on the Cytb gene, we used a Crocodylidae-specific substitution rate of complete mtDNA genomes of 0.01 site/million years (Eo and DeWoody 2010). Next, we implemented three independent runs in BEAST v2.6.4 (Bouckaert et al 2014), each consisting of 5×10^7 generations and sampled at every 5×10^3 generations. We discarded each run's initial 10% generations as burn-in and combined the results with LogCombiner v2.6.4 (Suchard et al 2018). Finally, we assessed convergence of runs based on effective sample sizes ($ESS \geq 200$) using Tracer v1.7 (Rambaut et al 2018).

Lineages delimitation and genetic statistics

We implemented a Bayesian Analysis of Population Structure (BAPS) to assess the presence and nature of spatial genetic structure in *Paleosuchus palpebrosus* in BAPS v6.0 (Corander et al 2008), with a population mixture model and k ranging from 1 to 10. Then, we conducted population admixture analysis 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” analyses, through the geographic locations of each sample (Corander et al 2013). In addition, we used the Generalized Mixed Yule Coalescent (GMYC; Pons et al 2006) implemented in the R package splits (Fujisawa and Barraclough, 2013) to delimit species. 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). Recently, Carstens et al (2022) argued that assessment of model adequacy is fundamental for robust phylogeographic inferences. Thus, we assessed the fit of the GMYC model to our empirical dataset using the P2C2M.GMYC R package (Fonseca et al 2021). P2C2M.GMYC implements a parametric bootstrap 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* 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 used the program Arlequin 3.5 (Excoffier and Lischer 2010) to perform Tajima's D neutrality tests (Tajima 1989) and Fu's F_s (Fu 1997). Tajima's D can be used as a test statistic to assess whether the data are consistent with the lineage being at a neutral mutation-drift equilibrium. When negative and significant, it suggests an excess of low frequency polymorphisms relative to expectation, indicating lineage size expansion, when positive and significant, signifies 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 for a deficiency of alleles, as would be expected from a recent bottleneck (Holsinger 2006). Also, with Arlequin v.3.5.2 (Excoffier and 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 and GMYC delimitation for grouping. 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 and 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 *Paleosuchus palpebrosus* using a Bayesian approach implemented in BEAST v1.8.2. 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 dispersion of lineages continuously in space and time. We implemented a coalescent prior with

constant population size, an HKY+G model of substitution, 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 chains of 50 million generations, assessed stability of the analysis in Tracer v1.7 ensuring that all ESS values were >200 (Rambaut et al 2018). In order to calibrate the tree, we used a Crocodylidae-specific substitution rate of complete mtDNA genomes of 0.01 site/million years (Eo and DeWoody 2010). The resulting tree was summarized with TreeAnnotator v1.8.2, the first 10% of samples discarded as burn-in. We used SPREAD v1.0.4 to generate a kml file (Bielejec et al 2011) which was plotted in Google Earth (<http://earth.google.com>).

To evaluate the lineages size changes through time, we used the Bayesian Skyline Plot method (BSP) in BEAST v2.6.4 applied separately for each lineage identified by gene tree analysis. To calibrate the BSP, we used the same strategy implemented in the BEAST analysis (see section above), but using a Coalescent Bayesian Skyline Model and running MCMC simulations of 20,000,000 generations, sampling topologies and parameters every 2,000 generations, discarding the first 10% generations as burn-in. Parameter convergence between runs and performance analysis (considered $ESS \geq 200$) were conducted in Tracer v1.7 (Rambaut et al 2018).

Identifying determinants of genetic differentiation

To test if landscape features (aridity, slope and river), geographic distance and climate differences could mediate the dispersion, genetic distance and local adaptations, respectively, we tested the effects of these five predictors and its consequences on species genetic variation and differentiation through the relative contributions of Isolation by Distance (IBD), Isolation by Environment (IBE), and Isolation by Resistance (IBR).

Our hypothesis of IBD posits a positive 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 used only locations with more than two samples. For IBE, our hypothesis postulates that climate differences can shape patterns of genetic differentiation in *P. palpebrosus*. For each sampling point, we extract values for 19 standardized climatic variables, downloaded from the WorldClim database (<https://www.worldclim.org/data/bioclim.html>) at a spatial resolution of 2.5 arc-minutes

(Hijmans et al 2005). Then, we resumed those variables with a principal components analysis (PCA), and retained the first PC (representing 85%). Pairwise environmental distances were calculated with *dist* function in R v.4.1.2. For testing the IBR scenario, 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 river as conductance surface (IBR). Our river network raster contains the presence of main rivers from South America (stream lines at 1:10m scale; <https://www.natureearthdata.com/>). 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.

Pairwise matrix of genetic differentiation among localities (ϕ_{st}) were obtained with an analysis of molecular variance (AMOVA) in Arlequin 3.5.2 (Excoffier and Lischer 2010). The significance of pairwise comparisons was assessed by 10,000 permutations. We replaced negative ϕ_{st} values ($n = 11$) with 0 because negative values of ϕ_{st} 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) and a commonality analysis (CA) implemented in the *ecodist* and *yhat* packages in R. 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 et al 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). In the regression, the predictor variables were the matrices of geographic distance, pairwise aridity, pairwise slope, pairwise river and the first climatic PCA, and the response variable was a pairwise genetic distance matrix.

We created a distance matrix derived from the first principal component scores (Patterson et al 2006; Shirk et al 2010). In order to evaluate the contribution of

geographic distance alone, we also included a pairwise distance matrix based on the Euclidean distance between the coordinates for each location, generated using the *Ecodist* package in R (Goslee and Urban 2007). The model was run 10.000 times and the regression coefficient and significance of each predictor variable and their shared contributions were estimated.

Results

Sampling and DNA extraction, amplification, and sequencing

We obtained a final alignment of 619 bp of the mitochondrial Cytochrome b gene of 227 individuals of *Paleosuchus palpebrosus* from 40 localities, and we found no stop codons in the electropherograms.

Phylogenetic analysis and demographic history

The topology of the mitochondrial Cytochrome b gene tree showed three strongly supported lineages for *Paleosuchus palpebrosus* (pp=1.00) (Figure 2). The beginning of *Paleosuchus* diversification into *P. trigonatus* and *P. palpebrosus* dates approximately at 5.39 Mya (95% HPD; 3.98–6.95 Mya). The divergence among lineages of *P. palpebrosus* started with a split between the Brazilian Shield and the other amazonian lineages at 1.45 Mya (95% HPD; 0.99–1.94 Mya), followed by a split between South and North Amazon lineages at 1.36 Mya (95% HPD; 0.8–1.61 Mya). However, there was only moderate support between amazonian lineages (0.35 in the superior node).

Lineages delimitation and genetic statistics

The BAPS and GMYC analyses recovered three mtDNA lineages in *P. palpebrosus*: one from the north of the Amazon basin, one from the south of the Amazon basin, including the samples from the Tocantins-Araguaia River basin, and one 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 nucleotide diversity of 0.6921 (Figure 3). Tajima's D and Fu's F showed negative values, but not significant (Table 1). All comparisons between the lineages show ϕ_{st} values greater than 0.9. The uncorrected *p*-distance among lineages showed genetic

differences of approximately 1.74%, as follows the pairwise comparisons: 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.

Spatiotemporal Phylogeographic Reconstruction and Demographic History

The phylogeographic analysis suggested an Amazonian origin for *Paleosuchus palpebrosus* (Figure 4), with subsequent dispersal to Tocantins-Araguaia River basin, then to La Plata River basin, while it increases dispersion to other regions of the Amazon basin. Finally, the species disperses to the São Francisco River basin, and Parnaíba River and Eastern Atlantic Northeast basins. The Bayesian Skyline Plot analyses for all lineages showed that effective population sizes were relatively constant through time (Figure 5).

Identifying determinants of genetic differentiation

The MRDM model explained almost 40% ($R^2 = 0.3968$, R^2 adjusted = 0.3881) of the total observed genetic variation, considering all five variables. From all these explained variables, three were significant: slope ($p = 1.22 \times 10^{-5}$), aridity ($p = 0.0329$), and river ($p = 0.0438$). Only spatial distance ($p = 0.0771$) and PC1 ($p = 0.4479$) had no significant contribution for the observed genetic differentiation.

Discussion

Phylogenetic analysis and demographic history

We found that *Paleosuchus palpebrosus* is spatially structured in three lineages: North Amazon, South Amazon, and Brazilian Shield. The three lineages recovered brought unique information about the genetic structure of the species for other areas where we reported genetic data for the first time. 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 at approximately 4.28 Mya (95% HPD = 2.2–7.1 Mya), while the North Amazon and South Amazon diverged at 3.32 Mya (95% HPD = 1.5–5.6 Mya), using

fossils to calibrate the gene tree. Differently, we chose to use Crocodylidae-specific substitution rate of complete mtDNA genomes. Even so, the dates of the two studies and this one correspond to the Pliocene/Pleistocene epochs, when orogenic events (i.e. Andean uplifts) contributed to shaping the Neotropical diversity and distribution (Antonelli and Sanmartín 2011), and as we observed, *P. palpebrosus* showed isolation by resistance. Other groups in South America also diversified in this period, such as the frog *Boana albopunctata*, where the uplift of the Chapada dos Guimarães plateau represented a complex topography for structuring (1.7–2.6 Mya) (Prado et al 2012). Likewise, the origin of the East-West lineage of the snake *Bothrops neuwiedi* took place at this period as well (3.06–1.71 Mya), after the final uplift of the Central Brazilian Plateau (Machado et al 2014).

The phylogeographic relationship among lineages indicates that they diversified recently, possibly during the Pliocene. 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 today (Toivonen et al 2007). This is interesting especially because Amazonian lowlands is characterized by the abundant dynamic headwaters of the Amazon River and went through geological events in its history that changed its water courses (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.

Lineages delimitation and genetic statistics

The GMYC and BAPS analyses assigned three lineages for the species, which can be ratified by the high level of genetic differentiation among lineages shown by AMOVA (Table 2). A similar scenario is found for the toad *Pipa carvalhoi*, where hydrographic basins reflect the genetic structure of three lineages that can be related to past paleodrainages that connected basins (Lima et al 2020). Similarly, the lineages of the fishes *Schizodon dissimilis* and *Prochilodus lacustris* are structured by headwater captures between different basins and coastal dispersal due to Pleistocene sea level fluctuations (Abreu et al 2020).

The species has a pattern of low haplotype diversity along its wide distribution through the hydrographic basins (Amazon, La Plata River, São Francisco River, Tocantins-Araguaia River, Parnaíba River and Eastern Atlantic Northeast). There is

haplotype sharing between them (Figure 3), which may reflect a similar hydrological history (Kozak et al 2005), with the Amazon basin being its origin center (Figure 4). In general, the hydrographic basins delimit the different lineages found. Nevertheless, this pattern is not unique with a single lineage for each basin, because the individuals may be able to move between basins. A similar pattern is also found in the pink dolphin (*Inia geoffrensis*), where the gene flow is maintaining a relative homogeneity between two lineages with shared haplotype (Hollatz et al 2011). There is also a connection between Morelet's crocodile (*Crocodylus moreletii*) lineages, where the lineages of one river are acting as a "mainland", while the surrounding smaller lineages resemble "islands" (Dever et al 2002).

Research carried out with Amazonian organisms studied how the past weather conditions have promoted barriers and corridors for dispersal (Prates et al 2016; Silva et al 2019; Pirani et al 2020). In this study, the Amazon basin showed seven unique haplotypes, in addition to two shared with other basins. The geology, hydrology and geomorphology of these basins may explain the biogeographic relationships between the aquatic and semi-aquatic fauna of these regions (Hubert and Renno 2006). Studies carried out with aquatic organisms have demonstrated affinity between the Amazon and Parnaíba River basins (Pinna and Wosiacki 2003; Garda and Cannatella 2007; Albert and Reis 2011), and there are records of endemic species from the Parnaíba River basin and Eastern Atlantic Northeast basin with Amazonian species (Vari 1989), possibly because the Parnaíba river was, in the past, a tributary of the Amazon River (Agassiz and Agassiz 2000). Presumably, the evolutionary history of landscape features promote isolation between lineages that, even when isolated, share haplotypes, given that the diversification of the species is very recent.

Spatiotemporal Phylogeographic Reconstruction and Demographic History

Besides the low genetic diversity throughout its distribution, our historical demography for this species found a recent history and no changes on effective lineage sizes during all time. Probably, the generalist habit (Magnusson et al 1987; Campos et al 1995; Vasconcelos et al 2021) and demographic stability despite environmental changes in the Pleistocene (Werneck et al 2012; Rossetti et al 2017) explain why *P. palpebrosus* lineages are continuously distributed without many genetic divergences throughout its wide distribution. Widely distributed species can be impacted by gradients in climatic variables and different historical processes throughout their range

limits (Mott 2010), but even so, *P. palpebrosus* showed demographic stability. The species occupies different aquatic environments throughout its distribution (Magnusson 1985, Campos and Mourão 2006), including primary and secondary streams (*igarapés*), that were not greatly affected by historical processes, and this may have contributed to this scenario. Thus, based on the demographic stability coupled with low genetic diversity, we may infer that lineages have sufficient geographical stability to preserve the phylogenetic characteristics (Zhang et al 2020). Pliocene and Pleistocene climate change occurred abruptly and radically, but were probably not enough to prevent the maintenance of the species in the areas where it occurs, given that climatic variables do not influence the structuring of the species (IBE; $p = 0.4479$), and as a widely distributed and generalist species, its population size has remained stable. Possibly, *P. palpebrosus* has behavioral flexibility that allows it to cope with climate changes (Beever et al 2017).

Identifying determinants of genetic differentiation

We found that Isolation by Resistance (IBR) significantly explains the genetic divergence of *P. palpebrosus*, where slope and aridity acts as landscape resistance, and rivers act as conductance between lineages. This findings agrees with previous studies on fauna diversification in Neotropics, in which IBR explained part of the genetic differentiation. IBR explained more genetic differentiation in *Podocnemis erythrocephala* and *P. sextuberculata*, two amazonian riverine turtle species where Amazon river acts as a barrier and water type of rivers acts as a resistance factor for connectivity, respectively (Oliveira et al 2019). Resistance isolation also explains the genetic divergence between lineages of the poison frog *Epipedobates anthonyi*, where the mountain range limits dispersal (Páez-Vacas et al 2021).

The Madeira River is one of the most important tributaries of the Amazon basin and is an important area of contact between the two Amazonian lineages. This river is the largest water volume and sediment flow among 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 great floods (Farias et al 2010) may be a difficulty for *P. palpebrosus* to cross it, or the species may be adapted to the type of water in Madeira River, due to ecological differences, and does not migrate from to other rivers. In addition, it is a transitional zone between the Ombrophilous dense forest and Ombrophilous open forest (Ortiz et

al 2018), which can be important for isolation by distance, since rivers have an effect on the structuring of *P. palpebrosus*. Further south of the Madeira River, there is the Parecis plateau, which separates the Amazonian lineages from the Brazilian Shield lineage. Studies with other organisms have found mountains acting as barriers to gene flow. Sierra de Guadarrama acts as a strong barrier to gene flow for the frogs *Pelobates cultripes* (Sanchez-Montes et al 2017) and the uplift of the Andes potentially acted as an effective barrier to *Bothrops*, leading to vicariance speciation (Hamdan et al 2019). Here, the Parecis mountains may represent one of the landscape elements that reflects the influence of slope in the structuring of *P. palpebrosus* (Isolation by resistance).

Recently, has been proposed the Riverine Thruway Hypothesis (RTH) (Fonseca et al 2021), that postulates rivers as facilitators of gene flow, allowing lineage connectivity. This pattern is observed in *Paleosuchus palpebrosus*, in which the rivers may act to structure the observed lineages. Here, the river acts as a facilitator, a scenario already observed in previous studies with species also dependent on aquatic environments (Lawson 2013; Oliveira et al 2019). Possibly the species dispersed from the Amazon basin to the Tocantins-Araguaia River basin, where there is haplotype sharing, and then towards the La Plata River basin and other basins of the Brazilian Shield lineage. Despite being strongly associated with aquatic environments, *Paleosuchus palpebrosus* can also be dispersed by land routes, a behavior already registered in the sister species, *Paleosuchus trigonatus* (Magnusson and Lima 1991). 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 dispersion and colonization of new areas, now outside the Amazon basin, during this period (Vasconcelos et al 2006). In addition, as mentioned earlier, the low diversity found may reflect the recent separation of hydrographic basins.

Conclusion

We present that *P. palpebrosus* corresponds to a single and widespread species, composed of three structured lineages strongly supported that diverged at the Pleistocene and experienced a stable demographic history. We shed light on the main drivers of the evolutionary history of the species, showing that mainly isolation by resistance and isolation by distance shaped the genetic structure of this alligator.

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References

Abreu JMS, Waltz BT, Albert JS et al (2020) Genetic differentiation through dispersal and isolation in two freshwater fish species from coastal basins of Northeastern Brazil. *Neotropical Ichthyology* 18:e190114

Amavet PS, Zucoloto RB (2021) Perspectives and Final Considerations about the Molecular Ecology of New-World Crocodylians. In: Zucoloto RB, Amavet PS, Verdade LM et al (ed) *Conservation Genetics of New World Crocodylians*, 1st edn. Springer, New York, pp 215–222

Anantharaman R, Hall K, Shah V et al (2019) Circuitscape in Julia: High performance connectivity modelling to support conservation decisions. ArXiv Preprint. Available from <http://arxiv.org/abs/1906.03542> (accessed February 2022)

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

Beever EA, Hall LE, Varner J et al (2017) Behavioral flexibility as a mechanism for coping with climate change. *Frontiers in Ecology and the Environment* 15:299–308

Bielejec F, Rambaut A, Suchard MA et al (2011) SPREAD: Spatial Phylogenetic Reconstruction of Evolutionary Dynamics. *Bioinformatics* 27:2910–2912

Bittencourt PS, Campos Z, Muniz FDL et al (2019) Evidence of cryptic lineages within a small South American crocodylian: The Schneider's dwarf Caiman *Paleosuchus trigonatus* (Alligatoridae: Caimaninae). *PeerJ* 7:e6580

Bouckaert R, Heled J, Kuhnert D et al (2014) BEAST 2: A software platform for bayesian evolutionary analysis. *Plos Computational Biology* 10:e1003537

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. Amphibia-Reptilia 31:439–442

Camurugi F, Gehara M, Fonseca EM et al (2020) Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed Neotropical treefrog. Journal of Biogeography 2020:1–13

Carstens BC, Smith ML, Duckett DJ et al (2022) Assessing model adequacy leads to more robust phylogeographic inference. Trends in Ecology & Evolution. doi: 10.1016/j.tree.2021.12.007

Coutinho ME, Marioni B, Farias IP et al (2013) Avaliação do risco de extinção do jacaré-de-papo-amarelo *Caiman latirostris* (Daudin, 1802) no Brasil. Biodiversidade Brasileira 3:13–20

Diniz-Filho JAF, de Campos Telles MP, Bonatto SL et al (2008) Mapping the evolutionary twilight zone: molecular markers, populations and geography. Journal of Biogeography 35:753–763

Duminil J, Fineschi S, Hampe A et al (2007) Can population genetic structure be predicted from life-history traits? The American Naturalist 169:662–672

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797

Eo SH, DeWoody JA (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 277:3587–3592

Ersts PJ (2011) Geographic distance matrix generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567

Farias IP, Torrico JP, García-Dávila C et al (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

Farias IP, Willis S, Leão A et al (2019) The largest fish in the world's biggest river: Genetic connectivity and conservation of *Arapaima gigas* in the Amazon and Araguaia-Tocantins drainages. *PLOS ONE* 14:e0220882

Fonseca EM, Duckett DJ, Carstens BC (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 EM, Garda AA, Oliveira EF et al (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 YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–925

Fujisawa TT, Barraclough TG (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

Funk DJ, Nosil P, Etges WJ (2006) Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences* 103:3209–3213

Garda AA, Cannatella DC (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, Barth A, Oliveira EF et al (2017) Model-based analyses reveal insular population diversification and cryptic frog species in the *Ischnocnema parva* complex in the Atlantic forest of Brazil. *Molecular Phylogenetics and Evolution* 112:68–78

Gehara M, Summers K, Brown JL (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 SC, Urban DL (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*. Csiro Publishing, Clayton South

Hall KR, Anantharaman R, Landau VA et al (2021) Circuitscape in julia: Empowering dynamic approaches to connectivity assessment. *Land* 10:301

Hamdan B, Guedes TB, Carrasco PA et al (2019) A complex biogeographic history of diversification in Neotropical lancehead pitvipers (Serpentes, Viperidae). *Zoologica Scripta* 49:145–158

Harvey MG, Seeholzer GF, Smith BT (2017) Positive association between population genetic differentiation and speciation rates in New World birds. *PNAS* 114:6328–6333

Hijmans RJ, Cameron SE, Parra JL et al (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965–1978

Hollatz C, Vilaça ST, Redondo RAF et al (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 KE (2006) *Lecture Notes in Population Genetics*. University of Connecticut, Storrs

Hoorn C, Wesselingh FP, Steege H et al (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330:927–931

Hrbek T, Meliciano NV, Zuanon J et al (2008) Remarkable Geographic Structuring of Rheophilic Fishes of the Lower Araguaia River. *Frontiers in Genetics* 295:1–12

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

Lanfear R, Calcott B, Ho SYW et al (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29:1695–1701

Layman CA, Giery ST, Buhler S et al (2015) A primer on the history of food web ecology: fundamental contributions of fourteen researchers. *Food Webs* 4:14–24

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

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

Leite NK, Krusche AV, Ballester MVR et al (2011) Intra and interannual variability in the Madeira River water chemistry and sediment load. *Biogeochemistry* 105:37–51

Li D, Moran E, da Silva RFB (2020) Examining Water Area Changes Accompanying Dam Construction in the Madeira River in the Brazilian Amazon. *Water* 12:1921

Lichstein JW (2007) Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecology* 188:117–131

Lima LR, Brushci DP, Nascimento FAC et al (2020) Below the waterline: cryptic diversity of aquatic pipid frogs (*Pipa carvalhoi*) unveiled through an integrative taxonomy approach. *Systematics and Biodiversity* 18:771–783

Machado T, Silva VX, Silva MJJ (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

Magalhães FM, Lyra ML, Carvalho TR et al (2020) Taxonomic review of South American Butter Frogs: Phylogeny, geographic patterns, and species delimitation in the *Leptodactylus latrans* species group (Anura: Leptodactylidae). *Herpetological Monographs* 34:131–177

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

Magnusson WE, Da Silva EV, Lima AP (1987) Diets of amazonian crocodylians. *Journal of Herpetology* 21:85–95

Manel S, Schwartz MK, Luikart G et al (2003) Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology and Evolution* 18:189–197

McRae BH (2006) Isolation by distance. *Evolution* 60:1551–1561

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

Mullis K, Faloona F, Scharf S et al (1986) Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology* 25:263–273

Muniz F, Bittencourt PS, Hernández-Rangel SM et al (2021) Biogeography and Comparative Phylogeography of New-World Crocodylians. In: Zucoloto RB, Amavet PS, Verdade LM et al (ed). *Conservation Genetics of New World Crocodylians*. 1st edn. Springer Nature Switzerland AG, New York, pp 95–122

Muniz FL, Campos Z, Hernandez Rangel SM et al (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

Myers EA, Xue AT, Gehara M et al (2019) Environmental heterogeneity and not vicariant biogeographic barriers generate community-wide population structure in desert-adapted snakes. *Molecular Ecology* 28:4535–4548

Oliveira EF, Gehara M, São Pedro VA et al (2018) Phylogeography of Muller's termite frog suggests the vicariant role of the Central Brazilian Plateau. *Journal of Biogeography* 45:2508–2519

Oliveira EF, Martinez PA, São-Pedro VA et al (2018) Climatic suitability, isolation by distance and river resistance explain genetic variation in a Brazilian whiptail lizard. *Heredity* 120:251–265

Oliveira JA, Farias IP, Costa GC et al (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

Páez-Vacas M, Trumbo DR, Funk WC (2021) Contrasting environmental drivers of genetic and phenotypic divergence in an Andean poison frog (*Epipedobates anthonyi*). *Heredity* 128:33–44

Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. *PLoS Genet* 2:e2074–e2093

Phillipsen IC, Kirk EH, Bogan MT et al (2015) Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. *Molecular Ecology* 24:54–69

Pirani RM, Peloso PLV, Prado JR et al (2020) Diversification history of clown tree frogs in Neotropical rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group). *Molecular Phylogenetics and Evolution* 150:106877

Pirani RM, Werneck FP, Thomaz AT et al (2019) Testing main Amazonian rivers as barriers across time and space within widespread taxa. *Journal of Biogeography* 46:2444–2456

Prado CPA, Haddad CFB, Zamudio KR (2012) Cryptic lineages and Pleistocene population expansion in a Brazilian Cerrado frog. *Molecular Ecology* 21:921–941

Prunier JG, Colyn M, Legendre X et al (2015) Multicollinearity in spatial genetics: Separating the wheat from the chaff using commonality analyses. *Molecular Ecology* 24:263–283

Rambaut A, Drummond AJ, Xie D et al (2018). Posterior summarization in bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67:901–904

Ross JP (1998). Crocodiles: Status survey and conservation action plan. IUCN/SSC Crocodile Specialist Group, Gland

Rossetti DF, Cohen MCL, Pessenda LCR (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: Posada D (ed) *Bioinformatics for DNA Sequence Analysis*, 1st edn. Springer Protocols, New York, pp 337–350

Rueda-Almonacid JV, Carr JL, Mittermeier RA et al (2007) Las tortugas y los cocodrilianos de los países andinos del Trópico. *Conservación Internacional Série de Guías Tropicales de Campo*. Bogotá, Colombia

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

Rull V (2020) Neotropical Diversification: Historical overview and conceptual insights. In: Rull V, Carnaval AC (ed) *Neotropical diversification: Patterns and processes*, 1st edn. Springer Nature, Switzerland, pp 13–49

Sambrook J, Fritsch EF, Maniatis R (1989) *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, New York

Sanchez-Montes G, Wang J, Ari AH et al (2017). Mountains as barriers to gene flow in amphibians: Quantifying the differential effect of a major mountain ridge on the genetic structure of four sympatric species with different life history traits. *Journal of Biogeography* 45:318–331

Santos RC, Viana MNS, Monjeló LAS et al (2016) Testing the Effects of Barriers on the Genetic Connectivity in *Podocnemis erythrocephala* (Red-Headed Amazon River Turtle): Implications for Management and Conservation. *Chelonian Conservation and Biology* 15:12–22

Shirk AJ, Wallin DO, Cushman SA et al (2010) Inferring landscape effects on gene flow: A new model selection framework. *Molecular Ecology* 19:3603–3619

Shirley MH, Carr AN, Nestler JH et al (2018) Systematic revision of the living African slender-snouted crocodiles (*Mecistops* Gray, 1844). *Zootaxa* 4504:151–193

Shirley MH, Vliet KA, Carr AN et al (2014) Rigorous approaches to species delimitation have significant implications for African crocodylian systematics and conservation. *Proceedings of the Royal Society B* 281:20132483

Silva SM, Peterson AT, Carneiro L et al (2019) A dynamic continental moisture gradient drove Amazonian bird diversification. *Science Advances* 5:eaat5752

Smith BT, McCormack JE, Cuervo AM et al (2014) The drivers of tropical speciation. *Nature* 515:406–409

Suchard MA, Lemey P, Baele G et al (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4:vey016

Swenson NG (2014) *Functional and Phylogenetic Ecology in R*. Springer, New York

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

Thomassen HA, Cheviron ZA, Freedman AH et al (2010) Spatial modeling and landscape-level approaches for visualizing intra-specific variation. *Molecular Ecology* 19:3532–3548

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

Turchetto-Zolet AC, Pinheiro F, Salgueiro F et al (2012) Phylogeographical patterns shed light on evolutionary processes in South America. *Molecular Ecology* 22:1193–1213

Vasconcellos MM, Colli GR, Weber JN et al (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 BD, Abreu JMNS, Brandão RA (2020) História natural do jacaré-paguá (*Paleosuchus palpebrosus*), o pequeno notável das veredas dos sertões da Trijunção entre Bahia, Goiás e Minas Gerais. In: Brandão, R, Françoso RD, Machado TH et al (ed) *História Natural do Sertão da Trijunção do Nordeste, Centro-Oeste e Sudeste do Brasil*, 1st edn. PerSe, São Paulo, pp 149–164

Waddell EH, Crotti M, Lougheed SC et al (2018) Hierarchies of evolutionary radiation in the world's most species rich vertebrate group, the Neotropical *Pristimantis* leaf litter frogs. *Systematics and Biodiversity* 16:807–819

Werneck FP, Gamble T, Colli GR et al (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

Wright S (1943) Isolation by distance. *Genetics* 28:114–138.

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

Table 1. General genetic parameters of *Paleosuchus palpebrosus* clusters, where N = number of samples; D = Tajima's D with simulated p-value (alpha = 0.05, 10.000 simulations); Fs = Fu's fs with simulated p-value (alpha < 0.02, 10.000 simulations).

Groups	N	D (p)	Fs (p)
North Amazon	47	-1.65128 (0.02670)	-0.92808 (0.22620)
South Amazon	112	-1.01895 (0.10690)	-1.92505 (0.09260)
Brazilian Shield	68	-1.33542 (0.07310)	-2.57542 (0.06540)

Table 2. Genetic distance observed between *Paleosuchus palpebrosus* lineages.

Pairwise ϕ_{st}	North Amazon	South Amazon	Brazilian Shield
North Amazon	-		
South Amazon	0.97713	-	
Brazilian Shield	0.95218	0.97408	-

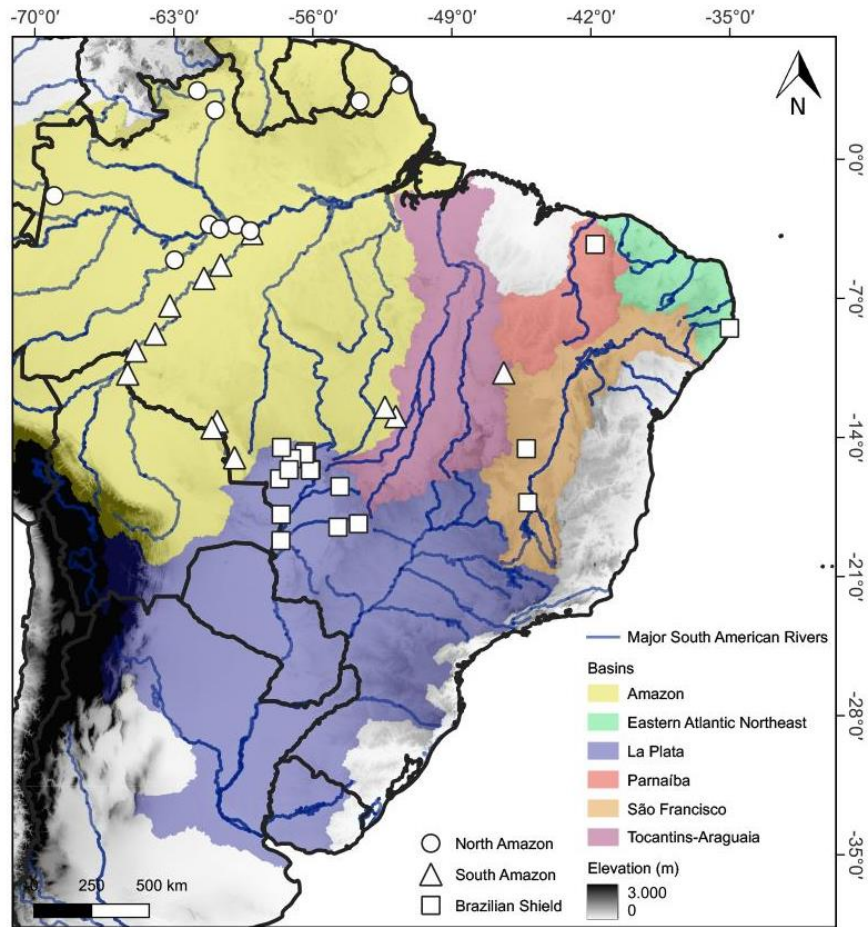


Figure 1. Distribution of sampled localities for *Paleosuchus palpebrosus* based on the Cytb mitochondrial dataset, including samples sent by scientific collections and available on GenBank. Colors represent the basins sampled in the study. The polygons represent each of the three recovered lineages.

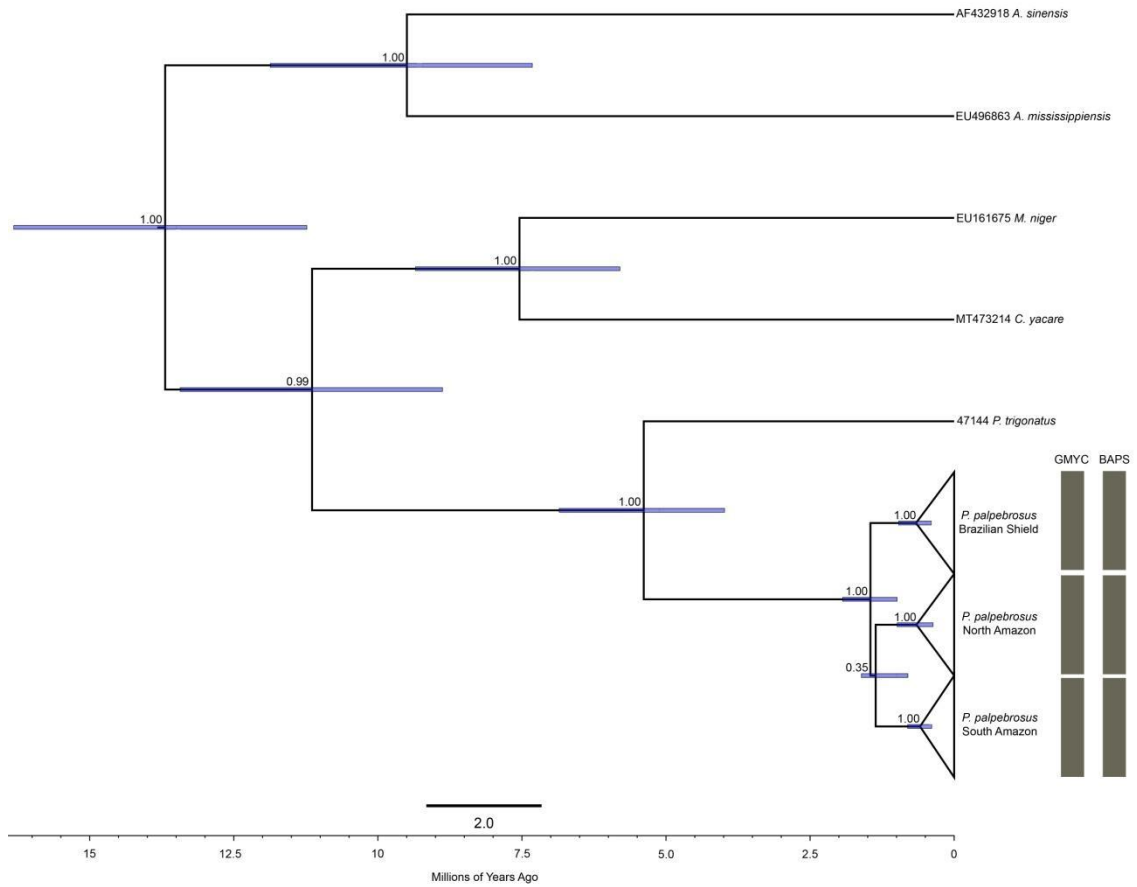


Figure 2. Tree topology generated using BEAST based on the Cytb mitochondrial dataset composed of 227 *Paleosuchus palpebrosus* sequences and 5 sequences of the species used as outgroup (*Alligator sinensis*, *Alligator mississippiensis*, *Melanosuchus niger*, *Caiman yacare* and *Paleosuchus trigonatus*) (619 bp). BAPS and GMYC analysis indicate the three recovered lineages.

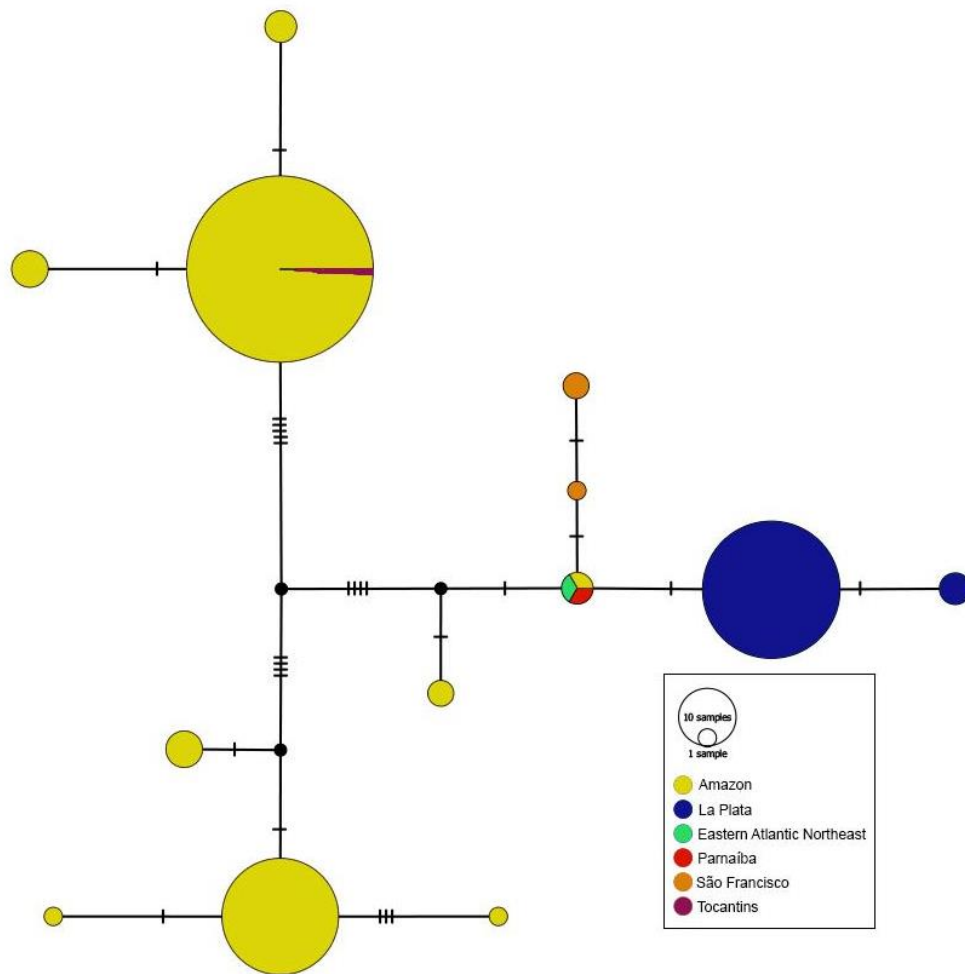


Figure 3. Haplotype network showing the low genetic diversity among *Paleosuchus palpebrosus* individuals throughout their distribution based on the Cytb mitochondrial dataset. Each colored circle represents a haplotype. Black circles represent unsampled haplotypes and the dashes represent mutational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

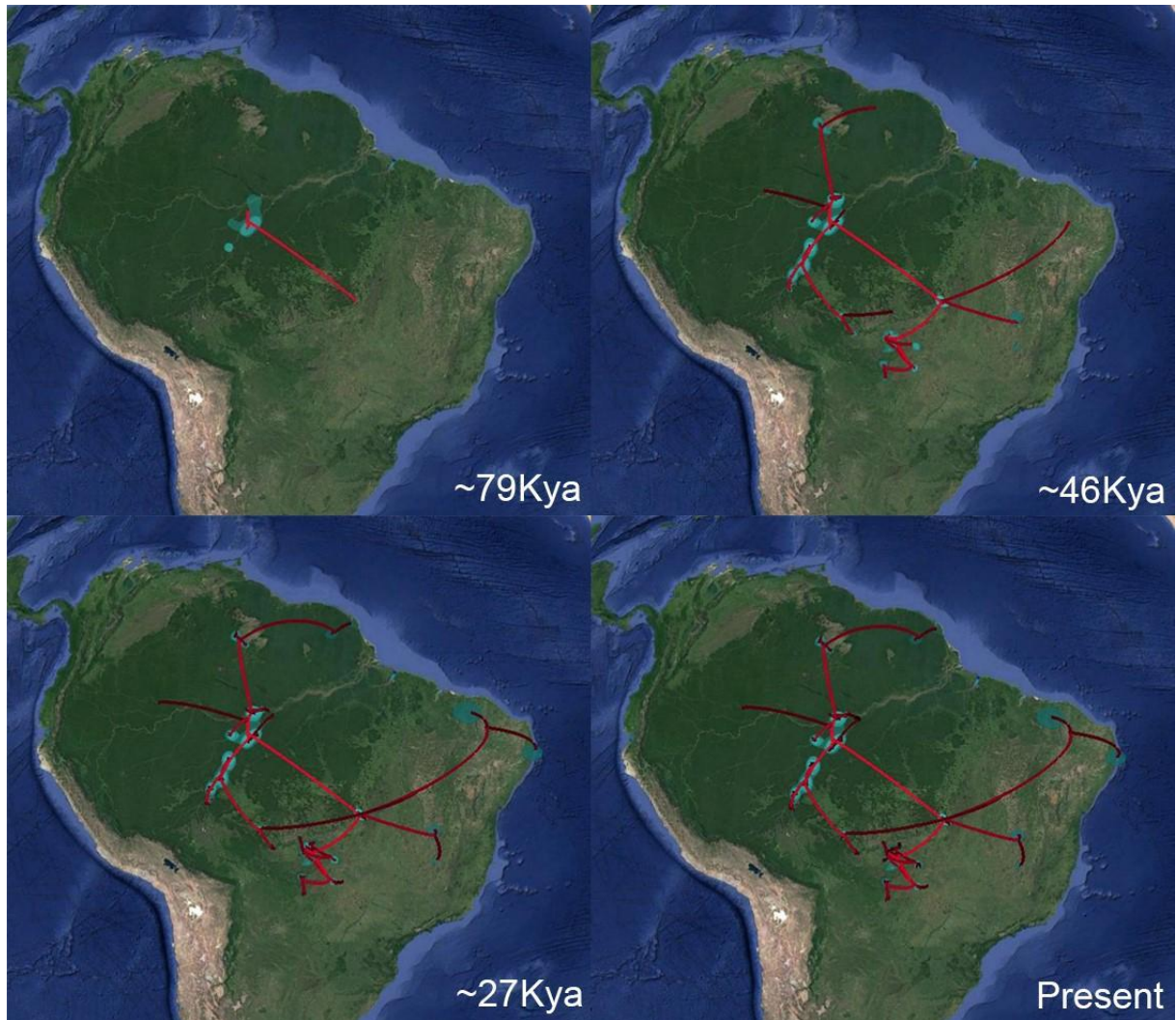


Figure 4. Phylogeographic reconstruction of *Paleosuchus palpebrosus*. Phylogeographical analysis of the species based on the Cytb mitochondrial dataset using a Relaxed Random Walk model for continuous trait reconstruction in Beast software. In the upper left corner, center of origin of *P. palpebrosus*. 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).

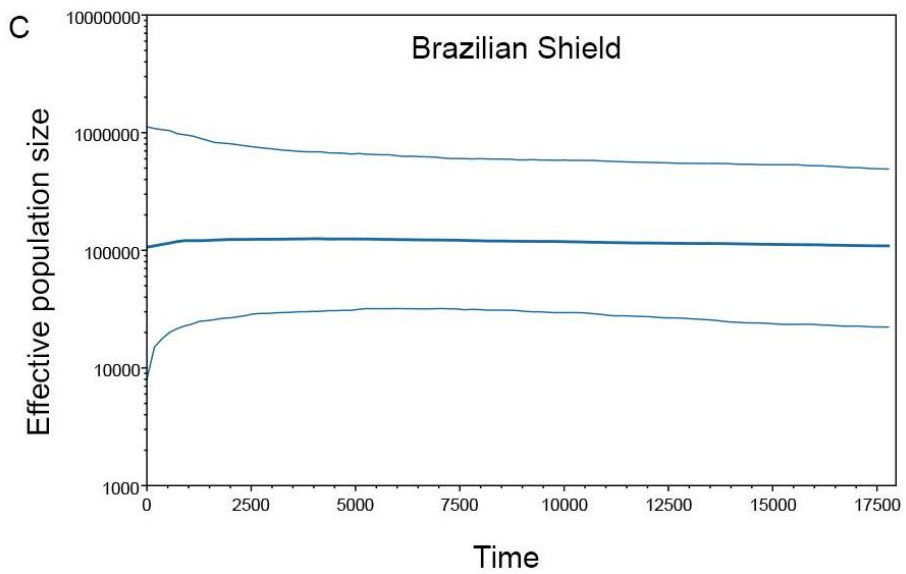
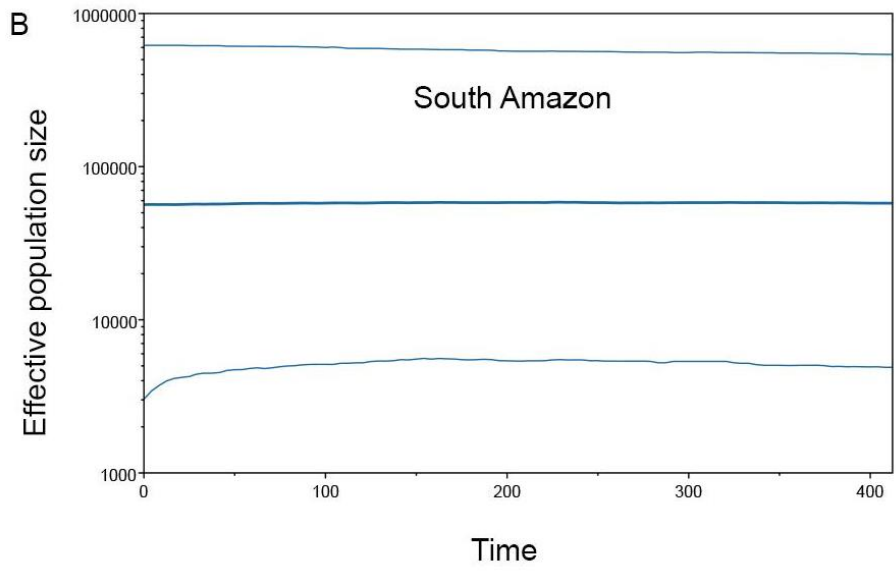
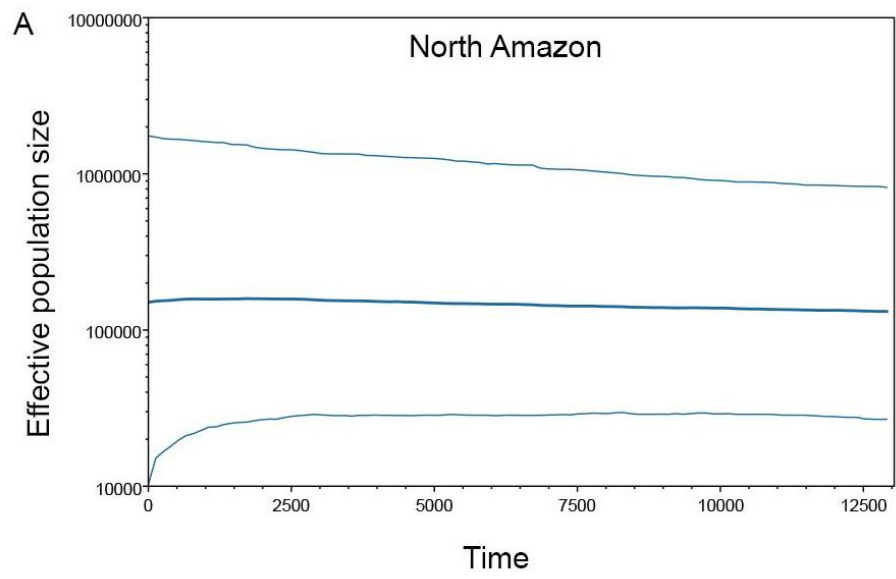


Figure 5. Bayesian skyline plots for each mtDNA (Cytb) lineage of *Paleosuchus palpebrosus*, where A is North-Amazon lineage, B is South-Amazon lineage, and C is Brazilian Shield lineage. The x-axis represents years from present to past, while y-axis represents the effective lineage size (N_e). The blue line in the middle indicates the median of N_e , while the blue lines at the ends indicate a 95% higher posterior probability.

Appendix 1. Voucher information and GenBank numbers. Specimens used for the molecular analyses, including GenBank numbers for mitochondrial Cytb sequences. * data not available in the original references.

Species	Voucher	Locality	Genbank Accession number	Reference
<i>Paleosuchus palpebrosus</i>	CHUNB44538	Buritizeiro, Minas Gerais	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB42271	Mateiros, Tocantins	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB50779	Cerejeiras, Rondônia	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB50780	Cerejeiras, Rondônia	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB53280	Cocos, Bahia	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB53282	Cocos, Bahia	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB61181	Piripiri, Piauí	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB68957	Ribeirão Cascalheira, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB68958	Ribeirão Cascalheira, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	CHUNB71118	Querência, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	900032001008857	Recife, Pernambuco	-	This study
<i>Paleosuchus palpebrosus</i>	JM87	Rosário Oeste, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM12	Cuiabá, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM36	Cuiabá, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM35	Várzea Grande, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM54	Nobres, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM56	Nobres, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM57	Cáceres, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM59	Cáceres, Mato Grosso	-	This study
<i>Paleosuchus palpebrosus</i>	JM81	Rosário Oeste, Mato Grosso	-	This study

<i>Paleosuchus palpebrosus</i>	MAP 6214	Alcinópolis, Mato Grosso do Sul	-	This study
<i>Paleosuchus palpebrosus</i>	CTGAH4406_Jpr	Rio Japurá, Amazonas	MH846341	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1676_Amp	Rio Uaçá, Oiapoque, Amapá	MH846342	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4398_GuF	Rio Tanpok, Kaw, Guiana Francesa	MH846343	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH0887_Mrc	Ilha de Maracá, Roraima	MH846344	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH0890_Mrc	Ilha de Maracá, Roraima	MH846345	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH0885_Mrc	Ilha de Maracá, Roraima	MH846346	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH0884_Mrc	Ilha de Maracá, Roraima	MH846347	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH0886_Mrc	Ilha de Maracá, Roraima	MH846348	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH0902_Mrc	Ilha de Maracá, Roraima	MH846349	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4005_Mcj	Mucajaí, Roraima	MH846350	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4007_Mcj	Mucajaí, Roraima	MH846351	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1714_01Mcp	Manacapuru, Amazonas	MH846352	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1715_02Mcp	Manacapuru, Amazonas	MH846353	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1716_03Mcp	Manacapuru, Amazonas	MH846354	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1717_04Mcp	Manacapuru, Amazonas	MH846355	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1718_05Mcp	Manacapuru, Amazonas	MH846356	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1719_06Mcp	Manacapuru, Amazonas	MH846357	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1723_07Mcp	Manacapuru, Amazonas	MH846358	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1724_08Mcp	Manacapuru, Amazonas	MH846359	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1725_09Mcp	Manacapuru, Amazonas	MH846360	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH1780_11Mcp	Manacapuru, Amazonas	MH846361	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1785_12Mcp	Manacapuru, Amazonas	MH846362	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1792_14Mcp	Manacapuru, Amazonas	MH846363	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1793_19Mcp	Manacapuru, Amazonas	MH846364	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4444_Autz	Autazes, Amazonas	MH846365	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1760_MAO	Manaus, Amazonas	MH846366	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1762_MAO	Manaus, Amazonas	MH846367	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1763_MAO	Manaus, Amazonas	MH846368	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1761_MAO	Manaus, Amazonas	MH846369	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1765_MAO	Manaus, Amazonas	MH846370	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1769_MAO	Manaus, Amazonas	MH846371	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1771_MAO	Manaus, Amazonas	MH846372	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2940_CAA	Capiranga, Amazonas	MH846373	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2942_CAA	Capiranga, Amazonas	MH846374	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2943_CAA	Capiranga, Amazonas	MH846375	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2941_CAA	Capiranga, Amazonas	MH846376	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2948_CAA	Capiranga, Amazonas	MH846377	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2947_CAA	Capiranga, Amazonas	MH846378	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2944_CAA	Capiranga, Amazonas	MH846379	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2950_CAA	Capiranga, Amazonas	MH846380	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4411_PUR	Rio Purus, Amazonas	MH846381	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4435_PUR	Rio Purus, Amazonas	MH846382	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH4439_PUR	Rio Purus, Amazonas	MH846383	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4410_PUR	Rio Purus, Amazonas	MH846384	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4413_PUR	Rio Purus, Amazonas	MH846385	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4415_PUR	Rio Purus, Amazonas	MH846386	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH4426_PUR	Rio Purus, Amazonas	MH846387	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2089_01NON	Nova Olinda do Norte, Amazonas	MH846388	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2090_02NON	Nova Olinda do Norte, Amazonas	MH846389	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2091_03NON	Nova Olinda do Norte, Amazonas	MH846390	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2140_04NON	Nova Olinda do Norte, Amazonas	MH846391	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2141_05NON	Nova Olinda do Norte, Amazonas	MH846392	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2142_06NON	Nova Olinda do Norte, Amazonas	MH846393	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2143_07NON	Nova Olinda do Norte, Amazonas	MH846394	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2144_08NON	Nova Olinda do Norte, Amazonas	MH846395	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2145_09NON	Nova Olinda do Norte, Amazonas	MH846396	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2147_11NON	Nova Olinda do Norte, Amazonas	MH846397	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2148_12NON	Nova Olinda do Norte, Amazonas	MH846398	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2149_13NON	Nova Olinda do Norte, Amazonas	MH846399	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH2254_01NAP	Novo Aripuanã, Amazonas	MH846400	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2256_03NAP	Novo Aripuanã, Amazonas	MH846401	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2257_04NAP	Novo Aripuanã, Amazonas	MH846402	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2258_05NAP	Novo Aripuanã, Amazonas	MH846403	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2260_06NAP	Novo Aripuanã, Amazonas	MH846404	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2261_07NAP	Novo Aripuanã, Amazonas	MH846405	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2262_08NAP	Novo Aripuanã, Amazonas	MH846406	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2263_09NAP	Novo Aripuanã, Amazonas	MH846407	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2264_10NAP	Novo Aripuanã, Amazonas	MH846408	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2265_11NAP	Novo Aripuanã, Amazonas	MH846409	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2126_01Mnc	Manicoré, Amazonas	MH846410	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2127_02Mnc	Manicoré, Amazonas	MH846411	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2128_03Mnc	Manicoré, Amazonas	MH846412	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2129_04Mnc	Manicoré, Amazonas	MH846413	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2130_05Mnc	Manicoré, Amazonas	MH846414	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2131_06Mnc	Manicoré, Amazonas	MH846415	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2132_07Mnc	Manicoré, Amazonas	MH846416	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2133_08Mnc	Manicoré, Amazonas	MH846417	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2134_09Mnc	Manicoré, Amazonas	MH846418	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2135_10Mnc	Manicoré, Amazonas	MH846419	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2136_11Mnc	Manicoré, Amazonas	MH846420	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2139_12Mnc	Manicoré, Amazonas	MH846421	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH2099_01HuA	Humaitá, Amazonas	MH846422	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2100_02HuA	Humaitá, Amazonas	MH846423	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2101_03HuA	Humaitá, Amazonas	MH846424	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2102_04HuA	Humaitá, Amazonas	MH846425	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2103_05HuA	Humaitá, Amazonas	MH846426	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2104_06HuA	Humaitá, Amazonas	MH846427	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2186_07HuA	Humaitá, Amazonas	MH846428	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2193_09HuA	Humaitá, Amazonas	MH846429	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2171_01HuB	Humaitá, Amazonas	MH846430	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2172_02HuB	Humaitá, Amazonas	MH846431	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2179_03HuB	Humaitá, Amazonas	MH846432	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2180_04HuB	Humaitá, Amazonas	MH846433	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2181_05HuB	Humaitá, Amazonas	MH846434	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2182_06HuB	Humaitá, Amazonas	MH846435	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2184_08HuB	Humaitá, Amazonas	MH846436	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2185_09HuB	Humaitá, Amazonas	MH846437	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2188_11HuB	Humaitá, Amazonas	MH846438	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2189_12HuB	Humaitá, Amazonas	MH846439	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2190_13HuB	Humaitá, Amazonas	MH846440	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2191_14HuB	Humaitá, Amazonas	MH846441	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2013_02Pvco	Porto Velho, Rondônia	MH846442	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2014_03Pvco	Porto Velho, Rondônia	MH846443	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH2017_04Pvco	Porto Velho, Rondônia	MH846444	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2019_05Pvco	Porto Velho, Rondônia	MH846445	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2020_06Pvco	Porto Velho, Rondônia	MH846446	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2023_08Pvco	Porto Velho, Rondônia	MH846447	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2071_09Pvco	Porto Velho, Rondônia	MH846448	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2072_10Pvco	Porto Velho, Rondônia	MH846449	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2073_11Pvco	Porto Velho, Rondônia	MH846450	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2074_12Pvco	Porto Velho, Rondônia	MH846451	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2075_13Pvco	Porto Velho, Rondônia	MH846452	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1682_01EC	Jaci Paraná, Rondônia	MH846453	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1691_03EC	Jaci Paraná, Rondônia	MH846454	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1813_04EC	Jaci Paraná, Rondônia	MH846455	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1864_05EC	Jaci Paraná, Rondônia	MH846456	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1865_06EC	Jaci Paraná, Rondônia	MH846457	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2016_08EC	Jaci Paraná, Rondônia	MH846458	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2062_09EC	Jaci Paraná, Rondônia	MH846459	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2063_10EC	Jaci Paraná, Rondônia	MH846460	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2064_11EC	Jaci Paraná, Rondônia	MH846461	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2065_12EC	Jaci Paraná, Rondônia	MH846462	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2067_13EC	Jaci Paraná, Rondônia	MH846463	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2069_15EC	Jaci Paraná, Rondônia	MH846464	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1854_01GM	Guajará-Mirim, Rondônia	MH846465	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH2105_02GM	Guajar-Mirim, Rondnia	MH846466	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2106_03GM	Guajar-Mirim, Rondnia	MH846467	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2107_04GM	Guajar-Mirim, Rondnia	MH846468	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2168_05GM	Guajar-Mirim, Rondnia	MH846469	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2169_06GM	Guajar-Mirim, Rondnia	MH846470	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2170_07GM	Guajar-Mirim, Rondnia	MH846471	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2407_01GuMa	Guajar-Mirim, Rondnia	MH846472	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2409_02GuMa	Guajar-Mirim, Rondnia	MH846473	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2410_03GuMa	Guajar-Mirim, Rondnia	MH846474	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2426_04GuMa	Guajar-Mirim, Rondnia	MH846475	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2429_05GuMa	Guajar-Mirim, Rondnia	MH846476	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2430_06GuMa	Guajar-Mirim, Rondnia	MH846477	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2431_07GuMa	Guajar-Mirim, Rondnia	MH846478	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1755_01VB	Vila Bella da Santssima Trindade, Mato Grosso	MH846479	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1756_02VB	Vila Bella da Santssima Trindade, Mato Grosso	MH846480	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1801_04VB	Vila Bella da Santssima Trindade, Mato Grosso	MH846481	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1803_05VB	Vila Bella da Santssima Trindade, Mato Grosso	MH846482	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1805_06VB	Vila Bella da Santssima Trindade, Mato Grosso	MH846483	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH1853_07VB	Vila Bella da Santíssima Trindade, Mato Grosso	MH846484	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1888_08VB	Vila Bella da Santíssima Trindade, Mato Grosso	MH846485	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2309_09VB	Vila Bella da Santíssima Trindade, Mato Grosso	MH846486	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2310_10VB	Vila Bella da Santíssima Trindade, Mato Grosso	MH846487	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1758_01PO	Pimenteiras do Oeste, Rondônia	MH846488	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1759_02PO	Pimenteiras do Oeste, Rondônia	MH846489	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1806_03PO	Pimenteiras do Oeste, Rondônia	MH846490	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1889_04PO	Pimenteiras do Oeste, Rondônia	MH846491	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1890_05PO	Pimenteiras do Oeste, Rondônia	MH846492	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1891_06PO	Pimenteiras do Oeste, Rondônia	MH846493	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1892_07PO	Pimenteiras do Oeste, Rondônia	MH846494	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1894_09PO	Pimenteiras do Oeste, Rondônia	MH846495	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1895_10PO	Pimenteiras do Oeste, Rondônia	MH846496	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2302_01BB	Barra do Bugres, Mato Grosso	MH846497	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2303_02BB	Barra do Bugres, Mato Grosso	MH846498	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2304_03BB	Barra do Bugres, Mato Grosso	MH846499	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2305_04BB	Barra do Bugres, Mato Grosso	MH846500	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2306_05BB	Barra do Bugres, Mato Grosso	MH846501	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH2307_06BB	Barra do Bugres, Mato Grosso	MH846502	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2108_01SU	Serra do Urucum, Corumbá, Mato Grosso do Sul	MH846503	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2110_03SU	Serra do Urucum, Corumbá, Mato Grosso do Sul	MH846504	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2111_04SU	Serra do Urucum, Corumbá, Mato Grosso do Sul	MH846505	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2112_05SU	Serra do Urucum, Corumbá, Mato Grosso do Sul	MH846506	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2783_07SU	Serra do Urucum, Corumbá, Mato Grosso do Sul	MH846507	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2784_08SU	Serra do Urucum, Corumbá, Mato Grosso do Sul	MH846508	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1741_01Ro	Rondonópolis, Mato Grosso	MH846509	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1883_02Ro	Rondonópolis, Mato Grosso	MH846510	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1884_03Ro	Rondonópolis, Mato Grosso	MH846511	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1885_04Ro	Rondonópolis, Mato Grosso	MH846512	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1886_05Ro	Rondonópolis, Mato Grosso	MH846513	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1887_06Ro	Rondonópolis, Mato Grosso	MH846514	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2298_07Ro	Rondonópolis, Mato Grosso	MH846515	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2299_08Ro	Rondonópolis, Mato Grosso	MH846516	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2301_10Ro	Rondonópolis, Mato Grosso	MH846517	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1742_01Co	Coxim, Mato Grosso do Sul	MH846518	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH1743_02Co	Coxim, Mato Grosso do Sul	MH846519	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1744_03Co	Coxim, Mato Grosso do Sul	MH846520	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1745_04Co	Coxim, Mato Grosso do Sul	MH846521	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1746_05Co	Coxim, Mato Grosso do Sul	MH846522	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1747_06Co	Coxim, Mato Grosso do Sul	MH846523	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1754_07Co	Coxim, Mato Grosso do Sul	MH846524	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1880_08Co	Coxim, Mato Grosso do Sul	MH846525	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1881_09Co	Coxim, Mato Grosso do Sul	MH846526	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2295_11Co	Coxim, Mato Grosso do Sul	MH846527	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2296_12Co	Coxim, Mato Grosso do Sul	MH846528	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1734_01SAM	Serra do Amolar, Corumbá, Mato Grosso do Sul	MH846529	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1736_03SAM	Serra do Amolar, Corumbá, Mato Grosso do Sul	MH846530	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1737_04SAM	Serra do Amolar, Corumbá, Mato Grosso do Sul	MH846531	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1739_05SAM	Serra do Amolar, Corumbá, Mato Grosso do Sul	MH846532	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1748_07SAM	Serra do Amolar, Corumbá, Mato Grosso do Sul	MH846533	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1727_02SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846534	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1730_05SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846535	Muniz et al (2018)

<i>Paleosuchus palpebrosus</i>	CTGAH1733_08SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846536	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1749_09SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846537	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2001_10SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846538	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2003_11SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846539	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2004_12SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846540	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2006_14SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846541	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2007_15SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846542	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2008_16SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846543	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH2009_17SAR	Serra das Araras, Coxim, Mato Grosso do Sul	MH846544	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1787_02TS	Tangara da Serra, Mato Grosso	MH846545	Muniz et al (2018)
<i>Paleosuchus palpebrosus</i>	CTGAH1790_05TS	Tangara da Serra, Mato Grosso	MH846546	Muniz et al (2018)
<i>Paleosuchus trigonatus</i>	CHUNB47144	Alta Floresta, Mato Grosso	-	This study
<i>Caiman yacare</i>	CTGAH2239	Humaitá, Amazonas	MT473214	Roberto et al (2020)

<i>Melanosuchus niger</i>	*	*	EU161675	Hrbek et al (2008)
<i>Alligator mississippiensis</i>	ALmi111USA	Estados Unidos da América	EU496863	Venegas-Anaya et al (2008)
<i>Alligator sinensis</i>	*	*	AF432918	Glenn et al (2002)
