



The Role of Vicariance and Paleoclimatic Shifts in the Diversification of *Uranoscodon superciliosus* (Squamata, Tropiduridae) of the Amazonian Floodplains

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Abstract

Wetlands are present in different parts of the globe, holding a significant portion of the local biodiversity, and have been under the constant influence of climatic changes worldwide on different time scales. Investigations with a phylogeographic approach have revealed the role of Quaternary climatic shifts on a global scale, favoring constant changes in the amplitude of floodplains and affecting the evolutionary history of multi-taxa. This pattern was observed in the Amazonian biota, but due to the complex geological history, some of these models alone are insufficient, especially in widespread species that live in specific regions of the biome. Herein, we investigated the species delimitation and biogeographic history of the semi-aquatic lizard *Uranoscodon superciliosus* (*U. superciliosus*), widely distributed in Amazonian floodplains, a habitat that was for a long time considered a corridor to gene flow. Our results support a high genetic diversity with five well-supported lineages within *U. superciliosus*: North of the Amazon River, East Xingu, Solimões, Purus, and Tapajós-Madeira, with the basal split within this group in the Miocene and others in the Plio-Pleistocene. These results corroborated a mixture of distinct processes that shaped the diversity of *U. superciliosus* with rivers as vicariant barriers and the already known role of paleoclimatic shifts during the Quaternary promoting diversification. Among tetrapods, our work is one of the first to describe the genetic structure in a widespread taxon along river-edge environments, thus supporting both patterns of diversification with the compartmentalized lineages along different Amazonian rivers (floodplains) and also the more common pattern found in upland forest species, with main rivers acting as vicariant barriers and playing a role in allopatric speciation.

Keywords Phylogeography · Species Delimitation · Genetic Diversity · Population dynamics · Miocene

Introduction

Wetlands in different countries and continents cover 6% of the global surface (Junk et al., 2013). These environments hold a significant portion of the local biodiversity and have been under the constant influence of climatic changes worldwide on different time scales, thus making most floodplains an ephemeral system, with regimes of wet and dry cycles affecting their range (Gopal et al., 2000; Junk et al., 2013). Being multidisciplinary, the use of a phylogeographic approach is indicated to disentangle the relation between the historical processes of landscape formation and patterns of diversification in time and space (Avice, 2009; Leal et al., 2016). Studies using this approach with endemic organisms from flooded environments are increasing, and enlightening on how the evolution of the landscape shaped this biodiversity (Barbosa et al., 2021; Dalapicolla et al., 2021; Luna et al., 2021; Márquez et al., 2006; Silva et al., 2019; Thom

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et al., 2018, 2020, 2022). These investigations have revealed the role of Quaternary climatic shifts worldwide favoring constant changes in the amplitude of floodplains and affecting the evolutionary history of multiple taxa, such as the cases of the rodent *Mastomys huberti* (Wroughton, 1909) from West Africa (Mouline et al., 2008), the marsh deer *Blastocerus dichotomus* (Illiger, 1815) and three species of waterbirds, all from the Brazilian Pantanal in South America (Lopes et al., 2007; Márquez et al., 2006).

Inserted in the Neotropics, Amazonia is the biome that harbors the highest number of species in the world and still has an underestimated intraspecific genetic diversity to be described (Antonelli et al., 2018; de Groot et al., 2012). Studies have investigated the complex evolution of the Amazonian landscape and proposed that several historical processes have generated and maintained this diversity (Bicudo et al., 2019; Hoorn et al., 2017; Pupim et al., 2019). Among them, paleoclimatic and geomorphological episodes that occurred during the Neogene and Quaternary, such as sea-level fluctuation and orogenic events (e.g. the uplift of the Andes) are considered key factors that shaped the current Amazon drainage system (Antonelli et al., 2018; Leite & Rogers, 2013; Turchetto-Zolet et al., 2013). Related to these historical processes two main hypotheses of diversification were proposed, one postulated that rivers acted as the main barriers to speciation (Riverine Barrier Hypotheses—RBH) while the other supports the forest dynamics (retraction and expansion) as a result of paleoclimatic fluctuations during the Quaternary (Forest Refuges Hypotheses—FRH; Wallace, 1854; Haffer, 1969). The role of the RBH has not been observed for specialized taxa from flooded regions, although being well known for species that occupy the upland non-flooded forests (*terra firme*) (Ribas et al., 2012; Dal Vechio et al., 2020; Pirani et al., 2019; Quaresma et al., 2022). Notwithstanding, case studies have proven that the use of more than one biogeographic hypothesis is necessary to explain diversification patterns for several cases, given the complexity of the Amazonian landscape with a mosaic of heterogeneous environments (Ribas et al., 2018).

Geomorphological features of the Amazon basin, with high precipitation levels and slope terrain, provided the scenario for the establishment of an outstanding system of seasonally flooded regions, that have a current extension of approximately 840.000 km² (Hess et al., 2015), comprising two habitats (Junk et al., 2011): (1) “várzea”, which occurs along whitewater rivers rich in suspended sediments deposited on riverbanks; and (2) “igapó”, associated with clear and blackwater rivers with low sediment loads (Salo et al., 1986; Wittmann et al., 2010). These areas hold a considerable number of specialized and endemic tetrapods, including amphibians, birds, mammals, and squamates (Ávila-Pires, 1995; Cohn-Haft et al., 2007; Matocq et al., 2000; Miralles et al., 2006). Both areas are very dynamic, under

the constant influence of pluvial seasonality, acting directly on the amplitude and extension of these wetlands (Junk et al., 2011).

First investigations with specialized taxa from flooded regions in Amazonia were focused on widely distributed species and showed no signals or low levels of genetic differentiation between populations (Aleixo, 2006; Cadena et al., 2011; Harvey et al., 2017; Matocq et al., 2000). Their results proposed that floodplains were homogeneous habitats with dynamic characteristics allowing species to colonize both river banks and maintain gene flow. However, new studies of comparative phylogeography found strong evidence of recent diversification and genetic structure, suggesting the influence of Quaternary paleoclimatic shifts affecting the evolutionary history of taxa (Luna et al., 2021; Silva et al., 2019; Thom et al., 2020), like in other wetlands in the World (Lopes et al., 2007; Márquez et al., 2006; Mouline et al., 2008). In this climate-driven scenario, changes in rainfalls, water level, and sedimentary budget led to subsequent episodes of contraction and expansion of the wetlands, consequently affecting the distribution of taxa by creating a mechanism of expansion followed by isolation (Irion, 1997; Sawakuchi et al., 2022).

Investigating and understanding the evolution of the biodiversity that inhabits different ecological regions enhances the comprehension of landscape formation, how these events might affect species historically, and reveals an underestimated diversity. Few studies have been focusing on species that inhabit seasonally flooded environments, which cover approximately 30% of the Amazon basin. Thus, new evidence from the Amazonian *várzea* and *igapó* might represent an important source of further findings in evolutionary patterns applicable to other wetlands and species discovery. Until now, phylogeographic studies addressing the Amazonian wetland biota were mainly focused on birds (Barbosa et al., 2021; Luna et al., 2021; Silva et al., 2019; Thom et al., 2018, 2020, 2022), which opens the question of whether other groups associated with these environments are being affected by the paleoclimatic dynamism already described or by other processes.

In the present study, we aim to infer the molecular diversity and processes involved in the diversification of the semi-aquatic lizard *Uranoscodon superciliosus* (Linnaeus, 1758), currently recognized as a single species that belongs to a monotypic genus in Tropicoduridae. This arboreal and diurnal species is strikingly associated with water and is commonly found on rivers, lakes and stream edges, and flooded areas, and is rarely found away from water (Ávila-Pires, 1995; Howland et al., 1990). *U. superciliosus* is widely distributed throughout the wetlands in Amazonia, occurring from the northeast of South America to the eastern Andes (Bolivia, eastern Colombia, Venezuela, French Guiana, Suriname, and Brazil) (Ribeiro-Júnior & Amaral, 2015). This species is an

interesting model to investigate how the complex evolutionary history of the Amazon drainage system influences the genetic structure of the biota associated with floodplains.

Herein, combining data from mitochondrial and nuclear loci of *U. superciliosus*, we intend to: (i) investigate possible genetic structure, based on a species delimitation approach and their phylogenetic relationship; (ii) propose a scenario attempting to understand the diversification and distribution of possible lineages within *U. superciliosus*, based on divergence times and biogeographic reconstructions; and (iii) evaluate the possible correspondence of diversification events with climatic and/or the geological history of Amazonia.

Material and Methods

Molecular Protocols, Alignment, and Data Partition

We analyzed 95 tissue samples of *U. superciliosus*, thus covering almost the entire known geographic distribution. The samples are deposited in the following Brazilian institutions: Coleção Herpetológica Osvaldo Rodrigues da Cunha, Museu Paraense Emílio Goeldi, Belém, Pará (MPEG); Instituto de Biociências da Universidade de São Paulo, São Paulo, São Paulo (IB-USP); and Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo (MZUSP). The complete list of samples, with details on collection number, locality and coordinates can be accessed in Online Resource 1.

We extracted genomic DNA from muscle, liver, or tail tips using the Wizard® Genomic DNA Purification Kit (Promega; Animal Tissue—Mouse Tail). The DNA extraction was quantified on a Qubit fluorometer (Thermo Fisher Scientific). Three mitochondrial (mtDNA: 12S—small subunit ribosomal (Benavides et al., 2007), 16S—large subunit ribosomal (Geurgas et al., 2008; Whiting et al., 2003), and ND4—NADH dehydrogenase subunit 4 (Arévalo et al., 1994) and three nuclear genes (nuDNA: KIF24—Kinesin Family Member 24 (Groth & Barraclough, 1999), PRLR—Prolactin Receptor Precursor, and RAG-1—Recombination Activating Gene 1 (both described by Portik et al., 2012) were amplified with the Promega MasterMix. For RAG-1 we applied the Qiagen HotStarTaq Master Mix Kit. We followed the manufacturer's recommended protocol for all procedures PCR amplification reactions followed a standard 4 min initial denaturation at 94 °C and a final extension of 6 min at 72 °C, adjusting the annealing temperature to optimize the reaction for five markers (see details of primer sequences in Online Resource 2). We purified PCR products using the Polyethylene Glycol PEG BioUltra 8000 protocol (Sambrook & Russell, 2006). Purified reactions were then sequenced using the specific primers and BigDye 3.1 cycle sequencing chemistry (Applied Biosystems) following the

manufacturer's recommended protocol and cycling conditions on an Applied Biosystems 3100 automated capillary sequencer, producing two strands (forward and reverse).

We visualized and edited the obtained sequences using Geneious 7.1.3 (Kearse et al., 2012), then each marker was aligned using the MAFFT online software (Katoch et al., 2019), choosing the following methods: G-INS-i for protein-coding genes and Q-INS-I for the ribosomal 12S and 16S, for considering the secondary structure of RNA. We phased sequences from nuclear markers to avoid the effect of heterozygotes using Phase, version 2.1 (Stephens et al., 2001), generating the input and output files with the assistance of the SeqPHASE online software (Flot, 2010). We concatenated all alignments datasets using the software Sequence Matrix, version 1.8 (Vaidya et al., 2011).

We estimated the best partition schemes and evolutionary models, considering ribosomal genes and codon positions, in the case of coding genes, as a minimum partition, under a greedy search and linking branch lengths with Partition-Finder, version 2.1.1 (Lanfear et al., 2017), limiting the models search for “mrbayes” (for Bayesian Inference) and “beast” (for Species Tree), under the Akaike Information Criterion corrected (AICc). These analyses were performed in the CIPRES Science Gateway, version 3.3 (Miller et al., 2012).

Phylogenetic Analysis

We reconstructed phylogenetic relationships in *U. superciliosus* lineages through a Bayesian Inference in MrBayes, version 3.2.6 (Ronquist & Huelsenbeck, 2003), with two independent runs, four parallel Markov chain Monte Carlo (MCMC), and 10 million generations, collecting a tree every 1000 generations and discarding a 10% burn-in, assuming ESS values ≥ 200 which were visualized with Tracer version 1.7.1 (Rambaut et al., 2018). This analysis was also implemented in the CIPRES Science Gateway, version 3.3 (Miller et al., 2012). We used *Microlophus quadrivittatus* (Tschudi, 1845) to root the tree, with *Plica plica* (Linnaeus, 1758) and *Tropidurus oreadicus* Rodrigues, 1987 as additional outgroups, based on the most recent phylogenetic hypothesis for Tropiduridae, proposed by Carvalho et al. (2016). The outgroup sequences were obtained from data available on the GenBank database (Benson et al., 2016).

Species Delimitation and Population Analysis

We tested the presence of distinct lineages in *U. superciliosus* with a Bayesian Statistics Group Analysis performed in the software BAPS version 6.0 (Corander et al., 2007), with two distinct concatenated datasets, one with exclusively mitochondrial loci and one with only the phased nuclear markers, to evaluate the information from both heritages

distinctively. For both datasets, we first ran the mixture analysis with the maximal number of groups ($K = 10$) and tested ten times for each, we then ran the admixture analysis with the previously described data, implementing 1000 interactions.

We implemented a multi-locus coalescent delimitation analysis (A10) in the software BPP version 4.3.8 (Flouri et al., 2018) to test the support of the clusters obtained previously from the less conservative result of BAPS (in this case with mtDNA) and also recovered as monophyletic clades in the phylogenetic tree. We followed the four scenarios with distinct inverse gamma prior combinations for Θ and τ described by Ramos et al. (2019), as follows: (1) large ancestral population size and deep divergence [(3, 0.04), (3, 0.02)]; (2) small ancestral population size and shallow divergence [$\Theta \sim \text{IG}(3, 0.004)$, $\tau \sim \text{IG}(3, 0.002)$]; (3) small ancestral population size and deep divergence [$\Theta \sim \text{IG}(3, 0.004)$, $\tau \sim \text{IG}(3, 0.02)$]; and (4) large ancestral population size and shallow divergence [$\Theta \sim \text{IG}(3, 0.04)$, $\tau \sim \text{IG}(3, 0.002)$]. All scenarios were run twice with both algorithms in BPP (1 and 0, Yang & Rannala, 2010) and 10 million MCMC generations, sampling every 100 generations with 10,000 generations as burn-in.

For all population analyses, we defined the lineages recovered in the delimitation results. We constructed a Haplotype Network for each marker under an infinite site model (i.e., uncorrected or Hamming distance) using the package *pegas* version 1.1 in the R software (Paradis, 2010). We inferred Statistical Summaries in DNAsp version 6 (Rozas et al., 2017) to evaluate for all loci: the number of haplotypes, nucleotide and haplotype diversity, the number of polymorphic sites, the standard deviation of nucleotide diversity, and the standard deviation haplotype diversity. We performed an Analysis of Molecular Variance (AMOVA) per loci based on 20,000 random permutations and an F_{st} Pairwise Distance matrix based on the mitochondrial concatenate dataset with 10,000 bootstrap replicates, to evaluate the levels of genetic structure and differentiation among and within lineages in the software Arlequin, version 3.5.2.2 (Excoffier & Lischer, 2010).

Species Tree, Divergence Time Estimation, and Ancestral Range Reconstruction

We estimated the divergence time of the five lineages resulting from the phylogenetic inference and results from BAPS and BPP through a coalescent species tree using the StarBEAST template of BEAST version 1.8.4 (Drummond et al., 2012). Because of the lack of fossil records for the group, we calibrated the tree using a rate widely used for mtDNA in squamates dating analysis, originally described by Macey et al., (1998) and corrected by Bryson et al., (2012), setting for the mitochondrial DNA a normal prior distribution with a mean of

0.00805 substitutions/my and standard deviation of 0.001 for the *ucl.d.mean* parameter. For nuDNA, we selected the default gamma prior (*ucl.d.mean*) and exponential prior with a mean of 0.5 (*ucl.d.stdev*) following the parameters proposed by Werneck et al., (2012). We selected the Yule Process and relaxed model (uncorrelated normal log) to accommodate the possibility of independent rates of molecular evolution in the different branches (Drummond et al., 2006). We implemented two independent runs of 200 million generations, sampling one tree every 20,000 generations. We used Tracer, version 1.7.1 (Rambaut et al., 2018) for stationary visualization, to verify the convergence of independent runs and to assure the ESS values (≥ 200). We combined runs and trees after a 10% burn-in using LogCombiner version 1.8.4 and obtained a maximum credibility clade tree (MCC) using Tree Annotator version 1.8.4 (Drummond et al., 2012).

We performed an ancestral area reconstruction under a maximum likelihood approach, using the BioGeoBEARS version 1.1.2 R package (BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts) (Matzke, 2013; Matzke & Sidje, 2013; R Core Team, 2016) combined to the ultrametric species tree obtained in BEAST to reconstruct the possible center of origin, biogeographic scenarios and possible occupation routes of the five lineages within *U. superciliosus*. We performed six independent analyses comparing distinct alternative models under the Akaike Information Criterion corrected (AICc): (1) Bayesian Inference of Biogeography for Discrete Areas (BayArea; Landis et al., 2013); (2) Dispersal-Vicariance Analysis (DIVA; Ronquist, 1997); and (3) Dispersal-Extinction Cladogenesis (DEC; Ree & Smith, 2008) and implementing the founder event speciation with the “+J” for all three models. We defined five biogeographic areas based on the current distribution range of each of the five lineages, limiting to both banks of the Tapajós and Madeira Rivers, the course of the Solimões River, the course of the Purus River, the North of the Amazon River, and east of Xingu River (including Marajó Island). We also implemented an area adjacency matrix where the possibility of the non-adjacent regions of eastern Xingu, Purus, and Solimões representing a single ancestral area was set to zero. Recently, Ree and Sanmartín (2018) reported a critical review on the DEC+J model implemented in BioGeoBEARS. However, Klaus and Matzke (2020) replied to this critique by showing that comparisons between DEC and DEC+J models had no statistical issues. Because of that, we implemented all six models in our analysis.

Results

Phylogenetic Inference, Genetic Structure, and Molecular Diversity

The concatenated molecular dataset included 95 terminals resulting in 3,643 aligned base pairs (mtDNA:

12S = 414 bp, 16S = 555 bp, and ND4 = 711 bp; nDNA: KIF24 = 549 bp, PRLR = 538 bp and RAG-1 = 876 bp). Details on partition schemes and best substitution models are presented in Online Resource 3. The Bayesian phylogenetic analysis recovered *U. superciliosus* as a monophyletic group with five major and geographically congruent clades (Fig. 1): (1) Northern Amazon River (NAR); (2) both banks of the Solimões River (SO); (3) Eastern Xingu River, including the Marajó Island (EX); (4) course

of Purus River (PU); and (5) both banks of Tapajós and Madeira rivers (TM). The phylogenetic tree recovered the clade NAR (EX + SO) as the sister of PU + TM. We obtained four well supported clades (with posterior probability ≥ 0.99), and PU + TM was weakly supported (PP = 0.54).

The BAPS analysis using the dataset with the concatenated mitochondrial data corroborated our phylogenetic inference, thus recovering the same five groups (Fig. 1). In

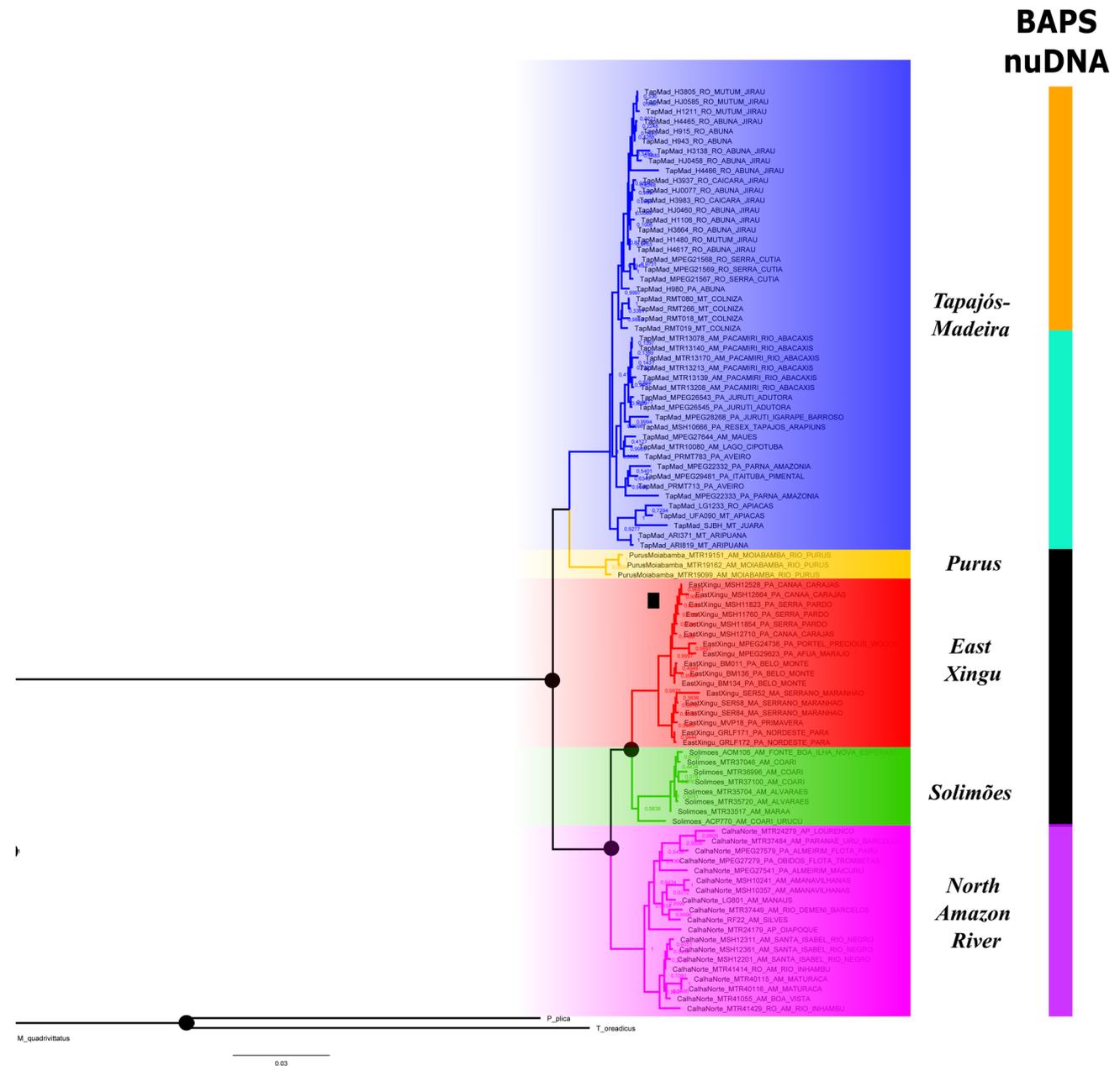


Fig. 1 Bayesian Inference showing phylogenetic relationships of the five lineages recovered within *U. superciliosus*, based on four loci (mtDNA and nuDNA); colored bars represent the population structure delimited by the mitochondrial and nuclear BAPS analysis and BPP. Numbers Black dots represent nodes with the posterior prob-

abilities of nodes with high support ($pp \geq 0.95$) and black square represents node with $pp = 0.54$ from the main clades. For more details on the geographic distribution and admixture charts of the nuclear BAPS see Online Resource 4 (Color figure online)

TM) (Fig. 3). Four terminals were supported by posterior probability ≥ 0.88 , except Purus River (PU; PP = 0.57). The low support of Purus (PU) might reflect in the species-tree topology changing the placement and relation for some lineages. The diversification within *U. superciliosus* began during the late Miocene, with TM diverging from the others at approximately 6.6 Ma (95% HPD: 11.2–3.4 Mya), followed by the divergence of NAR at approximately 4.5 Ma (95% HPD: 7.3–2.5 Mya), PU at approximately 3.3 Ma (95% HPD: 5.7–1.6 Mya) and the sister group EX and SO at approximately 2.0 Ma (95% HPD: 3.6–0.8 Mya).

Of the six tested models in the BioGeoBEARS, the Dispersal-Vicariance Analysis (DIVA) had the best value of log-likelihood and best fit to our data (AICc_{tw} = 0.91) (Table 2). This analysis recovered the ancestral area of all lineages of *U. superciliosus* as the northern Amazon River (NAR) and courses of the Madeira and Tapajós Rives (TM) (Fig. 3). The first vicariance event split this ancestral area in TM and NAR in the late Miocene (6.7 Mya). The ancestral area of the remaining lineages of *U. superciliosus* was inferred as NAR, indicating the occurrence of a sympatric speciation event, splitting the Northern Amazon River clade from all others (PU, SO, and EX) in the Early Pliocene (4.5 Mya). The ancestral lineages from PU, SO, and EX geo-dispersed to Purus banks with a vicariant event occurring in the Late Pliocene (3.3 Mya), resulting in one clade restricted to both Purus banks and the others located on the northern Amazon River. This later ancestral clade geo-dispersed to Solimões banks and a vicariant event occurred in the Early Pleistocene (2.0 Mya), splitting the lineage from Solimões banks from the northern Amazon River clade which range-switched to eastern Xingu River.

Discussion

The high genetic diversity in widely spread Amazonian species is a direct response to the complex and dynamic evolutionary history of the landscape, where patterns of diversification in multiple taxa seem to be concordant with historical processes in distinct temporal scales (Bicudo et al., 2019). Studies have shown the link between the biogeographic history of Amazonian organisms with the establishment of the drainage system in the Neogene (Ribas et al., 2012; Dal Vechio et al., 2020), the paleoclimatic fluctuations during the Quaternary (Luna et al., 2021; Thom et al., 2020) and the role of both processes in the Plio-Pleistocene (Quaresma et al., 2022).

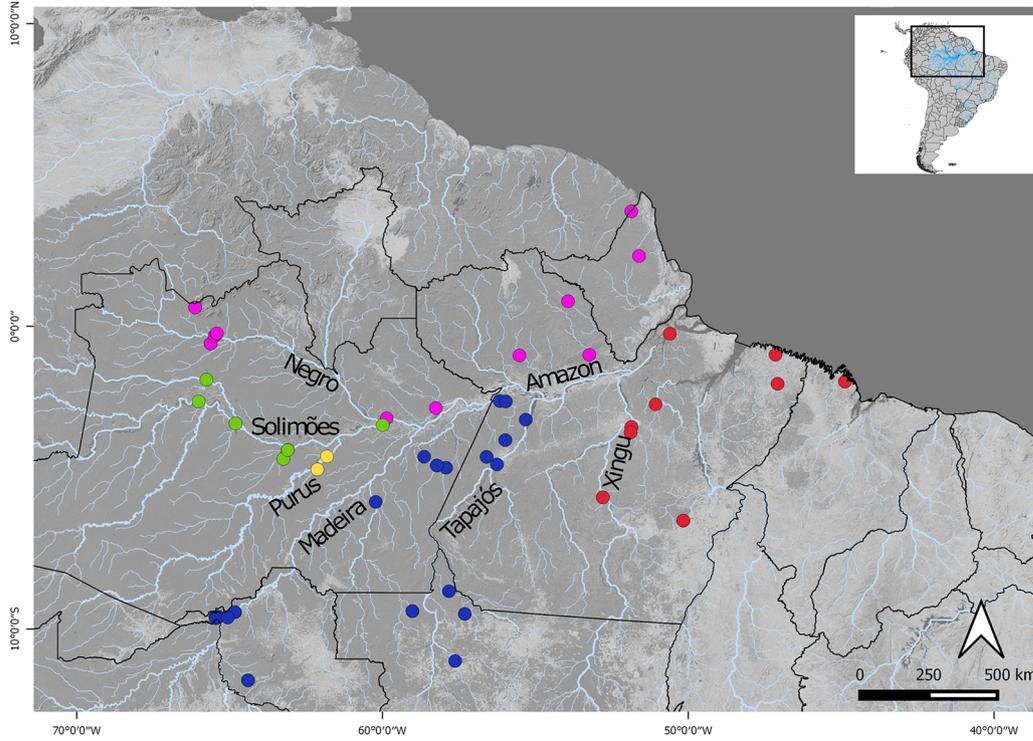
Our work is among the first to investigate the evolutionary processes associated with the patterns of genetic structure in a lizard that occupies river-edge environments, a habitat that was for a long time considered a corridor to gene flow (Aleixo, 2006). We added a different perspective to the

diversification of this biota, recovering an older divergence time in the Miocene. Our results are also the first to report the role of rivers as vicariant barriers to populations from seasonally flooded environments. This study corroborates the already known role of paleoclimatic shifts during the Quaternary in the dynamism of floodplains, isolating populations following speciation, thus reinforcing that a single biogeographic event cannot fully explain the origin of the high and complex biodiversity within Amazonia (Ribas et al., 2018; Turchetto-Zolet et al., 2013). The results of the phylogenetic, population (with mtDNA), and species delimitation analyses recovered high diversity in *Uranoscodon superciolosus*, with five geographically structured clades which corroborated others studies that show the diversity in Amazon region is still underestimated (Dal Vechio et al., 2020; Thom et al., 2020; Luna et al., 2021). Many factors could influence the different resolution in the BAPS analysis using nuclear genes (four clusters), such as the slow evolutionary rate and ancestral information present in the nuclear loci (Moore, 1995), different effective population sizes, incomplete lineage sorting, hybridization, and demographic asymmetries (Prugnolle et al., 2002; Toews & Brelsford, 2012). This disagreement in the number of clusters between different loci have been previously observed in other taxa (Peres et al., 2017; Turchetto-Zolet et al., 2013), but still shows the underestimation of the biodiversity in Amazonia.

Several molecular studies have revealed underestimated diversity in lizards with a widespread distributed within the Amazonian *terra firme* forest (Domingos et al., 2017; Gamble et al., 2012; Geurgas & Rodrigues, 2010; Ribeiro et al., 2020; Sturaro et al., 2018). Moreover, the role of the Amazonian drainage system in limiting the geographic distribution (*Anolis chrysolepis*; D'Angiolella et al., 2011) and acting as an effective barrier to gene flow (*Gonatodes humeralis*; Pirani et al., 2019) for the *terra firme* lizards is well known. Our results recovered population structure indicating that the Amazon and Xingu Rivers limit lineages along opposite margins, a previously undocumented pattern for river-edge taxa, that brings a new perspective to how the drainage system influences this biota.

Rivers acting as physical barriers to gene flow in a semi-aquatic species were tested in a recent study with *Salamandra salamandra* (Figueiredo-Vázquez et al., 2021). The authors found a strong effect of rivers impeding gene flow, for both semi-aquatic and terrestrial reproductive modes. In addition, dispersal capability was directly linked with the levels of genetic interchange (Avise et al., 1986; Zamudio et al., 2016), where highly dispersive species have continuous gene flow, impeding speciation. On the other hand, species with low mobility tend to show phylogeographic structure, as in the case of the leaf-litter lizard *Loxopholis oswaldoi* (Marques-Souza et al., 2020). The lineages of *U. superciliosus* along both banks of the rivers Purus and

A)



B)

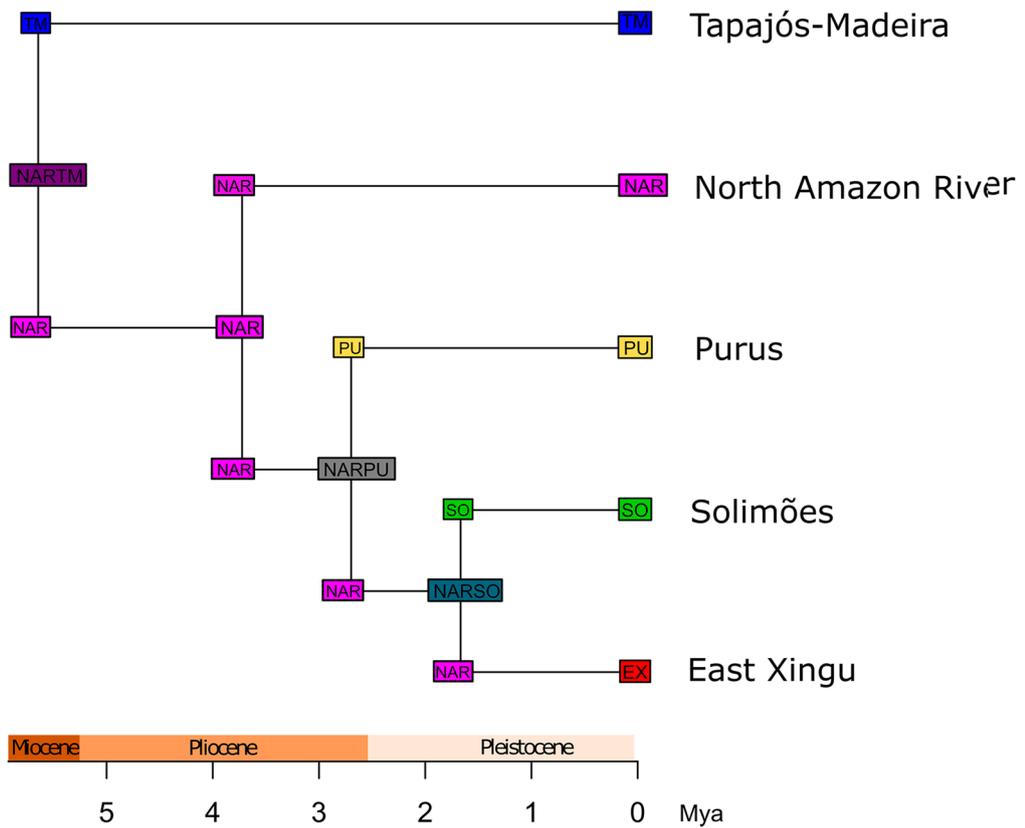


Fig. 3 **A** Geographic distribution of all samples analyzed in the present study. **B** Topology inferred in a coalescent species tree for all five lineages within *U. superciliosus* and biogeographic reconstruction based on the best fit DIVALIKE model. *TM* Tapajós-Madeira; *NAR* North Amazon River; *PU* Purus; *SO* Solimões, and *EX* East Xingu

Solimões suggest a dispersive capacity and also a sectoral diversification, with no clear physical barrier. Similar to our results, Thom et al. (2020) found genetic compartmentalization in floodplain birds; although populations did not differ on opposite margins, they were structured in different sectors along with the courses of Amazonian rivers, including the Solimões.

Besides the intrinsic characteristics of each organism, the ecological factor of seasonally flooded regions seems to be influencing the genetic structure and limiting the geographic distribution of several organisms that inhabit these areas. This pattern is explained by the Isolation by Environment hypothesis (IBE; Wang & Bradburd, 2014), where changes in physical characteristics in transitional areas between várzea (whitewater) and igapó (clear/black water) promote local adaptation followed by speciation events, as observed in some species of fishes and birds (Beheregaray et al., 2015; Laranjeiras et al., 2020). Although working with a floodplain organism, our results do not show signals of ecological speciation, with vicariant distributions concentrated mostly along whitewater rivers, as observed by Thom et al. (2020).

Placing in time the establishment of the modern Amazonian drainage system is the key factor to link diversification patterns with evolutionary processes. Scenarios that post-date this establishment reveal rivers as secondary barriers, or the role of different factors, such as changes in the landscape caused by paleoclimatic shifts, precipitation, or physical features (Baker et al., 2020; Cowman & Bellwood, 2013; Pirani et al., 2019). Interpretations based on geological and paleontological evidence point to the onset of the transcontinental Amazon–Solimões in the late Miocene to early Pliocene (Hoorn et al., 2017; Latrubesse et al., 2010). Also, a dynamic history of continuously changing environments combining geology, climate, and tectonics shaped the biotic diversification (Bicudo et al., 2019).

Our historical biogeography analysis suggests that the initial cladogenetic event took place in the late Miocene (6.7 Mya), separating populations north–south of the Amazon River. This period coincides with the establishment of the transcontinental Solimões–Amazonas, thus suggesting a vicariant event related to the drainage evolution. North–south lineages divided by the Amazon River were previously observed in the recent case of the pit viper *Bothrops jararacussu* species group (Dal Vechio et al., 2020), with time intervals similar to *U. superciliosus*, and the classic phylogeographic study with the widespread trumpeters

Psophia during the Pleistocene, this having a more recent date (Ribas et al., 2012).

The latest studies with river-edge birds suggest recent diversifications, with populations diverging during the Mid – Late Pleistocene (Thom et al., 2020) and Holocene (Choueri et al., 2017), a period marked by a very dynamic water system (Pupim et al., 2019). Diversification within *U. superciliosus* was older than that obtained for floodplain taxa until now, but interestingly coincident with upland taxa in Amazonia (Pavan & Marroig, 2017; Ribas et al., 2012; Rull, 2011). These results corroborate the complex and heterogeneous history of the Amazonia landscape, where different taxa may have distinct diversification histories in the flooded area (Bicudo et al., 2019).

Our biogeographic reconstruction placed the ancestral area of all *U. superciliosus* lineages in the current Guiana Shield and modern Tapajós and Madeira Rivers. This area was also observed for the Clown Tree Frog *Dendropsophus leucophyllatus* species group (Pirani et al., 2020) and the avian fauna from different landscapes, both *terra firme* (Silva et al., 2019) and floodplains (Thom et al., 2020). This suggests a possible stability in humid conditions in western Amazonia, holding a stable flooded environment for a long period, thus favoring the occurrence of taxa associated with watercourses and being a possible source of species richness (Bicudo et al., 2019).

Besides the episode of vicariance previously described, dispersal and range-switching events were also suggested in the biogeographic analysis (DIVA). Although little information is available on the dispersal capability of *U. superciliosus*, we suggest that the combination of individuals observed on floating logs with the affinity to water that includes morphological adaptations to swim and dive might act as a facilitator to the diffusion of the organism through its distribution (Ávila-Pires, 1995; Howland et al., 1990).

The evolution of the Amazonian drainage system was marked by episodes of climate shifts in orbital and millennial time scales, according to paleoenvironmental studies (Baker et al., 2020; Häggi et al., 2019). Same studies highlight these episodes occurring in the Quaternary and late Neogene, influencing the landscape, and resulting in cycles of expansion and contraction that favored alternated connection and isolation of populations. As an example of this dynamism, factors such as high levels of precipitation, increased the levels of sediment discharge, breaking the connections along seasonally flooded regions and isolating populations (Pupim et al., 2019). This isolation followed a floodplain expansion in the Amazon–Solimões mainstem, thus allowing a secondary contact among previously isolated populations, especially in white water tributaries, such as the Madeira and Solimões Rivers (Pupim et al., 2019; Thom et al., 2018).

Table 2 Biogeographic models and values of log-likelihood of the six compared models in BioGeoBears

	LnL	nump	d	e	j	AICc	AICc_wt
DEC	− 11.47	2	0.014	1.0e-12	0	32.94	0.069
DEC+J	− 5.33	3	1.0e-12	1.0e-12	1.21	40.67	0.0014
DIVALIKE*	− 8.89*	2*	0.016*	1.0e-12*	0*	27.77*	0.91*
DIVALIKE+J	− 4.87	3	1.0e-12	1.0e-12	0.48	39.74	0.0023
BAYAREALIKE	− 12.8	2	0.063	0.36	0	35.6	0.018
BAYAREALIKE+J	− 5.54	3	1.0e-07	0.0051	1.00	41.08	0.0012

The values of the best-fit model are marked by asterisks (DIVA)

LnL ln(likelihood); nump number of parameters; d dispersal; e extinction; j founder-event speciation; AICc corrected Akaike Information Criterion; AICc_wt weight of the models based on the AICc

In a recent comparative phylogeographic study with widely distributed birds in Amazonian floodplains, the authors reported the link between the effects of climatic and rainfall shifts during the Mid–Late Pleistocene with the diversification in these environments (Thom et al., 2020). Their results pointed to the Heinrich Stadial and the Last Maximum Glacial (LGM) in the landscape having a main role in the diversification within the three species complex of birds.

Here we suggest that in addition to the latest climate-sedimentary dynamic processes already described, older episodes might have also acted in the diversification of the Amazonian floodplain biota. For example, prior events of warm-cold variations, rainfall cycles of 40 kyr initiating approximately at 2.6 Mya in the early Quaternary and paleoclimate in the late Neogene (Lisiecki & Raymo, 2005; Vonhof & Kaandorp, 2010).

A dynamic climate and sediment discharge might have promoted an expansion of the floodplains along the Amazon-Solimões mainstem. This scenario may provide a connection between these areas and enabled dispersion to colonize southwestern and eastern Amazonia. We could suggest this scenario to the lineage from East Xingu, which includes individuals from Marajó Island. The current area that encompasses the archipelago might represent a possible migration route from the Guiana Shield to Eastern Amazonia, since the separation of the islands from the mainland occurred approximately in the Late Pleistocene to Holocene (Rossetti et al., 2008), postdating the lineage diversification (2.03 Mya).

The floodplains in the main stem of the Amazon River are directly influenced by sediment discharge and water flow, thus presenting high heterogenic areas (Filizola et al., 2009; Gualtieri et al., 2018). This influence makes a narrower confluence region in central Amazonia, since it carries different levels of sediment discharge, breaking the distribution of several birds (Thom et al., 2020). Also, the lineages found in the present study had the same breaking point, between the mouths of the Negro and Madeira Rivers, thus suggesting

that this region might act as an interruption zone also for lizards that inhabit floodplains.

Recently, authors proposed a division of Amazonia into “overdispersed”, comprising areas of long-term stability (in the Guiana and Brazilian Shields) and “underdispersed” with highly dynamic areas with continuously changing habitats, holding younger lineages (Bicudo et al., 2019; Crouch et al., 2019). The diversification within *U. superciliosus* shows an interesting pattern that partially agrees with this division, having one of the oldest lineages in the Guyana Shield (NAR–4.5 Mya) and two of the youngest lineages in the more unstable region (PU–3.3 Mya and SO–2.0 Mya).

The increasing number of investigations on the biota from seasonally flooded regions opened discussion for new patterns and historical processes that influenced the diversification and evolution in Amazonia (Choueri et al., 2017; Luna et al., 2021; Silva et al., 2019; Thom et al., 2018, 2020). Based on the shared patterns found by Thom et al. (2020) for birds and in the present study for lizards, we support that future investigation might also show a sectorized genetic structure following the course of rivers and a genetic diversity yet to be discovered.

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Author Contributions All authors contributed to the study's conception and design. MVS and MJS performed the material preparation, data collection, and formal analysis. The first draft of the manuscript was written by MVS and all authors commented on previous versions of the manuscript. ALCP, MTR and MJS contributed to the writing, review, and editing of the final version. Resources were provided by ALCP, MTR and MJS. Funding acquisition and supervision were undertaken by ALCP and MJS. All authors read and approved the final manuscript.

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Data Availability All data generated and analyzed during this study are included in this published article and its supplementary information files. Further data are available from the authors upon request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All samples used in the present study were collected under license and are formally deposited in scientific collections.

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