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Original Article

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The influence of historical climate cycles on the diversification and demography of species from the Neotropical lizard group *Anolis chrysolepis*

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ABSTRACT

Historical shifts in climate have significantly influenced the establishment of ecosystems and the evolution of species. Phylogeographical studies integrated with niche modelling can provide insights into divergence events and demography. This study explores the evolutionary history, demographic patterns, and spatiotemporal dynamics of seven South American lizard species of the *Anolis chrysolepis* species group, which has been proposed to be strongly impacted by Pleistocene climatic fluctuations under the Refugia Hypothesis. We utilized mitochondrial and nuclear DNA sequences to delineate lineages, estimate divergence times, and examine demographic dynamics. Ecological niche models were employed to assess historical range shifts and identify climatic stability zones during the Quaternary. Our results identified 40 distinct lineages within the group. The species *A. chrysolepis* and *A. tandai* exhibited the highest lineage diversity, with seven and 12 lineages, respectively, while others showed fewer divergences. In the Cerrado savannas, species diversification aligns with Miocene geological and climatic changes, supporting the plateau/depression theory. In Amazonia, diversification during the Miocene was tied to Andean orogenesis, with Pleistocene range expansions influenced by climatic cycles. All species demonstrated demographic declines during the Pleistocene. Niche models highlighted stability areas in the Cerrado, and western and northern Amazonia, consistent with proposed refugia. These findings offer new insights into the biogeographical and evolutionary processes shaping biodiversity in these megadiverse regions.

Keywords: phylogeography; Amazonia; Cerrado; population dynamics; spatiotemporal reconstruction; anoles lizards

INTRODUCTION

Historical climatic changes have had significant impacts on the evolution of the environment and species. During the Pleistocene (~2.6 million to 11.7 thousand years ago), glacial periods of low temperature and humidity alternated with interglacial periods of high temperature and humidity (Hewitt 2000). These climatic fluctuations not only reshaped global landscapes but also influenced the distribution and demographic dynamics of various animal groups, having significant consequences for their diversity and genetic structure (Silva *et al.* 2018, Vasconcellos *et al.* 2019).

The major environmental changes stemming from climate fluctuations have impacted the existing biodiversity in the

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Neotropical region (de Lima *et al.* 2014, Silva *et al.* 2018). A quasidipole pattern in the Amazon rainforest, named the South American Precipitation Dipole, has been suggested through the analysis of oxygen isotope records (Cheng *et al.* 2013). Such a pattern postulated that western Amazonia, a region richer in biodiversity, showed no significant signals of climatic change in the last 250 000 years, while eastern Amazonia, a region with comparatively lower biodiversity levels, showed signs of oscillation between dry and wet periods during the Pleistocene, thus exhibiting climatic instability. The authors suggested that this instability may have led to a loss of diversity rather than the previously postulated increase by Haffer (1969) under the Pleistocene Refugia Hypothesis.

Studies focusing on the Neotropical dry diagonal, which encompasses biomes that are seasonally stressed by drought—Seasonally Dry Tropical Forests (SDTFs), Cerrado, and Chaco (Werneck 2011, Werneck *et al.* 2012)—showed signals of vegetational retraction during the Last Interglacial (120 000 kya) to the Last Glacial Maximum (21 kya). During this period, peripheral depression areas became drier and lost their characteristic vegetation (Ab'Saber 1983). In contrast, plateau areas maintained their savannah vegetation throughout the late Pleistocene, thus showing greater climatic and environmental stability (Werneck *et al.* 2011). During wetter periods, riparian vegetation expanded into depression areas, leading to the establishment of gallery forests connected to adjacent forest biomes (i.e. the Amazonia and the Atlantic forests), thereby allowing gene flow among biotic communities (Ledo and Colli 2017). Thus, the Plateau-Depression hypothesis (Werneck 2011) predicts that the climatic dynamics of the Cerrado during the Quaternary created biological patterns for the plateau and depression regions. More specifically, it proposes that the plateau regions (climatically more stable) would be associated with older lineages, displaying greater genetic structure, demographic stability, and genetic diversity. Meanwhile, depressions (climatically more unstable) would be associated with younger lineages, exhibiting lower genetic structure, diversity, and demographic instability (Werneck 2011, Guarnizo *et al.* 2016).

The processes underlying the origin, distribution, and maintenance of biodiversity at shallow evolutionary levels can be elucidated through phylogeographical research (Turchetto-Zolet *et al.* 2013, Fonseca *et al.* 2018). Phylogeography is an integrative discipline that combines molecular and geographical data to unravel how historical events have shaped the distribution of genetic variability, thus investigating lineage evolution (Avise *et al.* 2016). Given that genetic structure is influenced by the demographic history of biological groups and that signals of demographic changes are imprinted in the DNA of contemporary lineages (Barrick and Lenski 2013, Prates *et al.* 2016), genetic data coupled with coalescent analyses can also be used to test hypotheses regarding changes in the demographic dynamics and historical distribution of different groups (Collevatti *et al.* 2013, Pirani *et al.* 2019).

Integrating phylogeographical analyses with geospatial data adds robustness to phylogeographical investigations (Werneck *et al.* 2012, Perktaş *et al.* 2019). Ecological niche modelling (ENM) is a technique that uses environmental data from known occurrence points of species to determine their potential distribution over space and time through projections in different periods (Pearson 2010, Machado *et al.* 2019). In this context, ENM aids in elucidating the mechanisms influencing lineage structure (Alvarado-Serrano and Knowles 2014, Peterson and Anamza 2015), as well as in verifying quantitative and qualitative changes in demography and ranges through time.

The diversity, reduced mobility, strong association with local climates, and susceptibility to environmental changes of Squamata make them excellent model organisms for evolutionary and phylogeographical studies (Camargo *et al.* 2010). The lizard genus *Anolis* has been the focus of various classic works in ecology and evolution (Williams 1983, Losos 2011), such as the mechanisms behind adaptive radiation and convergent evolution in the Caribbean islands (McGlothlin *et al.* 2018, Stroud and Losos 2020). Early studies proposed that the diversification of the Amazonian *Anolis chrysolepis* species group was strongly impacted by Pleistocene climatic fluctuations and suggested the Refugia Hypothesis, with four refugia areas proposed for the group (Vanzolini and Williams 1970). The proposed refugia areas were distributed as follows: Area I covered the eastern regions of the Guianas and Suriname to Northern Brazil; Area II encompassed Venezuela, Trinidad, and the northern part of Guyana; Area III represented the Amazonia regions of Colombia, Ecuador, and Peru; and finally, Area IV included central Brazil. This hypothesis, however, was subsequently challenged based on initial molecular evidence and traditional phylogenetic approaches (Glor *et al.* 2001).

In this study we focused on seven species that comprise the *A. chrysolepis* species group (*sensu* D'Angiolella *et al.* 2011), a clade with a wide Neotropical distribution, with representatives in areas of the Amazon rainforest and the Cerrado savanna (D'Angiolella *et al.* 2011, Ribeiro-Júnior 2015). Previous studies have conducted extensive sampling of occurrence data (Avila-Pires *et al.* 2009, Ribeiro-Júnior 2015) and revealed the main phylogenetic relationships of the group (D'Angiolella *et al.* 2011). However, these studies have not integrated spatially explicit approaches into phylogeographical and demographic inferences, making such a group and datasets good models for testing hypotheses on the effects of Pleistocene climatic changes on the Neotropical fauna.

Because of their wide distributions, in biomes with different historical climatic dynamics, studying the historical demographic and distributional patterns of the *A. chrysolepis* group in response to climatic changes may help elucidate broader diversification processes in the Neotropics (Prates *et al.* 2016, Sheu *et al.* 2020). Furthermore, knowledge about the responses of species of the group to past climatic changes can help us infer potential impacts and responses to future climatic changes (Fordham *et al.* 2020).

Through the use of molecular data and ENM, this study aimed to investigate the evolutionary history and potential effects of past climatic cycles on the *A. chrysolepis* species group. More specifically, we (i) inferred the delimitation of evolutionarily independent intraspecific lineages, phylogenetic relationships, and divergence times, followed by historical demographic and the spatiotemporal phylogeographical history of each species in the group, seeking evidence of changes in effective population size and distribution areas through time; and (ii) inferred, through paleomodelling, how the species distribution patterns were affected throughout the Quaternary climatic fluctuations and whether the predicted areas of stability coincide geographically with the four refugial areas previously proposed based on the Refugia Hypothesis.

MATERIALS AND METHODS

Genetic data

The sequences used in this study were obtained from multiple sources: 257 sequences from D'Angiolella *et al.* (2011), 200 sequences from GenBank, and 36 new sequences generated for this study. Specifically, we utilized sequences from the mitochondrial gene (mtDNA) NADH dehydrogenase subunit 2 (*ND2*), as well as fragments from three nuclear genes (nuDNA): megakaryoblastic leukaemia 1 (*MKL1*), nerve growth factor (*NGFB*), and recombination activating gene 1 (*RAG1*) (Supporting Information Table S2). The collected data encompass sequences of all seven species within the *A. chrysolepis* species group, five of which are from Amazonia (*Anolis chrysolepis*, *A. tandai*, *A. scypheus*, *A. planiceps*, and *A. bombiceps*), and two from the Cerrado (*Anolis brasiliensis* and *A. meridionalis*). We also used samples of *Anolis auratus*, *A. lineatus*, and *A. fuscoauratus* as outgroups in the analysis because of their close relationships with the ingroup (D'Angiolella *et al.* 2011). In summary, our dataset consisted of 308 sequences encompassing 1015 bp of *ND2*, 58 sequences covering 233 bp of *MKL1*, 64 sequences encompassing 426 bp of *NGFB*, and 63 sequences spanning 854 bp of *RAG1*.

The sequences were aligned using the MUSCLE algorithm (Edgar 2004) with its default settings on MEGA11 (Tamura *et al.* 2021). We employed PHASE v.2.1.1 (Stephens *et al.* 2001) with default settings to address the gametic phase of heterozygous individuals of the nuDNA data.

Lineage delimitation and dated phylogenetic inference

To delimit lineages within the *A. chrysolepis* species group and evaluate their evolutionary independence, we used a combination of distance-based and tree-based delimitation methods, using the *ND2* alignment for all available specimens in each putative species group.

As a distance-based method, we used Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al.* 2021), a hierarchical clustering approach that exclusively relies on pairwise genetic distances. ASAP does not require distance thresholds (Puillandre *et al.* 2021). The analysis was performed using the substitution model K80 (Kimura 1980) on the ASAP analysis platform (available at https://bioinfo.mnhn.fr/abi/public/asap/).

The first tree-based method used was the multirate extension of the Poisson Tree Process (mPTP) (Kapli *et al.* 2017). This method examines the number of lineages based on the accumulated substitutions between branching events in a nonultrametric tree. It operates under the assumption that the number of substitutions between species is significantly greater than that within species (Zhang *et al.* 2013). This method allows for multiple speciation rates across the different lineages, instead of assuming that all branches of the phylogenetic tree have the same speciation rate, which allows us to handle more complex scenarios (Kapli *et al.* 2017).

To infer the species delimitation using mPTP, we first inferred the best fitting nucleotide substitution model using ModelTest-NG v.0.2.0 (Flouri *et al.* 2015, Darriba *et al.* 2020) under the corrected Akaike Information Criterion (AICc; Hurvich and Tsai 1989). Then we used the resulting substitution model (TIM2+I+G) to produce a maximum likelihood tree using the tool RAxML-NG v.1.1.0 (Kozlov *et al.* 2019). The resulting tree was utilized as an input in the software mPTP v.0.2.4 (Kapli *et al.* 2017) in two steps. First, to avoid oversplitting the dataset (Kapli *et al.* 2017), we estimated the minimum branch length along the tree. We used those results during the second step, where we performed two independent runs of 10^8 generations, sampling every 10^4 steps, with a burn-in of 10%.

The second tree-based delimitation method used was the Bayesian implementation of the generalized mixed Yule-coalescent model (bGMYC) (Reid and Carstens 2012). The bGMYC method utilizes an ultrametric tree's branching pattern to determine species groups, using the likelihood score to dictate the optimal transition point from a Yule model (i.e. cladogenesis event) to a coalescent model (i.e. population divergence) (Pons *et al.* 2006, Fujisawa and Barraclough 2013). It uses Markov chain Monte Carlo (MCMC) simulation to account for the error in phylogenetic and parameter estimation (Reid and Carstens 2012).

We constructed an ultrametric tree in BEAST2 v.2.7.5 (Bouckaert *et al.* 2019) using unique haplotypes because identical sequences result in zero-length branches, causing the model to oversplit the dataset (Reid and Carstens 2012). We employed bModelTest to infer the best nucleotide substitution model (Bouckaert and Drummond 2017) and used the Optimized Relaxed Clock model (Douglas *et al.* 2021) with a rate equal to 0.0065 substitutions per million years (Macey *et al.* 1998), a rate commonly applied in molecular dating studies for the *ND2* gene within Squamata. To ensure convergent results, we ran three independent MCMC runs, each consisting of 2×10^8 generations, with samples collected every 2×10^4 steps. We assessed convergence of each of the runs using Tracer v.1.7.2 (Rambaut *et al.* 2018) and considered a parameter well-sampled when its effective sample size (ESS) was ≥200. We combined the results of the different runs using LogCombiner v.2.7 (Bouckaert *et al.* 2019) and discarded 10% of the generations as burn-in.

We randomly selected 100 trees from the posterior distribution and performed the bGMYC analysis using the package bGMYC in R 3.6.3 (R Core Team 2021). As recommended in the bGMYC package manual (available at https://nreid.github.io/assets/bGMYC_instructions_14.03.12.txt), we performed independent GMYC MCMC runs on the 100 selected trees consisting of 5×10^4 generations, discarding the first 4×10^4 as burn-in and choosing a threshold of 0.5 to delineate lineages.

From the preceding analyses, we established a consensus on hypothetical lineages by visually checking for congruent breaks across different methods of mitochondrial lineage delimitation.

We used STACEY (Species Tree and Classification Estimation, Yarely) (Jones 2017), implemented in BEAST 2.7.5 (Bouckaert *et al.* 2019), to validate delimited lineages, assigning individuals a priori to a lineage according to the consensus. STACEY integrates species delimitation and phylogenetic inference in a Bayesian framework (Jones 2017), allowing for the acquisition of a time-calibrated species tree as we validated the species groups. The species are analysed using the collapse weight parameter. A collapse weight of zero maintains the a priori lineages as they are, without grouping them into fewer species. In contrast, a collapse weight of one will group two or more lineages into a single partition, effectively reducing the number of inferred species (Jones 2017). We used the default value for collapse weight, a beta distribution with $\alpha = 1$ and $\beta = 1$ (equivalent to a uniform distribution with a minimum of zero and a maximum of one). All remaining parameters were left at their default settings, except for popPriorScale, which was set with a lognormal distribution with a mean equal to −7.0 and a standard deviation of 2.0 to reflect our assumptions about effective population sizes.

Our dataset for this analysis comprised mitochondrial and nuclear genes for the entire *A. chrysolepis* species group. We conducted the analysis across these four independent partitions over three independent runs, each with an MCMC chain for 10^8 generations, sampling every 10^4 generations. Because STACEY is a parameter-rich model, we employed the HKY site model (Hasegawa *et al.* 1985) with estimated frequencies for each gene and a strict clock model for all partitions to achieve convergence. The clock rate for mtDNA was set at 0.0065 substitutions per million years (Macey *et al.* 1998) and substitution rates for each nuclear gene were estimated based on the mtDNA substitution rate using a broad prior (1/*x* distribution). Convergence and adequate mixing of the MCMC chains were confirmed using Tracer v.1.7.2 (Rambaut *et al.* 2018). The runs were combined, after removing a 10% burn-in, with LogCombiner v.2.7 (Bouckaert *et al.* 2019). We also used TreeAnnotator v.2.7 (Bouckaert *et al.* 2019) to determine the maximum clade credibility (MCC) tree, utilizing a burn-in of 10%.

Historical diffusion reconstructions and historical demography

To reconstruct the phylogeographical history of the *A. chrysolepis* group we used the Relaxed Random Walk model (RRW; Lemey *et al.* 2010) implemented in BEAST v.2.7.5 (Bouckaert *et al.* 2019).

We conducted the RRW analyses using mtDNA (*ND2*) sequences for six of the seven species delimited in the previous analyses in the *A. chrysolepis* species group. We excluded *A. bombiceps* due to an insufficient number of available sequences for this analysis. Geographical coordinates were added as traits, obtained from D'Angiolella *et al.* (2011) and from our molecular sampling and treated as spherical geography data to account for the Earth's curvature. To introduce random noise to identical coordinates, we applied a jitter of 0.001 in RStudio v.4.2.1 (RStudio Team 2021).

For each species, we conducted an analysis with two independent runs, with MCMC chain of 2×10^8, sampling every 2×10^4 generations. We used bModelTest to identify the site model for our data. We implemented an Optimized Relaxed Clock (Douglas *et al.* 2021) for the mtDNA sequences with a clock rate of 0.0065 substitutions per million years (Macey *et al.* 1998). We also implemented an Optimized Relaxed Clock for the coordinates trait and the clock rate was set to be estimated based on the mtDNA substitution rate. Because we also wanted to analyse the historical demography of each species, we used Coalescent Bayesian Skyline as a tree prior (Drummond *et al.* 2005). All other parameters were kept as default.

For *A. meridionalis* we ran two independent runs with an MCMC chain of 5×10^8, sampling every 5×10^4 generations, and setting the dwindowsize and twindowsize parameters to 0.05 to account for convergence issues.

We used Tracer v.1.7.2 (Rambaut *et al.* 2018) to check for the convergence and mixing of our data and to visualize our Coalescent Bayesian Skyline Plots. We combined our runs with LogCombiner v.2.7 (Bouckaert *et al.* 2019), removing a burn-in of 10%. We used TreeAnnotator v.2.7 (Bouckaert *et al.* 2019) to achieve an MCC tree, also utilizing a burn-in of 10%. We created a visualization of the results using the R package seraphim (Dellicour *et al.* 2016).

Palaeomodelling

To access the potential historical distributions of the seven species in the *A. chrysolepis* species group, and to search for areas of historical climatic stability, we modelled their current ecological niches and predicted projections across four climatic periods: the present, Mid-Holocene (MD, ~6 kya), Last Glacial Maximum (LGM, ~21 kya), and Last Interglacial (LIG, ~120 kya).

We obtained occurrence data from D'Angiolella *et al.* (2011) and Ribeiro-Junior (2015). To decrease sampling bias and minimize spatial autocorrelation, we spatially thinned the occurrence records using the function *thin()* available in the R package 'spThin' (Aiello-Lammens *et al.* 2015), excluding occurrences that were less than 15 km from each other. After the spatial thinning, we retained 27 unique occurrences for *A. bombiceps*, 64 for *A. scypheus*, 97 for *A. planiceps*, 71 for *A. chrysolepis*, 98 for *A. tandai*, 96 for *A. brasiliensis*, and 96 for *A. meridionalis*.

Environmental predictor variables were selected based on their relevance for the species' ecology. We used three types of variables: (i) bioclimatic variables, obtained from the CHELSA v.2.0 database (https://chelsa-climate.org) (Karger *et al.* 2017) for current time periods, and Paleoclim (Brown *et al.* 2018) for past bioclimatic variables, given that the *A. chrysolepis* species complex is sensitive to climatic variations; (ii) elevation data obtained from The Shuttle Radar Topography Mission (SRTM) (Farr *et al.* 2007), from which we derived the variable roughness (Wilson *et al.* 2007) (the absolute difference between the elevation of a grid cell and its neighbours), which indicates elevation variability—an important factor since species within the complex can inhabit plateau and depression areas; and (3) Normalized Difference Vegetation Index (NDVI), a measure of the greenness of the vegetation (median value throughout the year), downloaded from the COPERNICUS Global Land Service (https://land.copernicus.eu), also relevant considering that species occur in lowland forests and open vegetation formations. All variables were aggregated to 10 arcmin spatial resolution.

To avoid multicollinearity issues when modelling with multiple potentially correlated variables, we used Variance Inflation Factor (VIF) analysis, where variables with VIF > 10 were removed (Sillero *et al.* 2021). Subsequently, the five most important variables for each species were identified using permutation-based methods implemented in the SDMtune package (Vignali *et al.* 2020).

After selecting the variables with high permutation importance for each species, we kept five consensus variables that most frequently appeared across all species for modelling. These variables were: Mean Diurnal Air Temperature Range (bio2), Isothermality (bio3), Precipitation of the Wettest Month (bio13), Precipitation of the Driest Month (bio14), and Mean Monthly Precipitation of the Warmest Quarter (bio18). We obtained the same variables estimated for the Mid-Holocene (~6 kya), LGM (~21 kya), and LIG (~120 kya) in the PaleoClim database (Brown *et al.* 2018).

We trained our ENMs using the Maximum Entropy algorithm (MaxEnt) (Phillips and Dudík 2008), adjusting the algorithm parameters through a model selection with the ENMeval package (Muscarella *et al.* 2014) in R (RStudio Team 2021). Among the parameters evaluated in the model selection, the regularization multiplier (RM) is selected to reduce the chances of data overfitting by prioritizing simpler models (Merow *et al.* 2013). The values for RM were evaluated over a range from 0.5–5 with increments of 0.5. We also evaluated the feature classes, which are mathematical transformations of environmental covariates. They were linear (L), linear and quadratic (LQ), linear, quadratic, and product (LQP), threshold (T), and linear, quadratic, and threshold (LQT) (Merow *et al.* 2013).

MaxEnt is a machine learning technique that uses data of known occurrence records against a background data (Phillips and Dudík 2008). To set the background data, we set the area for modelling and projection to a 10° buffer around a minimum convex polygon based on the presence records, and then we randomly sampled points that were equal in number to 10% of the total number of cells of the environmental layers.

For our model fitting, we used the checkerboard2 method for data partitioning. This method divides the background and presence records into four distinct bins (25% for testing and 75% for training). By utilizing this, we ensure impacts of spatial autocorrelation will not be significant (Roberts *et al.* 2017).

We then used delta-AIC < 2 to select the models that had the best trade-off between model fitness and complexity (Richards 2008), considering the regularization multiplier and feature class selection. To evaluate model performances, we used the Area Under the Curve (AUC), with values >0.75 indicating reasonably good models and the True Skill Statistic (TSS) (Allouche *et al.* 2006), with values >0.5 indicating reasonably good models, and partial Receiver Operating Characteristic Curve (pROC) (Peterson *et al.* 2008), with values >1.5 indicating reasonably good models.

After model selection, we projected the model to the distinct time periods (LIG, LGM, MD, and present). Then, we created a binary map to represent potential species presences and absences. This was achieved by applying a threshold method, specifically selecting the maximum specificity and sensitivity threshold, which minimizes omission and commission errors in the prediction (Liu *et al.* 2005). This binary map allowed for the delineation of stable areas for each species across different climatic periods by summing up the predicted presences (0–4 periods with suitable habitats/predicted presences).

Finally, we combined the stability maps of all species in one map that represents the average areas of stability for the *A. chrysolepis* species group, by summing the stability values (0–4 for each species) and dividing by the number of species in each grid cell to achieve a map that shows the stability of the habitat relative to the number of species present.

RESULTS

Lineage delimitation and dated phylogenetic inference

The lineage delimitation analysis using ASAP identified 43 potential lineages for the entire dataset, achieving a high best ASAP score of 18.00, indicating strong support for these groupings. Within the Cerrado region, the method delineated eight distinct lineages for both *A. brasiliensis* and *A. meridionalis*. In the Amazon, the analysis identified three lineages for *A. planiceps*, 14 for *A. tandai*, eight for *A. chrysolepis*, and one for both *A. scypheus* and *A. bombiceps* (Supporting Information Figs S1–S3).

The mPTP analysis yielded similar results, identifying 42 lineages in total. The main differences compared to the ASAP results were observed in the number of lineages for certain species: two fewer lineages for *A. tandai*, two more for *A. brasiliensis*, and one fewer for *A. meridionalis* (Supporting Information Figs S1–S3).

The bGMYC analysis, which tends to recover more fine-grained genetic divergences (Reid and Carstens 2012), identified a much larger number of lineages, 82 in total (Supporting Information Figs S1–S3). It was particularly less conservative in the Cerrado region, delineating 20 different lineages for *A. meridionalis* and 24 for *A. brasiliensis*. This highlights significant disparities with the earlier analyses, emphasizing the sensitivity of the bGMYC method to within-species variation.

The consensus delimitation that we created from the results of the previous analyses was validated by STACEY (Fig. 1A), which recovered 40 lineages, with high posterior probabilities for most lineages (> 0.90), except for *A. chrysolepis* and *A. tandai*.

Within the Cerrado, six distinct lineages were identified for the open-area species *A. meridionalis*, labelled as ME1–ME6. These lineages exhibited a geographically dispersed distribution across the region, with ME1 and ME6 predominantly occurring in the core of the Cerrado, and Cerrado plus the transition zone Cerrado–Caatinga, respectively. Lineage ME3 was concentrated primarily in the state of Rondônia in Brazil, an Amazon rainforest area. Meanwhile, ME2 extended eastward from Mato Grosso in Brazil to Bolivia and Paraguay, encompassing regions characterized by the Cerrado, Chaco, and Pantanal. The ME5 lineage was positioned on the border between Mato Grosso and Mato Grosso do Sul, while the ME4 lineage exhibited the widest distribution, ranging from the core of the Cerrado to the xeric biome of the Caatinga (see Fig. 1B).

The seven lineages of *A. brasiliensis* (BR1–BR7) exhibited wide distributions, with BR2 found in the Cerrado but also extending its distribution to the south of Amazonia, and BR3 found in the Caatinga and in the ecotonal region between the Caatinga and Cerrado. The remaining lineages appeared to be distributed in the Cerrado along different points of the Tocantins River (Fig. 1C).

In Amazonia, the analysis identified seven lineages for *A. chrysolepis* (CR1–CR7). Almost all the lineages of *A. chrysolepis* are constrained to distinct interfluves in the Guiana Shield. Lineages CR1 and CR2 occur on opposite margins of the

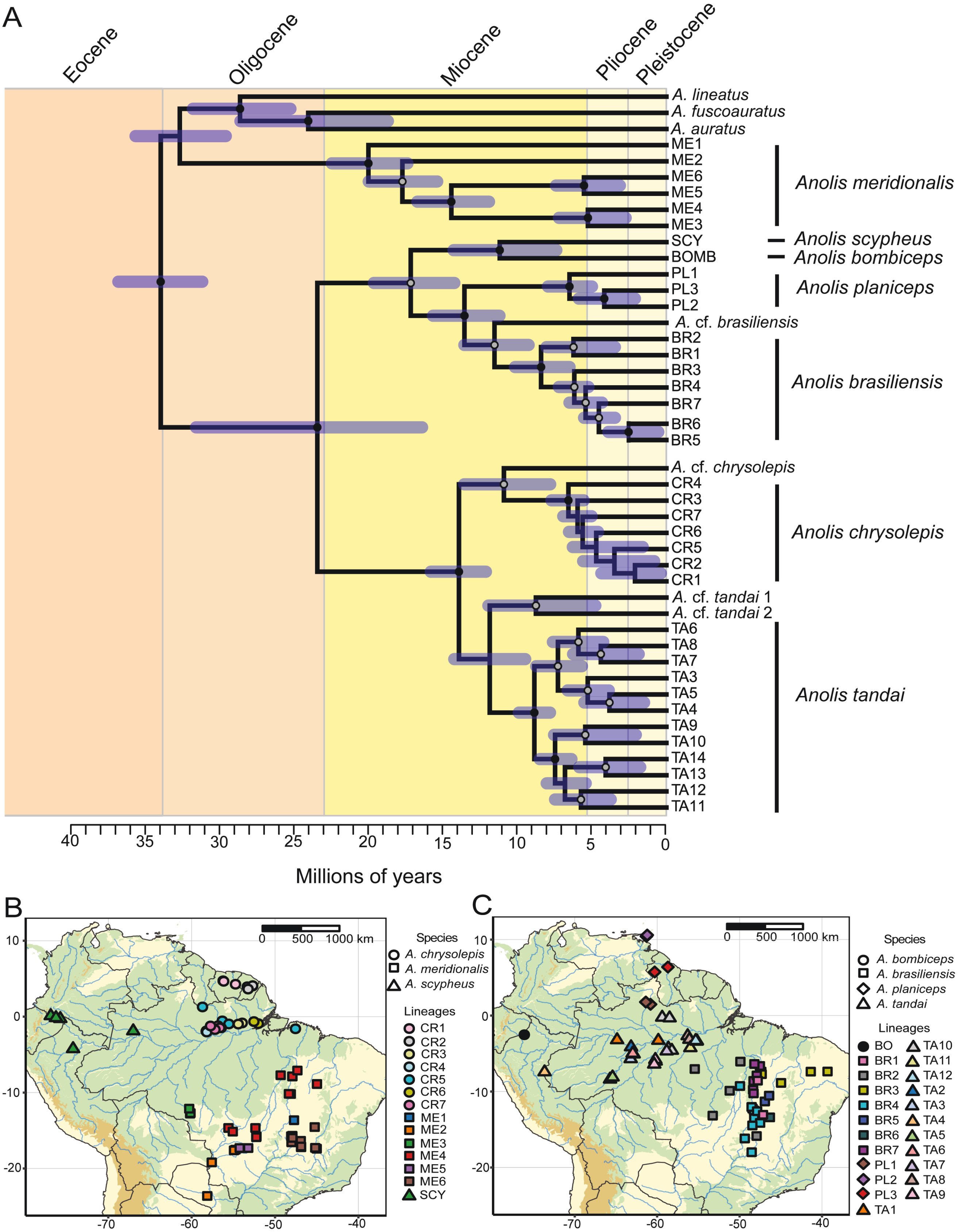

Figure 1. A, Species Tree of the *Anolis chrysolepis* species group estimated through STACEY, with divergence time estimates based on four loci. Blue bars represent 95% highest posterior density (HPD). Black circles indicate support >0.95, gray dots indicate support between 0.5 and 0.95, and branches without dots represent posterior probability <0.5. The external group used in the analysis is *Anolis auratus*, *Anolis lineatus*, and *Anolis fuscoauratus*; B, Map showing the distribution of lineages estimated for three species of the *Anolis chrysolepis* species group. Circles represent *A. chrysolepis*, triangles represent *A. scypheus*, and squares represent *A. meridionalis*; C, Map showing the distribution of lineages estimated for four species of the *Anolis chrysolepis* species group. Squares represent *A. brasiliensis*, diamond shapes represent *A. planiceps*, circles represent *A. bombiceps*; and triangles represent *A. tandai*.

Maroni River, with CR1 being found in Suriname and CR2 in French Guyana. Lineage CR3 was found in the south of the state of Amapá in Brazil. Other lineages predominantly occur in the interfluves of the northern tributaries of the Amazon River (Fig. 1B). For *A. tandai* (TA1–TA12), on the other hand, with the exception of lineage TA10, all others seem to occur in the Amazon sedimentary basin (Fig. 1C).

Anolis planiceps was divided into three lineages (PL1, PL2, and PL3), with PL1 found in northern Brazil, PL2 in Trinidad and Tobago, and PL3 in Guyana.

Anolis scypheus was classified as a single lineage, referred to as SCY, covering Ecuador, Northern Peru, and Northwestern Amazonian region in Brazil (Fig. 1C). However, *A. bombiceps* lacked sufficient data for conclusive delimitation of intraspecific lineages and distributions and requires further investigation (Fig. 1C).

The origin of the *A. chrysolepis* species group is estimated to have occurred during the late Eocene or early Oligocene, ~34 Mya [95% highest posterior density (HPD) interval 37–31 Mya]. Overall, the phylogeny exhibited strong support (> 0.95) for nodes representing older divergence events, whereas it displayed weaker support (< 0.95) for more recent events (Fig. 1A).

The phylogeny revealed that the *A. chrysolepis* species group may be paraphyletic due to the uncertain placement of *A. meridionalis*, based on the low posterior probability of its basal node. *Anolis meridionalis* lineages are older, with longer branches than those found for other species (Fig. 1A). The lineages diverged mostly during the Miocene and early Pliocene.

The divergence event that led to the emergence of the other species in the *A. chrysolepis* group occurred ~23 Mya (95% HPD interval 32–16 Mya), with strong support (posterior probability of 0.99). This branching event created two clades. The first one is composed by the Amazonian species *A. bombiceps*, *A. scypheus*, and *A. planiceps*, plus the Cerrado species *A. brasiliensis*. *Anolis bombiceps* and *A. scypheus* diverged in the Miocene at around 11 Mya (95% HPD interval 14–9 Mya). The divergence between *A. planiceps* and *A. brasiliensis* is estimated to have occurred during the Miocene at around 13 Mya (95% HPD interval 16–11 Mya). The lineages estimated for each of these species emerged around the late Miocene and Pliocene. The second clade is composed of *A. chrysolepis* and *A. tandai*, two sister species that diverged in the Miocene around 14 Mya (95% HPD interval 16–12 Mya).

The analysis also recovered lineages for the nominal species *A. tandai* and *A. chrysolepis* with long branches positioned as sister groups to those species. We therefore decided to identify them separately from the nominal species as *A.* cf. *tandai* and *A.* cf. *chrysolepis*, respectively. We also obtained a lineage positioned as a sister group to the nominal species *A. brasiliensis* that we decided, for the purposes of this paper, to name as *A.* cf. *brasiliensis*.

Historical diffusion reconstruction

According to the RRW analysis, the confidence intervals for the oldest diversification events for *A. brasiliensis* began around 16.3 Mya, in the Amazonia/Cerrado ecotone. By 6.88 Mya, the species had expanded eastward, extending its distribution to the area closer to ecotonal regions entering the Caatinga xeric biome. This expansion continued southward, reaching the central Cerrado at Goiás state by ~2.70 Mya, and then diffused northwards again over the last 65 000 years (Fig. 2A).

For *A. meridionalis*, the confidence intervals for the oldest diversification events are at ~53 Mya, to the south of the area of the current distribution in the Cerrado. Analysing the nodes, we can see signs of expansion eastward to the area in the central Cerrado (currently known as Brasília) around 26.7 Mya. A significant shift in its expansion occurred subsequently, as it extended westward towards Peru and northward towards Rondônia around 5.51 Mya. Over the past 2.65 Myr, this species has further expanded its range to encompass regions in the Amazonia/Cerrado ecotone (Fig. 2B).

The species *A. planiceps* emerged in northeastern Guyana around 9.58 Mya and expanded northward 1.8 Mya to colonize Trinidad and Tobago, later diffusing southwards into Roraima ~117 000 years ago (Fig. 2C).

For *A. scypheus*, although there are indications that the species originated in northeastern Ecuador around 2.59 Mya, the lack of additional data creates significant uncertainty, preventing us from more confidently determining the ancestral area of the species. Subsequently, the species spread eastward to northern Brazil and Peru by about 80 000 years ago (Fig. 2D; Supporting Information Fig. S5).

The diversification path of *A. chrysolepis* also presents an interesting pattern. The first diversification events occurred at the centre of the current distribution around 8.45 Mya, and *A. chrysolepis* extended east to colonize the south of Amapá in Brazil by about 5.76 Mya. Approximately 400 000 years ago, the species moved west towards the state of Amazonas and north to colonize Suriname and French Guiana. By around 120 000 years ago, it had extended its area to Guyana (Fig. 2E).

Finally, for *A. tandai*, the analysis inferred that the first events of diversification were in central Amazonia (currently state of Amazonas near the city of Coari) on the southern bank of the Solimões River at around 12.8 Mya. The species first diffused eastwards (~9.19 Mya) and later westwards reaching the state of Acre in Brazil. At ~3.44 Mya, the species colonized the northern bank of the Amazon River, reaching the northeast region of the state of Amazonas in Brazil (Fig. 2F).

Historical demography

According to the Bayesian Skyline Plots generated, we observed that the gallery forest species, *A. brasiliensis*, seems to have undergone an historical population expansion process around 3 Mya, followed by a period of population stability and decline over the last 200 000 years. (Fig. 3A). *Anolis meridionalis*, the open area species, appears to have experienced relative stability until the last 1 Myr, when it underwent a population decline event (Fig. 3B).

For the Amazonian forest species, it appear that all experienced a population decline event in the last 1 Myr, such as *A. planiceps* (Fig. 3C). However, this event is more pronounced for *A. chrysolepis* (Fig. 3D), while *A. tandai*, besides the recent population decline, also had a subtle tendency of population expansion around 10 Mya (Fig. 3E). *Anolis scypheus* was relatively stable throughout the analysed period, but also showed a subtle sign of recent decline, synchronous with the population decline of other Amazonian species (Fig. 3F).

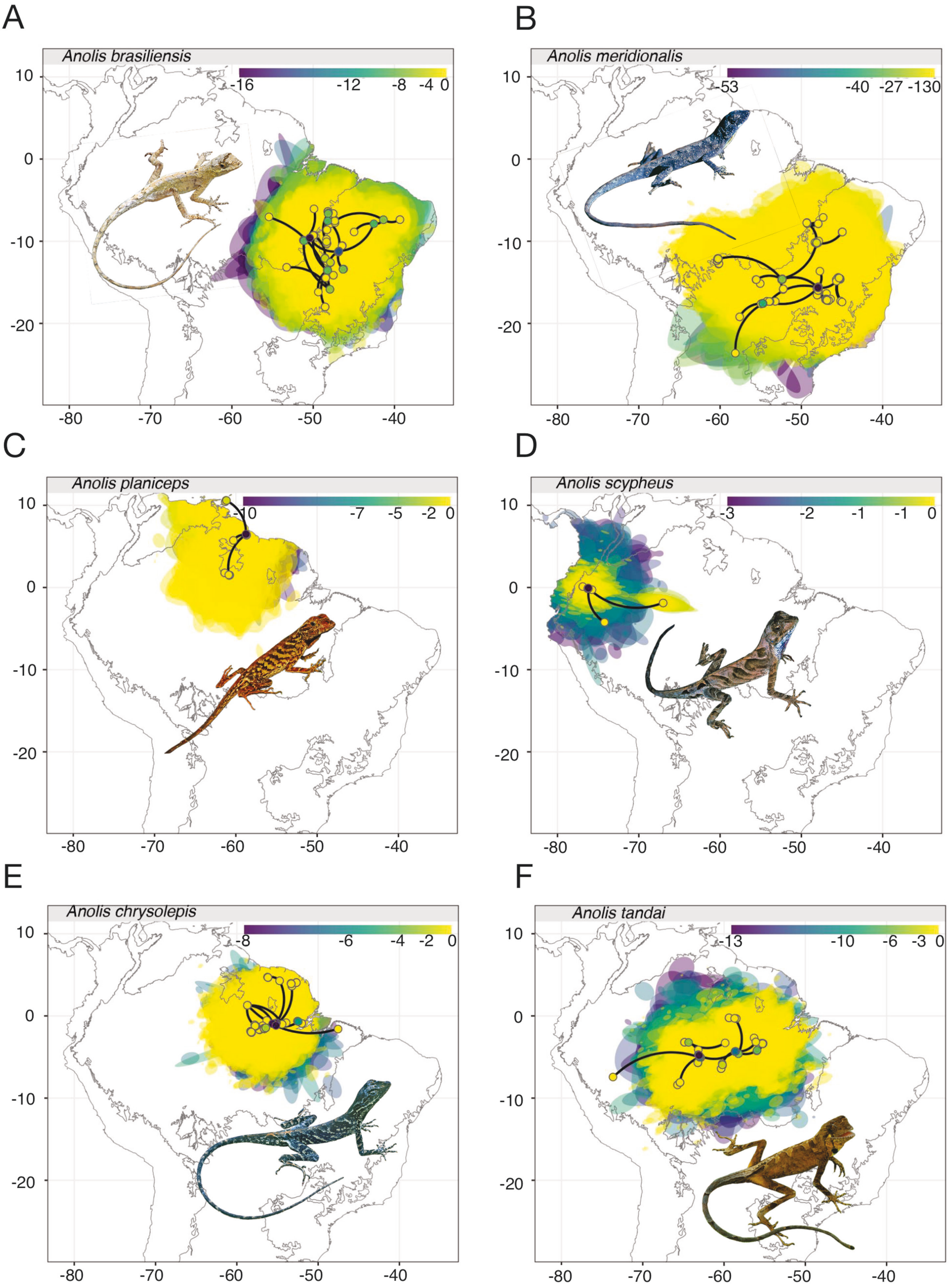

Figure 2. Relaxed Random Walk model inferred from mtDNA data of six species of the *Anolis chrysolepis* species group. Coloured bar represents the time of the events (in millions of years), with purple representing older events and yellow representing more recent events. Circles represent nodes of the Maximum Clade Credibility tree and are coloured according to their time of occurrence. The lines represent the geographical spread of the nodes through time. A, *Anolis brasiliensis*; B, *Anolis meridionalis*; C, *Anolis planiceps*; D, *Anolis scypheus*; E, *Anolis chrysolepis*; F, *Anolis tandai*. Photos by Laurie J. Vitt (A, B, D, E, F) and Pedro P. G. Taucci (C).

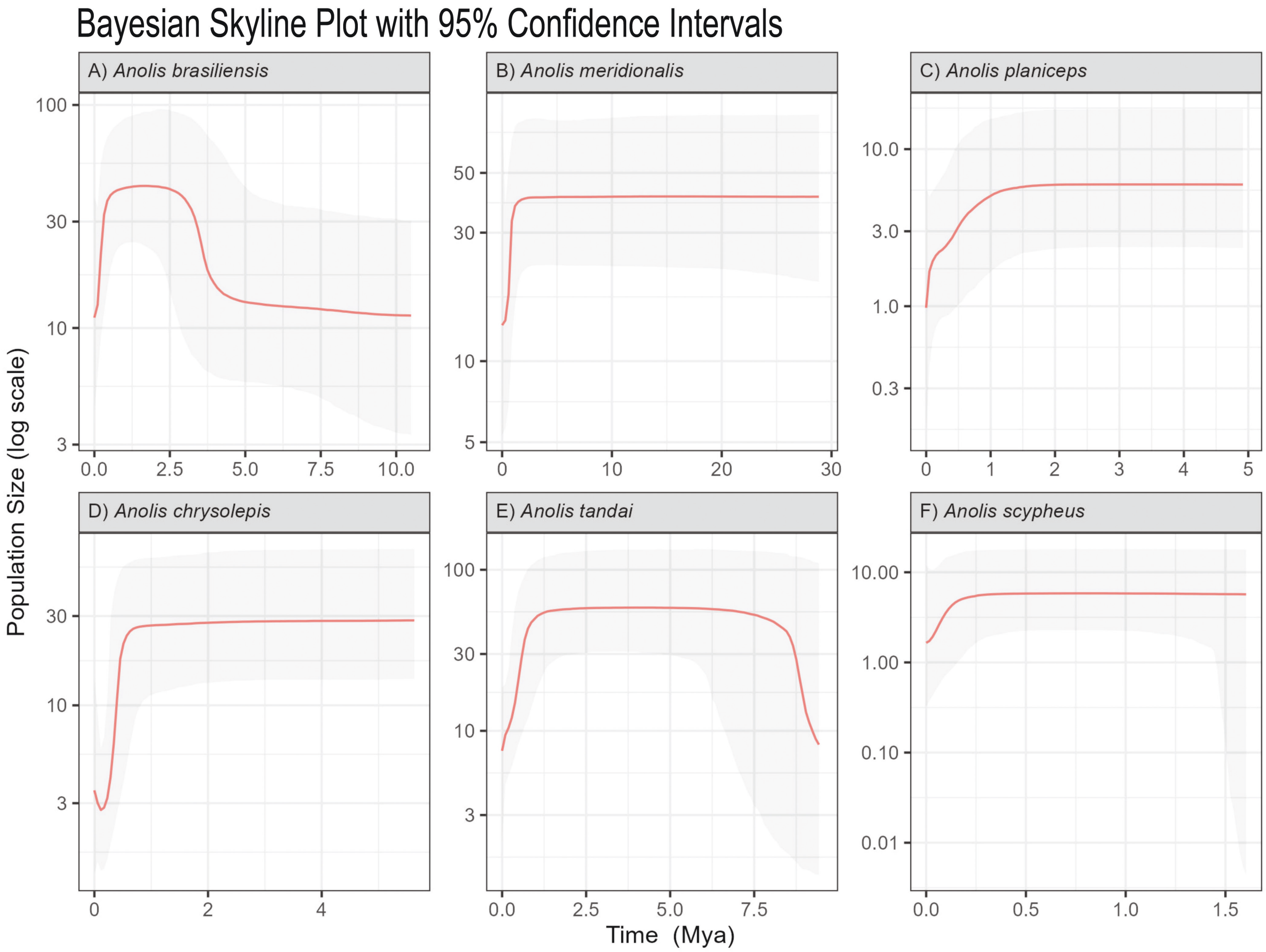

Figure 3. Bayesian skyline plots (BSP) inferred from the mtDNA datasets of the six species of the *Anolis chrysolepis* species group. BSP—redlines and shadows represent respectively the median and the 95% HPD ranges of the estimates.

Palaeomodelling

All species-specific ENMs had strong performance, with AUC values exceeding 0.9 and pROC values indicating reasonably good models (all species except *A. tandai* had pROC values > 1.5), yet TSS scores for most species indicate moderate to low model fit (Supporting Information Table S1). For detailed information on ENMs by species and period, please refer to the Supporting Information.

Both Cerrado species (*A. brasiliensis* and *A. meridionalis*) were inferred to have expanded their suitability areas into Amazonia during the LGM. Suitable areas for the savanna-associated species, *A. meridionalis*, appear to penetrate even further into southern Amazonia than *A. brasiliensis*, the gallery forest-associated species (Supporting Information Figs S2, S3). However, by the Mid-Holocene the suitable areas for both species seem to be concentrated in the core of the Cerrado once again.

Amazonian species (i.e. *A. chrysolepis*, *A. planiceps*, *A. scypheus*, *A. bombiceps*, and *A. tandai*) reveal contrasting dynamics in the distribution of suitable areas across time periods. Species currently distributed in the northeastern side of the biome (*A. chrysolepis* and *A. planiceps*) exhibit a very limited distribution of suitability areas during the LGM and LIG, expanding notably during the Mid Holocene (Supporting Information Figs S4, S5). Conversely, species currently found in the western portion of the biome, *A. bombiceps* and *A. scypheus*, have different historical distributions. *Anolis bombiceps* shows minimal suitability during the LIG, improving slightly during the LGM but expanding significantly in the Mid-Holocene (Fig. S6). In contrast, *A. scypheus* exhibits an expanded suitability area during the LGM but contracts again in the Holocene (Fig. S7). The suitability area for *Anolis tandai* during the LIG appears north of the present model coverage, significantly reducing during the LGM and expanding again in the Holocene (Fig. S8).

Species-specific stability maps indicate that some areas retained suitable habitats across all studied periods. *Anolis meridionalis* exhibits a stability area that extends throughout the Cerrado core (Fig. 4D). *Anolis brasiliensis* presents stability areas encompassing the entire Cerrado (Fig. 4B). *Anolis scypheus* shows a stability area that encompasses parts of Peru, Ecuador, Colombia, and the northwestern region of the Brazilian Amazonia (Fig. 4F). By contrast, *A. planiceps*, *A. chrysolepis*, *A. bombiceps*, and *A. tandai* do not present any extensive areas that were stable across all periods. Overall, stability areas for all species are concentrated in the Cerrado, the western portion of the Amazonia rainforest, and a small area in the state of Amapá near the Amazon River mouth (Fig. 4H).

DISCUSSION

In this study, we have investigated the evolutionary history, demographic dynamics, and spatial distribution of the *A. chrysolepis* species group. It diverged in the late Miocene and Pliocene, with lineages of *A. chrysolepis* and *A. tandai* having originated in the Pleistocene. Regarding demographic history, all the species exhibit populational retraction in the last 1 Myr, which may be correlated with glaciation cycles. However, *A. meridionalis* in the Cerrado and *A. scypheus* in Amazonia exhibited more stable demographic histories when compared to the other species codistributed in their respective biomes. Furthermore, ENM analysis identified zones of climatic stability in western Amazonia and in the core of the Cerrado, partially coincident with proposed refugia areas (Vanzolini and Williams 1970), which could explain the demographic trends recovered.

Our findings regarding the phylogenetic relationships within the *A. chrysolepis* species group largely align with previous studies (D'Angiolella *et al.* 2011). Two distinctive clades were identified: one composed of the Amazonian species *A. tandai* and *A. chrysolepis*, and another consisting of one of the Cerrado species, *A. brasiliensis*, and the Amazonian species *A. bombiceps*, *A. scypheus*, and *A. planiceps*. Our results, however, did not support the monophyly of the *A. chrysolepis* species group, showing *A. meridionalis* within a sister clade to the other species within the complex (Fig. 1A). Yet, it is important to highlight that such placement was not strongly supported by our analyses, indicating a more complex evolutionary history than previously understood and need of further genomic-level datasets and analyses to clarify the placement of *A. meridionalis* with respect to the *A. chrysolepis* species group.

Our analysis also uncovered extensive cryptic diversity, particularly within *A. meridionalis*, which we found to consist of six distinct, well-structured lineages with deep divergences times. This finding aligns with Guarnizo *et al.* (2016), who proposed that *A. meridionalis* is not one species but a complex of several undescribed species. Additionally, D'Angiolella *et al.* (2011) found morphological differences between *A. meridionalis* representatives in Bolivia, further supporting the existence of multiple potential species within this complex. By contrast, our analysis found only one lineage for *A. bombiceps* and *A. scypheus*, though this result is limited by the low availability of samples for each species. Therefore, further studies with more comprehensive datasets are essential to accurately define the limits of these lineages and explore their intraspecific structure.

Regarding divergence times, while Vanzolini and Williams (1970) postulated a Quaternary origin for Neotropical anoles, subsequent genus-level studies have suggested such diversification occurred well before the Pleistocene (Glor *et al.* 2001, Prates *et al.* 2015). Our evolutionary scenario supports an older origin for the *A. chrysolepis* species group. According to our results, *A. meridionalis* was probably the first species to diverge, during the Oligocene (~32 Mya), an epoch marked by a drop in atmospheric CO^2, leading to global climatic drying and cooling related to a global expansion of open biomes (Pound and Salzmann 2017, Azevedo *et al.* 2020, Antoine *et al.* 2021). This climatic shift probably influenced species diversification through altered fire regimes and environmental dynamics (Selkin *et al.* 2015).

Subsequently, the events related to the Miocene (~23–5 Mya) were pivotal for the diversification of the *A. chrysolepis* species group. During the early to mid-Miocene, the rising Central Andes significantly altered regional climates, increasing aridity and shifting seasonal precipitation patterns (Uba *et al.* 2006, Mulch *et al.* 2010), which might have led to the diversification of all the *A. meridionalis* lineages recovered by the present study. Later, from the late Miocene to the Pliocene transition, warmer climates and the expansion of savanna vegetation across South America further shaped the landscape (Pound *et al.* 2011, Spriggs *et al.* 2014, Azevedo *et al.* 2020) and might have led to the origin of the species *A. brasiliensis*.

The warming events of the Miocene might also have led to changes in habitat availability and species dispersal routes for *A. meridionalis* and *A. brasiliensis*. Both species show spatial diffusion during this period with early diversification of *A. meridionalis* occurring in the central Cerrado, with subsequent spatial diffusion primarily northwestards towards the Amazon basin and towards the southeast, and *A. brasiliensis* originating around 16 Mya around the Amazonia–Cerrado ecotonal zone and experiencing spatial diffusion eastwards towards the Caatinga.

Besides its origin in the Amazonia–Cerrado ecotonal zone, more details connect *A. brasiliensis* to the Amazonia biome. The species is closely related to the Amazonian species *A. scypheus*, *A. bombiceps*, and *A. planiceps* and is itself a species associated with forest riparian vegetation within the Cerrado. Therefore, it is feasible to infer that their common ancestor was probably also an Amazonian species. Additionally, palaeomodelling studies suggest that during the late Miocene, drier, savanna-like vegetation potentially extended its distribution to the eastern Amazon basin and the central and southern areas of the current Cerrado domain (Pound *et al.* 2011). This could have potentially aided the ancestor of *A. brasiliensis* in colonizing the Cerrado region yet maintaining an association with more mesic conditions.

The varying divergence times and positions within the phylogeny of the two Cerrado species suggest that it is possible that the *A. chrysolepis* species group had two events of colonization of the Cerrado: first with the open vegetation species *A. meridionalis*, and later with the riparian forest species *A. brasiliensis*. However, the timing and support of such a double colonization scenario is contingent on a better resolution and support of the phylogenetics placement of *A. meridionalis* (see above).

Regarding demography, our study revealed quite distinct demographic histories over the past few million years across the Cerrado species. We detected expansion signals for *A. brasiliensis*, at ~3 Mya, followed by a retraction around 1 Mya. In contrast, *A. meridionalis*, the open vegetation Cerrado species found in plateau areas, displayed a relatively stable population dynamic. These demographic changes are probably linked to the climatic cycles of the Pleistocene. It is also during the Pleistocene that we see a second event of spatial diffusion for both Cerrado species, which suggests that the climatic cycles of the Pleistocene could also have affected the species distribution.

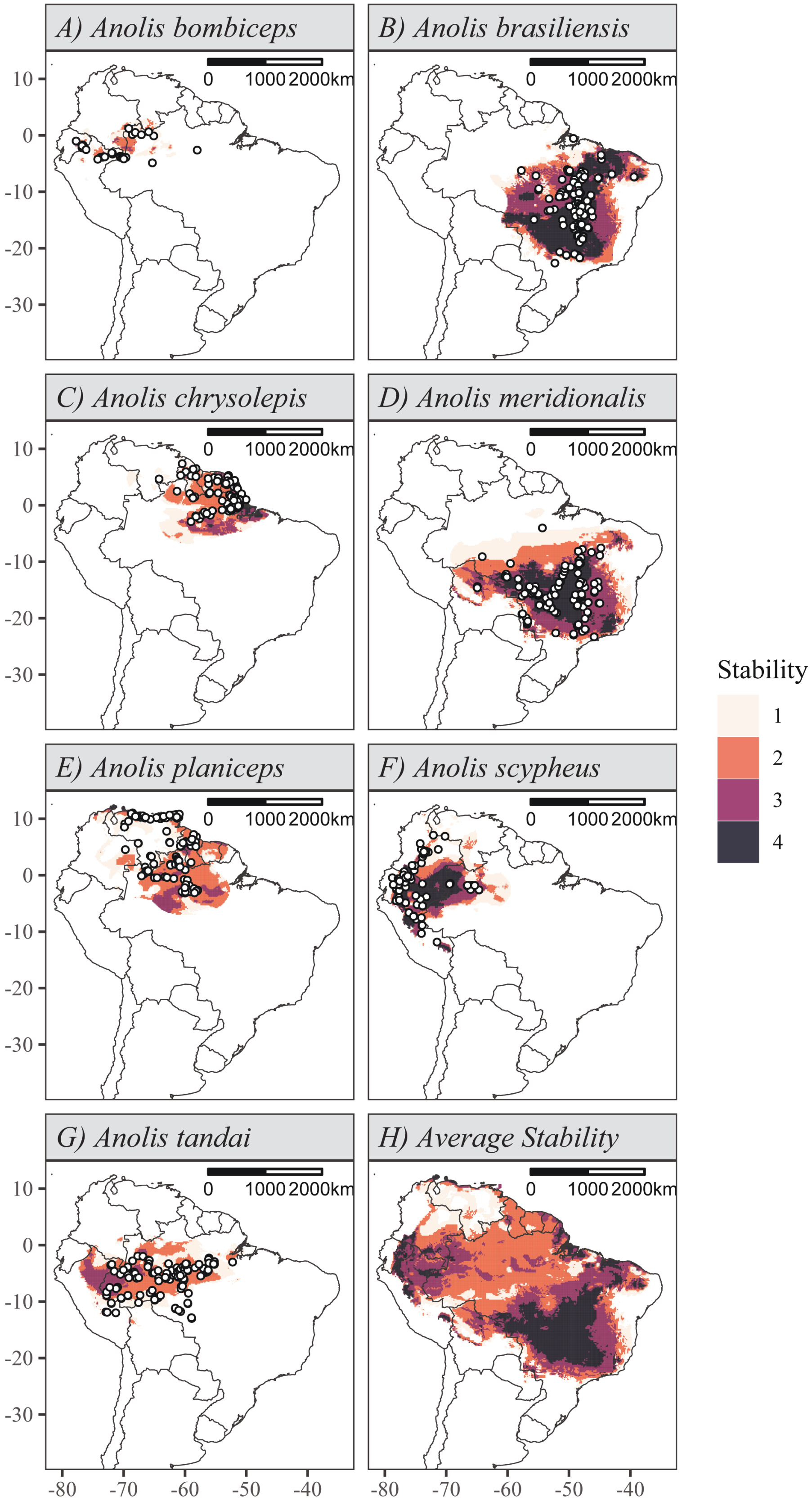

Figure 4. Stability area maps derived from paleomodeling results for seven species in the *Anolis chrysolepis* species group. Projections were made for the present, mid-Holocene (~6 k), Last Glacial Maximum (LGM, ~21k), and Last Interglacial (LIG, ~120k), and stacked, with results indicating the sum of the predicted suitable areas across all periods, representing the stability areas inferred for: A, *Anolis bombiceps*; B, *Anolis brasiliensis*; C, *Anolis chrysolepis*; D, *Anolis meridionalis*; E, *Anolis planiceps*; F, *Anolis scypheus*; G, *Anolis tandai*; H, the combined maps for all species across all periods, weighted by the species richness in each cell. The color scale indicates the number of studied periods for which the area remained stable. White dots indicate the current distribution of species.

During the drier periods of the Pleistocene, riparian vegetation in the Cerrado valleys retracted, while plateau areas maintained their savanna-like vegetation, indicating greater climatic stability (Werneck 2011, De Melo *et al.* 2016). Werneck (2011) proposed that these recent geological processes have led to riparian forests being characterized by species with weaker genetic structure, lower phylogeographical resolution, and evidence of population expansion. Conversely, species in plateau and open vegetation areas are expected to exhibit older diversification ages, higher genealogical structure, and greater genetic diversity due to the plateau's stable and ancient geological history. This was named the Plateau/Depression hypothesis. Our data support this hypothesis: *A. brasiliensis* is a younger, less structured species with signs of population size changes, while *A. meridionalis* is an older, more structured species with a lesser change in effective population size. Our palaeomodels for both *A. meridionalis* and *A. brasiliensis* indicate an increase in suitable areas across the Cerrado during the LGM, followed by a decrease in suitability during the Holocene, matching the current habitat suitability. This decrease in suitability areas in the Holocene probably explains the recent population retraction observed in both species.

The diversification of species within the Amazonia occurred concurrently with landscape and regional climate changes induced by the Andean orogeny (Hoorn *et al.* 2010). During the Miocene, uplift of the Andes intensified precipitation in the western Amazon. This, coupled with subsidence in certain areas and the rising sea levels that promoted marine water intrusions, formed an extensive wetland system characterized by shallow lakes and swamps called the Pebas system (Hoorn *et al.* 2010, 2022).

At around 11 Mya, when *A. bombiceps* and *A. scypheus* are believed to have diversified, the transition between the Pebas and Acre systems occurred, draining these wetlands and transforming the hydrological dynamics of the western Amazon. This shift may have acted as a diversification driver for taxa in both eastern and western Amazonia, potentially leading to the divergence of these species (Hoorn *et al.* 2010, 2022).

The divergence time of the sister species *A. chrysolepis* and *A. tandai* (~13 Mya), along with their geographical ranges—*A. chrysolepis* in the Guiana Shield and *A. tandai* in the Brazilian Shield—suggests a link to the formation of the Amazon River. The establishment of the transcontinental Amazon River probably facilitated population isolation and subsequent speciation, leading to the diversification of these sister species (Hoorn *et al.* 2010).

Spatial–temporal reconstruction showed that *A. chrysolepis* originated in the south of the Guiana Shield ~8 Mya and expanded its range to the north. *Anolis tandai*, on the other hand, originated in the Brazilian Shield at around 14 Mya, and its expansion was first eastwards and then it had a more radial-like expansion. These expansion events occurred in the last 1 Myr and are probably related to Pleistocene climatic cycles. Lastly, *A. planiceps* originated around 13 Mya and the three intraspecific lineages diversified during the late Miocene and early Pliocene, a period marked by climatic changes (Wesselingh and Salo 2006).

The demographic analyses for Amazonian forest species indicate that all species experienced population retraction in the past 1 Myr, although those in eastern Amazonia showed more pronounced declines. *Anolis chrysolepis* exhibited the most significant population retraction. Conversely, *A. scypheus*, found in western Amazonia, demonstrated relative demographic stability. The palaeomodels complement these findings. During the LGM all species in eastern Amazonia had very small suitability areas or they disappeared altogether. *Anolis scypheus* and *A. bombiceps*, however, two species distributed in western Amazonia, maintained relatively high suitability during the entire period observed.

Such results agree with the South American Precipitation Dipole findings of Cheng *et al.* (2013), and consequent demographic expectations for the affected biota. The quasidipole pattern indicates that eastern Amazonia experienced alternating dry and wet periods during the Pleistocene, resulting in climatic instability that may have caused population reductions and extinctions in eastern areas. In contrast, western Amazonia showed no significant climatic changes in the last 250 000 years, so the species inhabiting this region should have more stable demographic histories, as recovered here for Amazonian anoles.

We found three areas of historical climatic stability in South America indicating long-term suitability for anole species: one in western Amazonia, a second in the Cerrado, and, lastly, a small area in eastern Amazonia, near the Amazon River mouth. It is important to point out that these stability areas match three of the 'core refugia areas' of diversification proposed by Vanzolini and Williams (1970). In this way, although the refugia hypothesis does not explain the diversification of the group, it is still an important concept to understand the influence of the climatic cycles of the Pleistocene in the *A. chrysolepis* species group.

The study by Vanzolini and Williams (1970) on *A. chrysolepis* was conceived and published at a time when molecular biology was still in its infancy. At that time, they only had morphological characters at their disposal to understand the evolutionary process, which often did not allow them to distinguish intraspecific from interspecific variation. It is therefore natural that, using modern molecular analysis tools, our study points to very different conclusions. However, they were pioneers in focusing their work on neglected widely distributed species in the Amazon, which, as we now know, hide a diversity that is still unknown.

In summary, our dated historical reconstructions indicate that the divergence of the *A. chrysolepis* species group was driven by a series of geomorphological and climatic events over an extended period, ranging from the Oligocene throughout the Miocene, reaching the Pleistocene. The varied origins and evolutionary pathways of each species highlight the complexity of the group's evolutionary history. These findings underscore the importance of considering multiple factors and time periods when studying the evolutionary dynamics of species groups with a continent-wide distribution and contrasting ecological biomes.

CONCLUSIONS

This study has deepened our understanding of how historical climate changes and geological transformations have influenced biological diversity in the Cerrado and Amazonia, emphasizing the complex evolutionary and demographic dynamics of the *A.*

chrysolepis species group. Our findings reveal a phylogeny consistent with previous works and an uncertain placement for *A. meridonalis*. We found 40 distinct lineages and demonstrated varied patterns of diversification in response to climatic and geological changes that occurred mainly during the Miocene. Furthermore, our historical demographic analysis indicates patterns of demographic decline during the Pleistocene, while spatial–temporal diffusion shows considerable range expansion during the same period. This highlights the significant impact of Pleistocene climatic fluctuations on the *A. chrysolepis* species group. Palaeomodelling corroborates this by showing changes in habitat suitability for most species in the study during the Pleistocene. Additionally, the identification of climatic stability areas in this study aligns with the stability areas proposed by the refugia hypothesis.

Despite these advances, several questions remain unanswered. The uncertain phylogenetic placement of *A. meridionalis* suggests a complex evolutionary history that could be explored with more comprehensive genomic data, and high-resolution datasets. Additionally, morphological studies with taxonomic approaches should be employed to determine if the large number of lineages found represent cryptic species. Furthermore, given the ongoing climate crisis, studying how *Anolis* species might respond to current and future environmental changes could inform conservation efforts aimed at preserving biodiversity in these critical biomes.

In summary, these findings underscore the intricate interplay between climatic events and evolutionary processes, providing valuable insights into the historical factors that have shaped the biodiversity of the *A. chrysolepis* species group and reinforcing the importance of considering past environmental changes in understanding current biological diversity. However, much remains to be discovered, particularly regarding unresolved phylogenetic relationships of these species. Addressing these knowledge gaps will be crucial for fully understanding the evolutionary dynamics that continue to shape the rich biodiversity of the Neotropics.

SUPPLEMENTARY DATA

Supplementary data are available at *Biological Journal of the Linnean Society* online.

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CREDIT STATEMENT

A.P.V.O. (conceptualization, methodology, data curation, original draft preparation, visualization, reviewing and editing the manuscript), A.B.D'A. (conceptualization, methodology, data curation, supervision, reviewing and editing the manuscript), J.A.R.A. (conceptualization, methodology, visualization, supervision, reviewing and editing the manuscript), L.J.V. (conceptualization, reviewing and editing the manuscript), G.R.C. (conceptualization, reviewing and editing the manuscript), EL.C. (conceptualization, methodology, reviewing and editing the manuscript), T.G. (conceptualization, reviewing and editing the manuscript), P.P.G.T. (conceptualization, methodology, visualization, reviewing and editing the manuscript), M.T.R. (conceptualization, reviewing and editing the manuscript), B.N. (conceptualization, reviewing and editing the manuscript), T.C.S.A.-P. (conceptualization, reviewing and editing the manuscript), and F.P.W. (conceptualization, methodology, supervision, reviewing and editing the manuscript)

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

The data that support the findings of this study are derived from the following sources: 1. Published data from D'Angiolella *et al.* (2011), available at 10.3099/0027-4100-160.2.35. 2. Data from a forthcoming publication by Choueri *et al.* (in prep) will be made available upon publication.

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