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Spatio-temporal evolution of the catuaba clade in the Neotropics: Morphological shifts correlate with habitat transitions

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Abstract

Aim: The biotic assembly of one of the most species-rich savannas, the Brazilian Cerrado, has involved recruitment of lineages from several surrounding regions. However, we lack a clear understanding about the timing and pathways of biotic exchanges among these regions and about the role those interchanges had in the assembly of Neotropical biodiversity. We investigated the timing and routes of species movements between wet or seasonally dry habitats across Neotropical regions and assessed the potential for ecological adaptation by evaluating the habitat transitions correlated with morphological shifts.

Location: Neotropics.

Taxon: The plant genus *Anemopaegma* (Bignonieae, Bignoniaceae).

Methods: We inferred a Bayesian molecular phylogeny of *Anemopaegma* using one nuclear and two chloroplast markers. We sampled more than 90% of the known species diversity of *Anemopaegma*, covering its full geographical range. We estimated divergence times using a Bayesian relaxed-clock approach and inferred ancestral ranges as well as shifts in habitat and morphological characters.

Results: Phylogenetic analyses recovered seven main clades within *Anemopaegma*. The genus likely originated in Amazonia in the late Oligocene. Early-diverging lineages diversified *in situ* in Amazonia, particularly during the Miocene, with independent dispersal

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events to the Andes, Atlantic Forest and Cerrado. Shifts from seasonally dry forest to savanna habitats were correlated with shifts from liana to shrub and the loss of tendrils.

Main Conclusions: The timing of diversification of major lineages within *Anemopaegma* is consistent with major geological and climatic events that occurred during the late Palaeogene and Neogene, such as the Andean uplift and the Middle Miocene Climatic Optimum. Movements across different regions within the Neotropics were relatively common but shifts between habitats were not. The correlation in the evolution of the shrubby habit, the loss of tendrils and the shifts from forest to savanna are consistent with a scenario of ecological adaptation.

KEYWORDS

biogeography, Brazilian flora, Cerrado, divergence times, Neotropical biota, phylogeny, plants, trait evolution

1 | INTRODUCTION

The unevenness of Neotropical plant distribution patterns is remarkable (Antonelli & Sanmartín, 2011). As in other parts of the world, certain lineages are narrowly distributed and endemic to particular phytogeographic domains or biomes, while others are broadly distributed and shared among multiple regions (BFG, 2021; Gentry, 1982). These uneven patterns are determined by different factors such as life history traits, lineage evolution and ecology, as well as geological and climatic events that led to the formation of the regions where plant species occur (Hughes et al., 2013; Rull, 2011).

Recent studies have reported the importance of major geological and climatic events on the spatio-temporal evolution of Neotropical lineages, especially during the Palaeogene and Neogene (Antonelli et al., 2018; Hoorn et al., 2010). Among the most important geological and climatic events that have been associated with the diversification of species-rich lineages in the Neotropics are: (i) the formation of a dry corridor that separated the Amazonian and Atlantic forests since the Oligocene and eventually formed distinct, individual regions (i.e. Cerrado, Caatinga and Chaco) during the Neogene (Costa, 2003; Hoorn et al., 2010; Sobral-Souza et al., 2015); (ii) the Andean uplift, which started during the Palaeogene and intensified during the Oligocene, with the most intense peaks having occurred during the mid-late Miocene and early Pliocene (Garzione et al., 2008; Hoorn et al., 2010). The Andean orogeny led to drastic changes in the climate, hydrology and biomes of northern South America, including the formation of the Western Amazonian 'mega wetlands' (between 23 and 7 million years ago [Ma]; Bernal et al., 2019; Hoorn et al., 2010); (iii) the Mid-Miocene Climatic Optimum (MMCO; 17–15 Ma), an exceptionally warm period followed by global cooling associated with aridification and expansion of South American savannas and grasslands (Antonelli et al., 2015; Arakaki et al., 2011; Azevedo et al., 2020; Zachos et al., 2001); and (iv) the closure of the Central American Seaway and Panama Isthmus (between 15 and 3.5 Ma; Montes et al., 2012, 2015), which led to massive biotic interchanges between South and North America (Bacon et al., 2015a, 2015b).

All of these geological and climatic events have been associated with the creation of new habitats and niches that hugely impacted biotic movements among Neotropical regions (Antonelli et al., 2018; Fine & Lohmann, 2018). Shifts between regions have been suggested as a driver of plant evolution, in some cases leading to adaptation to new climates and/or habitats (Donoghue & Edwards, 2014). Neotropical habitats are highly heterogeneous, including several different forested habitats (e.g. tropical rain forests, seasonally dry topical forests) and non-forested, open habitats (e.g. Cerrado, Caatinga) (Fiaschi et al., 2016; Hughes et al., 2013). While biotic exchanges among similar habitats are expected, the high frequency of interchange between forested and open habitats is more intriguing given the significant changes in morphology and ecophysiology required (e.g. Bacon et al., 2017; Cássia-Silva et al., 2019; Freitas et al., 2019; Zizka et al., 2020). Nevertheless, further studies are needed to explain why some lineages respond by moving and others by adapting (Donoghue & Edwards, 2014).

The Cerrado is the most species-rich savanna in the world (Sarmiento, 1983) and harbours a biota that seems to have diversified in the late Miocene/early Pliocene (Simon et al., 2009; Simon & Pennington, 2012). Community assembly in this region involved recruitment of lineages from different surrounding habitats (Antonelli et al., 2018; Cássia-Silva et al., 2020; Simon et al., 2009). However, we still lack a clear understanding about the exact timing and routes of biotic exchanges among these regions (Fine & Lohmann, 2018) and a deep understanding of the role that those interchanges played for the assembly of Neotropical biodiversity. While a multi-taxon approach is certainly necessary to investigate broad patterns of biotic assembly, an accurate overall synthesis will only be achieved once detailed case studies focusing on well-sampled clades are available (Donoghue & Edwards, 2014; Fine & Lohmann, 2018; Hughes et al., 2013).

In this study, we use the plant genus *Anemopaegma* (Bignonieae, Bignoniaceae) to address the timing and routes of biotic exchanges within the Neotropics. *Anemopaegma* is a clade of lianas and shrubs that is broadly distributed across the Neotropics and composed of 46 species with varied levels of endemism in different Neotropical habitats (Firetti-Leggieri et al., 2015; Lohmann, 2003;

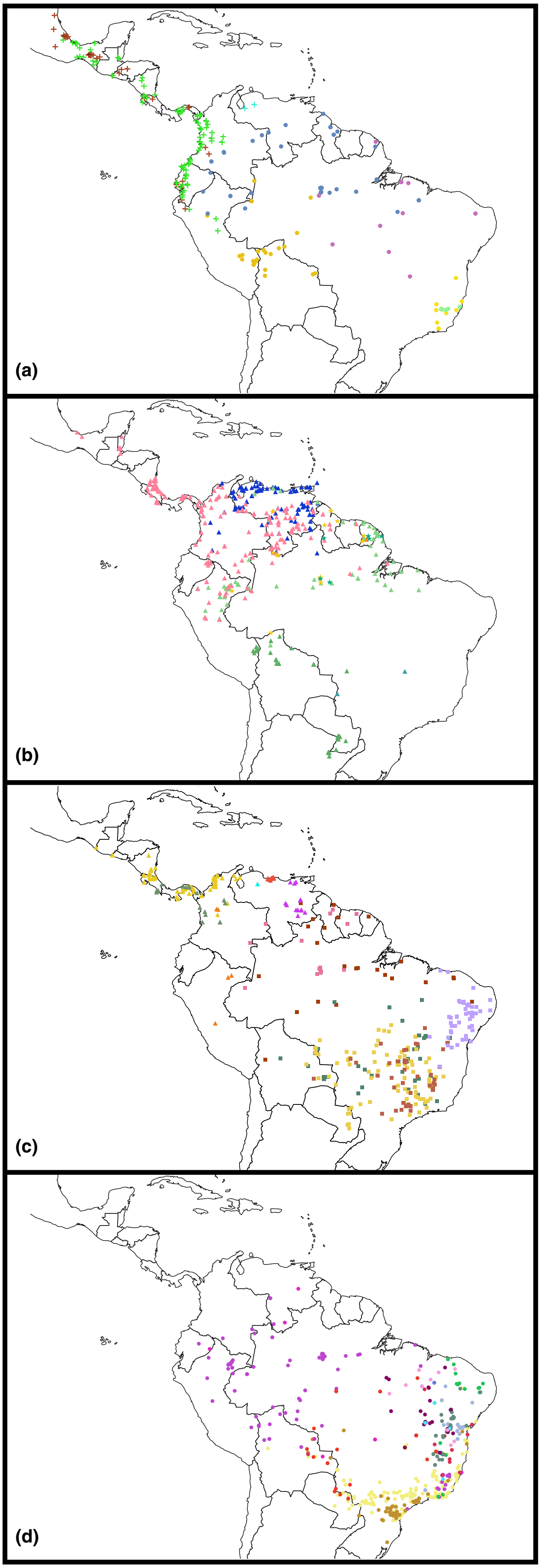

FIGURE 1 Distribution of the *Anemopaegma* species sampled in this study based on a dataset of georeferenced records compiled during herbarium work: (a) Clades 1 (cross) and 2 (circle); (b) Clades 3 (triangle) and 4 (star); (c) Clades 5 (square) and 6 (triangle); and (d) Clade 7 (circles). Clades according to Figure 2. A larger version of the maps and colour legends for each species can be found in Figure S1.1 of Appendix S1. Maps were generated using the software QGIS 3.10 (QGIS, 2019) and an EPSG:4326-WGS 84 projection

Lohmann, 2006; Lohmann et al., 2013; Lohmann & Taylor, 2014) (Figure 1, see Figure S1.1 of Appendix S1). Specifically, the genus includes 24 species that occur exclusively in wet forests, seven that are restricted to seasonal forests and seven that are endemic to savannas (Lohmann & Taylor, 2014). The genus further includes eight species that occur simultaneously in two different habitats, five of them occurring in wet and seasonal forests and three in seasonal forests and savannas. We expect that ancestral reconstructions will recover a higher number of lineage movements among ecologically similar Neotropical regions than among habitats, which would entail morphological shifts. Because shifts from wet forested habitats directly into savanna habitats require significant changes in morphology and ecophysiology and considering that there are no species within the genus that occur simultaneously in wet forests and savannas, we expect ancestral character state reconstructions to recover a pattern consistent with gradual shifts into savanna habitats, preceded by shifts into seasonally dry forested habitats. Our results expand our knowledge on the correlation of traits and the assembly of the Neotropical biota.

2 | MATERIALS AND METHODS

2.1 | The study group

The genus *Anemopaegma* includes 46 species that occur in forested habitats such as wet forests and seasonally dry topical forests, as well as open habitats such as the Cerrado and the Caatinga (Firetti-Leggieri et al., 2015; Lohmann & Taylor, 2014). While species of *Anemopaegma* share a similar flower morphology, species of this genus are diverse in terms of life-form (lianas and shrubs) and the presence or absence of tendrils. Specifically, the shrubby habit and lack of tendrils in some species are hypothesized to represent adaptations to open seasonally dry habitats (Lohmann, 2003) (see Figure S1.2 of Appendix S1).

Less than 10% of the *Anemopaegma* species diversity (5 of 46 species) were sampled within a study that aimed at reconstructing phylogenetic relationships among members of the tribe Bignonieae (Lohmann, 2006). In addition, whole chloroplast genome sequences have been used to study relationships among three species of the genus belonging to the *Anemopaegma arvense* species complex (Firetti et al., 2017). However, detailed studies based on a comprehensive sampling of taxa are still lacking.

2.2 | Taxon and molecular sampling

We sampled 42 species of *Anemopaegma*, representing about 90% of the diversity currently recognized (Firetti-Leggieri et al., 2015; Lohmann & Taylor, 2014). We also sampled members of the closely related *Mansoa* (8 of 12 species) and *Pyrostegia* (the two species recognized). Samples were collected during extensive fieldwork carried on by LGL, MFC and collaborators or taken from herbarium specimens. For the 52 taxa sampled, we generated new sequences of the *ndh*F chloroplast gene, the *rp*L32-*trn*L intergenic spacer and the *Pep*C nuclear gene. A complete list of the species sampled, specimen localities and vouchers is available in Table S1.1 of Appendix S1. We also used previously published *ndh*F and *Pep*C sequences available for members of the tribe Bignonieae and three other Bignoniaceae outgroups, matching the sampling used in Lohmann et al. (2013), which is referred to here as the Bignonieae dataset. Genomic DNA was isolated from silica-dried tissue or herbarium material using a modified CTAB DNA extraction protocol (Doyle & Doyle, 1987), or the DNeasy Plant DNA Extraction Kit (QIAGEN). For *Pep*C, two fragments with different lengths were often amplified; therefore, PCR products were separated by agarose gel electrophoresis, the larger band excised and used in cloning procedures, after purification, following the procedures described by Lohmann (2006). Whenever possible, up to four colonies per species were sequenced to ensure that the multiple copies of *Pep*C were evolving in concert. PCR amplification, cloning and sequencing followed Zuntini et al. (2013).

2.3 | Alignment and phylogenetic analyses

Multiple sequence alignment for each region was carried out in Geneious 6.1.6 (Biomatters Ltd.), using the Muscle algorithm with default settings (Edgar, 2004). We verified and edited the alignment manually in Geneious. Furthermore, jModelTest 0.1.1 (Darriba et al., 2012; Guindon & Gascuel, 2003) was used to select the best substitution model of DNA evolution for each data partition under the Akaike information criterion. The best-fitting model of DNA substitution for *ndh*F and *rp*L32-*trn*L was TVM + G + I, and for *Pep*C was GTR + G.

Bayesian phylogenetic analyses were performed in MrBayes 3.2 (Ronquist et al., 2012), on the Cipres Science Gateway (Miller et al., 2010), using Metropolis-coupled Markov chain Monte Carlo (MC^3). We ran two independent analyses, with four chains each (one cold and three heated), starting from random trees for 10×10^7 generations, sampling every 1000 generations. The convergence, the effective sample size and posterior distributions were examined in Tracer 1.6 (Rambaut et al., 2013), and by examining the potential scale reduction factor, with values approaching 1 as runs converged in MrBayes. To summarize the posterior probability of trees, we discarded 25% of the samples as burn-in and calculated a 50% majority rule consensus tree. Since preliminary analyses using the three markers separately and combined did not indicate significant differences between topologies (see Appendix S2), the chloroplast and nuclear datasets were concatenated in a data matrix that was used in the final phylogenetic inference.

2.4 | Calibration and divergence time estimation

We used fossil calibrations and a relaxed molecular clock approach to estimate the phylogeny and divergence times using Beast 1.7.5 (Drummond et al., 2012; Drummond & Rambaut, 2007). We used the Bignonieae dataset and the same fossils from Lohmann et al. (2013) as calibration points plus the newly generated data for *Anemopaegma*, *Pyrostegia* and *Mansoa*. The molecular dataset was partitioned by region, each analysed with its own evolutionary model as in the MrBayes analysis described above. To verify the effects of missing data and taxa, we performed analyses under different approaches and datasets to test for consistency among inferred ages (Appendix S3). The analyses were run using an uncorrelated lognormal relaxed molecular clock model and a birth–death prior on the distribution of branching times (Gernhard, 2008). The Markov chains Monte Carlo were run for 50 million generations, sampling every 1000th generation. After verifying that the effective sample sizes for all parameters had exceeded 200, and that chains had reached stationarity in Tracer, we used TreeAnnotator 1.7.5 to remove 10% of the trees as burn-in and to estimate mean node height and the 95% highest posterior density (HPD). All analyses were carried out in Cipres.

2.5 | Biogeographic analyses

We used a georeferenced dataset that includes 2985 records of *Anemopaegma*, *Mansoa* and *Pyrostegia* compiled by LGL from herbarium records; for a detailed description of the dataset see Narváez-Gomez et al. (2021). This dataset follows the generic delimitation and species circumscription of Lohmann and Taylor (2014).

We uploaded all georeferenced species localities into the QGIS software (http://www.qgis.org/) to plot the distribution of each species (Figure 1). With these distribution maps, information on topographic relief, Neotropical habitats and literature on bioregionalization (e.g. Cabrera & Willink, 1973; Morrone, 2014), we delimited 10 areas that best represented our study taxa (Figure 2), namely: (A) Atlantic Forest, (B) Interior Atlantic Forest, (C) Cerrado, (D) Caatinga, (E) Chaco, (F) Amazonia, (G) Andes, (H) Chocó, (I) Northern North-West Amazonia and (J) Mexico plus Central America. We used SpeciesGeoCoder (Töpel et al., 2017) to bin species occurrences into these 10 areas.

Ancestral ranges were inferred using the dispersal–extinction–cladogenesis model (DEC; Ree et al., 2005) implemented in the software Lagrange (C++ version; Ree & Smith, 2008). Differently from other models, DEC allows speciation via the biologically relevant mechanisms of widespread vicariance and subset sympatry to be

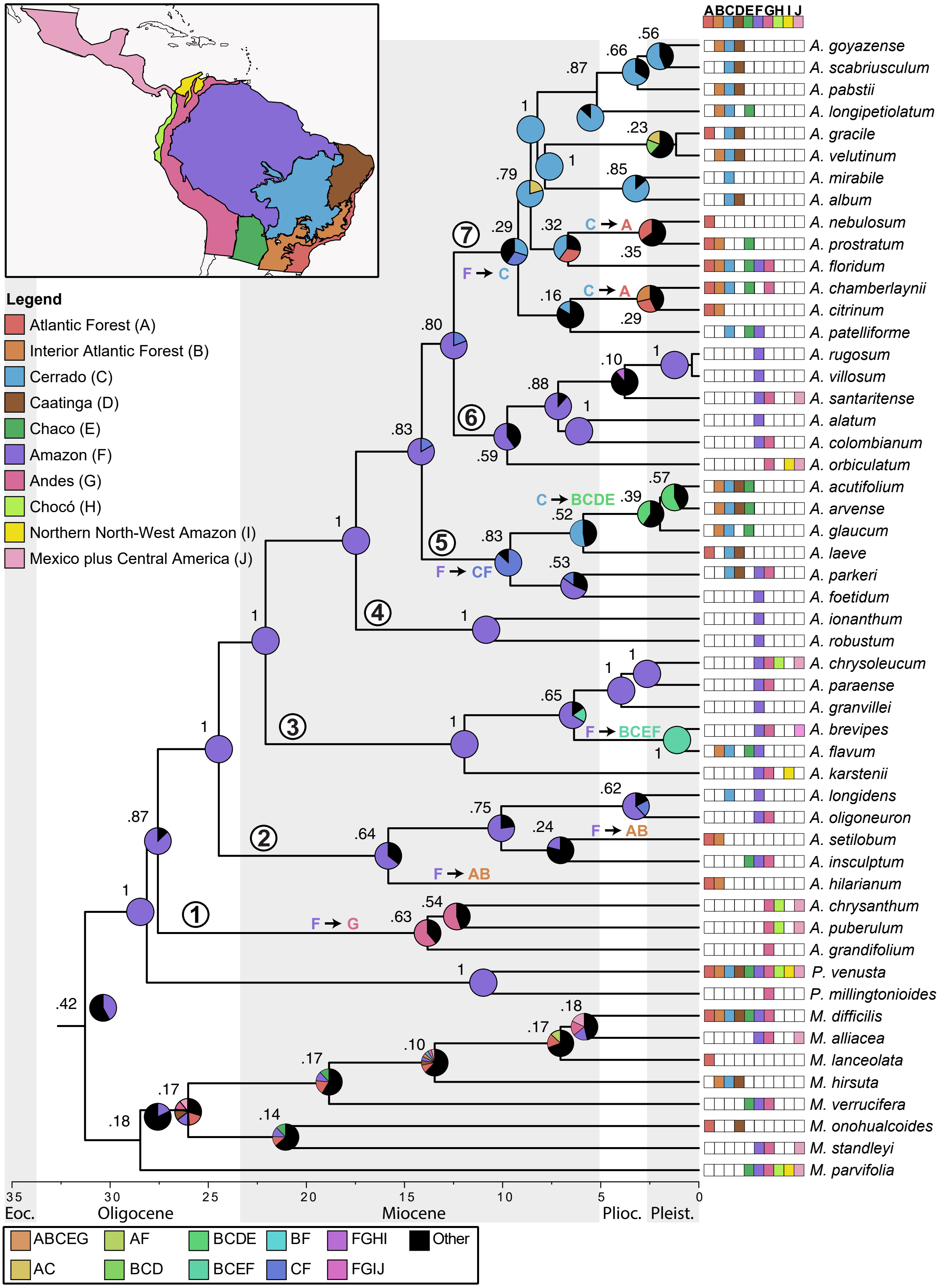

FIGURE 2 Trimmed Bayesian dated maximum clade credibility tree for *Anemopaegma* based on an uncorrelated lognormal relaxed clock with ancestral state estimates reconstructed using dispersal–extinction–cladogenesis model in LAGRANGE. Map shows the biogeographical regions used in this study. Coloured squares to the right of the tree indicate the geographical range of each extant species and those at the bottom of the tree indicate combined ranges. Pie charts represent the relative probabilities of ancestral ranges. Branches are labelled with posterior probability for the most likely ancestral state. Arrows indicate shifts between areas. Ancestral area probabilities <0.1 were combined (black sections of the pie charts). Circled numbers on branches indicate main clades discussed in the text

incorporated into the analyses. Tips of the tree were coded as the current distribution areas of each species. Except for *Pyrostegia venusta*, which is broadly distributed in all 10 areas, the *Anemopaegma* species sampled do not occur in more than six areas. To reduce the size of the instantaneous transition rate matrix and reflect the maximum number of areas occupied by the extant *Anemopaegma* species, we therefore set the maximum number of ancestral areas to six. We used the maximum clade credibility (MCC) tree generated in BEAST with the complete dataset (Bignonieae dataset plus the three newly sequenced markers), maintaining only species of *Anemopaegma*, *Mansoa* and *Pyrostegia* (see Appendix S3). The other Bignonieae genera were trimmed using BAYESRATE 1.5 (Silvestro et al., 2011; Silvestro & Schnitzler, 2011).

2.6 | Habitat and morphological character evolution

Although biomes and other large-scale biotic assemblages (such as bioregions; Antonelli, 2017; Vilhena & Antonelli, 2015) are useful for investigating geographic range evolution, they mask fine-scale habitat heterogeneity that can drive the occurrence and local adaptation of plant species. We therefore also estimated the ancestral habitats of *Anemopaegma* and inferred the evolution of two morphological characters that were hypothesized to be associated with habitat shifts (Lohmann, 2003), that is, life-form and tendrils. Based on information provided on herbarium labels, personal field observations and literature (Lohmann & Taylor, 2014; Pool, 2008), we categorized the habitats in which the species of *Anemopaegma*, *Mansoa* and *Pyrostegia* occur as: (0) wet forests, which are tall evergreen forests with a closed canopy and multiple strata. These forests include tropical rain forests and forests with lower rainfall, although soil moisture is constant via edaphic factors such as proximity to rivers (e.g. gallery forests) or water cycling (Dexter et al., 2018; Guan et al., 2015), (1) seasonally dry forests that include annually recurring drought stress, with many plants shedding their leaves during the dry season (Murphy & Lugo, 1986; Reich & Borchert, 1984) and (2) savannas, which are open habitats, with a tree component that does not form a closed canopy. Differently from seasonally dry forests, the savannas are covered by a significant grass component and its species are exposed to regular fires, which are key for the maintenance of its biodiversity (Abreu et al., 2017; Dexter et al., 2018; Durigan & Ratter, 2016; Parr et al., 2014; Simon & Pennington, 2012). Within *Anemopaegma*, species are generally restricted to a single habitat, although some species may be found in up to two different habitats as defined here. In these cases, we considered all applicable states when coding habitat types. We also categorized species according to two selected morphological characters: (i) life-form, with species being liana or shrub, and (ii) the presence or absence of tendrils.

We used BAYESRATE to extract a random set of 1000 trees from the post burn-in trees produced in the divergence time estimation with the complete dataset (see section Calibration and divergence time estimation and Appendix S3), and then trimmed all trees to include only species of *Anemopaegma*, *Mansoa* and *Pyrostegia*. As input for the ancestral habitat, life-form and tendril reconstructions, we used this set of 1000 trees and the trimmed MCC tree (the same used in the ancestral range reconstruction).

We performed ancestral state reconstructions using the 'corHMM' R package 1.22 (http://cran.r-project.org/web/packages/corHMM/; Beaulieu et al., 2013). We tested two models of trait evolution involving a different number of transition rates between character states. Specifically, we tested an equal-rates model (ER) and an all-rate-different matrix (ARD), in which each transition rate is optimized as an independent parameter. To select the best-fitting model, we calculated AICc using the distribution of trees and chose the model with the lowest average AICc. Based on the best model, we then calculated the mean, minimum and maximum probabilities of ancestral states for selected clades.

We used Pagel's (1994) method to test whether habitat and morphological traits are correlated. We conducted these analyses using the Discrete module implemented in BAYESTRAITS 3.0.2 (Pagel & Meade, 2019) under a Bayesian (MCMC) approach. Marginal log likelihoods were estimated for each run using stepping-stone sampling (Xie et al., 2011), with 100 steps, each run for 1000 iterations. We averaged the marginal log likelihoods across 10 independent runs and then calculated the log Bayes factor to compare the averages of marginal likelihoods between a complex (dependent) and a simple (independent) model. We considered the standard thresholds of 2 log BF higher than 6 as strong statistical support for the best model and higher than 10 as very strong statistical support for the best model (Kass & Raftery, 1995).

For the character reconstruction analysis, we categorized the habitat in three states: (i) wet forest, (ii) seasonally dry forest and (iii) savanna. The Discrete module only takes binary states, however. For this reason, for the correlation analyses, habitat states were regrouped in two different ways: (a) forest (including wet and seasonally dry forest states) and savanna; (b) wet habitat and seasonal habitat (including seasonally dry forest and savanna states). We analysed the following pairs of characters: (1) habitat (forest/savanna) and life-form (liana/shrub), (2) habitat (forest/savanna) and tendrils (presence/absence), (3) habitat (wet/seasonally dry) and life-form (liana/shrub), (4) habitat (wet/seasonally dry) and tendrils (presence/absence) and (5) life-form (liana/shrub) and tendrils (presence/absence).

3 | RESULTS

3.1 | Phylogenetic analyses

In total, new sequences from 40 taxa were newly generated for this study for the *ndh*F marker, 49 taxa for *rp*L32-*trn*L and 29 taxa for *PepC* (Table S1.1 of Appendix S1). The complete dataset included 42 species of *Anemopaegma*, eight species of *Mansoa* and two species of *Pyrostegia*, corresponding to 91%, 67% and 100% of the total species diversity of each genus respectively. The *ndh*F aligned dataset included 2104 nucleotides (nt), the *rp*L32-*trn*L included 1117 nt and the *Pep*C included 633 nt.

The Bayesian analysis with the three datasets concatenated inferred in MrBayes produced a well-resolved and strongly supported majority rule consensus tree (Figure S2.5 of Appendix S2). *Anemopaegma*, *Mansoa* and *Pyrostegia* form a strongly supported clade (posterior probability [pp] = 1.0). *Mansoa* is monophyletic (pp = 1.0) and sister to a clade that includes *Pyrostegia* and *Anemopaegma* (pp = 1.0). The two species of *Pyrostegia* were recovered as a clade (pp = 1.0) that is sister to a small clade, composed of three species of *Anemopaegma* (pp = 1.0), specifically *A. chrysanthum*, *A. grandifolium* and *A. puberulum* (Clade 1). The clade composed of *Pyrostegia* and three *Anemopaegma* species (pp = 0.99) is sister to the core *Anemopaegma* clade (pp = 1.0). The remaining *Anemopaegma* species, in turn, are divided into six strongly supported clades (all with pp = 1.0), referred here as Clades 2 to 7. The relationships among and within clades are generally strongly supported, except for four subclades within Clades 5, 6 and 7 that were poorly supported (Figure 2, Figure S2.5 of Appendix S2).

3.2 | Calibration and divergence time estimation

There was no well-supported incongruence between the results of the analyses using different datasets performed to test for consistency among inferred ages or for the effects of missing data and taxa (see Appendix S3). Comparisons between resulting ages for nodes of interest from the two approaches tested are summarized in Table S3.1 of Appendix S3. A trimmed MCC chronogram only including species from the three focal genera, based on the Bignonieae dataset (Lohmann et al., 2013) plus the dataset generated here, is shown in Figure S3.3 of Appendix S3.

The Beast analyses yielded a topology that is similar to that derived from the MrBayes analyses, differing on the most recent common ancestor (MRCA) of both *Anemopaegma* and *Pyrostegia* due to the constraint forcing to render *Anemopaegma* monophyletic (Figure 2, Appendix S3). The MRCA of the *Mansoa* + *Pyrostegia* + *Anemopaegma* clade dates to the Early Oligocene, about 31 Ma (95% HPD: 36.9–26.1 Ma). The estimated crown node ages for *Mansoa* and *Anemopaegma* are about 28 Ma (95% HPD: 34.1–23.2 Ma) and 27 Ma (95% HPD: 32.9–22.7 Ma), respectively, suggesting that these groups likely originated towards the end of the Early Oligocene. A late Miocene origin was inferred for the crown node of *Pyrostegia* (11 Ma, 95% HPD: 16.5–5.9 Ma) (Figure 2, see also Figure S3.3 in Appendix S3).

3.3 | Biogeographic analyses

Ancestral ranges estimated under the DEC model using Lagrange are depicted in Figure 2. The most likely areas and values of relative posterior probabilities (p) of the region for selected nodes are represented in Table S3.1 of Appendix S3. The most likely ancestral range for the MRCA of the *Mansoa* + *Pyrostegia* + *Anemopaegma* clade is Amazonia (*p* = 0.42). Amazonia was also recovered as the most likely area for the MRCA of *Pyrostegia* (*p* = 1) and *Mansoa* (*p* = 0.18, but *p* = 0.15 for Atlantic Forest). Nevertheless, the reconstructions close to the root were largely uncertain.

Biogeographic reconstructions within *Anemopaegma* were all well-supported. Amazonia was reconstructed as the most likely ancestral range for the MRCA of *Anemopaegma* (*p* = 1) during the Oligocene. Our results indicate that *Anemopaegma* most likely diverged *in situ* in Amazonia during the late Oligocene–Miocene, where its main lineages were formed. The first split in the genus gave rise to Clade 1, although diversification within this clade did not start until the middle Miocene (ca. 13 Ma), with a dispersal event from Amazonia to the Andes, which is the most likely ancestral range for the MRCA of Clade 1 (*p* = 0.63). Amazonia was inferred as the most likely ancestral area for the MRCA of most of the main clades, including Clades 2, 3, 4 and 6 (p between 0.60 and 1). Subsequent dispersal events occurred within these clades from Amazonia to other regions: within Clade 2 to the Interior Atlantic Forest (ca. 7 Ma), and within Clade 3 to the Interior Atlantic Forest, Cerrado and Chaco (ca. 6 Ma). Dispersal from Amazonia to the Cerrado occurred within Clades 5 and 7 (between 14 and 9 Ma). Within Clade 7, independent dispersal events occurred from the Cerrado to the Atlantic Forest (between 6 and 2 Ma). Further dispersal events, especially from Amazonia, the Andes and the Cerrado, also took place with the colonization of the other biogeographical areas (i.e. Atlantic Forest, Caatinga, Chaco, Chocó, Northern North-West Amazonia and Mexico plus Central America), leading to their current distribution patterns (Figure 2).

3.4 | Habitat and morphological character evolution

A comparison of the AIC scores calculated over the distribution of trees indicated that the ARD model better fit the character state distribution of the ancestral habitat and life-form reconstructions, while an ER model was a better fit for tendril reconstructions (Figure S4.1 of Appendix S4). Ancestral state reconstructions are depicted in Figure 3. The mean, minimum and maximum probabilities of each state inferred across the distribution of trees for selected clades are summarized in Table S4.1 of Appendix S4. Only nodes with high support in the Bayesian dating analysis conducted in BEAST are described and discussed. Our discussion therefore excludes the poorly supported relationships at the base of Clade 7. Instead, we evaluate and discuss shifts in habitats and life-forms for the strongly supported subclades.

Ancestral reconstructions of habitat type recovered equal mean probabilities (p) around 0.33 for all three states for the MRCA of the *Anemopaegma* + *Pyrostegia* + *Mansoa* clade (Figure 3a). Wet forests were reconstructed as the most likely character state for each genus individually, that is, *Mansoa* (*p* = 0.45), *Pyrostegia* (*p* = 0.80) and *Anemopaegma* (*p* = 0.47). Within *Anemopaegma*, two radiations from wet to seasonally dry forest were identified: one within Clade 3, resulting in the diversification of *A. brevipes* and *A. flavum* (p = 0.97), and another for the MRCA of clades 5, 6 and 7 (p = 0.40). Shifts from seasonally dry forest back to wet forest were inferred for the MRCA of *A. parkeri* and *A. foetidum* (p = 0.63), for the MRCA of Clade 6 (p = 0.59) and for the MRCA of *A. floridum*, *A. prostratum* and *A. nebulosum* (p = 0.48). Three shifts from seasonally dry forests to savannas were inferred for the MRCA of a subclade within Clade 5 (i.e. *A. leave*, *A. glaucum*, *A. acutifolium* and *A. arvense*; p = 0.53), within Clade 7 for the MRCA of *A. mirabile* and *A. album* (0.65) and for the MRCA of *A. longipetiolatum*, *A. pabstii*, *A. goyazense* and *A. scabriusculum* (p = 0.75; Figure 3a).

Ancestral reconstructions of life-form recovered liana as the most likely character state for the MRCA of each of the three genera individually, that is, *Mansoa* (p = 0.66), *Pyrostegia* (p = 0.93) and *Anemopaegma* (p = 0.67). Four shifts to shrubs were observed in Clade 5 within the subclade containing *A. glaucum*, *A. acutifolium* and *A. arvense* (p = 1) and in three subclades within Clade 7: (i) *A. album* + *A. mirabile* (p = 0.69), (ii) *A. gracile* + *A. velutinum* (p = 0.60) and (iv) *A. longipetiolatum* + *A. pabstii* + *A. goyazense* + *A. scabriusculum* (p = 0.55; Figure 3b).

The presence of tendrils was recovered as the most likely character state for the MRCA of each of the three genera individually, as well as for most of the nodes within the whole clade (p = 1). The absence of tendrils was recovered as the most likely state for the MRCA of the subclades containing *A. glaucum*, *A. acutifolium* and *A. arvense* (within Clade 5; p = 1), *A. album* plus *A. mirabile* (p = 0.59) and *A. goyazense* plus *A. scabriusculum* (p = 0.96; within Clade 7; Figure 3c).

For the correlation analysis between habitat (forest/savanna) and life-form (shrub/liana), the log Bayes factor comparing the complex (dependent) and the simple (independent) model was 21.26, indicating that shifts in habitat and life-form are strongly correlated. For the correlation analysis between habitat (forest/savanna) and tendrils, the log Bayes factor was 16.18. For the correlation analysis between habitat (wet/seasonally dry) and life-form, the log Bayes factor was 8.56. For the correlation analysis between habitat (wet/seasonally dry) and tendrils, the log Bayes factor was 6.43. For correlation analysis between life-form and tendrils, the log Bayes factor was 26.86. The detailed results of the analyses are listed in Table S4.2 of Appendix S4.

4 | DISCUSSION

Many studies have searched for explanations to current plant distribution patterns in the Neotropics by focusing on shifts between broadly defined regions (e.g. Antonelli et al., 2018; Fine & Lohmann, 2018). Other studies have focused on finer-scale dynamics, examining the frequency of interchange between forested and open habitats and the associated changes in morphology, anatomy and ecophysiology (Cássia-Silva et al., 2019; Kreft & Jetz, 2007; Liesenfeld et al., 2019; Simon & Pennington, 2012). In this study, we bridged the gap between these two approaches

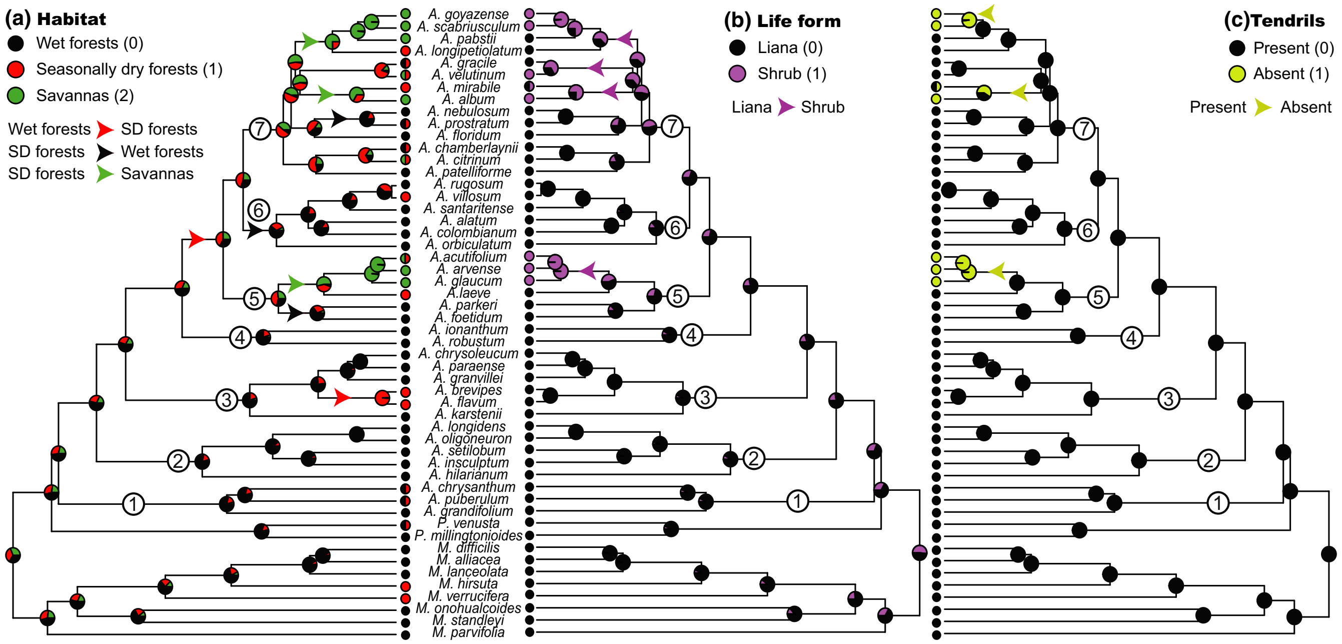

FIGURE 3 Ancestral character state reconstructions for *Anemopaegma* on the trimmed maximum credibility tree, reconstructed using 'corHMM' R package. Current states are shown at the tips of the trees. The inferred ancestral character states are shown for each node. Character states are indicated in the legend. Arrows indicate likely shifts between states (there is some uncertainty around their exact placement, as highlighted by the relative probabilities of the ancestral states shown in the pie charts at the nodes of the tree). (a) habitat; (b) life-form; and (c) tendrils. Circled numbers on branches indicate main clades discussed in the text

by reconstructing the phylogenetic relationships within *Anemopaegma* and inferring temporal, spatial and morphological patterns of evolution in this broadly distributed clade of Neotropical lianas and shrubs.

4.1 | Spatio-temporal evolution of *Anemopaegma*

Our ancestral range reconstructions suggest that *Anemopaegma* originated in Amazonia during the late Oligocene at ca. 32–22 Ma (Figure 2), a period of intensive global climatic cooling (Zachos et al., 2001). Between 65 and 33 Ma, the area known today as Amazonia was once part of a much larger 'pan-Amazonian' region, which once extended over most of northern South America (Hoorn et al., 2010). During this time, the breakup of the Pacific plate caused the uplift of the Andes (Garzione et al., 2008). This change in geography later led to intensive changes in the Amazonian landscape, triggering biotic diversification (Bernal et al., 2019; Hoorn et al., 2010; Jaramillo, Romero, et al., 2017; Wesselingh et al., 2010). Mountain building in the northern Andes intensified between 23 and 10 Ma (Hoorn et al., 2010). However, the most intense peaks of Andean orogeny followed during the late–middle Miocene (ca. 12 Ma) and early Pliocene (ca. 4 Ma; Hoorn et al., 2010), which is consistent with the diversification of the Andean Clade 1 (*A. chrysanthum* + *A. puberulum* + *A. puberulum*) at around 13 Ma. *Anemopaegma* lineages only colonized the Cerrado, Caatinga and Chaco after the MMCO (17–15 Ma), a period of higher temperature that was followed by global cooling, which led to the aridification and expansion of the savannas and grasslands in South America (Arakaki et al., 2011; Zachos et al., 2001).

Species within Clade 1 also occur in Mexico plus Central America and the Chocó, indicating dispersal events from the Andes to these areas after 13 Ma and providing additional support for the hypothesis of older biotic interchange between South and Central America (Bacon et al., 2015a, 2015b, 2016; Hoorn et al., 2010; Montes et al., 2015). Indeed, it has been estimated that the closure of the Central American Seaway may have occurred at around 15–13 Ma (Jaramillo, Montes, et al., 2017; Montes et al., 2015). While the fossil record indicates that mammal exchanges intensified at around 2.6–2.4 Ma (Woodburne, 2010), plant exchanges between South and Central America started at around 25 Ma (Bacon et al., 2013; Bacon et al., 2015a, 2015b; Thode et al., 2019). The other *Anemopaegma* species that colonized Mexico plus Central America seem to have originated in Amazonia and expanded into the Andes, after ca. 12 Ma (e.g. within Clades 3 and 6), without movements to this area from other regions.

The split between Clade 2 and the remaining lineages of *Anemopaegma* (Clades 3 to 7) was inferred at 24 Ma, during the late Oligocene, followed by *in situ* speciation in Amazonia during the Miocene. Dispersal to other areas occurred independently within each major clade, except for Clade 4, which only includes two Amazonian species. Diversification within Clade 2 involved a split between Amazonia and the Atlantic Forest, with the mean crown age at ca. 15 Ma. Species within this clade occur either in the Atlantic Forest or in Amazonia (sometimes extending into neighbouring areas such as the Cerrado, Andes and Chaco), but never in both. A similar pattern is observed within Clade 5, with one of its subclades being restricted to Amazonia and surrounding areas, and another restricted to the Atlantic Forest and neighbouring areas. These results suggest that the diversification within these clades might have been influenced by the formation of the dry corridor, which started in the Oligocene and progressed through the Neogene. Another interesting pattern within Clade 5 is that most of the Atlantic Forest species also occur in the Interior Atlantic Forest and other seasonally dry areas (i.e. Cerrado, Caatinga and Chaco), suggesting historical connections among these regions (Dexter et al., 2018).

Clade 3 diversified mostly within Amazonia at around 12 Ma, extending into north-western Amazonia (i.e. Andes, Chocó, Northern North-West Amazonia and Mexico plus Central America). One exception is *A. flavum*, which occurs in Amazonia and dispersed to eastern seasonally dry regions (i.e. Interior Atlantic Forest, Cerrado and Chaco). The diversification of this clade may be associated with the Andean orogeny, which had the most intense peaks during the late-mid Miocene (ca. 12 Ma) and early Pliocene (ca. 4.5). Andean uplift had a profound impact on the geology and climate, resulting in water and sediment deposition and modification of river systems and regional habitats in South America (Antonelli et al., 2018; Hoorn et al., 2010).

The split between Clades 6 and 7 at ca. 12 Ma involved a divergence between north-western South America (Clade 6) and eastern South America (Clade 7). More specifically, Clade 6 may have diversified *in situ* in Amazonia and later expanded into the Andes, Northern north-western Amazonia and Mexico plus Central America. In contrast, Clade 7 diversified mainly in the Atlantic Forest and in the seasonally dry areas (i.e. Interior Atlantic Forest, Cerrado, Caatinga and Chaco). Speciation within Clade 7 occurred mostly *in situ* in the Cerrado with subsequent movements to other regions, especially to the Atlantic Forest and Interior Atlantic Forest. Within Clade 7, some species are restricted to moist areas, including the Amazonian and Atlantic Forest, while others occur in seasonally dry areas, such as the Cerrado, Caatinga and Interior Atlantic Forest. The current distribution of *Anemopaegma* species appears to be related to niche divergence between species occurring in seasonally dry or wet regions.

Within *Anemopaegma*, three independent dispersals from Amazonia to the Cerrado coincide with the global increase in savanna biomes since the late Miocene (Orme, 2007; Simon et al., 2009), namely species movements within Clade 7 (12–9 Ma), Clade 5 (14–9 Ma) and Clade 3 (6–1 Ma). As aridity increased in the Oligocene, the belt of xeromorphic formations between Amazonia and the Atlantic Forest, which includes the Caatinga, Cerrado and Chaco regions (Bigarella et al., 1975), started to separate the once continuous Neotropical forest (Sobral-Souza et al., 2015). This belt, which is also known as the 'South American Dry Diagonal' or 'major South American disjunction' (Brieger, 1969), potentially played an important role in the diversification of *Anemopaegma*.

4.2 | Habitat and morphological evolution of *Anemopaegma*

We used *Anemopaegma* to investigate the timing and routes of biotic exchanges within the Neotropics. We expected that ancestral reconstructions would indicate more movements among Neotropical regions than shifts in habitat and morphology. While uncertainties remain on the extant timing and topological placement of the transitions, we observed at least nine shifts or range expansions among Neotropical regions, supporting our expectations. We further observed eight habitat shifts, four shifts from liana to shrub and three tendril losses. Shifts from forest into savanna habitats were expected to be preceded by shifts into seasonally dry forested habitats. Our habitat reconstruction did not infer any direct shifts from wet forest to savanna but identified two shifts from wet forest to seasonally dry forest, which also supports our expectations. Namely, shifts from wet forest to seasonally dry forests were recovered within Clade 3 [6–1 Ma] and at the divergence of Clades 5–7 [17–14 Ma], followed by three shifts from seasonally dry forest to savanna, one within Clade 5 [9–5 Ma] and two within Clade 7 [9–3 and 9–5 Ma] (Figure 3a).

The timing of radiation within the savanna habitat by *Anemopaegma* species corroborates earlier findings that suggested that Cerrado lineages originated less than 10 Ma, coinciding with the expansion of savanna biomes worldwide (Simon et al., 2009). Furthermore, three reversals from seasonally dry forest back to wet forest were also inferred within Clade 5 [9–6 Ma], Clade 6 [12–9 Ma] and Clade 7 [6–2 Ma]. The idea that connections between Amazonian and Atlantic forests were available through humid patches or gallery forests that crossed the Cerrado, Chaco and Caatinga (Costa, 2003; Sobral-Souza et al., 2015) is consistent with the patterns reconstructed within *Anemopaegma*, where forests may have acted as dispersal routes, allowing for range expansions across different Neotropical regions.

Shifts in life-form, specifically from liana to shrub, the loss of tendrils and shifts from forest to savanna habitats were reconstructed mostly for the same lineages (Figures 3a–c). The correlation analyses indicated that these traits have all been strongly correlated throughout their evolutionary history. Other studies have associated movements to the Cerrado with the acquisition of innovations, in particular traits associated with fire and drought resistance, rather than dispersal of lineages pre-adapted to these conditions (Simon et al., 2009; Simon & Pennington, 2012). Given that most habitats within the Cerrado usually do not form a closed canopy, the loss of tendrils and switch from a liana to a shrubby habit is important for colonizing savanna habitats. Hence, our results are compatible with a scenario of ecological adaptation. Moreover, traits that were favoured when lineages shifted from wet to seasonally dry forest may have functioned as pre-adaptations, subsequently enabling a further shift to the harsher savanna habitat (Donoghue, 2008).

5 | CONCLUSIONS

Overall, our results indicate that the orogeny of the Andes and the formation of the 'Dry Diagonal' that separated Amazonia and the Atlantic Forest played an important role in the diversification history of *Anemopaegma*. Movements across different regions within the Neotropics were relatively common, while shifts between habitats were rare. The fact that shifts to the savanna were preceded by shifts to seasonally dry forest habitat indicates that the acquisition of preadaptive traits likely enabled subsequent shifts to savanna. The evolution of the shrubby habit and loss of tendrils, along with shifts to savanna habitats, are consistent with a scenario of ecological adaptation.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The molecular data used in this study are available in Genbank (see Table S1.1 for accession numbers).

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REFERENCES

Abreu, R. C. R., Hoffmann, W. A., Vasconcelos, H. L., Pilon, N. A., Rossatto, D. R., & Durigan, G. (2017). The biodiversity cost of carbon sequestration in tropical savanna. *Science Advances*, *3*, e1701284.

Antonelli, A. (2017). Biogeography: Drivers of bioregionalization. *Nature Ecology and Evolution*, *1*, 0114.

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

Antonelli, A., Zizka, A., Carvalho, F. A., Scharn, R., Bacon, C. D., Silvestro, D., & Condamine, F. L. (2018). Amazonia is the primary source of Neotropical biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 6034–6039.

Antonelli, A., Zizka, A., Silvestro, D., Scharn, R., Cascales-Miñana, B., & Bacon, C. D. (2015). An engine for global plant diversity: highest evolutionary turnover and emigration in the American tropics. *Frontiers in Genetics*, *6*, 130.

Arakaki, M., Christin, P.-A., Nyffeler, R., Lendel, A., Eggli, U., Ogburn, R. M., Spriggs, E., Moore, M. J., & Edwards, E. J. (2011). Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 8379–8384.

Azevedo, J. A. R., Collevatti, R. G., Jaramillo, C. A., Strömberg, C. A. E., Guedes, T. B., Matos-Maraví, P., Bacon, C. D., Carillo, J. D., Faurby, S., & Antonelli, A. (2020). On the young savannas in the land of ancient forests. In V. Rull & A. C. Carnaval (Eds.), *Neotropical diversification: patterns and processes* (pp. 271–298). Springer.

Bacon, C. D., Molnar, P., Antonelli, A., Crawford, A. J., Montes, C., & Vallejo-Pareja, M. C. (2016). Quaternary glaciation and the Great American Biotic Interchange. *Geology*, *44*, 375–378.

Bacon, C. D., Mora, A., Wagner, W. L., & Jaramillo, C. A. (2013). Testing geological models of evolution of the Isthmus of Panama in a phylogenetic framework. *Botanical Journal of the Linnean Society*, *171*, 287–300.

Bacon, C. D., Moraes, M., Jaramillo, C., & Antonelli, A. (2017). Endemic palm species shed light on habitat shifts and the assembly of the Cerrado and Restinga floras. *Molecular phylogenetics and evolution*, *110*, 127–133.

Bacon, C. D., Silvestro, D., Jaramillo, C. A., Smith, B. T., Chakrabarty, P., & Antonelli, A. (2015a). Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 6110–6115.

Bacon, C. D., Silvestro, D., Jaramillo, C. A., Smith, B. T., Chakrabarty, P., & Antonelli, A. (2015b). Reply to Lessios and Marko et al.: Early and progressive migration across the Isthmus of Panama is robust to missing data and biases. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, E5767–E5768.

Beaulieu, J. M., O'Meara, B. C., & Donoghue, M. J. (2013). Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in Campanulid Angiosperms. *Systematic Biology*, *62*, 725–737.

Bernal, R., Bacon, C. D., Balslev, H., Hoorn, C., Bourlat, S. J., Tuomisto, H., Salamanca, S., van Manen, M. T., Romero, I., Sepulchre, P., & Antonelli, A. (2019). Could coastal plants in western Amazonia be relicts of past marine incursions? *Journal of Biogeography*, *46*, 1749–1759.

BFG, The Brazil Flora Group. (2021). Brazilian Flora 2020: Leveraging the power of a collaborative scientific network. *Taxon*, *71*, 178–198.

Bigarella, J. J., Andrade-Lima, D., & Riehs, P. J. (1975). Considerações a respeito das mudanças paleoambientais na distribuição de algumas espécies vegetais e animais no Brasil. *Anais da Academia Brasileira de Ciências*, *47*, 411–464.

Brieger, F. G. (1969). Patterns of evolutionary and geographical distribution in neotropical orchids. *Biological Journal of the Linnean Society*, *1*, 197–217.

Cabrera, A. L., & Willink A. (1973). Biogeografía de América Latina. Monografía 13, Serie de Biología. Secretariat General OEA, Washington, D.C., USA.

Cássia-Silva, C., Cianciaruso, M. V., Dias, P. A., Freitas, C. F., Souza-Neto, A. C., & Collevatti, R. G. (2020). Among cradles and museums: seasonally dry forest promotes lineage exchanges between rain forest and savanna. *Plant Ecology & Diversity*, *13*, 1–13.

Cássia-Silva, C., Freitas, C. G., Alves, D. M. C. C., Bacon, C. D., & Collevatti, R. G. (2019). Niche conservatism drives a global discrepancy in palm species richness between seasonally dry and moist habitats. *Global Ecology and Biogeography*, *28*, 814–825.

Costa, L. P. (2003). The historical bridge between the Amazon and the Atlantic forest of Brazil; a study of molecular phylogeography with small mammals. *Journal of Biogeography*, *30*, 71–86.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*, 772.

Dexter, K. G., Pennington, R. T., Oliveira-Filho, A. T., Bueno, M. L., Silva de Miranda, P. L., & Neves, D. M. (2018). Inserting tropical dry forests into the discussion on biome transitions in the tropics. *Frontiers in Ecology and Evolution*, *6*, 104.

Donoghue, M. J. (2008). A phylogenetic perspective on the distribution of plant diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 11549–11555.

Donoghue, M. J., & Edwards, E. J. (2014). Biome shifts and niche evolution in plants. *Annual Review of Ecology, Evolution, and Systematics*, *45*, 547–572.

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin*, *19*, 11–15.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, *7*, 214.

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

Durigan, G., & Ratter, J. A. (2016). The need for a consistent fire policy for Cerrado conservation. *Journal of Applied Ecology*, *53*, 11–15.

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

Fiaschi, P., Pirani, J. R., Heiden, G., & Antonelli, A. (2016). Floristic biogeography of South America. In C. J. B. de Carvalho & E. A. B. Almeida (Eds.), *Biogeografia da América do Sul / Análise de Tempo, Espaço e Forma* (pp. 215–226). RJ: Roca.

Fine, P. A. V., & Lohmann, L. G. (2018). The importance of dispersal in the assembly of the Neotropical biota. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 5829–5831.

Firetti, F., Zuntini, A. R., Gaiarsa, J. W., Oliveira, R. S., Lohmann, L. G., & Van Sluys, M. A. (2017). Complete chloroplast genome sequences contribute to plant species delimitation: A case study of the *Anemopaegma* species complex. *American Journal of Botany*, *104*, 1493–1509.

Firetti-Leggieri, F., Demarco, D., & Lohmann, L. G. (2015). A new species of *Anemopaegma* (Bignonieae, Bignoniaceae) from the Atlantic Forest of Brazil. *Phytotaxa*, *219*, 174–182.

Freitas, C. G., Bacon, C. D., Souza-Neto, A. C., & Collevatti, R. G. (2019). Adjacency and area explain species bioregional shifts in Neotropical palms. *Frontiers in Plant Science*, *10*, 55.

Garzione, C. N., Hoke, G. D., Libarkin, J. C., Withers, S., MacFadden, B., Eiler, J., Ghosh, P., & Mulch, A. (2008). Rise of the Andes. *Science*, *320*, 1304–1307.

Gentry, A. H. (1982). Neotropical floristic diversity: Phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden*, *69*, 557–593.

Gernhard, T. (2008). The conditioned reconstructed process. *Journal of Theoretical Biology*, *253*, 769–778.

Guan, K., Pan, M., Li, H., Wolf, A., Wu, J., Medvigy, D., Caylor, K. K., Sheffield, J., Wood, E. F., Malhi, Y., Liang, M., Kimball, J. S., Saleska, S. R., Berry, J., Joiner, J. & Lyapustin, A. I. (2015). Photosynthetic seasonality of global tropical forests constrained by hydroclimate. *Nature Geoscience*, *8*, 284–289.

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*, 696–704.

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

Hughes, C. E., Pennington, R. T., & Antonelli, A. (2013). Neotropical plant evolution: Assembling the big picture. *Botanical Journal of the Linnean Society*, *171*, 1–18.

Jaramillo, C., Montes, C., Cardona, A., Silvestro, D., Antonelli, A., & Bacon, C. D. (2017). Comment (1) on "Formation of the Isthmu s of Panama" by O'Dea et al. *Science Advances*, *3*, e1602321.

Jaramillo, C., Romero, I., D'Apolito, C., Bayona, G., Duarte, E., Louwye, S., Escobar, J., Luque, J., Carrillo-Briceño, J. D., Zapata, V., Mora, A., Schouten, S., Zavada, M., Harrington, G., Ortiz, J., & Wesselingh, F. P. (2017). Miocene flooding events of western Amazonia. *Science Advances*, *3*,e1601693.

Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, *90*, 773–795.

Kreft, H., & Jetz, W. (2007). Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 5925–5930.

Liesenfeld, V., Gentz, P., De Freitas, E. M., & Martins, S. (2019). Leaf morphology and anatomy of Asteraceae of the Pampas biome (sandfields). *Flora*, *258*, 151418.

Lohmann, L. G. (2003). Phylogeny, classification, morphological diversification and biogeography of Bignonieae (Bignoniaceae). PhD thesis, University of Missouri-St. Louis, USA.

Lohmann, L. G. (2006). Untangling the phylogeny of neotropical lianas (Bignonieae, Bignoniceae). *American Journal of Botany*, *93*, 304–318.

Lohmann, L. G., Bell, C., Calió, M. F., & Winkworth, R. (2013). Pattern and timing of biogeographic history in the Neotropical tribe Bignonieae (Bignoniaceae). *Botanical Journal of the Linnean Society*, *171*, 154–170.

Lohmann, L. G., & Taylor, C. M. (2014). A new generic classification of tribe Bignonieae (Bignoniaceae). *Annals of the Missouri Botanical Garden*, *99*, 348–489.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Institute of Electrical and Electronics Engineers (Ed.), *Proceedings of the gateway computing environments workshop (GCE)* (pp. 1–8). New Orleans, Louisiana: IEEE Xplor.

Montes, C., Cardona, A., Jaramillo, C., Pardo, A., Silva, J. C., Valencia, V., Ayala, C., Pérez-Angel, L. C., Rodriguez-Parra, L. A., Ramirez, V., & Niño, H. (2015). Middle Miocene closure of the Central American Seaway. *Science*, *384*, 226–228.

Montes, C., Cardona, A., McFadden, R., Morón, S. E., Silva, C. A., Restrepo-Moreno, S., Ramírez, D. A., Hoyos, H., Wilson, J., Farris, D., Bayona, G. A., Jaramillo, C. A., Valencia, V., Bryan, J., & Flores, J. A. (2012). Evidence for middle Eocene and younger land emergence in central Panama: Implications for Isthmus closure. *Geological Society of America Bulletin*, *124*, 780–799.

Morrone, J. J. (2014). Biogeographical regionalisation of the Neotropical region. *Zootaxa*, *3782*, 1–110.

Murphy, P. G., & Lugo, A. E. (1986). Ecology of tropical dry forest. *Annual Review of Ecology and Systematics*, *17*, 67–88.

Narváez-Gomez, J. P., Guedes, T. B., & Lohmann, L. G. (2021). Recovering the drivers of sampling bias in Bignonieae (Bignoniaceae) and identifying priority areas for new survey efforts. *Biodiversity and Conservation*, *30*, 2319–2339.

Orme, A. R. (2007). Tectonism, climate, and landscape change. In T. T. Veblen, K. R. Young, & A. R. Orme (Eds.), *The Physical Geography of South America* (pp. 23–44). Oxford University Press.

Pagel, M. (1994). Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society B. Biological Sciences*, *255*, 37–45.

Pagel, M., & Meade, A. (2019). BayesTraits, version 3.0.2. Available at http://www.evolution.rdg.ac.uk/BayesTraitsV3.0.2/BayesTraitsV3.0.2.html

Parr, C. L., Lehmann, C. E., Bond, W. J., Hoffmann, W. A., & Andersen, A. N. (2014). Tropical grassy biomes: misunderstood, neglected, and under threat. *Trends in Ecology and Evolution*, *29*, 205–213.

Pool, A. (2008). A revision of the genus *Pyrostegia* (Bignoniaceae). *Annals of the Missouri Botanical Garden*, *95*, 495–510.

QGIS Development Team (2019). QGIS Geographic Information System, version 3.10. Open Source Geospatial Foundation Project. Available at http://qgis.osgeo.org

Rambaut, A., Suchard, M., & Drummond, A. (2013). Trace v1.6. http://tree.bio.ed.ac.uk/software/tracer

Ree, R. H., Moore, B. R., Webb, C. O., & Donoghue, M. J. (2005). A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, *59*, 2299–3231.

Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, *57*, 4–14.

Reich, P. B., & Borchert, R. (1984). Water stress and tree phenology in a tropical dry forest in the lowlands of Costa Rica. *Journal of Ecology*, *72*, 61–74.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542.

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

Sarmiento, G. (1983). The savannas of tropical America. In Bourlière, F. (Ed.), *Ecosystems of the World: tropical savannas* (pp. 245–288). Elsevier.

Silvestro, D., & Schnitzler, J. (2011). BayesRate: Bayesian estimation of diversification rates. http://sourceforge.net/projects/bayesrate.

Silvestro, D., Schnitzler, J., & Zizka, G. (2011). A Bayesian framework to estimate diversification rates and their variation through time and space. *BMC Evolutionary Biology*, *11*, 311.

Simon, M. F., Grether, R., de Queiroz, L. P., Skema, C., Pennington, R. T., & Hughes, C. E. (2009). Recent assembly of the Cerrado, a Neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 20359–20364.

Simon, M. F., & Pennington, R. T. (2012). Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado. *International Journal of Plant Sciences*, *173*, 711–723.

Sobral-Souza, T., Lima-Ribeiro, M. S., & Solferini, V. N. (2015). Biogeography of Neotropical Rainforests, past connections between Amazon and Atlantic Forest detected by ecological niche modeling. *Evolutionary Ecology*, *29*, 643–655.

Thode, V. A., Sanmartín, I., & Lohmann, L. G. (2019). Contrasting patterns of diversification between Amazonian and Atlantic forest clades of Neotropical lianas (*Amphilophium*, Bignonieae) inferred from plastid genomic data. *Molecular Phylogenetics and Evolution*, *133*, 92–106.

Töpel, M., Zizka, A., Calió, M. F., Scharn, R., Silvestro, D., & Antonelli, A. (2017). SpeciesGeoCoder: Fast categorization of species occurrences for analyses of biodiversity, biogeography, ecology and evolution. *Systematic Biology*, *66*, 145–151.

Vilhena, D., & Antonelli, A. (2015). A network approach for identifying and delimiting biogeographical regions. *Nature Communications*, *6*, 6848.

Wesselingh, F. P., Hoorn, C., Kroonenberg, S. B., Antonelli, A., Lundberg, J. G., Vonhof, H. B., & Hooghiemstra, H. (2010). On the origin of Amazonian landscapes and biodiversity: a synthesis. In C. Hoorn & F. P. Wesselingh (Eds.), *Amazonia, Landscape and Species Evolution* (pp. 421–431). Blackwell Publishing.

Woodburne, M. O. (2010). The Great American Biotic Interchange, dispersals, tectonics, climate, sea level and holding pens. *Journal of Mammalian Evolution*, *17*, 245–264.

Xie, W., Lewis, P. O., Fan, Y., Kuo, L., & Chen, M.-H. (2011). Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, *60*, 150–160.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to Present. *Science*, *292*, 686–693.

Zizka, A., Carvalho-Sobrinho, J. G., Pennington, R. T., Queiroz, L. P., Alcantara, S., Baum, D. A., Bacon, C. D., & Antonelli, A. (2020). Transitions between biomes are common and directional in Bombacoideae (Malvaceae). *Journal of Biogeography*, *47*, 1310–1321.

Zuntini, A. R., Fonseca, L. H. M., & Lohmann, L. G. (2013). Primers for phylogeny reconstruction in Bignonieae (Bignoniaceae) using herbarium samples. *Applications in Plant Sciences*, *1*, 1300018.

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Author contributions: MFC and LGL designed the research and collected the samples. LGL identified all samples and compiled the database with morphological data and georeferenced occurrence data. MFC carried out the molecular work. MFC, VAT and DS performed the analyses. MFC, VAT, CDB, DS, AA and LGL interpreted the results. MFC led the writing of the manuscript with contributions from all authors. All authors have read and approved the final version of the manuscript.

SUPPORTING INFORMATION

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