diversity

MDPI

Article

Molecular Phylogeny and Historical Biogeography of *Byrsonima* (Malpighiaceae) Corroborates the Mid-Miocene Origins of Neotropical Savannas

Rafael Felipe de Almeida [1,*], Augusto Francener [2], Maria Candida Henrique Mamede [2] and Cássio van den Berg [3]

[1] Royal Botanic Gardens Kew, Richmond, Surrey TW9 3AE, UK
[2] Instituto de Pesquisas Ambientais, Programa de Pós-Graduação em Biodiversidade Vegetal e Meio Ambiente, Água Funda, São Paulo 04301-902, São Paulo, Brazil; augustofng@gmail.com (A.F.); mcmamede@gmail.com (M.C.H.M.)
[3] Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Feira de Santana 44036-900, Bahia, Brazil; vcassio@gmail.com
* Correspondence: dealmeida.rafaelfelipe@gmail.com

Abstract: We present a dated and calibrated molecular phylogeny for one of the most characteristic genera of Neotropical savannas, *Byrsonima* (Malpighiaceae), based on the ETS, ITS, and *psbA-trnH* markers. We sampled 33 species of *Byrsonima* and four species of the outgroups *Blepharandra*, *Diacidia*, and *Pterandra* to test the monophyly of the infrageneric classification of the genus. Bayesian inference (BI) analysis was performed for the combined molecular dataset. Seven morphological characters were optimized on the obtained tree. Calibration points derived from a published chronogram for Malpighiaceae were used alongside a relaxed, uncorrelated molecular clock on Beast 1.8.4. Ancestral range reconstructions focusing on four main Neotropical biomes (Cerrado, Atlantic rainforest, Amazon rainforest, and Caatinga dry forests) were performed on BioGeoBEARS. Our phylogenetic results corroborated the monophyly of *Byrsonima*, but all of its subgenera and sections were polyphyletic, with all morphological characters circumscribing these infrageneric ranks being highly homoplastic. The most recent common ancestor of *Byrsonima* was widespread in South American biomes at 11.41 Ma, posteriorly diversifying in the Amazon rainforests up to 7.72 Ma, when it started massively diversifying in Neotropical savannas. A few re-colonization events from savannas to rain or dry forests occurred from 2.95–0.53 Ma. These results corroborate the mid-Miocene origins of Neotropical savannas, and future studies should aim to sample Mesoamerican species of *Byrsonima*.

Keywords: Byrsonimeae; Cerrado; Malpighiales; systematics; taxonomy

Citation: de Almeida, R.F.; Francener, A.; Mamede, M.C.H.; van den Berg, C. Molecular Phylogeny and Historical Biogeography of *Byrsonima* (Malpighiaceae) Corroborates the Mid-Miocene Origins of Neotropical Savannas. *Diversity* **2024**, *16*, 488. https://doi.org/10.3390/d16080488

Academic Editor: Mario A. Pagnotta

Received: 17 July 2024
Revised: 7 August 2024
Accepted: 8 August 2024
Published: 10 August 2024

1. Introduction

Byrsonima Rich. ex Kunth is the second largest genus in Malpighiaceae, comprising 164 species that are endemic to dry or rain forests and savannas in the Neotropical region [1]. The genus is currently placed in the subfamily Byrsonimoideae and the tribe Byrsonimeae, alongside *Blepharandra* Griseb. and *Diacidia* Griseb [1]. Its diversity center is currently found in South America, with Brazil being the richest country, with 99 species and 55 endemics [2]. The genus is easily recognized by its shrubby to tree habit, the presence of interpetiolar stipules, leaves, eglandular bracts and bracteoles, its elongated thyrsi with 1-4-flowered cincinni (i.e., compound inflorescences resembling racemes), its slender styles that are subulate at the apex, its minute stigmas, and its fleshy, indehiscent drupes, with three-locular pyrenes [2].

The first infrageneric classification for *Byrsonima* was proposed by Niedenzu [3], including two subgenera (*B.* subg. *Byrsonima* and *B.* subg. *Macrozeugma* Nied.), distinguished based on the length of the connectives in relation to the length of the locules (i.e., *B.* subg.

Byrsonima equaling locules and *B.* subg. *Macrozeugma* longer than locules). *Byrsonima* subg. *Byrsonima* comprises 70 yellow-flowered species, mostly distributed throughout the Cerrado biome (i.e., Neotropical savannas) and dry and rain forests [1]. The subgenus is divided into two sections, mostly based on the type of indumentum on leaf blades [3]. *Byrsonima* subg. *Byrsonima* sect. *Eriolepsis* Nied. comprises 30 species, with leaves usually covered by tomentose to velutine indumenta [3]. On the other hand, *Byrsonima* subg. *Byrsonima* sect. *Sericolepsis* Nied. comprises 40 species, with leaves covered by sericeous indumenta that might become deciduous at maturity [3,4].

Byrsonima subg. *Macrozeugma* comprises 70 white- to pink-flowered species, mostly distributed throughout rain to dry forests and savannas [1,5]. The subgenus is divided into two sections, mostly based on the shape of the apex of the anthers and the presence of indumentum in the ovary [3]. *Byrsonima* subg. *Macrozeugma* sect. *Colobotheca* Nied. comprises 60 species, characterized by anthers bearing rounded apices and a glabrous ovary [3,5]. On the other hand, *Byrsonima* subg. *Macrozeugma* sect. *Acrotheca* Nied. comprises ten species, with anthers bearing caudate, mucronate, or acuminate apices and a pubescent ovary [3]. This section is further divided into two subsections, *Brachyceras* Nied. and *Uroceras* Nied., based on the presence of indumentum on the anthers [3].

In the last molecular phylogeny for Malpighiaceae, Davis and Anderson [6] sampled all the genera from the family but only included ten species of *Byrsonima*, with most of them belonging to *B.* subg. *Macrozeugma*. Additionally, the monophyly of the genus was not tested in that study, since the authors did not sample the type species, *B. spicata* (Cav.) DC. We present the first molecular phylogeny for *Byrsonima*, testing the monophyly of the genus and assessing its infrageneric classification. Additionally, we dated and calibrated the obtained tree to reconstruct biome ancestral ranges to elucidate its historical biogeography in the Neotropics.

2. Materials and Methods

2.1. Taxon Sampling and Molecular Protocols

We sampled 38 taxa, including outgroups (*Blepharandra cachimbensis* W.R.Anderson, *Diacidia aracaensis* W.R.Anderson, *Pterandra hatschbachii* W.R.Anderson, and *Pterandra pyroidea* A.Juss.), and 33 species of *Byrsonima* (Supplementary Table S1). Silica gel-dried leaves or herborized leaves (12–80 mg) were used for DNA extraction. Genomic DNA was extracted using the CTAB 2× protocol, modified from Doyle and Doyle [7]. Fragments were amplified by PCR (polymerase chain reaction). A single plastid (*psbA-trnH*) and two nuclear regions (internal and external transcribed spacers) were sequenced based on their variability and number of informative characters in Malpighiaceae [8,9]. Protocols to amplify *psbA-trnH* followed Shaw et al. [10,11], and the ETS and ITS regions followed Almeida et al. [12]. The amplification mix that achieved success for ETS, ITS, and *psbA-trnH* regions was a TopTaq (Qiagen) mix following the standard protocol in the kit manual, with the addition of 1.0 M betaine and 2% DMSO for the ETS and ITS regions. PCR products were purified using PEG 11% precipitation (polyethylene glycol) [13] and were sequenced directly with the same primers used for the PCR amplification, with the exception of the ITS region, in which we used primers 92 and ITS4. Sequence electropherograms were produced in an automatic sequencer (ABI 3130XL Genetic Analyzer) using Big Dye Terminator 3.1 (Applied Biosystem). All newly generated sequences were submitted to GenBank and have been publicly available since 2018. Sequences were edited using Geneious 4.84 [14] and aligned using Muscle v3 [15], with subsequent adjustments in the preliminary matrices made manually by eye. The complete data matrices are available in Supplementary Table S2.

2.2. Phylogenetic Analyses

All trees were rooted in representatives from the tribe Acmanthereae (i.e., *Pterandra hatschbachii* and *P. pyroidea*, according to Almeida et al. [1]). The model of nucleotide evolution was selected using hierarchical likelihood ratio tests using J Modeltest 2 [16].

A Bayesian inference analysis (BI) was conducted with a mixed model and unlinked parameters using MrBayes 3.1.2 [17]. The Markov chain Monte Carlo (MCMC) was run using two simultaneous independent runs with four chains each (one cold and three heated), saving one tree every 1000 generations for a total of ten million generations. We excluded 25% of retained trees as 'burn in' and checked for a stationary phase of likelihood, checking for ESS values higher than 200 for all parameters on Tracer 1.6 [18]. The posterior probabilities (PP) of clades were based on the majority rule consensus using the remaining trees, calculated with MrBayes 3.1.2 [17].

2.3. Character Selection, Coding, and Morphological Analysis

Characters were scored mainly from herbarium samples from ALCB, CEPEC, CGMS, CNMT, COR, CPAP, DDMS, ESA, ESAL, FUEL, HB, HCF, HERBAM, HPAN, HRC, HRCB, GTO, HUCP, HUEFS, HEPH, HEUM, HUFSJ, HUFU, HUPG, HUTO, IAN, IBGE, INPA, IPA, MBM, NY, NX, P, R, RB, SP, SPF, SPSF, TANG, UB, UEC, UESC, UFG, UFMT, UFRN, UNOP, UPCB, and US herbaria [19]. Character coding followed the recommendations of Sereno [20] for morphological phylogenies. Primary homology hypotheses [21] were proposed for leaf and floral characters. A total of seven characters representing the morphological-based classification of *Byrsonima* were scored and optimized on the consensus tree using maximum likelihood on Mesquite 2.73 [22] and visualized on Winclada [23].

2.4. Calibration

Estimates were conducted using BEAST 1.8.4 [24] based on the Bayesian combined tree generated by MrBayes. This analysis used a relaxed uncorrelated lognormal clock and Yule process speciation prior to inferring trees [24]. The calibration parameters were based on previous estimates derived from a comprehensive study of the whole Malpighiaceae family [25]. We used five calibration points based on a dated molecular phylogeny sampling all Malpighiaceae genera [25]: the most recent common ancestor (MRCA) of *Byrsonima* (mean = 10.0 Mya), *Blepharandra* + *Diacidia* (mean = 28.0 Mya), Byrsonimeae (mean = 40.0 Mya), Acmanthereae (mean = 26.0 Mya), and subfamily Byrsonimoideae (root, mean = 53.0 Mya) using a normal prior for each node. Two separate and convergent runs were conducted, with 10,000,000 generations, sampling every 1000 steps and 2000 trees as burn-in. We checked for ESS values higher than 200 for all parameters on Tracer 1.6 [18]. Tree topology was assessed using TreeAnnotator and FigTree 1.4.0 [26].

2.5. Ancestral Range Reconstruction

Species distribution data were compiled from Almeida et al. [2] and herbarium collections of GBIF [27]. Ancestral range reconstructions were defined as: (A) Cerrado savannas, (B) Atlantic rainforest, (C) Amazon rainforest, and (D) Caatinga dry forests. Ancestral ranges of *Byrsonima* were estimated using the R package BioGeoBEARS version 1.1.1 [28] and the models DEC, DIVALIKE, and BAYAREALIKE. Considering the possibility of underestimation of extinction due to the null ranges of species allowed by the models, analyses with the "*" option were also performed. The "j" option was used to consider founder speciation events. Different combinations of the three models and the two options generated a total of 12 models. The best-fitting models for analyses were determined by AIC weight ratios.

3. Results

3.1. Phylogenetics and Character Mapping

The nuclear dataset represented 1120 characters of the dataset, the plastid dataset represented 316 characters, and the combined plastid + nuclear dataset included 1436 analyzed characters (Supplementary Table S2). Topologies produced by BI based on the individual nuclear and plastid datasets did not exhibit incongruences among the topologies produced, so we performed a combined analysis of plastid + nuclear datasets. The BI analysis recovered a fully resolved tree with mostly well-supported clades (>PP 0.95;

Figure 1). The monophyly of *Byrsonima* was tested and corroborated for the first time in a molecular phylogeny, with its species emerging into 12 informal clades: *B. triopterifolia* clade (*B. rigida* + *B. triopterifolia*); *B. coniophylla*, *B. japurensis*, *B. punctulata*, and *B. umbellata* clades (*B. melanocarpa* + *B. umbellata*); *B. brachybotria* and *B. affinis* clades (*B. affinis* + *B. poeppigiana*); *B. intermedia* clade (*B. intermedia* + *B. stannardii* + *B. verbascifolia*); *B. stipulacea* clade (*B. crispa* + *B. lanulosa* + *B. stipulacea*); *B. coccolobifolia* clade (*B. cipoensis* + *B. coccolobifolia* + *B. correifolia* + *B. gardneriana* + *B. macrophylla* + *B. vacciniifolia*); *B. spicata* clade (*B. chrysophylla* + *B. laxiflora* + *B. linguifera* + *B. spicata*); and *B. crassifolia* clade (*B. crassifolia Amapá* + *B. crassifolia Goiás* + *B. cydoniifolia* + *B. linearifolia* + *B. pachyphylla* + *B. salzmanniana* + *B. subterranea* + *B. sp Emas*; Figure 1). The seven morphological characters mapped into the molecular phylogeny were recovered as highly homoplastic (Figure 1).

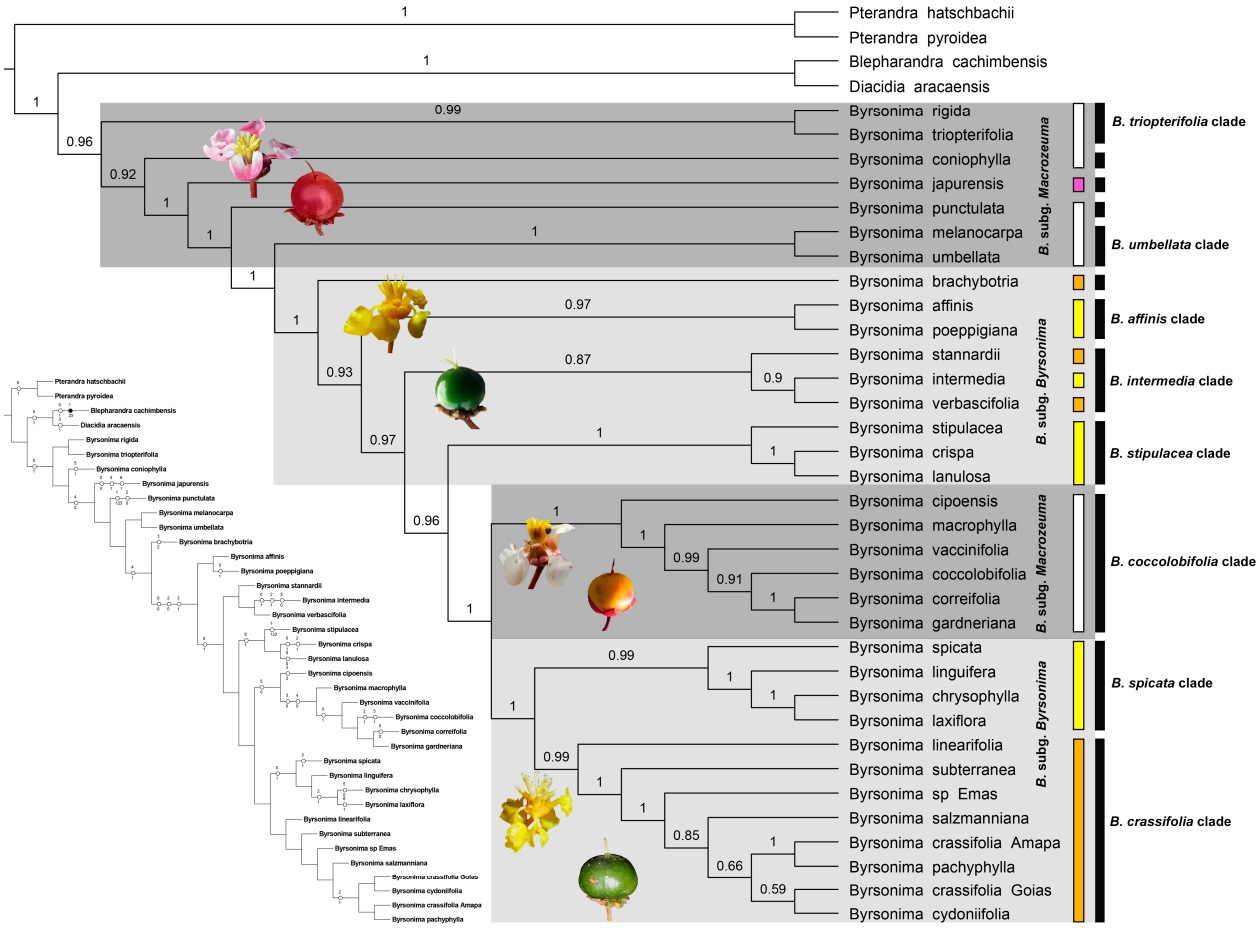

Figure 1. Molecular phylogeny of *Byrsonima*: (**right tree**) posterior probabilities of the Bayesian inference shown above the branches. Yellow bars = *B.* subg. *Byrsonima* sect. *Sericolepsis*. Orange bars = *B.* subg. *Byrsonima* sect. *Eriolepsis*. White bars = *B.* subg. *Macrozeugma* sect. *Colobotheca*. Pink bar = B. subg. Macrozeugma sect. Acrotheca. Black bars = informal clades proposed here (**left tree**); character-mapping showing homoplasies are in white, and synapomorphies or autapomorphies are shown in black. The number above the circles refers to the character number, and the number below the circles refers to the character state, both found in Supplementary Table S3.

3.2. Divergence Times and Ancestral Ranges of Byrsonima

Estimates of divergence times and ancestral range reconstructions showed that the most recent common ancestor (MRCA) of *Byrsonima* emerged at 11.41 Ma and was widespread in the Amazon rainforest, Caatinga dry forest, and Cerrado savanna biomes. The *B. triopterifolia* clade was the first Cerrado colonization in the genus but diversified only at 5.96 Ma. Several early diverging lineages (*B. coniophylla*, *B. japurensis*, and *B. punctulata*)

diversified in the Amazon rainforest until 8.53 Ma. A second colonization event of the Cerrado savanna took place at 7.72 Ma, with most lineages diversifying in this biome until 0.45 Ma. Three recolonization events of the Amazon rainforest from a Cerrado MRCA took place at 3.28, 2.81, and 2.95 Ma in the *B. affinis*, *B. stipulacea*, and *B. spicata* clades, respectively. Three colonization events of the Atlantic rainforest from a Cerrado MRCA happened at 2.81, 1.22, and 0.53 Ma in the *B. stipulacea* and *B. spicata* clades, respectively. Two colonization events of the Caatinga dry forest took place at 5.96 and 0.56 Ma in the *B. triopterifolia* and *B. coccolobifolia* clades, respectively (Figures 2 and 3).

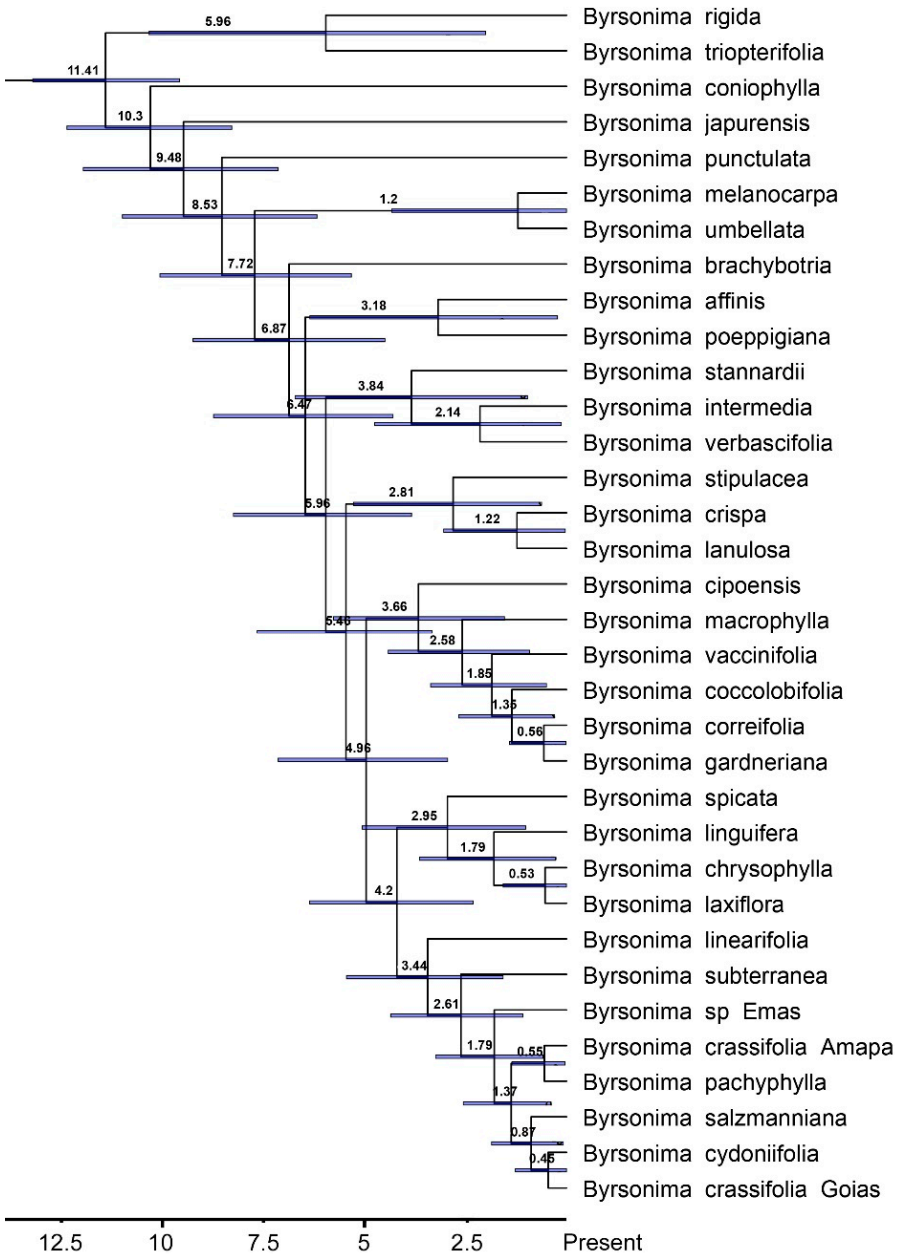

Figure 2. Chronogram of *Byrsonima* showing the mean node ages estimated on branches. Blue bars represent 95% confidence intervals (CI) for the estimated median dates.

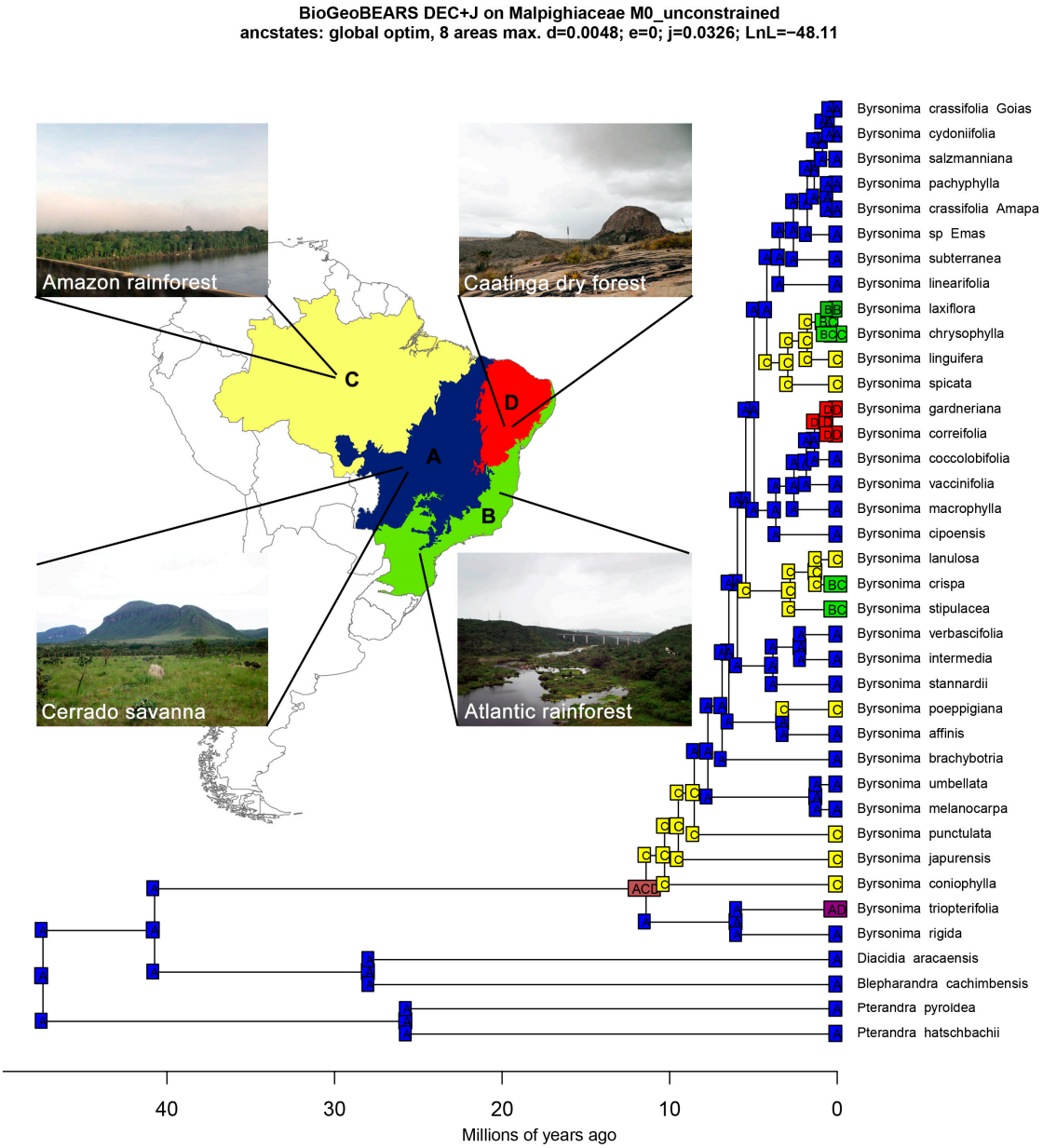

Figure 3. Ancestral range reconstruction for *Byrsonima*: (**A**) Cerrado savanna, (**B**) Atlantic rainforest, (**C**) Amazon rainforest, and (**D**) Caatinga dry forest.

4. Discussion

4.1. Phylogenetics and Systematics of Byrsonima

Our results recovered *Byrsonima* to be monophyletic, with strong support corroborating previous studies [1,6,9,29–31]. However, the infrageneric ranks of the classification system of *Byrsonima*, traditionally divided into two subgenera with two sections each, was evidenced as being grossly polyphyletic. Metabolomic studies in *Byrsonima* also showed that species from each subgenus did not group with each other based on secondary metabolites, corroborating the polyphyletic nature of these taxonomic ranks [32]. In general, we can separate these subgenera based on petal color, with *B.* subg. *Byrsonima* bearing yellow to orange petals and *B.* subg. *Macrozeugma* bearing white to pink petals [5], but petal color was also revealed to be a polyphyletic character in our results.

An early diverging clade comprising *B. rigida* and *B. triopterifolia* was sister to the remaining species of *Byrsonima*. Those species were distinguished by pink or white corollas with glabrous bracts, bracteoles, and anthers and connectives not exceeding the anther

locules [5]. *Byrsonima melanocarpa* was recovered as being sister to *B. umbellata*, sharing several morphological characters, but most remarkably, the globose connective surpassed the thecae [5].

The *B. coccolobifolia* clade was composed of *B. coccolobifolia*, *B. correifolia*, *B. gardneriana,* and *B. vacciniifolia*. These closely related species possessed pink or white petals, with connectives surpassing the thecae in more than ¼ of their length [5]. Nevertheless, *B. cipoensis* diverged from the remaining species in possessing yellow posterior petals + white to pink lateral and posterior petals, and the connectives did not surpass the anther locules in more than ¼ of their length [5]. The only characteristic shared by all species was the glabrous ovary, but it was a homoplastic condition, due to other species also showing this trait.

The *B. spicata* clade includes *B. laxiflora*, *B. chrysophylla*, *B. spicata* and *B. linguifera*. These species are commonly found in several biomes throughout Brazil [2], with *B. chrysophylla* and *B. spicata* extending to the Guyana Highlands and Central America [33]. With the exception of *B. linguifera*, the other species present a unique set of characteristics, such as posterior petals bearing two or more glands at the apex of the claw or on the base of the limb and a leaf architecture with fine, parallel secondary veins. According to Anderson [34], *Byrsonima linguifera*, with prominent secondary veins and an absence of glands at the posterior petals, is closely related to *B. arthropoda*, *B. poeppigiana*, and *B. schunkei*. Nevertheless, we recovered *B. poeppigiana* as being sister to *B. affinis*. These species have very distinct morphologies, in addition to *B. affinis* being native to the Brazilian Cerrado, while *B. poeppigiana* is found in the Amazon forest. The inclusion of *B. schunkei* and *B. arthropoda* in future studies is necessary to clarify their relation. *Byrsonima stipulacea* was initially described by Jussieu [35] but was later transferred to *Alcoceratothrix* by Niedenzu [3], based solely on the large, unusual, deciduous stipules. Based on a profound study of *Byrsonima* and on the comparison to closely related species, Anderson [34] reestablished the name *B. stipulacea* and considered this character to be exclusive to it. Our results support the decision made by Anderson [34], recovering that *B. stipulacea* nested within the core of *Byrsonima* and is closely related to *B. crispa* and *B. lanulosa*. However, no morphological synapomorphies could be indicated for this clade based on the present sampling. The inclusion of species morphologically related to *B. stipulacea*, such as *B. fanshawei* and *B. duckeana*, in future studies may help us understand this peculiar relation.

The *B. crassifolia* clade includes numerous species placed in *Byrsonima* sect. *Eriolepsis*. This section is circumscribed by their connectives not surpassing the anther locules (when surpassing, no more than ¼ of their length); usually deciduous, long, and acuminate bracts; adaxially glabrous, abaxially lanate tomentose; bracteoles similar to the bracts (only smaller); coriaceous to sub-coriaceous leaves; and drupes with hard stones that are smooth or minutely verrucose [3]. All species in the *B. crassifolia* clade possess yellow flowers, hairy anthers, and thecae with a rounded apex. Nevertheless, these characteristics are found separately in other species outside the clade. Thus, they could be considered homoplastic synapomorphies for this clade. Further sampling and genetic markers are needed to confirm the monophyly of *B.* sect. *Eriolepsis*.

4.2. Historical Biogeography of Byrsonima

The diversification of *Byrsonima* in Neotropical biomes seems to corroborate main patterns of biome diversification in South America. The early to mid-Paleogene origins of Cerrado savannas are suggested by molecular phylogenetic studies on oil-collecting bees [36] and Vochysiaceae [37]. However, most Cerrado savanna lineages started to diversify in the Eocene, with most of them greatly diversifying from the mid-Miocene onwards (Figure 4). *Byrsonima* started diversifying in the Cerrado savanna from 7.72 Ma, corroborating the period from 12.0–5.0 Ma, in which most woody plants diversified in Cerrado savannas, according to the literature (Figure 4). *Byrsonima* also colonized the Amazon rainforest at least three times from 10.0–2.80 Ma (mid to late-Miocene), which also corroborates diversification patterns in the Amazon rainforest [38]. Finally, *Byrsonima*

colonized the Atlantic rainforest at least three times starting in the Pleistocene, always from an Amazonian MRCA, corroborating the long-known Pleistocene connections between both rainforests [39].

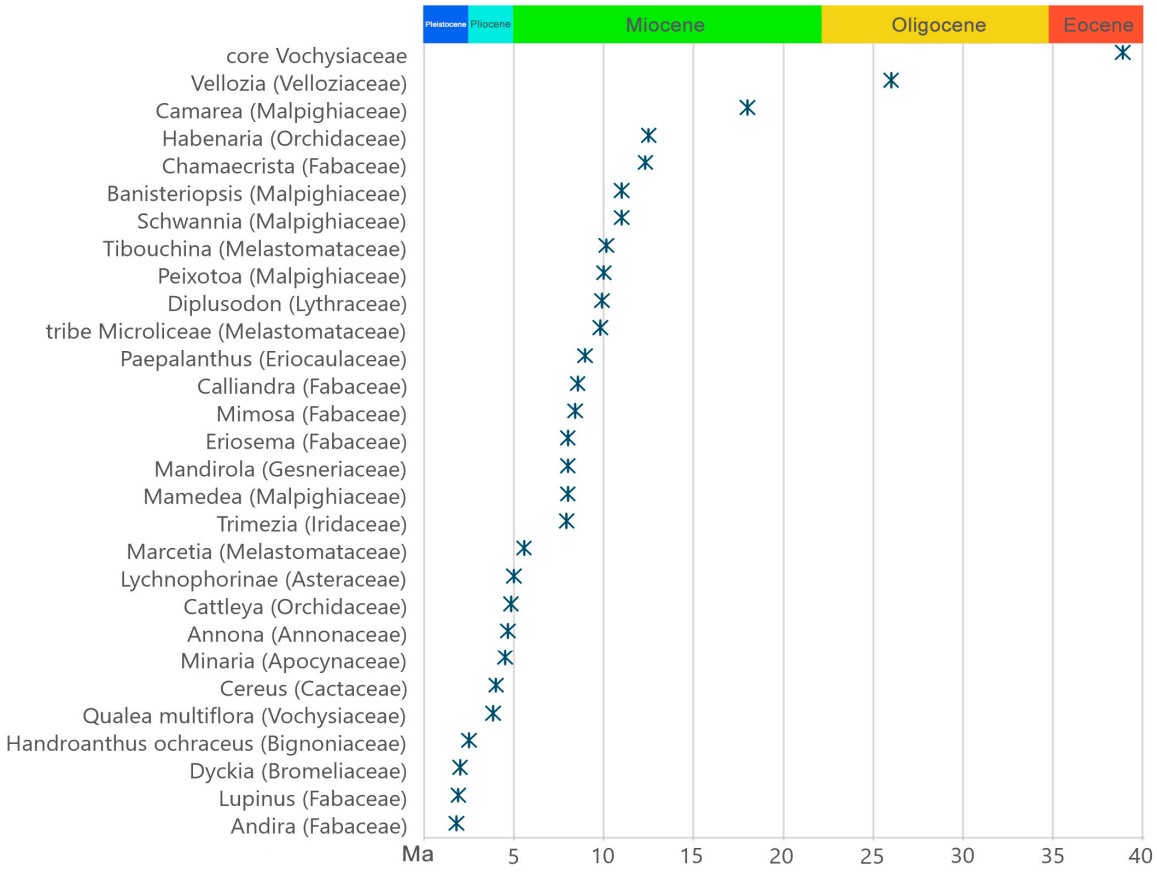

Figure 4. Divergence times for the MRCAs of several lineages of flowering plants diversified in the Cerrado savanna based on dated calibrated trees from molecular phylogenetic studies [25,37,40–46].

5. Conclusions

Our study is the first to test and corroborate the monophyly of *Byrsonima* by sampling its type species alongside one-fourth of its global species richness. The monophyly of all infrageneric ranks currently accepted in *Byrsonima* was rejected. The ancestral line of *Byrsonima* seems to have arisen at about 11.41 Ma; it was initially widespread, and then was only in the Amazon rainforest; then, it greatly diversified in the Cerrado savanna from the mid- to late-Miocene period. An increased sampling and further molecular markers are still needed for a better understanding of the morphological evolution and diversification of this genus in the Neotropics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16080488/s1, Table S1: DNA sampling; Table S2: Alignment; Table S3: Morphological matrix.

Author Contributions: Conceptualization, R.F.d.A. and A.F.; methodology, C.v.d.B.; software, C.v.d.B.; validation, R.F.d.A., A.F., and C.v.d.B.; formal analysis, R.F.d.A.; investigation, A.F.; resources, C.v.d.B.; data curation, M.C.H.M.; writing—original draft preparation, R.F.d.A.; writing—review and editing, all authors; visualization, C.v.d.B.; supervision, C.v.d.B. and M.C.H.M.; project administration, C.v.d.B.; funding acquisition, C.v.d.B. All authors have read and agreed to the published version of the manuscript.

Funding: RFA was sponsored by Programa de Desenvolvimento Científico e Tecnológico Regional CNPq/FAPEG (grants #317720/2021-0 and #202110267000867). AF was sponsored by a CAPES doctoral fellowship. Fieldwork and laboratory analysis were supported by FAPESB (grant #PNX0014/2009), Reflora Malpighiales Project (grant #563548/2010–0), and Universal Project (grant #486079/2013–9).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data used in this study are available in Supplementary Tables S1–S3.

Acknowledgments: We thank the staff and curators of all the visited herbaria for their assistance on loans and on getting DNA samples; the staff and students from Plant Molecular Systematics Laboratory (LAMOL) for their assistance on molecular protocols; Climbiê Ferreira Hall, Helisvânia Gomes, and André Amorim for collecting samples; and Climbiê Ferreira Hall and Marco Octávio de Oliveira Pellegrini for their comments on an early version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. de Almeida, R.F.; de Morais, I.L.; Alves-Silva, T.; Antonio-Domingues, H.; Pellegrini, M.O.O. A new classification system and taxonomic synopsis for Malpighiaceae (Malpighiales, Rosids) based on molecular phylogenetics, morphology, palynology, and chemistry. *PhytoKeys* **2024**, *242*, 69–138. [CrossRef] [PubMed]
2. de Almeida, R.F.; Francener, A.; Pessoa, C.; Sebastiani, R.; Oliveira, Y.R.; Amorim, A.M.A.; Mamede, M.C.H. Malpighiaceae in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. 2020. Available online: https://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB155 (accessed on 7 December 2023).
3. Niedenzu, F. *De Genere Byrsonima (Pars Posterior)*; Arbeiten aus dem botanischen Institut des Kgl; Lyceum Hosianum: Braunsberg, Poland, 1901; pp. 1–45.
4. Niedenzu, F. Malpighiaceae. In *Das Pflanzenreich*; Engler, A., Ed.; Wilhelm Engelmann: Leipzig, Germany, 1928; Volume 141, p. 870.
5. Rolim, S.I.E. Revisão e Redefinição de *Byrsonima* Rich. ex Kunth Subg. *Macrozeugma* Nied. (Malpighiaceae). Ph.D. Thesis, University of São Paulo, São Paulo, Brazil, 2004. Available online: https://bdpi.usp.br/item/001449938 (accessed on 7 July 2024).
6. Davis, C.C.; Anderson, W.R. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *Amer. J. Bot.* **2010**, *97*, 2031–2048. [CrossRef] [PubMed]
7. Doyle, J.J.; Doyle, J.S. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **1987**, *19*, 11–15.
8. de Almeida, R.F.; Amorim, A.M.A.; van den Berg, C. Timing the origin and past connections between Andean and Atlantic Seasonally Dry Tropical Forests in South America: Insights from the biogeographical history of *Amorimia* (Malpighiaceae). *Taxon* **2018**, *67*, 739–751. [CrossRef]
9. Almeida, R.F.; Pellegrini, M.O.O.; de Morais, I.L.; Simão-Bianchini, R.; Rattanakrajang, P.; Cheek, M.; Simões, A.R.G. Barking up the wrong tree: The dangers of taxonomic misidentification in molecular phylogenetic studies. *Plant Ecol. Evol.* **2023**, *156*, 146–159.
10. Shaw, J.; Lickey, E.B.; Beck, J.T.; Farmer, S.B.; Liu, W.; Miller, J.; Siripun, K.C.; Winder, C.T.; Schilling, E.E.; Small, R.L. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* **2005**, *92*, 142–166. [CrossRef]
11. Shaw, J.; Lickey, E.B.; Schilling, E.E.; Small, R.L. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Amer. J. Bot.* **2007**, *94*, 275–288. [CrossRef]
12. de Almeida, R.F.; Amorim, A.M.A.; Correa, A.M.S.; van den Berg, C. A new infrageneric classification for *Amorimia* (Malpighiaceae) based on morphological, phytochemical and molecular evidence. *Phytotaxa* **2017**, *313*, 231–248.
13. Paithankar, K.R.; Prasad, K.S.N. Precipitation of DNA by polyethylene glycol and ethanol. *Nucl. Acids Res.* **1991**, *19*, 1346. [CrossRef]
14. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [CrossRef]
15. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* **2004**, *32*, 1792–1797. [CrossRef] [PubMed]
16. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [CrossRef] [PubMed]
17. Ronquist, F.; Huelsenbeck, J.P. Mr Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **2003**, *19*, 1572–1574. [CrossRef] [PubMed]

18. Rambaut, A.; Suchard, M.A.; Xie, D.; Drummond, A.J. Tracer v1.6. Available online: http://beast.bio.ed.ac.uk/Tracer (accessed on 7 February 2023).
19. Thiers, B. Index Herbariorum. Available online: https://sweetgum.nybg.org/science/ih/ (accessed on 7 February 2023).
20. Sereno, P.C. Logical basis for morphological characters in phylogenetics. *Cladistics* **2007**, *23*, 565–587. [CrossRef] [PubMed]
21. De Pinna, M.C.C. Concepts and tests of homology in the cladistic paradigm. *Cladistics* **1991**, *7*, 367–394. [CrossRef]
22. Maddison, W.P.; Maddison, D.R. Mesquite: A Modular System for Evolutionary Analysis. Version 3.61. Available online: http://www.mesquiteproject.org (accessed on 7 February 2023).
23. Nixon, K.C. Winclada (Beta) Ver. 0.9. Published by the Author, Ithaca, NY. Available online: http://www.cladistics.com (accessed on 7 February 2023).
24. Drummond, A.J.; Suchard, M.A.; Xie, D.; Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol Evol.* **2012**, *29*, 1969–1973. [CrossRef]
25. Davis, C.C.; Schaefer, H.; Xia, Z.; Baum, D.A.; Donoghue, M.J.; Harmon, L.J. Long-term morphological stasis maintained by a plant–pollinator mutualism. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5914–5919. [CrossRef]
26. Rambaut, A. FigTree Version 1.3.1: Tree Figure Drawing Tool. Computer Program and Documentation Distributed by the Author. Available online: http://tree.bio.ed.ac.uk/software/figtree (accessed on 7 February 2023).
27. GBIF. Global Biodiversity Information Facility. Available online: http://gbif.org (accessed on 7 February 2023).
28. Matzke, N.J. BioGeoBEARS: Biogeography with Bayesian (And Likelihood) Evolutionary Analysis in R Scripts, CRAN: The Comprehensive R Archive Network, Berkeley, 2013, CA. Available online: http://CRAN.R-project.org/package=BioGeoBEARS (accessed on 7 February 2023).
29. Cameron, K.M.; Chase, M.W.; Anderson, W.R.; Hills, H.G. Molecular Systematics of Malpighiaceae: Evidence from plastid *rbcL* and *matK* sequences. *Amer. J. Bot.* **2001**, *88*, 1847–1862. [CrossRef]
30. Davis, C.C.; Anderson, W.R.; Donoghue, M. Phylogeny of Malpighiaceae: Evidence from chloroplast *ndhF* and *trnL-F* nucleotides sequences. *Amer. J. Bot.* **2001**, *88*, 1830–1846. [CrossRef]
31. Davis, C.C.; Bell, C.D.; Mathews, S.; Donoghue, M.J. Laurasian migration explains Gondwanan disjunctions: Evidence from Malpighiaceae. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6833–6837. [CrossRef]
32. Santos-Zanuncio, V.S.; Alves, F.M.; Silva, D.B.; Carollo, C.A. Chemosystematic implications based on metabolic profiling of the genus Byrsonima (Malpighiaceae). *Folia Geobot.* **2020**, *55*, 289–300. [CrossRef]
33. Anderson, W.R. Malpighiaceae. In: The Botany of Guayana Highland—Part IX. *Mem. N. Y. Bot. Gard.* **1981**, *32*, 129–131.
34. Anderson, W.R. Notes on Neotropical Malpighiaceae - I. *Contr. Univ. Michigan. Herb.* **1982**, *16*, 55–108.
35. Jussieu, A. Malpighiacearum synopsis, monographiae mox edendae prodromus. *Ann. Sci. Nat. Botanique* **1840**, *13*, 247–291, 321–338.
36. Aguiar, A.J.C.; Melo, G.A.R.; Vasconcelos, T.N.C.; Gonçalves, R.B.; Giugliano, L.; Martins, A.C. Biogeography and early diversification of Tapinotaspidini oil-bees support presence of Paleocene savannas in South America. *Mol. Phylogenetics Evol.* **2020**, *143*, 106692. [CrossRef]
37. Gonçalves, D.J.P.; Shimizu, G.H.; Ortiz, E.M.; Jansen, R.K.; Simpson, B.B. Historical biogeography of Vochysiaceae reveals an unexpected perspective of plant evolution in the Neotropics. *Amer. J. Bot.* **2020**, *107*, 1004–1020. [CrossRef]
38. Antonelli, A.; Sanmartín, I. Why are there so many plant species in the Neotropics? *Taxon* **2011**, *60*, 403–414. [CrossRef]
39. Carnaval, A.C.; Waltari, E.; Rodrigues, M.T.; Rosauer, D.; VanDerWal, J.; Damasceno, R.; Prates, I.; Strangas, M.; Spanos, Z.; Rivera, D.; et al. Prediction of phylogeographic endemism in an environmentally complex biome. *Proc. R. Soc. B* **2014**, *281*, 20141461. [CrossRef]
40. Amaral, D.T.; Minhós-Yano, I.; Oliveira, J.V.M.; Romeiro-Brito, M.; Bonatelli, I.A.S.; Taylor, N.P.; Zappi, D.C.; Moraes, E.M.; Eaton, D.; Franco, F.F. Tracking the xeric biomes of South America: The spatiotemporal diversification of Mandacaru cactus. *J. Biogeogr.* **2021**, *48*, 3085–3103. [CrossRef]
41. Buzatti, R.S.O.; Pfeilsticker, T.R.; de Magalhães, R.F.; Bueno, M.L.; Lemos-Filho, J.P.; Lovato, M.B. Genetic and historical colonization analyses of an endemic savanna tree, *Qualea grandiflora*, reveal ancient connections between Amazonian savannas and Cerrado core. *Front Plant Sci.* **2018**, *9*, 981. [CrossRef]
42. Cândido, E.S.; Vatanparast, M.; Vargas, W.; Bezerra, L.M.P.A.; Lewis, G.L.; Mansano, V.F.; Simões, A.O.; Silva, M.J.; Stirton, C.; Tozzi, A.M.G.A.; et al. Molecular phylogenetic insights into the evolution of *Eriosema* (Fabaceae): A recent tropical savanna-adapted genus. *Bot. J. Linn. Soc.* **2020**, *194*, 439–459. [CrossRef]
43. Fiorini, C.F.; Peres, E.A.; da Silva, M.J.; Araujo, A.O.; Borba, E.L.; Solferini, V.N. Phylogeography of the specialist plant *Mandirola hirsuta* (Gesneriaceae) suggests ancient habitat fragmentation due to savanna expansion. *Flora* **2020**, *262*, 151522. [CrossRef]
44. Simon, M.F.; Grether, R.; Queiroz, L.P.; Skema, C.; Pennington, R.T.; Hughes, C.E. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 20359–20364. [CrossRef] [PubMed]

45. Vasconcelos, T.N.C.; Alcantara, S.; Andrino, C.O.; Forest, F.; Reginato, M.; Simon, M.F.; Pirani, J.R. Fast diversification through a mosaic of evolutionary histories characterizes the endemic flora of ancient Neotropical mountains. *Proc. R. Soc. B* **2020**, *287*, 20192933. [CrossRef] [PubMed]
46. Vitorino, L.C.; Lima-Ribeiro, M.S.; Terribile, L.C.; Collevatti, R.G. Demographical expansion of *Handroanthus ochraceus* in the Cerrado during the Quaternary: Implications for the genetic diversity of Neotropical trees. *Biol. J. Linn. Soc.* **2018**, *123*, 561–577. [CrossRef]