Biogeography of *Stigmaphyllon* (Malpighiaceae) and a Meta-Analysis of Vascular Plant Lineages Diversified in the Brazilian Atlantic Rainforests Point to the Late Eocene Origins of This Megadiverse Biome

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Abstract: We investigated the biogeography of *Stigmaphyllon*, the second-largest lianescent genus of Malpighiaceae, as a model genus to reconstruct the age and biogeographic history of the Brazilian Atlantic Rainforest (BAF). Few studies to date have focused on the tertiary diversification of plant lineages in the BAFs, especially on *Stigmaphyllon*. Phylogenetic relationships for 24 species of *Stigmaphyllon* (18 ssp. from the Atlantic forest (out of 31 ssp.), three ssp. from the Amazon Rainforest, two ssp. from the Caatinga biome, and a single species from the Cerrado biome) were inferred based on one nuclear DNA (PHYC) and two ribosomal DNA (ETS, ITS) regions using parsimony and Bayesian methods. A time-calibrated phylogenetic tree for ancestral area reconstructions was additionally generated, coupled with a meta-analysis of vascular plant lineages diversified in the BAFs.

Our results show that: (1) *Stigmaphyllon* is monophyletic, but its subgenera are paraphyletic; (2) the most recent common ancestor of *Stigmaphyllon* originated in the Brazilian Atlantic Rainforest/Caatinga region in Northeastern Brazil ca. 26.0 Mya; (3) the genus colonized the Amazon Rainforest at two different times (ca. 22.0 and 6.0 Mya), the Caatinga biome at least four other times (ca. 14.0, 9.0, 7.0, and 1.0 Mya), the Cerrado biome a single time (ca. 15.0 Mya), and the Southern Atlantic Rainforests five times (from 26.0 to 9.0 Mya); (4) a history of at least seven expansion events connecting the Brazilian Atlantic Rainforest to other biomes from 26.0 to 9.0 Mya, and (5) a single dispersion event from South America to Southeastern Asia and Oceania at 22.0 Mya via Antarctica was proposed. Compared to a meta-analysis of time-calibrated phylogenies for 64 lineages of vascular plants diversified in the Brazilian Atlantic Rainforests, our results point to a late Eocene origin for this megadiverse biome.

Keywords: ancestral area reconstruction; Atlantic rainforest; time-calibrated phylogenies; Malpighiales; neotropical flora

1. Introduction

Malpighiaceae Juss. is one of the most diverse plant families of Neotropical shrubs, trees, and lianas [1], with most species confined to this region [2]. Its species are easily recognized by their remarkable floral conservatism, with flowers frequently bearing a pair of oil-secreting glands at the base of sepals, petals clawed at the base, and a posterior petal differentiated from the remaining lateral four [1,2]. This family has received broad phylogenetic attention in the past few years [2–8], including more focused investigations on generic delimitations and the phylogenetic position of
Old-World clades [2,6]. However, there have been few efforts to determine finer-scale patterns of molecular analyses and historical biogeography in Neotropical Malpighiaceae [4,6,8,9].

*Stigmaphyllon* A.Juss. is one of the several genera in Malpighiaceae re-circumscribed due to recent molecular phylogenetic studies [2,6]. It is currently the second-largest lianescent genus in Malpighiaceae and the only occurring in forested habitats in the tropics and subtropics of America, Africa, Asia, and Oceania [10]. Most species are woody lianas with long-petioled, elliptical to cordate leaves, corymb or umbels of yellow flowers arranged in dichasia, styles holding laterally expanded appendages in the apex, and schizocarpic fruits splitting into three-winged mericarps, bearing a sizeable dorsal wing (Figure 1 a–h) [10,11]. This genus is currently divided into two subgenera, *Stigmaphyllon* and *Ryssopterys*, treated as separate genera before molecular phylogenies [10]. *Stigmaphyllon* subgenus *Stigmaphyllon* includes ca. 90 species restricted to the Neotropics, except for *Stigmaphyllon bannisterioides* A.Juss., which is also found in West Africa [11]. On the other hand, *S.* subgenus *Ryssopterys* includes ca. 20 species restricted to Southeast Asia and Oceania [10]. The monophyly of *Stigmaphyllon* and its subgenera has been suggested by previous phylogenetic studies but never adequately corroborated because its type species was never sampled [2,3,5,6].

Previous phylogenetic studies for Malpighiaceae suggested a Brazilian Atlantic Rainforest (herein treated as BAF) origin for *Stigmaphyllon*, with *S. paralias* A.Juss. being consistently recovered in several studies as the first lineage to diverge in the genus [2,6]. Even though the BAFs currently comprise more than 15,000 known vascular plant species [12], they are regarded as one of the most threatened hotspots for biodiversity worldwide due to being mostly fragmented and disturbed by most of Brazil’s human population and economic activity [13]. Several biogeographic hypotheses have been proposed to explain the origins of the great biodiversity of the BAFs, such as: 1. Miocene to Pleistocene forest corridors between BAFs and the Amazon rainforests via Cerrado’s gallery forests and/or via the coastal region in Northeastern Brazil [14]; and 2. Pleistocene refugia hypothesis, which suggested that Pleistocene climatic fluctuations led to rainforest fragmentation and promoted divergence of lineages or species in isolated forest fragments or refugia [15]. Pleistocene diversifications in BAFs have been greatly criticized due to the lack of concordance with empirical phylogenetic data, as well as by the evidence that shifts in forest species distribution, rather than fragmentation, have been the main consequences of global glaciations in the Neotropics [16]. Although most previous studies have focused on explaining BAFs biodiversity through Miocene to Pleistocene climatic/geological events, no study to date has focused on timing the age of vascular plant lineages diversification in BAFs.

In this study, we focus on timing the biogeographic history of BAFs using *Stigmaphyllon* as a model genus, supplemented by a meta-analysis of vascular plant lineages diversified in this biome to infer the age of BAFs. More specifically, we: (1) test the monophyly of *Stigmaphyllon* and its subgenera; (2) time-calibrate the phylogenetic tree; (3) estimate the ancestral areas of *Stigmaphyllon*; and (4) shed some light on the age and biogeographic history of BAFs using *Stigmaphyllon* and a meta-analysis of vascular plant lineages diversified in this biome.
Figure 1. Phylogram from the combined analysis of nuclear and ribosomal markers for *Stigmaphyllon*. Posterior probabilities are shown above branches and bootstrap values below branches. Black bars represent *S. subg. Stigmaphyllon* and the white bar represents *S. subg. Ryssopterys*. (dark blue bar) *S. paralias* group; (yellow bar) *S. ciliatum* group; (dark green bar) *S. subg. Ryssopterys*; (orange bar) *S. finlayanum* group; (purple bar) *S. auriculatum* group; (light green bar) *S. urenifolium* group; (light blue bar) *S. lalandianum* group; (red bar) *S. sinuatum* group; (pink bar) *S. blanchetii* group; (grey bar) *S. saxicola* group. (A) adaxial surface of a leaf of *S. angustilobum* A.Juss. (B) Umbel of *S. ciliatum* (Lam.) A.Juss. in side view evidencing ciliate reduced leaves associated with the inflorescence. (C) open flower of *S. ciliatum* in frontal view. (D) androecium and gynoecium of *S. ciliatum* in side view, (E) mericarp of *S. paralias* A.Juss. in side view. (F) winged mericarp of *S. ciliatum* in side view. (G) winged mericarp of *S. saxicola* (Cav.) A.Juss. in side view. (H) winged mericarp of *S. saxicola* C.E.Anderson in side view. (I) winged mericarps of *S. blanchetii* in side view (photographs by R.F.Almeida).

2. Results

2.1. Phylogenetic Analysis

Our combined dataset for ribosomal (ETS, ITS) and nuclear (PHYC) markers contains a total of 2283 characters, of which 1724 characters are constant, 257 characters are variable, but parsimony uninformative, and 302 characters are parsimony informative. The combined analysis of nuclear and ribosomal markers provides higher support for more clades than those based on independent nuclear and ribosomal datasets. Overlapping peaks (paralogous copies) for ETS and ITS were not recorded during electrophoresis and sequencing. The heuristic search for the combined dataset found
15 trees (consistency index, CI = 0.80, retention index, RI = 0.70), and the strict consensus tree includes 17 moderately supported clades (bootstrap percentage, BP > 75; Figure 1). The Bayesian analysis recovered 25 well-supported to moderately supported clades (posterior probabilities, PP > 0.95 and >0.80, respectively; Figure 1).

The monophyly of *Stigmaphyllon* (Figure 1) is strongly supported by Maximum Parsimony (MP) and Bayesian Inference (BI) analyses, being recovered as sister to *Diplopterys*. The clade *Stigmaphyllon* + *Diplopterys* is well supported (BS100/PP1.0) as sister to *Bronwenia* (Figure 1). Within *Stigmaphyllon*, *Stigmaphyllon* subg. *Stigmaphyllon* is recovered as polyphyletic, with its representatives placed in three separate lineages (Figure 1, black bars), named: the early-diverging *Stigmaphyllon paralias* group (Figure 1, dark blue bar), *Stigmaphyllon ciliatum* group (Figure 1, yellow bar) and core *Stigmaphyllon* (here represented by the common ancestor that the lineage *S. finlayanum* + *S. puberulum* share with the remaining species (Figure 1, black bar)). *Stigmaphyllon* subg. *Ryssopterys* (Figure 1, dark green bar) is recovered as monophyletic in our analyses, being sister to the *Stigmaphyllon ciliatum* group (Figure 1, white bar).

Within core *Stigmaphyllon*, seven major lineages are recovered, here designated as 1. *S. puberulum* group, 2. *S. auriculatum* group, 3. *S. urenifolium* group, 4. *S. lalandianum* group, 5. *S. sinuatum* group, 6. *S. blanchetii* group, and 7. *S. gayanum* group. The *S. puberulum* group (Figure 1, orange bar) is well-supported (BS100/PP1.0), comprising only two species, *S. finlayanum* A.Juss. and *S. puberulum* Griseb., while the *S. auriculatum* group (Figure 1, purple bar) is moderate to well-supported (BS77/PP1.0), comprising three species, *S. angustilobum* A.Juss., *S. arenicola* C.E.Anderson, and *S. auriculatum* (Cav.) A.Juss. The *S. urenifolium* group (Figure 1, light green bar) is weak to well-supported (BS/PP0.60), comprising four species, *S. blanchetii* C.E.Anderson, *S. lalandianum* A.Juss., and *S. vitifolium* A.Juss. The *S. sinuatum* group (Figure 1, red bar) is well-supported (BS100/PP1.0), represented by only two species, *S. lindenianum* A.Juss. and *S. sinuatum* (DC.) A.Juss., while the *S. blanchetii* group (Figure 1, rose bar) is weakly-supported (BS-/PP0.60), comprising four species, *S. blanchetii* C.E.Anderson, *S. caatingicola* R.F.Almeida and Amorim, *S. jatrophiolium* A.Juss., and *S. salzmannii* A.Juss. Finally, the *S. gayanum* group (Figure 1, gray bar) is weakly to well-supported (BS-/PP1.0), and represented by four species, *S. cavernulosum* C.E.Anderson, *S. gayanum* A.Juss., *S. macropodum* A.Juss., and *S. saxicola* C.E.Anderson.

### 2.2. Ancestral Area Reconstruction and Divergence Times Estimation

The S-DEC reconstruction suggests that the most recent common ancestor (MRCA) of *Stigmaphyllon* was widespread in the northern portion of the BAFs ca. 26.0 Mya (Figures 2 and 3, node 3, Table 1). A dispersal event (node 3) took place then, splitting the MRCA of the *Stigmaphyllon paralias* group from the remaining species, which dispersed from Seasonally Dry Tropical Forests (SDTFs) to Campos Rupestres in northeastern Brazil ca. 14.0 Mya (Figures 2 and 3, node 4, Table 1). The MRCA of the *S. ciliatum* and *S. timoriense* (DC.) C.E.Anderson clade diverged ca. 22.0 Mya (node 5) and split into those lineages ca. 10.0 Mya (node 6), colonizing dunes vegetation on the Atlantic and Australasian rainforests (Figures 2 and 3, node 6, Table 1). The MRCA of core *Stigmaphyllon* dispersed to the southern portion of the BAFs ca. 19.0 Mya (Figures 2 and 3, node 7, Table 1). The MRCA of the *S. puberulum* group arose 6.25 Mya, being widespread over both the Amazon and Atlantic rainforests, with a vicariant event giving rise to its current lineages (Figures 2 and 3, node 8, Table 1). The MRCA of the remaining species (node 9) remained distributed in the BAFs ca. 17.0 Mya, giving rise to the MRCA of the *S. auriculatum* group ca. 9.4 Mya (node 10). A dispersal event followed by the colonization of dunes vegetation in Southern Brazil gave rise to the *S. arenicola* lineage (Figures 2 and 3, node 10, Table 1). In the Pleistocene, ca. 0.72 Mya, the MRCA of the *S. auriculatum* and *S. angustilobum* arose in the BAFs from Southeastern Brazil, giving rise to those lineages via a dispersal event from rainforests to SDTFs (Figures 2 and 3, node 11, Table 1). The MRCA of *S. urenifolium* (Figures 2 and 3, node 12, Table 1)
arose ca. 15.0 Mya via a dispersal and a vicariant event from the BAFs to the Cerrado. The MRCA of the remaining species (Figures 2 and 3, node 13, Table 1) arose ca. 13.0 Mya in the BAFs and, probably, in the Amazon forest, as well. The MRCA of node 14 remained distributed within the Southeastern portion of the BAFs ca. 7.0 Mya (Figures 2 and 3, node 14, Table 1) and diversified into its four main lineages ca. 4.0 Mya (Figures 2 and 3, nodes 15–16, Table 1). Both a dispersal and a vicariant event took place ca. 12.0 Mya giving rise to the MRCA of node 17, which was distributed within the BAFs, Caatinga, and the Amazon rainforest. The lineage of node 18 colonized the Amazon rainforest for the first time, but only diversified ca. 0.5 Mya (Figures 2 and 3, node 18, Table 1).

Figure 2. Chronogram and Statistical-Dispersal-Extinction-Cladogenesis (S-DEC) ancestral area reconstructions for *Stigmaphyllon*. Nodes are numbered from 1 to 26. Numbers above branches represent estimated ages (Mya). Branch letters on the left represent the reconstructed ancestral area(s): (A) Atlantic rainforest; (B) Seasonally Dry Tropical Forest; (C) Amazon rainforest; (D) Cerrado; (E) Australasian rainforests. Branch letters on the right represent dispersal (d) or vicariant (v) events.
Figure 3. Times of dispersal/vicariance events leading to biome shifting in Stigmaphyllon. (blue) Atlantic rainforest; (purple) Seasonally Dry Tropical Forest; (orange) Amazon Rainforest; (red) Cerrado. Green circles represent the original ancestral populations of Stigmaphyllon. Dark circles represent populations of Stigmaphyllon colonizing new biomes over different time periods.

Table 1. Divergence times estimates (maximum/mean/minimum), ancestral area reconstructions, and dispersal/vicariance events reconstructed for all nodes in this study.

| Nodes | Max  | Age  | Min  | Ancestral Area Reconstruction       | Dispersal/Vicariance Event |
|-------|------|------|------|-------------------------------------|---------------------------|
| 1     | 40.0 | 39.95| 35.0 | Atlantic Forest/Caatinga            | Dispersal                 |
| 2     | 41.29| 36.39| 29.92| Atlantic Forest/Caatinga            | Dispersal                 |
| 3     | 34.09| 26.47| 19.35| Atlantic Forest                    | Dispersal                 |
| 4     | 22.14| 14.06| 6.76 | Caatinga                           | Dispersal                 |
| 5     | 30.14| 22.41| 15.43| Atlantic Forest                    | Dispersal                 |
| 6     | 19.87| 10.18| 2.18 | Atlantic Forest/Asian Rainforests   | Vicariance                |
| 7     | 26.14| 19.19| 12.71| Atlantic Forest                    | Dispersal                 |
| 8     | 12.35| 6.25 | 1.06 | Atlantic Forest/Caatinga/Amazon Forest | Vicariance               |
| 9     | 24.05| 17.45| 11.64| Atlantic Forest                    | -                         |
| 10    | 16.22| 9.4  | 3.18 | Atlantic Forest                    | Dispersal                 |
2.3. Meta-Analysis

We identified 113 genera of ferns/lycophytes, gymnosperms, magnoliids, monocots, and eudicots comprising lineages exclusively diversified in the BAF biome (out of a total of 2224 genera currently recorded by the Flora do Brasil Project; Table 2). Only 64 of those genera have estimated diversification ages available from 34 phylogenetic studies published from 2004 to 2020 (Figure 4, Table 2). Most of those studies were published from 2015 to 2020 when molecular clocks and time-calibrated trees were already widespread in phylogenetic literature.

Table 2. Age of Brazilian Atlantic Rainforests (BAF) lineages of vascular plants based on phylogenetic literature and distribution data from Flora do Brasil [13] and Plants of the World Online [17]. ± refers to ages unavailable from the consulted literature.
Table 2. Cont.

| Family                | Genus/Lineage                      | BAF spp./Genus | Phytophysiognomy | Max    | Mean  | Min    | Reference |
|-----------------------|------------------------------------|----------------|------------------|--------|-------|--------|-----------|
| Magnoliids            |                                     |                |                  |        |       |        |           |
| Monimiaceae           | *Macroturus* Perkins                | 1/1            | Rainforest       | ?      | 11.26 | ?      | [22]      |
| Monimiaceae           | *Mollinedia* Ruiz and Pav.          | 32/55          | Rainforest       | ?      | 2.20  | ?      | [22]      |
| Monocots              |                                     |                |                  |        |       |        |           |
| Amaryllidaceae        | *Griffinia* Ker Gawl.               | 17/22          | Grassland        | ?      | ?     | ?      | -         |
| Araceae               | *Asterestigma* Fisch. and C.A. Mey. | 8/10           | Rainforest       | ?      | ?     | ?      | -         |
| Araceae               | *Dracontias* Engl.                 | 2/2            | Rainforest       | ?      | ?     | ?      | -         |
| Asparagaceae          | *Herreria* Ruiz and Pav.           | 6/8            | Rainforest       | ?      | ?     | ?      | -         |
| Bromeliaceae          | *(E.Morren ex Mez)* Harms           | 30/40          | Rainforest       | 5.5    | 3.3   | 1.5    | [23]      |
| Bromeliaceae          | *Araeococcus* Brong.               | 6/9            | Rainforest       | ?      | 3.5   | ?      | [24]      |
| Bromeliaceae          | *Billbergia* Thunb.                | 35/63          | Rainforest       | ?      | 4.5   | ?      | [24]      |
| Bromeliaceae          | *Canistropis* (Mez) Leme            | 11/12          | Rainforest       | ?      | 3.5   | ?      | [24]      |
| Bromeliaceae          | *(Stigmatodon Leme, G.K.Br. and Barfuss)* | 18/18        | Rainforest       | 6.4    | 5.5   | 2.8    | [23]      |
| Bromeliaceae          | *Vriesea* Lindl.                   | 167/255        | Rainforest       | 6.8    | 5.0   | 3.3    | [23]      |
| Commelinaceae         | *Siderasis* Raf.                   | 6/6            | Rainforest       | 16.69  | 8.57  | 2.26   | [25]      |
| Commelinaceae         | *(Dichorisandra J.C.Mikan)* Leme    | 40/52          | Rainforest       | 6.38   | 2.78  | 0.32   | [25]      |
| Dioscoreaceae         | *Dioscora* L.                      | 81/628         | Rainforest       | 30.0   | 22.0  | 15.0   | [26]      |
| Iridaceae             | *Neomarica* Sprague                | 27/27          | Grassland        | ?      | 6.5   | ?      | [27]      |
| Marantaceae           | *Ctenanthe* Eichler                | 11/15          | Rainforest       | ?      | ?     | ?      | -         |
| Marantaceae           | *Maranta* L.                       | 20/37          | Rainforest       | ?      | ?     | ?      | -         |
| Marantaceae           | *Saranthe Eichler*                 | 8/10           | Rainforest       | ?      | ?     | ?      | -         |
| Marantaceae           | *Thalia* L.                        | 4/6            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *Bifrenaria* Lindl.                | 17/21          | Rainforest       | ?      | 13.0  | ?      | [28]      |
| Orchidaceae           | *Capanemia*                        | 6/9            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Centroglossa Barb.Rodr.)*        | 6/6            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *Cirrhaa* Lindl.                   | 7/7            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *Hoehnelia Ruschi*                 | 2/2            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Isabelia Barb. Rodr.)*           | 3/3            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Lankesterella Ames)*             | 7/11           | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Loefgrenianthus Hoehne)*         | 1/1            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Miltonia Lindl.)*                | 19/19          | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Phymatidium Lindl.)*             | 9/9            | Rainforest       | ?      | ?     | ?      | -         |
| Orchidaceae           | *(Pseudolaelia Porto and Brade)*   | 10/15          | Rainforest       | ?      | ?     | ?      | -         |
| Poaceae               | *(Chusquea Kunth)*                 | 45/185         | Rainforest       | ?      | 9.0   | ?      | [29]      |
| Poaceae               | *(Merostachys Spreng.)*            | 44/53          | Rainforest       | ?      | ?     | ?      | -         |
| Poaceae               | *(Olyra L.)*                       | 9/15           | Rainforest       | ?      | 14.0  | ?      | [30]      |
| Poaceae               | *(Raddia Bertol.)*                 | 9/12           | Rainforest       | ?      | 22.0  | ?      | [30]      |
Table 2. Cont.

| Family           | Genus/Lineage                             | BAF spp./Genus | Phytophysiognomy | Max  | Mean  | Min  | Reference |
|------------------|------------------------------------------|----------------|-------------------|------|-------|------|-----------|
| **Eudicots**     |                                          |                |                   |      |       |      |           |
| Acanthaceae      | Herpetocanthus Nees                       | 14/21          | Rainforest        | ?    | ?     | ?    | -         |
| Apocynaceae      | Bahiella J.F.Morales                      | 2/2            | Rainforest        | ?    | ?     | ?    | -         |
| Aristolochiaceae | Peplonia Decne. Barrosa R.M.King and H.Rob.| 8/13           | Rainforest        | ?    | 13.0  | ?    | [31]      |
| Asteraceae       | Disynaphia Hook. and Arn. ex DC. Grazelia R.M.King and H.Rob. | 7/11           | Rainforest        | ?    | ?     | ?    | -         |
| Asteraceae       | Pamphilea DC.                             | 8/9            | Rainforest        | ?    | ?     | ?    | -         |
| Asteraceae       | Stiffia J.C.Mikan                         | 4/6            | Rainforest        | ?    | 34.0  | ?    | [32]      |
| Asteraceae       | Piptocarpha R.Br.                         | 24/50          | Rainforest        | ?    | ?     | ?    | -         |
| Bignoniaceae     | Paraatecoma Kuhl.                         | 1/1            | Rainforest        | ?    | ?     | ?    | -         |
| Bignoniaceae     | Zeyheria Mart.                            | 2/2            | Rainforest        | ?    | ?     | ?    | -         |
| Cactaceae        | Rhipsalis Gaertn.                         | 37/43          | Rainforest        | 11.82| 7.67  | 4.26 | [34]      |
| Callophyllaceae  | Kielmeiera Mart. and Zucc.                | 24/50          | Rainforest        | 23.0 | 15.54 | 10.0 | [35]      |
| Cleomaceae       | Tarenuya Raf. Toconitosis                 | 14/14          | Rainforest        | 18.39| 16.60 | 14.7 | [36]      |
| Clusiaceae       | Planch. and Triana                        | 2/2            | Rainforest        | 34.0 | 20.46 | 13.0 | [37]      |
| Erythroxylaceae  | Erythroxylum P.Browne Brasiliocroton      | 71/259         | Rainforest        | ?    | ?     | ?    | -         |
| Euphorbiaceae    | P.E.Berry and Cordeiro                    | 2/2            | Rainforest        | 58.42| 48.13 | 39.6 | [38]      |
| Euphorbiaceae    | Astraea Klotzsch                          | 10/14          | Rainforest        | 34.38| 19.05 | 4.46 | [38]      |
| Euphorbiaceae    | Croton L.                                 | 98/1157        | Rainforest        | 40.92| 39.03 | 37.0 | [38]      |
| Fabaceae         | Dahlstedtia Malme                         | 9/16           | Rainforest        | ?    | ?     | ?    | -         |
| Fabaceae         | Holocalyx Micheli Schrad.                | 1/1            | Rainforest        | 52.1 | 1.3   | 28.8 | [39]      |
| Fabaceae         | Maldenhawera Schrad. Parapiptadenia Brenan | 8/11           | Rainforest        | ?    | 48.0  | ?    | [40]      |
| Fabaceae         | Paubrasilia Paubrasilia                   | 5/6            | Rainforest        | ?    | 11.0  | ?    | [41]      |
| Fabaceae         | Gagnon, H.C.Lima and G.P.Lewis            | 1/1            | Rainforest        | ?    | 48.0  | ?    | [41]      |
| Fabaceae         | Schizolobium Vogel                        | 1/1            | Rainforest        | ?    | 40.5  | ?    | [41]      |
| Gentianaceae     | Calolisianthus (Griseb.) Gilg             | 4/4            | Rainforest        | ?    | ?     | ?    | -         |
| Gentianaceae     | Chelonanthus (Griseb.) Gilg               | 4/5            | Rainforest        | ?    | ?     | ?    | -         |
| Gentianaceae     | Deianira Cham. and Schidl.                | 4/7            | Rainforest        | ?    | ?     | ?    | -         |
| Gentianaceae     | Prepusa Mart.                             | 5/6            | Rainforest        | ?    | ?     | ?    | -         |
| Gentianaceae     | Senasa Taub. Tetrapolinia                 | 1/2            | Rainforest        | ?    | ?     | ?    | -         |
| Gentianaceae     | Maguire and B.M.Boom                      | 1/1            | Rainforest        | ?    | ?     | ?    | -         |
| Gesneriaceae     | Codonanthe (Mart.) Hanst.                 | 8/9            | Rainforest        | ?    | 7.0   | ?    | [42]      |
| Gesneriaceae     | Nematanthus Schrad.                      | 32/32          | Rainforest        | ?    | 7.0   | ?    | [42]      |
| Gesneriaceae     | Palavanu Vand.                            | 4/6            | Rainforest        | ?    | 7.0   | ?    | [42]      |
Table 2. Cont.

| Family          | Genus/Lineage               | BAF spp./Genus | Phytophysiognomy | Max | Mean | Min | Reference |
|-----------------|-----------------------------|----------------|------------------|-----|------|-----|-----------|
| Eudicots        |                             |                |                  |     |      |     |           |
| Gesneriaceae    | Sinningia Nees              | 70/75          | Rainforest       | 14.0| 14.0 | 10.0| [42]     |
| Gesneriaceae    | Vanhouttea Lem.             | 9/10           | Rainforest       | 7.0 | 7.0  | 5.0 | [42]     |
| Loasaceae       | Blumenbachia Schrad. Barnebya (Griseb.) | 7/12 | Rainforest       | 4.22| 27.5 | 20.7| [43]     |
| Malpighiaceae   | W.R. Anderson and B.Gates   | 1/2            | Rainforest       | 62.0| 62.0 | 50.0| [44]     |
| Melastomataceae | Behuria Cham.               | 17/17          | Rainforest       | 8.50| 8.50 | 6.50| [45]     |
| Melastomataceae | Bertolonia Raddi            | 27/27          | Rainforest       | 9.26| 9.26 | 7.26| [45]     |
| Melastomataceae | Hueria DC. Physeterostemon  | 13/17          | Rainforest       | 8.50| 8.50 | 6.50| [45]     |
| Malpighiaceae   | R.Goldenb. and Amorim       | 5/5            | Rainforest       | 2.50| 2.50 | 1.50| [45]     |
| Melastomataceae | Pleochiton Naudin ex A. Gray| 12/12          | Rainforest       | 4.43| 4.43 | 3.43| [45]     |
| Moraceae        | Clarisia Ruiz and Pav.      | 2/2            | Rainforest       | 7.0 | 7.0  | 5.0 | -        |
| Moraceae        | Ficus L.                    | 38/874         | Rainforest       | 25.0| 25.0 | 20.0| [46]     |
| Myrtaceae       | Accara Landrum Blepharocalyx | 1/1           | Rainforest       | 32.0| 32.0 | 28.0| [47]     |
| Myrtaceae       | O. Berg.                    | 3/4            | Rainforest       | 40.0| 40.0 | 35.0| [47]     |
| Myrtaceae       | Calyptranthes Sw.           | 35/35          | Rainforest       | 24.0| 24.0 | 20.0| [47]     |
| Myrtaceae       | Curitha Salywon and Landrum | 1/1            | Rainforest       | 40.0| 40.0 | 35.0| [47]     |
| Myrtaceae       | Eugenia L.                  | 2/54/1149      | Rainforest       | 44.0| 44.0 | 34.0| [47]     |
| Myrtaceae       | Myrocygienia                | 33/45          | Rainforest       | 25.0| 25.0 | 20.0| [47]     |
| Myrtaceae       | Myrcia DC.                  | 200/609        | Rainforest       | 28.0| 28.0 | 22.0| [48]     |
| Myrtaceae       | Myrcaria O. Berg.           | 18/27          | Rainforest       | 26.0| 26.0 | 20.0| [48]     |
| Myrtaceae       | Myrthanium Schott           | 1/1            | Rainforest       | 29.0| 29.0 | 25.0| [47]     |
| Myrtaceae       | Neomitrantes D. Legrand     | 15/15          | Rainforest       | 12.0| 12.0 | 8.0 | [47]     |
| Myrtaceae       | Plinia L.                   | 29/78          | Rainforest       | 37.0| 37.0 | 30.0| [47]     |
| Myrtaceae       | Psilium L.                  | 39/91          | Rainforest       | 20.0| 20.0 | 15.0| [47]     |
| Rubiaceae       | Balgyaa C. Presl Bradea Standl. ex Brade | 6/10 | Rainforest       | 20.0| 20.0 | 15.0| [48]     |
| Rubiaceae       | Bradea Standl. ex           | 6/6            | Rainforest       | ?   | ?   | ?   | -        |
| Rubiaceae       | Coccocyptelum P. Browne     | 14/22          | Rainforest       | ?   | ?   | ?   | -        |
| Rutaceae        | Conocarpus J.C. Mikans Mettrodora A. St.-Hil Neoraputia | 40/48 | Rainforest       | ?   | ?   | ?   | -        |
| Rutaceae        | Emmerich ex Kallunki        | 5/6            | Rainforest       | ?   | ?   | ?   | -        |
| Sapindaceae     | Cardospermum L. Thinsia Triana and Planck. | 6/10 | Rainforest       | 23.0| 23.0 | 18.0| [49]     |
| Sapindaceae     | Petunia Juss.               | 7/11           | Rainforest       | ?   | ?   | ?   | -        |
| Solanaceae      | Callisthene Mart.           | 10/16          | Grassland        | 11.5| 8.49 | 5.5 | [50]     |
| Vochysiaceae    |                             | 8/8            | Rainforest       | 30.0| 22.0 | 14.0| [51]     |
3. Discussion

3.1. Phylogenetics of Stigmaphyllon

The topology recovered from the combined dataset (i.e., ribosomal + nuclear markers) evidenced that the subgenera of *Stigmaphyllon* proposed by Anderson [10] are paraphyletic. All previous phylogenetic studies of Malpighiaceae sampled mostly Mesoamerican and Amazonian species of the genus [2,3,5,9,44], making it difficult to properly test the monophyly of its subgenera. The only BAF species of *Stigmaphyllon* sampled in previous studies were *S. ciliatum* and *S. paralias*. However, in all these studies *S. paralias* (a Brazilian Atlantic Rainforest lineage) is consistently recovered as sister to all remaining lineages of *Stigmaphyllon*. Additionally, this is the first time *S. auriculatum*, the type species of the genus, is included in a phylogenetic study. On the other hand, our results highly corroborate the previous topologies recovered for *Stigmaphyllon*, with the *S. paralias* group recovered as the first lineage to diverge, followed by the clade comprising the *S. ciliatum* group + *S. subg. Ryssopterys* sister to a large clade consisting of species from the *S. tomentosum* group (core *Stigmaphyllon*) [2,9]. Additional species sampling allied to a thorough morphological study on a phylogenetic perspective in *Stigmaphyllon* is urgently required to shed some light on its infragenic classification.

3.2. Divergence Times and Biogeography of Stigmaphyllon

Our divergence times estimation for the MRCA of *Stigmaphyllon* (ca. 26.5 Mya) is similar to the age of 22.5 Mya estimated by Davis et al. [44] and of 21.0 Mya estimated by Willis et al. [9]. The estimated ages for the MRCA obtained by us for the *S. paralias* group and the *S. ciliatum* + *S. subg. Ryssopterys* clade strongly corroborates those previously reported by Willis et al. [9] (17.0, 15.0, and 10.0 Mya, respectively). The only exception was the age of 19.0 Mya estimated by us for core-*Stigmaphyllon* when Willis et al. [9] recovered the estimation of 10.0 Mya for this clade. This apparent difference in age estimates might be due to the more comprehensive sampling of this clade in the present study.
Over these last 26.0 Mya, the lineages of *Stigmaphyllon* went through 13 dispersals and four vicariance events (VE). Worth noting is that VEs in this genus were always followed by a biome shifting event (BSE) from the BAFs to another tropical biome. The first VE followed by a BSE in *Stigmaphyllon* occurred ca. 15.0 Mya in the MRCA of *S. urenifolium*, from the BAFs to the Brazilian Cerrado. Several plant lineages present the same diversification pattern with older ancestors arising in the BAFs and colonizing the Cerrado biome ca. 15 Mya, such as clade 5 of *Amphilophium* Kunth (Bignoniaceae) [52], *Astraea* Klotzsch (Euphorbiaceae) [38], *Dolichandra* Cham. (Bignoniaceae) [33], *Fridericia* Mart. (Bignoniaceae) [52], and *Xylophragma* Sprague (Bignoniaceae) [53]. Dispersal events from forest to open habitats is one of the main factors explaining richness in Neotropical biodiversity [16]. Even though in *Stigmaphyllon*, those dispersal events occurred mostly among forested biomes, its single Cerrado lineage seems to point to an older diversification of this biome, such as in those from Vochysiaceae (20.0–15.0 Mya) [51]. Few species of *Stigmaphyllon* successfully colonized the Cerrado biome, such as *S. jobertii*, *S. occidentale*, *S. tomentosum*, and *S. urenifolium*. Future studies sampling those species in the molecular phylogeny of *Stigmaphyllon* are crucial to test if the genus colonized the Cerrado biome more than a single time. However, several studies seem to present the same pattern pointed by us of forest lineages occupying and diversifying in the Cerrado biome [16], with the opposite rarely recorded in biogeographic studies. We hypothesize that Cerrado lineages colonizing forested biomes might be rare due to the recent diversification of several plant lineages of Neotropical savannas [54].

The second VE followed by a BSE in *Stigmaphyllon* occurred ca. 12.0 Mya in the MRCA of *S. sinuatum* group, from the BAFs to the Amazon rainforest. The same pattern of diversification was recorded in other plant lineages, with Atlantic rainforest MRCAcolonizing the Amazon rainforest at this time, such as *Eugenia* L. clade G [55]. At least 50 dispersal events occurred between the Atlantic and Amazon rainforests [16]. The number of lineage exchanges between these biomes fluctuated over the last 60.0 Mya, with its previous increase starting ca. 12.0 Mya and peaking ca. 6.0–3.0 Mya [16], corroborating our results with *Stigmaphyllon*. The MRCA of *S. finlayanum* group was the fourth VE, followed by a BSE occurring in the genus, ca. 6.30 Mya, from the BAFs to the Amazon rainforest. The same pattern of diversification was recorded in other plant lineages, with Atlantic rainforest MRCAcolonizing the Amazon rainforest at this time, such as the clade 3 of *Amphilophium* Kunth (Bignoniaceae) [52] and the abovementioned study by Antonelli et al. [16].

The third VE followed by a BSE in *Stigmaphyllon* occurred ca. 10.0 Mya in the MRCA of *S. subg. Ryssopterys* + *S. ciliatum* group, from the BAFs to the Asian rainforests. This lineage diversified ca. 22.0 Mya, so we hypothesize that its MRCA was widely distributed among dunes vegetation in the Atlantic and Asian rainforests via the Antarctic route. Antarctica’s glaciation process started ca. 34.0 Mya, during the Eocene/Oligocene transition, on high altitude regions of this continent [56]. From 34.0–12.0 Mya, Antarctica had intermittent ice sheet coverage, leaving intact Tertiary pockets of pre-glaciation fauna and flora that only went completely extinct ca. 12.0 Mya with the formation of permanent ice sheets in this continent [57], the same confidence interval recovered in our study for the MRCA of *S. subg. Ryssopterys* + *S. ciliatum* group. The same pattern of vicariance event is observed in the *Drymophila* (Australian) + *Luzuriaga* (South American) clade (Alstromeriaceae), in which its MRCA split ca. 23.0 Mya, giving rise to these genera from ca. 10.0 to 4.0 Mya [58].

On the other hand, 14 dispersal events (DE) occurred within *Stigmaphyllon*, mostly within the North-South corridors of the BAFs, and at least three DEs from the BAFS to the Caatinga biome. From these latter three DEs, the first occurred ca. 14.0 Mya in the MRCA of *S. paralias* group, the second occurred ca. 9.0 Mya in the MRCA of *S. saxicola* group and the third occurred ca. 1.0 Mya in the MRCA of the *S. auriculatum* group. From the remaining DEs that happened within the BAFs, at least five of them occurred from North to South ca. 22.0, 17.0, 12.0, 11.0, and 9.0 Mya. The same pattern with Northern BAF lineages colonizing Southern portions of this biome is observed in *Aechmea* (Bromeliaceae) [59]. However, the North-South dispersals in this genus started only ca. 6.0 Mya (Bromeliaceae) [59], being much younger than those in *Stigmaphyllon*. 
3.3. Time and Diversification of the Atlantic Rainforest

Several implications for understanding the BAFs historical biogeography might be postulated from the biogeographical study of *Stigmaphyllon*. Our results suggest a late-Eocene origin for these forests, that seems to be partially corroborated by the literature. Until the late Paleocene and early Eocene, the Earth’s climate was mostly warmer and more humid than today, suggesting that South America was probably covered by continuous rainforests [60,61]. When comparing the mean ages recovered in published studies for several lineages of ferns, gymnosperms, and angiosperms diversified in the BAFs, we bring new evidence that vascular plants started colonizing these forests over the last 60.0 Mya (Figure 4; Table 2), corroborating the abovementioned authors. The oldest lineage to occupy the BAFs might have been the genus *Barnebya* W.R.Anderson and B.Gates ca. 60 Mya (Malpighiaceae, Eudicots), with the diversification of most Eudicot lineages in these forests occurring from 40.0 to 15.0 Mya (Figure 4; Table 2). During the late Eocene and Oligocene, global episodes of cooling and dryness favored the expansion of grasslands in the southern and central regions of the continent [60,62], which culminated in the formation of a diagonal belt of more open and drier biomes (also known as “dry diagonal”) [63]. The formation of the dry diagonal marked the formation of the Atlantic forest in the east and Amazonia in the west [64]. On the other hand, the colonization and diversification of Magnoliid lineages took place from 18.0 to 3.0 Mya (Figure 4; Table 2), followed by gymnosperm lineages that diversified from 15.0 to 11.0 Mya, and finally from monocot lineages diversifying from 12.0 to 3.0 Mya in these rainforests (Figure 4; Table 2). Fossil records and paleoclimate studies suggest that the BAFs and the Amazon rainforests were re-connected multiple times in the Miocene and Pliocene [64]. Mean ages regarding the colonization and diversification of ferns in the BAFs are still incipient, with a single study on the family Cyatheaceae evidencing its initial diversification in these forests at 30.0 Mya (Figure 4; Table 2). Additionally, from the 113 BAF lineages presented in Table 2, only 64 show mean ages based on time-calibrated phylogenies available in the literature. At least 18 families of angiosperms (Acanthaceae, Amaryllidaceae, Apocynaceae, Araceae, Asparagaceae, Asteraceae, Bignoniaceae, Erythroxylaceae, Fabaceae, Gentianaceae, Lauraceae, Marantaceae, Moraceae, Orchidaceae, Poaceae, Rubiaceae, Rutaceae, and Sapindaceae) with lineages diversified in the Atlantic forest still lack published time-calibrated phylogenies.

Another worth mentioning factor that might have played an important role in the diversification of the BAFs was the uplift of Serra do Mar and Serra da Mantiqueira Mountain Ranges in Eastern Brazil. Those mountains were previously thought to have uplifted around 120.0 Mya, but geological studies from the past decade have pointed to an earlier uplifting age for these mountains, from 60.0 to 30.0 Mya [65]. The tertiary uplift age of these mountains in Eastern Brazil was a direct result of the Andean uplift, coinciding with our results for the colonization of the BAFs by 64 lineages of vascular plants [65].

4. Material and Methods

4.1. Taxon Sampling and Plant Material

We sampled 24 species of *Stigmaphyllon* (ca. ¼ of the Neotropics’ genus diversity: 18 spp. from the Brazilian Atlantic Rainforest (out of 31 spp.), three spp. from the Amazon Rainforest, two spp. from the Caatinga, and one spp. from the Cerrado biomes), including outgroups *Bronwenia* W.R.Anderson and C.C.Davis and *Diplopterys* A.Juss. From this total, 23 species represent *S* subg. *Stigmaphyllon* and a single species *Stigmaphyllon timoriense* (DC) C.E.Anderson represents *S* subg. *Ryssopterys* (Table 3). For DNA extraction, we used mainly field-prepared silica dried leaves (12–80 mg) and herbarium specimens as necessary (Table 3).
Table 3. Species and DNA regions sampled in this study. Genbank accession numbers are presented for columns ETS (External Transcribed Spacer), ITS (Internal Transcribed Spacer), and PHYC (Phytochrome C gene). * Sequences were obtained from GenBank.

| Species                          | Voucher (Herbarium Acronym) | ETS          | ITS          | PHYC           |
|----------------------------------|-----------------------------|--------------|--------------|----------------|
| *Bronwenia cinerascens* (Benth.) | Nee 48, 658 (SP)            | KR054586.1   | HQ246821.1   | HQ246987.1     |
| W.R. Anderson and C.C. Davis     | Nee 48, 658 (SP)            | KR054586.1   | HQ246821.1   | HQ246987.1     |
| *Diplopterys pubipetala* (A.Juss.) | Francener 1126 (SP)         | KR092986     | HQ246821.1   | HQ247045.1     |
| W.R. Anderson and C.C. Davis     | Francener 1126 (SP)         | KR092986     | HQ246821.1   | HQ247045.1     |
| *Stigmaphyllon alternifolium* A.Juss. | Almeida 501 (SP)            | KR054612.1   | -            | -              |
| W.R. Anderson and C.C. Davis     | Almeida 501 (SP)            | KR054612.1   | -            | -              |
| *Stigmaphyllon angustilobum* A.Juss. | Almeida 503 (SP)            | KR054591.1   | MT559811     | -              |
| C.E. Anderson                    | Almeida 503 (SP)            | KR054591.1   | MT559811     | -              |
| *Stigmaphyllon arenicola* C.E. Anderson | Sebastani 5 (SP)            | KR054589.1   | -            | -              |
| C.E. Anderson                    | Sebastani 5 (SP)            | KR054589.1   | -            | -              |
| *Stigmaphyllon auriculatum* (Cav.) | Almeida 584 (HUEFS)         | KR054592.1   | MT559812     | -              |
| A.Juss.                          | Almeida 584 (HUEFS)         | KR054592.1   | MT559812     | -              |
| *Stigmaphyllon cancellatum* C.E. Anderson | Almeida 552 (SP)            | KR054615.1   | MT559813     | -              |
| C.E. Anderson                    | Almeida 552 (SP)            | KR054615.1   | MT559813     | -              |
| *Stigmaphyllon cancellatum* 1   | Queirós 13, 530 (HUEFS)     | KR054607.1   | -            | -              |
| R.F. Almeida and Amarim          | Queirós 13, 530 (HUEFS)     | KR054607.1   | -            | -              |
| *Stigmaphyllon cancellatum* 2   | Almeida 577 (HUEFS)         | KR054595.1   | MT559814     | -              |
| R.F. Almeida and Amarim          | Almeida 577 (HUEFS)         | KR054595.1   | MT559814     | -              |
| *Stigmaphyllon caatingicola* C.E. Anderson | Cardoso 2083 (HUEFS)        | MT490608     | MT559815     | -              |
| C.E. Anderson                    | Cardoso 2083 (HUEFS)        | MT490608     | MT559815     | -              |
| *Stigmaphyllon ciliatum* (Lam.)  | Almeida 541 (SP)            | KR054590.1   | -            | HQ247151.1     |
| A.Juss.                          | Almeida 541 (SP)            | KR054590.1   | -            | HQ247151.1     |
| *Stigmaphyllon finlayanum* A.Juss. | Pace 457 (SPF)              | KR054599.1   | -            | HQ247152.1     |
| Stigmaphyllon gayanum A.Juss.    | Pace 457 (SPF)              | KR054599.1   | -            | HQ247152.1     |
| *Stigmaphyllon harleyi* C.E. Anderson | Almeida 500 (SP)            | KR054610.1   | -            | -              |
| C.E. Anderson                    | Almeida 500 (SP)            | KR054610.1   | -            | -              |
| *Stigmaphyllon jatrophiophilum* A.Juss. | Santos 378 (HUEFS)         | KR054594.1   | MT581513     | -              |
| C.E. Anderson                    | Santos 378 (HUEFS)          | KR054594.1   | MT581513     | -              |
| *Stigmaphyllon jandalanum* A.Juss. | Filho s.n. (SP369102)       | KR054614.1   | -            | -              |
| *Stigmaphyllon lindemanianum* A.Juss. | Kollmann 4279 (CEPEC)       | KR054596.1   | -            | -              |
| C.E. Anderson                    | Kollmann 4279 (CEPEC)       | KR054596.1   | -            | -              |
| *Stigmaphyllon macropodum* A.Juss. | Aguilar 718 (SP)            | KR054603.1   | -            | HQ247153.1     |
| C.E. Anderson                    | Aguilar 718 (SP)            | KR054603.1   | -            | HQ247153.1     |
| *Stigmaphyllon paralas* A.Juss.  | Almeida 538 (SP)            | KR054602.1   | MT559816     | -              |
| C.E. Anderson                    | Almeida 538 (SP)            | KR054602.1   | MT559816     | -              |
| *Stigmaphyllon puberulum* A.Juss. | Almeida 509 (SP)            | KR054593.1   | KY241909.1   | AF500566.1     |
| C.E. Anderson                    | Almeida 509 (SP)            | KR054593.1   | KY241909.1   | AF500566.1     |
| *Stigmaphyllon salzmannii* A.Juss. | Perdiz 732 (HUEFS)         | KR054600.1   | -            | -              |
| C.E. Anderson                    | Perdiz 732 (HUEFS)          | KR054600.1   | -            | -              |
| *Stigmaphyllon saxicola* C.E. Anderson | Badini 24,261 (HUEFS)       | KR054605.1   | MT559818     | -              |
| C.E. Anderson                    | Badini 24,261 (HUEFS)       | KR054605.1   | MT559818     | -              |
| *Stigmaphyllon sinuatum* (DC.)   | Amorim 3159 (CEPEC)         | KR054608.1   | -            | -              |
| A.Juss.                          | Amorim 3159 (CEPEC)         | KR054608.1   | -            | -              |
| *Stigmaphyllon timoriense* (DC)  | Anderson 796 (US)           | -            | -            | AF500545.1     |
| C.E. Anderson                    | Anderson 796 (US)           | -            | -            | AF500545.1     |
| *Stigmaphyllon unerfolium* A.Juss. | Guedes 13,932 (HUEFS)      | KR054604.1   | -            | -              |
| C.E. Anderson                    | Guedes 13,932 (HUEFS)       | KR054604.1   | -            | -              |
| *Stigmaphyllon vitifolium* A.Juss. | DalCol 233 (HUEFS)         | KR054598.1   | -            | -              |

4.2. Molecular Protocols

Genomic DNA was extracted using the 2 × CTAB protocol, modified from Doyle and Doyle [66]. Three DNA regions (nuclear PHYC gene, and the ribosomal external and internal transcribed spacers (ETS and ITS)) were selected based on their variability in previous Malpighiaceae studies [2,5,6,8,67]. Protocols to amplify and sequence ETS and ITS followed Almeida et al. [8]. For amplification, we used the TopTaq (Qiagen) mix following the manufacturer’s standard protocol, with the addition of betaine (1.0 M final concentration) and 2% DMSO for the ETS region. PCR products were purified using PEG (polyethylene glycol) 11% and sequenced directly with the same primers used for PCR amplification.
Sequence electropherograms were produced on an automatic sequencer (ABI 3130XL genetic analyzer) using the Big Dye Terminator 3.1 kit (Applied Biosystems). Additional sequences for PHYC were retrieved from GenBank (Table 3). Newly generated sequences were edited using the Geneious software [68], and all datasets were aligned using Muscle [69], with subsequent adjustments in the preliminary matrices made by eye. The complete data matrices are available at TreeBase (accession number S21218). This study was authorized by the Genetic Heritage and Associated Traditional Knowledge Management National System of Brazil (SISGEN #A3B8F19).

4.3. Phylogenetic Analysis

Analyses were rooted in Bronwenia, according to Davis and Anderson [2]. Individual analyses for each marker were performed, and since no significant incongruencies were found, analyses of combined matrices (i.e., nuclear + ribosomal markers) were performed using maximum parsimony (MP) conducted with PAUP 4.0b10a [70]. A heuristic search was performed using TBR swapping (tree-bisection reconnection), and 1000 random taxon-addition sequence replicates with TBR swapping limited to 15 trees per replicate to prevent extensive searches (swapping) in suboptimal islands, followed by TBR in the resulting trees with a limit of 1000 trees. In all analyses, the characters were equally weighted and unordered [71]. Relative support for individual nodes was assessed using non-parametric bootstrapping [72], with 1000 bootstrap pseudoreplicates, TBR swapping, simple taxon addition, and a limit of 15 trees per replicate.

For the model-based approach, we selected the model GTR + I + G using hierarchical likelihood ratio tests (HLRT) on J Modeltest 2 [73]. A Bayesian analysis (BA) was conducted with mixed models and unlinked parameters, using MrBayes 3.1.2 [74]. The Markov chain Monte Carlo (MCMC) analysis was performed 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 as ‘burn-in’ trees from the first two million generations, and tree distributions were checked for a stationary phase of likelihood. The posterior probabilities (PP) of clades were based on the majority-rule consensus produced with the remaining trees in MrBayes 3.1.2 [74].

4.4. Calibration

Estimates were conducted based on a simplified ultrametric Bayesian combined tree generated with BEAST 1.8.4 [75]. This analysis used a relaxed uncorrelated lognormal clock and Yule process speciation prior to inferring trees. The calibration parameters were based on previous estimates derived from a comprehensive fossil-calibrated study of the whole Malpighiaceae [44,76]. We opted for calibrating at the root, using a normal prior with mean initial values of 40.0 Mya (representing the age estimated for the MRCA of the Stigmaphylloid clade) and a standard deviation of 1.0 [44,67,76]. 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 400 for all parameters on Tracer 1.6 [77]. Tree topology was assessed using TreeAnnotator and FigTree 1.4.0 [78].

4.5. Ancestral Area Reconstruction

Species distribution data were compiled from the taxonomic revision of Bronwenia [79], Diplopterys [80], and Stigmaphyllon [10,11] (Figure 3). Occurrences were categorized according to a modified version of the biome domains adopted by IBGE [81] and WWF [82], which reflects distribution patterns in Stigmaphyllon, namely: (A) Brazilian Atlantic Rainforest, (B) Seasonally Dry Tropical Forest [Caatinga], (C) Amazon Rainforest, (D) Cerrado, and (E) Australasian Rainforests (Figure 2). Ancestral areas of Stigmaphyllon and its relatives were estimated using a maximum likelihood analysis of geographic range evolution using software Rasp 3.2 [83]. Estimates were conducted using the statistical dispersal-extinction-cladogenesis (S-DEC) [84], according to the parameters proposed by Ree and Sanmartín [85].
4.6. Meta-Analysis

Ages of BAF lineages of vascular plants (ferns/lycophytes, gymnosperms, magnoliids, monocots, and eudicots) were compiled based on the phylogenetic literature and distribution data from Flora do Brasil [12] and Plants of the World Online [17]. Online repositories such as GBIF, BIEN and Species Link were not used since specimen identification is not usually updated or are not identified by a Malpighiaceae specialist. Additionally, the main problem in using all the above-mentioned repositories is that only Flora do Brasil present reliable information on the biome distribution of species sampled in our study. We considered a genus or lineage within a genus diversified in the BAF when at least 50% of its total number of species occurred in this biome. Data from BAF lineages of vascular plant species are presented in Table 2, alongside estimated ages, and references. A boxplot graphic is also presented in Figure 4, showing the diversification of vascular plants through time in the BAF based on data presented in Table 2.

5. Conclusions

Even though dispersal events from forested to open habitats have been recently identified as one of the main factors explaining richness in Neotropical biodiversity [16], the same pattern was not recovered for Stigmaphyllon. A late-Eocene origin for this genus is suggested, with its MRCA originating in the Northeastern BAFs, with several dispersal events taking place to other Neo- and Paleotropical biomes from 22.0 to 1.0 Mya, alongside several dispersals from Northern to Southern portions of the BAFs. When comparing our results with published divergence times for BAFs’ vascular plant lineages, a late-Eocene origin for these forests was evidenced. The immense gap in time-calibrated phylogenies focusing on BAFs’ vascular plant lineages is still the most significant impediment for a more comprehensive understanding of the plant diversification timeframe in these forests. Additionally, the recent evidenced tertiary uplift of Serra do Mar and Serra da Mantiqueira Mountain Ranges might also have played an important role in the diversification of the megadiverse BAFs.

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References

1. Xi, Z.; Ruhfel, B.R.; Schaefer, H.; Amorim, A.M.A.; Sugumaran, M.; Wurdack, K.J.; Endress, P.K.; Matthews, M.L.; Stevens, P.F.; Mathews, S.; et al. Phylogenomics and a posteriori data partitioning resolve Cretaceous angiosperm radiation Malpighiales. Proc. Natl. Acad. Sci. USA 2012, 109, 17519–17524. [CrossRef] [PubMed]

2. Davis, C.C.; Anderson, W.R. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. Am. J. Bot. 2010, 97, 2031–2048. [CrossRef] [PubMed]

3. Cameron, K.M.; Chase, M.W.; Anderson, W.R.; Hills, H.G. Molecular systematics of Malpighiaceae: Evidence from plastid rbcL and matK sequences. Am. J. Bot. 2001, 88, 1847–1862. [CrossRef] [PubMed]

4. Davis, C.C. Madagasikaria (Malpighiaceae): A new genus from Madagascar with implications for floral evolution in Malpighiaceae. Am. J. Bot. 2002, 89, 699–706. [CrossRef]
5. Davis, C.C.; Anderson, W.R.; Donoghue, M.J. Phylogeny of Malpighiaceae: Evidence from chloroplast ndhF and trnL-F nucleotide sequences. *Am. J. Bot.* 2001, 88, 1830–1846. [CrossRef]
6. Davis, C.C.; Bell, C.D.; Fritsch, P.W.; Mathews, S. Phylogeny of Acridocarpus-Brachyolphon (Malpighiaceae): Implications for tertiary tropical floras and Afroasian biogeography. *Evolution* 2002, 56, 2395–2405. [CrossRef]
7. Davis, C.C.; Fritsch, P.W.; Bell, C.D.; Mathews, S. High-latitude tertiary migrations of an exclusively tropical clade: Evidence from Malpighiaceae. *Int. J. Plant. Sci.* 2004, 165, S107–S121. [CrossRef]
8. Almeida, R.F.; Amorim, A.M.A.; Correa, A.M.S.; van den Berg, C. A new infragenetic classification for *Amorimia* (Malpighiaceae) based on morphological, phytochemical, and molecular evidence. *Phytotaxa* 2017, 313, 231–248. [CrossRef]
9. Willis, C.G.; Franzone, B.F.; Xi, Z.; Davis, C.C. The establishment of Central American migratory corridors and the biogeographic origins of seasonally dry tropical forests in Mexico. *Front. Genet.* 2014, 5, 433. [CrossRef]
10. Anderson, C.E. Revision of *Ryssopterys* and transfer to Stigmaphyllon (Malpighiaceae). *Blumea* 2011, 56, 73–104. [CrossRef]
11. Anderson, C.E. Monograph of Stigmaphyllon (Malpighiaceae). *Syst. Bot. Monographs* 1997, 51, 1–313. [CrossRef]
12. Flora do Brasil. 2020. Available online: http://floradobrasil.jbrj.gov.br/reflora/listaBrasil/ConsultaPublicaUC/ResultadoDaConsultaNovaConsulta.do#CondicaoTaxonCP (accessed on 10 November 2020).
13. Scarano, F.R.; Ceotto, P. Brazilian Atlantic forest: Impact, vulnerability, and adaptation to climate change. *Biodivers. Conserv.* 2015, 24, 2319–2331. [CrossRef]
14. Ledo, R.; Colli, G.R. The historical connections between the Amazon and the Atlantic forest revisited. *J. Biogeogr.* 2017, 44, 2551–2563. [CrossRef]
15. Vanzolini, P.E.; Williams, E.F. The vanishing refuge: A mechanism for ecogeographic speciation. *Pap. Avulsos Zool.* 1981, 34, 251–255.
16. Antonelli, A.; Zizka, A.; Carvalho, F.A.; Scharm, R.; Bacon, C.D.; Silvestro, D.; Condamine, F.L. Amazonia is the primary source of Neotropical biodiversity. *Proc. Natl. Acad. Sci. USA* 2018, 115, 6034–6039. [CrossRef][PubMed]
17. POWO-Plants of the World Online. 2020. Available online: http://www.plantsofttheworldonline.org (accessed, on 10 November 2020).
18. Korall, P.; Pryer, K.M. Global biogeography of scaly tree ferns (Cyatheaceae): Evidence for Gondwanan vicariance and limited transoceanic dispersal. *J. Biogeogr.* 2014, 41, 402–413. [CrossRef]
19. Kranitz, M.L.; Biffin, E.; Clark, A.; Hollingsworth, M.L.; Ruhsam, M.; Gardner, M.F.; Thomas, P.; Mill, R.R.; Ennos, R.A.; Gaudeul, M.; et al. Evolutionary diversification of New Caledonian *Araucaria*. *PLoS ONE* 2014, 9, e110308. [CrossRef]
20. Quiroga, M.P.; Mathiasen, P.; Iglesias, A.; Mill, R.R.; Premoli, A.C. Molecular, and fossil evidence disentangle the biogeographical history of *Podocarpus*, a key genus in plant geography. *J. Biogeogr.* 2016, 43, 372–383. [CrossRef]
21. Richardson, J.E.; Chatrou, L.W.; Mols, J.B.; Erkens, R.H.J.; Prie, M.D. Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Phil. Trans. R. Soc. Lond. B* 2004, 359, 1495–1508. [CrossRef]
22. Renner, S.S.; Strijk, J.S.; Strasberg, D.; Thèbaud, C. Biogeography of the Monimiaceae (Laurales): A role for East Gondwana and long-distance dispersal, but not West Gondwana. *J. Biogeogr.* 2010, 37, 1222–1238. [CrossRef]
23. Kessous, I.M.; Neves, B.; Couto, D.R.; Paixão-Souza, B.; Pederneiras, L.C.; Moura, R.L.; Barfuss, M.H.J.; Salgueiro, F.; Costa, A.F. Historical biogeography of a Brazilian lineage of Tillandsioideae (subtribe Vrieseinae, Bromeliaceae): The Paranaean Sea hypothesized as the main vicariant event. *Bot. J. Linn. Soc.* 2020, 192, 625–641. [CrossRef]
24. Givnish, T.J.; Barfuss, M.H.J.; Van Ek, B.; Riina, R.; Schulte, K.; Horres, R.; Gonsisika, P.A.; Jabaily, R.S.; Crayn, D.M.; Smith, J.A.C.; et al. Phylogeny, adaptative radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. *Am. J. Bot.* 2011, 98, 872–895. [CrossRef][PubMed]
25. Pellegrini, M.O.O. Systematics of Commelinaceae, Focusing on Neotropical Lineages. Ph.D. Thesis, Instituto de Biociências, São Paulo, Brazil, 2019; 650p.
26. Couto, R.S.; Martins, A.C.; Bolson, M.; Lopes, R.C.; Smidt, E.C.; Braga, J.M.A. Time calibrated tree of *Dioscorea* (Dioscoreaceae) indicates four origins of yams in the Neotropics since the Eocene. *Bot. J. Linn. Soc.* 2018, 188, 144–160. [CrossRef]

27. Vasconcelos, T.N.C.; Alcantara, S.; Andrino, C.O.; Forest, F.; Reginato, M.; Simon, M.E.; 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]

28. Gustafsson, A.L.S.; Verola, C.F.; Antonelli, A. Reassessing the temporal evolution of orchids with new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). *BMCEvol. Biol.* 2010, 10, 177. [CrossRef]

29. Ruiz-Sanchez, E. Biogeography and divergence time estimates of woody bamboos: Insights in the evolution of Neotropical bamboos. *Bot. Soc. Bot. Mex.* 2011, 88, 67–75. [CrossRef]

30. Zhang, X.Z.; Zeng, C.X.; Ma, P.F.; Haevermans, T.; Zhang, Y.X.; Zhang, L.N.; Guo, Z.H.; Li, D.Z. Multi-locus plastid phylogenetic biogeography supports the Asian hypothesis of the temperate woody bamboos (Poaceae: Bambusoideae). *Mol. Phylogenet. Evol.* 2016, 96, 118–129. [CrossRef]

31. Rapini, A.; van den Berg, C.; Liede-Schumann, S. Diversification of Asclapiadoideae (Apocynaceae) in the New World. *Ann. Missouri Bot. Gard.* 2007, 94, 407–422. [CrossRef]

32. Mandel, J.R.; Dikow, R.B.; Siniscalchi, C.M.; Thapa, R.; Watson, L.E.; Funk, V.A. A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. *Proc. Natl. Acad. Sci. USA* 2019, 116, 14083–14088. [CrossRef]

33. Fonseca, L.H.M.; Lohmann, L.G. Biogeography, and evolution of *Dolichandra* (Bignoniaceae, Bignoniaceae). *Bot. J. Linn. Soc.* 2015, 179, 403–420. [CrossRef]

34. Hernández-Hernández, T.; Brown, J.W.; Schlumberger, B.O.; Eguiarte, L.E.; Magallón, S. Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytol.* 2014, 202, 1382–1397. [CrossRef] [PubMed]

35. Meseguer, A.S.; Lobo, J.M.; Cornuault, J.; Beerling, D.; Ruhfel, B.R.; Davis, C.C.; Jousselin, E.; Sammartin, I. Reconstructing deep-time paleoclimate legacies in the clusioid Malpighiales unveil their role in the evolution and extinction of the boreotropical flora. *Glob. Ecol. Biogeogr.* 2018, 27, 616–628. [CrossRef]

36. Soares-Neto, R.L.; Thomas, W.W.; Barbosa, M.R.V.; Ralson, E.H. Diversification of New World Cleomaceae with emphasis on *Tarenaya* and the description of *Illisella*, a new genus. *Taxon* 2020, 69, 321–336. [CrossRef]

37. Ruhfel, B.R.; Bove, C.P.; Philbrick, C.T.; Davis, C.C. Dispersal largely explains the Gondwanan distribution of the ancient tropical clusioid plant clade. *Am. J. Bot.* 2016, 103, 1117–1128. [CrossRef] [PubMed]

38. Silva, O.L.M.; Riina, R.; Cordeiro, I. Phylogeny and biogeography of *Astraea* with new insights into the evolutionary history of Croton-eae (Euphorbiaceae). *Mol. Phylogenet. Evol.* 2020, 145, 106738. [CrossRef] [PubMed]

39. Lavin, M.; Herendeen, P.S.; Wojciechowski, M.F. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Syst. Biol.* 2005, 54, 575–594. [CrossRef]

40. Bruneau, A.; Mercure, M.; Lewis, G.P.; Herendeen, P.S. Phylogenetic patterns and diversification in the caesalpinioi legumes. *Botany* 2008, 86, 697–718. [CrossRef]

41. Gagnon, E.; Ringelberg, J.J.; Bruneau, A.; Lewis, G.P.; Hughes, C.E. Global Succulent Biome phylogenetic conservatism across the pantropical Caesalpinia group (Leguminosae). *New Phytol.* 2019, 222, 1994–2008. [CrossRef] [PubMed]

42. Roalson, E.H.; Roberts, W.R. Distinct processes drive diversification in different clades of Gesneriaceae. *Syst. Biol.* 2016, 65, 662–684. [CrossRef]

43. Castillo, R.A.; Luebert, F.; Henning, T.; Weigend, M. Major lineages of Loasaceae subfam. Loasoideae diversified during the Andean uplift. *Mol. Phylogenet. Evol.* 2019, 141, 106616. [CrossRef]

44. Davis, C.C.; Schaefer, H.; Xi, 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] [PubMed]

45. Reginato, M.; Vasconcelos, T.N.C.; Kriebel, R.; Simões, A.O. Is dispersal mode a driver of diversification and geographical distribution in the tropical plant family Melastomataceae? *Mol. Phylogenet. Evol.* 2020, 148, 106815. [CrossRef] [PubMed]
46. Machado, A.F.P.; Ronsted, N.; Bruun-Lund, S.; Pereira, R.A.S.; Queiroz, L.P. Atlantic forest trees to the all Americas: Biogeographical history and divergence times of Neotropical Ficus (Moraceae). Mol. Phylogenet. Evol. 2018, 122, 46–58. [CrossRef] [PubMed]
47. Vasconcelos, T.N.C.; Proença, C.E.B.; Ahmad, B.; Aguilar, D.S.; Aguilar, R.; Amorim, B.S.; Campbell, K.; Costa, I.R.; De-Cardvalho, P.S.; Faria, J.E.Q.; et al. Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species-rich tribe of Myrtaceae. Mol. Phylogenet. Evol. 2017, 109, 113–137. [CrossRef] [PubMed]
48. Santos, M.F.; Lucas, E.; Sano, P.T.; Buerki, S.; Staggemeier, V.G.; Forest, F. Biogeographical patterns of Myrcia s.l. (Myrtaceae) and their correlation with geological and climatic history in the Neotropics. Mol. Phylogenet. Evol. 2017, 108, 34–48. [CrossRef] [PubMed]
49. Buerki, S.; Forest, F.; Alvarez, N.; Nylander, J.A.A.; Arrigo, N.; Sanmartín, I. An evaluation of new parsimony-based versus parametric inference methods in biogeography: A case study using the globally distributed plant family Sapindaceae. J. Biogeogr. 2011, 38, 531–550. [CrossRef]
50. Särkinen, T.; Bohs, L.; Olmstead, R.G.; Knapp, S. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. BMC Evol. Biol. 2013, 13, 214. [CrossRef]
51. 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. Am. J. Bot. 2020, 107, 1–17. [CrossRef]
52. Thode, V.A.; Sanmartín, I.; Lohmann, L.G. Contrasting patterns of diversification between Amazonian and Atlantic forest clades of Neotropical lianas (Amphilophium, Bignonieae) inferred from plastid genomic data. Mol. Phylogenet. Evol. 2019, 133, 92–106. [CrossRef]
53. Lohmann, L.G.; Bell, C.D.; Caliò, M.F.; Winkworth, R.C. Pattern, and timing of biogeographical history in the Neotropical tribe Bignonieae (Bignoniaceae). Bot. J. Linn. Soc. 2013, 171, 154–170. [CrossRef]
54. 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]
55. Mazine, F.F.; Faria, J.E.Q.; Giaretta, A.; Vasconcelos, T.; Forest, F.; Lucas, E. Phylogeny and biogeography of the hyper-diverse genus Eugenia (Myrtaceae: Myrteae), with emphasis on E. sect. Umbellatae, the most unmanageable clade. Taxon 2018, 67, 752–769. [CrossRef]
56. Davies, B.J.; Hambrey, M.J.; Smelle, J.L.; Carrivick, J.L.; Glasser, N.F. Antarctic Peninsula ice sheet evolution during the Cenozoic era. Quat. Sci. Rev. 2012, 31, 30–66. [CrossRef]
57. Lewis, A.R.; Marchant, D.R.; Ashworth, A.C.; Hedenäs, L.; Hemming, S.R.; Johnson, J.V.; Leng, M.L.; Machlus, M.L.; Newton, A.E.; Raine, J.I.; et al. Mid-Miocene cooling and the extinction of tundra in continental Antarctica. Proc. Natl. Acad. Sci. USA 2008, 105, 10676–10680. [CrossRef]
58. Chacón, J.; Assis, M.C.; Meerow, A.W.; Renner, S.S. From East Gondwana to Central America: Historical biogeography of the Alstroemeriaceae. J. Biogeogr. 2012, 39, 1806–1818. [CrossRef]
59. Maciel, J.R. Estudos Taxonômicos, Filogenéticos e Biogeográficos em Achmea (Bromeliaceae). Ph.D. Thesis, Universidade Federal de Pernambuco, Recife, Brazil, 2017; 213p.
60. Morley, R.J. Origin and Evolution of Tropical Rain Forests; Wiley: Chichester, UK, 2000; p. 362.
61. Zachos, J.; Pagani, M.; Sloan, L.; Thomas, E.; Billups, K. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 2001, 292, 686–693. [CrossRef]
62. Flower, B.P.; Kennett, J.P. The middle Miocene climate transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 1994, 108, 537–555. [CrossRef]
63. Prado, D.E.; Gibbs, P.E. Patterns of species distributions in the dry seasonal forests of South America. Ann. Mo. Bot. Gard. 1993, 80, 902–927. [CrossRef]
64. Peres, E.A.; Pinto-da-Rocha, R.; Lohmann, L.G.; Michelangeli, F.A.; Miyaki, C.Y.; Carnaval, A.C. Patterns of species and lineage diversity in the Atlantic Rainforest of Brazil. In Neotropical Diversification: Patterns and Processes; Rull, V., Carnaval, A.C., Eds.; Springer: Cham, Switzerland, 2020; pp. 415–520.
65. Karl, M.; Glaßmacher, U.A.; Kollenz, S.; Franco-Magalhães, A.O.B.; Stockli, D.F.; Hackspacher, P.C. Evolution of the South Atlantic passive continental margin in southern Brazil derived from zircon and apatite (U–Th–Sm)/He and fission-track data. Tectonophysics 2013, 604, 224–244. [CrossRef]
66. Doyle, J.J.; Doyle, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem.* Bull. 1987, 19, 11–15.

67. 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 *Amorinia* (Malpighiaceae). *Taxon* 2018, 67, 739–751. [CrossRef]

68. 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] [PubMed]

69. Edgar, R.C. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Inform.* 2004, 5, 113.

70. Swofford, D.L. *PAUP*: Phylogenetic Analysis Using Parsimony and Other Methods, Version 4.0b10; Sinauer: Sunderland, UK, 2002.

71. Fitch, W.M. Towards defining the course of evolution: Minimum change for a specific tree topology. *Syst. Zool.* 1971, 20, 406–416. [CrossRef]

72. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 1985, 39, 783–791. [CrossRef]

73. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics, and parallel computing. *Nat. Methods* 2012, 9, 772. [CrossRef]

74. Ronquist, F.; Huelsenbeck, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003, 19, 1572–1574. [CrossRef]

75. 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]

76. Cai, L.; Xi, Z.; Peterson, K.; Rushworth, C.; Beaulieu, J.; Davis, C.C. Phylogeny of Elatinaceae and the tropical Gondwanan origin of the Centroplacaceae (Malpighiaceae, Elatinaceae) clade. *PLoS ONE* 2016, 11, e0161881. [CrossRef]

77. Rambaut, A.; Suchard, M.A.; Xie, D.; Drummond, A.J. Tracer v1.6. 2014. Available online: http://beast.bio.ed.ac.uk/Tracer (accessed on 10 November 2020).

78. FigTree. 2020. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 10 November 2020).

79. Anderson, W.R.; Davis, C.C. Generic adjustments in Neotropical Malpighiaceae. *Contr. Univ. Michigan Herb.* 2007, 25, 137–166.

80. Gates, B. *Banisteriopsis*, *Diplopterys* (Malpighiaceae). *Flora Neotrop.* 1982, 30, 1–238.

81. IBGE-Instituto Brasileiro de Geografia e Estatística. Mapa de vegetação do Brasil. Rio de Janeiro, Brasil. 2012. Available online: http://www.ibge.gov.br/home/presidencia/noticias/21052004biomashtml.shtml (accessed on 10 November 2020).

82. WWF-World Wildlife Fund. Tropical and Subtropical Dry Broadleaf Forests. 2020. Available online: http://www.worldwildlife.org/biomes/tropical-and-subtropical-dry-broadleaf-forests (accessed on 10 November 2020).

83. Yu, Y.; Harris, A.J.; Blair, C.; He, X.J. RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. *Mol. Phylogenet. Evol.* 2015, 87, 46–49. [CrossRef] [PubMed]

84. Ree, R.H.; Smith, S.A. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 2008, 57, 4–14. [CrossRef] [PubMed]

85. Ree, R.H.; Sammartín, I. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *J. Biogeogr.* 2018, 45, 741–749. [CrossRef]

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