Molecular phylogeny and evolution of inflorescence types in Eperua

Hans ter Steege, Elenice A Fortes, Danaë M. A. Rozendaal, Roy H. J. Erkens, Daniel Sabatier, Gerardo Aymard, Elza Duijm, Marcel Eurlings, Felix Grewe, Maihyra M Pombo, et al.

Received: 31 March 2023 | Accepted: 28 July 2023

DOI: 10.1002/ajb2.16229

RESEARCH ARTICLE

American Journal of Botany

Molecular phylogeny and evolution of inflorescence types in *Eperua*

Hans ter Steege[1,2] | **Elenice A. Fortes**[3] | **Danaë M. A. Rozendaal**[4,5] | **Roy H. J. Erkens**[6] | **Daniel Sabatier**[7] | **Gerardo Aymard**[8,9] | **Elza Duijm**[1] | **Marcel Eurlings**[1] | **Felix Grewe**[10] | **Maihyra M. Pombo**[11] | **Vitor F. Gomes**[12,13] | **Vidal F. de Mansano**[14] | **Sylvia M. de Oliveira**[1]

[1]Naturalis Biodiversity Center, Leiden, The Netherlands

[2]Quantitative Biodiversity Dynamics, Dept. of Biology, Utrecht, Utrecht University, The Netherlands

[3]Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil

[4]Plant Production Systems Group, Wageningen University, P.O. Box 430, Wageningen 6700 AK, the Netherlands

[5]Centre for Crop Systems Analysis, Wageningen University, P.O. Box 430, Wageningen 6700 AK, the Netherlands

[6]Maastricht Science Programme, Faculty of Science and Engineering, Maastricht University, P.O. Box 616. 6200 MD, Maastricht, The Netherlands

[7]AMAP, IRD, Cirad, CNRS, INRA, Université de Montpellier, Montpellier F-34398, France

[8]UNELLEZ-Guanare, Programa de Ciencias del Agro y del Mar, Herbario Universitario (PORT), Mesa de Cavacas, estado Portuguesa, 3350, Venezuela

[9]Compensation International Progress S.A. Ciprogress–Greenlife, Bogotá D.C., Colombia

[10]Grainger Bioinformatics Center, Field Museum, 1400 S. Lake Shore Drive, Chicago 60605-2496, IL, USA

[11]Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia – INPA, Av. André Araújo, 2936, Manaus 69067-375, AM, Brazil

[12]Escola de Negócios Tecnologia e Inovação, Centro Universitário do Pará, Belém, PA, Brazil

[13]Universidade Federal do Pará, Rua Augusto Corrêa 01, Belém 66075-110, PA, Brazil

[14]Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão, 915, Rio de Janeiro 22460-030, RJ, Brazil

Correspondence
Hans ter Steege, Naturalis Biodiversity Center, Leiden, The Netherlands.
Email: hans.tersteege@naturalis.nl

Abstract

Premise: The Amazonian hyperdominant genus *Eperua* (Fabaceae) currently holds 20 described species and has two strongly different inflorescence and flower types, with corresponding different pollination syndrome. The evolution of these vastly different inflorescence types within this genus was unknown and the main topic in this study.

Methods: We constructed a molecular phylogeny, based on the full nuclear ribosomal DNA and partial plastome, using Bayesian inference and maximum likelihood methods, to test whether the genus is monophyletic, whether all species are monophyletic and if the shift from bat to bee pollination (or vice versa) occurred once in this genus.

Results: All but two species are well supported by the nuclear ribosomal phylogeny. The plastome phylogeny, however, shows a strong geographic signal suggesting strong local hybridization or chloroplast capture, rendering chloroplast barcodes meaningless in this genus.

Conclusions: With our data, we cannot fully resolve the backbone of the tree to clarify sister genera relationships and confirm monophyly of the genus *Eperua*.

Within the genus, the shift from bat to bee and bee to bat pollination has occurred several times but, with the bee to bat not always leading to a pendant inflorescence.

KEYWORDS

Eperua, Fabaceae, inflorescence morphology, molecular phylogeny, pollen, pollination, pollinator shift

Trees of the genus *Eperua* Aubl. (Fabaceae) are a well-known sight to travelers in Northern Amazonia and the Guiana Shield and can be dominant in forests on poor soils and along rivers in the Guianas and Upper Rio Negro (Fanshawe, 1952; Huber, 1995; Aymard et al., 2009). At the plot level, *E. grandiflora* (Aubl.) Baill. and *E. leucantha* Benth. may comprise more than 10% of the forest stand, while *E. falcata* Aubl. has been found to achieve a mono-dominance of 64% on 1-ha plots (ter Steege et al., 2019). With estimated 3.65 billion individuals, *Eperua* is the 15th most-abundant genus in Amazonia and has been classified as hyperdominant (ter Steege et al., 2013, 2020). Among the 18 currently accepted, three are also hyperdominant: *E. falcata* ranks 18 with c. 1.3 billion individuals; *E. leucantha* ranks 27 with c. 1.1 billion individuals; and *E. purpurea* Benth. ranks 99 with c. 0.5 billion individuals (ter Steege et al., 2020).

The distribution of *Eperua* can broadly be subdivided into three main areas: the three Guianas, the upper Rio Negro area, and central/northeastern Amazonia. *Eperua* species occur mainly in forests in white-sand areas and in clay soils along creeks and rivers (Fanshawe, 1952; Lindeman and Moolenaar, 1959; Janzen, 1974; Cowan, 1975; Klinge and Medina, 1979; Berry et al., 1998). Trees have to cope with drought in both habitats; soil in white-sand areas has low water availability, and a lack of oxygen in the soil in flooded riverine forests makes water uptake impossible (Kubitzki, 1989; ter Steege, 1990). *Eperua* has anatomical adaptations to drought: thick leaves in several species, a revolute leaf margin in *E. grandiflora* subsp. *guyanensis* R.S. Cowan and *E. glabriflora* (Ducke) R.S. Cowan, and a wax layer on the abaxial surface of leaflets in *E. purpurea*.

In the latest classification of Fabaceae, *Eperua* is placed within the Detarioideae (Legume Phylogeny Working Group, 2017). Its sister genus is *Eurypetalum* Harms (Bruneau et al., 2001; Fougère-Danezan et al., 2007; Bruneau et al., 2008; Legume Phylogeny Working Group, 2017; de la Estrella et al., 2018), with two African species, *Eu. tessmannii* Harms and *Eu. unijugum* Harms (Obiang-Mbomio and Breteler, 2007). *Eperua* was thoroughly revised based on morphology in 1975 (Cowan, 1975) when it included 14 species, four subspecies, and four varieties. Six new species have been described since: *E. praesagata* R.S. Cowan (Cowan, 1985), *E. banaensis* G.A. Romero & Aymard. G.A. (Romero-González and Aymard, 2019), *E. cerradoensis* E.A. Fortes, G.S. da Silva & Mansano and *E. manausensis* E.A. Fortes & Mansano (Fortes et al., 2023a), and most recently *E. froesii* E.A. Fortes, Aymard, H. ter Steege, & Mansano and *E. reddeniae* E.A. Fortes & Mansano (Fortes et al., 2023b). *Eperua* is characterized by evenly pinnate leaves, 2–6 pairs of leaflets (most with a clear drip-tip, except for *E. obtusata* R.S. Cowan and *E. banaensis*), flowers with one petal and four scale-like petalodes, (5–)10 fertile stamens, anthers dehiscing by longitudinal slits, and non-arillate seeds (Cowan, 1975; Cowan and Berry, 1998; Romero-González and Aymard, 2019). For names and authors, we follow Fortes and Mansano (2022).

Eperua possesses two entirely different inflorescence morphologies. Long pendant inflorescences with faintly colored flowers (e.g., *E. falcata*, *E. rubiginosa* Miquel, *E. leucantha*, Figure 1) are a common sight along roads and rivers in the Guianas and upper Rio Negro region. Canopies with striking purple or pale violet flowers on short peduncles (Figure 2) are also a well-known sight in the upper Rio Negro (*E. purpurea*) and Guianas (*E. grandiflora*). The flowers of the species with long, pendant inflorescences are considered bat-pollinated (Cowan, 1975; Fleming et al., 2009), which has been observed for *E. rubiginosa* and *E. falcata* (Irwin, personal communication in Cowan, 1975; Delaval et al., 2005; Geiselman, 2010). In fact, pollen of *E. falcata* was found in feces of 12 bat species, which rely on this species for pollen and nectar in the dry season (Geiselman, 2010). Species of *Eperua* are also visited by hummingbirds (*E. glabra* R.S. Cowan; H. ter Steege, personal observations and Irwin, personal communication in Cowan, 1975) and *E. falcata* (S. Mori, New York Botanical Garden, personal observations). The species with purple and pale violet flowers on a short peduncle are considered bee-pollinated (Vogel, 1968; Cowan, 1975) but are also sometimes visited by honeycreepers (*Cyanerpes* spp., H. ter Steege, personal observations). The two inflorescence types illustrate contrasting strategies of flower positioning in the canopy, arguably related to pollinator accessibility: Flowers pendant below branches and canopy leaves or upright above the canopy may facilitate either bat or hummingbird access.

Moreover, autochorous ballistic dispersal by the explosive opening of the pods, common in the first group, is likely more efficient in the obstacle-free space reached by pendant inflorescences. A third inflorescence type, in *E. schomburgkiana* Benth., *E. duckeana* (Ducke) R.S. Cowan, is intermediate; the nontubular corolla of white flowers are in a somewhat longer, elongated downward-pointing inflorescence, and anthers and stigmas are exserted (Figure 1E).

Cowan (1975) assumed that the original state of the inflorescence of *Eperua* was the short type with purple, bee-pollinated flowers and suggested that the long, pendant inflorescence evolved within the genus. The "ground-plan diagram of phylogenetic relationships" of Cowan (1975: Fig. 10, p. 19), however, was based on the characters of the

FIGURE 1 Bat-pollinated *Eperua* species. (A) *E. falcata*, flowers, Paracou, French Guiana © Hans ter Steege; (B) *E. leucantha*, inflorescence, road to Vitina km 7, Amazonas, Venezuela © Francisco Castro-Lima; (C) *E. rubiginosa* var. *rubiginosa*, flowers, Rive Tonnegrande, French Guiana © Hans ter Steege; (D) *E. rubiginosa* var. *rubiginosa*, fruits, Rive Tonnegrande, French Guiana © Hans ter Steege; (E, F) *E. duckeana*, inflorescence and fruit, Cachoeira Natal, Amazonas, Brazil © Hans ter Steege.

FIGURE 2 (See caption on next page).

flowering structures, extant species were placed on nodes, and the outgroup was a self-constructed hypothetical outgroup with bee-pollinated flowers. Hence, the evolution of the inflorescence was preconceived. Banks and Rico (1999) constructed a phylogeny of *Eperua*, based on the pollen morphology of the genus, using *Umtiza* and *Cynometra* as outgroup, genera not closely related to *Eperua*.

Here, using molecular data of all but four known *Eperua* species and subspecies, and including one new species (Fortes et al., 2023b), we aimed to establish whether *Eperua*, with its three types of inflorescences, is a monophyletic group and if molecular data support all species within its circumscription. We describe the flowering structure and leaf morphology of all *Eperua* species and use a molecular phylogeny to form a hypothesis of the evolution of the inflorescence type in the genus based on data independent of the inflorescence and flower structure. We also provide distribution maps for all species.

MATERIALS AND METHODS

We examined 3096 herbarium sheets, which amounted to 2258 unique collections. We manually checked all species identifications and localities and added locality coordinates if not present on herbarium labels but findable through the internet. As a result, 1727 specimens had location information (coordinates), which was used to produce species occurrence maps with custom R scripts (R Core Team, 2019).

The morphological data used in this study were compiled from the taxonomic literature (Cowan, 1975, 1985; Romero-González and Aymard, 2019). We selected a set of quantitative characters, including those with a clear diagnostic value (Appendix S1: Table S1A). For *E. froesii*, we measured the same characters. The quantitative flower characters were also scaled to a fixed flower character—petal length—to prevent scoring interdependent floral characters. Pollen data were taken from Banks and Rico (1999) and Cowan (1975); pollen data are lacking for *E. praesagata* R.S. Cowan. Due to the proximity (and possible synonymy) of *E. praesagata* to *E. glabra* R.S. Cowan, pollen data from *E. glabra* were used for *E. praesagata*. For the pollen of *E. froesii* (Prance 1458, NHN), we used a Zeiss Axio imager M2 (Zeiss, Jena, Germany) and measured polar diameter for 15 pollen grains and equatorial diameter for seven. For SEM images, pollen exines were acetolyzed in 95% v/v ethanol, pipetted onto specimen stubs, air dried, then sputter-coated with gold (150 nm thick) and examined using a JEOL JSM-6480 SEM (Oxford Instruments, Abingdon, UK). Herbarium material was not available for *E. banaensis* and three species not yet described during the laboratory work for this study (*E. cerradoensis*, *E. manausensis*, *E. reddeniae*).

A standard principal component analysis (PCA) was carried out to find the main structure among all flower characters and group all species based on their main flower/inflorescence morphology. All data were standardized ($\mu = 0$, $\sigma = 1$) before analysis. For the PCA, we used the rda() function of the R package vegan (Oksanen et al., 2020) with default parameters. *Eperua banaensis* and *E. obtusata* had limited pollen data and none for fruit and were not used in the PCA. A priori, we divided the genus into species with an evidently pendant inflorescence, those with an intermediate type, and those with an erect inflorescence (Appendix S1, Table S1A). The first two were considered bat-pollinated.

For the phylogenetic analyses, we applied genome skimming of the full genome of each accession, targeted to produce at least 5 Gb per accession, which was used to make de-novo sequences of the plastome and the nuclear ribosomal region (NRb).

At the Naturalis Biodiversity Center (Leiden, The Netherlands), leaf samples were taken from 62 specimens, including herbarium material and leaves that had been collected in silica in the field (DNA voucher specimen data in Appendix S1, Table S2). DNA was extracted using a modified CTAB protocol (Doyle and Doyle, 1987). DNA concentration was determined using the Dropsense spectrophotometer (Trinean NV, Gentbrugge, Belgium), and the quality of the DNA was checked using the QIAxcel system and DNA Screening kit (QIAGEN, Valencia, CA, USA). DNA was sonicated using the Covaris M220 (Covaris, Woburn, MA, USA) and microTUBE-59 AFA Fiber Screw-Cap according to the manufacturer's program for an insert size of 350 bp, followed by a Dual index library prep with the DNA NEBNext Ultra II Library Prep Kit (New England Biolabs, Ipswich, MA, USA), using a quarter of the recommended volumes with the Dual Index Primers Set, NEBNext Multiplex Oligos for Illumina (New England Biolabs). The libraries were checked for concentration with the QIAxcel and pooled equimolarly using the QIAgility instrument. Using a High Sensitivity chip, we did a final quantity and quality check with the Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequence reads of 150 bp were generated using the Illumina HiSeq 4000 and later the Illumina NovaSeq 6000 system (Illumina, San Diego, CA, USA).

At the Field Museum of Natural History (Chicago, IL, USA), DNA was isolated from 15 leaves by first homogenizing

FIGURE 2 Bee-pollinated *Eperua* species. (A) *E. glabriflora*, terminal branch with inflorescences, cultivated at Rio de Janeiro Botanical Garden, Brazil, Fortes 150 © Elenice Fortes; (B) *E. purpurea*, terminal branch with inflorescences, São Gabriel da Cachoeira, Amazonas, Brazil © Hans ter Steege; (C) *E. grandiflora* subsp. *grandiflora*, flower front view, Piste de Saint Élie, French Guiana. © Daniel Sabatier; (D, E) *E. grandiflora* subsp. *guyanensis*, Mabura Hill, Guyana © Hans ter Steege (D) flower lateral view, (E) fruit; (F) *E. bijuga* fruit, Bahia de Caxiuaña, Pará, Brazil © Hans ter Steege; (G) *E. jenmanii* subsp. *jenmanii*, fruit, Groete Ck., Essequibo, Guyana, Tiwari 924, New York Botanical Garden; (H) *E. jenmanii* subsp. *jenmanii*, flowers, Kamarang, Pakaraima Mts., Guyana. Maas 3981 © Lubbert Westra.

60–80 mg of leaf tissue on a single 3.2 mm chrome-coated steel bead for 1–2 min at 25 Hz in a TissueLyser II (QIAGEN, Hilden, Germany). We extracted genomic DNA using a 2× CTAB protocol with 3% w/v PVP and 2% v/v 2-mercaptoethanol in the extraction buffer (Khanuja et al., 1999) and an initial incubation for 14 h at 65°C. Finally, the DNA was recovered with 50 µL elution buffer from the Invisorb kit (Invitek Diagnostics Germany, Berlin, Germany). DNA concentration was quantified using the Qubit fluorometer (Thermo Fisher, Waltham, MA, USA), and DNA quality was evaluated by running 5 µL in a 1% w/v high-melt agarose gel and looking for evidence of degradation. The sequencing library was produced with the Swift 1S kit (Integrated DNA Technologies, Coralville, IA, USA) and sequenced on a NextSeq 500 Illumina sequencer.

We used GetOrganelle (Jin et al., 2020) for de novo assembly of two data sets per specimen: (1) the nuclear ribosomal (NRb) DNA (5800–5900 bp: 26S-ITS1-5.8S-ITS2-18S), plus an offset of 1000 bp before the 26S region that included the ETS region (Appendix S1, Table S3), (2) the entire plastome (Cp). Assembled contigs of the different specimens were imported to Geneious Prime (version 2021.2.2, Dotmatics, Bishop's Stortford, UK). When de novo assembly failed to produce a contig for a given specimen, we used the function Map to Reference in Geneious Prime, using one of the successfully de novo assembled specimens as reference. This procedure was carried out for both NRb and the plastome. In total, we obtained 73 high-quality NRb sequences and 61 plastomes (Appendix S1, Table S4). Because nucleotide repeats in the plastome often create problems in de-novo mapping (Dierckxsens et al., 2017), we used concatenated reading frames of the coding DNA sequences (CDS) of the plastome for phylogenetic analysis (Chave et al., 2020) (Appendix S1, Table S5).

Sequences were aligned with MAFFT (Katoh and Standley, 2013) using the plug-in of Geneious Prime (and the web-based version of EMBL-EBI, https://www.ebi.ac.uk/Tools/msa/). We used maximum likelihood to construct phylogenetic trees, making use of the PACA Bioinfo platform at phylogeny.fr (Dereeper et al., 2008), with standard settings (4 substitution rate categories; gamma distribution parameter estimated; the proportion of invariable sites estimated; transition/transversion ratio estimated) using an approximate likelihood ratio Test (aLRT) to estimate bootstrap values, and the HKY-85 substitution model. We also inferred phylogenetic trees using Bayesian inference (BI) (Huelsenbeck and Ronquist, 2001), as implemented in Geneious Prime, with standard settings, a chain length of 1,100,000, and burn-in of 110,000, and a subsampling frequency of 200. We used the GTR+I+G substitution model as suggested by Abadi et al. (2019). We assessed convergence by analyzing the effective sample size (ESS) after the burn-in for all parameters.

To place all species of *Eperua* in the subfamily Detarioideae (de la Estrella et al., 2018), we extracted from our CDS regions the plastid regions *matK* (~1500 bp, GenBank name "matK gene", without the *trnK* of de la Estrella et al., because it is not part of the CDS), *rpL16* (~1056 bp, GenBank name "rpl 16 intron"), *trnG-UCC* (~784 bp, GenBank name "trnG UCC gene", which overlaps for 95% with the trnG-trnG2G [a.k.a. *trnS*] of de la Estrella et al.) from our plastomes. Areas of the DNA regions of de la Estrella et al. that were not covered by our data and created gaps were deleted from the alignment. We also cut ITS from our Nrb sequences and aligned it with the sequence data of de la Estrella et al. (2018) from GenBank (Appendix S1, Table S6). We used maximum likelihood and Bayesian inference to infer phylogenetic relationships with settings as above.

RESULTS

Eperua has a distinct geographic distribution, mainly confined to the northern Amazon (Appendix S1, Maps S1–S7). Four species have a distribution extending to the south of the Amazon River (*E. oleifera* Ducke, *E. bijuga* Mart. ex Benth., *E. cerradoensis*, and *E. froesii*). In the Guianas, the species attain high dominance in forests (13% of all individuals in areas of 100 × 100 km), mainly because of the high local dominance of *E. falcata* and *E. grandiflora* in that region (Appendix S3 of ter Steege et al., 2015, 2019). Similar abundances are found in the Upper Rio Negro area, mainly due to high densities of *E. leucantha* and to a lesser extent, *E. purpurea* (Aymard et al., 2009; ter Steege et al., 2015).

Inflorescence type (pendant, intermediate, erect) strongly affects flower size and morphology (Appendix S1, Table S1A, Figure S1). Species with a pendant inflorescence have a larger hypanthium, shorter petals, longer stamen filaments and anthers, and a longer style. They also have longer fruits with more seeds. The species with an intermediate inflorescence have some characteristics in common with the species with pendant inflorescences (e.g., hypanthium size, filament length, ovary length, long fruit, 3–4 seeds per fruit), and some with erect inflorescences (pollen size, style length). For some characters, they are intermediate (pedicel length, anther length). Species with pendant inflorescences have much larger pollen than species with the other two inflorescence types (see pollen polar length and equatorial diameter; Appendix S1, Table S1A, Figure S1).

With the flower characteristics relative to the petal length (Appendix S1, Table S1B), the species with the intermediate-type inflorescence generally had the characteristics of those with pendant inflorescences, except for the sepal shape. Sepal, stamen, and pistils are all longer, relative to the petal, in species with pendant inflorescences than in those with erect inflorescences. The intermediate species have higher values for filament and gynophore length than the other two groups (Appendix S1, Figure S2).

The PCA of original data neatly separated the species with pendant vs. erect inflorescences based on the long peduncle and the flower morphology (Appendix S1, Figure S3). The first axis explained 48% of the variation, and the second axis explained 23%. Species with a pendant inflorescence have

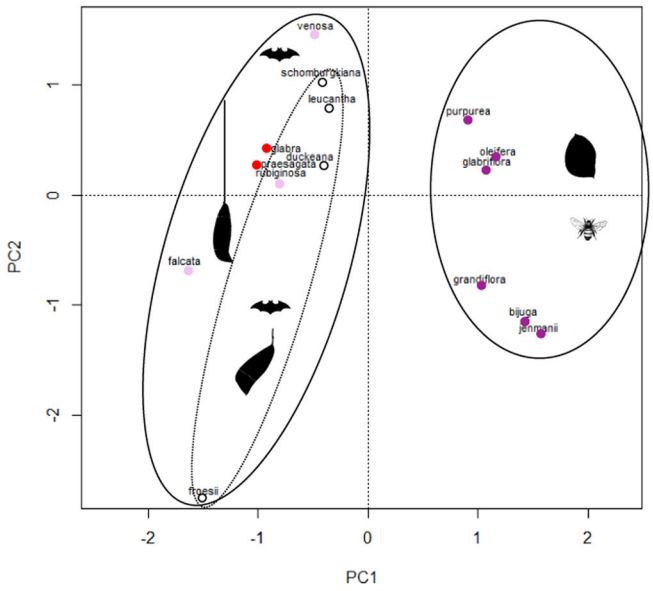

FIGURE 3 PCA with flower data relative to petal length. Fruit shape and flower color and suggested pollination syndrome indicated. Axis 1 has an eigenvalue of 12.7 and explains 67% of the variation; axis 2 has an eigenvalue of 2.0 and explains 10% of the variation in the data.

shorter petals, longer filaments, and more robust anthers. The PCA of data relative to petal length separated the two flower morphologies even better (Figure 3), with the first axis explaining 67% of all variation, axis 2 explaining 10%. In species with a pendant inflorescence, the petals are barely longer than the sepals, and the gynophore, ovary, and style are longer than the petal so that the anthers and stigma are exserted well out of the flower (Figure 2A–C). The exsertion is also the same for the intermediate species (Figure 2E), which forms a group with the species with a pendant inflorescence. In the species with the erect, purple inflorescences, the stamens remain enclosed by the large pink/purple/violet petal (Figure 2A–D, H).

Genome skimming

The genome skimming resulted in an average of 49 million sequences of 150 bp per accession, with a minimum of 144,111 and maximum of 482 million (Appendix S1, Table S1). We were able to reproduce 44 NRb sequences with GetOrganelle (Appendix S1: Table S3), a total of 72 NRb sequences (Appendix S1, Tables S2, S4), and a total of 58 plastomes (Appendix S1, Tables S2, S5). We were not able to extract other nuclear regions from our genome skims.

Phylogenetic relationships based on full ribosomal DNA

The whole nuclear ribosomal DNA phylogeny was based on a matrix of alignment length of 7452 bp (minimum sequence length 6789 bp, maximum sequence length 6901), with 78.6% identical and 12.2% parsimony-informative sites. The BI result is already very stable before the end of the burn-in and remains so for the entire run, resulting in a very low deviation among the solutions. All ESS values were above 450 after the burn-in, suggesting good convergence among the two runs in the BI. A second BI analysis resulted in the same topology with nearly identical PP values and similarly high ESS values (data not shown). The BI (Figure 4) and ML (Appendix S1, Figure S4) analyses resulted in trees with very similar topologies, except for the position of *E. froesii*, which was not well supported in the phylogeny. Three main well-resolved and supported clades within *Eperua* were recovered as described below.

As sister to the rest of the genus, a clade formed by *E. oleifera* and *E. purpurea* (Figure 4, clade 1) is a lineage diverging from the, here called, core *Eperua* clade (PP = 1, BS = 1), formed by clade 2 (PP = 1, BS = 0.96) and clade 3 (PP = 1, BS = 1). The two species in clade 1 are distributed in Central Amazonia in the Madeira River basin (*E. oleifera*) and upper Rio Negro basin (*E. purpurea*) in white sand ecosystems (Map S1). They share an erect inflorescence and a flower with a tubular corolla and inserted stamens. Besides that, they share fused and non-foliaceous stipules and secondary venation with one intramarginal vein very close to the margin. These two characters are, however, also found in clade 2. The species also differ from the other *Eperua* species by having concave triangular pollen (as does the genus *Eurypetalum*) and a similar outer pollen structure (Appendix S1, Figure S5). No synapomorphy was identified.

In the core *Eperua* (clades 2+3), clade 2 comprises *E. falcata*, *E. leucantha*, and *E. venosa* R.S. Cowan, species distributed in the Guiana Shield, Orinoco Basin, and Central Amazon (upper Rio Negro Basin) (Map S2). Clade 2 is the only clade supported by a synapomorphy; it groups all the species in the genus that have falcate leaflets. In addition, the species of clade 2 share secondary venation with one intramarginal vein very close to the margin, a pendant inflorescence, flowers with a nontubular corolla, and exserted stamens. Clade 3 includes species that occur throughout the geographical range of *Eperua*, except in the upper Rio Negro Basin, and does not show a common characteristic among the taxa. Its 10 species are grouped into two subclades:

Species in clade 3a (*E. rubiginosa*, *E. praesagata*, and *E. glabra*) are distributed in the Guiana Shield and Central Amazon (Trombetas River) (Map S3). The clade is characterized by a pendant inflorescence, nontubular corolla, and exserted stamens. The lateral racemes of the inflorescence are erect to patent, a characteristic also shared with *E. venosa* and *E. leucantha*, species with a pendant inflorescence in clade 2. Contrasting with clade 2 species, clade 3a species have leaves with straight leaflets. All species of clade 3a also share caducous bracteoles attached to the lower portion of the pedicels; this character can, however, also be found in clade 3b (*E. jenmanii* Oliv.). The species

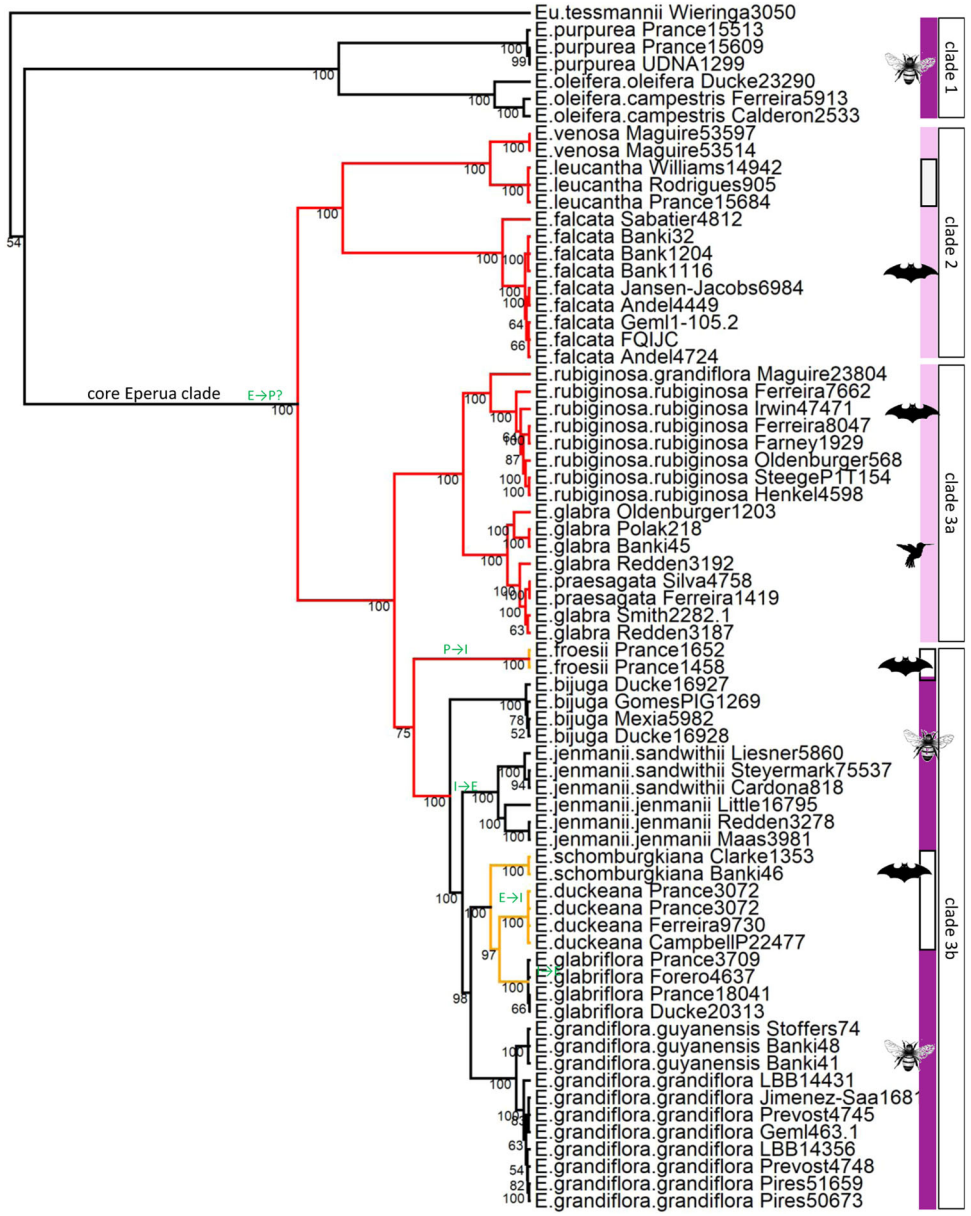

FIGURE 4 Phylogenetic tree of *Eperua* spp, based on Bayesian inference of the full nuclear ribosomal DNA (alignment of 7452 bp). Colors of the branches indicate the suggested/hypothesized inflorescence type: red, pendant; black, erect; orange, intermediate. Bars on the right indicate petal color, suggested pollinator, and clade. Green text: suggested transformation from erect to hanging inflorescence and reverse. Black values at branches: posterior probabilities.

also share triangular convex pollen with globular structures, a palynological characteristic restricted to this clade and *E. froesii* (Appendix S1, Figure S5).

Clade 3b (*E. froesii*, *E. bijuga*, *E. jenmanii*, *E. grandiflora*, *E. schomburgkiana*, *E. duckeana*, and *E. glabriflora*): distributed in the Guiana Shield, Orinoco Basin, Central Amazon, and Eastern Amazon (Maps S4-7). It groups species with an erect inflorescence, a tubular corolla, and inserted stamens that are joined in a diadelphous sheath of 9+1 (*E. bijuga*, *E.* glabriflora, *E. jenmanii*, *E. grandiflora*) and species with an intermediate inflorescence, a nontubular corolla and exserted stamens joined in a diadelphous sheath of 9+1, or in a tube (*E. froesii*, *E. schomburgkiana*, *E. duckeana*). But the lineages are not neatly separated into these two floral types. In the BI, the subgrouping in clade 3b is well supported, but in the ML analysis, there are several poorly supported divisions.

Pollen in *Eperua* is strongly triangular. It is concave in clade 1 and convex in the other species. Clade 1 has small pollen size (63–73 μm), while the two clades with pendant inflorescences (2, 3a) have larger pollen (91–102 μm) with different external structures (Appendix S1, Table S1A, Figure S5). Pollen is convex and triangular in clade 3b, but the ornamentation is not as rough, and the pollen is smaller (Appendix S1, Table S1A, Figure S5). Pollen of *E. froesii* is similar in size to that of the other species of clade 3b but has

a rough surface with globules as seen in the species of clade 3a (Appendix S1, Figure S5). Pollen of *E. schomburgkiana* is slightly rougher than that of the other species of clade 3b, but is of similar size (71 μm).

Except for *E. glabra* and *E. praesagata*, all *Eperua* species formed monophyletic groups with maximum phylogenetic support (PP = 1, BS = 1). Subspecies and varieties were also recovered as monophyletic groups with high phylogenetic support (PP > 0.987, BS > 0.89). In the BI topology, clade 3 is neatly separated into two lineages, one grouping *E. rubiginosa*, *E. glabra*, and *E. praesagata* (PP = 1, clade 3a), and another grouping all species with short and intermediate inflorescences except for *E. oleifera* and *E. purpurea* (PP = 0.937), which comprise clade 1. The ML topology did not recover these two highly supported lineages, and the relationship of *E. froesii* is uncertain, being more related to clade 3a formed by *E. rubiginosa*, *E. praesagata*, and *E. glabra*, but with low phylogenetic support (BS = 0.53).

The clades are not geographically structured throughout the distribution range of *Eperua*, and most clades do not have apomorphies or many shared taxonomic characteristics.

Placing Eperua within the Detarioideae

In the BI phylogeny, based on nuclear ribosomal regions ITS1, ITS2, and 5.8S (Appendix S1, Figure S6), extracted from our NRb sequences, and including *Eurypetalum unijugum* and *Stemonocoleus micranthus* from de la Estrella et al. (2018), *Eperua* and *Eurypetalum* are monophyletic groups with moderately high support (PP = 0.979 and 0.871, respectively). The two genera are not sister clades but form a polytomy with *S. micranthus*.

The topology within *Eperua* showed just a few differences from the full nuclear ribosomal DNA phylogeny. Clade 1 remained a supported sister clade of the core *Eperua* clade (PP = 0.979); in clade 3, there is a polytomy formed by *Eperua bijuga*, a second clade grouping *E. jenmanii*, *E. grandiflora*, *E. schomburgkiana*, *E. duckeana*, and *E. glabriflora* and a third clade grouping *E. rubiginosa*, *E. praesagata*, *E. glabra*, and *E. froesii*; *E. froesii* is a sister group with moderate support (PP = 0.815) of the clade *E. rubiginosa*, *E. glabra*, and *E. praesagata* (clade 3a of the full ribosomal DNA). Overall, the position of *E. jenmanii*, *E. grandiflora*, *E. duckeana*, and *E. glabriflora* changed, with the most significant changes being *E. glabriflora* forming a sister group to *E. schomburgkiana* with high support (PP = 0.932) and *E. grandiflora* being a sister clade (PP = 1) of *E. jenmanii*, *E. duckeana*, *E. glabriflora*, and *E. schomburgkiana*.

Phylogenetic relationships based on the plastome

The phylogeny constructed with the concatenation of the plastome coding DNA sequences (CDS) was based on a matrix of 82,045-bp alignment length (minimum sequence length 75,676 bp, maximum sequence length 78,737), with 77.2% identical sites and 3.4% informative sites (Appendix S1, Figure S7). The result did not differ from that using only the *matK-trnK*, *rpL16*, and *trnG-UCC/trnS* regions, so we show only those results because we can combine that data with that of de la Estrella et al. (2018). We included *Eu. unijugum*, *Augouardia letestui*, and *Stemonocoleus micranthus* because they are part of the *Eperua* s.l. clade (Fougère-Danezan et al., 2007; Bruneau et al., 2008; The Legume Phylogeny Working Group, 2017; de la Estrella et al., 2018). In the BI analysis (Figure 5), *Eperua* is a monophyletic genus (BS = 0.99) sister of *Eurypetalum*. We distinguished 11 clades within *Eperua* (Figure 5). Only *E. purpurea* (BS = 1) and *E. oleifera* (BS = 1) forming clade 1, *E. leucantha* (BS = 1), and *E. bijuga* (BS = 1) were recovered as monophyletic species. The clade formed by *E. leucantha* and *E. venosa* (BS = 0.991) was the only one that was also recovered in the full nuclear ribosomal DNA phylogeny.

Clade 2 consisted of only *E. falcata* individuals and clade 9 of only *E. grandiflora* individuals. Most of the other clades, however, grouped lineages with a common geographical distribution. Clade 3 had five species from eastern Guyana and Venezuela, clade 6 had two species from southern Suriname, clade 7 had three from central-eastern Amazonia, clade 8 had two species from central Amazonia, clade 10 had two species from southern Guyana, and clade 11 had five species from central Guyana.

DISCUSSION

We sought to answer three main questions. Is *Eperua* monophyletic? Are the current species circumscriptions supported by the molecular data? And how did the change of inflorescence occur within the genus? We can only answer the second question with some certainty. We included a substantial number of accessions per species, and with the full ribosomal DNA (~6800 bp), each species, subspecies, and variety were found to be monophyletic with very high bootstrap support (Figure 4), except in the case of *E. glabra* and *E. praesagata*, which also share many morphological characteristics (Fortes et al., 2023b). Based on the NRb results, we conclude that *E. praesagata* is best recognized as a synonym of *E. glabra*; this taxonomic modification is described in a monograph of the genus (Fortes et al., 2023b).

The first question is a bit more challenging. Our NRb phylogeny suggests that three clades diverged early, but the separation between *Eurypetalum*, clade 1 (*E. purpurea*, *E. oleifera*), and the core *Eperua* clade is not strongly supported. The tree based on the ITS1, ITS2, and 5.8S regions of the nuclear ribosomal genome, and including *Eu. unijugum* and *S. micranthus*, helped to resolve part of that polytomy with *Eperua* and *Eurypetalum* being monophyletic (PP = 0.979, 0.871, respectively) but forming a polytomy with *S. micranthus*.

The plastome did not solve the evolutionary history of *Eperua*, because we obtained a poorly resolved tree of the

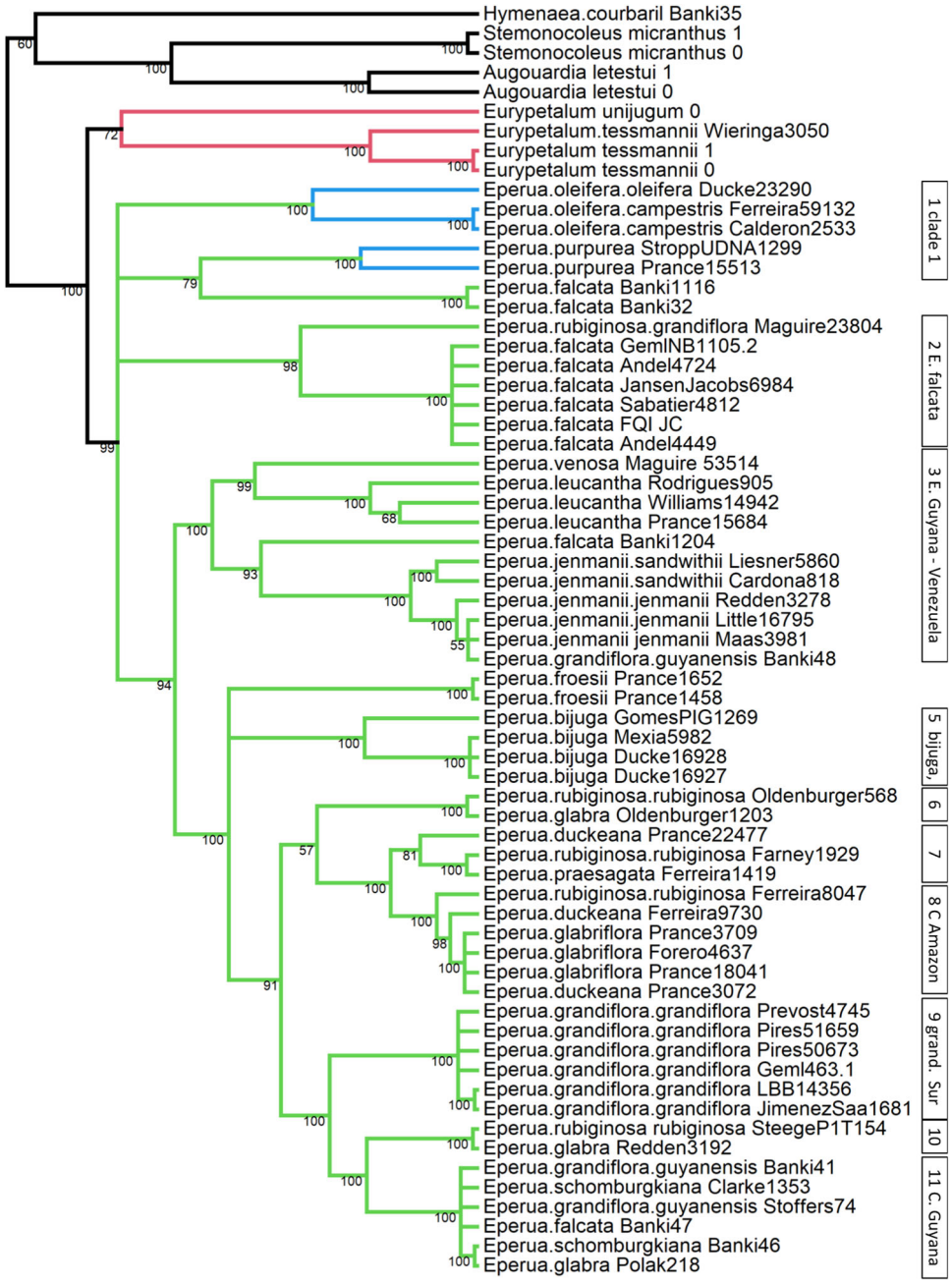

FIGURE 5 Phylogenetic tree of *Eperua* spp, based on Bayesian inference of three plastome regions (Appendix 4, alignment of 3615 bp, including data from de la Estrella et al. [2018]). Blocks on the right demarcate groups of sequences and are numbered from top to bottom (1–11). Black values at branches: posterior probabilities.

lineages within the genus. Although the same clades as the nuclear ribosomal genome phylogeny were not recovered, some taxa were recovered in a similar position: *E. oleifera* and *E. purpurea* are early-diverging lineages, and *E. duckeana*, *E. glabriflora*, *E. grandiflora*, *E. schomburgkiana*, *E. froesii*, and *E. bijuga* are grouped in a late-diverging lineage. But, the non-monophyly of *E. duckeana*, *E. falcata*, *E. glabriflora*, *E. grandiflora*, *E. jenmanii*, *E. rubiginosa*, and *E. schomburgkiana* does not corroborate the results of the nuclear ribosomal genome phylogeny. Surprisingly, the accessions of these species formed clades according to the geographical area where they occurred. This result could be due to hybridization or chloroplast capture, which can happen through hybridization or somatic exchange (Acosta and Premoli, 2010; Stegemann et al., 2012). The first author (H. ter Steege) has seen hummingbirds visit *E. glabra* inflorescences and honeycreepers (*Cyanerpes*) visit *E. grandiflora* flowers, so perhaps birds could be the intermediaries between the species, but it is difficult to imagine hybrids that can backcross with the original species. While hybridization seems unlikely among the bee- and bat-pollinated species, the somatic exchange might be possible

because many species of *Eperua* are very common or dominant in northeastern Amazonia (ter Steege et al., 2013, 2015). If the process of chloroplast capture is more common among species within one genus, then DNA barcodes that are based on chloroplast markers are less useful and not useful at all for the genus *Eperua*.

Cowan (1975) suggested that the pendant inflorescence originated once within the genus from an intermediate form (*E. schomburgkiana*). Cowan, however, developed his phylogenetic relationships hypothesis based on a hypothetical outgroup with a short erect inflorescence (sensu Figure 2). All purple-flowered *Eperua* taxa were placed in a second group in his monograph. In our phylogeny, pendant inflorescences are restricted to two lineages in *Eperua*. One of these lineages is our clade 2, which groups all three species with falcate leaves: *E. falcata*, *E. leucantha*, and *E. venosa* (Figure 5). This clade is one of the first lineages to diverge in *Eperua*, and groups species with very similar morphology (Cowan, 1975; Fortes et al., 2023b). It is not closely related to the other lineage that shares the pendant inflorescence type. This second lineage, clade 3a (Figure 5), groups *E. rubiginosa*, *E. glabra*, and *E. praesagata*. It is sister to clade 3b, the most speciose clade in *Eperua*, which contains species with short, erect inflorescences (Figures 2, 5) and purple flowers and those with intermediate forms (Figures 1, 5) with white flowers.

All species with a pendant inflorescence (clades 2 and 3b) have a nontubular corolla and exserted stamens, relatively large (91–128 μm) and rough pollen grains, characteristics associated with bat pollination (Vogel, 1968; Fleming et al., 2009). The short corolla results in a somewhat cup-shaped flower (Figure 1) that may assist in echolocation by bats (Simon et al., 2021). Other taxa in the genus that also have a nontubular, white corolla, and exserted stamens, also associated with bat pollination, are *E. duckeana*, *E. schomburgkiana*, and *E. froesii*, but they have a shorter and more erect inflorescence and are more related to the species with a tubular corolla. The pollen grains of these species have a similar size (58–73 μm) but somewhat rougher ornamentation compared to the pollen of the other species in clade 3b (Figure S5).

The short, erect inflorescence is not unique to the genus *Eperua* within the *Eperua* s.l. clade of detarioid legumes; the other three genera of the *Eperua* s.l. clade (*Eurypetalum*, *Augouardia*, and *Stemonocoleus*) also have this characteristic, but the tubular corolla with inserted stamens is an apomorphy of *Eperua* and occurs in both early- and late-diverging lineages of the genus. Unlike the species with a nontubular corolla, those with a tubular corolla and inserted stamens are associated with bee pollination (Vogel, 1968). The tubular corolla is exclusive to two lineages of *Eperua*, clade 1 and clade 3b (Figures 2, 5), which also have a short, erect inflorescence, but the two lineages are not closely related. The first is an early-diverging lineage, and the second is late-diverging. In addition, clade 3b mixes lineages with tubular and nontubular corollas.

Pollen grains (data from Cowan, 1975; Banks et al., 2013) are all strongly triangular in core *Eperua*, especially in the putative bat-pollinated clades (2 and 3a), which have relatively large pollen with a rough surface, consistent with bat pollination (Vogel, 1968; Stroo, 2000). The pollen shape and surface ornamentation of *E. purpurea* and *E. oleifera* is similar (supporting their inclusion in the same clade) and differs from species in the core genus.

Most species have a restricted geographical range (Maps S2–S7), except *E. rubiginosa* and *E. praesagata/glabra*. The restricted ranges may be the result of large seeds (fresh mass of 7–10 g in *E. falcata*; 47.6 g in *E. grandiflora* subsp. *grandiflora*, up to 60 g in *E. glabra*) in the genus and short-distance autochorous dispersal, or barochorous dispersal, and potentially high persistence of local seedlings despite seed predation (Forget, 1989, 1992). Despite the generally poor dispersal mechanisms, sister species can occur at the opposite edges of the genus distribution range (e.g., *E. falcata/venosa-E. leucantha*, and *E. oleifera-E. purpurea*). Whether these are cases of historical long-distance dispersal (Dexter et al., 2017) or remnants of a previous wider distribution range is unknown.

The greatest species diversity within the genus is found in central Guyana, where six species occur. In the upper Rio Negro area of Brazil, four species are found. Outside these areas, just one or two species occur in sympatry. We find few surprises in species range when compared to those presented by Cowan (1975), except that *E. glabra* has a much-expanded range into central Guyana, South Suriname, and Brazil, especially if *E. praesagata* is considered as conspecific.

Our original hypothesis concerning inflorescence and flower morphology was that only one shift from bee to bat or from bat to bee pollination had occurred within the genus. Our phylogeny, however, suggests several shifts. Fabaceae is one of the most species-rich angiosperm families and has a wide diversity of pollination syndromes. While bees are the main pollinator of Fabaceae, butterflies, beetles, moths, birds, bats, primates, and wind are also pollinators (Banks and Rudall, 2016). In almost all subclades of Fabaceae, the transition to bat and bird pollination has occurred (Banks and Rudall, 2016). In contrast to the pollen within the early-branching lineages of Fabaceae (Cercidoideae, Detarioideae, Duparquetioideae, Dialioideae, and Caesalpinioideae) that ranges from 30–50 μm (Banks and Rudall, 2016), the pollen of *Eperua* is relatively large (Figure S1). *Eperua venosa* possibly has the largest pollen in this group (130 μm, Banks and Rudall, 2016). Bat pollination is rare among flowering plants but widespread in Fabaceae, especially in subfamily Detarioideae (Banks and Rudall, 2016), in which *Eperua* is included. Bat pollination in the genus *Eperua* is no surprise. Whether bat pollination is the plesiomorphic state in the genus is debatable because separation of the early-branching lineages in the *Eperua* phylogeny has low BS support (Figure 5). In any case, all species of clade 2 and 3b have characteristics of bat pollination: a pendant inflorescence, short petals,

exserted stamens and stigma, and large and rough pollen. A shift to these characteristics occurred early in the clade; later, within clade 3b, a shift to smaller, smoother pollen took place, with flower form and color more associated with bee pollination. Nevertheless, within this clade, a reversal to bright white, bat-pollination-type flowers (*E. schomburgkiana*, *E. duckeana*) took place. These species have pollen with a rougher surface but not an increase in size (Appendix S1: Figure S6). Subsequently, in *E. glabriflora*, flower morphology reverted back to a bee pollination syndrome, including a color shift. *Eperua froesii* is somewhat anomalous; it is an early-diverging taxon in clade 3b with a flower morphology of a bat-pollinated species, pollen resembling that of the species of clade 3a, but pollen size equal to that of the other species of clade 3b.

One remaining phenomenon worthy of note is the consistent difference in fruit shape and number of seeds between bat-pollinated and bee-pollinated species. All bat-pollinated species have fruits that are more than twice as long as wide and contain more than one seed (Appendix S1, Table S1A), regardless of their species position in the phylogeny. Furthermore, bat-pollinated species have a longer ovary than bee-pollinated species (species with long, pendant, and intermediate inflorescences combined, data from Appendix S1, Table S1A, R^2 adjusted [adj.] = 33%, $P < 0.05$). For length of ovary and stamens relative to the petal, the effect is much stronger (data from Appendix S1, Table S1B, ovary: R^2 adj. = 77%, $p << 0.001$; stamens: R^2 adj. = 85%, $P << 0.001$). It is noteworthy that all these changes occur together and in different clades across the phylogeny. Because the expression of petals, stamens, and carpels are regulated by the C-factor of the ABC model of floral development (Irish, 2017), perhaps one regulatory gene causing lengthening carpel and stamen structures but reduction of petal size (all bat pollinates species have exserted stamens and stigma) may have such an effect, producing a longer ovary with space for more ovules, and subsequently a longer fruit with more seeds. Both latest species (Fortes et al., 2023a) also adhere neatly to this "rule", with *E. manausensis* (bee-pollinated morphology) having a squarish fruit with one seed and *E. cerradoensis* (bat-pollinated morphology) having a fruit that is longer than wide with four seeds per fruit.

AUTHOR CONTRIBUTIONS

H.t.S, R.H.J.E., and D.M.A.R. conceived the study; H.t.S. and S.m.d.O. designed the genome skimming; E.D., M.E., and F.G. carried out the lab work; M.M.P. and V.F.G. collected additional leaf material; H.t.S. and E.A.F. wrote the manuscript; all authors contributed text and commented on the various manuscript versions.

ACKNOWLEDGMENTS

The authors thank all collectors who collected the many specimens of this beautiful genus. Olaf Bánki and Tinde van Andel collected some new leaf samples in silica for this study. We thank Sebastien Santini (CNRS/AMU IGS UMR7256) and the PACA Bioinfo platform (supported by IBISA) for the availability and management of the phylogeny.fr website that was used to perform all ML analyses. Bertie Joan van Heuven of Naturalis Biodiversity Center assisted with the light microscopy and took SEM photographs of *Eperua froesii*. Werner de Gier provided scripts for the construction of the colored phylogenies. Francisco Castro-Lima provided the image for *E. leucantha*. The comments of two anonymous reviewers and Associate Editor Hanno Schaefer helped to improve our manuscript. E.A.F. thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for the Ph.D. thesis scholarship (process 870360/1997-3). V.F.M. is grateful to CNPq (process number 303053/2018-6) and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; process number E-26-/203.007/2017).

DATA AVAILABILITY STATEMENT

The specimen data of all *Eperua* species are available at GBIF and SpeciesLink. NRb and plastome data have been uploaded to GenBank (GenBank accession numbers can be found in Appendix S1, Tables S4, S5). All character data are provided in Appendix S1 (Tables S1A, B). A list of all collections with coordinates used in mapping and fasta files with the alignment of the NRb sequences and full coding DNA sequences of the plastome are available at Dryad Digital Repository: https://doi.org/10.5061/dryad.7d7wm3813.

ORCID

Hans ter Steege http://orcid.org/0000-0002-8738-2659
Elenice A. Fortes http://orcid.org/0000-0001-8307-6534
Danaë M. A. Rozendaal http://orcid.org/0000-0002-3007-3222
Roy H. J. Erkens http://orcid.org/0000-0002-1093-0370
Daniel Sabatier http://orcid.org/0000-0003-0883-1530
Gerardo Aymard http://orcid.org/0000-0001-9405-0508
Elza Duijm http://orcid.org/0000-0002-6383-3419
Marcel Eurlings http://orcid.org/0000-0002-1786-9883
Felix Grewe http://orcid.org/0000-0002-2805-5930
Maihyra M. Pombo http://orcid.org/0000-0002-0329-9736
Vitor F. Gomes http://orcid.org/0000-0002-3855-5584
Vidal F. de Mansano http://orcid.org/0000-0002-7204-0744
Sylvia M. de Oliveira http://orcid.org/0000-0002-1440-9718

REFERENCES

Abadi, S., D. Azouri, T. Pupko, and I. Mayrose. 2019. Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* 10: 934.

Acosta, M. C., and A. C. Premoli. 2010. Evidence of chloroplast capture in South American Nothofagus (subgenus *Nothofagus*, Nothofagaceae). *Molecular Phylogenetics and Evolution* 54: 235–242.

Aymard, G., R. Schargel, P. Berry, and B. Stergios. 2009. Estudio de los suelos y la vegetación (estructura, composición florística y diversidad)

en bosques macrotérmicos no-inundables, estado Amazonas Venezuela (aprox. 01° 30′–05° 55′ N; 66° 00′–67° 50′ O). *Biollania, Edición Especial* 9: 6–251.

Banks, H., and L. Rico. 1999. Pollen morphology and phylogenetic analysis of Eperua Aublet (Detarieae: Caesalpinioideae: Leguminosae). *Grana* 38: 261–276.

Banks, H., and P. J. Rudall. 2016. Pollen structure and function in caesalpinioid legumes. *American Journal of Botany* 103: 423–436.

Banks, H., F. Forest, and G. Lewis. 2013. Palynological contribution to the systematics and taxonomy of *Bauhinia* s.l. (Leguminosae: Cercideae). *South African Journal of Botany* 89: 219–226.

Berry, P. E., B. K. Holst, and K. Yatskievych. 1998. Flora of the Venezuelan Guayana. Missouri Botanical Garden Press, St. Louis, MO, USA.

Bruneau, A., F. Forest, P. S. Herendeen, B. B. Klitgaard, and G. P. Lewis. 2001. Phylogenetic relationships in the Caesalpinioideae (Leguminosae) as inferred from chloroplast *trnL* intron sequences. *Systematic Botany* 26: 487–514.

Bruneau, A., M. Mercure, G. P. Lewis, and P. S. Herendeen. 2008. Phylogenetic patterns and diversification in the caesalpinioid legumes. *Botany* 86: 697–718.

Chave, J., C. Sothers, A. Iribar, U. Suescun, M. W. Chase, and G. T. Prance. 2020. Rapid diversification rates in Amazonian Chrysobalanaceae inferred from plastid genome phylogenetics. *Botanical Journal of the Linnean Society* 194: 271–289.

Cowan, R. S. 1975. A monograph of the genus *Eperua* (Leguminosae: Caesalpinioideae). Smithsonian Institution Press, Washington, D.C., USA.

Cowan, R. S. 1985. Studies in tropical American Leguminosae-IX. *Brittonia* 37: 291–304.

Cowan, R. S., and P. E. Berry. 1998. *Eperua*. In P. E. Berry, K. Yatskievych, and B. K. Holst [eds.], Flora of the Venezuelan Guayana, 64–67. Missouri Botanical Garden, St. Louis, MO, USA.

de la Estrella, M., F. Forest, B. Klitgård, G. P. Lewis, B. A. Mackinder, L. P. de Queiroz, J. J. Wieringa, and A. Bruneau. 2018. A new phylogeny-based tribal classification of subfamily Detarioideae, an early branching clade of florally diverse tropical arborescent legumes. *Scientific Reports* 8: 6884.

Delaval, M., M. Henry, and P. Charles-Dominique. 2005. Interspecific competition and niche partitioning example of a neotropical rainforest bat community. *Revue d'Ecologie (Terre et Vie)* 60: 149–166.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J. F. Dufayard, et al. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36: W465–W469.

Dexter, K. G., M. Lavin, B. M. Torke, A. D. Twyford, T. A. Kursar, P. D. Coley, C. Drake, et al. 2017. Dispersal assembly of rain forest tree communities across the Amazon basin. *Proceedings of the National Academy of Sciences, USA* 114: 2645–2650.

Dierckxsens, N., P. Mardulyn, and G. Smits. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Research* 45: e18.

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Fanshawe, D. B. 1952. The vegetation of British Guyana. A preliminary review. Imperial Forestry Institute, Oxford, UK.

Fleming, T. H., C. Geiselman, and W. J. Kress. 2009. The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany* 104: 1017–1043.

Forget, P.-M. 1989. La regeneration naturelle d'une espèce autochore de la forêt guyanaise: *Eperua falcata* Aublet (Caesalpiniaceae). *Biotropica* 21: 115–125.

Forget, P.-M. 1992. Regeneration ecology of *Eperua grandiflora* (Caesalpiniaceae), a large-seeded tree in French Guiana. *Biotropica* 24: 146–156.

Fortes, E. A., and V. F. Mansano. 2022. Untangling nomenclatural issues of some Amazonian trees of *Eperua* Aubl. (Leguminosae, Detarioideae, Detariae). *PhytoTaxa* 542: 189–198.

Fortes, E. A., G. S. Da Silva, and V. F. Mansano. 2023a. Two new species of *Eperua* (Leguminosae, Detarioideae, Detarieae) from the Amazon and Cerrado Biomes discovered in a botanical garden and a backyard. *Phytotaxa* 591: 196–208.

Fortes, E. A., I. P. Dos Reis, H. ter Steege, G. Aymard, R. D. S. Secco, R. C. V. Martins-Da-Silva, and V. F. Mansano. 2023b. A taxonomic revision of the genus *Eperua* Aubl (Fabaceae, Detarioideae, Detarieae). *Phytotaxa*. 617: 1–127.

Fougère-Danezan, M., S. Maumont, and A. Bruneau. 2007. Relationships among resin-producing *Detarieae* s.l. (Leguminosae) as inferred by molecular data. *American Society of Plant Taxonomists* 32: 748–761.

Geiselman, C. K. 2010. Diet and reproduction of sympatric nectar-feeding bat species (Chiroptera: Phyllostomidae) in French Guiana. Ph.D. dissertation, Columbia University, NY, NY, USA.

Huber, M. O. 1995. Vegetation. *In* J. A. Steyermark, P. E. Berry, and B. K. Holst [eds.], Flora of the Venezulean Guyana, 1, 97–160. Timber Press, Portland, OR, USA.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Irish, V. 2017. The ABC model of floral development. *Current Biology* 27: R887–R890.

Janzen, D. H. 1974. Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica* 6: 69–103.

Jin, J.-J., W.-B. Yu, J.-B. Yang, Y. Song, C. W. DePamphilis, T.-S. Yi, and D.-Z. Li. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology* 21: 241.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Khanuja, S. P. S., A. K. Shasany, M. P. Darokar, and S. Kumar. 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant Molecular Biology Reporter* 17: 74.

Klinge, H., and E. Medina. 1979. Rio Negro caatingas and campinas, Amazonas states of Venezuela and Brazil. *In* R. L. Specht [ed.], Heathlands and related shrublands of the world. A. Descriptive studies, 483–488. Elsevier, Amsterdam, Netherlands.

Kubitzki, K. 1989. The ecogeographical differentiation of Amazonian inundation forests. *Plant Systematics and Evolution* 162: 285–304.

Legume Phylogeny Working Group. 2017. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon* 66: 44–77.

Lindeman, J. C., and S. P. Moolenaar. 1959. Preliminary survey of the vegetation types of northern Suriname. *In* I. A. De Hulster, J. Lanjouw, and F. W. Ostendorf [eds.], The vegetation of Suriname, 1, part 2, 1–45. Van Eedenfonds, Amsterdam, Netherlands.

Obiang-Mbomio, D., and F. J. Breteler. 2007. Révision du genre *Eurypetalum* Harms (Fabaceae, Caesalpinioideae). *Adansonia, série 3* 29: 67–76.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson, M. Henry, H. Stevens, and H. Wagner. 2020. vegan: Community ecology package. R package version 2.5-7. Website: https://CRAN.R-project.org/package=vegan

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: https://www.r-project.org

Romero-González, G. A., and G. A. Aymard. 2019. A new species of *Eperua* (Leguminosae, Detarioideae) from Amazonas State, Venezuela. *Harvard Papers in Botany* 24: 341–347.

Simon, R., K. Bakunowski, A. E. Reyes-Vasques, M. Tschapka, M. Knörnschild, J. Steckel, and D. Stowell. 2021. Acoustic traits of bat-pollinated flowers compared to flowers of other pollination syndromes and their echo-based classification using convolutional neural networks. *PLoS Computational Biology* 17: e1009706.

Stegemann, S., M. Keuthe, S. Greiner, and R. Bock. 2012. Horizontal transfer of chloroplast genomes between plant species. *Proceedings of the National Acadamy of Sciences, USA* 109: 2434-2438.

Stroo, A. 2000. Pollen morphological evolution in bat pollinated plants. *Plant Systematics and Evolution* 222: 225–242.

ter Steege, H. 1990. A monograph of Wallaba, Mora and Greenheart. Tropenbos International, Wageningen, Netherlands.

ter Steege, H., T. W. Henkel, N. Helal, B. S. Marimon, B. H. Marimon-Junior, A. Huth, J. Groeneveld, et al. 2019. Rarity of monodominance in hyperdiverse Amazonian forests. *Scientific Reports* 9: 13822.

ter Steege, H., N. C. A. Pitman, T. J. Killeen, W. F. Laurance, C. A. Peres, J. E. Guevara, R. P. Salomão, et al. 2015. Estimating the global conservation status of over 15,000 Amazonian tree species. *Science Advances* 1: e1500936.

ter Steege, H., N. C. Pitman, D. Sabatier, C. Baraloto, R. P. Salomao, J. E. Guevara, O. L. Phillips, et al. 2013. Hyperdominance in the Amazonian tree flora. *Science* 342: 1243092.

ter Steege, H., P. I. Prado, R. A. F. d. Lima, E. Pos, L. de Souza Coelho, D. de Andrade Lima Filho, R. P. Salomão, et al. 2020. Biased-corrected richness estimates for the Amazonian tree flora. *Scientific Reports* 10: 10130.

Vogel, S. 1968. Chiropterophilie in der Neotropischen Flora. *Flora, Abteilung B* 157: 562–602.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Additional Supporting Information may be found online in the supporting information section at the end of the article in Appendix S1 (including Tables S1–S6, Figures S1–S7, Maps S1–S7, Literature cited, R code to plot species maps).

How to cite this article: ter Steege, H., E. A. Fortes, D. M. A. Rozendaal, R. H. J. Erkens, D. Sabatier, G. Aymard, E. Duijm, et al. 2023. Molecular phylogeny and evolution of inflorescence types in Eperua. *American Journal of Botany* 110(10): e16229. https://doi.org/10.1002/ajb2.16229