Morphological and Molecular Evidence Support Elevating Erythroxylum macrophyllum var. savannarum (Erythroxylaceae) to Specific Status

Authors: Jara-Muñoz, Orlando A., White, Dawson M., and Rivera-Díaz, Orlando

Source: Systematic Botany, 47(2) : 467-476

Published By: The American Society of Plant Taxonomists

URL: https://doi.org/10.1600/036364422X16512572274990

Systematic Botany (2022), 47(2): pp. 467–476
ISSN 0363-6445 (print); ISSN 1548-2324 (online)

DOI 10.1600/036364422X16512572274990
Date of publication June 14, 2022

Morphological and Molecular Evidence Support Elevating *Erythroxylum macrophyllum* var. *savannarum* (Erythroxylaceae) to Specific Status

Orlando A. Jara-Muñoz,[1,3,4] Dawson M. White,[2,3,4] and Orlando Rivera-Díaz[1]

[1]Universidad Nacional de Colombia, Instituto de Ciencias Naturales, Cra. 30 No. 45-03, Edificio 425, Bogotá, D.C., Colombia; oajaram@unal.edu.co
[2]Grainger Bioinformatics Center, Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605, USA; dawson.white@gmail.com
[3]Authors contributed equally to this work
[4]Authors for correspondence

Communicating Editor: Martin F Wojciechowski

Abstract*—Erythroxylum macrophyllum* is a morphologically variable and widely distributed species complex in Central and South America with several sub-specific taxa and numerous species included in its synonymy. A single variety grows in the Colombo-Venezuelan savanna region which can be distinguished from the rest of the *E. macrophyllum* complex by the size of leaves, cataphyll and stipule characteristics, and shape of calyx lobes. A molecular phylogeny reconstructed from 519 nuclear genes also reveals that the savanna variety is more closely related to *E. acuminatum* and *E. pauciflorum* than *E. macrophyllum*. This phylogenomic evidence also suggests *Erythroxylum* sect. *Macrocalyx,* to which *E. macrophyllum* belongs, is a polyphyletic taxonomic section. We thus propose elevating this variety to specific status, as ***Erythroxylum savannarum***. We provide an updated taxonomic description, information about its habitat and distribution, and justify its informal IUCN categorization of Near Threatened (NT).

Keywords*—Erythroxylum* sect. *Macrocalyx*; exon-capture DNA sequencing, integrative taxonomy, Llanos, Neotropical *Erythroxylum*.

The Erythroxylaceae is a middle size family of angiosperms composed of ca. 280 species of trees, treelets, and shrubs (White 2019, 2020). Four genera are recognized in the family, three of which are restricted to Africa and contain only 11 species. The fourth is *Erythroxylum* P.Browne, which is distributed in lowland tropical and subtropical dry and humid forests of America, Africa, Asia, Australia, and Oceania (Daily 2004). Furthermore, about three-quarters of the ca. 270 described *Erythroxylum* species are Neotropical, with centers of diversity in northeastern Brazil and the Venezuelan Guayana (Plowman and Hensold 2004). The genus is also well known as the natural source of the alkaloid cocaine, which is extracted from two species, *Erythroxylum coca* Lam. and *Erythroxylum novogranatense* (D.Morris) Hieron. These are both very important plants for native cultures from Costa Rica to northern Argentina, with at least an 8000-yr history of use as quotidian stimulant and medicine (Dillehay et al. 2010; White et al. 2021). The traditional use of coca leaf is sharply contrasted with public health problems arising from recreational cocaine abuse and narcotrafficking (Stolberg 2011; Restrepo et al. 2019).

Given the large number of species in *Erythroxylum*, a practical intrageneric taxonomy is valuable for species identification as well as ecological and systematic investigations. The genus is currently structured according to the sectional classification system of Schulz (1907), wherein 19 sections are distinguished by geographic distributions (e.g. African; Australian; Neotropical) and several primary morphological characters describing the calyx, fruit shape, stipule, and/or style. However, this sectional classification must be revised after several recent phylogenetic studies have shown most sections are not monophyletic (Emche et al. 2011; Islam 2011; White et al. 2019). This study is the first to present the phylogeny of species in *Erythroxylum* section *Macrocalyx* O.E.Schulz, a small section of six Neotropical species.

Erythroxylum macrophyllum Cav. is one of the most broadly distributed and morphologically variable species of *Erythroxylum*, ranging from Brazil to southern Mexico between 0 and ~2000 m a. s. l., but it is absent in the Caribbean islands (Plowman and Hensold 2004). The species was included in *Erythroxylum* section *Macrocalyx* because of the presence of striate stipules and broad to foliose calyx (Schulz 1907). According to Plowman (1988b), *E. macrophyllum* can be considered a polymorphic species complex, in which different morphotypes grow in proximity but under different ecological conditions, such as different soils or microclimates. He ultimately described three varieties with distinct morphological and/or ecological traits: *E. macrophyllum* var. *macrocnemium* (Mart.) Plowman, a large leaved (> 30 cm) form from the Peruvian and Ecuadorian Amazon, *E. macrophyllum* var. *ecuadorense* Plowman, from the Ecuadorian Amazon, and *E. macrophyllum* var. *savannarum* Plowman (1988b) from the Llanos, a tropical grassland ecoregion shared by Colombia and Venezuela. The savanna variety was distinguished from the others by its smaller leaves, persistent cataphylls and stipules, slender pedicels, and lanceolate, oblong, or narrowly ovate calyx lobes (vs. subfoliose, broadly ovate, or obovate calyx lobes that overlap at the margins in *E. macrophyllum* var. *macrophyllum*). More recently, Jara-Muñoz (2011) conducted an extensive morphological analysis of the *E. macrophyllum* complex and showed anatomical and morphological evidence for the non-monophyly of *E. macrophyllum* varieties, most importantly that *E. macrophyllum* var. *savannarum* is more similar to *Erythroxylum daphnites* Mart. than to the type variety of *E. macrophyllum* and to any other species in *E.* section *Macrocalyx*.

We propose elevating *E. macrophyllum* var. *savannarum* to the taxonomic rank of species based on a thorough morphological analysis and a molecular phylogeny that shows it is evolutionarily distinct from *E. macrophyllum*. We compare important morphological and anatomical characters among the revised taxon and its close relatives and provide a dichotomous key for morphological diagnosability. Lastly, we discuss the implications for the current intrageneric taxonomy, considering our phylogenetic hypothesis.

Materials And Methods

Morphological Data—We determined relevant taxonomic characters for this *Erythroxylum* clade through years of experience in *Erythroxylum* taxonomy and understanding previous literature, especially the contributions from Schulz (1907, 1931), Plowman (1984, 1986, 1988a, 1988b), Loiola (2001), and Oviedo (2002). Some leaf anatomy characters for the species from Cerrado, *Erythroxylum campestre* A.St.-Hil. and *Erythroxylum suberosum* A.St.-Hil., were obtained from Bieras and Sajo (2004). We recorded macro-morphological characters by observing herbarium specimens from COL, US, F, and MO. For micro-morphological and anatomical characters, we destructively sampled herbarium leaf fragments with permission from curators. Observations of epicuticular waxes were obtained by scanning electron microscopy (SEM), FEI, Quanta 200 from the Centro de Equipos Inter Facultades (CEIF) of the Universidad Nacional de Colombia; leaf surfaces were sputter coated with gold/palladium for the SEM-micrograph. Classification and terminology of these waxes follows Barthlott et al. (1998). We made anatomical slides by free-hand cutting rehydrated leaves and staining them with chlorine dioxide, washing with tap water, and staining with carmine-thionine before mounting them with glycerinated gelatin (Becerra et al. 2002). Slides were observed and photographed using a Leica DM500 microscope and Kodak M530 camera. Descriptive morphology follows Font Quer (2000), with translation to English following Hornak (2011). A particular case is the term cataphyll, which typically names achlorophyllous, scale-like structures homologous to leaves, but in *Erythroxylum* descriptions beginning with Plowman (1984) this term was applied to describe a stipule subtending a leaf rudiment ("spinule") as opposed to a fully developed leaf. Cataphylls and/or stipules grouped together at the base of branches are termed ramenta.

Informal Conservation Status—Assessment of the informal conservation status of the species follows the IUCN guidelines (IUCN 2019). For the estimation of extent of occurrence (EOO) and area of occupancy (AOO), we employed the online tool GeoCAT (Bachman et al. 2011). Each locality was georeferenced based on information from herbarium labels.

Molecular Data—We extracted genomic DNA from 19 *Erythroxylum* herbarium specimens representing 13 species using a modified 2 × CTAB protocol with 3% PVP and 2% 2-mercaptoethanol in the extraction buffer (Khanuja et al. 1999). Sample voucher information including GenBank SRA accession numbers is presented in Appendix 2. Discolored samples were cleaned with the MOBIO DNA Clean-Up Kit (QIAGEN, Hilden, Germany). DNA concentration was measured using a Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts). All genomic DNA samples with fragment sizes > 500 bp, as determined from gel electrophoresis, were acoustically sheared to 400 bp (PiP 50 W, duty factor 10%, 200 cycles per burst, 70 sec, 20°C) with the Covaris M220 Focused-ultrasonicator in 130 µL snap-cap tubes (Covaris Inc., Woburn, Massachusetts).

Starting with 10–20 ng/ul input DNA, we prepared samples for exon capture using the KAPA Hyper-Prep kit (Kapa Biosystems, Inc., Wilmington, Massachusetts) with the Illumina TruSeq HT dual-indexed adapters (96 samples; Illumina Inc., San Diego, California) or Adapterama indexed adapters with iTru7_101 and iTru5_101 primer combinations (65 samples; Glenn et al. 2019). DNA libraries were pooled in groups of 10–16 (together with other *Erythroxylum* samples not presented here) for hybridization-based exon capture using custom RNA probes that capture 547 low-copy nuclear genes (see White et al. 2019; Arbor Biosciences, Ann Arbor, Michigan). Baits were diluted 1:2 in water to enable more pools, but we did not dilute any other reagents. Our target-capture protocol followed the myBaits v3 user manual, with 22 hr of hybridization at 65°C and 12–14 cycles of post-capture amplification using 2 × KAPA HiFi HotStart ReadyMix. All samples were sequenced on the Illumina HiSeq4000 platform in a single lane (2 × 100 bp) at the University of Oregon.

Reads were filtered, trimmed, and demultiplexed with bbduk (Bushnell 2015) by removing bases with a q-score < 20 and hard trimming 5–20 bases from both ends as judged from the FASTQC base composition graph (Andrews 2010). PCR duplicates were removed with SuperDeduper (github.com/dstreett/Super-Deduper). Cleaned reads were mapped to the 547 target sequences (exons concatenated) with BWA (Li and Durbin 2009) and de novo assembled with SPAdes (Bankevich et al. 2012) within the HybPiper pipeline (Johnson et al. 2016) using a minimum coverage threshold of six.

The cladogram presented in this paper is derived from the large phylogenomic reconstruction of 202 *Erythroxylum* species from White's (2019) dissertation and will be presented in its entirety in a forthcoming paper. We removed samples from individual gene alignments if the assembled supercontig (exon + intron) DNA sequence was shorter than 500 bp. We removed all genes that HybPiper flagged as containing paralogs. Final gene sequences were aligned using the MAFFT local pairwise algorithm with up to 1000 refinement iterations (Katoh et al. 2002). Lastly, we used trimAl (automatic setting) to remove sites with a high proportion of gaps (Capella-Gutiérrez et al. 2009). Alignments are available the Dryad Digital Repository (Jara-Muñoz et al. 2022) and at github.com/Erythroxylum/datasets.

We reconstructed a maximum-likelihood tree from each alignment with RaxML v. 8 (Stamatakis 2014), taking the best tree from 20 random starting trees inferred under a GTRCAT + GAMMA model of nucleotide substitution (Tavaré 1986; Yang 1993). We inferred the final phylogenetic hypothesis using the coalescent-based reconciliation algorithm in ASTRAL-III v. 5.7.5, which produces a 'summary' tree representing the maximum number of quartet topologies observed from the 519 gene trees (Zhang et al. 2018). We scored statistical support for the ASTRAL summary tree inference by calculating gene concordance factors (gCF) from the 519 gene trees and site concordance factors (sCF) from the 519 gene alignments using IQ-TREE 2.0 (Minh et al. 2020a, 2020b). To test statistical support for the monophyly of the savanna variety with *E. macrophyllum* var. *macrophyllum*, we used IQ-TREE to score a constrained topology placing the savanna variety sister to the four *E. macrophyllum var. macrophyllum* samples, and otherwise retained the same topology as the ASTRAL summary tree.

Results

Morphological Evidence—A summary of the morphological data is presented in Table 1 and voucher information for the specimens studied for morphological comparison is presented in Appendix 1. There are three macro-morphological characters that separate *E. macrophyllum* var. *savannarum* from *E. macrophyllum* var. *macrophyllum*: 1) persistent stipules vs. deciduous in *E. macrophyllum* var. *macrophyllum*, 2) distichous cataphylls vs. polystichous, and 3) clavate stigma vs. capitate. In addition, leaves of the savanna variety are usually smaller (3.4–12.5(17.5) vs. 15–23 cm long) and typically coriaceous (vs. chartaceous in *E. macrophyllum* var. *macrophyllum*).

Anatomical characters of the midvein are generally uniform in *Erythroxylum* with most taxa, including *E. macrophyllum* var. *savannarum*, exhibiting two collateral vascular bundles (Table 1). However, *E. macrophyllum* var. *macrophyllum*, as well as *Erythroxylum citrifolium* A.St.-Hil. differs by having an additional adaxial bundle, first observed by Rury (1982). Additionally, the epicuticular wax on abaxial leaf surfaces forms membranous platelets in *E. macrophyllum* var. *savannarum* and *E. daphnites*, but forms rosette platelets in *E. macrophyllum* var. *macrophyllum, E. campestre, E. citrifolium, Erythroxylum durum* Moore, *Erythroxylum pauciflorum* Rusby, and *E. suberosum* (Table 1; Fig. 1; Jara-Muñoz 2011).

Phylogenetic Inference—Exon-capture sequencing produced 290,449 to 13,604,762 reads per sample (median 2.1 M) and each of the seventeen samples recovered between 391 and 543 genes at a minimum of 50% target gene length (median 543 genes; Fig. 2). Twenty-eight genes were dropped for containing paralogs, leaving 519 genes in the phylogenetic reconstruction. Gene alignments ranged from 510 to 14,829 bp with a median length of 2446 bp, and a total length of 1,397,910 bp with 13.77% missing sites (gaps or N's).

Our coalescent-based reconciliation of 519 nuclear gene trees inferred the strongest support for a species tree that split *E. macrophyllum* var. *macrophyllum* and *E. macrophyllum* var. *savannarum* into two separate clades (Fig. 3A). This position justifies the species status of *E. macrophyllum* var. *savannarum* based on a general lineage or species as ranked taxa concept (De Queiroz 1998; Baum 2009). The savanna variety forms a clade with (*E. acuminatum, E. pauciflorum*) with a posterior probability (PP) of 1, gCF of 6.18%, and sCF of 46.1%, while

TABLE 1. Summary of morphological and anatomical differences between *E. savannarum* and close congeners.

Character	*E. macrophyllum* var. *macrophyllum*	*E. savannarum*	*E. campestre*	*E. citrifolium*	*E. daphnites*	*E. durum*	*E. pauciflorum*	*E. suberosum*
Stipule persistence	Deciduous	Persistent	Deciduous	Deciduous	Persistent	Deciduous	Persistent	Persistent
Cataphyll disposition	Polistichous, not imbricate	Distichous and imbricate	Polistichous, not imbricate	Polistichous, not imbricate	Distichous and imbricate	Polistichous, not imbricate	Polistichous, not imbricate	Polistichous, not imbricate
Form of calyx lobes	Widely ovate or oblong	Triangular	Triangular	Triangular	Triangular	Widely ovate or oblong	Widely ovate or oblong	Widely ovate or oblong
Form of stigma	Capitate	Clavate	Capitate	Capitate	Capitate	Capitate	Clavate	Capitate
Type of abaxial epicuticular wax	Rosette platelets	Membranous platelets	Rosette platelets	Rosette platelets	Membranous platelets	Rosette platelets	Rosette platelets	Rosette platelets
Midvein vascular bundles	Two collateral bundles plus one adaxial bundle	Two collateral bundles	Two collateral bundles	Two collateral bundles plus one adaxial bundle	Two collateral bundles	Two collateral bundles	Two collateral bundles	Two collateral bundles

FIG. 1. Types of epicuticular waxes on the abaxial surface of *Erythroxylum* species. A. Membranous platelets in *E. savannarum*. B. Rosette platelets in *E. pauciflorum*. C. Membranous platelets in *E. daphnites*. D. Rosette platelets in *E. amplum*.

E. macrophyllum var. *macrophyllum* and *E. durum* form a sister clade with a PP of 1, gCF of 8.58%, and sCF of 49.5% (Fig. 3A). Relationships with the other clades are not well supported, leaving two candidate clades to be sister to the proposed *E. savannarum* and *E. macrophyllum* var. *macrophyllum* clades: one containing (*E. daphnites*, (*E. citrifolium,* (*Erythroxylum mucronatum* Benth., *E. campestre*))), or a clade containing (*E. suberosum*, *E. rimosum*). External to these is a clade containing (*E. amazonicum*, *E. amplum*), with a PP of 1, gCF 10.8%, and sCF 42.4%. The root of the tree is placed in Clade III of White et al. (2019) and has posterior probability of 1.

DISCUSSION

Even though Plowman (1988b) pointed out that *E. macrophyllum* var. *savannarum* "is distinctive both ecologically and morphologically," he decided upon the varietal classification stating the presence of intermediate forms. However, the thorough investigation of the *E. macrophyllum* complex by Jara-Muñoz (2011) showed the savanna variety and *E. macrophyllum* var. *macrophyllum* are morphologically distinct; and our phylogenetic hypothesis further justifies the separation between these taxa. Perhaps Plowman would have updated this classification himself had his prosperous career not been cut so short by his untimely death in 1989. According to the species-as-taxa criteria (Baum 2009), *E. macrophyllum* var. *savannarum* should be recognized as a species because of the clear evidence for non-monophyly with *E. macrophyllum* var. *macrophyllum* (Fig. 3), as well as the fact that it is a morphologically distinct and diagnosable lineage (Baum and Donoghue 1995).

Erythroxylum savannarum is morphologically most similar to *E. daphnites*, a species from the distant Brazilian and Bolivian cerrado (savanna) that differs in its short, shrubby habit and shorter pedicels and fruits. However, our phylogenetic reconstruction places *E. savannarum* closer to *E. acuminatum* and *E. pauciflorum*, the first from seasonal forests in the

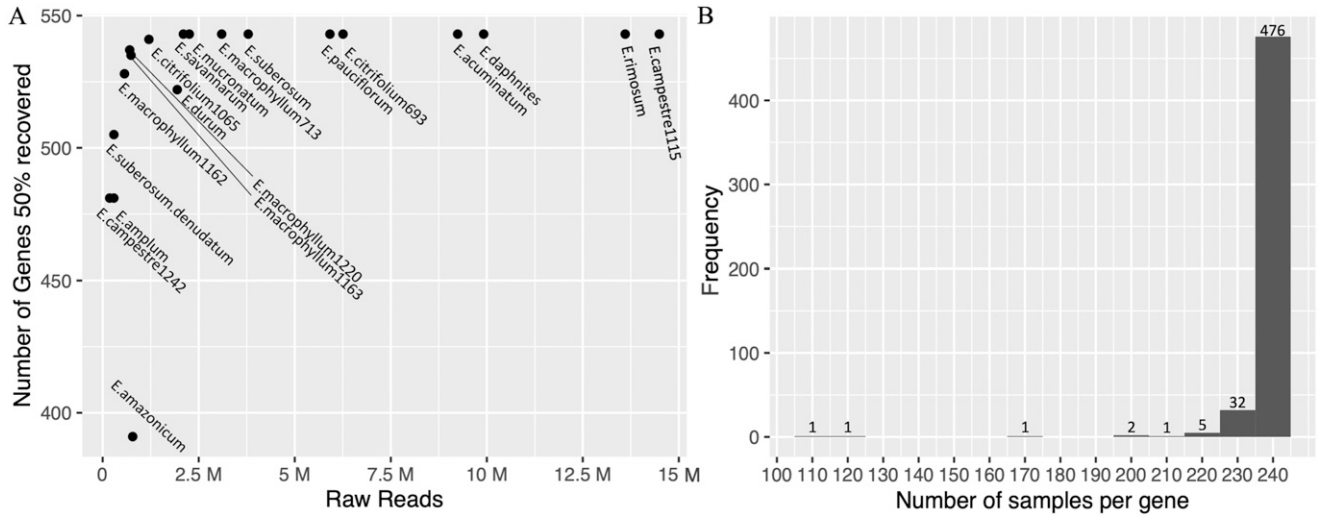

FIG. 2. Exon-capture sequencing and gene assembly statistics for 519 genes. A. The number of raw reads sequenced, and the number of genes successfully assembled to at least 50% of target length per sample. B. Histogram of the number of samples in each gene alignment.

Peruvian and Ecuadorian Andes/Amazon region and the second from the Bolivian Cerrado.

The phylogenomic reconstruction revealed several unexpected relationships between taxa in this clade, such as: 1) the non-sister relationship of *E. savannarum* and *E. daphnites*, 2) the distant relationship of two morphologically very similar species, *E. amplum* and *E. macrophyllum* var. *macrophyllum*, which have even been treated as synonyms (e.g. Jørgensen et al.

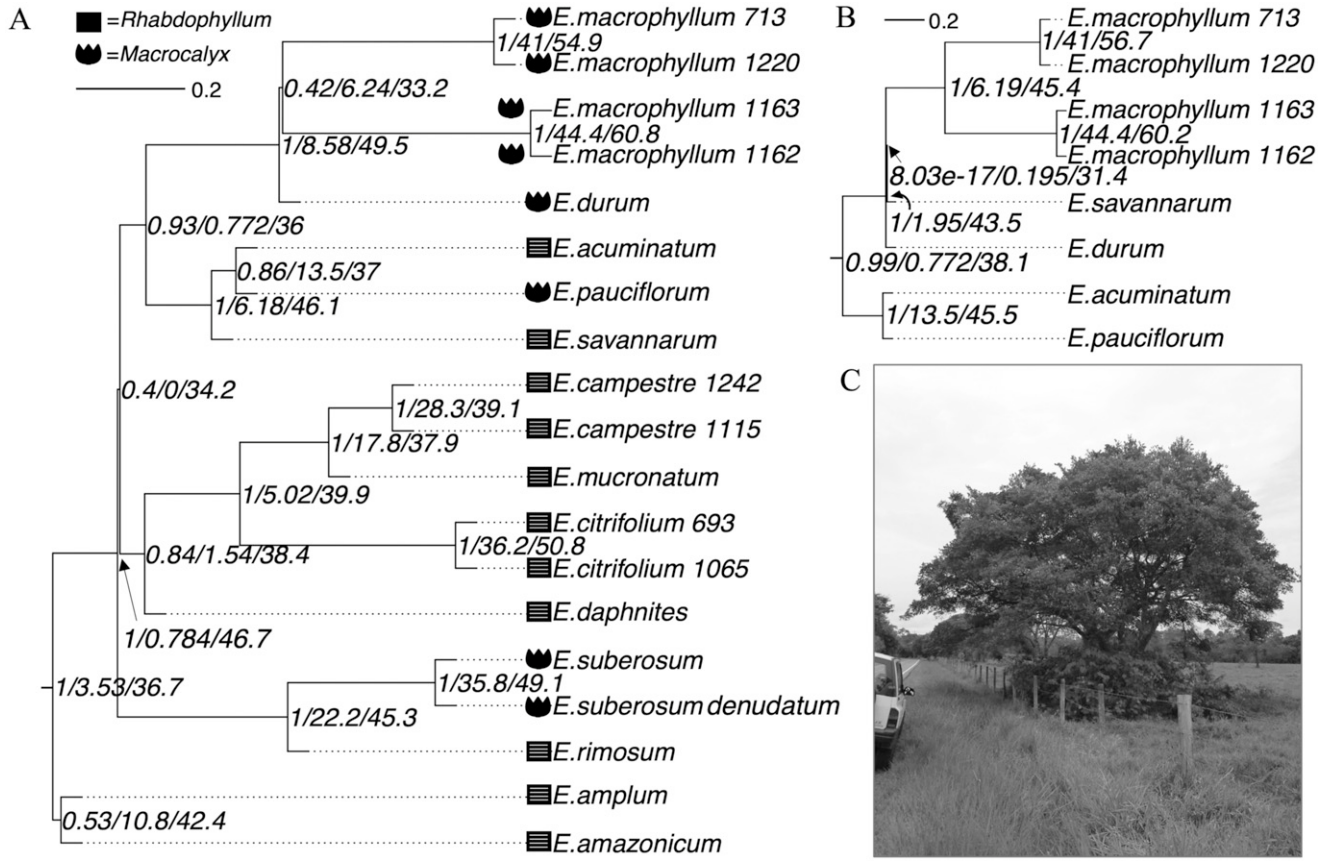

FIG. 3. Phylogenomic inferences of proposed *E. savannarum* and closest relatives. A. ASTRAL summary tree with symbols to the left of sample names indicating traditional sectional classification to *Erythroxylum* sect. *Macrocalyx* or *E.* sect. *Rhabdophyllum*. Node values report three support statistics: ASTRAL's local posterior probability, gene concordance factor, and site concordance factor. Branch lengths are in coalescent units, but terminal branch lengths are arbitrary. B. Constrained topology placing the proposed *E. savannarum* as sister to *E. macrophyllum* var. *macrophyllum* with node values and branch lengths as in A. C. A tree of the proposed *E. savannarum* in the Meta Department, Colombia (photo credit: D. M. White).

2014), and 3) the separation of the *E. amazonicum*, *E. citrifolium*, and *E. mucronatum* lineages, which can be (perhaps only in areas of hybrid formation) very difficult to differentiate morphologically. Overall, *Erythroxylum* is a morphologically intricate genus in which species discrimination relies heavily upon sets of quantitative traits and descriptive characteristics of vegetative organs (e.g. leaf shape, stipule margins; see dichotomous key). The phylogenomic reconstruction reveals that many morphological traits are convergent, and overall phenotypic similarities are not good estimates of species' evolutionary relationships within *Erythroxylum* clades.

While the three crucial nodes separating *E. macrophyllum* var. *macrophyllum* from *E. savannarum* have high PP support, and the PP of an alternative tree constraining the savanna species to be monophyletic with *E. macrophyllum* var. *macrophyllum* has a PP of 8.03 e^{-17} (Fig. 3), the very low gCF values across the tree reveal substantial gene tree heterogeneity. This must be caused by stochastic errors from limited information in single genes, even though our median gene alignment length was 2446 bp. The sCF values are also low, but they are high enough to support the species tree presented in Fig. 3A as the majority signal in the data, especially as it concerns the placement of *E. savannarum*. Introgression could also contribute to our observed gene tree heterogeneity, but since *E. acuminatum* and *E. pauciflorum* are allopatric with *E. savannarum*, we believe the confounding influence of introgression within our particular clade of interest is minimal. We did not conduct any explicit tests of admixture or reticulation, but this could be an informative line of inquiry for understanding the diversity of this clade overall. Our taxonomic proposal is clearly substantiated by the most concordant species tree topology.

These phylogenomic results also contribute to a growing number of studies (Islam 2011; White et al. 2019; White 2020) demonstrating the non-monophyly of several sections of *Erythroxylum* originally proposed by Schulz (1907) in his seminal taxonomic treatment of Erythroxylaceae. The presence of striations in the stipules (sclerified vascular bundles; Rury 1982) is a character used by Schulz (1907) in to distinguish *Erythroxylum* section *Rhabdophyllum, E.* sect. *Macrocalyx,* and the monotypic *E.* sect. *Pogonophorum* O.E.Schulz from the 16 other sections. *Erythroxylum* section *Macrocalyx* (six spp.) was distinguished from the larger *E.* sect. *Rhabdophyllum* (> 60 spp.) by the presence of foliose or broadly oblong to ovate calyx lobes (Schulz 1907; Loiola 2001). The foliose calyx lobe is present in individuals of *E. macrophyllum* var. *ecuadorense* and *E. macrophyllum* var. *macrocnemium*, and is frequent but not universal among *E. macrophyllum* var. *macrophyllum* individuals. However, *E. savannarum* individuals bear smaller, triangular calyx lobes representative of *E.* sect. *Rhabdophyllum*.

Representatives of four of the six *Erythroxylum* sect. *Macrocalyx* species are included in the phylogram in Fig. 3A, showing that *E.* sect. *Macrocalyx* is a polyphyletic section forming three separate lineages amongst nine species of *E.* sect. *Rhabdophyllum*. Individuals of the two remaining species, *E. hamigerum* O.E.Schulz and *E. macrocalyx* Mart., were also sampled in the large Erythroxylaceae species tree from White's dissertation (2020), and were placed as sister species within a distantly related clade comprised of other *E.* sect. *Rhabdophyllum* and *E.* sect. *Archerythroxylum* species. The three additional species originally placed in *Macrocalyx* by Schulz (1907) have been combined or placed in synonymy with *E. macrophyllum* (Plowman 1988a; Plowman and Hensold 2004). Future taxonomic revision should dissolve *Erythroxylum* sect. *Macrocalyx* into *E.* sect. *Rhabdophyllum* because it is clearly not a monophyletic group.

Taxonomic Treatment

Erythroxylum savannarum (Plowman) Jara & D.M. White, stat. nov. *Erythroxylum macrophyllum* var. *savannarum* Plowman. Brittonia 40(3): 263, Figs. 5–7, 1988. Type: Venezuela. Amazonas: Road NE from Puerto Ayacucho towards El Burro, 36.5 km from Puerto Ayacucho, 28 Apr 1984, *T. Plowman & F. Guánchez 13757* (holotype: F!, isotypes: B, CAY, COL!, F!, G, GB, GH, K, MA, MG, MY, NY!, P(photo!), TFAV, U, UC, US!, VEN).

Tree or small tree up to 10 m tall. **Branches** erect–patent, gray to brown, longitudinally striated, without lenticels. **Stipules** persistent, long triangular to lanceolate, 2.9–7.2 mm long, coriaceous, clearly striated, brown, apex acute with 2–3 setae, early caducous and ca. 1 mm long, margin entire. **Ramenta** present, distichous and imbricate, cataphylls similar to stipules. **Leaves** generally grouped to the tip of branches; petioles adaxially ribbed, 2.1–6.4 mm long; leaf blade oblong-elliptic or obovate, rarely ovate, 3.4–12.5 (17.5) × 1.2–4.7(5.6) mm, coriaceous, base cuneate, apex obtuse to rounded, margin plane to barely involute, abaxially pale brown, without vernation lines, adaxially dark brown or grayish, midvein prominent abaxially, with central elevation acute adaxially, secondary veins prominulous on both sides, reticulations obscure. **Flowers** generally axillary to cataphylls, 1–3 per fascicle; bracteoles triangular, ca. 1.5 mm long, membranous, apex acuminate, margin entire to slightly fimbriate; pedicels markedly pentagonal, gradually thickening to the apex, 6.7–10 mm long; calyx 1.5–2.1 mm long, deeply divided, lobes triangular, ovate, oblong or lanceolate, 1.2–1.5 mm long; petals oblong-ovate, membranous, limb distally concave, 2.1–2.3 × 0.8–1.3 mm, apex obtuse, claw obovate, 0.5–0.7 × 0.8–0.9 mm, ligules ca. 1.1 mm long, anterior auricles well-formed, 0.2–0.4 mm long, posterior auricles 0.4–0.5 mm long, medial appendix 0.2 mm long; staminal crown urceolate, generally shorter than the calyx, 0.8–0.9 long. **Ovary** ellipsoid, 1–1.1 × 0.6–0.7 mm. **Brevistylous flowers**: filaments sub-equal 2.2–3.2 mm long or 1–1.4 mm long, styles 3, free, 1–1.6 mm long. **Longistylous flowers**: Antisepal filaments, 1.2–1.4 mm long, antipetal filaments 1.7–2 mm long; styles 3, free, 2.3–3 mm long. **Drupes** ovoid-ellipsoid, 4.9–8.8 × 2–5.5 mm, red when ripe, endocarp terete.

Distribution and Habitat—This species grows in the Llanos ecoregion between Colombia and Venezuela. It is most abundant in gallery forests and in the transitional zone with the Colombian cordillera oriental or the Venezuelan Guayana (Fig. 4).

Etymology—The variety name *savannarum* is retained because the new combination has not been used before. This name refers to the habitat where this taxon is mainly distributed, the savanna or Llanos region between Colombia and Venezuela.

Common Names—Perhaps due to its local abundance and similarity to other *Erythroxylum* species, this tree has several common names. In Colombia it is called Ajicito, Arrabal, Arrayán sabanero, Coca, Coca de monte, and Piedrito.

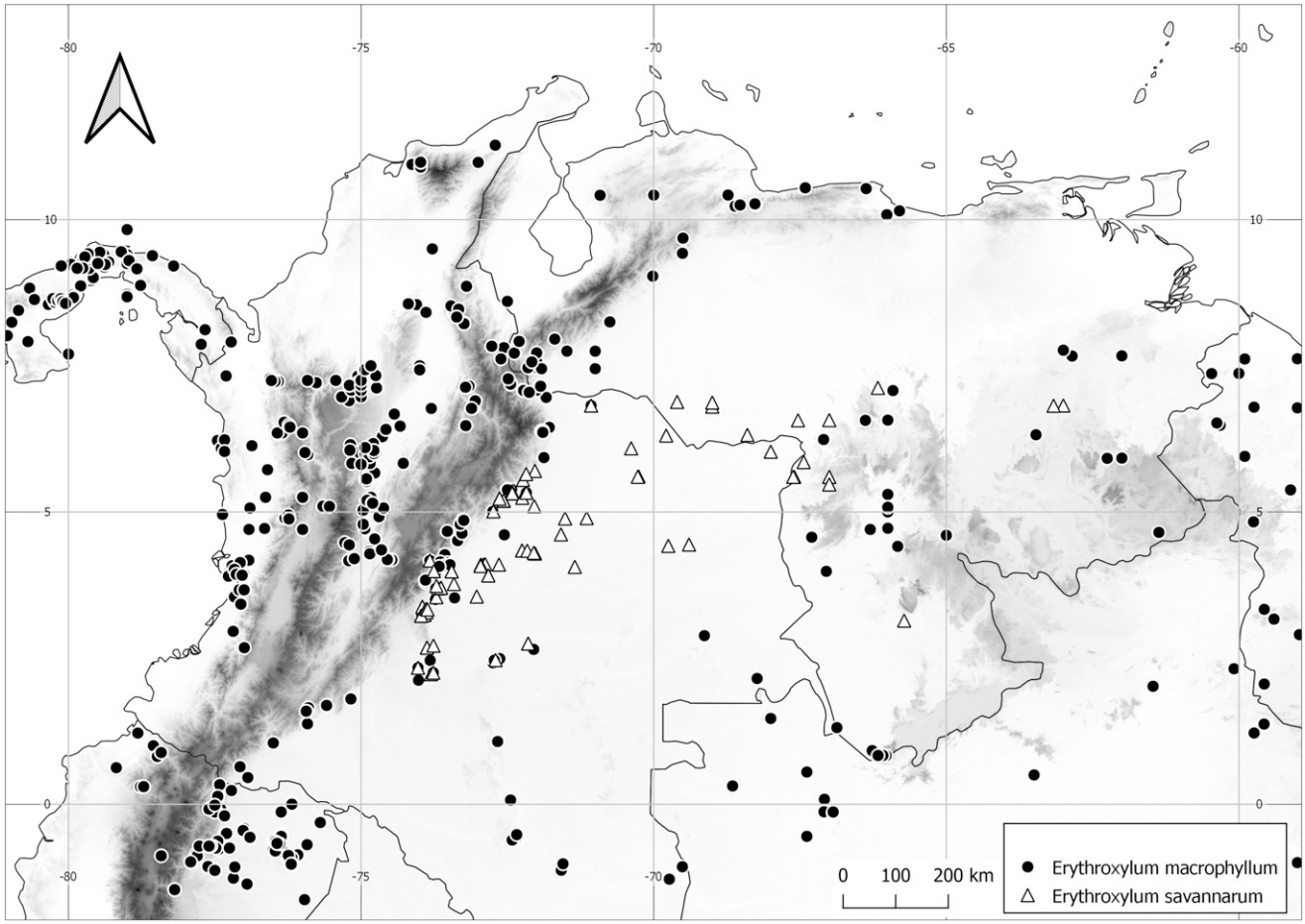

FIG. 4. Geographic distributions of *Erythroxylum savannarum* (white triangles) and *E. macrophyllum* including *E. macrophyllum* var. *ecuadorense* and *E. macrophyllum* var. *macrocnemium* (black circles), showing that most of the *E. savannarum* records come from the Colombo-Venezuelan Llanos region, where *E. macrophyllum* var. *macrophyllum* is absent. Specimen records were obtained from herbaria CAUP, CHOCO, COL, CUVC, F, HUA, HUQ, JAUM, MEDEL, MO, NY, UIS, and US.

In Venezuela it is called Pilón amarillo, Escobo, Socoroco negro, and Miel de pajarito (Plowman 1988b; pers. obs.).

***Informal Conservation Status*—**The extent of occurrence (EOO) is 470,506 km^2, and area of occupancy (AOO) is 316 km^2. Based on criteria B of the IUCN (2019), this AOO together with a severely fragmented habitat as well as declining population or habitat size would justify Endangered status. While habitat reduction from agricultural expansion is well documented in the Colombian Llanos (Romero-Ruiz et al. 2012; Lavelle et al. 2014), we cannot currently determine the effect of agricultural expansion on population sizes of *E. savannarum* because it is frequently encountered in fencerows and other disturbed sites. Thus, we hypothesize that if a formal conservation assessment were performed, the conservation status of this species would likely be Near Threatened (NT) with the expectation that future evidence of population decline could support changing the status to Endangered.

***Additional Specimens Examined*—Colombia.** —CASANARE: Cedros, Oct 1992, *E. Acero 206* (FMB), Nunchía, Vda. Puerto Payero, 02 Dec 1997, *A. Ayala 710* (COL); May 1990, *R. Castillo 31* (UPTC). Aguazul, May 1994, Correa, J. (FMB), Tauramena, Sep 1994, *J. Correa* (FMB), Vda. Buenavista-Rio Chiquito, 15 Nov 1997, *J. Correa 513* (COL); Vda. La Ñata, Finca de Ernesto Jimenez, 26 Nov 1997, *J. Correa 718* (COL); Yopal, 17 May 1990, *J. Gonzalez 17* (UPTC). Hacienda El Mango, Jul 1975, *R. Guarin 143* (UPTC), caño Pica-Pico, 22–25 Apr 1988, *G. Mahecha 5234* (COAH), Orocué, Resguardo Paravare, 110 m, 2 May 2006, *L. Miranda 135* (COAH), Paz de Ariporo, Corr. La Hermosa, finca Nicaragua, 113 m, 27 Oct 2004, *J. Ramírez 8832* (COAH). —GUAVIARE: San José del Guaviare, Trocha ganadera, La Bocana-Mipiripán, 16 Oct 1985, *J. Idrobo 11839* (COL), 16 Oct 1985, *J. Idrobo 11840* (COL). —META: San Martín, vereda La Castañeda, finca Santa Rosa, 3°36′51″N, 73°38′33″W, 362 m, 28 Feb 2005, *A.M. Aldana & P. Stevenson 29* (COL), Puerto Lopez, Parque Natural Municipal Yucao, cuenca del río Yucao, 185 m, 1 Jan 1997, *A. Fajardo 237* (CUVC), Finca Guaracu, 25 Feb 1997, *A. Fajardo 237* (COL, CUVC), Puerto Gaitán, Llanuras cercanas al río Manacacias y Alto de Neblinas, 15 Mar 1986, *J. Fernandez-Alonso 5772* (COL), San Juan de Arama, Serrania de la Macarena, camino entre Los Micos y San Juan de Arama, 27 Mar 1956, *A. Fernández-Pérez 5112* (COL), Puerto Gaitán, San Pedro de Arimena, Alto Neblinas, 200 m, 15 Mar 1986, *E. Forero 10231* (COL), San Juan de Arama, Alrededores de La Macarena, Sabanas de San Juan de Arama, Hacienda Los Micos, 500 m, 26 Apr 1957, *J. Idrobo 2620* (COL, MO); Vista Hermosa, Reserva Nacional de La Macarena, 18 Jan 1959, *R. Jaramillo 1289* (COL, HUA), Llanos orientales; entre Puerto Gaitán y Puerto Lopez, caño de La Emma, afluente del Rio Yacao, 15 Feb 1976, *R. Jaramillo 5128* (COL); Entre Puerto Gaitán y Puerto López, caño de la Emma, afluente del río Yacao, 15 Feb 1976, *R. Jaramillo 5129* (COL), Llanos orientales; caño Camoa al Oriente de San Martín, 23 Apr 1983, *R. Jaramillo 7851* (COL), Villavicencio, about 20 Km southeast of Villavicencio, 500 m, 17 Mar 1939, *E. Killip 34300* (COL, F, US); Puerto Lopez, 240 m, 29 Jul 1944, *E. Little, Jr. 8327* (COL, US). San Martín de los Llanos, 5 miles [ca. 8 km] north of San Martín on road to Villavicencio, 579 m, 14 Sep 1974, *T. Plowman 4199* (COL, F); Villavicencio, 579 m, 14 Sep 1974, *T. Plowman 4200* (COL, F); Northeast corner of Sierra de la Macarena, 426 m, 15 Sep 1974, *T. Plowman 4216* (COL, F, US); Northeast corner of Sierra de la Macarena, 15 Sep 1974, *T. Plowman 4245* (COL); Noreste de la Sierra de la Macarena, Hacienda Los Micos, El

Tablazo, 15 Sep 1974, *T. Plowman 4264* (COL); 731 m, 15 Sep 1974, *T. Plowman 4266* (COL, F, US); Carretera de la Serranía entre San Martín y Puerto López. 5 Mar 1987, *L. Quiñones 998* (COL); Granada, 26 Jul 1994, Rincón, R. 275 (COL); Margen derecha de caño Curía, bosque de galería muy degradado, Reserva La Macarena, 06 Jan 1987, *D. Rivera 1326* (COL); Vichada: Cumaribo, Varavaca, 23 Mar 1973, *I. Cabrera 2808* (COL). **Venezuela.** —Amazonas: Atures, road northeast from Puerto Ayacucho toward El Burro, 28 Apr 1984, *T. Plowman 13757* (COL).

Key for the Closely Related Species of *Erythroxylum savannarum*

1. Calyx lobes sub-foliose, widely ovate, or oblong 2
   2. Cortex suberous. Stipule 3–5 mm long. Apex of the leaves rounded ..... *E. suberosum* A.St.-Hil.
   2. Cortex not suberous. Stipules longer. Apex of the leaves rounded, truncate, or emarginated 3
      3. Stipules persistent. Leaves to 12 cm long. Apex of the leaves acute, acuminate, obtuse, or rounded 4
         4. Secondary venation evident below. Calyx lobes overlapping and fused to ca. ½ the length. Bolivia. ..... *E. pauciflorum* Rusby
         4. Secondary venation not evident below. Calyx lobes fused at the base but not overlapping. Northwestern Brazil. . *E. rimosum* O.E.Schulz
      3. Stipules deciduous. Leaves usually more than 12 cm long, except for some populations of *E. macrophyllum* from Central America. 5
         5. Small shrub to 1.5 m. Leaves whitish beneath. Western Brazil to Bolivia. ..... *E. durum* S.Moore
         5. Tree or treelet, more than 1.5 m. Leaves green beneath. Widely distributed in the Neotropics. ..... *E. macrophyllum* Cav.
1. Calyx lobes triangular. 6
   6. Stipules 12–40 mm long, margins ragged, almost amplexicaulous. Leaves up to 13 cm long. ..... *E. mucronatum* Benth.
   6. Stipules shorter, 2.5–20 mm long., margin entire, not amplexicaulous. Leaves up to 24 cm long 7
      7. Cataphylls distichous, imbricate, grouped at the base of short branches; stipules persistent. 8
         8. Shrub to 3 m high. Pedicels 4–7 mm long. Drupe 10–11 mm long. ..... *E. daphnites* Mart.
         8. Treelet or tree up to 10 m height. Pedicels 6.7–10 mm long. Drupe 4.9–8.8 mm long .... *E. savannarum* (Plowman) Jara & D.M.White
      7. Cataphylls rarely grouped, when grouped then polystichous; stipules deciduous or persistent 9
         9. Stipule apex obtuse 10
            10. Pedicel gradually increasing in thickness. Leaves coriaceous, usually obovate, with secondary venation of leaves not obvious beneath. Drupe 12–16 mm long. ..... *E. amazonicum* Peyr.
            10. Pedicel almost the same thickness. Leaves chartaceous, usually elliptic, with secondary venation obvious beneath. Drupe 9–10 mm long ..... *E. citrifolium* A.St.-Hil.
         9. Stipule apex acute 11
            11. Stipules persistent. Leaves with impressed secondary venation above, apex acuminate. ..... *E. acuminatum* Ruíz & Pav.
            11. Stipules deciduous or persistent. Leaves without impressed secondary venation above, apex rounded, obtuse, emarginate, acute, or rarely short-acuminate 12
               12. Stipules longer than the petioles, persistent. Leaves less than 16 cm long, with secondary venation obscure below. ..... *E. campestre* A.St.-Hil.
               12. Stipules shorter than the petioles, deciduous. Leaves more than 16 cm long, with secondary venation evident below. ..... *E. amplum* Benth.

Acknowledgments

We thank the curators and collection managers of the following herbaria CAUP, CHOCO, COL, CUVC, F, HUA, HUQ, JAUM, MEDEL, MO, NY, UIS, and US for permitting the examination of *Erythroxylum* specimens. DMW is supported by a National Science Foundation Fellowship (DBI 2010821). Computational resources were provided by the Grainger Bioinformatics Center at The Field Museum of Natural History. Finally, we thank M. F. Wojciechowski and two anonymous reviewers for their constructive comments.

Author Contributions

OAJ and ORD provided the morphological and anatomical data. DMW provided data and molecular analysis. OAJ, DMW, and ORD contributed to the redaction of the paper.

Literature Cited

Andrews, S. 2010. FastQC: A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Bachman, S., J. Moat, A. W. Hill, J. De La Torre, and B. Scott. 2011. Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126.

Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Lesin, S. I. Nikolenko, S. Pham, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi, G. Tesler, M. A. Alekseyev, and P. A. Pevzner. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19: 455–477.

Barthlott, W., C. Neinhuis, D. Cutler, F. Ditsch, I. Meusel, I. Theisen, and H. Wilhelmi. 1998. Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society* 126: 237–260.

Baum, D. A. 2009. Species as ranked taxa. *Systematic Biology* 58: 74–86.

Baum, D. A. and M. J. Donoghue. 1995. Choosing among alternative "phylogenetic" species concepts. *Systematic Botany* 20: 560–573.

Becerra, N., E. Barrera, and X. Marquinez. 2002. *Anatomía y Morfología de los Órganos Vegetativos de las Plantas Vasculares*. Bogotá, Colombia: Unibiblos.

Bieras, A. C. and M. G. Sajo. 2004. Anatomia foliar de *Erythroxylum* P. Browne (Erythroxylaceae) do cerrado do estado de São Paulo, Brasil. *Acta Botanica Brasílica* 18: 601–612.

Bushnell, B. 2015. BBMap. Version 37.75. https://sourceforge.net/projects/bbmap/.

Capella-Gutiérrez, S., J. M. Silla-Martínez, and T. Gabaldón. 2009. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25: 1972–1973.

Daly, D. C. 2004. Erythroxylaceae (Coca Family). Pp 143–145 in *Flowering Plants of the Neotropics*, eds. N. Smith, S. A. Mori, A. Henderson, D. W. Stevenson, and S. V. Heald. Princeton: Princeton University Press.

De Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation. Pp. 57–75 in *Endless Forms: Species and Speciation*, eds. D. J. Howard and S. H. Berlocher. New York: Oxford University Press.

Dillehay, T. D., J. Rossen, D. Ugent, A. Karathanasis, V. Vásquez, and C. P. J. Netherly. 2010. Early Holocene coca chewing in northern Peru. *Antiquity* 84: 939–953.

Emche, S. D., D. Zhang, M. B. Islam, B. A. Bailey, and L. W. Meinhardt. 2011. AFLP phylogeny of 36 *Erythroxylum* species: Genetic relationships among *Erythroxylum* species inferred by AFLP analysis. *Tropical Plant Biology* 4: 126–133.

Font Quer, P. 2000. *Diccionario de Botánica.* Barcelona: Ediciones Península.

Glenn, T. C., R. A. Nilsen, T. J. Kieran, J. G. Sanders, N. J. Bayona-Vásquez, J. W. Finger, T. W. Pierson, K. E. Bentley, S. L. Hoffberg, S. Louha, F. J. Garcia-De Leon, M. A. del Rio Portilla, K. D. Reed, J. L. Anderson, J. K. Meece, S. E. Aggrey, R. Rekaya, M. Alabady, M. Belanger, K. Winker, and B. C. Faircloth. 2019. Adapterama I: Universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorically-indexed Illumina libraries (iTru & iNext). *PeerJ* 7: e7755.

Hornak, K. A. 2011. *Dictionary of Botany: English-Spanish/Spanish-English = Diccionario de Botánica: Inglés-Castellano/Castellano-Inglés.* Lansdowne, Pennsylvania: Castilla La Vieja.

Islam, M. B. 2011. *Tracing the Evolutionary History of Coca* (Erythroxylum). Ph.D. dissertation. Boulder, Colorado: University of Colorado.

IUCN. 2019. Guidelines for using the IUCN Red List categories and criteria. Version 14. Prepared by the Standards and Petitions Committee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Jara-Muñoz, O. A. 2011. El complejo *Erythroxylum macrophyllum* (Erythroxylaceae): Delimitación taxonómica y posición filogenética. M.S. thesis. Bogotá: Universidad Nacional de Colombia.

Jara-Muñoz, O. A., D. M. White, and O. Rivera-Díaz. 2022. Data from: Morphological and Molecular Evidence Support Elevating *Erythroxylum macrophyllum* var. *savannarum* (Erythroxylaceae) to Specific Status. Dryad Digital Repository. https://doi.org/10.5061/dryad.1ns1rn8wn.

Johnson, M. G., E. M. Gardner, Y. Liu, R. Medina, B. Goffinet, A. J. Shaw, N. J. C. Zerega, and N. J. Wickett. 2016. HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Applications in Plant Sciences* 4: 1600016.

Jørgensen, P. M., M. H. Nee, and S. G. Beck. 2014. Catálogo de las plantas vasculares de Bolivia. *Monographs in Systematic Botany from the Missouri Botanical Garden* 127: 1–1744.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

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.

Lavelle, P., N. Rodríguez, O. Arguello, J. Bernal, C. Botero, P. Chaparro, Y. Gómez, A. Gutiérrez, M. P. Hurtado, S. Loaiza, S. X. Pullido, E. Rodríguez, C. Sanabria, E. Velásquez, and S. J. Fonte. 2014. Soil ecosystem services and land use in the rapidly changing Orinoco River Basin of Colombia. *Agriculture, Ecosystems & Environment* 185: 106–117.

Li, H. and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25: 1754–1760.

Loiola, M. I. B. 2001. *Revisão taxonômica de* Erythroxylum *P. Browne sect.* Rhabdophyllum *O. E. Schulz (Erythroxylaceae Kunth)*. Ph.D. dissertation. Recife, Brazil: Universidade Federal Rural de Pernambuco.

Minh, B. Q., M. W. Hahn, and R. Lanfear. 2020a. New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution* 37: 2727–2733.

Minh, B. Q., H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. von Haeseler, and R. Lanfear. 2020b. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534.

Oviedo, R. P. 2002. *Contribución al Conocimiento de Erythroxylaceae Kunth en Cuba y las Antillas*. Thesis. La Habana, Cuba: Universidad de La Habana.

Plowman, T. 1984. New taxa of *Erythroxylum* (Erythroxylaceae) from the Amazon basin. *Acta Amazonica* 14: 117–144.

Plowman, T. 1986. Four new species of *Erythroxylum* (Erythroxylaceae) from Northeastern Brazil. *Brittonia* 38: 189–200.

Plowman, T. 1988b. New species and a new combination of *Erythroxylum* (Erythroxylaceae) from Amazonian Peru: Contribution to the study of the flora and vegetation of Peruvian Amazonia XIV. *Candollea* 43: 421–430.

Plowman, T. 1988a. New taxa of *Erythroxylum* (Erythroxylaceae) from the Venezuelan Guayana. *Brittonia* 40: 256–268.

Plowman, T. and N. Hensold. 2004. Names, types, and distribution of neotropical species of *Erythroxylum* (Erythroxylaceae). *Brittonia* 56: 1–53.

Restrepo, D. A., E. Saenz, O. A. Jara-Muñoz, I. F. Calixto-Botía, S. Rodríguez-Suárez, P. Zuleta, B. G. Chavez, J. A. Sanchez, and J. C. D'Auria. 2019. Erythroxylum in focus: An interdisciplinary review of an overlooked genus. *Molecules* 24: 3788.

Romero-Ruiz, M. H., S. G. A. Flantua, K. Tansey, and J. C. Berrio. 2012. Landscape transformations in savannas of northern South America: Land use/cover changes since 1987 in the Llanos Orientales of Colombia. *Applied Geography (Sevenoaks, England)* 32: 766–776.

Rury, P. M. 1982. *Systematic Anatomy of the Erythroxylaceae.* Ph.D. dissertation. Chapel Hill: University of North Carolina.

Schulz, O. E. 1907. Erythroxylaceae. Pp. 1–176 in *Pflanzenreich*, vol. IV, ed. H. G. A. Engler. Leipzig: Verlag von Wilhelm Engelmann.

Schulz, O. E. 1931. Erythroxylaceae. Pp. 227–658 in *Die Natürlichen Pflanzenfamilien*, eds. A. Engler and K. Prantl. Leipzig: Verlag von Wilhelm Engelmann.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Stolberg, V. B. 2011. The use of coca: Prehistory, history, and ethnography. *Journal of Ethnicity in Substance Abuse* 10: 126–14.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 7: 57–86.

White, D. M. 2019. *Biogeography, Diversification, and Domestication in the Coca Family (Erythroxylaceae)*. Ph.D. dissertation. Chicago: University of Illinois.

White, D. M. 2020. A new variety of *Erythroxylum ulei* from the Cordillera Escalera of Peru (*Archerythroxylum*, Erythroxylaceae). *Phytotaxa* 449: 279–286.

White, D. M., M. B. Islam, and R. J. Mason-Gamer. 2019. Phylogenetic inference in section *Archerythroxylum* informs taxonomy, biogeography, and the domestication of coca (*Erythroxylum* species). *American Journal of Botany* 106: 154–165.

White, D. M., J.-P. Huang, O. A. Jara-Muñoz, S. Madriñán, R. H. Ree, and R. J. Mason-Gamer. 2021. The origins of coca: Museum genomics reveals multiple independent domestications from progenitor *Erythroxylum gracilipes*. *Systematic Biology* 70: 1–13.

Yang, Z. 1993. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* 10: 1396–1401.

Zhang, C., M. Rabiee, E. Sayyari, and S. Mirarab. 2018. ASTRAL-III: Polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* 19: 153.

Appendix 1. Voucher information of the specimens studied for morphological and anatomical observations.

***Erythroxylum campestre* Peyr**. *L. Williams 8065* (COL), *S. Jung 95* (COL), *E. Forero 8225* (COL), *E. Forero 8346* (COL). ***Erythroxylum citrifolium* A.St-Hil**. *Kejakai 850* (COL), *Graville 4660* (COL). ***Erythroxylum daphnites* Mart.**, *S. Altamirano 4102* (MO), *T. Killen 6044* (MO). *A. Carrión 343* (MO), *H. Irwin 17150* (MO). ***Erythroxylum durum* S.Moore**, *R. Quevedo 1064* (MO), *R. Foster 13702* (F), *C. Jordán 162* (MO), *M. Saldias 3350* (MO), *C. Cid 6247* (NY), *G. Hatschbach 65439* (F), *M. Silva 3376* (NY), *M. Vieira 980* (NY). ***Erythroxylum macrophyllum* Cav., Belize.** *P. Gentle 5333* (F), *W. Schipp* (MO, NY), *W. Schipp 1345* (NY), *P. Gentle 6016* (MO, US), *M. Nee 46946* (NY), *Peck, M. 831* (NY). **Bolivia.** *R. Quevedo 1064* (MO), *T. Killen 3515* (MO), *G. Prance 8425* (COL, US), *M. Nee 31854* (NY), *M. Saldias 3350* (MO), *J. Steinbach 1568* (NY), *M. Nee 50370* (NY), *M. Nee 39665* (NY), *M. Nee 39699* (NY), *C. Jordán 162* (MO). **Brazil.** *S. Lowrie 375* (NY, US), *B. Krukoff 5526* (NY, US), *B. Krukoff 5555* (NY, US), *B. Krukoff 5748* (NY, US), *B. Krukoff 5385* (NY, US), *C. Cid 5185* (NY, US), *G. Prance 2915* (US), *C. Cid 5081* (US), *D. Daly 11032* (MO, NY), *C. Cid 2898* (NY, US), *G. Prance 7561* (US), *E. Poeppig 2742* (US), *L. Alencar 91* (US), *G. Prance 25591* (US), *G. Prance 22980* (NY, US), *G. Prance 3220* (MO), *G. Prance 24246* (NY, US), *G. Prance 15940* (COL, NY, US), *O. Nascimento 137* (MO), *C. Cid 8541* (NY), *G. Prance 2455* (US), *G. Prance 3219* (NY, US), *B. Krukoff 6851* (NY, US), *B. Krukoff 6247* (US), *G. Prance 22696* (COL, MO, US), *E. Killip 30112* (NY, US), *G. Prance 28156* (US), *M. Nee 42501* (NY, US), *G. Prance 3773* (US), *G. Prance 11425* (NY, US), *W. Rodrigues 2550* (F), *F. Mello s.n.* (F), *G. Prance 22897* (US), *B. Krukoff 9014* (NY), *G. Prance 8241* (COL, NY, US), *R. De Lemos 20817* (US), *J. Murça 589* (US), *T. Plowman 12424* (US), *C. Cid 3291* (US), *I. Amaral 737* (US), *G. Prance 8152* (NY, US), *W. Thomas 4658* (US), *C. Cid 6247* (NY), *D. Daly 1129* (NY, US), *C. Sperling 6086* (US), *D. Daily 816* (US), *J. Pires 3950* (US), *J. Pires 8132* (US), *G. Black 50* (US), *J. Pires 2648* (US), *J. Murça 16* (US), *A. Ducke 964* (MO, US), *E. Pareira 2552* (US), *E. Pereira 3252* (F), *A. Silva 151* (US), *W. Andrew 8236* (US), *G. Prance 58719* (US), *G. Prance 26430* (MO, US), *C. Cid 1620* (US), *M. Silva 3998* (F), *R. De Lemos 20595* (US), *T. Plowman 9909* (US), *G. Prance 58719* (COL, MO), *G. Prance 5186* (US), *M. Vieira 980* (US). **Colombia.** *J. Mutis 2399* (US), *R. López 4434* (COAH), *P. Toro 761* (COAH), *T. Plowman 6355* (F), *J. Pipoly 15784* (FMB, UIS), *A. Rudas 4048* (UIS), *A. Rudas 1327* (FMB, MO, UIS), *A. Rudas 1278* (FMB, MO, UIS), *A. Rudas 4049* (FMB, HUA), *A. Posada 2836* (COAH), *D. Cárdenas 10820* (COAH), *P. Toro 749* (COAH), *J. Shepherd 916* (HUA), *J. Shepherd 843* (COL, HUA, MO), *R. Callejas 8697* (F, HUA), *R. Callejas 8731* (F, HUA), *R. Callejas 4484* (F, HUA, MO), *R. Callejas 8173* (F, HUA), *T. Espinal 4787* (MEDEL), *A. Gentry 78914* (F), *R. Fonegra 1765* (F, HUA, MO), *R. Fonegra 8550* (HUA), *A. Cogollo 211* (MO), *A. Cogollo 4957* (COL, MO), *L. E. Vera 483* (MEDEL), *J. Ramirez 174* (COL, HUA, MO), *E. Rentería 2702* (MO), *A. Cogollo 4496* (MO), *M. Celis 235* (COL), *H. Cuadros 2189* (MO, US), *J. L. Fernandez-Alonso 24094* (COL, MO), *M. Balcázar 371* (COL), *J. Betancur 7783* (COL), *J. L. Fernandez-Alonso 22933* (COL), *J. L. Fernandez-Alonso 23562* (COL), *J. L. Fernandez-Alonso 24750* (COL), *J. Betancur 9080* (COL), *T. Arias 102* (HUA), *M. Correa 2228* (HUA), *R.*

Bernal 2915 (COL, HUA), *H. David 1145* (HUA), *J. Duivenvoorden 2439* (COAH), *M. Sánchez 923* (COAH), *P. Palacios 2898* (MO), *J. Phillips 266* (COAH), *H. Hernández 660* (COL), *N. Castaño 1676* (COAH, COL), *H. Bernal 530* (COL), *H. Bernal 501* (COL), *G. Lozano 7088* (COL), *G. Lozano 5254* (COL, FMB, MO), *L. Rubiano 620* (COL), *J. L. Fernandez-Alonso 13857* (COL), *H. García-Barriga 20576* (COL, US), *J. Duke 15389* (MO, US), *A. Gentry 20235* (COL, MO), *A. Gentry 20235* (COL, F, MO), *F. García 1208* (FMB), *F. García 1416* (FMB), *J. Espina 3698* (F), *J. Espina 3875* (FMB), *J. Espina 2003* (COL, F), *E. Forero 5309* (COL, F), *E. Forero 4574* (COL, F, HUA), *E. Forero 2773* (COL), *N. Bastidas 04* (CHOCO), *F. Cuesta 02* (CHOCO, HUA), *L. Forero 216* (MO), *S. Diaz-Piedrahita 3569* (COL), *H. García-Barriga 12334* (COL, US), *H. García-Barriga 11736* (COL, US), *H. García-Barriga 12307* (COL, US), *E. Montenegro 64* (COL), *J. García 456* (COL), *J. Betancur 9667* (COL), *J. Rangel-Ch. 3242* (COL), *M. Cordoba 387* (COL), *E. Carbonó 380* (COL), *Jr. Kirkbride 2045* (COL, US), *D. Restrepo 1282* (COAH), *T. Plowman 136231* (COL), *R. Cortés 1863* (COAH, HUA, UDBC), *J. Cuatrecasas 4576* (F, US), *J. Cuatrecasas 13207* (COL, F, US), *M. Balick 24* (COL), *J. García 81* (COL), *J. Betancur 11379* (COL), *R. Galindo 854* (UIS), *L. Forero 1847* (CUVC), *L. Mora-Osejo 1050* (COL, PSO), *R. Fonegra 8115* (HUA), *J. Betancur 10941* (COL, HUA), *H. García-Barriga 8406* (COL, US), *M. Koie 5125* (US), *R. Sanchez 3309* (COL), *H. García-Barriga 12032* (COL, F), *J. Triana 5597* (COL), *E. Echeverry 1309* (COL), *H. García-Barriga 18750* (COL, US), *J. Idrobo 11044* (COL), *J. Idrobo 11009* (COL), *A. Gentry 48002* (F, MO, CUVC), *F. Silva 15* (CUVC), *D. Faber-Langendoen 1277* (MO), *A. Gentry 40228* (COL, MO), *H. Cuadros 960* (MO), *H. Cuadros 899* (MO), *J. Cuatrecasas 21329* (COL, F, US, VALLE), *L. Clavijo 512* (COAH), *L. Clavijo 215* (COL), *J. Mutis 2386* (US), **Costa Rica.** *O. Brenes 14393* (US), *B. Hammel 19800* (NY), *A. Smith 1881* (MO), *A. Brenes 13658* (NY), *A. Carvajal 233* (MO, NY), *J. Gomez 9755* (F), *A. Brenes 5476* (NY), *A. Brenes 5453* (F, NY), *I. Chacón 1667* (NY, US), *R. Lent 2380* (NY), *A. Brenes 4460* (NY), *A. Brenes 4905* (NY), *A. Brenes 17020* (NY), *A. Brenes 6816a* (NY), *A. Brenes 6839* (NY), *A. Brenes 3657* (NY), *P. Standley 44515* (US), *R. Espinoza 1241* (NY), *L. Acosta 1003* (NY), *G. Rivera 1112* (F), *G. River 1011* (MO, NY), *J. Chaves 410* (NY), *R. Wilbur 16655* (MO), *G. Hartshrorn 1198* (F), *B. Hammel 16595* (MO), *F. Almeda 7168* (NY), *S. Koptur 133* (F), *W. Haber 1379* (NY), *W. Burger 10578* (NY), *A. Skutch 5023* (F, HUA, US). **Ecuador.** *G. Tipaz 1862* (COL, MO), *W. Palacios 5189* (COL, MO), *G. Villa 850* (US), *E. Little, Jr. 530* (COL, US), *L. Suin 1534* (COL, MO), *C. Cerón 19083* (COL, MO), *M. Aulestia 1796* (COL, MO), *H. Baslev 60542* (US), *W. Palacios 4662* (US), *C. Cerón 2912* (MO, US), *H. Vargas 1143* (COL, MO), *B. Ollgaard 38857* (US), *W. Palacios 2277* (US), *H. Lugo 2368* (US), *M. Aulestia 3050* (COL), *M. Aulestia 3523* (COL, MO), *M. Aulestia 2088* (COL, MO), *M. Aulestia 1872* (COL, MO), *W. Palacios 5593* (COL, MO), *D. Neill 7650* (MO), *H. Lugo 2343* (MO), *W. Palacios 6053* (COL, MO), *W. Palacios 12835* (COL, MO), *A. Dik 720* (COL, MO), *M. Aulestia 1711* (COL, MO), *Y. Mexia 7300* (US), *M. Nee 31406* (US), *C. Cerón 20767* (COL, MO), *W. Palacios 6884* (COL, MO). **Guatemala.** *E. Contreras 4457* (F), *J. Steyermark 44553* (F, US), *J. Steyermark 38213* (NY), *C. Lundell 19360* (MO, US), *P. Standley 73076* (F), *E. Contreras 10833* (MO, US), *G. Jones 3216* (NY, US), *G. Jones 3166* (NY, US), *R. Tún 778* (NY), *E. Contreras 6701* (F). **Guyana.** *M. Jansen-Jacobs 32160* (F), *T. McDowell 4176* (HUA, US), *J. Pipoly 8083* (COL, US), *L. Gillespie 2073* (COL, US), *J. Pipoly 8083* (US), *J. Pipoly 8025* (US), *P. Mutchnick 1030* (US), *P. Mutchnick 1104* (US), *N. Sandwith 571* (US), *M. Jansen-Jacobs 821* (MO), *D. Clarke 4067* (US), *L. Gillespie 1599* (US), *L. Gillespie 2048* (MO, US), *B. Maguire 45992* (MO), *M. Polak 502* (US), *M. Jansen-Jacobs 4225* (US), *N. Laug 101* (US), **French Guyana.** *G. Wachenheim 441* (US), *C. Leg 4022* (US), *S. Mori 23988* (US), *S. Mori 22975* (US), *S. Mori 22753* (US). **Honduras.** *C. Nelson 2737* (MO), *C. Nelson 4768* (F). **Mexico.** *T. Croat 40624* (MO), *D. Breedlove 25194* (NY), *D. Breedlove 15134* (NY), *E. Martínez 20693* (MO, NY), *E. Martínez 19868* (NY), *E. Matuda 1816* (NY), *D. Breedlove 10342* (F), *R. Cedillo 1373* (MO), *G. Marínez-Calderón 153* (US), *D. Lorence 4059* (MO), *H. Hernández 989* (MO), *C. Cowan 3116* (NY), *J. Panero 5760* (CUVC), *M. Nee 29948* (NY), *M. Nee 22675* (NY), *M. Nee 25077* (NY), *M. Nee 24726* (NY), *Liebmann 791C* (US). **Nicaragua.** *P. Moreno 20182* (MO, NY), *P. Moreno 10240* (F), *P. Moreno 21953* (NY), *P. Moreno 25400* (NY), *D. Neill 4112* (MO), *D. Stevens 8438* (MO), *J. Pipoly 3924* (MO), *E. Little 25434* (F), *A. Molina 14997* (NY), *A. Molina 14919* (NY, US), *A. Molina 15143* (NY). **Panama.** *H. Wedel 2405* (MO, US), H. Churchill 4601 (F), *P. Cooper 494* (NY), *A. Gentry 2801* (MO, NY), *B. Hammel 6249* (NY), *M. Nee 10681* (F, NY), *M. Davidson 629* (US), *B. Hammel 14414* (F), *R. Woodson 1105* (NY), *G. McPherson 8726* (F), *J. Kirkbride, Jr. 869* (MO), *G. McPherson 9113* (MO), *G. McPheterson 9074* (COL, NY, US), *B. Hammel 1769* (MO), *R. Williams 543* (NY), *R. Woodson 1305* (NY), *R. Wilbur 11758* (NY), *P. Allen 1772* (NY, US), *P. Allen 1802* (NY, US), *S. Castroviejo 14720* (COL), *S. Castroviejo 13051* (COL), *J. Cuadras 8053* (COL), *S. Castroviejo 13425* (COL), *S. Castroviejo 7113* (COL), *S. Castroviejo 8151* (COL), *M. Ballesteros 1688* (COL), *M. Velayos 8210* (COL), *S. Knapp 4451* (F), *P. Standley 30218* (NY, US), *R. Foster 2223* (F, US), *T. Antonio 3887* (NY), *J. Duke 14556* (F), *E. Tyson 3960* (MO), *A. Gentry 5815* (MO, NY, US), *R. Liesner 701* (NY, US), *J. Miller 954* (F), *P. Allen 2447* (MO), *D. Porter 4304* (NY), *J. Folsom 2549* (NY), *S. Mori 5144* (MO), *A. Gentry 5079* (NY), *D. LeDoux 2628* (MO, NY), *G. Nevers 7565* (F), *G. Barclay 2822* (NY, US), *C. Galdames 2001* (F), *T. Antonio 2394* (NY). **Peru.** *R. Vásquez 24786* (MO). *S. Tunqui 493* (MO), *P. Acevedo 9288* (US), *P. Nuñez 23807* (US), *A. Weberbauer 6962* (US), *J. Schunke 5797* (US), *J. Schunke 11228* (US), *J. Schunke 6580* (US), *M. Mathias 5946* (US), *J. Schunke 835* (COL, US), *J. Schunke 2905* (COL, US), *J. Schunke 2198* (COL, US), *T. Plowman 5896* (F), *R. Ferreyra 4935* (US), *R. Ferreyra 4960* (US), *S. McDaniel 2584* (US), *L. Williams 4685* (US), *S. McDaniel 16831* (US), *G. Klug 1144* (US), *M. Rimachi 8571* (US), *R. Ferreyra 4934* (US), *T. Croat 20191* (MO), *R. Vásquez 7938* (US), *T. Plowman 7130* (US), *J. Revilla 107* (MO), *D. Daly 5805* (US), *G. Klug 3185* (MO, US), *Spichiger s.n.* (US), *G. Prance 24181* (US), *T. Plowman 2595* (US), *L. Angulo 7* (MO), *E. Killip 27540* (US), *L. Williams 3196* (US), *R. Vasquez 15578* (COL, MO), *R. Vasquez 18209* (COL), *R. Vasquez 17657* (COL, MO), *J. Schunke 2613* (COL, US), *R. Vasquez 18242* (F), *S. McDaniel 13635* (F), *A. Gentry 25011* (F), *C. Díaz 9205* (COL, MO), *M. Aguilar 1029* (COL, MO), *R. Vásquez 18242* (MO), *D. Simson 728* (COL, US), *H. van der Werff 18001* (COL, MO), *A. Monteagudo 5289* (COL, MO), *S. Vilca 366* (COL, MO), *R. Vásquez 29878* (COL, MO), *A. Monteagudo 5394* (COL, MO), *R. Foster 8980* (COL, MO), *L. Williams 5635* (US), *S. Knapp 8548* (COL, US), *S. Knapp 8248* (US), *S. Knapp 7175* (MO), *G. Klug 2738* (US), *T. Plowman 5803* (US), *T. Plowman 5796* (US), *M. Mathias 5975* (US), *S. Knapp 8558* (US), *C. Belshaw 3355* (MO, US), *A. Gentry 45551* (MO), *J. Schunke 3932* (COL). **Surinam.** *V. Westgoff 7096* (US), *B. Hammel 21533* (US), *L. Elburg 9431* (COL), *R. Evans 2434* (COL, MO, US), *M. Jansen-Jacobs 6242* (US). **Venezuela.** *L. Williams 13238* (US), *A. Gentry 46683* (US), *R. Liesner 15834* (US), *G. Davidse 27330* (US), *R. Cowan 31451* (US), *G. Davidse 12462* (MO), *J. Steyermark 89871* (US), *F. Breteler 3705* (US), *F. Breteler 3525* (MO, US), *J. Steyermark 86245* (US), *J. Steyermark 90012* (US), *G. Davidse 13721* (COL), *F. Ortega 1704* (F), *L. Aristeguieta 4786* (US), *G. Bunting 13575* (US), *L. Valverde 1074* (MO), *H. van der Werff 5404* (MO), *J. Steyermark 100345* (F), *E. Tejera 180* (US), *G. Bunting 8889* (F), *G. Bunting 6779* (F). ***Erythroxylum pauciflorum*** **Peyr.**, *L. Cayola 2123* (COL). ***Erythroxylum suberosum*** **A.St.-Hil., Brazil.** *A. Ducke 1321* (US), *J. dos Santos 282* (COL), *P. Heriger 81* (COL), *S. Mori 15706* (US), *G. Prance 3404* (US), *R. Harley 15597* (US), *P. Silva s.n.* (US), *T. Plowman 12702* (US), *L. Cobra 159* (US), *E. Heringer 5646* (US), *L. Fielder 65* (US), *E. Heringer 18272* (US), *E. Heringer 18278* (US), *E. Heringer 18226* (US), *A. Enilton 68* (US), *B. Pereira 45* (US), *Kirkbride Jr. 15* (US), *Kirkbride Jr. 3601* (US), *L. Queiroz 260* (US), *T. Plowman 9186* (US), *P. Ezechias 14084* (US), *T. Plowman 9137* (US), *G. Eiten 10032* (US), *F. Oliveira 854* (US), *T. Plowman 8208* (US), *T. Plowman 8396* (US), *G. Schartz 1005* (US), *T. Plowman 9366* (US), *G. Eiten 10542* (US), *F. Segadas 1055* (US), *H. Irwin 28678* (US), *J. Pirani 8673* (US). *E. Heringer 3580* (US), *J. Evangelista 234* (US), *G. Eiten 6815* (US), *L. Williams 7432* (US), *M. Barreto 2230* (US), *L. Williams 7526* (US), *T. Plowman 9022* (US), *W. Davis 1850* (US), *J. Coelho 964* (US), *M. Vieira 652* (US), *J. Pruski 3431* (US), *I. Amaral 1442* (US), *S. Jung 74* (COL), *E. Forero 8218* (COL), *E. Forero 8468* (COL), *S. Jung 147* (COL), *E. Forero 8351* (COL). **Guyana.** *A. Smith A2264* (US), *M. Jansen-Jacobs 2086* (US), *T. McDowell 2156* (US), *H. Irwin 584* (US), *H. Clarke 2135* (US), *H. Clarke 2089* (US), *L. Gillespie 1795* (US). **Venezuela.** *F. Tamayo 2823* (US), *J. Steyermark 29938* (US).

APPENDIX 2. Voucher specimens used in phylogenetic analysis. Information is given in the following format: Species, voucher specimen, herbarium and catalogue number, GenBank SRA accession number.

Erythroxylum acuminatum **Ruiz & Pav.,** *D.M. White 509*, F2320297, SAMN12054919. ***Erythroxylum amazonicum*** **Peyr.**, *R. Vasquez 24660*, F2198813, SAMN09809311. ***Erythroxylum amplum*** **Benth.**, *A. Gentry 22350*, F1854023, SAMN12054925. ***Erythroxylum campestre*** **Peyr.**, *H.S. Irwin 18705*, MO3481417, SAMN12055082. *J.L. Costa-Lima 2566*, HUEFS292739, SAMN12054936. ***Erythroxylum citrifolium*** **A.St.-Hil.**, *J.L. Costa-Lima 2715*, HUEFS242887, SAMN12054945. *D.M. White 573*, F2324346, SAMN12054944. ***Erythroxylum daphnites*** **Mart.**, *J.L. Costa-Lima 2524*, HUEFS242697, SAMN12054959. ***Erythroxylum durum*** **S.Moore**, *Cid Ferreira 6247*, INPA137882, SAMN12054964.

***Erythroxylum macrophyllum* Cav.**, *S. Mori 21153*, F2033387, SAMN12055002. *J.C. Lindeman 277*, F1903555, SAMN12055001. *D.M. White 575*, F2324347, SAMN12054999. *E. Velasco-Sinaca 644*, MO6356781, SAMN12055000. ***Erythroxylum mucronatum* Benth**, *D.M. White 494*, F2320309, SAMN12055012. ***Erythroxylum pauciflorum* Rusby**, *L.E. Cayola Pérez 2129*, MO2909699, SAMN12055027. ***Erythroxylum rimosum* O.E.Schulz**, *J.L. Costa-Lima 646*, UPF78185, SAMN12055046. ***Erythroxylum savannarum* (Plowman) Jara & D.M.White**, *D.M. White 555*, F2324371, SAMN09809377. ***Erythroxylum suberosum* A.St.-Hil.**, *J.L. Costa-Lima 2713*, HUEFS242885, SAMN12055075. ***Erythroxylum suberosum* var. *denudatum* O.E.Schulz**, *W.R. Anderson 36306*, F1842063, SAMN12055076.