

Phylogenetic Position of the Monotypic Genus *Verbenoxylum* (Verbenaceae) and New Combination Under *Recordia*

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Abstract—In spite of the recent studies on the phylogeny of Verbenaceae, the position of the monotypic *Verbenoxylum*, endemic to the Atlantic rainforest in southeastern Brazil, remains unsolved. Molecular data were here analyzed to infer the phylogenetic placement of this genus; furthermore morphological data was studied in order to examine traits that support relationships among taxa. Sequences of the plastid regions of *ndhF* gene and *trnL-trnF* intergenic spacer were analyzed to conduct phylogenetic studies with maximum parsimony, maximum likelihood, and Bayesian inference. Morphological traits that had been traditionally used to distinguish tribes within Verbenaceae, as well as those employed to characterize *Verbenoxylum*, were examined. *Verbenoxylum* is nested within the tribe Duranteae, sister to *Recordia*, a monotypic genus endemic to Bolivia, a placement never reported before. The morphological traits analyzed prove not to be useful to distinguish tribes but are important at lower taxonomic levels. Based on the sister relationship and morphological similarities between the genera *Verbenoxylum* and *Recordia*, we propose the inclusion of *Verbenoxylum reitzii* into *Recordia*, forming the new combination *Recordia reitzii*.

Keywords—Bolivia, Brazil, character evolution, molecular phylogeny, *ndhF*, *trnL-trnF*.

Verbenaceae (Lamiales) comprises about 35 genera and 800 species of herbs, shrubs, trees, and lianas that can occur in a broad variety of habitats. The genera are distributed mostly in the New World, with only *Chascanum* E. Mey. and *Coelocarpum* Balf. f. exclusive to Africa and the Indian Ocean Rim, respectively (Atkins 2004; Marx et al. 2010). Most of the classification systems proposed for Verbenaceae were based on morphological characters (Schauer 1847; Briquet 1895; Junell 1934; Troncoso 1974; Sanders 2001; Atkins 2004), whereas Marx et al. (2010) proposed a classification based on molecular markers (Table 1).

Verbenoxylum Tronc., as currently circumscribed, has only one species, *Verbenoxylum reitzii* (Moldenke) Tronc., endemic to southeastern Brazil. It occurs in the southern limit of the Atlantic rainforest in the Brazilian states of Rio Grande do Sul and Santa Catarina (Troncoso 1974; Reitz et al. 1978, 1983; Sobral et al. 2006) from 10–550 m above sea level (Fig. 1; Appendix 1). *V. reitzii* is considered vulnerable to extinction in the List of threatened species of Rio Grande do Sul Brazilian state (SEMA 2003), and according to the classification of rarity proposed by Rabinowitz (1981), which is based on geographical distribution, habitat distribution, and local population size, this species would belong to form 7, the most restricted form of rarity (Caiafa and Martins 2010). There are few biological studies on this species (Troncoso 1971; von Poser et al. 1997; Bueno and Leonhardt 2011).

Verbenoxylum was first described by Moldenke (1949) under *Citharexylum* L. However, Troncoso (1971), based on flower characters (corolla tube, thecal orientation, style length, and stigma) and fruit type, segregated it from *Citharexylum* under the new genus *Verbenoxylum*. This genus has been traditionally placed within the tribe Citharexyleae Briquet (Troncoso 1974; Sanders 2001; Atkins 2004) because of its previous relation to *Citharexylum*. Troncoso (1974) included *Verbenoxylum* in Citharexyleae, which also comprised the genera *Baillonia* Bocq., *Citharexylum*, *Duranta* L., and *Rhaphithamnus* Miers. Sanders (2001) expanded this tribe including genera

Coelocarpum, *Rehdera* Moldenke, *Rhaphithamnus*, and *Recordia* Moldenke. This concept of Citharexyleae was later followed by Atkins (2004) with the exception of *Coelocarpum*, which was not assigned to any tribe. However, based on molecular evidence Marx et al. (2010) identified a clade they recognized as Citharexyleae, comprising *Citharexylum*, *Baillonia*, and *Rehdera*. Since *Verbenoxylum* was the only genus of Verbenaceae not represented in the molecular phylogeny of Verbenaceae (Marx et al. 2010), its phylogenetic placement remains uncertain.

We present here a phylogenetic analysis using the plastid regions *ndhF* and *trnL-trnF* for all Verbenaceae genera, and examine twelve morphological traits in an evolutionary context to answer the following questions: 1) What is the phylogenetic position of *V. reitzii* within Verbenaceae? 2) Does it belong to the tribe Citharexyleae as proposed in previous taxonomic treatments for the family? 3) Are the morphological characters used in traditional classifications, here evaluated, useful to delimit tribes or genera within Verbenaceae?

MATERIALS AND METHODS

Taxon and Gene Sampling—The plastid regions *ndhF* gene, *trnL* intron, and *trnL-trnF* intergenic spacer were sequenced for field collected *V. reitzii* and combined with sequences from a previous molecular phylogeny of Verbenaceae (Marx et al. 2010). Sampling in the species-rich tribes Lantaneae and Verbenae was reduced in relation to Marx et al. (2010). All Verbenaceae genera were included in this analysis, among a total of 79 species. The outgroup was composed of 18 species representing other families in Lamiales. Voucher information and GenBank accession numbers can be found in Appendix 2.

DNA Extraction, Amplification, and Sequencing—Total genomic DNA was extracted from silica-gel dried tissue using a modified Doyle and Doyle (1987) CTAB protocol. Amplification and sequencing were performed using protocols described in Olmstead et al. (2008, 2009) with primers listed in Olmstead and Sweere (1994) and Taberlet et al. (1991). The PCR products were purified by precipitation from a 20% polyethylene glycol solution and washed in 70% ethanol (Dunn and Blattner 1987).

Phylogenetic Analyses—Sequences were assembled and edited with Sequencher 4.5 (Gene Codes Corp., Ann Arbor, Michigan, U. S. A.) and manually aligned using Se-Al 2.0a11 (Rambaut 2002) or with MAFFT v.6

TABLE 1. Classification systems for Verbenaceae s. s. and characters that they used.

Authors	Schauer (1847)	Briquet (1895)	Junell (1934)	Troncoso (1974)	Sanders (2001)	Atkins (2004)	Marx et al. (2010)
Infrafamilial division	5 subtribes Casseliinae Durantinae Lantaniinae Petreinae Verbeninae	5 subfamilies Citharexyleae Euverbeneae Lantaneae Monochileae Petreeae Priveae	6 tribes Casseliae Citharexyleae Lantaneae Petreeae Priveae Verbeneae	7 tribes Casseliae Citharexyleae Lantaneae Parodiantheae Petreeae Priveae Verbeneae	4 tribes Citharexyleae Lantaneae Petreeae Verbeneae	6 tribes Casseliae Citharexyleae Lantaneae Petreeae Priveae Verbeneae	8 tribes Casseliae Citharexyleae Duranteae Lantaneae Neospartaneae Petreeae Priveae Verbeneae
Characters	Calyx and corolla Inflorescence Fruit	Inflorescence Gynoecium	Gynoecium	Gynoecium Fruit	Calyx and corolla Inflorescence Anther and connective Staminode Style Carpel number Fruit	Inflorescence Anther and connective Staminode Style Carpel number Fruit	Seven DNA markers

(<http://mafft.cbrc.jp/alignment/server/>) followed by manual adjustments. Nucleotide composition and variable sites were estimated using Mega 5 (Tamura et al. 2011).

Gaps from the *ndhF* and *trnL-trnF* sequences were coded as binary characters (Graham et al. 2000; Simmons and Ochoterena 2000) using

GapCoder (Young and Healy 2003) and combined into a single dataset with the plastid regions in all analyses.

Maximum parsimony (MP) analyses were conducted in PAUP* 4.0b10 (Swofford 2002). Heuristic searches were performed with 1,000 replicates of random sequence additions, maximum of 10 trees saved per replicate,

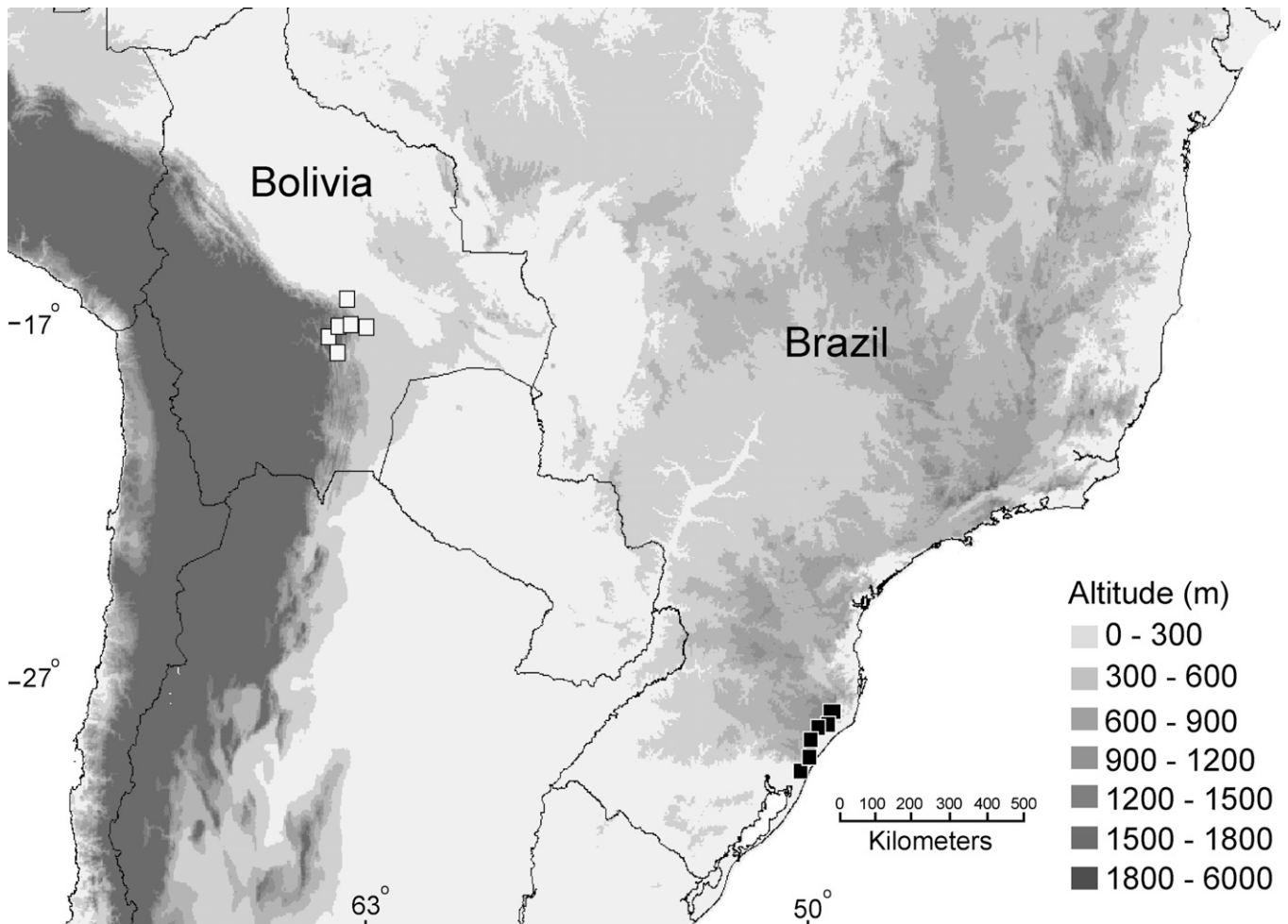


FIG. 1. Map of South America showing known localities of distribution of *Verbenoxylum reitzii* (black squares) and *Recordia boliviana* (white squares). Voucher information can be found in Appendix 1.

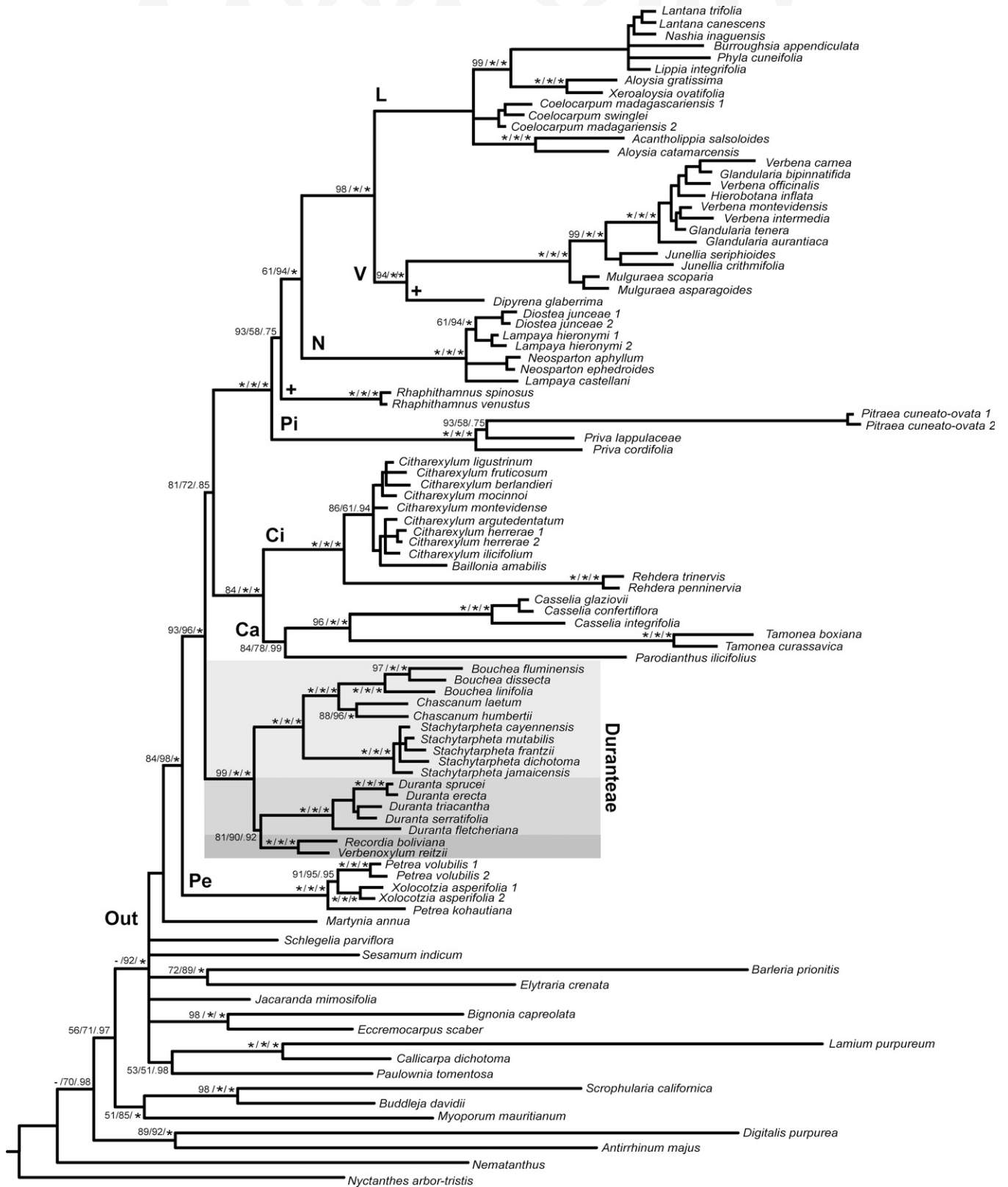


FIG. 2. Bayesian consensus tree topology based on combined data from the plastid markers *ndhF* and *trnL-trnF* inferred with MrBayes. Branches with MP/ML bootstrap support (BSP/BSL) and BI posterior probability (PP). Asterisk indicates maximum support and “-” clade not obtained in the tree. Letters on branches indicate Verbenaceae tribes: L. Lantaneae. V. Verbeneae. N. Neospartoneae. Pi. Priveae. Ci. Citharexyleae. Ca. Casseliae. Pe. Petreeae. Out. outgroups. Cross indicates genera not assigned to tribe.

and tree-bisection-reconnection (TBR) branch swapping. Characters were unordered and equally weighted. Statistical support was estimated using 1,000 bootstrap (BSP) replicates (Felsenstein 1985) with three random addition replicates.

For maximum likelihood (ML) analyses and Bayesian inference (BI), the evolutionary models were selected using jModelTest 0.1.1 (Guion and Gascuel 2003; Posada 2008) with Akaike Information Criterion (AIC). A TVM + G model was determined to best-fit both *ndhF* and *trnL-trnF*. Because the same model was selected and they are both noncoding plastid regions, they were concatenated. The gaps were treated as binary characters in the analyses. Maximum likelihood analyses were conducted using GARLI 2.0 (Zwickl 2006) with two independent search replicates and 1,000 bootstrap (BSL) replicates. The consensus tree was constructed in PAUP* 4.0b10 (Swofford 2002).

The BI analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with 10 million generations sampled every 100 generations in two independent runs, each with four simultaneous Markov chains initiated with a random tree. The convergence between runs was checked with Tracer 1.5 (Rambaut and Drummond 2009). The first 25% of the sampled trees were discarded as burn-in and the remaining were used to build a consensus tree.

Morphological Ancestral State Reconstruction—We analyzed twelve morphological characters (Appendix 3) that were important in the taxonomy of the family or were used to distinguish taxa from *Verbenoxylum* (Schauer 1847; Briquet 1895; Junell 1934; Troncoso 1971, 1974; Sanders 2001; Atkins 2004) (Table 1). The character states were mapped into the Bayesian consensus tree obtained in the molecular analyses, using parsimony in Mesquite 2.75 (Maddison and Maddison 2011), according to the literature and herbaria specimens. The tree was collapsed to tips that represent the Verbenaceae genera and with outgroup taxa removed.

RESULTS

Phylogenetic Analyses—The gene region *ndhF* presented sequence lengths from 2,071–2,140 bp, with the aligned sequence including 1,164 conserved, 940 variable, 660 parsimony informative characters, a consistency index (CI) of 0.51, and a retention index (RI) of 0.75. Sequence lengths of *trnL-trnF* ranged from 891 bp to 1,232 bp, with the aligned sequence including 605 conserved, 447 variable, 276 parsimony informative characters, a CI of 0.64, and a RI of 0.8. The total length of the combined data unambiguously aligned was 3,372 bp, being 1,769 conserved, 1,387 variable, and 936 parsimony informative sites and 209 gaps that were scored as binary characters in the analyses. The combined dataset presented a CI of 0.56 and a RI of 0.78.

The MP, ML, and BI trees have similar topologies and are consistent with respect to relationships among genera within Verbenaceae. The analyses using the two plastid regions confirm the results previously obtained by Marx et al. (2010) for the phylogenetic relationships within Verbenaceae. *Verbenoxylum* forms a well-supported clade (BSP = 100, BSL = 100, PP = 1.00) with genus *Recordia*, within the tribe Duranteae Bentham, with these two genera sister to *Duranta* (BSP = 81, BSL = 90, PP = 0.92) (Fig. 2). The rest of the tribe forms a second clade comprising *Bouchea* Cham., *Chascanum*, and *Stachytarpheta* Vahl (BSP = 100,

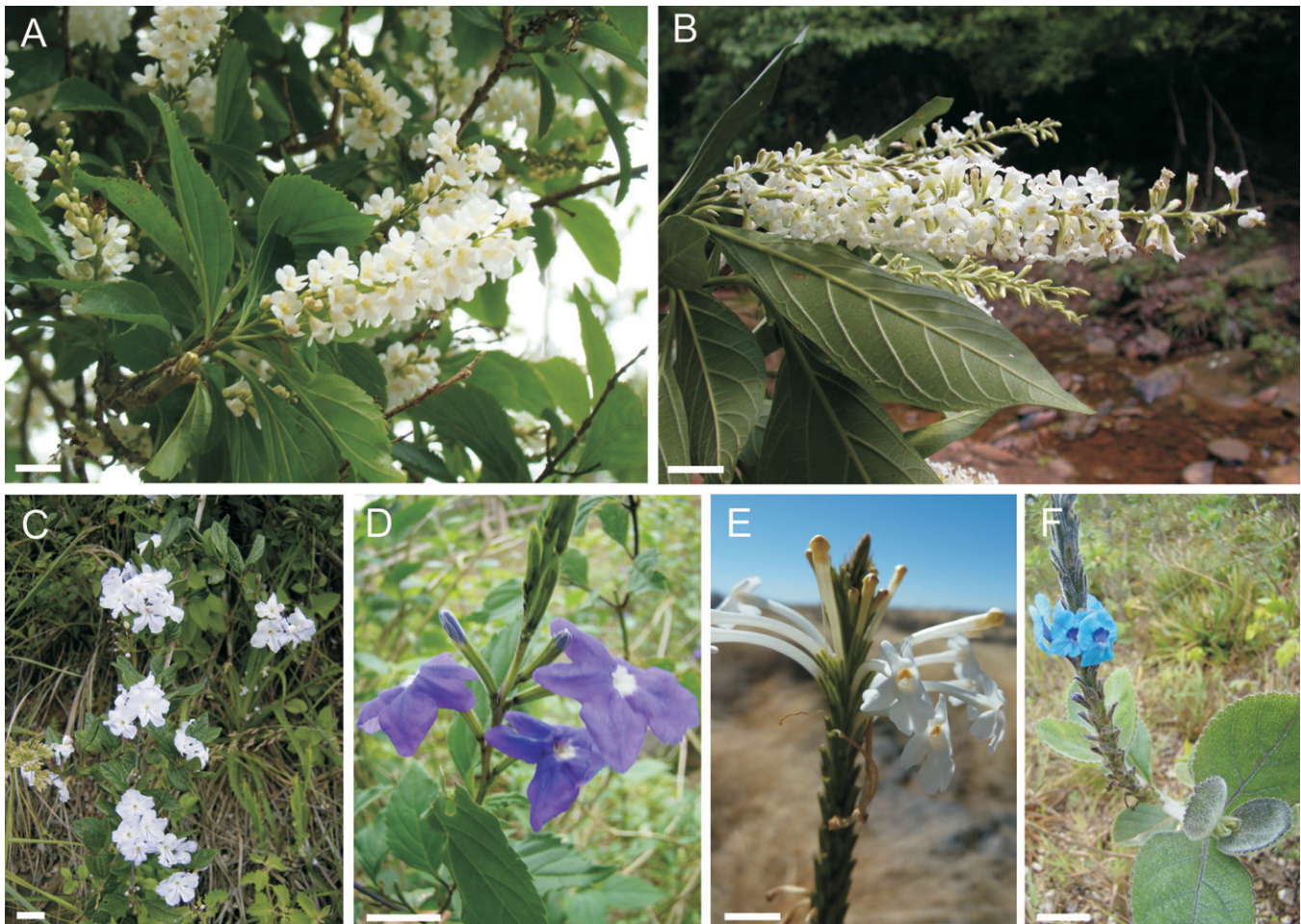


FIG. 3. Members of the tribe Duranteae (all scales = 1 cm). A. *Verbenoxylum reitzii*. B. *Recordia boliviana*. C. *Duranta vestita* Cham. D. *Bouchea fluminensis* (Vell.) Moldenke. E. *Chascanum* sp. F. *Stachytarpheta reticulata* Mart. ex Schauer. A, C, D, and F. Verónica Thode; B. Luzmila Arroyo, Museo Noel Kempff; E. Erin Tripp, Rancho Santa Ana Botanic Garden.

BSL = 100, PP = 1.00). Genera from the tribe Duranteae as circumscribed here are illustrated in Fig. 3A–F. Matrices and final tree files can be accessed on TreeBASE (study number S13802).

Morphological Analyses—We mapped character states for twelve discrete morphological traits for all Verbenaceae genera (Fig. 4A–L). All characters are shown to be homoplastic among tribes within the family, but can be important to distinguish genera within a tribe. Characters are described in Appendix 3, and ancestral state reconstructions along with their utility for distinguishing genera in Duranteae are summarized in Table 2. Some characters have ambiguous reconstructions (more than one state can be the basal condition) for the common ancestor (for the family or tribe) due to the lack of information for a taxon or because the states are equally parsimonious. Morphological details of *V. reitzii* are shown in Fig. 5.

The tribe Duranteae is composed of two clades, one formed by *Duranta*, *Recordia*, and *Verbenoxylum* (D + R + V) and the other by *Bouchea*, *Chascanum*, and *Stachytarpheta* (B + C + S). Many morphological differences were found between these two clades, contributing to the lack of a single morphological synapomorphy that could distinguish this tribe from the rest (Fig. 4A–L).

Within Duranteae, the D + R + V clade shares one synapomorphic trait: presence of linear floral bracts (Fig. 4H). Pedicellate flowers (Fig. 4I) are found in all members of the D + R + V clade, constituting a plesiomorphic trait for this clade, however it helps to distinguish the latter from the B + C + S clade, where sessile flowers constitute a synapomorphy.

Duranta has two synapomorphic traits: fleshy fruits (Fig. 4B) and four carpels (Fig. 4C). The presence of a short style (Fig. 4E) differentiates *Duranta* from the rest of Duranteae. However, this trait is ambiguously reconstructed for the ancestor of the tribe, thus it is not possible to distinguish this as the synapomorphic or the plesiomorphic condition for *Duranta*.

The *Recordia* + *Verbenoxylum* clade has four synapomorphic traits: they are trees (Fig. 4A), have long stamen filaments (Fig. 4J), divergent thecae (Figs. 4K, 5O), and exerted anthers (Figs. 4L, 5N). A bicarpellate ovary (Fig. 4C) is also shared by the *Recordia* + *Verbenoxylum* clade, but this character is ambiguously reconstructed, so the state for the ancestor of Duranteae is unknown.

The B + C + S clade is supported by two synapomorphic traits: herbs or suffrutescent shrubs (Fig. 4A) with sessile flowers (Fig. 4I). Presence of one carpel (Fig. 4C) also characterizes this clade but it is not possible to distinguish which state of this trait is derived or plesiomorphic due to the ambiguous reconstruction for the ancestor of Duranteae. The *Bouchea* + *Chascanum* clade shares two synapomorphies: an oblique stigma (Fig. 4D) and absence of a staminode (Fig. 4G). Genus *Stachytarpheta* has two synapomorphic traits: two fertile stamens (Fig. 4F), and vertical thecal orientation (Fig. 4K).

DISCUSSION

Phylogenetic Position of *Verbenoxylum* Within Verbenaceae—Our results strongly support the placement of *V. reitzii* within the tribe Duranteae, as sister to *Recordia boliviana* Moldenke (Fig. 2). These two species are sister to *Duranta*, composing a clade sister to the rest of the tribe, represented by genera

Bouchea, *Chascanum*, and *Stachytarpheta*. Marx et al. (2010) showed that Citharexyleae of earlier classifications (Troncoso 1974; Sanders 2001; Atkins 2004) was not monophyletic, which left open the question of which clade *Verbenoxylum* belonged to. Troncoso (1971) mentioned affinities between *Verbenoxylum* and *Recordia*, such as similar habit and similar flowers. However, a placement near *Citharexylum*, rather than *Duranta*, was implied in those classifications. The tribe Duranteae was established by Bentham (1839), composed of four genera: *Citharexylum*, *Duranta*, *Petrea* L., and *Rhaphithamnus*. In the molecular phylogeny of Verbenaceae proposed by Marx et al. (2010), the tribe is circumscribed to include *Bouchea*, *Chascanum*, *Stachytarpheta*, *Duranta*, and *Recordia*. Our phylogenetic analyses suggest that *V. reitzii* also belongs to this tribe (Appendix S1, see online Supplemental Data). This expands the composition of Duranteae and resolves the placement of *Verbenoxylum* to tribe.

Morphological Characters Within Duranteae—With a well-resolved phylogeny for the family it is possible to interpret the evolution of morphological traits and to evaluate the characters used traditionally to distinguish tribes or genera. According to our results, none of the twelve characters here studied are informative for distinguishing tribes within the family but they can be important to distinguish genera or suprageneric groups within a tribe. These traits probably have multiple origins within Verbenaceae (Fig. 4A–L). Marx et al. (2010) mentioned that none of the traditional treatments for Verbenaceae matches their molecular phylogeny, suggesting that homoplasy is frequent in the characters used in those classifications. A synapomorphic trait to support Duranteae, as here circumscribed, was not identified. However, the morphological characters here studied can be important to define groups within this tribe (Fig. 4A–L; Table 2).

Recordia and *Verbenoxylum* (Durateae), and *Citharexylum* and *Rehdera* (Citharexyleae) are the only genera in Verbenaceae with species that are trees. Within Duranteae, this character is a synapomorphy for the *Recordia* + *Verbenoxylum* clade, having evolved from ancestors that were shrubs or small trees. On the other hand, in the ancestor of the B + C + S clade there was a shift to herbs or suffrutescent shrubs (Fig. 4A).

Dry fruits are present in the majority of Verbenaceae genera. However, *Duranta* has fleshy fruits, and constitutes the only genus in Duranteae without dry fruit. Sanders (2001) associated *Duranta* with *Citharexylum* by the presence of fleshy fruits. Nevertheless, our study shows that fleshy fruits have evolved independently in both genera. Fruit type was one of the morphological differences noticed by Troncoso (1971) to distinguish *Verbenoxylum* from *Citharexylum*, being dry in the first (Fig. 5E–F) and fleshy in the latter (Fig. 4B).

In Verbenaceae, plants can have one, two, or four carpels. In Duranteae, the B + C + S clade is characterized by one carpel, *Recordia* + *Verbenoxylum* clade has two carpels (Fig. 5H), and *Duranta* is the only genus in the family with four carpels. A shift from two to four carpels is the most probable explanation for the carpel condition in *Duranta*. This means that the ancestor of the D + R + V clade probably had two carpels and this might constitute a synapomorphy for this clade. However, the ancestral carpel condition in Duranteae remains uncertain in this analysis (Fig. 4C).

A capitate stigma is plesiomorphic in Verbenaceae and is the state shared by most Duranteae (Fig. 5I). However, an oblique stigma, which also is found independently in several

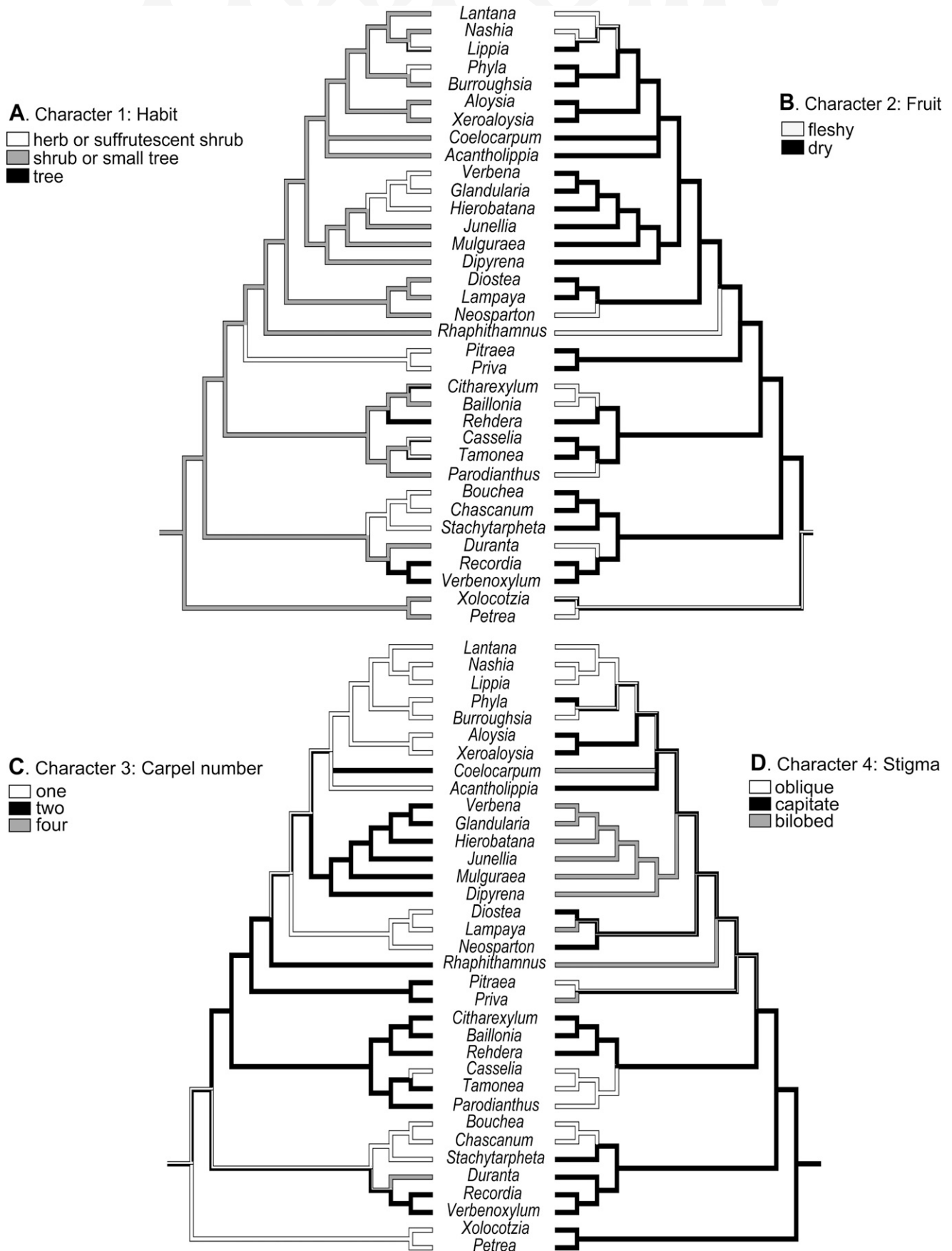


FIG. 4. Ancestral state reconstruction mapped on the Bayesian consensus tree with terminal collapsed to tips that represent the Verbenaceae genera, using parsimony in Mesquite of A. Habit. B. Fruit. C. Carpel number. D. Stigma. E. Style length. F. Fertile stamens. G. Staminode. H. Floral bracts. I. Pedicel. J. Stamen filament. K. Thecal orientation. L. Anther exertion.

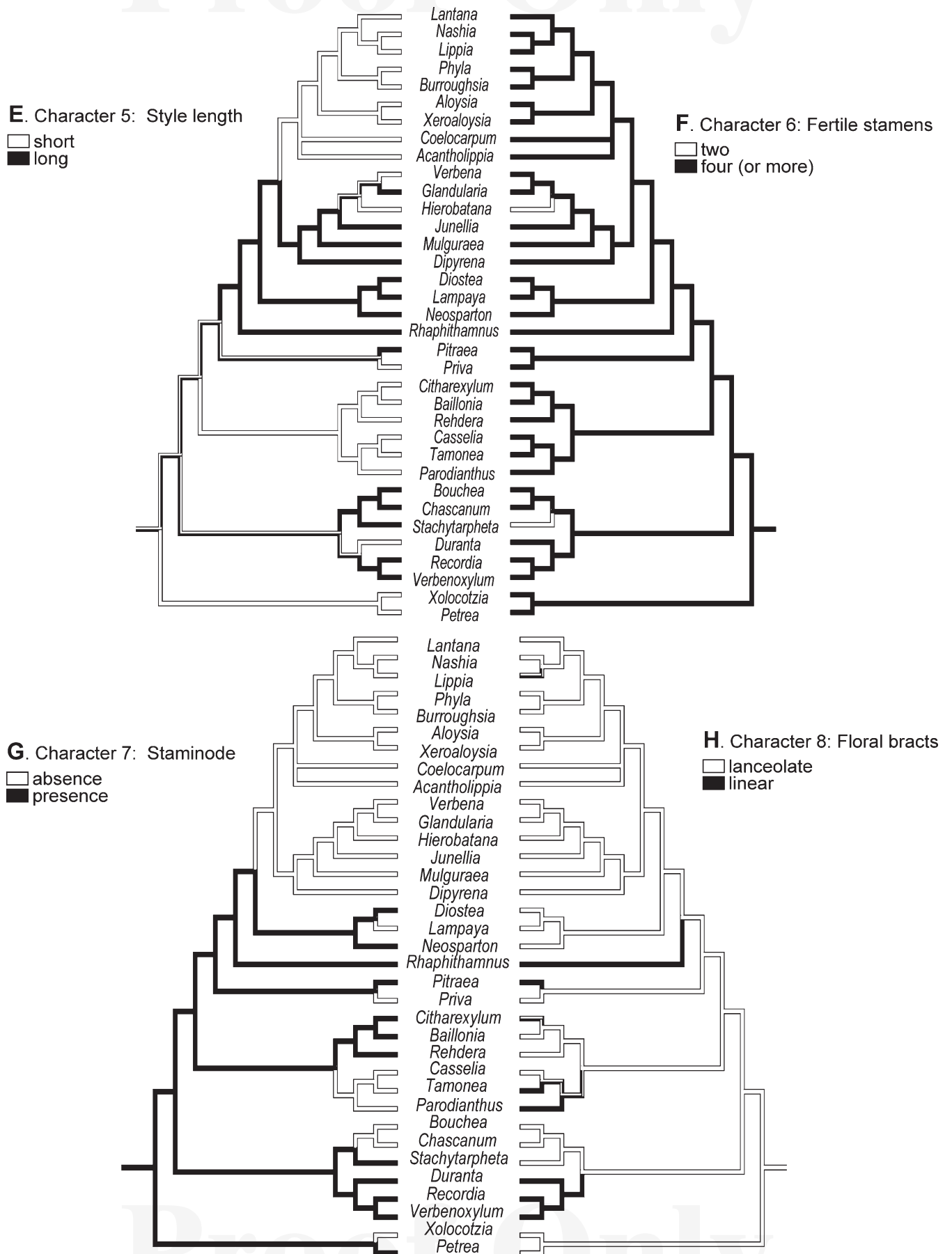


FIG. 4. Continued.

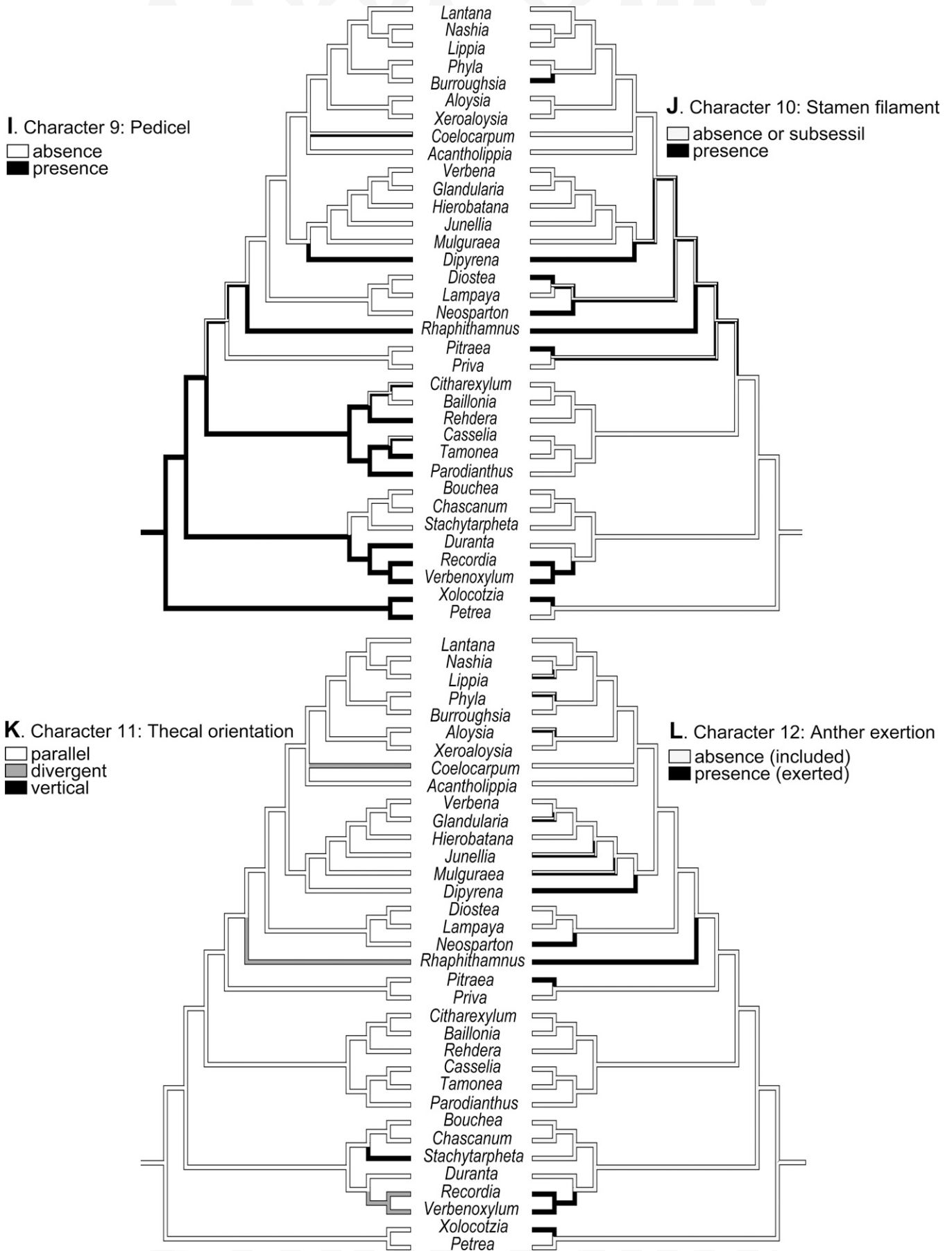


FIG. 4. Continued.

TABLE 2. Results for the ancestral state reconstruction using parsimony analyzed with Mesquite. B = *Bouchea*; C = *Chascanum*; D = *Duranta*; R = *Recordia*; S = *Stachytarpheta*; V = *Verbenoxylum*.

Character	Number of steps	Ancestral state for Verbenaceae	Ancestral state for Duranteae	State for <i>Recordia</i> + <i>Verbenoxylum</i>	Taxa distinguished within Duranteae
1	10	Shrub or small tree	Shrub or small tree	Tree	(B + C + S)/D/(R + V)
2	8	Ambiguous	Dry	Dry	D
3	7	Ambiguous	Ambiguous	Two	(B + C + S)/D/(R + V)
4	10	Capitate	Capitate	Capitate	(B + C)
5	7	Ambiguous	Ambiguous	Long	D
6	2	Four (or +)	Four (or +)	Four (or +)	S
7	6	Presence	Presence	Presence	(B + C)
8	7	Lanceolate	Lanceolate	Linear	(B + C + S)/(D + R + V)
9	8	Presence	Presence	Presence	(B + C + S)/(D + R + V)
10	8	Absence or subsessile	Absence or subsessile	Presence	(R + V)
11	4	Parallel	Parallel	Divergent	(B + C + D)/S/(R + V)
12	12	Absence (included)	Absence (included)	Presence (exerted)	(R + V)

other tribes, is a synapomorphy for the *Bouchea* + *Chascanum* clade (Fig. 4D).

Style length is ambiguously reconstructed for the ancestor of Duranteae, nevertheless the presence of a short style (less than three times the ovary length) in *Duranta* separates this genus from the rest of the tribe, which have long styles (more than three times the ovary length). Style length was another trait mentioned by Troncoso (1971) to segregate *Verbenoxylum*, with a long style, from *Citharexylum*, with short styles. However, in extraordinary cases, species of *Citharexylum* from Central America can have long styles (Gibson 1970). This character has been used traditionally to distinguish the genus *Glandularia* J.F. Gmel. from *Verbena* L. (Botta 1993; O'Leary et al. 2010) (Fig. 4E).

Most Verbenaceae have four fertile stamens (Fig. 5O), only the monotypic genus *Hierobotana* Briq., from the tribe Verbeneae, and *Stachytarpheta* have two fertile stamens, thus distinguishing *Stachytarpheta* within Duranteae (Fig. 4F).

Presence of a staminode is less frequent within Verbenaceae than absence. Within Duranteae, presence of staminode is a plesiomorphic state found in *Stachytarpheta*, *Recordia* (Fig. 5O), *Verbenoxylum*, and certain species of *Duranta*. This last genus has species with and without staminodes, as well as taxa with five fertile stamens, showing that this trait is variable within *Duranta*. Absence of a staminode is a synapomorphy for the *Bouchea* + *Chascanum* clade (Fig. 4G).

The shape of the floral bracts in Verbenaceae is quite variable and often is used to distinguish species within a genus (e.g. Atkins 2005; Thode and Mentz 2010). However, linear floral bracts constitute a synapomorphy for the D + R + V clade. The linear floral bracts found in the *Recordia* + *Verbenoxylum* clade (Fig. 5D, L) are usually difficult to observe in herbarium material because are early deciduous. The genera from the B + C + S clade has lanceolate floral bracts (Fig. 4H).

Within Duranteae, the presence of a floral pedicel (longer than 2 mm) is a plesiomorphic state for the D + R + V clade, and sessile flowers is a synapomorphy for the B + C + S clade (Fig. 4I).

Anthers subtended by a long filament (more than 2 mm long) are found in a few taxa within Verbenaceae, with sessile or subsessile anthers occurring most frequently in the family. In Duranteae, presence of long filaments constitutes a synapomorphy for the *Recordia* + *Verbenoxylum* clade (Fig. 5O).

Duranta species have subsessile filaments; however there is one species, *Duranta serratifolia* (Griseb.) Kuntze, which has long filaments. Troncoso (1971) used this character to distinguish *Verbenoxylum* from *Citharexylum*, with sessile or subsessile anthers in the latter (Fig. 4J).

The most common thecal orientation in Verbenaceae is parallel. Divergent thecae are present in *Rhaphithamnus*, *Coelocarpum*, and within Duranteae is a synapomorphy for the *Recordia* + *Verbenoxylum* clade (Fig. 5O). Vertical thecae are a synapomorphy for *Stachytarpheta*. The rest of the tribe have parallel thecae. Troncoso (1971) used this trait to segregate *Verbenoxylum*, with divergent thecae, from *Citharexylum*, which has parallel thecae (Fig. 4K).

In Verbenaceae anthers are most frequently included in the corolla tube. Nevertheless, exerted anthers appear independently several times in all Verbenaceae tribes, except in the tribe Citharexyleae. In Duranteae, exerted anthers state is a synapomorphy for the *Recordia* + *Verbenoxylum* clade (Fig. 5N), thus this character is useful to differentiate these taxa from the rest of the tribe (Fig. 4L).

The twelve characters studied here showed to be informative to distinguish taxa within Duranteae (Table 2). The *Recordia* + *Verbenoxylum* clade is morphologically distinct from the rest of the genera in the tribe (Figs. 3A–F, 4A–L). *Verbenoxylum* and *Recordia* differ from the other genera by five traits: both are trees, have a bicarpellate ovary, flowers that have anthers with long filaments, with divergent, and exerted thecae. The floral traits of the *Recordia* + *Verbenoxylum* clade seem to show a distinct reproductive strategy comparing with the rest of the tribe, possibly associated with different pollinators, but further biological studies on both taxa are necessary. The differences between the two genera lie in floral bract, calyx, and leaf pubescence and margins, with *Verbenoxylum* being almost glabrous and having serrated leaves, and *Recordia* being hirsute and having entire leaf margins (Fig. 5A–F). Both are narrow endemics and monotypic; *Verbenoxylum* is distributed in the subtropical Atlantic rainforest in Rio Grande do Sul and Santa Catarina, Brazil. *Recordia* is endemic to Bolivia, common in the low mountains west of Santa Cruz de la Sierra, on the highway between Samaipata and Cochabamba (M. Nee, pers. comm.), occurring between 500 and 1,850 m above sea level in dry subtropical semi-deciduous forest (Fig. 1).

New Combination—Our molecular and morphological analyses strongly support the combination of *Verbenoxylum reitzii* and *Recordia boliviana* as a single genus, despite the

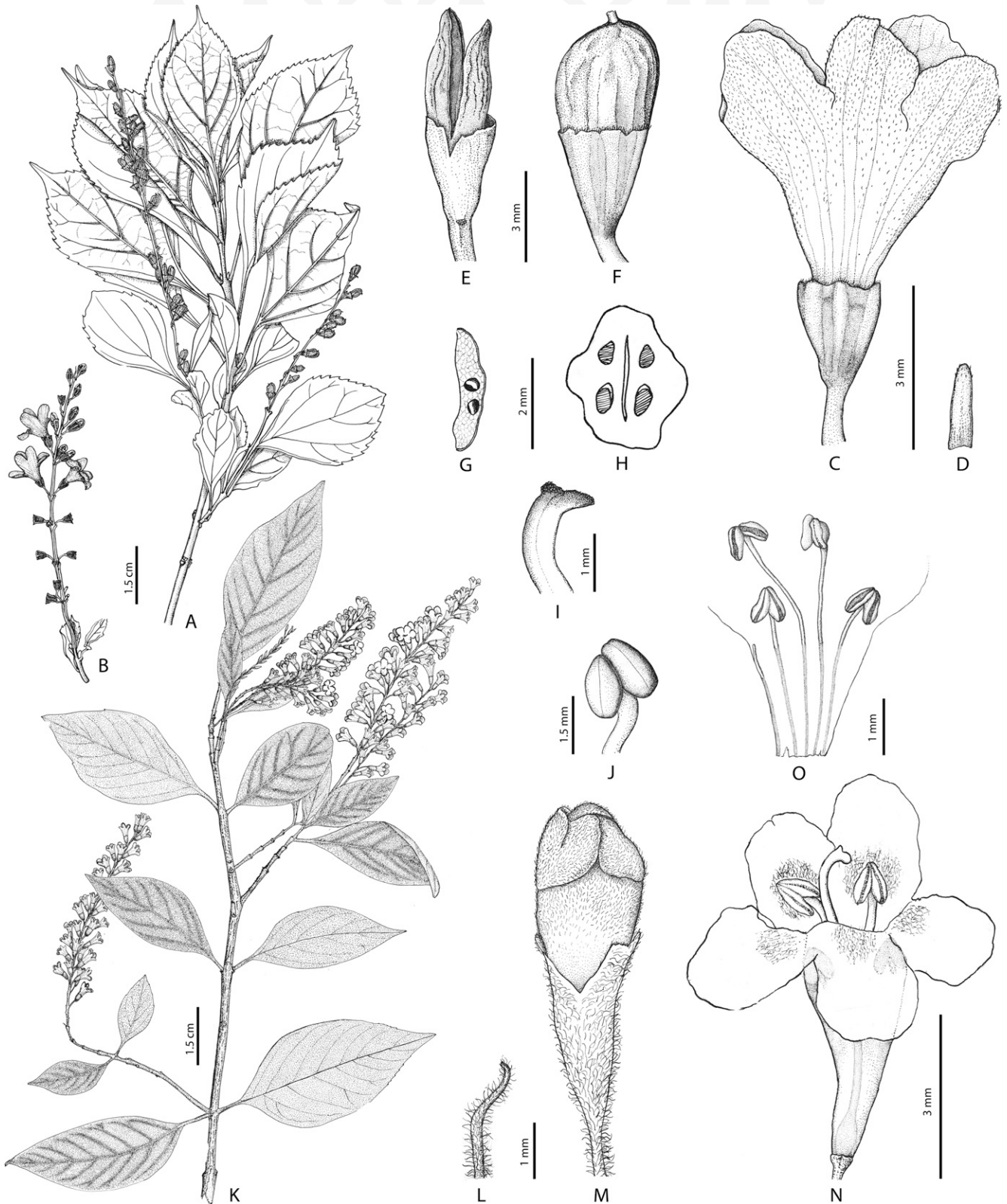


FIG. 5. *Verbenoxylum reitzii*. A. Plant branch in fruit. B. Florescence with flowers. C. Flower. D. Floral bract. E. Mature fruit with calyx. F. Fruit with calyx. G. Mericarp, cross section. H. Ovary, cross section. I. Stigma. J. Anther. *Recordia boliviana*. K. Plant branch. L. Floral bract. M. Flower in blossom. N. Flower with exerted anthers. O. Open corolla with androecium (Modified from Troncoso 1974).

large distributional gap. Our results show that the molecular and morphological differences do not justify different generic rank. We propose a new combination for *Verbenoxylum reitzii*, transferring it to the genus *Recordia*, and reducing *Verbenoxylum* to synonymy. Consequently, *Recordia* is now circumscribed to include two species, *Recordia reitzii* endemic to Brazil and *Recordia boliviana* endemic to Bolivia.

TAXONOMIC TREATMENT

Recordia reitzii (Moldenke) Thode & O'Leary, comb. nov.
Verbenoxylum reitzii (Moldenke) Tronc. *Darwiniana* 16: 626. 1971. *Citharexylum reitzii* Moldenke, *Phytologia* 3: 59. 1949.—TYPE: BRAZIL. Santa Catarina: Arar (Araranguá), Rodeio da Areia, 12 Nov 1943, R. Reitz c175 (holotype: NY!; isotypes: NY!, RB!, SI!).

KEY TO THE SPECIES OF *RECORDIA*

1. Plants mostly glabrous, calyx scarcely pubescent, leaf margins serrated, endemic to Brazil *R. reitzii*
 1. Plants mostly puberulous, calyx hirsute, leaf margins entire, endemic to Bolivia *R. boliviana*

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APPENDIX 1. Vouchers corresponding to the localities plotted on the map (Fig. 1).

Recordia boliviana. BOLIVA. Santa Cruz: Florida, 06 Nov 2005, D. Villarroel 182 (MO); Florida, 12 Dec 2007, D. Villarroel 1660 (MO); Vallegrande, 28 Nov 2011, G. A. Parada, Y. Inturias & M. Betancur 3846 (MO); Vallegrande, s.d., M. Nee 38482 (MO); the plain around Santa Cruz that lies within the Flora de la Región del Parque Nacional Ambaró, s.d., M. Nee 38027 (MO); within the Flora de la Región del Parque Nacional Ambaró, but above the 700 m contour, s.d., M. Nee 47855 (MO).

Verbenoxylum reitzii. BRAZIL. Santa Catarina: Criciúma, 19 Jan 2010, V. Thode 314 (ICN); Orleans, 12 Nov 2009, V. Thode 291 (ICN); Praia Grande, 06 Nov 2009, V. Thode 278 (ICN); Timbé do Sul, 07 Dec 2009, V. Thode 284 (ICN). Rio Grande do Sul: Osório, 05 Nov 2009, V. Thode 269 (ICN); Três Forquilhas, 06 Nov 2009, V. Thode 282 (ICN).

APPENDIX 2. List of specimens sampled: Taxon, place of collection, vouchers, and GenBank accession numbers (ndhF, trnLF). Sequences not available are indicated by —. Abbreviations of herbaria follow Holmgren et al. (1990).

Ingroup—Acantholippia salsoloides Griseb. ARGENTINA. Salta: R. Olmstead 2007–23 (WTU), HM216682, HM216586. **Aloysia catamarcensis** Moldenke. ARGENTINA. La Rioja: R. Olmstead 2007–82 (WTU), HM216683, HM216587. **Aloysia gratissima** (Gillies & Hook.) Tronc. Cultivated, K.-J. Kim 12803 (TEX), AF130154, HM216592. **Baillonia amabilis** Bocq. ex Baill. BOLIVIA. El Poston-Chiqueta. M. Cardenas 4522 (US), HM216691, HM216595. **Bouchea dissecta** S. Watson. MEXICO. Municipio de Nogales: A. L. Reina G., T. R. Van Devender, P. Merlin 2004–951 (TEX), HM216692, HM216596. **Bouchea fluminensis** (Vell.) Moldenke. Cultivated, H. Rimpler 1141 (FB), HM216693, HM216597. **Bouchea linifolia** A. Gray. U. S. A. Texas: B. L. Turner 20–423 (TEX), HM216694, HM216598. **Burroughsia appendiculata** (B.L.Rob. & Greenm.) Moldenke. MEXICO. Coahuila: J. Henrickson 14273 (LL), HM216695, HM216600. **Casselia confertiflora** (Moldenke) Moldenke. BRAZIL. Goiás: R. C. Mendonça et al. 2859 (US), HM216697, HM216602. **Casselia glaziovii** var. **serrata** Moldenke. BRAZIL. Minas Gerais: M. A. Silva et al. 3630 (US), HM216698, HM216603. **Casselia integrifolia** Nees & Mart. BRAZIL. Espírito Santo: J. R. Pirani et al. 3449 (US), HM216699, HM216604. **Chascanum humbertii** Moldenke. MADAGASCAR. Miller and Randrianasola 6127 (MO), HM216700, HM216605. **Chascanum laetum** Walp.. ETHIOPIA. Wollo: J. DeWilde 6923 (MO), HM216701, HM216606. **Citharexylum argutedentatum** Moldenke. PERU. Cusco: Urubamba, R. Olmstead 2009–32 (WTU), —, HM216607. **Citharexylum argutedentatum** Moldenke. PERU. Cusco: Calca, R. Olmstead 2009–36 (WTU), HM216702, HM216608. **Citharexylum berlandieri** B. L. Rob. Cultivated: Fairchild Tropical Gardens 78169B, J. Francisco-Ortega (FTG), HM216703, HM216609. **Citharexylum fruticosum** L. Cultivated: Kew 000–69–14013, no voucher, HM216704, —. **Citharexylum fruticosum** L. CUBA. Pinar del Rio: R. Olmstead 96–113 (WTU), —, HM216610. **Citharexylum herrerae** Mansf. PERU. Apurímac: Abancay, R. Olmstead 2009–11 (WTU), HM216705, HM216611; PERU. Apurímac: Abancay, R. Olmstead 2009–21 (WTU), HM216706, HM216612. **Citharexylum ilicifolium** Kunth. PERU. Cusco: Urubamba, R. Olmstead 2009–31 (WTU), HM216707, HM216613. **Citharexylum ligustrinum** van Houtte. Cultivated: Kew 000–69.51235, no voucher, HM216708, HM216614. **Citharexylum moccinoi** D. Don. NICARAGUA. Jinotega: S. Grose 151 (HULE), HM216709, HM216615. **Citharexylum montevidense** (Spreng.) Moldenke. ARGENTINA. Buenos Aires: R. Olmstead 2004–102 (WTU), HM216710, HM216616. **Coelocarpum madagascariense** Scott-Elliott. MADAGASCAR. Schatz 2977 (MO), HM216712, HM216618; MADAGASCAR. Phillipson and Milijaona 3569 (MO), HM216711, HM216617. **Coelocarpum swinglei** Moldenke. MADAGASCAR. Phillipson et al. 3443 (MO), HM216713, HM216619. **Diostea juncea** Miers. Cultivated: RBG Edinburgh 19300262, no voucher, HM216715, HM216621; Cultivated: RBG Kew 1969–35347, no voucher, HM216714, HM216620. **Dipyrrena glaberrima** (Gillies & Hook.) Hook. ARGENTINA. Mendoza: R. Olmstead 2004–179 (WTU), HM216716, HM216622. **Duranta erecta** L. Cultivated: Jardín Botánica Nacional, Havana, Cuba, R. Olmstead 1996–100 (WTU), HM216717, HM216623. **Duranta fletcheriana** Moldenke. CUBA. Topes de Collantes: R. Olmstead 1996–71 (WTU), HM216718, HM216624. **Duranta serratifolia** (Griseb.)

Kuntze. ARGENTINA. Salta: R. Olmstead 2007–009 (WTU), HM216719, HM216625. **Duranta sprucei** Briq. Cultivated: Waimea Bot. Gard. 75S356, R. Olmstead 1992–221 (WTU), HM216720, HM216626. **Duranta triacantha** Juss. PERU. Apurímac: R. Olmstead 2009–20 (WTU), HM216721, HM216627. **Glandularia aurantiaca** (Speg.) Botta. ARGENTINA. Mendoza: R. Olmstead 2004–196 (WTU), HM216722, EF571554. **Glandularia bipinnatifida** (Nutt.) Nutt. U. S. A. Colorado: R. Olmstead 92–133 (WTU), HM216723, —; U. S. A. Texas: Y.-W. Yuan 2005–12 (WTU), —, EF571535. **Glandularia tenera** (Spreng.) Cabrera. Cultivated: Waimea Bot. Gard. 74P1415, R. Olmstead 92–222 (WTU), HM216728, —; ARGENTINA. Mendoza: R. Olmstead 2004–148 (WTU), —, EF571556. **Hierobotana inflata** Briq. ECUADOR. Pichineha: E. Asplund 17069 (US), HM216729, HM216628. **Junellia crithmifolia** (Gillies & Hook.) N. O’Leary & P. Peralta. ARGENTINA. Mendoza: R. Olmstead 2004–169 (WTU), HM216730, EF571558. **Junellia seriphoides** (Gillies & Hook.) Moldenke. ARGENTINA. Mendoza: R. Olmstead 2004–147 (WTU), HM216732, EF571561. **Lampaya castellani** Moldenke. ARGENTINA. Jujuy: R. Olmstead 2007–063 (WTU), HM216736, HM216630. **Lampaya hieronymi** Schum. ex Moldenke. ARGENTINA. Catamarca: E. Marilínez Carretero 2092 (MERL), HM216737, HM216631; Catamarca: F. Biurrin et al. 4960 (SI), HM216738, HM216632. **Lantana canescens** Kunth. ARGENTINA. Salta: R. Olmstead 2007–006 (WTU), HM216740, HM216634. **Lantana trifolia** L. Cultivated: Jardín Botánica Nacional Havana, Cuba, R. Olmstead 1996–98 (WTU), HM216745, HM216639. **Lippia integrifolia** Hieron. ARGENTINA. Catamarca: R. Olmstead 2007–78 (WTU), HM216749, HM216643. **Mulguraea asparagoides** (Gillies & Hook.) O’Leary & Peralta. ARGENTINA. Mendoza: R. Olmstead 2004–192 (WTU), HM216756, EF571567. **Mulguraea scoparia** (Gillies & Hook.) O’Leary & Peralta. ARGENTINA. Mendoza: R. Olmstead 2004–178 (WTU), HM216758, EF571566. **Nashia inaguensis** Millsp. Cultivated: Fairchild Tropical Gardens 8655, no voucher, HM216759, HM216650. **Neosparton aphyllum** (Gillies & Hook.) Kuntze. ARGENTINA. Mendoza: R. Olmstead 2004–193 (WTU), HM216760, HM216651. **Neosparton ephedroides** Griseb. ARGENTINA. Catamarca: R. Olmstead 2007–077 (WTU), HM216761, HM216652. **Parodianthus ilicifolius** (Moldenke) Troncoso. ARGENTINA. San Luis: R. Olmstead 2004–181 (WTU), HM216762, HM216653. **Petrea kohautiana** C. Presl. ECUADOR. J. L. Clark 6554 (US), HM216763, HM216654. **Petrea volubilis** L. Cultivated: RBG Kew 000.73.17818, no voucher, AY919283, HM216655; Cultivated: RBG Kew 326.75.03134, no voucher, FJ887872, FJ870052. **Phyla cuneifolia** (Torr.) Greene. U. S. A. Colorado: R. Olmstead 1992–134 (WTU), HM216765, HM216657. **Pitreaa cuneato-ovata** (Cav.) Caro. ARGENTINA. Mendoza: R. Olmstead 2004–186 (WTU), HM216768, HM216661; Catamarca: R. Olmstead 2007–81 (WTU), HM216769, HM216662. **Priva cordifolia** Druce. SOUTH AFRICA. Natal: W. Vos 391 (NU), HM216770, HM216663. **Priva lappulacea** (L.) Pers. CUBA. Villa Clara: R. Olmstead 1996–86 (WTU), HM216771, HM216664. **Recordia boliviana** Moldenke. BOLIVIA. Santa Cruz: M. Nee 24092 (TEX), HM216772, HM216665. **Rehdera penninervia** Standl. & Moldenke. GUATEMALA. El Petén: C. L. Lundell and E. Contreras 19938 (TEX), HM216773, —; El Petén, M. Pena-Chocarro and N. Bonilla 1378 (MO), —, HM216666. **Rehdera trinervis** (Blake) Moldenke. MEXICO. Compeche: E. Martínez S., D. Alvarez M., S. Ramírez A. 31706 (TEX), HM216774, HM216667. **Rhaphithammus spinosus** (Juss.) Moldenke. Cultivated: RBG Kew 128–83.01596, no voucher, L36409, FJ870056. **Rhaphithammus venustus** B. L. Rob. CHILE. Juan Fernández Islands: T. F. Stuessy 11855 (OS), HM216775, HM216668. **Stachytarpheta cayennensis** (Rich.) Vahl. ARGENTINA. Corrientes: R. Olmstead 2004–113 (WTU), HM216776, HM216669. **Stachytarpheta dichotoma** (Ruiz & Pav.) Vahl. U. S. A. Hawaii: R. Olmstead 951 (WTU), L36414, HM216670. **Stachytarpheta frantzii** Polak. Cultivated: Fairchild Tropical Gardens 2001–0533B, no voucher, HM216777, HM216671. **Stachytarpheta jamaicensis** (L.) Vahl. CUBA. Topes de Collantes: R. Olmstead 1996–68 (WTU), HM216778, HM216672. **Stachytarpheta mutabilis** (Jacq.) Vahl. Cultivated: Waimea Bot. Gard. 75C1444, R. Olmstead 1992–207 (WTU), HM216779, HM216673. **Tamonea boxiana** (Moldenke) R. A. Howard. U. S. A. Puerto Rico: R. Olmstead 2003–12 (WTU), HM216780, HM216674. **Tamonea curassavica** (L.) Pers. Cultivated: Germany, H. Rimpler 1917 (FB), HM216781, HM216675. **Verbena camea** Medik. U. S. A. Florida: Zomlefer 693 (WTU), HM216783, HM216676. **Verbena intermedia** Gillies & Hook. ARGENTINA. Entre Ríos: R. Olmstead 2004–106 (WTU), HM216785, EF571522. **Verbena montevidensis** Spreng. ARGENTINA. Corrientes: R. Olmstead 2004–112 (WTU), HM216788, EF571521. **Verbena officinalis** L. Cultivated, R. Olmstead 98–55 (WTU), HM216789, EF571525. **Verbenoxylum reitzii** (Mold.) Tronc. BRAZIL. Rio Grande do Sul: V. Thode 162 (ICN), KC466433, KC466432. **Xeroaloyisia ovatifolia** (Moldenke) Troncoso. ARGENTINA. San Luis: R. Olmstead 2004–184 (WTU), HM216792, HM216678. **Xolocotzia asperifolia** Miranda. MEXICO. Chiapas: D. Neill 5477 (MO), HM216793, HM216680; NICARAGUA. Matagalpa: W. D. Stevens 22332 (MO), HM216794, HM216679.

Outgroup—*Antirrhinum majus* L. Cultivated, no voucher, L36413, —; Cultivated, *Erixon and Bremer 10* (UPS), —, AJ430929. *Barleria prionitis* L. Cultivated: Uppsala Bot. Gard. 1977–3036 (UPS), U12653, —; *R. Scotland s.n.*, —, AF063118. *Bignonia capreolata* L. Cultivated: RBG Kew 1980–3846, no voucher, J887855/DQ222566, FJ870021. *Buddleja araucana* Phil. ARGENTINA. Neuquén: *R. Olmstead 2007–94* (WTU), —, HM216599. *Buddleja davidii* Franch. Cultivated, *R. Olmstead 88–007* (WTU), L36394, —. *Callicarpa dichotoma* Raeusch. Cultivated: Beal Bot. Gard., *R. Olmstead 88–012* (WTU), L36395, AF363665. *Digitalis purpurea* L. Cultivated, *K.-J. Kim 13943* (YNUH), AF130150, —; Cultivated, *E. Freeman s.n.*, —, AF034871. *Ecchremocarpus scaber* Ruiz & Pav. Cultivated: RBG Kew 1988–132, *M. W. Chase 2999* (K), AF102630, FJ870030. *Elytraria crenata* Vahl. *R. Scotland s.n.*, U12657, —. *Elytraria imbricata* (Vahl.) Pers. U. S. A. Arizona: *McDade and Jenkins 1155* (ARIZ), —, AF061819. *Jacaranda mimosifolia* D. Don. BRAZIL. *L. Lohmann 369* (MO), EF105012, EF105070. *Lamium purpureum* L. U. S. A. Ohio: *Wagstaff 88–031* (BHO), U78694, AF363664. *Martynia annua* L. U. S. A. Arizona: *P. Jenkins 97–149* (ARIZ), HM216755, HM216649. *Myoporum mauritianum* A. DC. Cultivated: RBG Kew 1984–4220, no voucher, L36403, AJ299257. *Nematanthus hirsutus* (Mart.) Wiehler. Cultivated, no voucher, L36404, —. *Nematanthus strigillosus* (Mart.) H. E. Moore. Cultivated: USBRG, *J. Skog 7751* (US), —, AY047148. *Nyctanthes arbor-tristis* L. Cultivated: RBG

Kew 099–86.00993, no voucher, U78708, —; *K. Dahlstrand s.n.* (GB), —, AF231863. *Paulownia tomentosa* (Thunb.) Steud. Cultivated, *R. Olmstead 88–008* (WTU), L36406, —; Cultivated, *Erixon and Bremer 22* (UPS), —, AJ430926. *Schlegelia parviflora* (Oerst.) Monach. VENEZUELA. (Cultivated at MO), *Gentry 14221* (MO), L36410, AJ608570. *Scrophularia californica* Cham. & Schldtl. U. S. A. California: *C. W. dePamphilis s.n.* (PAC), L36411, —; *E. Freeman s.n.*, —, AF118802. *Sesamum indicum* L. Cultivated, no voucher, L36413, —; Cultivated, *P. Jenkins 97–141* (ARIZ), —, AF067067.

APPENDIX 3. Characters and corresponding states used in this study for the morphological ancestral state reconstruction.

Habit: Tree (0); shrub or small tree (1); herb or suffrutescent shrub (2).
2. Fruit: Dry (0); fleshy (1). **3. Carpel number:** One (0); two (1); four (2).
4. Stigma: Capitate (0); bilobed (1); oblique (2). **5. Style length:** Long = more than three times the ovary length (0); short = less than three times (1).
6. Fertile stamens: Two (0); four or more (1). **7. Staminode:** Absence (0); presence (1). **8. Floral bracts:** Linear (0); lanceolate (1). **9. Pedicel:** Absence = sessile or subsessile (0); presence = longer than 2 mm (1). **10. Stamen filament:** Absence or subsessile = less than 2 mm (0); presence = more than 2 mm (1). **11. Thecal orientation:** Divergent (0); vertical (1); parallel (2). **12. Anther exertion:** Absence/included (0); presence/exserted (1).