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Author(s): Patricia Lu-Irving, Nataly O'Leary, Anna O'Brien, and Richard G. Olmstead
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Resolving the Genera *Aloysia* and *Acantholippia* within tribe Lantaneae (Verbenaceae), using Chloroplast and Nuclear Sequences

Patricia Lu-Irving,[1,4] Nataly O'Leary,[2] Anna O'Brien,[1,3] and Richard G. Olmstead[1]

[1]Department of Biology and Burke Museum, University of Washington, Seattle, Washington, 98195, U. S. A.
[2]Instituto de Botánica Darwinion, Labardén 200, San Isidro, Argentina.
[3]New address: Section of Evolution and Ecology, University of California, Davis, California 95616, U. S. A.
[4]Author for correspondence (pli2@uw.edu)

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Abstract—Species belonging to the genera *Aloysia* and *Acantholippia* are difficult to place within Lantaneae due to gene tree incongruence and limited sampling in previous studies. We use an expanded sample of both genera, and DNA sequence data from six loci, to reveal that *Aloysia* and *Acantholippia* species occur in five consistently inferred, well-supported clades. The precise relationships of these clades to one another are still enigmatic, due to gene tree incongruence. However, coalescent-based species tree inference supports the inclusion of most of *Acantholippia* in an expanded *Aloysia* sensu lato, with a 4-lobed calyx as its defining feature. Five new combinations are proposed to reflect this relationship: ***Aloysia deserticola***, ***Aloysia riojana***, ***Aloysia salsoloides***, ***Aloysia tarapacana***, and ***Aloysia trifida***. Geographic range shifts from subtropical South America to North America have occurred at least twice in *Aloysia*. Shifts between determinate and indeterminate inflorescence arrangement have occurred at least twice independently. The elongate, lax inflorescence, which is characteristic of most of *Aloysia*, is hypothesized to be derived from a condensed inflorescence.

Keywords—Chloroplast loci, ETS, gene tree incongruence, phylogeny, PPR loci, species tree inference

Species-level systematics can be challenging when the species under consideration have a tangled evolutionary history. If morphological traits are not true to lineages, and if evolutionary processes obscure phylogenetic inference from molecular data, then satisfactory taxonomic schemes are difficult to achieve. This study focuses on resolving the phylogenetic relationships among a group of species in which morphological parallelisms have confounded traditional classification, and which have been difficult to resolve in previous molecular systematics studies, due to gene tree incongruence. We use expanded sampling and coalescent-based phylogenetic inference from multiple, independent loci to provide a basis for the revision of the genera *Aloysia* Paláu and *Acantholippia* Griseb.

Aloysia is a genus of shrubs and small trees in tribe Lantaneae (Verbenaceae). Members of *Aloysia* are endemic to the New World, where they are mainly found in subtropical regions, and in the Andes. The medicinal and culinary herb *Aloysia citrodora* Paláu ("lemon verbena"; the more commonly spelled "*Aloysia citriodora*" is an orthographic variant) is cultivated worldwide. The monotypic genus *Xeroaloysia* Tronc., endemic to Argentina, is thought to be closely related to *Aloysia*. *Acantholippia* is a small genus of shrubs that occur in Argentina, Chile, and Bolivia, where they inhabit dry, open environments, including the Altiplano.

The generic boundaries between *Aloysia* and *Lippia* L., and between *Acantholippia* and *Lippia*, are historically somewhat blurred, with Bentham and Hooker (1876) treating *Aloysia* and *Acantholippia* as part of *Lippia*, while Moldenke (1959) maintained them as separate genera. Among the defining features of both *Aloysia* and *Acantholippia* is a four-lobed calyx (whereas the calyces of *Lippia* species are bifid or truncate), with the exception of some *Aloysia* species with bifid calyces. This has been interpreted as progressive reduction in the number of calyx teeth (from five, the condition in the rest of Verbenaceae; O'Leary et al. 2012). Additionally, *Aloysia* species characteristically possess lax inflorescences (racemes or spikes in which the rachis is visible and the floral bracts inconspicuous; Fig. 1D , E, F, H, I), in contrast with the tightly condensed, capitate or spicate inflorescences of *Lippia*, which often feature relatively large, foliaceous or showy floral bracts. Again, there are exceptions, with condensed inflorescences occurring in some *Aloysia* species, and with a few *Lippia* species featuring rather lax inflorescences. *Acantholippia* has *Lippia*-like condensed inflorescences, but is recognized primarily by (in addition to a 4-lobed calyx) xerophytic adaptations such as spines and/or reduced leaves (Fig. 1G); several species of *Lippia* and *Nashia* Millsp. (another segregate from *Lippia*) found in dry habitats possess similar adaptations. Previous studies have suggested that traits traditionally used to characterize genera in Lantaneae do not define monophyletic groups (Marx et al. 2010; O'Leary et al. 2012; Lu-Irving and Olmstead 2013). However, uncertainty in previous phylogenetic reconstructions means that the pattern of evolution of many traits within Lantaneae remains unclear.

Background Information—Paláu (1784) erected the genus *Aloysia* as a note appended to a translation of Linnaeus' work, describing a single species, *Aloysia citrodora* (the obscurity of this publication has caused confusion over the authorship of *Aloysia*; Armada and Barra 1992). Subsequently, *Aloysia* was treated as a subgenus or section within *Lippia* (e.g. Schauer 1847; Bentham and Hooker 1876; Briquet 1897, 1904), but has most often been accepted as an independent genus (Chamisso 1832; Moldenke 1959; Troncoso 1974; Atkins 2004). Botta (1979) treated the Argentine species of *Aloysia*, but an unpublished thesis by Siedo (2006) is the most complete treatment to date, in which 30 species and 14 varieties are recognized across the geographic range of the group. New species have since been described (e.g. Wood 2009), but the results of a recent revision call for 29 species and eight varieties in *Aloysia* (O'Leary et al. ined.), broadly similar to Siedo's (2006) treatment. Three widespread species, *Aloysia gratissima* (Gillies & Hook.) Tronc., *Aloysia scorodonioides* Cham., and *Aloysia virgata* (Ruiz & Pav.) Pers., are particularly variable and circumscribed differently according to different treatments (Siedo 2006; O'Leary et al. ined.). *Aloysia* is most diverse in South America, with 22 species occurring there; seven are endemic to North America (O'Leary et al. ined.). One species,

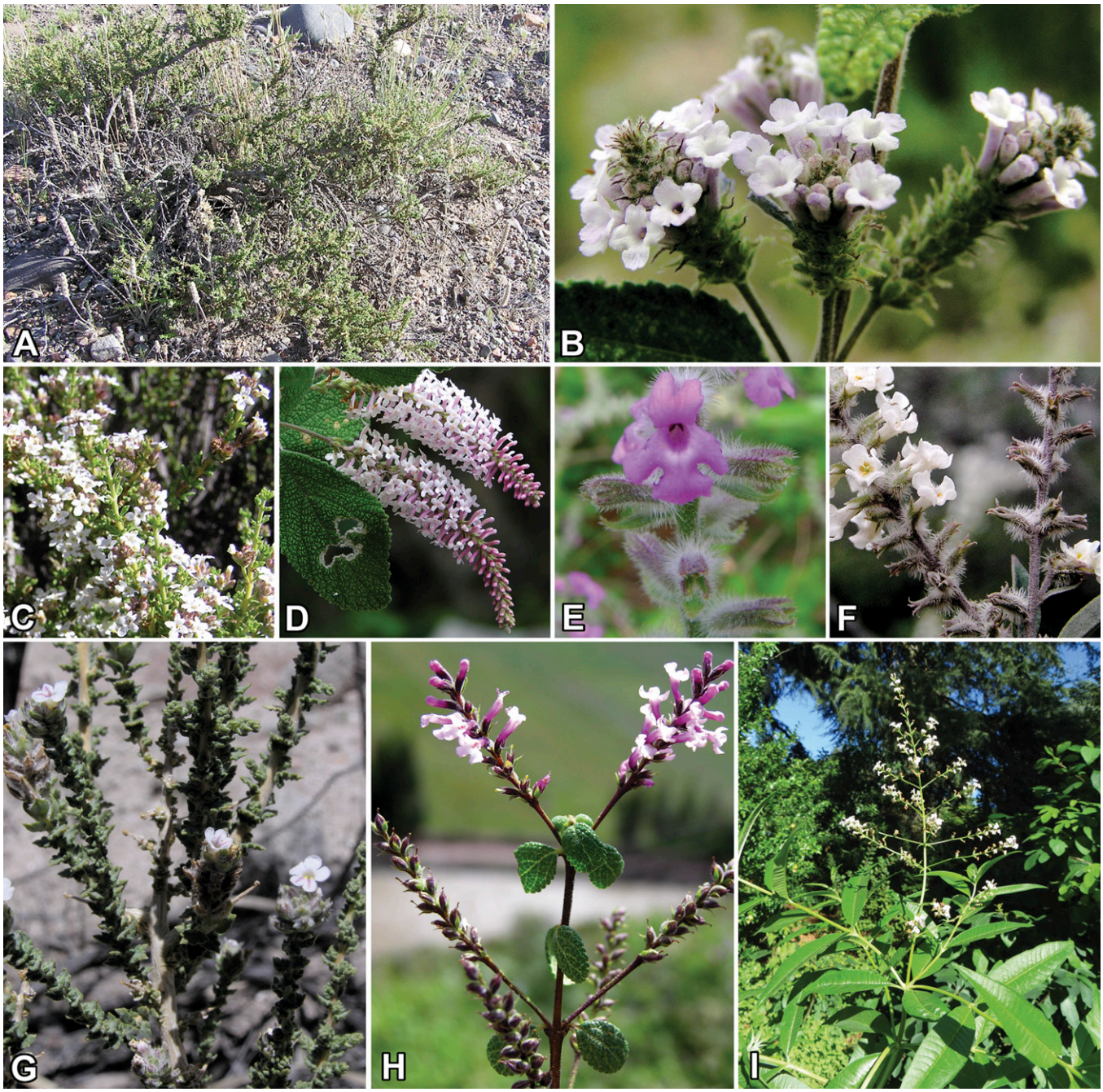

FIG. 1. Selected species of *Aloysia* and *Acantholippia*. A. *Acantholippia seriphioides*. B. *Aloysia catamarcensis*. C. *Acantholippia salsoloides*, inflorescence. D. *Aloysia velutina*, inflorescence. E. *Aloysia macrostachya*. F. *Aloysia citrodora*, flowers. G. *Acantholippia salsoloides*, habit. H. *Aloysia scorodonioides* var. *hypoleuca*, inflorescence arrangement. I. *Aloysia citrodora*, inflorescence.

A. gratissima, is found in both North and South America, with a disjunction in distribution across the tropics.

Xeroaloysia was separated from *Aloysia* by Troncoso (1960), and is currently accepted as distinct from it based on fruit anatomy; the fruits in *Xeroaloysia ovatifolia* (Moldenke) Tronc. are one-seeded drupes, whereas fruits in *Aloysia* are typically dry schizocarps separating into two, one-seeded units (cluses) at maturity, similar to fruits in *Lippia*.

Acantholippia was established by Grisebach in 1874, and is currently accepted as distinct from *Lippia* based on the presence of albumen in the seeds, subactinomorphic corollas, and xerophytic adaptations. The most recent treatments of *Acantholippia* recognize either six or seven species (Botta 1980; Caro 1982).

The most recent and complete phylogenetic treatment of Verbenaceae (Marx et al. 2010) found *Aloysia* to be non-monophyletic: *Aloysia* species formed two clades, with *Xeroaloysia* and *Acantholippia* species nesting within them. Marx et al. (2010) were concerned with reconstructing broad relationships across the family, so they included only a limited sample of Lantaneae, and were unable to achieve good resolution within Lantaneae. With increased sampling, Lu-Irving and Olmstead (2013) confirmed the findings of Marx et al. (2010), and revealed a third distinct clade of *Aloysia* species,

derived within a clade of *Lantana* L. and *Lippia* species. However, the relationships between *Aloysia*, *Acantholippia*, and the rest of Lantaneae could not be resolved with confidence, and no taxonomic revisions were made.

The relationships inferred from chloroplast data by Marx et al. (2010) and Lu-Irving and Olmstead (2013) provided the basis for a detailed study of the evolution of morphological traits in Verbenaceae (O'Leary et al. 2012). The most important morphological characters found to vary among major groups in Lantaneae were the loss of the terminal unit in inflorescence arrangement (converting a determinate compound inflorescence to an indeterminate structure, or the transition from heterothetic to homothetic pleiobotrya sensu O'Leary et al. 2012), and reduction in number of calyx teeth. Because this was based on a chloroplast reconstruction, without taking conflicting signal from nuclear loci into account, a more complete phylogenetic study might prompt reinterpretation of the evolution of these traits.

Objectives—When different genes have different histories, efforts to obtain a correct phylogeny can be misled. Whereas gene trees are often implicitly assumed to reflect the species tree, this is not always the case (Maddison 1997). Lantaneae have been shown to be a difficult group, with a tangled evolutionary history (Lu-Irving and Olmstead 2013); therefore, a multi-locus approach is needed to resolve the phylogenetic positions of *Aloysia* and *Acantholippia*.

Herein we present a molecular phylogenetic study of Lantaneae focusing on *Aloysia* and its related genera, *Acantholippia* and *Xeroaloysia*. Our goal is to uncover the extent to which generic revision is needed, and to provide a basis for that revision. We use a larger and broader sampling of *Aloysia* and *Acantholippia* than has been used previously, and DNA sequence data from six loci shown to be useful in phylogenetic studies in Lantaneae (Lu-Irving and Olmstead 2013): the high-copy external transcribed spacer (ETS) locus of the nuclear rDNA, two low-copy independent loci of the nuclear pentatricopeptide repeat containing gene family (PPR 81 and PPR 123; Yuan et al. 2009, 2010), and three intergenic chloroplast loci (*trnT-trnL*, *rpl32-trnL*, *trnQ-rps16*).

Materials And Methods

Sampling—We sampled 45 accessions (individuals; Appendix 1) from 24 species of *Aloysia*, four species of *Acantholippia*, and *Xeroaloysia ovatifolia*. We use several synonymized names throughout this paper; synonymy according to Siedo (2006) and O'Leary et al. (ined.) is detailed in Appendix 2. The sampled *Aloysia* species span the North American, Andean, and subtropical South American distribution of this genus. Fifteen species belonging to *Lantana*, *Lippia*, *Phyla* Lour., and *Nashia* were chosen to represent the *Lantana-Lippia* clade. The outgroup consisted of seven species belonging to different tribes within Verbenaceae, and one species of *Coelocarpum*, the sister group to core Lantaneae.

DNA Extraction, Amplification, and Sequencing—DNA was extracted from dried leaf tissue. The source tissue was either collected in the field and preserved in silica gel, or sampled from herbarium specimens. Extractions were carried out following a standard CTAB method (modified from Doyle and Doyle 1987); DNA was purified by precipitation in 100% isopropanol, and some extractions were further purified using a Promega DNA clean-up kit. Amplification of target loci was carried out by PCR, using equipment, primers, and reaction conditions as described by Lu-Irving and Olmstead (2013). Amplification products were purified by precipitation in polyethylene glycol. Cycle sequencing reactions were carried out using standard Applied Biosystems reagents and protocols for dye terminator dideoxy sequencing. The internal sequencing primers used to obtain overlapping reads for each locus were those described by Lu-Irving and Olmstead (2013). Products of sequencing reactions were purified by precipitation in sodium acetate and ethanol, or by passing through Sephadex G-50 columns. Raw sequence data were generated using Applied Biosystems PRISM Genetic Analyzers, and processed using Sequencher (Gene Codes Corp.).

Alignment and Phylogenetic Inference—Sequences were aligned using MAFFT v.6 (Katoh et al. 2002), and minor adjustments were made manually using SeAl v.2.0a11. Data from the six target loci were assembled into six data sets: ETS, PPR 81, PPR 123, concatenated chloroplast sequences, concatenated nuclear sequences, and all data in concatenation. Primary phylogenetic analyses were model-based, but maximum parsimony analysis was also performed using PAUP* v.4b.10 (Swofford 2002) to evaluate its consistency with the model-based analyses.

To determine the most appropriate model of evolution, each data set was evaluated using jModeltest v.0.1 (Posada 2008), under both the Akaike information criterion (AIC) and Bayesian information criterion (BIC). The partition homogeneity test (PHT; Farris et al. 1995) as implemented in PAUP* v.4b.10 (Swofford 2002) was carried out as a gauge of incongruence between data sets. Phylogeny was then inferred from each data set using the maximum likelihood (ML) criterion as implemented in GARLI v.2.0 (Zwickl 2006), and Bayesian analysis as implemented in MrBayes v.3.2 (Ronquist and Huelsenbeck 2003). Data sets consisting of concatenated loci were treated as single partitions. Shimodaira-Hasegawa (SH) tests of topology (Shimodaira and Hasegawa 1999) were carried out using PAUP* to further assess the level of incongruence between data sets. Species tree reconstructions were carried out using the coalescence-based Bayesian approach implemented in *BEAST (via BEAST v.1.7.2; Heled and Drummond 2010).

Maximum likelihood analyses in GARLI were carried out with termination conditions at 20,000 generations, and a threshold score 0.05. Each analysis was run with two replicates. Bootstrapping was carried out with 1,000 replicates, with termination after 10,000 generations. Analyses in MrBayes used two replicate runs, each consisting of four chains, sampling every 1,000 generations. Convergence between runs was assessed by observing standard deviations of split frequencies of less than 0.01, and/or by examining plots of split frequencies between runs using the AWTY online interface (Wilgenbusch et al. 2004). If convergence diagnostics did not indicate stationarity after one million generations, analyses were allowed to continue up to 50 million generations, with periodic monitoring, and were stopped after runs had converged. Processing power for longer MrBayes analyses was provided by the NSF TeraGrid via the CIPRES portal (Miller et al. 2010). A burn-in fraction of 25% was specified when summarizing trees.

For species tree analyses, four independent loci were specified; concatenated chloroplast sequences, ETS, PPR 81, and PPR 123. A large analysis including all taxa was run, and a smaller analysis using a reduced sample of taxa (10 species) was also run, to gauge robustness of the inferred topology to

the quantity of input data. Because chloroplast capture through hybridization is common in plants, and is not a mechanism taken into account by the coalescent approach, *BEAST analyses were run both with and without the chloroplast data included. The chloroplast data were treated as an organellar (haploid) locus (with half the effective population size of a bi-parentally inherited locus), and other loci were treated as autosomal. The final analysis used an HKY model for all data sets, default speciation and clock models, and the priors for mean population size and birth rate were set to gamma distributions with shape=2 (additional test analyses were performed using more complex models and various priors). Replicate runs were performed for at least 100 million generations, sampling every 10,000; runs were considered converged when effective sample size (ESS) values were above 200 as assessed using Tracer v.1.5 (Rambaut and Drummond 2007).

Results

Sequences gathered for each DNA accession at each locus were submitted to GenBank (Appendix 1). Chloroplast loci varied in size among individuals, from 640–700 base pairs (bp) for *trnT-trnL*, 825–1,030 bp for *rpl32-trnL*, and 1,075–1,665 bp for *trnQ-rps16*. After alignment, the total number of positions in each data set was: 514 for ETS, 1,221 for PPR 81, 1,325 for PPR 123, 4,266 for chloroplast data combined, 3,060 for nuclear data combined, and 7,326 for all data combined. Due to difficulty in amplifying and sequencing target regions from DNA extracted from herbarium specimens, a few sequences for target loci were partial or missing from the final data sets. The proportion of all sequences that were partial or missing was less than 6% of the total number of sequences in the matrix, and with a few exceptions were from accessions of species represented by another individual (Appendix 1). The total proportion of sites scored as missing data in the final data sets was approximately 20%, including gaps. The concatenated data matrix was submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S14117).

The models of evolution implemented for each data set were: SYM + G for ETS, GTR + G for PPR 81, HKY + G for PPR 123, TVM + G for chloroplast, TVM + I + G for nuclear, and TVM + G for all. Partition homogeneity tests indicated significant differences ($p = 0.01$) between partitions (data sets). Convergence diagnostics indicated that replicate runs over all final Bayesian-based phylogenetic analyses reached stationarity.

Summarized results of phylogenetic analyses of individual loci and chloroplast sequences are depicted in Fig. 2. These trees are largely resolved with support for major clades; topologies from ML and Bayesian analyses were broadly congruent, with minor disagreements over poorly-supported nodes (Figs. S1-S4). The trees inferred from all data are fully resolved with strong support along the backbone of the ingroup; for the concatenated data set, ML and Bayesian analyses inferred identical topologies (Fig. 3: concatenated sequences; Fig. 4: coalescent species tree). Maximum parsimony analyses yielded trees with similar topologies and bootstrap support values to those inferred using model-based methods (results not shown). The results of all analyses identify the same major clades, but reconstruct the relationships between and within them differently (Figs. 2–4).

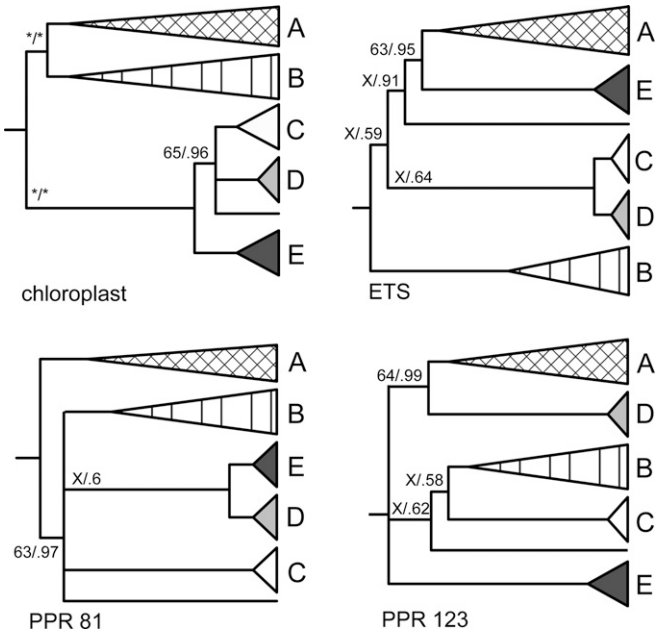

Fig. 2. Schematic summarizing the results of phylogenetic analyses of individual loci, showing conflicting positions of major clades. A. *Lantana-Lippia* clade. B. *Aloysia gratissima* clade. C. *Aloysia citrodora* clade. D. *Aloysia catamarcensis* + *Aloysia polystachya*. E. *Acantholippia salsoloides* + *Acantholippia deserticola*. Single tip represents *Acantholippia trifida*. Support values for the arrangement of major clades are ML bootstrap values/Bayesian posterior probabilities greater than 50%/0.50. Stars denote 100% support, Xs denote bootstrap values below 50%. Phylogenetic reconstructions from individual loci are shown in detail in Figs. S1–S4 (supplementary information).

Topology tests indicate significant incompatibility between the results of analyses of different data sets (Table 1). The results of species tree reconstructions were robust to varying the number of taxa and loci analyzed, and the same topology was inferred from the data using different models and priors (results not shown).

Discussion

Five major clades are consistently inferred from all subsets of the data: 1) the majority of *Aloysia* species are grouped together in a clade that also includes *Xeroaloysia* (hereafter referred to as the *A. gratissima* clade; Figs. 3B, 4B); 2) the type species of *Aloysia*, *A. citrodora*, occurs in a small clade (hereafter referred to as the *A. citrodora* clade; Figs. 3C, 4C); 3) *Aloysia catamarcensis* Moldenke and *Aloysia polystachya* (Griseb.) Moldenke are each other's closest relatives (Figs. 3D, 4D); 4) the type species of *Acantholippia*, *A. salsoloides* Griseb., is reconstructed in a sister relationship with *Acantholippia deserticola* (Phil.) Moldenke (Figs. 3E, 4E); 5) there is a well-supported clade of *Lippia* and *Lantana* species, including the small genera *Phyla* and *Nashia* (Figs. 3A, 4A), consistent with the results of previous studies (Marx et al. 2010; Lu-Irving and Olmstead 2013). Three Mexican species of *Aloysia* form a clade nested within the *Lantana-Lippia* clade (the remaining North American endemics sampled, *A. macrostachya* (Torr.) Moldenke and *A. wrightii* A. Heller, are sister species belonging to the *A. gratissima* clade). *Acantholippia seriphioides* (A. Gray) Moldenke is sister to the *Lantana-Lippia* clade (the *Lantana-Lippia* clade is hereafter described as including *A. seriphioides*, and the three *Aloysia*

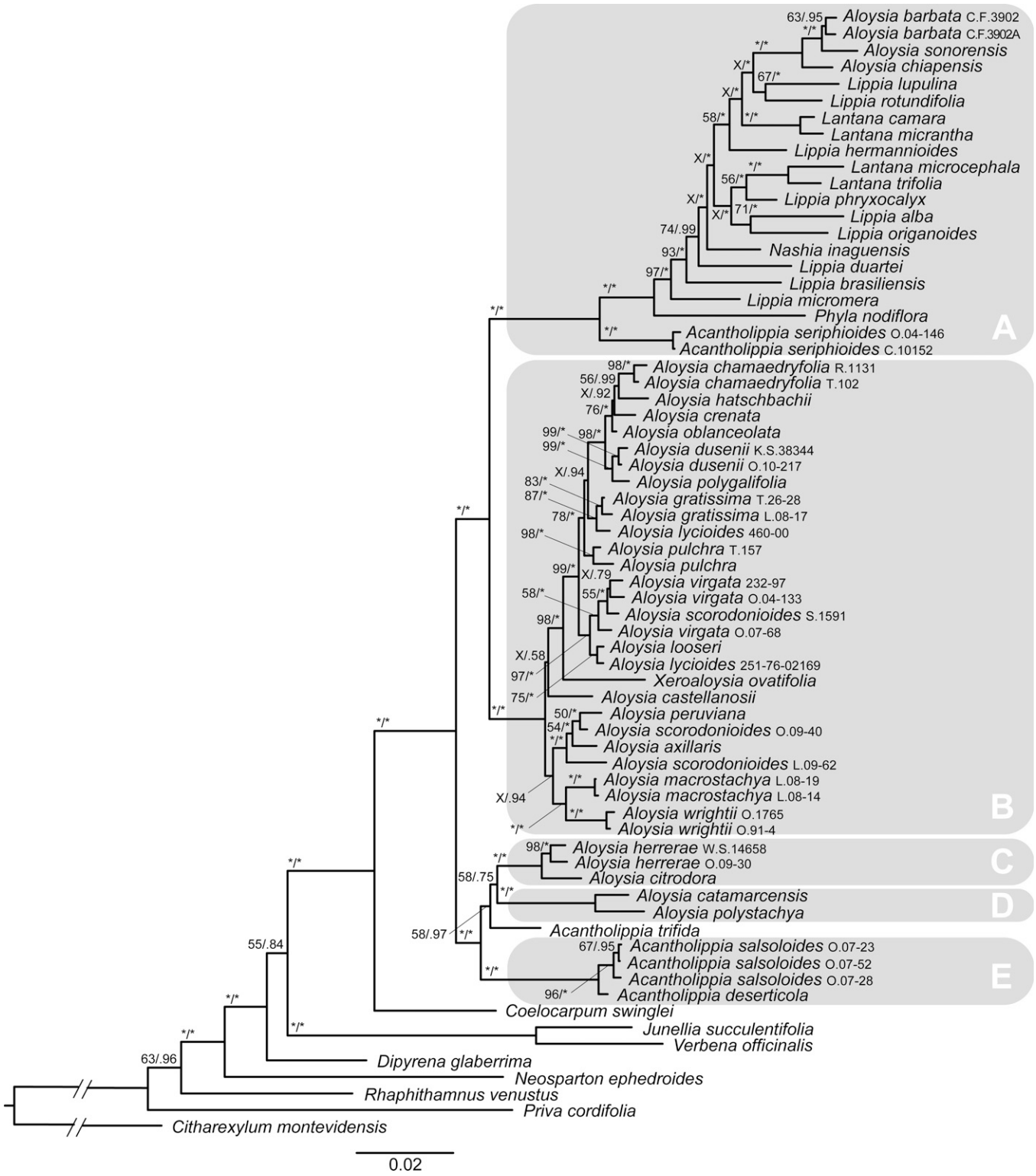

FIG. 3. Phylogeny inferred from 7,326 aligned positions of DNA sequence data from 3 chloroplast and 3 nuclear loci in combination. Topology inferred by ML and Bayesian analyses, branch lengths inferred by Bayesian analysis. Branches are labeled with ML bootstrap values/Bayesian posterior probabilities greater than 50%/0.50. Stars denote 100% support, Xs denote bootstrap values below 50%. A. *Lantana-Lippia* clade. B. *Aloysia gratissima* clade. C. *Aloysia citrodora* clade. D. *Aloysia catamarcensis* + *Aloysia polystachya*. E. *Acantholippia salsoloides* + *Acantholippia deserticola*.

species that nest within it). *Acantholippia trifida* (Gay) Moldenke is positioned on its own, not consistently part of a larger clade.

Major Clades of Aloysia and Acantholippia species—These results provide the first sufficiently representative sample of *Aloysia* and *Acantholippia* to allow us to identify and describe the clades to which these species belong.

THE *ALOYSIA GRATISSIMA* CLADE—Figures 3B, 4B. This clade includes the majority of *Aloysia* species, including *Xeroaloysia ovatifolia*. These species have more or less elongate, lax

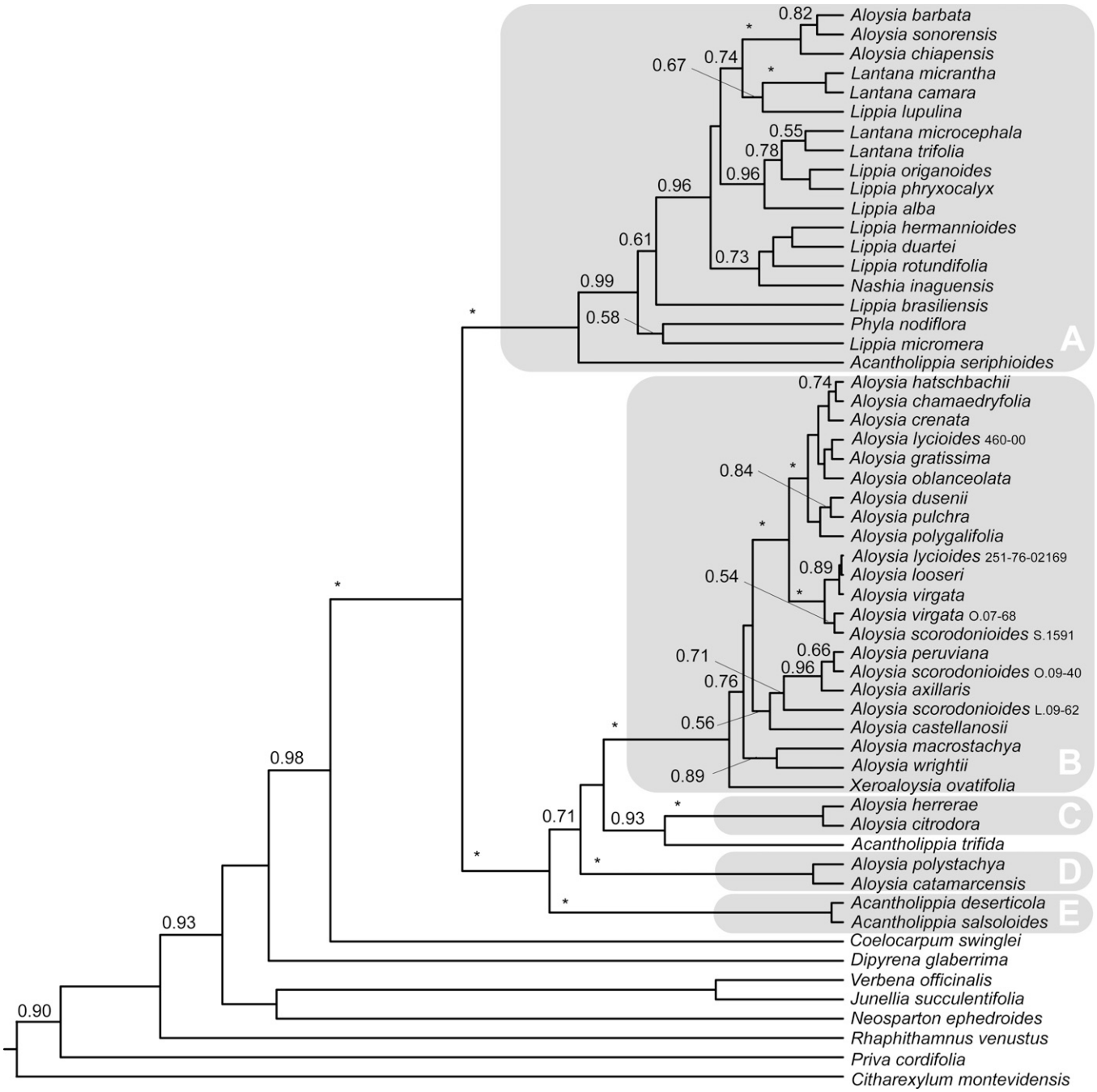

FIG. 4. Maximum clade credibility tree inferred using *BEAST, from 3 combined chloroplast loci and 3 individual nuclear loci. Branches are labeled with posterior probabilities greater than 0.50, rounded to two decimal places; stars denote posterior probabilities of 1. A. *Lantana-Lippia* clade. B. *Aloysia gratissima* clade. C. *Aloysia citrodora* clade. D. *Aloysia catamarcensis* + *Aloysia polystachya*. E. *Acantholippia salsoloides* + *Acantholippia deserticola*.

inflorescences, occurring in axillary arrangements (homothetic pleiobotrya sensu O'Leary et al. 2012; Fig. 5). Three subclades within the *A. gratissima* clade, corresponding with geographic distribution, are consistently recovered (Figs. 3–5): a North American subclade (two species: *A. macrostachya* and *A. wrightii*), an Andean subclade (*A. axillaris* J. R. I. Wood and *A. peruviana* (Turcz.) Moldenke, together with Peruvian accessions of *A. scorodonioides*), and a predominantly subtropical South American subclade. Support for the positions of *A. castellanosii* Moldenke and *X. ovatifolia* within the *A. gratissima* clade is consistently low. The Andean and North American subclades are reconstructed as sister to one another in the analysis of concatenated data (Fig. 3), but this relationship is not found in the analyses of individual loci (Figs. S2–S4); they are not sister to one another in the species tree, but support for their positions is low (Fig. 4). At least two independent range shifts into North America are evident in *Aloysia* (excluding the species nesting within the *Lantana-Lippia* clade; Fig. 5), but it is unclear from these results whether North American distributions are due to northward migration via the Andes, or to long-distance dispersal.

Individuals identified morphologically as *A. scorodonioides* and *A. virgata* do not form monophyletic groups, confirming the suspicion that the boundaries of these species are not yet

TABLE 1. The results of Shimodaira-Hasegawa test comparisons between trees inferred from different data sets.

	Tree					
Data set	ETS	PPR81	PPR123	Combined cp	Combined nr	All combined
ETS	(best)	0	0	0	0.125	0
PPR81	0	(best)	0	0	0.190	0.044
PPR123	0	0	(best)	0	0.006	0
Combined cp	0	0	0	(best)	0	0.008
Combined nr	0	0.015	0	0	(best)	0.073
All combined	0	0	0	0	0.002	(best)

well understood. Likewise, taxa that have been considered synonymous with *A. gratissima* (*A. looseri* Moldenke and *A. lycioides* Cham.) do not cluster together. Branch lengths are short throughout the *A. gratissima* clade, indicative of recent radiation. A population-level approach to sampling, data gathering, and analysis may be required to gain insight into the identities and evolutionary histories of species belonging to this clade.

THE *ALOYSIA CITRODORA* CLADE—Figures 3C, 4C. This clade includes the type species of *Aloysia*, *A. citrodora*, together with *A. herrerae*. A third species, *A. fiebrigii* (Hayek) Moldenke, morphologically similar to *A. herrerae*, is expected to belong to this clade. Inflorescences in these species are arranged in both axillary and terminal positions (heterothetic pleiobotrya sensu O'Leary et al. 2012; Fig. 5). These species are found naturally in allopatric distributions from Argentina and southern Bolivia (*A. citrodora* and *A. fiebrigii*) to southern Peru (*A. herrerae*), but *A. citrodora* is cultivated worldwide.

ALOYSIA POLYSTACHYA AND *ALOYSIA CATAMARCENSIS*—Figures 3D, 4D. These species, both from northern Argentina, have condensed, axillary inflorescences (homothetic pleiobotrya sensu O'Leary et al. 2012; Fig. 5). Their geographic distributions include some overlap, but they are not suspected to form hybrids (Siedo 2006). *Aloysia polystachya* and *Acantholippia salsoloides* are the only members of Lantaneae with alternate leaves.

ACANTHOLIPPIA SALSOLOIDES AND *ACANTHOLIPPIA DESERTICOLA*—Figures 3E, 4E. These species have condensed inflorescences, both axillary and terminal (heterothetic pleiobotrya sensu O'Leary et al. 2012; Fig. 5), and occur in semi-arid to arid habitats in subtropical South America, near the borders between Argentina, Chile, and Bolivia. This clade is also predicted to include *Acantholippia tarapacana* Botta and *Acantholippia riojana* Hieron. ex Moldenke, which share morphological features (such as inflorescence arrangement and spiny branches) in common with *A. salsoloides* and *A. deserticola*.

ACANTHOLIPPIA TRIFIDA—This species is reconstructed as discrete from any other clade. It is superficially similar to members of the *A. salsoloides* clade, but lacks spines, and its condensed inflorescences are axillary only (homothetic pleiobotrya sensu O'Leary et al. 2012; Fig. 5). *Acantholippia trifida* is endemic to north-central Chile, ranging just across the border into Argentina.

ACANTHOLIPPIA SERIPHIOIDES—This species is consistently and confidently reconstructed in a sister relationship with the *Lantana-Lippia* clade. It possesses xerophytic adaptations in common with other species of *Acantholippia*, such as reduced leaves, but several characters unite it morphologically with the *Lantana-Lippia* clade: its inflorescences are condensed and axillary only (homothetic pleiobotrya sensu O'Leary et al. 2012; Fig. 5), and its calyx is bilabiate (Botta 1980). *Acantholippia seriphioides* is widespread and abundant in dry habitats in southern Argentina.

ALOYSIA BARBATA AND RELATIVES—This subclade comprises five species (only three sampled here) with condensed inflorescences featuring conspicuous floral bracts, and bifid calyces, indicative of their common ancestry with the rest of the *Lantana-Lippia* clade (Fig. 5). It is unclear why these species have been considered members of *Aloysia*; the first so named was transferred from *Lippia* without accompanying justification by Moldenke (1940), who then described the remainder under *Aloysia*. All five are endemic to Mexico; two (*A. nahuire* Gentry & Moldenke and its segregate, *A. coalcomana* Siedo) are known only from single collections and may be extinct (Siedo 2006).

***Gene Tree Incongruence and Species Tree Inference*—**We find incongruence between loci with regards to reconstructing the relationships between major clades (Fig. 2; Figs. S1–S4). The chloroplast tree identifies the *A. gratissima* clade in a sister relationship with the *Lantana-Lippia* clade, with high confidence. Also inferred from chloroplast data is a strongly-supported clade consisting of the *A. citrodora* clade, *Aloysia catamarcensis* + *A. polystachya*, *Acantholippia salsoloides* + *A. deserticola*, and *Acantholippia trifida*. This clade is placed sister to the rest of Lantaneae (excluding *Coelocarpum*), with high confidence (Fig. 2, Fig. S1). None of the analyses of individual nuclear loci recover these relationships. Trees inferred from individual nuclear loci disagree on the sister group of the *Lantana-Lippia* clade, with moderate support in each case. It is variously reconstructed as *Acantholippia salsoloides* + *A. deserticola* (ETS; Fig. 2, Fig. S2), a monophyletic group consisting of all other major clades (PPR 81; Fig. 2, Fig. S3), or *A. catamarcensis* + *A. polystachya* (PPR 123; Fig. 2, Fig. S4).

These strongly-supported, yet conflicting topologies suggest different phylogenetic histories among loci (rather than stochastic effects arising from data sampling as the only source of incongruence). The significant differences between data sets indicated by the PHT and SH tests are consistent with this interpretation. Inconsistency between nuclear and chloroplast regions may be due to chloroplast transfer between lineages, occurring when ancestral hybridization events are followed by introgression, resulting in fixation of the captured chloroplast (reviewed by Rieseberg and Soltis 1991; an example in Verbeneae is documented by Yuan and Olmstead 2008a, 2008b). This might have occurred among the major lineages of *Aloysia* and *Acantholippia* species, but a more complicated picture of incomplete lineage sorting and/or gene duplication, perhaps in addition to hybridization, cannot be ruled out (Pamilo and Nei 1988; Maddison 1997).

In cases of incongruence between phylogenetic estimates from independent loci, two approaches to infer the species tree are commonly employed. Concatenation of sequences from different loci into a supermatrix, analyzed as a single data set, is one approach (the "total evidence" argument of Kluge 1989), and may be preferred when differences among gene trees derive only from stochastic sampling effects (Olmstead and Sweere 1994; Gadakgar et al. 2005). An alternative approach, which has become popular over the last decade, is to consider each gene tree as a data point from which a species tree may be inferred (Doyle 1992; Maddison

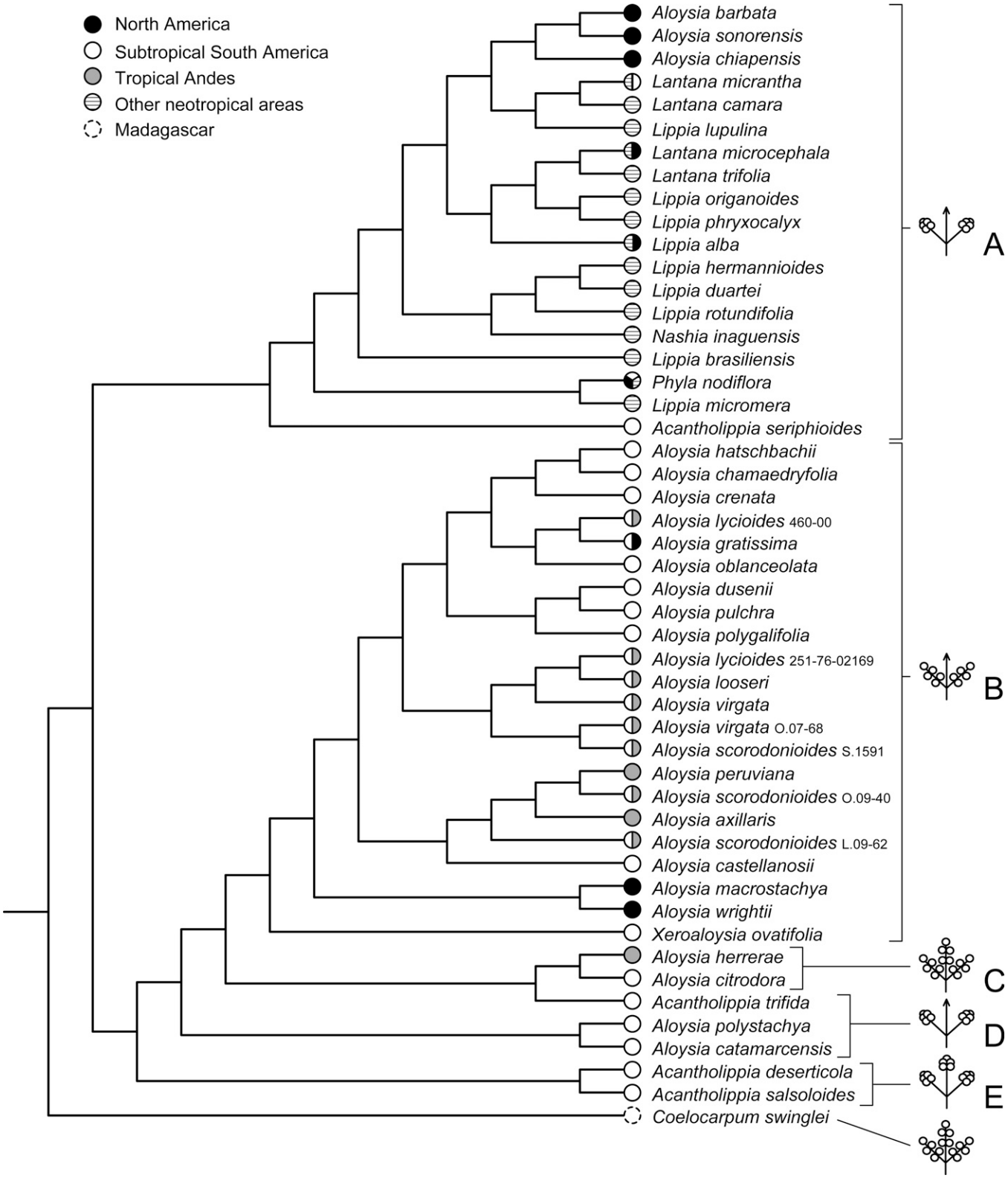

FIG. 5. Geographic distributions and inflorescence types mapped on to the *BEAST tree. A. Condensed inflorescence; terminal inflorescence absent. B. Lax inflorescence; terminal inflorescence absent. C. Lax inflorescence; terminal inflorescence present. D. Condensed inflorescence; terminal inflorescence absent. E. Condensed inflorescence; terminal inflorescence present.

1997; Slowinski and Page 1999). The most well-developed computational tools to do this are based on coalescent theory (reviewed by Degnan and Rosenberg 2009) and assume that incongruence between genes is due to lineage sorting effects, as might be expected when ancestral population sizes are large and branch lengths are short (Pamilo and Nei 1988). Coalescent-based approaches explicitly account for potentially different phylogenetic histories between loci;

these have been shown to recover the species tree more reliably than concatenation (Edwards et al. 2007; Leaché and Rannala 2011).

Here we have explored both a concatenation and a coalescent approach. The combined analysis of all data echoes the chloroplast tree, finding strong support for a sister relationship between the *A. gratissima* clade and the *Lantana-Lippia* clade, and strong support for a third monophyletic group, comprising the remainder of the major clades, as sister to both, with high confidence. There is a lack of signal for any of these relationships among individual nuclear loci, and also in the combined nuclear data. Given the relatively large quantity of chloroplast data, and its strong phylogenetic signal, it seems likely that the chloroplast gene history is masking the conflicting histories of the nuclear loci in the combined analysis. In contrast, the tree inferred from all data using *BEAST strongly supports a sister relationship between the *Lantana-Lippia* clade and a monophyletic group consisting of *Aloysia* and *Acantholippia* clades. This result is consistent with the topology of one nuclear gene tree (PPR 81), implying that the phylogenetic history of this locus is the same as the species tree. Neither analysis of all data (concatenated or coalescent) reconstructs shallower relationships between major clades with high confidence (Fig. 3C–E, Fig. 4C–E).

Patterns of Trait Evolution—Consideration of morphological trait evolution in light of these phylogenetic results might yield further insight into the relationships among major clades of *Aloysia* and *Acantholippia* species. O'Leary et al. (2012) identified two traits that varied in potentially informative ways among major clades within Lantaneae: the presence or absence of a terminal unit in the arrangement of inflorescences, resulting in either determinate or indeterminate compound structures (heterothetic vs. homothetic pleiobotrya), and the number of calyx lobes.

Homothetic pleiobotrya sensu O'Leary et al. (2012) are found in the *Lantana-Lippia* clade, the *A. gratissima* clade, *A. catamarcensis* + *A. polystachya*, and *Acantholippia trifida* (Fig. 5). This pattern was interpreted as resulting from two parallel losses of the terminal inflorescence, based on chloroplast topology and limited sampling, where one shift from heterothetic to homothetic pleiobotrya was interpreted as a synapomorphy for the *A. gratissima* clade + *Lantana-Lippia* clade (O'Leary et al. 2012). Our results, based on increased data and sampling, suggest a more complicated pattern of trait evolution (Fig. 5), involving at least three shifts between heterothetic and homothetic pleiobotrya.

The number of calyx teeth has traditionally been used to separate *Aloysia* and *Acantholippia* (with 4-lobed calyces) from members of the *Lantana-Lippia* clade (with bifid or truncate calyces). This was interpreted by O'Leary et al. (2012), based on a chloroplast phylogeny, as a progressive reduction in the number of calyx teeth from five in the rest of Verbenaceae, to four in Lantaneae, to two in the *Lantana-Lippia* clade. Our findings prompt re-interpretation of the evolution of this trait. Close examination of the morphology of *Acantholippia seriphioides* reveals that the calyx is bilobed, with each lobe only minutely 2-toothed (Botta 1980). This suggests homology with the 2-lobed calyx characterizing the rest of the *Lantana-Lippia* clade, rather than with the evenly 4-lobed calyces of the other species of *Acantholippia*, to which this species is unrelated. Thus, according to the species tree topology (Fig. 4), the *Aloysia* + *Acantholippia* clade is characterized by the synapomorphy of an evenly 4-lobed calyx (with one exception, *A. dusenii* Moldenke, representing an independent shift to a bilobed calyx).

Based on the results presented here, the condensed inflorescence found in *Acantholippia* species, *Aloysia polystachya* + *Aloysia catamarcensis*, and the *Lantana-Lippia* clade (Fig. 5) is most parsimoniously interpreted as representative of the ancestral condition in core Lantaneae (excluding *Coelocarpum*). Both of our combined data analyses suggest that the lax inflorescence characteristic of *Aloysia* as traditionally circumscribed is derived twice independently: in the *A. gratissima* clade, and in the *A. citrodora* clade.

Taxonomic Recommendations—*Aloysia* and *Acantholippia* are not monophyletic, requiring revision. *Xeroaloysia ovatifolia* nests within a clade of *Aloysia* species and cannot be maintained in its own genus (without fragmenting *Aloysia*). Interpreting gene tree incongruence with the intent to realign generic boundaries to coincide with monophyletic groups is challenging. To produce a revision that best reflects what is known about the phylogeny of these genera, we outline and discuss three potential approaches:

1) Discount the potential problems caused by incompatible gene histories, and accept the tree inferred from concatenated loci (Fig. 3) as the best estimate of the species tree. Recognizing the three major clades reconstructed by the chloroplast tree would require the absorption of most *Acantholippia* species into *Aloysia*, and the transfer of the majority of *Aloysia* species (those belonging to the *A. gratissima* clade) into *Xeroaloysia*. *Acantholippia seriphioides* and the Mexican *Aloysia* species nested within the *Lantana-Lippia* clade would require new names or combinations, pending a detailed revision of *Lantana* and *Lippia*. This scheme would require around 25 new combinations (not including *Acantholippia seriphioides* and the *Aloysia* species nesting within the *Lantana-Lippia* clade).

This is inadvisable because the relationships between clades inferred on the combined tree are only compatible with the chloroplast gene tree, and it is apparent that the chloroplast genome and the nuclear regions sampled here have different phylogenetic histories. For this reason, it cannot be assumed that the tree inferred from concatenated data is a good estimate of the species tree. Furthermore, diagnostic morphological traits to satisfactorily define the newly circumscribed *Aloysia* and *Xeroaloysia* are lacking.

2) Circumscribe genera to match only the well supported monophyletic groups consistently inferred among all independent loci. This would result in a much-reduced *Aloysia* and *Acantholippia*, while requiring the species belonging to the *Aloysia gratissima* clade to be transferred to *Xeroaloysia*, as described above. It would require a new genus to be erected for *A. catamarcensis* + *A. polystachya* and another new genus for *Acantholippia trifida*. *Acantholippia seriphioides* and the Mexican *Aloysia* species nested within the *Lantana-Lippia* clade would require new names or combinations, pending a detailed revision of *Lantana* and *Lippia*. This scheme would require two new genera, and around 25 new combinations (not including *Acantholippia seriphioides* and the *Aloysia* species nesting within the *Lantana-Lippia* clade).

As with the previous solution, there is the problem of defining the recircumscribed genera morphologically. Morphological traits simply do not provide good indication of evolutionary relationships among these species, with variation being either homoplastic or uninformative within the major clades outlined above. Furthermore, it is our opinion that splitting the species of *Aloysia* and *Acantholippia* among

five genera would be a poor representation of their close affiliation with one another. Another potential problem with this plan is that the evolutionary relationships of species not represented in our phylogenetic analyses might be other than predicted, which would result in a need for additional generic revisions in the future.

3) Accept the results of the *BEAST analysis as the best estimate of the species tree. According to this phylogenetic reconstruction, most *Aloysia* and *Acantholippia* species belong to a monophyletic lineage sister to the *Lantana-Lippia* clade. This prompts the absorption of some species of *Acantholippia* and *Xeroaloysia ovatifolia* into *Aloysia*, leaving the majority of names in *Aloysia* unchanged. *Acantholippia seriphioides* and the Mexican *Aloysia* species nested within the *Lantana-Lippia* clade would require new names or combinations, pending a detailed revision of *Lantana* and *Lippia*. This scheme would require five new combinations (not including *Acantholippia seriphioides* and the *Aloysia* species nesting within the *Lantana-Lippia* clade).

This is, in our opinion, the best solution. We consider the coalescent approach to provide the best estimate of the species tree, for reasons argued above. The monophyletic lineage comprising most of *Aloysia* (including *Xeroaloysia*) and *Acantholippia* reconstructed in species tree analyses is strongly supported (Fig. 4) and robust to varying the models, taxa, and loci analyzed (results not shown). The expanded *Aloysia* can be recognized, and distinguished from the *Lantana-Lippia* clade, by the morphological synapomorphy of the 4-lobed calyx. *Acantholippia seriphioides* should be excluded from *Aloysia* s. l., as should the North American *Aloysia* species nested within the *Lantana-Lippia* clade. These species could be transferred to *Lippia*, but this would be premature because *Lippia* and its affiliated genera are not monophyletic and will need extensive revision. We defer the creation of new combinations for these species until a detailed phylogenetic study of the *Lantana-Lippia* complex is completed.

Taxonomic Treatment

Based on the results and arguments presented here, we propose expanding the definition of *Aloysia* to include all members of Lantaneae with evenly 4-lobed calyces. These include all the species currently described under *Aloysia* (except the North American species with 2-lobed calyces), *Xeroaloysia ovatifolia*, and all but one of the species of *Acantholippia* (excluding *Acantholippia seriphioides*, but including the type, *A. salsoloides*). The following five new combinations and one resurrected taxon name are proposed at this time:

Aloysia ovatifolia Moldenke, Lilloa 5: 379. 1940. *Xeroaloysia ovatifolia* (Moldenke) Troncoso, Darwiniana 12: 51. 1960.

Aloysia salsoloides (Griseb.) Lu-Irving and O'Leary, comb. nov. *Acantholippia salsoloides* Griseb., Abh. Königl. Ges. Wiss. Göttingen 19: 244. 1874. *Lippia salsoloides* (Griseb.) Benth. & Hook. f., Gen. Pl. 2(2): 1143. 1876.

Aloysia deserticola (Phil.) Lu-Irving and O'Leary, comb. nov. *Lippia deserticola* Phil., Anales Univ. Chile 2: 350. 1865. *Acantholippia deserticola* (Phil.) Moldenke, Lilloa 5: 370. 1940.

Aloysia trifida (Gay) Lu-Irving and O'Leary, comb. nov. *Lippia trifida* Gay, Fl. Chil. 5: 29. 1849. *Acantholippia trifida* (Gay) Moldenke, Lilloa 5: 371. 1940.

Aloysia riojana (Hieron. ex Moldenke) Lu-Irving and O'Leary, comb. nov. *Acantholippia riojana* Hieron. ex Moldenke, Phytologia 3: 106. 1949.

Aloysia tarapacana (Botta) Lu-Irving and O'Leary, comb. nov. *Acantholippia tarapacana* Botta, Hickenia 1: 197. 1979.

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Appendix 1. Voucher information of species sampled in this study, and GenBank accession numbers for DNA sequences. Information is as follows: species and authority, specimen geographic origin, voucher information, GenBank numbers for six loci: *trnT-trnL*, *rpl32-trnL*, *trnQ-rps16*, ETS, PPR 81, PPR 123. Dashes denote missing data.

Acantholippia deserticola (Phil.) Moldenke. ARGENTINA. *Biurrun 4963* (SI), KF688743, KF688781, KF688856, KF688820, -, KF688924. ***Acantholippia salsoloides*** Griseb. ARGENTINA. *Olmstead 07-23* (WTU), KF688744, KF688782, KF688857, -, KF688890, KF688925; *Olmstead 07-28* (WTU), JX966953, JX966845, JX966899, JX966792, JX966695, JX966746; *Olmstead 07-52* (WTU), KF688745, KF688783, KF688858, KF688821, KF688891, KF688926. ***Acantholippia seriphioides*** (A.Gray) Moldenke. ARGENTINA. *Correa et al. 10152* (SI), KF688746, KF688784, KF688859, KF688822, -, -; *Olmstead 04-146* (WTU), JX966954, JX966846, JX966900, JX966793, JX966696, JX966747. ***Acantholippia trifida*** (Gay) Moldenke. ARGENTINA. *Biurrun 7706* (SI), KF688747, KF688785, KF688860, KF688823, KF688892, KF688927. ***Aloysia axillaris*** J. R. I.Wood. BOLIVIA. *Wood & Atahuachi 21575* (KEW), KF688749, KF688787, KF688862, KF688825, KF688894, KF688929. ***Aloysia barbata*** (Brandegee) Moldenke. MEXICO. *Carter & Ferris 3902* (US), KF688750, KF688788, KF688863, KF688826, KF688895, KF688930; *Carter & Ferris 3902A* (TEX), JX966955, JX966847, JX966901, JX966794, JX966697, JX966748. ***Aloysia castellanosii*** Moldenke. ARGENTINA. *Ferriencia 41191* (MERL), KF688751, KF688789, KF688864, KF688827, KF688896, KF688931. ***Aloysia catamarcensis*** Moldenke. ARGENTINA. *Olmstead 07-82* (WTU), KF688752, KF688790, KF688865, KF688828, KF688897, KF688932. ***Aloysia chamaedryfolia*** Cham. location unknown. *Rimpler 1131* (FB), KF688753, KF688791, KF688866, KF688829, KF688898, KF688933; BRAZIL. *Thode 102* (ICN), KF688754, KF688792, -, KF688830, KF688899, KF688934. ***Aloysia chiapensis*** Moldenke. MEXICO. *Martinez 932* (TEX), KF688755, KF688793, KF688867, KF688831, KF688900, KF688935. ***Aloysia citrodora*** Paláu. ARGENTINA. *Olmstead 07-13* (WTU), JX966956, JX966848, JX966902, JX966795, JX966698, JX966749. ***Aloysia crenata*** Moldenke. ARGENTINA. *Cabrera 29106* (SI), KF688756, KF688794, KF688868, KF688832, KF688901, -. ***Aloysia dusenii*** Moldenke. BRAZIL. *Krapovickas & Schinini 38344* (TEX), KF688757, KF688795, KF688869, KF688833, KF688902, KF688936; *Olmstead 10-217* (WTU), KF688758, KF688796, KF688870, KF688834, KF688903, KF688937. ***Aloysia gratissima*** (Gillies & Hook.) Tronc. U. S. A. Texas: *Lu-Irving 08-17* (WTU), KF688761, KF688799, KF688873, KF688837, KF688906, KF688940; *Turner 26-28* (TEX), KF688760, KF688798, KF688872, KF688836, KF688905, KF688939. ***Aloysia hatschbachii*** Moldenke. BRAZIL. *Hatschbach 51897* (US), KF688764, KF688802, -, KF688840, KF688909, KF688943. ***Aloysia herrerae*** Moldenke. PERU. *Olmstead 09-30* (WTU), KF688765, KF688803, JX966903, JX966796, KF688910, JX966750; BOLIVIA. *Wood & Serrano 14658* (KEW), KF688759, KF688797, KF688871, KF688835, KF688904, KF688938. ***Aloysia looseri*** Moldenke. ECUADOR. *Roig 9847* (MERL), KF688766, KF688804, KF688876, KF688841, -, KF688944. ***Aloysia lycioides*** Cham. cultivated. *R.B.G. Kew #251-76-02169* (living), KF688767, KF688805, KF688877, KF688842, KF688911, KF688945; cultivated. *J.B. Valencia #460-00* (living), JX966958, JX966850, JX966904, JX966797, JX966700, JX966751. ***Aloysia macrostachya*** (Torr.) Moldenke. U. S. A. Texas: *Lu-Irving 08-14* (WTU), KF688769, KF688807, JX966905, JX966798, JX966701, JX966752; *Lu-Irving 08-19* (WTU), KF688768, KF688806, JX966905, KF688843, KF688912, KF688946. ***Aloysia oblanceolata*** Moldenke. BRAZIL. *Thode 96* (ICN), KF688763, KF688801, KF688875, KF688839, KF688908, KF688942. ***Aloysia peruviana*** (Turcz.) Moldenke. PERU. *Olmstead 09-45* (WTU), KF688748, KF688786, KF688861, KF688824, KF688893, KF688928. ***Aloysia polygalifolia*** Cham. BRAZIL. *Thode 398* (ICN), KF688770, KF688808, KF688878, KF688844, KF688913, KF688947. ***Aloysia polystachya*** (Griseb.) Moldenke. ARGENTINA. *Kranz 817* (CESJ), KF688771, KF688809, KF688879, KF688845, KF688914, KF688948. ***Aloysia pulchra*** (Briq.) Moldenke. ARGENTINA. *Olmstead 04-129* (WTU), KF688772, KF688810, KF688880, KF688846, KF688915, KF688949; BRAZIL. *Thode 157* (ICN), KF688762, KF688800, KF688874, KF688838, KF688907, KF688941. ***Aloysia scorodonioides*** Cham. PERU. *Lu-Irving 09-62* (WTU), KF688775, KF688813, KF688883, KF688849, KF688917, KF688952; *Olmstead 09-40* (WTU), KF688777, KF688815, KF688885, KF688851, KF688919, KF688954; ARGENTINA. *Saravia 1591* (SI), KF688773, KF688811, KF688881, KF688847, -, KF688950. ***Aloysia sonorensis*** Moldenke. MEXICO. *Reichenbacher 85-1108* (TEX), KF688776, KF688814, KF688884, KF688850, KF688918, KF688953. ***Aloysia virgata*** (Ruiz & Pav.) Pers. ARGENTINA. *Olmstead 04-133* (WTU), EF571570, KF688816, KF688886, KF688852, KF688920, FJ549276; *Olmstead 07-68* (WTU), KF688774, KF688812, KF688882, KF688848, KF688916, KF688951; cultivated. *J.B. Valencia #232-97* (living), JX966960, JX966852, JX966906, JX966799, JX966702, JX966753.

Aloysia wrightii A.Heller. cultivated. *Ocampo 1765* (RSA), KF688778, KF688817, KF688887, KF688853, KF688921, KF688955; U. S. A. Arizona: *Olmstead 91-004* (WTU), KF688779, KF688818, KF688888, KF688854, KF688922, KF688956. ***Citharexylum montevidense*** (Spreng.) Moldenke. ARGENTINA. *Olmstead 04-102* (WTU), JX966962, JX966854, JX966908, JX966801, JX966703, FJ549285. ***Coelocarpum swinglei*** Moldenke. MADAGASCAR. *Phillipson et al. 3443* (MO), JX966963, JX966855, JX966909, JX966802, JX966704, JX966755. ***Dipyrena glaberrima*** (Gillies & Hook.) Hook. ARGENTINA. *Olmstead 04-179* (WTU), JX966964, JX966856, JX966910, JX966803, JX966705, FJ549277. ***Junellia succulentifolia*** (Kuntze) Moldenke. ARGENTINA. *Olmstead 10-1* (WTU), JX966965, JX966857, JX966911, JX966804, JX966706, JX966756. ***Lantana camara*** L. cultivated. *Lu-Irving 12-1* (WTU), JX966966, JX966858, JX966912, JX966805, JX966707, JX966757. ***Lantana micrantha*** Briq. ARGENTINA. *Olmstead 07-8* (WTU), JX966972, JX966864, JX966918, JX966811, JX966713, JX966762. ***Lantana microcephala*** A.Rich. cultivated. *Lu-Irving 08-7* (WTU), JX966973, JX966865, JX966919, JX966812, JX966714, JX966763. ***Lantana trifolia*** L. CUBA. *Olmstead 96-98* (WTU), JX966976, JX966868, JX966922, JX966815, JX966716, JX966766. ***Lippia alba*** (Mill.) N.E.Br. ex Britton & P.Wilson. cultivated. *Fairchild Tropical B.G. 71293A* (living), JX966978, JX966870, JX966924, JX966817, JX966718, JX966768. ***Lippia brasiliensis*** (Link) T.R.S. Silva. BRAZIL. *Lu-Irving 10-17* (WTU), JX966980, JX966872, JX966926, JX966819, JX966720, JX966770. ***Lippia duartei*** Moldenke. BRAZIL. *Lu-Irving 10-11* (WTU), JX966982, JX966874, JX966928, JX966821, JX966722, JX966772. ***Lippia hermannioides*** Cham. BRAZIL. *Thode 389* (ICN), JX966986, JX966878, JX966932, JX966825, JX966726, JX966775. ***Lippia lupulina*** Cham. BRAZIL. *Salimena 2941* (CESJ), JX966988, JX966880, JX966934, JX966827, JX966728, JX966777. ***Lippia micromera*** Schauer. cultivated. *Olmstead 92-225* (WTU), JX966990, JX966882, JX966936, JX966829, JX966730, JX966779. ***Lippia origanoides*** Kunth. cultivated. *Lu-Irving 10-18* (WTU), JX966991, JX966883, JX966937, -, JX966731, JX966780; cultivated. *Olmstead 92-210* (WTU), -, -, -, JX966830, -, -. ***Lippia phryxocalyx*** Briq. BRAZIL. *Eiten 4506* (US), KF688780, KF688819, KF688889, KF688855, KF688923, -. ***Lippia rotundifolia*** Cham. BRAZIL. *Salimena 2958* (CESJ), JX966995, JX966887, JX966941, JX966834, JX966734, JX966784. ***Nashia inaguensis*** Millsp. cultivated. *Lu-Irving s. n.* (WTU), JX967000, JX966892, JX966946, JX966839, JX966739, JX966789. ***Neosparton ephedroides*** Griseb. ARGENTINA. *Olmstead 07-77* (WTU), JX967001, JX966893, JX966947, JX966840, JX966740, FJ549279. ***Phyla nodiflora*** (L.) Greene. ARGENTINA. *Olmstead 07-65* (WTU), JX967002, JX966894, JX966948, JX966841, JX966741, JX966790. ***Priva cordifolia*** Druce. SOUTH AFRICA. *Vos 391* (NU), JX967003, JX966895, JX966949, JX966842, JX966742, FJ549281. ***Rhaphithamnus venustus*** B.L.Rob. CHILE. *Stuessy 11855* (OS), JX967004, JX966896, JX966950, JX966843, JX966743, FJ549282. ***Verbena officinalis*** L. cultivated. *Olmstead 03-156* (WTU), EF571525, JX966897, JX966951, FJ867561, JX966744, FJ549252. ***Xeroaloysia ovatifolia*** (Moldenke) Tronc. ARGENTINA. *Olmstead 04-184* (WTU), JX967005, JX966898, JX966952, JX966844, JX966745, JX966791.

Appendix 2. Status of species names used in this study, according to different taxonomic treatments of Aloysia.

Species and authority	O'Leary et al. unpublished	Siedo, 2006
Aloysia axillaris J.R.I.Wood	*A. scorodonioides*	[not included]
Aloysia barbata (Brandegee) Moldenke	accepted, not treated	accepted
Aloysia castellanosii Moldenke	accepted	accepted
Aloysia catamarcensis Moldenke	accepted	accepted
Aloysia chamaedryfolia Cham.	accepted	accepted
Aloysia chiapensis Moldenke	accepted, not treated	accepted
Aloysia citrodora Paláu	accepted	accepted
Aloysia crenata Moldenke	accepted	accepted
Aloysia dusenii Moldenke	accepted	accepted
Aloysia gratissima (Gillies & Hook.) Tronc.	accepted	accepted
Aloysia hatschbachii Moldenke	accepted	accepted
Aloysia herrerae Moldenke	accepted	accepted
Aloysia looseri Moldenke	*A. gratissima*	*A. virgata*
Aloysia lycioides Cham.	*A. gratissima*	accepted
Aloysia macrostachya (Torr.) Moldenke	accepted, not treated	accepted
Aloysia oblanceolata Moldenke	accepted	accepted
Aloysia peruviana (Turcz.) Moldenke	accepted	accepted
Aloysia polygalifolia Cham.	accepted	accepted
Aloysia polystachya (Griseb.) Moldenke	accepted	accepted
Aloysia pulchra (Briq.) Moldenke	accepted	*A. lycioides*
Aloysia scorodonioides Cham.	accepted	accepted
Aloysia sonorensis Moldenke	accepted, not treated	accepted
Aloysia virgata (Ruiz & Pav.) Pers.	accepted	accepted
Aloysia wrightii A.Heller	accepted, not treated	accepted