

**A MOLECULAR PHYLOGENY AND CLASSIFICATION OF
VERBENACEAE¹**

HANNAH E. MARX², NATALY O'LEARY³, YAO-WU YUAN^{2,4}, PATRICIA LU-IRVING²,
DAVID C. TANK⁵, MARÍA E. MÚLGURA³, AND RICHARD G. OLMSTEAD^{2,6}

²Department of Biology and Burke Museum, University of Washington, Seattle, Washington 98195, USA; ³Instituto de Botánica Darwinion, Labardén 200, San Isidro, Argentina; ⁴Present address: Department of Plant Biology, University of Georgia, Athens, Georgia 30602, USA; and ⁵College of Natural Resources and Stillinger Herbarium, University of Idaho, Moscow, Idaho 83844, USA

- *Premise of the study:* Verbenaceae consist of trees, shrubs, lianas, and herbs distributed primarily in Latin America, where they occur in a wide array of ecosystems. A second center of diversity exists in Africa. Competing morphology-based classifications that rely on different traits conflict in significant ways. A broad phylogenetic study was undertaken to assess those classifications and to examine the historical geography of the family.
- *Methods:* Analysis of seven chloroplast DNA regions for 109 species, representing all genera except one monotypic genus, provide inference into evolutionary relationships in Verbenaceae.
- *Key results:* The phylogeny shows that none of the traditional classifications reflect phylogenetic relationships very well. Eight clades are recognized as tribes (Casselleae, Citharexyleae, Duranteae, Lantaneae, Neospartoneae trib. nov., Petreeae, Priveae, and Verbeneae). Two genera, *Dipyrena* and *Rhaphithamnus*, remain unplaced in these larger clades. Petreeae, which consist of Neotropical lianas, are sister to the rest of the family. Lantaneae and Verbeneae together form a derived clade that comprises approximately two-thirds of the species in Verbenaceae.
- *Conclusions:* We present a new tribal classification, including one new tribe, Neospartoneae trib. nov., to accommodate three small genera of Argentine species (*Diostea*, *Neosparton*, and *Lampaya*). Phylogenetic inference suggests a South American origin for Verbenaceae, with approximately six colonization events having given rise to the Old World species.

Key words: biogeography; *ccsA*; classification; *matK*; molecular systematics; *ndhF*; phylogeny; *rbcL*; *rpoC2*; *rps3*; *trnLF*; Verbenaceae.

Verbenaceae are not the family they used to be. The family once was the “grab-bag” for asterid plants with irregular corollas and superior ovaries divided into two biovulate carpels, though not quite so divided as to have a gynobasic style and, thus, be placed in the Lamiaceae. Recent phylogenetic studies (e.g., Cantino, 1992; Olmstead et al., 1993, 2000, 2001; Wagstaff and Olmstead, 1997) have whittled Verbenaceae down to a core of approximately 35 genera and 1000 species (Atkins, 2004;

but see Sanders, 2001, for a lower estimate). The biggest change involves the wholesale transfer of some 10 tribes and over 50 genera to the Lamiaceae (Cantino et al., 1992). Several smaller groups have been segregated into their own or other families as well (e.g., Angiosperm Phylogeny Group, 1998; Beardsley and Olmstead, 2002; Schwarzbach and McDade, 2002). This represents a distinction recognized by Junell (1934) but not accepted by most plant taxonomists until the work of Cantino and colleagues (Abu-Asab and Cantino, 1987, 1989; Cantino, 1990, 1992).

What remains in Verbenaceae s.s. comprises most of Briquet's (1895) subfamily Verbenoideae. The plants in Verbenaceae can usually be recognized in the field by a combination of traits, including being woody trees or shrubs (a few genera, including *Verbena* and *Glandularia*, are herbaceous, and *Petrea* species are lianas) with opposite leaves and flowers with slightly bilateral corolla symmetry. Their fruits are fleshy or dry, generally with two or four seeds, often dividing into two or four segments. The difficulty that led to confusion in distinguishing Verbenaceae from Lamiaceae had to do with the degree of separation of the locules and the position of the style, used by many treatments and keys (e.g., Cronquist, 1981) to distinguish the families. However, the fundamental distinction has to do with where the ovules attach in relation to the false partitions that divide each carpel into two locules. In Verbenaceae, the ovules attach directly to the margins of the false carpel septa, whereas in Lamiaceae, the ovules attach to the sides of the inrolled carpel walls. This distinction is subtle and it is understandable that it was not generally recognized as an important taxonomic trait,

¹ Manuscript received 22 April 2010; revision accepted 25 August 2010.

The authors thank the curators and staff of the herbaria MERL, MO, OS, SI, TEX/LL, and US; the curators and staff of Fairchild Tropical Botanical Garden, Jardín Botánico Nacional de Cuba, Royal Botanical Gardens Edinburgh, Royal Botanical Gardens Kew, Jardín Botánico de Valencia, and Waimea Botanical Garden; and R. Jansen, P. Jenkins, H. Rimpler, and W. Vos for providing materials for this research. Special thanks to I. Peralta, D. Panuaga, E. Santiago-Valentin, and S. Leiva for assistance in the field; to C. Donovan and P. Lingwood for assistance in the laboratory; to R. Pozner for help with the Latin diagnosis; and to two anonymous reviewers for comments on the manuscript. Funding was provided by a University of Washington (UW) Howard Hughes Medical Institute Undergraduate Research Internship and a Mary Gates Research Scholarship from the UW Department of Biology to H.E.M.; a Molecular Plant Systematics Fellowship from the UW Department of Biology to Y.Y. and P.L.-I.; a CONICET postdoctoral fellowship to N.O.; a Smithsonian Senior Fellowship and National Science Foundation (NSF) grants DEB 0542493 and EF-0431184 to R.G.O.; NSF grant DEB 0710026 to R.G.O. and Y.Y.; and a CONICET grant to M.M. and N.O.

⁶ Author for correspondence (e-mail: olmstead@u.washington.edu)

which makes Junell's (1934) emphasis on this distinction all the more insightful.

The placement of Verbenaceae within Lamiales remains uncertain. Prior studies of Lamiales placed the latter within a core of families, including Acanthaceae, Bignoniaceae, Lamiaceae, Orobanchaceae, Scrophulariaceae, and a few small families, among which relationships have been only weakly resolved (Olmstead et al., 2000, 2001; Bremer et al., 2002; Oxelman et al., 2005). Most studies do not support a placement of Verbenaceae with Lamiaceae, although Bremer et al. (2002) found a clade comprising *Lamium* and *Verbena*, the only representatives of the two families in that study. More commonly, Lamiaceae form a clade with Orobanchaceae, Phrymaceae, and Paulowniaceae and a few other small genera (Olmstead et al., 2001; Oxelman et al., 2005; N. Refulio and R. G. Olmstead, unpublished data), while Verbenaceae fall elsewhere, either with Bignoniaceae and Martyniaceae (Olmstead et al., 2001; Oxelman et al., 2005) or with the small African family Thomandersiaceae (N. Refulio and R. G. Olmstead, unpublished data).

Despite the attention paid to Lamiaceae and other families of Lamiales (Cantino, 1992; Scotland et al., 1995; Smith et al., 1997; Wagstaff and Olmstead, 1997; Wagstaff et al., 1998; McDade and Moody, 1999; Oxelman et al., 1999, 2005; Young et al., 1999; Wallander and Albert, 2000; Olmstead et al., 2001, 2009; Albach et al., 2005; Rahmzadeh et al., 2005; Wolfe et al., 2005; Bennett and Mathews, 2006; Tank et al., 2006; Grose and Olmstead, 2007; McDade et al., 2008; Tank and Olmstead, 2008), Verbenaceae have gone largely unexamined phylogenetically. Many of Junell's (1934) insights into the systematics of Lamiaceae and Verbenaceae have been confirmed in studies of the Lamiales but have not been tested in the remaining Verbenaceae. Sanders (2001) alluded to an unpublished morphological cladistic analysis and outlined the basis for a new tribal classification in his treatment of the Verbenaceae of the southeastern United States. A cladistic analysis of iridoid glucosides (von Poser et al., 1997) found taxa from Lamiaceae and Verbenaceae s.s. interdigitated on the tree, and a dissertation by von Mulert (2001), which included 66 Verbenaceae taxa and 61 Lamiales taxa (but none of the former Verbenaceae taxa transferred to Lamiaceae by Cantino et al. [1992] were included), found a monophyletic Verbenaceae s.s. sister to Lamiaceae.

Wagstaff and Olmstead (1997) conducted a study using *rbcL* sequences designed to examine whether the former concept of Verbenaceae and Lamiaceae was valid or not. In that study, most members of Verbenaceae s.l. formed a clade with Lamiaceae, confirming Cantino's conclusions (Cantino, 1992). Representatives of subfamily Verbenoideae fell elsewhere on the tree, but not together forming a clade, because *Petrea* did not group with the other representatives (*Bouchea*, *Rhaphithamnus*, *Stachytarpheta*, and *Verbena*), thus leaving the question of monophyly of Verbenaceae s.s. uncertain. Evidence based on a multigene phylogeny showing that *Petrea* did indeed belong with other Verbenaceae s.s. was presented at the XVI Botanical Congress in St. Louis in 1999 (R. Olmstead, unpublished data). Recently, a representative sampling of Verbenaceae provided a case study for the application of the PPR (pentatricopeptide repeat) gene family, a novel source of nuclear genome data for plant phylogenetics, and, in so doing, also produced the first molecular phylogenetic analysis of Verbenaceae (Yuan et al., 2010). However, by including only a single outgroup species, that study did not constitute a test of monophyly of Verbenaceae.

Phylogenetic studies of groups within the family also are rare. Rueda (1994) conducted a morphological cladistic analysis of *Petrea* and the small tribe Petreeae as part of a monograph of the group. Atkins (2005) presented a morphological cladistic analysis of the Brazilian species of *Stachytarpheta*. As part of our work on Verbenaceae, a series of molecular systematic studies on *Verbena* and relatives (*Glandularia*, *Junellia*, and *Verbena*) have contributed to a better understanding of their evolution and biogeography (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009). This work has helped sort out generic boundaries in that difficult group, resulting in the transfer of one section of *Glandularia* to *Junellia* and segregation of a new genus, *Mulguraea*, from *Junellia* (O'Leary et al., 2009).

The treatment of Verbenaceae for *Families and Genera of Vascular Plants* (Atkins, 2004) includes a review of the taxonomic history of the family. Post-nineteenth-century treatments of the subfamily Verbenoideae (within a broad Verbenaceae s.l.), or Verbenaceae s.s., have recognized from four to seven tribes (Junell, 1934; Moldenke, 1971; Troncoso, 1974; Sanders, 2001; Atkins, 2004). These treatments exhibit broad disagreement about groupings at the deepest level in the family, reflecting the authors' reliance on different characters (e.g., fruit vs. inflorescence) to delineate tribes. The most detailed systematic work done in recent years includes studies of morphology, taxonomy, and floristics by co-authors of the present study and their colleagues at the Instituto Darwinion, Argentina (e.g., Troncoso, 1974, 1980; Botta, 1979, 1980, 1989; Martínez et al., 1996; Múlgura et al., 1998, 2002, 2003; Drewes and Martínez, 1999; Múlgura, 2000; Martínez and Múlgura, 2003; O'Leary and Peralta, 2007; O'Leary et al., 2007a, b, 2008, 2009, 2010; Peralta et al., 2008).

While much smaller than before, Verbenaceae are still a relatively large family and an important element in the flora of North and South America, especially in warm temperate and tropical regions. Verbenaceae include several economically important species used for wood, spices, and ornament. Apart from a few species of some of the largest genera (*Lantana*, *Lippia*, *Priva*, and *Verbena*) and a few small genera endemic to Africa and the Indian Ocean rim (e.g., *Chascanum* and *Coelocarpum*), Verbenaceae are New World in distribution. Verbenaceae include forest trees, shrubs, lianas, and herbs and occur in open and forested, xeric and mesic habitats. In many arid habitats in portions of Argentina, some species of *Junellia* and *Acantholippia* play roles as community dominants, and in some cloud forests of Andean southern Peru, species of *Citharexylum* also may share dominance. *Petrea* contributes to the rich liana flora of wet Neotropical forests (Rueda, 1994; Burnham, 2002). Species of *Glandularia*, *Lippia*, *Lantana*, and *Verbena* are common elements in disturbed sites in many habitats in Latin America, and a few species, most notably *Lantana camara*, have become widespread weeds.

One of the two major radiations in Verbenaceae is the *Verbena* complex (including *Glandularia*, *Junellia*, and *Mulguraea*). The distribution of this group exemplifies a pattern that has been observed in several plant groups, with disjunctions between the arid regions of temperate North and South America (e.g., Lewis and Oliver, 1961; Hunziker, 1975; Simpson et al., 2005). In Verbenaceae, *Glandularia* and *Verbena* both exhibit this distributional pattern (Lewis and Oliver, 1961; UMBER, 1979), whereas *Junellia* and *Mulguraea* are strictly South American. Alternative hypotheses have been raised to explain this pattern, including long-distance dispersal and migration along an Andean corridor (Solbrig, 1972). The direction this

migration has taken in various plant groups is often speculative, except in a few recent studies (e.g., *Larrea*—Lia et al., 2001; *Hoffmannseggia*—Simpson et al., 2005; and *Lycium*—Levin and Miller, 2005) in which south-to-north migration has been confirmed. In both *Glandularia* and *Verbena*, the direction of migration also has been confirmed to be from South America to North America (Yuan and Olmstead, 2008a), but evidence that might provide a test of a possible Andean migration route was missing from that study.

Our goal in this study is to produce a framework phylogeny of Verbenaceae. In so doing, we will identify the major clades within Verbenaceae that will provide the basis for a revised tribal classification of the family. Nearly complete generic sampling is included for two chloroplast DNA regions, *ndhF* and *trnLF*, that have been shown to be effective for generic phylogenies elsewhere in Lamiales and Solanales (Wagstaff et al., 1998; McDade and Moody, 1999; Beardsley and Olmstead, 2002; Olmstead et al., 2008, 2009). Five additional chloroplast DNA regions (*ccsA*, *matK*, *rbcL*, *rpoC2*, and *rps3*) were sequenced for a set of 33 taxa representing all of the suprageneric clades identified in the broader analysis to provide additional evidence for the main stem of the tree. A well-resolved phylogeny also permits interpretation of biogeographic history and provides the basis of future detailed studies of clades within Verbenaceae.

MATERIALS AND METHODS

Sampling in Verbenaceae included 121 samples representing 109 species (Appendix 1) and 18 outgroup species from other families in Lamiales. The 109 ingroup taxa represent 33 genera, including all but one (*Verbenoxylum*) recognized in the classification presented by Atkins (2004). Sampling within genera attempted to reflect generic and tribal diversity in the family, resulting in relatively less sampling of species-rich genera such as *Lippia*, *Lantana*, *Glandularia*, *Citharexylum*, and *Stachytarpheta*. Tissue samples from field collections in the United States, Peru, Cuba, Puerto Rico, Nicaragua, Argentina, and South Africa as well as herbarium specimens were used. In a few cases two accessions were used for a single taxon to obtain information for all of the gene regions. In one case, data from two closely related taxa (O'Leary et al., 2009) were combined into a single terminal in the analysis (data for *Junellia juniperina* are included with *J. uniflora*). Overall, the present study is the most extensive phylogenetic study in terms of both taxa and geographic distribution of the Verbenaceae.

Total genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1987) from silica-dried tissue and herbarium specimens. Amplification and sequencing of *ndhF* and *trnLF* followed protocols described in Olmstead et al. (2008, 2009), except that some new primers specific for Verbenaceae were constructed (for a complete list of primers, see Appendix S1 at <http://www.amjbot.org/cgi/content/full/ajb.1000144/DC1>). Only the more variable 3' half of *rbcL* was amplified and sequenced using standard protocols (Olmstead et al., 1992). Previously published primers were used for *matK*, *rbcL*, and *rpoC2*; for *ccsA* and *rps3*, "universal" primers were designed to work across angiosperms on the basis of the genomic-scale, angiosperm-wide alignments of Moore et al. (2007; see Appendix S1 for primer sequences). Amplified polymerase-chain-reaction products were purified by precipitation from a 20% polyethylene glycol solution and washed in 70% ethanol prior to sequencing.

Sequence chromatograms were edited using Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually using Se-Al 2.0a11 (Rambaut, 2002) or Muscle 3.6 (Edgar, 2004) followed by minor manual adjustments. Gaps were coded as binary characters when present in two or more ingroup taxa (Graham et al., 2000; Simmons and Ochoterena, 2000); gaps were otherwise treated as missing data. The *trnLF* and *ndhF* sequences and the gap matrix were combined into a single data set for analysis. Gaps were not scored for the seven-gene data set. All sequences used in our study have been submitted to GenBank (Appendix 1), and the data sets and trees have been submitted to TreeBASE (accession number S10736).

Three data sets were compiled and analyzed to accommodate the two sampling strategies: (1) all 139 accessions for two regions (*ndhF* and *trnLF*), (2) 33

accessions for all seven regions, and (3) all 139 accessions for all seven regions (with missing data scored for the taxa unsampled for the other five regions).

Parsimony analyses were conducted using PAUP* 4.0b10 (Swofford, 2002) with 500 initial-replicate heuristic searches using random-order-entry starting trees and TBR branch swapping and with MULPARS on, but keeping only five trees per replicate. A second round of analysis followed in which the strict consensus tree obtained from the first round was input as a constraint tree and only trees that were equal to it or shorter were kept. This inverse constraint search (Catalán et al., 1997) eliminates the need to run the heuristic search to completion by swapping on all trees. No additional trees were discovered, however. Support for clades was calculated by bootstrap analysis using 1000 bootstrap replicates following the procedure outlined by DeBry and Olmstead (2000).

We used Modeltest 3.7 (Posada and Crandall, 1998) to determine the model of sequence evolution best fit to the data according to Akaike's information criterion (AIC). Maximum likelihood (ML) analyses were conducted using RAXML 7.2.4 (Stamatakis, 2006; Stamatakis et al., 2008) and consisted of 1000 rapid bootstrap replicates with each gene region treated as a separate partition. Every fifth bootstrap tree generated by the rapid bootstrap analyses was used as a starting tree for full ML searches and the trees with the highest ML scores were chosen. Bayesian phylogenetic analyses were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), again with each gene region treated as a separate partition. Each analysis was conducted using the same models used for the ML analyses and consisted of two runs of 10 million generations from a random starting tree using a variable rate prior, six discrete rate categories to estimate the gamma distribution, and four Markov chains (using the default heating values) sampled every 1000 generations. For the two-gene, 139-taxon data set, parsimony-informative gaps were coded and analyzed as an additional partition under a binary model as implemented in MrBayes (coding = informative to account for only sampling parsimony informative indels). Convergence of the chains was determined by examining the plot of all parameter values and the $-\ln L$ against generation time using Tracer 1.5 (Rambaut and Drummond, 2004). Stationarity was assumed when all parameter values and the $-\ln L$ had stabilized. Burn-in trees were then discarded, and the remaining trees and their associated parameter values were saved. To explore more tree space and to decrease the chance of obtaining stationarity on local optima, two independent analyses were performed for each data set.

RESULTS

Complete sequences of the *trnLF* gene region were obtained for all accessions. Sequence lengths ranged from 903 nucleotides (nt) in *Pitreaea cuneato-ovata* to 941 nt in *Priva lappulacea* and yielded an aligned length of 1232 bp, of which 287 positions were parsimony informative. Several short regions totaling 38 nt could not be aligned unambiguously and were excluded from analysis. Thirty-nine alignment gaps were scored as binary characters for the two-gene analysis. Sequences of *ndhF* were obtained for all accessions, except for one of the two *Citharexylum argutudentatum* accessions. Partial sequences were obtained for 12 accessions that were derived from herbarium specimens. Approximately 700 nt were missing from the 5' end of one accession of *Coelocarpum madagascariense*, and ca. 500 nt were missing from the 3' end of three accessions (*Rhedera penninerva*, *Casselia confertifolia*, and *Verbena carnea*); approximately 100–200 nt were missing at the 3' end of eight other accessions. Sequences for the region amplified ranged from 2076 nt in *Junellia aspera* to 2098 nt in all *Stachytarpheta* species, with an aligned length of 2140 nt, of which 685 positions were parsimony informative. Three gaps were scored as binary characters. The combined data set for the two-gene analysis included 3334 unambiguously aligned positions (972 parsimony informative) and 42 gap characters.

The seven-gene data set had several missing sequences, as follows: *ccsA*—*Lantana canescens*; *rbcL*—*Duranta serratifolia*, *Parodianthus ilicifolius*; *rpoC2*—*Glandularia microphylla*, *Hierobotan inflata*, *Lantana canescens*, *Parodianthus ilicifolius*,

Rehdera trinervis, *Verbena officinalis*; *rps3*—*Aloysia virgata*, *Baillonia amabilis*, *Citharexylum montevidense*, *Glandularia microphylla*, *Hierobotana inflata*, *Lantana canescens*. The remaining three DNA regions, *matK*, *ndhF*, and *trnLF*, were complete for all taxa. Sequences of *ndhF* and *trnLF* for *Junellia uniflora* were combined with sequences of the other five regions for the closely related species *J. juniperina* (O'Leary et al., 2009) in the analyses.

The results of the ILD test (Farris et al., 1994; implemented in PAUP*) indicated that the *ndhF* and *trnLF* regions were not significantly different from two random partitions of the same size ($P = 0.19$), and the parsimony analyses of the individual gene regions yielded highly similar trees (not shown) that identified the same major clades and relationships among them, although with lower support values in several cases. Parsimony analysis yielded numerous equally most-parsimonious trees (length = 5605; consistency index = 0.63; retention index = 0.85).

To account for the inherent differences that exist between loci in base composition and among-site rate variation, both ML and Bayesian phylogenetic analyses were fully partitioned, with each gene region treated as a separate partition. For each partition in the 33-taxon data set, model selection using AIC, as implemented in Modeltest, resulted in one of the following models of sequence evolution: GTR+G (GTR = general time reversible, G = gamma-distributed variable sites; *ccsA*, *trnL*), TVM+G (TVM = transversional, a submodel of GTR with only five substitution types in the rate matrix, as opposed to six in the GTR model; *matK*, *rpoC2*, *rps3*), GTR+I+G (I = proportion of invariable sites; *rbcl*), or TVM+I+G (*ndhF*). For the 139-taxon data set, model selection resulted in the TVM+I+G model of sequence evolution for the *ndhF* partition and the TVM+G model for the *trnL* partition. Following the recommendation of Ronquist and Huelsenbeck (2003), the parameter for the proportion of invariable sites (I) was not implemented but, rather, the GTR+G model was implemented and the number of discrete rate categories used to estimate the gamma shape parameter was increased from four to six. Although runtime is increased linearly with an increase in the number of rate categories, this modification from the default parameters has been shown to improve convergence properties (Ronquist and Huelsenbeck, 2003). Likewise, Stamatakis (2006) discourages the use of the I parameter, and, therefore, our ML analyses used the GTR+G model of sequence evolution as implemented by the rapid bootstrap algorithm in RAxML (Stamatakis et al., 2008).

Bayesian analyses of the 33-taxon and 139-taxon data sets all achieved apparent stationarity after a maximum of ca. 250 000 generations; however, because this analysis contained long chains (10 million generations), a conservative burn-in of 1 million generations was used. To avoid the pitfall of achieving apparent stationarity on a local optimum, two independent Bayesian analyses were conducted for each data set; in both analyses all parameters reached stationarity at the same level, and these results represent the combination of these two independent analyses.

These analyses provide strong support for monophyly of Verbenaceae and identify a series of well-supported clades (Figs. 1–3), some of which correspond to tribal-level groups in recent classifications (Troncoso, 1974; Sanders, 2001; Atkins, 2004), but with many differences in relation to those treatments. The main stem of the tree also is moderately to well supported, and resolution is weak or missing only among terminal clades of closely related species (e.g., within tribe Lantaneae and

Citharexylum). The results of the two-gene by 139-taxon analyses (not shown) were consistent with the seven-gene by 139-taxon analyses with respect to all substantive relationships.

DISCUSSION

This study provides the first phylogeny of Verbenaceae with comprehensive, genus-level sampling. By including sufficient outgroup sampling, this study confirms the monophyly of Verbenaceae (ML bootstrap [bs] = 98%; maximum parsimony bs = 92%; posterior probability [pp] = 1.0; also a unique 6-nt deletion in *trnLF*). The results are consistent with the phylogenetic reconstruction based on limited taxonomic sampling and using five nuclear gene regions by Yuan et al. (2010). In that study, two isolated genera (*Diphyrena* and *Rhaphithamnus*) occur in slightly different positions than found here, but relationships elsewhere in our tree are congruent with that study. By contrast, the phylogeny of von Mulert (2001), based on morphology and secondary chemistry, is incongruent with our results in many respects. Similarities between that study and ours include his finding clades that match our Verbenaceae and Lantaneae (*Coelocarpum* was not sampled by von Mulert) and one that comprises *Bouchea*, *Chascanum*, and *Stachytarpheta*. Those clades all represent closely allied groups of genera recognized in almost all traditional classifications. Relationships among the main branches of von Mulert's tree do not coincide with our results in any way, including his finding the traditional group comprising *Citharexylum*, *Duranta*, and *Rhaphithamnus*, which we did not find. The phylogeny reconstruction presented here (Fig. 1) identifies eight major clades in addition to the two isolated genera just mentioned. The poor fit of clades discovered here with the composition of many recognized tribes from previous classifications (Briquet, 1895; Junell, 1934; Moldenke, 1971; Troncoso, 1974; Sanders, 2001; Atkins, 2004) indicates the need for a revised classification of Verbenaceae.

Clades of Verbenaceae—Relationships among the eight named clades are also well resolved in our phylogenetic reconstruction. The small group Petreeae is sister to the rest of the family, with Duranteae forming the second branch. Casseleeae and Citharexyleae together form a well-supported clade, which is sister to a large clade comprising Priveae, Neospartoneae trib. nov., Verbenaceae, Lantaneae, and the two small genera *Diphyrena* and *Rhaphithamnus*. Priveae, *Rhaphithamnus*, and then Neospartoneae diverge in sequence, leaving a clade comprising the two most species-rich clades, Verbenaceae and Lantaneae. In our cpDNA trees *Diphyrena* is sister to Verbenaceae, but in the nuclear DNA phylogeny of Yuan et al. (2010) it is sister to the clade comprising Verbenaceae and Lantaneae. Verbenaceae and Lantaneae represent a relatively recent diversification and account for nearly two-thirds of all extant species of Verbenaceae.

All traditional classifications have recognized a group that includes *Citharexylum*, *Duranta*, and *Rhaphithamnus*, alternatively named Duranteae (Bentham, 1839; Schauer, 1847) or Citharexyleae (e.g., Briquet, 1895; Moldenke, 1971; Troncoso, 1974; Sanders, 2001; Atkins, 2004). Similarities among these taxa include woody habit, fleshy fruits, and the presence of a staminode, all nonexclusive traits. The molecular phylogenetic results indicate that these three taxa belong in separate clades, each of which is more closely related to other groups that differ in one or more of these traits.

Tribes centered on *Verbena* and *Lantana/Lippia* have been a feature of all traditional classifications, but in each case they have been either narrowly circumscribed (e.g., Verbenaceae—Junell, 1934; Atkins, 2004; Lantaneae—Sanders, 2001) or broadly circumscribed (e.g., Verbenaceae—Schauer, 1847; Sanders, 2001; Lantaneae—Briquet, 1895; Moldenke, 1971; Sanders, 2001; Atkins, 2004). The small clade comprising *Diostea*, *Lampaya*, and *Neosparton*, herein called Neospartoneae, has not been recognized in any previous classification and is formally described here.

Petreeae Briquet—This clade is sister to the rest of Verbenaceae and is composed of two genera, *Petrea* and *Xolocotzia*, which share the characteristics of having fleshy drupaceous fruits consisting of two pyrenes derived from a unilocular ovary (one carpel having aborted) and large, showy calyces that exceed the corollas. *Petrea* is a group of about 11 species of Neotropical lianas distributed in wet tropical forests from southern Mexico to the Amazon basin (Rueda, 1994), whereas the monotypic *Xolocotzia* is a shrub or small tree from Mexico and Central America. Our results provide modest support for a paraphyletic *Petrea* with *Xolocotzia* nested within it, but any decision on whether to reject *Xolocotzia* should await further study to confirm this. On the basis of ovule placentation, Junell (1934) recognized the distinctiveness of *Petrea* from other taxa often classified with it previously (e.g., *Casselia*, *Lampaya*, and *Recordia* in Moldenke, 1971), and his concept of this group has been followed by Sanders (2001) and Atkins (2004).

Duranteae Benth—Benth (1839) first used this name to define a group containing *Citharexylum*, *Duranta*, *Rhaphithamnus*, and *Petrea*, but later removed *Petrea* (Benth and Hooker, 1876). Composition of the clade that bears this name here includes only *Duranta* from the original circumscription but also includes *Bouchea*, *Chascanum* (including *Svensonia*), *Recordia*, and *Stachytarpheta*. This clade represents a combination of taxa never previously recognized in any classification. They share inflorescences arranged in terminal spikes or compound racemes with terminal and axillary (only axillary in some *Duranta* specimens) flowering shoots (Martínez and Múlgura, 1997; Drewes and Martínez, 1999; Múlgura et al., 2002), a persistent enclosing calyx in fruit (not in *Bouchea*), and presence of two floral bracteoles in *Bouchea*, *Recordia*, some *Duranta* species, and a section of *Chascanum*. Duranteae are further divided in our results into two clades, one with *Duranta* and *Recordia*, and the other containing *Stachytarpheta*, *Bouchea*, and *Chascanum*.

Duranta is distributed from Mexico and the Caribbean south through the Andes to Argentina and is sister to the monotypic *Recordia*, a Bolivian endemic. The number of species in *Duranta* is poorly understood, with estimates ranging from 17 (Sanders, 1984) to 34 species (Troncoso, 1974). *Duranta* is unique in Verbenaceae in having a four-carpellate ovary, with eight ovules developing into a drupe with four two-seeded pyrenes (Caro, 1956) and inflorescences with presence of multiple axillary buds (Martínez and Múlgura, 1997). This clade is characterized by shrubs and small trees that are often spinose, with fleshy fruits and presence of a fifth staminode.

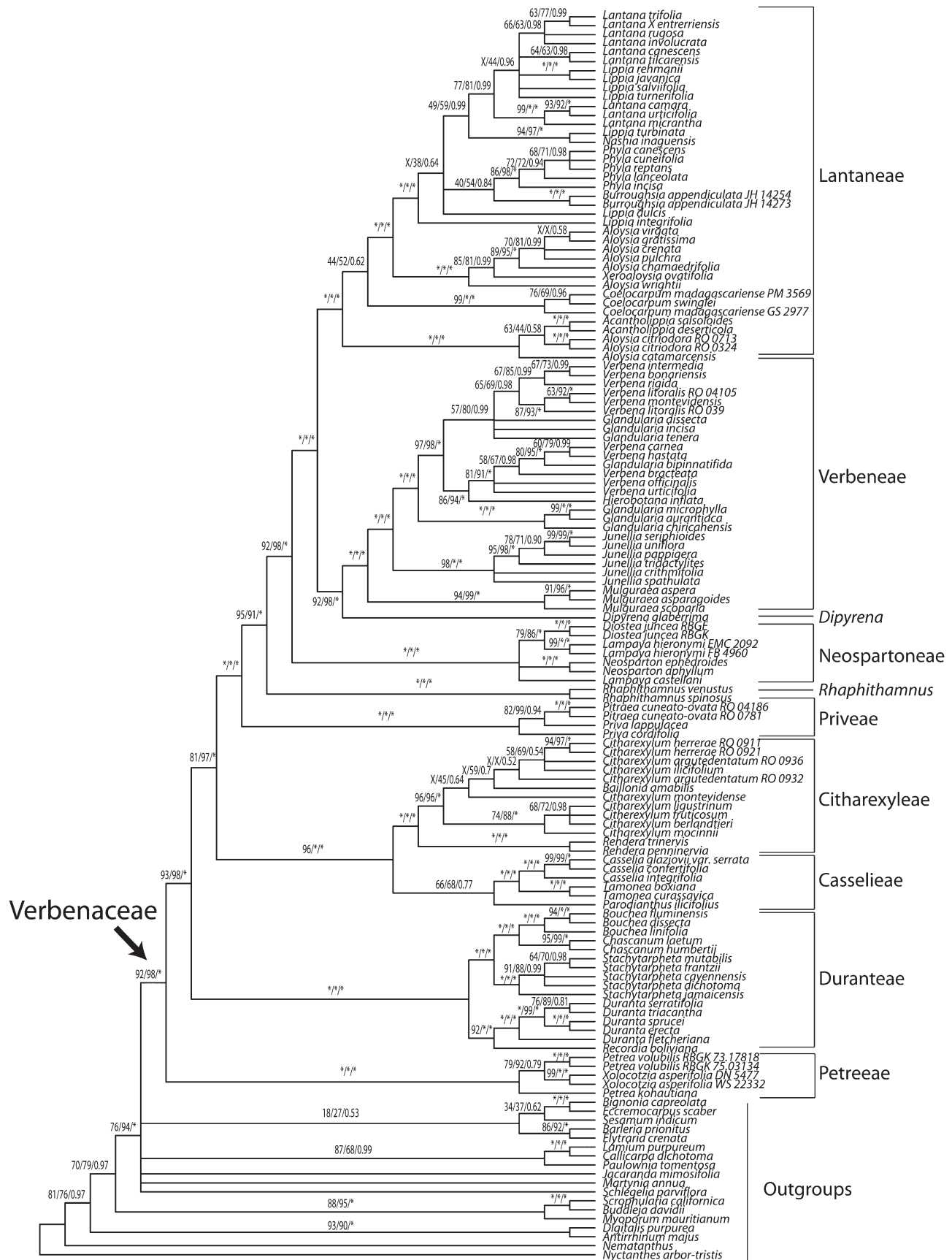
Plants in the clade of *Bouchea*, *Chascanum*, and *Stachytarpheta* differ principally from those in their sister clade by being herbs or low shrubs, with dry fruits divided into two mericarps at maturity, derived from a unilocular ovary (one carpel having aborted), and absence of a fifth staminal unit in the form of a

staminode. The inflorescence in this clade consists of a terminal spike subtended by leafy branches that also terminate in spikes, which may be interpreted as homologous with the compound racemes in *Duranta* (Martínez and Múlgura, 1997; Múlgura et al., 2002). *Stachytarpheta*, however, is unique in Verbenaceae in having only two functional stamens, with the abaxial stamens reduced to staminodes (*Hierobotana* has two stamens, but without staminodes). *Bouchea* apparently has lost the characteristic of an enclosing calyx in fruit. *Stachytarpheta* is the largest genus in this clade, with about 130 species distributed throughout warm regions of the New World and a center of diversity in Brazil (Atkins, 2005). In addition, there is one African species (*S. indica*), which may be indigenous to the Old World or a very early introduction (Atkins, 2005). *Bouchea* and *Chascanum* represent New World and Old World sister groups, with *Bouchea* having about 9 species distributed from the southern United States to Argentina, and *Chascanum* having about 27 species distributed from Africa and Madagascar through the Arabian peninsula to India. *Svensonia* is a segregate of *Chascanum* that has winged fruits (Moldenke, 1971), a trait that occurs occasionally in *Bouchea* as well, and is not recognized in recent treatments (Verdcourt, 1992; Atkins, 2004). *Chascanum laetum* (= *Svensonia laeta*) is included here and is strongly supported with *C. humbertii*.

Casseliaceae (Schauer) Troncoso—This clade comprises three small genera (*Casselia*, *Parodianthus*, and *Tamonea*) and 14 species. Support for this clade is modest in the seven-gene analysis (Fig. 1), where sequences of the five regions, which were sampled for a limited set of taxa, are missing for two *Casselia* and one *Tamonea* species and missing for *Parodianthus* for two of those gene regions (rpoC2 and ccsA), but is strong (pp = 0.99; ML bs = 90%) in the two-gene analysis (not shown). These taxa all have inflorescences consisting of lateral racemes reduced to two flowers in some species. Shared characters of ovule insertion and placentation were recognized first by Junell (1934), who placed *Casselia* and *Tamonea* together on this basis; *Parodianthus* was not described until later (Troncoso, 1941). However, differences in fruit type separate the genera: in *Tamonea* it is an entire, subdrupaceous four-seeded fruit; in *Parodianthus* it is a drupaceous fruit, with two two-seeded pyrenes; and in *Casselia* it is a subdrupaceous fruit, with two one-seeded pyrenes by abortion of the adaxial carpel (O'Leary and Múlgura, 2010). This misled other taxonomists (e.g., Moldenke, 1971; Troncoso, 1974; Sanders, 2001) until careful anatomical studies by Martínez and Múlgura (2003) confirmed Junell's results and showed that *Parodianthus* also belonged in this group, all sharing bicarpellate ovaries (one carpel in *Casselia*, by abortion) with false partitions fused to the carpel walls only at top and bottom and ovule insertion in the upper part of the locule, attached to the margins, and a broad placental line and placental bundles that enter the ovules from high in the locule.

The shrubby *Parodianthus*, with two species endemic to arid temperate regions of Argentina, is sister to a clade comprising the mostly herbaceous *Casselia* and *Tamonea*, in which woodiness, when present, is reduced to a low crown. *Casselia* and *Tamonea* have six species each and tropical distributions. *Casselia* is distributed in Brazil, Bolivia, and Paraguay, while *Tamonea* is widespread from Mexico and the Caribbean to Brazil and Paraguay.

Citharexyleae Briquet—Citharexyleae and Casseliaceae together form a well-supported clade (Fig. 1) of plants characterized by a bicarpellate ovary that generally develops into a



drupaceous to subdrupaceous fruit with four one-seeded pyrenes (*Tamonea*) or generally two two-seeded mericarps, with minute and often deciduous floral bracts and short pedicellate flowers. *Casselia* and *Baillonia* both have unicarpellate ovaries reduced by abortion of the adaxial carpel. Citharexyleae differ from Casselieae by the presence of a staminode and the fact that most plants are trees, whereas plants in Casselieae are herbs, shrubs, or subshrubs and lack staminodes.

Citharexyleae are dominated by the large genus *Citharexylum* (ca. 130 species), a genus of trees and shrubs that are widespread throughout the Neotropics. The small genus *Rehdera*, with three species in Central America, and the monotypic *Baillonia* from Brazil and Paraguay also belong to this clade. *Rehdera* appears to be sister to *Citharexylum* and *Baillonia*, with the latter nested within *Citharexylum*. Indeed, *Baillonia* appears to be a *Citharexylum* in which one carpel aborts during development, giving rise to a two-seeded fruit, a relationship noted by Junell (1934) and previously by Moore (1895). Another monotypic genus, *Verbenoxylum*, was segregated from *Citharexylum* by Troncoso (1971) on the basis of having a dry dehiscent fruit; this genus was not sampled in our study. The dry fruits that characterize *Rehdera* and *Verbenoxylum* appear to be a derived trait within the Casselieae/Citharexyleae clade. Similarly, inflorescences are reduced to a few-flowered raceme in *Rehdera* and several species of Casselieae.

Our sampling of eight species of *Citharexylum* includes species from Mexico, the Caribbean, Central America, Andean Peru, and Argentina. Greater sampling of both taxa and DNA sequence is needed to resolve relationships and to confirm whether *Rehdera* should be excluded or not, but the species from Central America, Mexico, and the Caribbean, which are geographically closest to *Rehdera*, form a moderately supported clade, which suggests that *Rehdera* is unlikely to be an offshoot of a recent diversification in this region.

Priveae Briquet—This small clade consists of two genera: *Priva*, with about 20 species, and the monotypic *Pitraea*. In the nuclear PPR gene tree of Yuan et al. (2010), Priveae and *Rhaphithamnus* switch places in relation to the present study, with moderate to strong support in both trees. Relatively long branches are exhibited by *Pitraea*, and to a lesser extent *Priva*, for all of the chloroplast genes, except *rbcL* (individual trees not shown), in contrast to the five nuclear PPR genes, in which no accelerated rate is observed. The internal branch on the tree subtending the attachment of Priveae and *Rhaphithamnus* is relatively long, which suggests that a long branch effect may be responsible for the conflict in placement between the chloroplast and nuclear trees, despite the fact that such effects are minimized by likelihood-based analyses.

Pitraea is unique in Verbenaceae in being a tuber-bearing perennial herb. It is frequently found in disturbed habitats and is a weed in cultivated fields in parts of South America where it is native. Both genera are perennial herbs, which may be woody at the base in some species of *Priva*, with dentate leaf margins, a short corolla, not much surpassing the calyx, and uncinat hairs on the rachis, stems, leaves, and peduncles of several species.

The fruit is dry, separating into two two-seeded mericarps, derived from a bicarpellate ovary.

Priva is one of only four genera (along with *Lantana*, *Lippia*, and *Verbena*) that have native distributions in both the New and Old Worlds, with species occurring in South America, Africa, and South Asia. In the New World, *Priva* is distributed from the southwestern United States to northern Argentina. Although our sampling is limited to one New World species (*P. lappulacea*) and one Old World species (*P. cordifolia*), it is sufficient to suggest that *Pitraea* may be derived from within *Priva*.

Neospartoneae Olmstead & O'Leary, trib. nov. Type: *Neosparton ephedroides* Griseb.

Frutices glabri. Folia plerumque parva. Racemus terminalis semper praesens, racemi lateralis nulli vel 2. Corollae triplo calyce longiores. Ovaria unicarpellata. Drupae unipyrenatae, 2-seminatae. Comprendit genus *Neosparton* et genera propinqua.

This small clade comprises three genera: *Neosparton*, with three species endemic to arid regions of Argentina; *Lampaya*, with two species of the high altiplano in Argentina and Chile; and the monotypic *Diostea*, from the Patagonian regions of Argentina and Chile (Fig. 4). Plants of all three genera are glabrous, typically with terminal spikes (lateral in some *Neosparton*). Flowers have relatively long corolla tubes, much surpassing the calyx, and are slightly curved in *Diostea* and *Neosparton*, but straight in *Lampaya*. They all have a fruit derived from a unicarpellate ovary, with a two-seeded pyrene.

Neosparton and *Diostea* exhibit an ephedroid habit, being many-branched shrubs with cylindrical, striate stems; leaves are reduced to deciduous bracts in *Neosparton* and small, often early deciduous leaves in *Diostea*. This habit has evolved independently more than once in Verbenaceae, with examples also in *Junellia* (e.g., *J. spathulata*). *Lampaya* is a low spreading shrub with thick fleshy leaves, an unusual trait in Verbenaceae. *Diostea* and *Neosparton* typically have staminodes, whereas *Lampaya* does not. Relationships among the three genera are not resolved, so it is uncertain whether the habit and staminode traits shared by *Diostea* and *Neosparton* are synapomorphies. This assemblage is unanticipated by previous classifications, although they often have been included in a large and variable Lantaneae (Troncoso, 1974; Sanders, 2001; Atkins, 2004). Despite the similarities in habit between *Diostea* and *Neosparton*, reliance on fruit characters in traditional taxonomies (fleshy in *Neosparton* and subdrupaceous in *Diostea* and *Lampaya*) is evident in the frequent placement of *Lampaya* and *Diostea* near each other (e.g., Troncoso, 1974; Atkins, 2004), but separate from *Neosparton*.

Verbeneae Dumortier—Detailed phylogenetic studies of this clade (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009) have led to revised generic circumscriptions (O'Leary et al., 2009). Atkins (2004) recognized five genera in Verbenaceae: *Verbena*, *Glandularia*, *Junellia*, *Urbania*, and *Hierobotana*. The above-cited studies have identified a monophyletic *Verbena* but found *Glandularia* and *Junellia* to be polyphyletic. *Junellia*, as traditionally circumscribed, consists of two distinct clades that

← Fig. 1. Bayesian consensus tree for the seven-gene, 139-taxon data set. Numbers on branches indicate bootstrap values or posterior probabilities for parsimony analysis/maximum likelihood/Bayesian analyses (asterisk indicates bootstrap of 100 or posterior probability of 1.0; "X" indicates clade not obtained in parsimony or maximum likelihood trees). Collector/botanical garden accession numbers referenced in appendix are listed with multiple accessions for individual species.



Fig. 2. The optimal maximum-likelihood tree for the seven-gene, 139-taxon data set with branch lengths.

form a paraphyletic grade at the base of Verbenaceae. The clade containing the type, *J. serpyllifolia*, includes four of the six sections recognized in a recent revision of *Junellia* (Peralta et al., 2008). This clade also includes *Glandularia* sect. *Paraglandularia*, which comprises approximately seven species, sharing several morphological traits, as well as a basic chromosome number ($x = 10$) with *Junellia* (Schnack and Covas, 1978; Botta, 1989). *Urbania pappigera*, the sole species in *Urbania*, which has been distinguished from *Junellia* by the presence of long hygroscopic hairs on the calyx, also was shown to belong to this clade (O'Leary et al., 2009). The two remaining sections of *Junellia* recognized by Peralta et al. (2008), including 11 species, are grouped together in a clade, now recognized as the new genus *Mulguraea* (O'Leary et al., 2009), and are sister to the rest of Verbenaceae. *Stylodon*, which sometimes is segregated from *Verbena* (e.g., Moldenke, 1971), was not sampled in the above-cited studies, but was included here (represented by *V. carnea*) and is shown to be nested within North American *Verbena*.

Verbenaceae are herbs or subshrubs, sometimes cushion-forming, with a bicarpellate ovary that develops into a dry fruit divided into four one-seeded mericarps. Flowers have corollas that are weakly zygomorphic, not much surpassing the calyx, anthers sometimes with a glandular connective, no staminode, and a bilobed style.

Verbena and *Glandularia* share a continental disjunction, with centers of diversity in the warm temperate and mostly arid regions of North and South America. *Verbena* also includes a few species distributed in Eurasia and North Africa, including the type, *V. officinalis*. The phylogenetic reconstruction of Verbenaceae (Yuan and Olmstead, 2008a, b) now permits a historical interpretation that includes an original diversification in South America, followed by colonization of North America at least once in *Verbena* and possibly two times in *Glandularia*. In *Verbena* (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009), the North American species form a clade in three of the four gene trees (a combined cpDNA tree and three nuclear loci: waxy, ITS/ETS, and PHOT1), in which case this clade is either sister to a clade comprising all of the sampled South American *Verbena* species (cpDNA—as seen here, with the caveat of a chloroplast transfer event transposing one branch; Yuan and Olmstead, 2008a; ITS/ETS—O'Leary et al., 2009; PHOT1—Yuan and Olmstead, 2008b), or is nested within a South American grade (waxy—Yuan and Olmstead, 2008a). In the fourth nuclear locus (PHOT2), neither the North American nor the South American species form clades (Yuan and Olmstead, 2008b). Taken together, these results illustrate the importance of obtaining multiple gene trees to understand the complex history of a recent diversification. The direction of migration/colonization in *Verbena* is clearly from South America to North America, but the more rapidly coalescing gene regions (in this case, cpDNA and ITS/ETS) yield a picture of sister groups on the two continents, whereas other, more slowly coalescing nuclear genes (e.g., waxy, PHOT2) depict a paraphyletic *Verbena* in South America retaining more of the ancestral molecular diversity within the clade (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009). *Junellia* and *Mulguraea* are restricted to the Andean region of South America from southern Peru to Patagonia.

Missing from the previous phylogenetic studies of *Verbena*, however, were samples of the central Andean species of *Verbena* and the monotypic *Hierobotana* from Ecuador. Taxonomic treatments of these *Verbena* species suggest that they are

part of the South American *Verbena* group (O'Leary et al., 2007b, 2010). Our results here and with a nuclear PPR locus (H. Marx, unpublished data) place *Hierobotana* as sister group to the North American clade, thus suggesting that *Hierobotana* may be a remnant of an Andean migration route by which *Verbena* colonized North America. Additional sampling of *Verbena* from Bolivia to Ecuador will be needed to test this hypothesis.

Lantaneae Endlicher—With about 275 species in nine genera, Lantaneae represent the largest radiation within Verbenaceae. The two principal genera are *Lantana* and *Lippia*, which together include about 80% of the species in Lantaneae. *Lippia* has about 120 species (N. Salimena and M. E. Múlgura, unpublished data), and *Lantana* has about 100 species (Rotman, 2009); these estimates are reduced considerably from previous published estimates (Verdcourt, 1992; Atkins, 2004). Other genera in Lantaneae, as defined here, are *Aloysia* (ca. 30 spp.), *Nashia* (7 spp.), *Acantholippia* (6 spp.), *Phyla* (5 spp.; N. O'Leary, unpublished data), *Coelocarpum* (5 spp.), *Burroughsia* (2 spp. often included in *Lippia*; Moldenke, 1940), and monotypic *Xeroaloyisia*.

Lantaneae sensu Briquet (1895) comprises Verbenaceae in which one carpel is aborted, resulting in a unicarpellate ovary. However, Junell (1934) showed that such reduction in carpel number has occurred multiple times within Verbenaceae (e.g., *Casselia*, *Baillonia*). Caro (1982) suggested that Lantaneae should be reduced to only those genera with a fleshy drupe, grouping genera with dry fruit separately under tribe Lippieae. Sanders's (2001) delimitation of Lantaneae matches ours with the exception that we include *Coelocarpum* based on the results of the present molecular phylogenetic study.

Lantaneae are small trees, woody shrubs, or even herbs (*Phyla*) with flowers having persistent calyces that more-or-less enclose the fruit, and predominantly five-lobed corollas, four-lobed in *Aloysia* (Rao, 1952). Staminodes are lacking, as is true for their sister group, Verbenaceae. Ovaries are unicarpellate, developing into fleshy two-seeded drupes (*Lantana*) or dry fruits dividing into two one-seeded mericarps (*Lippia*, *Aloysia*, *Phyla*). *Coelocarpum*, is the only genus placed here with a bicarpellate ovary; in *Coelocarpum*, the ovary develops into a fleshy drupe splitting into two two-seeded mericarps. Within Verbenaceae, essential oils are known only from members of Lantaneae and may be unique to this group (Atkins, 2004).

Lantaneae are well supported in this and other molecular phylogenetic studies as a monophyletic group sister to Verbenaceae (Yuan et al., 2010). However, the boundaries between genera in Lantaneae (most notably those separating *Lantana*, *Aloysia*, *Phyla*, and *Lippia*) are historically weak, and it has been suggested that many genera in Lantaneae may be nonmonophyletic (e.g., Sanders, 2001). Our results confirm nonmonophyly of *Aloysia* and are consistent with the idea that *Lantana* and *Lippia* also are nonmonophyletic, although relationships between *Lantana* and *Lippia* species are poorly resolved in the present study. Recent study of *Phyla* (N. O'Leary and M. E. Múlgura, unpublished data) has reduced it to five species, which here form a clade.

Despite the lack of resolution in the clade containing *Lippia* and *Lantana*, we can identify four well-supported clades. (1) *Lantana/Lippia* clade—Members of *Lantana*, *Lippia*, *Phyla*, *Nashia*, and *Burroughsia* sampled in this study form a well-supported clade, but relationships among these genera are unresolved. This clade is characterized by densely capitate or spicate

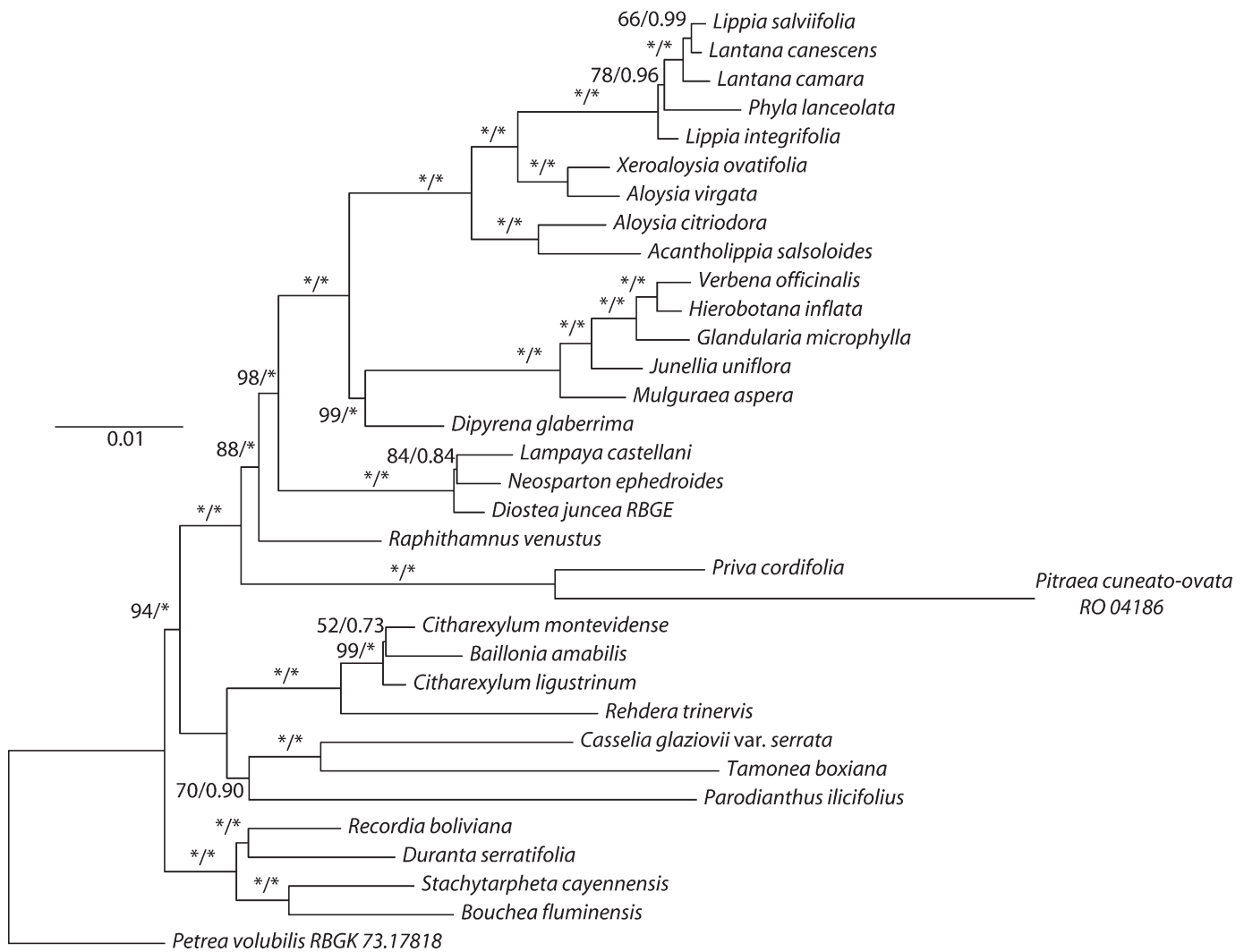


Fig. 3. Maximum likelihood tree from analysis of only those taxa with all seven regions available. Numbers on branches indicate maximum-likelihood bootstrap/Bayesian posterior probability.

inflorescences (if spicate, then rachis fully obscured by the overlapping floral bracts) and sessile flowers. Floral bracts are more or less conspicuous and sometimes colorful and show in species of *Lippia*. The trailing herbaceous species of the genus *Phyla* form a clade. Only single species of *Nashia* and *Burroughsia* were included. However, the relationship of these three small genera to *Lippia* and *Lantana* remains uncertain. (2) *Aloysia/Xeroaloyisia*—Most of the representatives of *Aloysia* that were sampled form a clade with *Xeroaloyisia*, sister to the *Lantana/Lippia* clade. Members of the *Aloysia/Xeroaloyisia* clade are woody shrubs or small trees with racemose or spicate inflorescences in which the rachis is exposed and generally exceeds the peduncle. Floral bracts are inconspicuous, calyces four-lobed, and fruits are dry in *Aloysia* and thinly fleshy drupes in *Xeroaloyisia*. (3) *Aloysia/Acantholippia* clade—the two species of *Acantholippia* sampled in this study form a clade with *Aloysia citriodora* and *Aloysia catamarcensis*. These are woody shrubs with inconspicuous floral bracts, four-lobed calyces, and dry fruit; some species have somewhat condensed inflorescences, reduced to only a few flowers in *Acantholippia*. Denser sampling across Lantaneae is needed to reveal other members

of this lineage, which may provide more insight into unifying traits. (4) *Coelocarpum*—a small genus of shrubs native to Madagascar, Socotra, and Somalia. *Coelocarpum* was placed in Citharexyleae by Briquet (1895) and Moldenke (1971) on the basis of fleshy fruits but was left unassigned to a tribe by Atkins (2004). The ovaries are bicarpellate, an uncharacteristic trait for Lantaneae. The five-lobed calyx and corolla and bicarpellate ovary are reminiscent of Verbeneae and may represent the ancestral condition in Lantaneae, which suggests that *Coelocarpum* is sister to the rest of Lantaneae. Lantaneae would be a more homogeneous group without *Coelocarpum*, but the very short branches connecting *Coelocarpum* with the *Aloysia/Acantholippia* clade and the clade comprising the remainder of Lantaneae mean that the molecular data are unable to confirm monophyly of Lantaneae excluding *Coelocarpum*, so we include *Coelocarpum* within Lantaneae.

Dipyrena and Raphithamnus—Chloroplast DNA sequences provide strong support for a phylogenetic position for the monotypic genus *Dipyrena* as sister to Verbeneae (Fig. 1; all seven individual cpDNA gene trees concur [not shown]). However,



Fig. 4. Neospartoneae. (A) *Diostea juncea* habit. (B) *Diostea juncea* inflorescence. (C) *Lampaya castallonii*. (D) *Neosparton aphyllum*. All photos by R. Olmstead.

nuclear DNA PPR sequence data contradict this placement and provide equally strong support (bs = 96%; pp = 1.0) for a position of *Dipyrena* as sister to the clade of Lantaneae plus Verbenae (Yuan et al., 2010; three of the five individual loci concur, with a fourth placing it one node lower and the fifth placing it sister to Verbenae, but with low support). *Dipyrena* is a shrub with habit similar to some species of *Mulguraea* (e.g., *M. aspera*), but with alternate leaves and a bicarpellate ovary developing into a subdrupaceous fruit divided into two two-seeded mericarps, whereas plants in tribe Verbenae typically have opposite leaves and dry fruit divided into four one-seeded mericarps. This has led to conflict in its placement in traditional classifications. Junell (1934), on the basis of ovary structure, placed *Dipyrena* in tribe Priveae, an arrangement followed by other authors (Troncoso, 1974; Atkins, 2004). Ravenna (2008) erroneously considered *Baillonia* and *Diostea* to be synonyms of a newly circumscribed *Dipyrena* because they all have long corolla tubes. The difference in the position of *Dipyrena* between the nuclear PPR tree (Yuan et al., 2010) and chloroplast tree here, along with the very short branch connecting *Dipyrena* to Verbenae, may reflect incomplete lineage sorting among three lineages that diverged closely in time, or possibly suggests evidence of an old hybridization event. Given the conflict also

in the molecular phylogenetic position, we prefer to leave *Dipyrena* unassigned to any more inclusive named group at this time.

Rhaphithamnus species are spinescent shrubs with bicarpellate ovaries that develop into a drupaceous fruit, and flowers with long straight corolla tubes, arranged into one- to five-flowered axillary racemes. The genus includes two species, one occurring in the Valdivian forests of Chile and Argentina and the other on the Juan Fernandez islands. *Rhaphithamnus* occurs in an isolated position within Verbenaceae in both this study (Fig. 1) and that of Yuan et al. (2010), but occupying slightly different positions in each study. The two species of *Rhaphithamnus* differ in several morphological traits and in pollinator preference (*R. venustus* on Juan Fernandez is hummingbird-pollinated) but exhibit very little genetic divergence, including having identical ITS sequences (Stuessy et al., 1994; D. Crawford, personal communication), which suggests recent colonization of the remote island archipelago. As noted in the discussion of Priveae, placement of *Rhaphithamnus* in relation to Priveae is uncertain, with the chloroplast tree possibly influenced by the long branch connecting Priveae to the tree.

Homoplasy in traditional tribal traits—Several traits have been used in traditional classifications to group taxa into tribes.

Emphasis on different traits has resulted in conflict between taxonomic systems in Verbenaceae, but none of the traditional systems align well with the molecular phylogeny, which suggests that homoplasy is rampant in all of the traits used in those classifications.

Briquet's (1895) treatment of the family has been the one most widely accepted. His classification is based on the number of locules in each carpel, the number of ovules in each locule, and inflorescence morphology. This classification was not accepted by Junell (1934), who suggested that abortion of a carpel had occurred independently several times. This is confirmed by our study, in which *Baillonia*, *Casselia*, tribe Petreeae, tribe Neospartoneae, a clade in tribe Duranteae, and tribe Lantaneae except *Coelocarpum* all have unicarpellate ovaries.

Some authors (e.g., Troncoso, 1974) have used fruits as a diagnostic trait for tribes; for example, fleshy or dry fruits, further divided, or not, into mericarps; this also has proved misleading for defining groups within Verbenaceae. All members of tribes Petreeae, Casselieae, Neospartoneae, and Citharexyleae (except *Rehdera*), as well as *Duranta* and *Lantana*, have fleshy fruits, and the rest have dry fruits, which shows that this character does not support suprageneric groups consistent with these phylogenetic results.

Inflorescence morphology and structure have been misunderstood and not correctly interpreted until recent studies (Martínez et al., 1996; Martínez and Múlgura, 1997; Múlgura et al., 1998, 2002; Drewes and Martínez, 1999). Consequently, classifications based on racemose or spicate flowering shoots in the terminal or axillary position (Schauer, 1847; Briquet, 1895) have been unnatural. A compound inflorescence with both terminal and branched lateral flowering shoots, termed a "heterothetic paniculiform pleiobotryum," has been suggested to be the primitive or central form in Verbenaceae, from which other forms were derived through processes such as condensation, reduction, and truncation (Martínez et al., 1996). Lantaneae generally have flowering shoots only in axillary positions (homothetic pleiobotrya), a derived condition from the heterothetic pleiobotrya found in most other tribes, except Casselieae.

Historical biogeography of Verbenaceae—Verbenaceae are predominantly New World in distribution. From our phylogeny we can infer at least five and possibly six Old World clades, all but one of which are primarily African or Malagasy in distribution. These include *Chascanum* (including *Svensonia*), a group within *Priva*, the Malagasy endemic *Coelocarpum*, a small group of *Verbena* in Europe, Asia, and North Africa, and one lineage each in *Lantana* and *Lippia*, which are poorly resolved in relation to each other in our results but appear likely to stem from distinct colonization events (P. Lu-Irving, unpublished data). *Stachytarpheta* has had several species named on the basis of collections from outside the New World, but all except *S. indica* have been shown to be synonyms of New World species that had colonized Old World locations following European contact (Atkins, 2005). However, *S. indica* is very similar to *S. angustifolia*, which also occurs in Africa as a weed, and Atkins (2005) speculated that *S. indica* is likely either a divergent phenotype of *S. angustifolia* that established early after contact or a hybrid with another weedy species, *S. jamaicensis*, which is also established in Africa. Thus, *Stachytarpheta* is probably restricted in its pre-Columbian distribution to the New World. *Lantana*, *Stachytarpheta*, and *Verbena* all have species that have become widely established as weeds outside their native ranges, mostly in tropical regions.

Distributions within the New World extend from Patagonia in southern South America to Canada, although very few species distributions extend north of the arid southwestern United States. Generic diversity is greatest in southern South America, especially in Argentina, where several clades have diversified in arid habitats, but where more mesic ecosystems nearby in northern Argentina also include representatives of many of the more wet-tropical groups. In contrast with the limited distribution outside the New World, each of the named clades, except for the very small Neospartoneae and the two isolated genera *Dipyrena* and *Rhaphithamnus*, have distributions that extend from Mexico, Central America, and/or the Caribbean in the north to Argentina, Bolivia, or Brazil in the south. In all of these clades, the initial diversification appears to be South American in distribution, as is the majority of the species diversity. The pattern of distribution described here for Verbenaceae is similar in many respects to that of two other asterid families, Bignoniaceae (Olmstead et al., 2009) and Solanaceae (Olmstead et al., 2008), which originated and diversified initially in the New World. As in Verbenaceae, both of those groups originated in South America, have colonized the Old World on multiple occasions (Bignoniaceae ca. 5 times, Solanaceae ca. 14 times), and many of their major clades are now widely distributed in the New World, but with limited representation in cool temperate North America (Olmstead et al., 2008, 2009).

Conclusions and future work—Verbenaceae are an important element in warm temperate and tropical floras of the New World, where they may become ecologically dominant in some communities. Traditional tribal classifications based on morphology have been misleading with respect to evolutionary relationships in Verbenaceae. The results presented here permit the realignment of genera into a new tribal classification, which recognizes a new tribe, Neospartoneae, comprising *Diostea*, *Lampaya*, and *Neosparton*. Additional work remains, most notably in Lantaneae, which include approximately 45% of the species in Verbenaceae, but also in Citharexyleae and Duranteae. The phylogeny presented here can serve as a basis for further work to better understand the evolution of traits, such as fruit and inflorescence architecture, which have misled previous systematists studying Verbenaceae.

LITERATURE CITED

- ABU-ASAB, M. S., AND P. D. CANTINO. 1987. Phylogenetic implications of leaf anatomy in subtribe Melittidinae (Labiatae) and related taxa. *Journal of the Arnold Arboretum* 68: 1–34.
- ABU-ASAB, M. S., AND P. D. CANTINO. 1989. Pollen morphology of *Trichostema* (Labiatae) and its systematic implications. *Systematic Botany* 14: 359–369.
- ALBACH, D. C., H. M. MEUDT, AND B. OXELMAN. 2005. Piecing together the "new" Plantaginaceae. *American Journal of Botany* 92: 297–315.
- ANGIOSPERM PHYLOGENY GROUP. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 399–436.
- ATKINS, S. 2004. Verbenaceae. In J. W. Kadereit [ed.], *The families and genera of flowering plants*, vol. 7, 449–468. Springer-Verlag, Berlin, Germany.
- ATKINS, S. 2005. The genus *Stachytarpheta* (Verbenaceae) in Brazil. *Kew Bulletin* 60: 161–272.
- BEARDSLEY, P. M., AND R. G. OLMSTEAD. 2002. Redefining Phrymaceae: The placement of *Mimulus*, tribe Mimuleae, and *Phryma*, character evolution and biogeography. *American Journal of Botany* 89: 1093–1102.
- BENNETT, J. R., AND S. MATHEWS. 2006. Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. *American Journal of Botany* 93: 1039–1051.

- BENTHAM, G. 1839. Enumeration of plants collected by Mr. Schomburgk in British Guiana. *Annals & Magazine of Natural History* 2: 441–451.
- BENTHAM, G., AND J. D. HOOKER. 1876. Verbenaceae. In *Genera plantarum*, vol. 2, 1131–1160. L. Reeve, London, England.
- BOTTA, S. M. 1979. Las especies del género *Aloysia* (Verbenaceae). *Darwiniana* 22: 67–108.
- BOTTA, S. M. 1980. Las especies del género *Acantholippia* (Verbenaceae). *Darwiniana* 22: 511–532.
- BOTTA, S. M. 1989. Studies in the South American genus *Junellia* (Verbenaceae Verbenoideae) I. Delimitation and infrageneric divisions. *Darwiniana* 29: 371–396.
- BREMER, B., K. BREMER, N. HEIDARI, P. ERIXON, R. G. OLMSTEAD, A. A. ANDERBERG, M. KALLERSJO, AND E. BARKHORDARIAN. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24: 274–301.
- BRIQUET, J. 1895. Verbenaceae. In A. Engler and K. Prantl [eds.], *Die natürlichen Pflanzenfamilien*, Tiel 4/3a, 132–182. Engelmann, Leipzig, Germany.
- BURNHAM, R. J. 2002. Diversity and distribution of lianas in Yasuní, Ecuador: Who's on top? *Journal of Tropical Ecology* 18: 845–864.
- CANTINO, P. D. 1990. The phylogenetic significance of stomata and trichomes in the Labiatae and Verbenaceae. *Journal of the Arnold Arboretum* 71: 323–370.
- CANTINO, P. D. 1992. Evidence for a polyphyletic origin of the Labiatae. *Annals of the Missouri Botanical Garden* 79: 361–379.
- CANTINO, P. D., R. M. HARLEY, AND S. J. WAGSTAFF. 1992. Genera of Labiateae: status and classification. In R. Harley [ed.], *Advances in Labiate science*, 511–522. Royal Botanic Gardens, Richmond, UK.
- CARO, J. A. 1956. Las especies de *Duranta* (Verbenaceae) silvestres y cultivadas en la República Argentina. *Revista Argentina de Agronomía* 23: 1–28.
- CARO, J. A. 1982. Sistematización del género *Acantholippia* Grisebach (Verbenaceae) y las especies de la Flora Argentina. *Dominguezia* 3: 1–31.
- CATALÁN, M. P., E. A. KELLOGG, AND R. G. OLMSTEAD. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndhF* gene sequences. *Molecular Phylogenetics and Evolution* 8: 150–166.
- CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, New York, USA.
- DEBRY, R. W., AND R. G. OLMSTEAD. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology* 49: 171–179.
- DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- DREWES, S. I., AND S. MARTÍNEZ. 1999. Morfología de las inflorescencias en Verbenaceae—Verbenoideae II: Tribu Petreeae. *Darwiniana* 37: 209–218.
- EDGAR, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.
- GRAHAM, S. W., P. A. REEVES, A. C. E. BURNS, AND R. G. OLMSTEAD. 2000. Microstructural changes in noncoding chloroplast DNA: Interpretation, evolution, and utility of indels and inversions in basal angiosperm phylogenetic inference. *International Journal of Plant Sciences* 161 (Supplement 6): S83–S96.
- GROSE, S. O., AND R. G. OLMSTEAD. 2007. Evolution of a charismatic neotropical clade: Molecular phylogeny of *Tabebuia* s.l., Crescentieae, and allied genera (Bignoniaceae). *Systematic Botany* 32: 650–659.
- HUNZIKER, J. H. 1975. On the geographical origin of *Larrea divaricata* (Zygophyllaceae). *Annals of the Missouri Botanical Garden* 62: 497–500.
- JUNELL, S. 1934. Zur Gynaceummorphologie und Systematik der Verbenaceen und Labiaten. *Symbolae Botanicae Upsaliensis* 4: 1–219.
- LEVIN, R. A., AND J. S. MILLER. 2005. Relationships within tribe Lycieae (Solanaceae): Paraphyly of *Lycium* and multiple origins of gender dimorphism. *American Journal of Botany* 92: 2044–2053.
- LEWIS, W. H., AND R. L. OLIVER. 1961. Cyto geography and phylogeny of the North American species of *Verbena*. *American Journal of Botany* 48: 638–643.
- LIA, V. V., V. A. CONFALONIERI, C. I. COMAS, AND J. H. HUNZIKER. 2001. Molecular phylogeny of *Larrea* and its allies (Zygophyllaceae): Reticulate evolution and the probable time of creosote bush arrival to North America. *Molecular Phylogenetics and Evolution* 21: 309–320.
- MARTÍNEZ, S., S. BOTTA, AND M. E. MÚLGURA. 1996. Morfología de las inflorescencias en Verbenaceae—Verbenoideae I: Tribu Verbenaeae. *Darwiniana* 34: 1–17.
- MARTÍNEZ, S., AND M. E. MÚLGURA. 1997. Yemas axilares múltiples, morfología y tipología de la inflorescencia en *Duranta* (Verbenaceae-Citharexyleae). *Boletín de la Sociedad Argentina de Botánica* 33: 113–122.
- MARTÍNEZ, S., AND M. E. MÚLGURA. 2003. The taxonomic position of *Parodianthus* (Verbenaceae): A morphological survey of the gynoecium and inflorescence. *Kew Bulletin* 58: 929–938.
- MCDADE, L. A., T. F. DANIEL, AND C. A. KIEL. 2008. Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales). *American Journal of Botany* 95: 1136–1152.
- MCDADE, L. A., AND M. L. MOODY. 1999. Phylogenetic relationships among Acanthaceae: Evidence from noncoding trnL-trnF chloroplast DNA sequences. *American Journal of Botany* 86: 70–80.
- MOLDENKE, H. N. 1940. Novelties in the Avicenniaceae and Verbenaceae. *Phytologia* 1: 409–432.
- MOLDENKE, H. N. 1971. A fifth summary of the Verbenaceae, Avicenniaceae, Stilbaceae, Dicrostylidaceae, Symphoremataceae, Nyctanthaceae, and Eriocaulaceae of the world as to valid taxa geographic distribution, and synonymy. 2 vols. Published by the author, Wayne, New Jersey, USA.
- MOORE, M. J., C. D. BELL, P. S. SOLTIS, AND D. E. SOLTIS. 2007. Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. *Proceedings of the National Academy of Sciences, USA* 104: 19363–19368.
- MOORE, SP. LE. M. 1895. The phanerogamic botany of the Matto Grosso Expedition, 1891–92. *Transactions of the Linnean Society of London (Botany)* Ser. 2 (4).
- MÚLGURA, M. E. 2000. Las especies de *Lippia* L. sect. *Dioicolippia* Tronc. (Verbenaceae). *Candollea* 55: 227–254.
- MÚLGURA, M. E., S. MARTÍNEZ, S. ATKINS, AND A. D. ROTMAN. 2002. Morfología de las inflorescencias en Verbenaceae—Verbenoideae II: Tribu Lantaneae p.p. *Darwiniana* 40: 1–15.
- MÚLGURA, M. E., S. MARTÍNEZ, AND A. SUYAMA. 1998. Morfología de las inflorescencias en *Lippia* (Verbenaceae). *Darwiniana* 36: 1–12.
- MÚLGURA, M. E., A. D. ROTMAN, AND S. ATKINS. 2003. Verbenaceae Parte 1. In A. T. Hunziker [ed.], *Flora Fanerogámica Argentina*. Conicet, Buenos Aires.
- O'LEARY, N., AND M. E. MÚLGURA. 2010. A taxonomic revision of *Casselia* (Verbenaceae), a genus endemic to the South American Cerrado and Mata Atlántica biogeographic provinces. *Journal of the Torrey Botanical Society* 137: 166–179.
- O'LEARY, N., M. E. MÚLGURA, AND O. MORRONE. 2007a. New combinations in South American *Glandularia* (Verbenaceae). *Novon* 17: 503–511.
- O'LEARY, N., M. E. MÚLGURA, AND O. MORRONE. 2007b. Revisión taxonómica de las especies del género *Verbena* (Verbenaceae): Serie *Pachystachyae*. *Annals of the Missouri Botanical Garden* 94: 571–621.
- O'LEARY, N., M. E. MÚLGURA, AND O. MORRONE. 2010. Revisión taxonómica de las especies del género *Verbena* L. (Verbenaceae) II: Serie *Verbena*. *Annals of the Missouri Botanical Garden* 97: in press.
- O'LEARY, N., AND P. PERALTA. 2007. Nuevas combinaciones en el género *Glandularia* (Verbenaceae). *Darwiniana* 45: 218–232.
- O'LEARY, N., P. PERALTA, AND M. E. MÚLGURA. 2008. A taxonomic revision of the genus *Tamonea* (Verbenaceae). *Botanical Journal of the Linnean Society* 157: 357–371.
- O'LEARY, N., Y.-W. YUAN, A. CHEMISQUY, AND R. G. OLMSTEAD. 2009. Reassignment of species of paraphyletic *Junellia* s.l. to the new genus

- Mulguraea* (Verbenaceae) and new circumscription of genus *Junellia*: Molecular and morphological congruence. *Systematic Botany* 34: 777–786.
- OLMSTEAD, R. G., L. BOHS, H. ABDEL MIGID, E. SANTIAGO-VALENTIN, V. F. GARCIA, AND S. M. COLLIER. 2008. A molecular phylogeny of the Solanaceae. *Taxon* 57: 1159–1181.
- OLMSTEAD, R. G., B. BREMER, K. SCOTT, AND J. D. PALMER. 1993. A parsimony analysis of the Asteridae sensu lato based on rbcL sequences. *Annals of the Missouri Botanical Garden* 80: 700–722.
- OLMSTEAD, R. G., C. W. DEPAMPHILIS, A. D. WOLFE, N. D. YOUNG, W. J. ELISENS, AND P. A. REEVES. 2001. Disintegration of the Scrophulariaceae. *American Journal of Botany* 88: 348–361.
- OLMSTEAD, R. G., R. K. JANSEN, K.-J. KIM, AND S. J. WAGSTAFF. 2000. The phylogeny of the Asteridae s.l. based on chloroplast *ndhF* sequences. *Molecular Phylogenetics and Evolution* 16: 96–112.
- OLMSTEAD, R. G., H. J. MICHAELS, K. SCOTT, AND J. D. PALMER. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcL*. *Annals of the Missouri Botanical Garden* 79: 249–265.
- OLMSTEAD, R. G., M. L. ZIHRA, L. G. LOHMANN, S. O. GROSE, AND A. J. ECKERT. 2009. A molecular phylogeny and classification of Bignoniaceae. *American Journal of Botany* 96: 1731–1743.
- OXELMAN, B., M. BACKLUND, AND B. BREMER. 1999. Relationships of the Buddlejaceae s.l. investigated using parsimony jackknife and branch support analysis of chloroplast *ndhF* and *rbcL* sequence data. *Systematic Botany* 24: 164–182.
- OXELMAN, B., P. KORNHALL, R. G. OLMSTEAD, AND B. BREMER. 2005. Further disintegration of the Scrophulariaceae. *Taxon* 54: 411–425.
- PERALTA, P., M. E. MÚLGARA, S. S. DENHAM, AND S. M. BOTTA. 2008. Revisión del género *Junellia* (Verbenaceae). *Annals of the Missouri Botanical Garden* 95: 338–390.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics (Oxford, UK)* 14: 817–818.
- RAHMANZADEH, R., K. MULLER, E. FISCHER, D. BARTELS, AND T. BORSCH. 2005. The Linderniaceae and Gratiolaceae are further lineages distinct from the Scrophulariaceae (Lamiales). *Plant Biology* 7: 67–78.
- RAMBAUT, A. 2002. Se-AL: Sequence Alignment Editor, v2.0a11. Computer program and documentation, available at <http://evolve.zoo.ox.ac.uk/>.
- RAMBAUT, A., AND A. J. DRUMMOND. 2004. Tracer. University of Edinburgh, Edinburgh, UK. Computer program and documentation, available at <http://tree.bio.ed.ac.uk/software/tracer/>.
- RAO, V. S. 1952. The floral anatomy of some Verbenaceae with special reference to the gynoeceum. *Journal of the Indian Botanical Society* 31: 297–315.
- RAVENNA, P. 2008. Studies in Verbenaceae V: *Dipyrena* Hook. a valid genus antedating *Diostea* Miers. including a new species and transfers. *Onira* 11: 40–45.
- RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, UK)* 19: 1572–1574.
- ROTMAN, A. D. 2009. El género *Lantana* L. (Verbenaceae-Verbenoideae) en Paraguay: sinopsis y novedades. *Candollea* 64: 297–301.
- RUEDA, R. M. 1994. Systematics and evolution of the genus *Petrea* (Verbenaceae). *Annals of the Missouri Botanical Garden* 81: 610–652.
- SANDERS, R. W. 1984. Provisional synopsis of the species and natural hybrids in *Duranta* (Verbenaceae). *Sida* 10: 308–318.
- SANDERS, R. W. 2001. The genera of Verbenaceae in the southeastern United States. *Harvard Papers in Botany* 5: 303–358.
- SCHAUER, J. C. 1847. Verbenaceae. In A. P. De Candolle [ed.], *Prodromus systematis naturalis regni vegetabilis* 11, 522–700. Fortin, Masson et Sociorum, Paris.
- SCHNACK, B. J. C., AND G. COVAS. 1978. Subgéneros de *Glandularia*. *Apuntes para la Flora de La Pampa* 57: 225–226.
- SCHWARZBACH, A. E., AND L. A. MCDADE. 2002. Phylogenetic relationships of the mangrove family Avicenniaceae based on chloroplast and nuclear ribosomal DNA sequences. *Systematic Botany* 27: 84–98.
- SCOTLAND, R. W., J. S. SWEERE, P. A. REEVES, AND R. G. OLMSTEAD. 1995. Higher level systematics of Acanthaceae determined by chloroplast DNA sequences. *American Journal of Botany* 82: 266–275.
- SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.
- SIMPSON, B. B., J. A. TATE, AND A. WEEKS. 2005. The biogeography of *Hoffmannseggia* (Leguminosae, Caesalpinoideae, Caesalpinieae): A tale of many travels. *Journal of Biogeography* 32: 15–27.
- SMITH, J. F., J. C. WOLFRAM, K. D. BROWN, C. L. CARROLL, AND D. S. DENTON. 1997. Tribal relationships in the Gesneriaceae: Evidence from DNA sequences of the chloroplast gene *ndhF*. *Annals of the Missouri Botanical Garden* 84: 50–66.
- SOLBRIG, O. T. 1972. The floristic disjunctions between the ‘Monte’ in Argentina and the ‘Sonoran Desert’ in Mexico and the United States. *Annals of the Missouri Botanical Garden* 59: 218–223.
- STAMATAKIS, A. 2006. RAXML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics (Oxford, UK)* 22: 2688–2690.
- STAMATAKIS, A., P. HOOVER, AND J. ROUGEMONT. 2008. A rapid bootstrap algorithm for the RAXML web servers. *Systematic Biology* 57: 758–771.
- STUESSY, T. F., D. J. CRAWFORD, T. SANG, AND R. RODRIGUEZ. 1994. Morphological and molecular divergence between the two species of *Rhaphithamnus* (Verbenaceae). *American Journal of Botany* 80 (Supplement): 190.
- SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4b10. Sinauer Associates, Sunderland, Massachusetts, USA.
- TANK, D. C., P. BEARDSLEY, S. KELCHNER, AND R. G. OLMSTEAD. 2006. Review of the systematics of Scrophulariaceae s.l. and their current disposition. L.A.S. Johnson Review no. 7. *Australian Systematic Botany* 19: 289–307.
- TANK, D. C., AND R. G. OLMSTEAD. 2008. From annuals to perennials: Phylogeny and diversification of subtribe Castillejinae (Orobanchaceae) and implications for chromosomal and morphological evolution. *American Journal of Botany* 95: 608–625.
- TRONCOSO, N. S. 1941. Un nuevo género de Verbenáceas de la Argentina: *Parodianthus*, nov. gen. *Darwiniana* 5: 31–40.
- TRONCOSO, N. S. 1971. *Verbenoxylum*, nuevo género de Verbenáceas arbóreas de Brasil austral. *Darwiniana* 16: 622–626.
- TRONCOSO, N. S. 1974. Los generos de Verbenacea de Sudamerica extra-tropical. *Darwiniana* 18: 295–412.
- TRONCOSO, N. S. 1980. Taxonomic novelties in the genera *Lantana* and *Lippia* (Verbenaceae). *Hickenia* 1: 227–231.
- UMBER, R. E. 1979. The genus *Glandularia* (Verbenaceae) in North America. *Systematic Botany* 4: 72–102.
- VERDCOURT, B. 1992. Verbenaceae. In R. M. Polhill [ed.], *Flora of tropical East Africa*. A.A. Balkema, Rotterdam, The Netherlands.
- VON MULERT, U. 2001. Phylogenie der Verbenaceae: Kladistische Untersuchungen mit morphologischen und chemischen Merkmalen. Dissertation zur Erlangung des Doktorgrades der Fakultät für Chemie und Pharmazie der Albert-Ludwigs-Universität Freiburg im Breisgau.
- VON POSER, G. L., M. E. TOFFOLI, M. SOBRAL, AND A. T. HENRIQUES. 1997. Iridoid glucosides substitution patterns in Verbenaceae and their taxonomic implication. *Plant Systematics and Evolution* 205: 265–287.
- WAGSTAFF, S. J., AND R. G. OLMSTEAD. 1997. Phylogeny of the Labiatae and Verbenaceae inferred from *rbcL* sequences. *Systematic Botany* 22: 165–179.
- WAGSTAFF, S. J., P. A. REEVES, L. HICKERSON, R. E. SPANGLER, AND R. G. OLMSTEAD. 1998. Phylogeny of Labiatae s.l. inferred from cp-DNA sequences. *Plant Systematics and Evolution* 209: 265–274.
- WALLANDER, E., AND V. A. ALBERT. 2000. Phylogeny and classification of Oleaceae based on *rps16* and *trnLF* sequence data. *American Journal of Botany* 87: 1827–1841.
- WOLFE, A. D., C. P. RANDLE, L. LIU, AND K. E. STEINER. 2005. Phylogeny and biogeography of Orobanchaceae. *Folia Geobotanica* 40: 115–134.
- YOUNG, N. D., K. E. STEINER, AND C. W. DEPAMPHILIS. 1999. The evolution of parasitism in the Scrophulariaceae/Orobanchaceae: Plastid gene sequences refute an evolutionary transition series. *Annals of the Missouri Botanical Garden* 86: 876–893.

- YUAN, Y.-W., C. LIU, H. E. MARX, AND R. G. OLMSTEAD. 2010. An empirical demonstration of using PPR (pentatricopeptide repeat) genes as phylogenetic tools: phylogeny of Verbenaceae and the *Verbena* complex. *Molecular Phylogenetics and Evolution* 54: 23–35.
- YUAN, Y.-W., AND R. G. OLMSTEAD. 2008a. A species-level phylogenetic study of the *Verbena* complex (Verbenaceae) indicates two independent intergeneric chloroplast transfers. *Molecular Phylogenetics and Evolution* 48: 23–33.
- YUAN, Y.-W., AND R. G. OLMSTEAD. 2008b. Evolution and phylogenetic utility of the PHOT gene duplicates in a recently diversified group: Dramatic intron size variation and footprint of ancestral recombination. *American Journal of Botany* 95: 1166–1176.

APPENDIX 1.

Taxa, geographic origin, vouchers, and GenBank accession numbers (*ndhF*, *trnLF*, *ccsA*, *matK*, *rbcL*, *rpoC2*, *rps3*). When only two GenBank numbers appear, these are *ndhF* and *trnLF*. Names in parentheses are names that have been assigned to other genera in some classifications. GenBank numbers beginning with “HM” indicate sequences used for the first time in this study. “na” indicates no sequence available for that accession.

- Acantholippia deserticola* (Phil.) Moldenke; Argentina: Jujuy; RGO 2007-52 (WTU); HM216681; HM216585. *Acantholippia salsoloides* Griseb.; Argentina: Salta; RGO 2007-23 (WTU); HM216682; HM216586. *Acantholippia salsoloides* Griseb.; Argentina: Salta; RGO 2007-28 (WTU); na; na; HM853831; HM853864; HM853897; HM853927; HM853953. *Aloysia catamarcensis* Moldenke; Argentina: La Rioja; RGO 2007-82 (WTU); HM216683; HM216587. *Aloysia chamaedryfolia* Cham.; Cultivated; H. Rimpler 1131 (FB); HM216684; HM216588. *Aloysia citriodora* Ortega ex Pers.; Cultivated; RGO 2003-24 (WTU); HM216685; HM216589. *Aloysia citriodora* Ortega ex Pers.; Argentina: Salta; RGO 2007-13 (WTU); HM216686; HM216590; HM853832; HM853865; HM853898; HM853928; HM853954. *Aloysia crenata* Moldenke; Argentina: Corrientes; Cabrera 29106 (SI); HM216687; HM216591. *Aloysia gratissima* (Gillies & Hook.) Tronc.; Cultivated; K.-J. Kim 12803 (TEX); AF130154; HM216592. *Aloysia pulchra* Moldenke; Argentina: Misiones; RGO 2004-129 (WTU); HM216688; HM216593. *Aloysia virgata* (Ruiz & Pav.) Pers.; Cultivated; Valencia Jard. Bot. 232-97; no voucher; HM216689; na. *Aloysia virgata* (Ruiz & Pav.) Pers.; Argentina: Misiones; RGO 2004-133 (WTU); na; EF571570; HM853830; HM853863; HM853896; HM853926; na. *Aloysia wrightii* A.Heller; USA: Arizona; RGO 1991-004 (WTU); HM216690; HM216594. *Baillonia amabilis* Bocq. ex Baill.; Bolivia: El Poston-Chiqueta; M. Cardenas 4522 (US); HM216691; HM216595; HM853846; HM853880; HM853912; HM853939; na. *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; HM853852; HM853886; HM853917; HM853943; HM853970. *Bouchea linifolia* A. Gray; USA: Texas; B.L. Turner 20-423 (TEX); HM216694; HM216598. *Burroughsia appendiculata* (B. L. Rob. & Greenm.) Moldenke; Mexico: Coahuila; James Henrickson 14254 (TEX); HM216696; HM216601. *Burroughsia appendiculata* (B. L. Rob. & Greenm.) Moldenke; Mexico: Coahuila; James Henrickson 14273 (LL); HM216695; HM216600. *Casselia confertifolia* (Moldenke) Moldenke; Brazil: Goias; R.C. Mendonca et al. 2859 (US); HM216697; HM216602. *Casselia glaziovii* (Briq. & Moldenke) Moldenke var. *serrata* Moldenke; Brazil: Minas Gerais; M.A. Silva et al. 3630 (US); HM216698; HM216603; HM853849; HM853883; HM853915; HM853941; HM853967. *Casselia integrifolia* Nees & Mart.; Brazil: Espirito Santo; J.R. Pirani et al. 3449 (US); HM216699; HM216604. *Chascanum humbertii* Moldenke; Madagascar; Miller & Randrianasola 6127 (MO); HM216700; HM216605. *Chascanum laetum* Walp. (= *Svensonia laeta* (Fenzl ex Walp.) Moldenke); Ethiopia: Wollo; J. DeWilde 6923 (MO); HM216701; HM216606. *Citharexylum argutedentatum* Moldenke; Peru: Cusco: Urubamba; RGO 2009-32 (WTU); na; HM216607. *Citharexylum argutedentatum* Moldenke; Peru: Cusco: Calca; RGO 2009-36 (WTU); HM216702; HM216608. *Citharexylum berlandieri* S. Watson; Cultivated; Fairchild Tropical Gardens 78169B, J. Francisco-Ortega (FTG); HM216703; HM216609. *Citharexylum fruticosum* L.; Cultivated; Kew 000-69-14013; no voucher; HM216704; na. *Citharexylum fruticosum* L.; Cuba: Pinar del Rio; RGO 96-113 (WTU); na; HM216610. *Citharexylum herrerae* Mansf.; Peru: Apurimac: Abancay; RGO 2009-11 (WTU); HM216705; HM216611. *Citharexylum herrerae* Mansf.; Peru: Apurimac: Abancay; RGO 2009-21 (WTU); HM216706; HM216612. *Citharexylum ilicifolium* Kunth; Peru: Cusco: Urubamba; RGO 2009-31 (WTU); HM216707; HM216613. *Citharexylum ligustrinum* van Houtte; Cultivated; Kew 000-69.51235; no voucher; HM216708; HM216614; HM853847; HM853881; HM853913; HM853940; HM853965. *Citharexylum mocinnii* D. Don; Nicaragua: Jinotega; S. Grose 151 (HULE); HM216709; HM216615. *Citharexylum montevidense* (Spreng.) Moldenke; Argentina: Buenos Aires; RGO 2004-102 (WTU); HM216710; HM216616; HM853845; HM853879; HM853911; HM853938; na. *Coelocarpum madagascariense* Scott-Elliott; Madagascar; Phillipson and Milijaona 3569 (MO); HM216711; HM216617. *Coelocarpum madagascariense* Scott-Elliott; Madagascar; Schatz 2977 (MO); HM216712; HM216618. *Coelocarpum swinglei* Moldenke; Madagascar; Phillipson et al. 3443 (MO); HM216713; HM216619. *Diostea juncea* Miessner; Cultivated; RBG Kew 1969-35347; no voucher; HM216714; HM216620. *Diostea juncea* Miessner; Cultivated; RBG Edinburgh 19300262; no voucher; HM216715; HM216621; HM853875; HM853907; HM853934; HM853961; HM853841. *Dipyrena glaberrima* Gill & Hook.; Argentina: Mendoza; RGO 2004-179 (WTU); HM216716; HM216622; HM853838; HM853872; HM853904; HM853931; HM853958. *Duranta erecta* L.; Cultivated; Jardín Botánica Nacional, Havana, Cuba; RGO 1996-100 (WTU); HM216717; HM216623. *Duranta fletcheriana* Moldenke; Cuba: Topes de Collantes; RGO 1996-71 (WTU); HM216718; HM216624. *Duranta serratifolia* (Griseb.) Kuntze; Argentina: Salta; RGO 2007-009 (WTU); HM216719; HM216625; HM853854; HM853870; na; HM853945; HM853972. *Duranta sprucei* Briq.; Cultivated; Waimea Bot. Gard. 75S356; RGO 1992-221 (WTU); HM216720; HM216626. *Duranta triacantha* Juss.; Peru: Apurimac; RGO 2009-20 (WTU); HM216721; HM216627. *Glandularia aurantiaca* (Speg.) Botta; Argentina: Mendoza; RGO 2004-196 (WTU); HM216722; EF571554. *Glandularia bipinnatifida* Nutt.; USA: Colorado; RGO 92-133 (WTU); HM216723; na. *Glandularia bipinnatifida* Nutt.; USA: Texas; Y.-W. Yuan 2005-12 (WTU); na; EF571535. *Glandularia chiracahensis* Umber; USA: Arizona; Y.-W. Yuan 2005-9 (WTU); HM216724; EF571546. *Glandularia dissecta* (Willd. ex Spreng.) Schnack & Covas; Argentina: Misiones; RGO 2004-122 (WTU); HM216725; EF571536. *Glandularia incisa* (Hook.) Troncoso; Argentina: Corrientes; RGO 2004-108 (WTU); HM216726; EF571537. *Glandularia microphylla* (Kunth) Cabrera; Argentina: Mendoza; RGO 2004-156 (WTU); HM216727; EF571552; HM853835; HM853868; HM853901; na; na. *Glandularia tenera* (Spreng.) Cabrera; Cultivated; Waimea Bot. Gard. 74P1415; RGO 92-222 (WTU); HM216728; na. *Glandularia tenera* (Spreng.) Cabrera; Argentina: Mendoza; RGO 2004-148 (WTU); na; EF571556. *Hierobotana inflata* Briq.; Ecuador: Pichineha; Erik Asplund 17069 (US); HM216729; HM216628; HM853834; HM853867; HM853900; na; na. *Junellia crithmifolia* (Gillies & Hook. ex Hook.) Schnack & Covas (= *Glandularia crithmifolia* Gillies & Hook.); Argentina: Mendoza; RGO 2004-169 (WTU); HM216730; EF571558. *Junellia juniperina* (Lag.) Moldenke; Argentina: Mendoza; RGO 2004-158 (WTU); na; na; HM853836; HM853869; HM853902; HM853929; HM853956. *Junellia pappigera* (Phil.) O’Leary & Peralta (= *Urbania pappigera* Phil.); Argentina: Salta; RGO 2007-36 (WTU); HM216731; FJ867544. *Junellia seriphoides* (Gillies & Hook.) Moldenke; Argentina: Mendoza; RGO 2004-147 (WTU); HM216732; EF571561. *Junellia*

- spathulata* (Gillies & Hook.) Moldenke; Argentina: Mendoza; RGO 2004-190 (WTU); HM216733; EF571565. *Junellia tridactylites* (Lag.) Moldenke; Cultivated: RBG Kew 1988-1195; no voucher; HM216734; HM216629. *Junellia uniflora* (Phil.) Moldenke; Argentina: Mendoza; RGO 2004-155 (WTU); HM216735; EF571562. *Lampaya castellani* Moldenke; Argentina: Jujuy; RGO 2007-063 (WTU); HM216736; HM216630; HM853840; HM853874; HM853906; HM853933; HM853960. *Lampaya hieronymi* Schum. ex Moldenke; Argentina: Catamarca; E. Marilínez Carretero 2092 (MERL); HM216737; HM216631. *Lampaya hieronymi* Schum. ex Moldenke; Argentina: Catamarca; F. Biurrún et al. 4960 (SI); HM216738; HM216632. *Lantana camara* L.; Cultivated; RGO 92-140 (WTU); HM216739; HM216633; HM853826; HM853859; HM853892; HM853922; HM853949. *Lantana canescens* Kunth; Argentina: Salta; RGO 2007-006 (WTU); HM216740; HM216634; na; HM853857; HM853890; na; na. *Lantana X enterriensis* Troncoso; Argentina: Corrientes; RGO 2004-126 (WTU); HM216747; HM216641. *Lantana involucrata* L.; Cuba: Pinar del Río; RGO 1996-124 (WTU); HM216741; HM216635. *Lantana micrantha* Briq.; Argentina: Salta; RGO 2007-8 (WTU); HM216742; HM216636. *Lantana rugosa* Willd. ex Schauer; South Africa, Natal; W. Vos 389 (NU); HM216743; HM216637. *Lantana tilcarensis* Troncoso; Argentina: Salta; RGO 2007-18 (WTU); HM216744; HM216638. *Lantana trifolia* L.; Cultivated: Jardín Botánica Nacional Havana, Cuba; RGO 1996-98 (WTU); HM216745; HM216639. *Lantana urticifolia* Mill.; Cuba: Topes de Collantes; RGO 1996-75 (WTU); HM216746; HM216640. *Lippia dulcis* Trevir.; Cultivated; RGO 1998-56 (WTU); HM216748; HM216642. *Lippia integrifolia* Hieron.; Argentina: Catamarca; RGO 2007-78 (WTU); HM216749; HM216643; HM853827; HM853860; HM853893; HM853923; HM853950. *Lippia javanica* Spreng.; South Africa: Natal; W. Vos 390 (NU); HM216750; HM216644. *Lippia rehmannii* H. Pearson; South Africa; H.H.W. Pearson s.n., 2/12/92 (voucher location unknown); HM216751; HM216645. *Lippia salvifolia* Cham.; Argentina: Misiones; RGO 2004-131 (WTU); HM216752; HM216646; HM853825; HM853858; HM853891; HM853921; HM853948. *Lippia turbinata* Griseb.; Argentina: Tucumán; RGO 2007-74 (WTU); HM216753; HM216647. *Lippia turnerifolia* Cham.; Argentina: Corrientes; RGO 2004-121 (WTU); HM216754; HM216648. *Mulguraea asparagoides* (Gillies & Hook.) O'Leary & Peralta (= *Junellia asparagoides* Gillies & Hook.); Argentina: Mendoza; RGO 2004-192 (WTU); HM216756; EF571567. *Mulguraea aspera* (Gillies & Hook.) O'Leary & Peralta (= *Junellia aspera* Gillies & Hook.); Argentina: Mendoza; RGO 2004-163 (WTU); HM216757; EF571568; HM853837; HM853871; HM853903; HM853930; HM853957. *Mulguraea scoparia* (Gillies & Hook.) O'Leary & Peralta (= *Junellia scoparia* Gillies & Hook.); Argentina: Mendoza; RGO 2004-178 (WTU); HM216758; EF571566. *Nashia inaguensis* Millsp.; Cultivated: Fairchild Tropical Gardens 8655; no voucher; HM216759; HM216650. *Neosparton aphyllum* (Gillies & Hook.) Kuntze; Argentina: Mendoza; RGO 2004-193 (WTU); HM216760; HM216651. *Neosparton ephedroides* Griseb.; Argentina: Catamarca; RGO 2007-077 (WTU); HM216761; HM216652; HM853839; HM853873; HM853905; HM853932; HM853959. *Parodianthus ilicifolius* (Moldenke) Troncoso; Argentina: San Luis; RGO 2004-181 (WTU); HM216762; HM216653; HM853851; HM853885; na; na; HM853969. *Petrea kohautiana* C. Presl; Ecuador; J. L. Clark 6554 (US); HM216763; HM216654. *Petrea volubilis* L. (rec'd as *P. racemosa* Nees); Cultivated: RBG Kew 000.73.17818; no voucher; AY919283; HM216655; HM853856; HM853889; HM853920; HM853947; HM853974. *Petrea volubilis* L.; Cultivated: RBG Kew 326.75.03134; no voucher; FJ887872; FJ870052. *Phyla canescens* (Kunth) Greene; Argentina: Mendoza; RGO 2004-159 (WTU); HM216764; HM216656. *Phyla cuneifolia* (Torr.) Greene; USA: Colorado; RGO 1992-134 (WTU); HM216765; HM216657. *Phyla incisa* Small; USA: Texas; K.-J. Kim 12801 (TEX); AF130153; HM216658. *Phyla lanceolata* (Michx.) Greene; McCormac 4090 (OS); HM216766; HM216659. *Phyla lanceolata* (Michx.) Greene; USA: Texas; P. Lu-Irving 2008-16 (WTU); na; na; HM853828; HM853861; HM853894; HM853924; HM853951. *Phyla reptans* (Kunth) Greene; Argentina: Jujuy; RGO 2007-65 (WTU); HM216767; HM216660. *Pitrea cuneato-ovata* (Cav.) Caro; Argentina: Mendoza; RGO 2004-186 (WTU); HM216768; HM216661; HM853844; HM853878; HM853910; HM853937; HM853964. *Pitrea cuneato-ovata* (Cav.) Caro; Argentina: Catamarca; RGO 2007-81 (WTU); HM216769; HM216662. *Priva cordifolia* Druce; South Africa: Natal; W. Vos 391 (NU); HM216770; HM216663; HM853843; HM853877; HM853909; HM853936; HM853963. *Priva lappulacea* (L.) Pers.; Cuba: Villa Clara; RGO 1996-86 (WTU); HM216771; HM216664. *Recordia boliviana* Moldenke; Bolivia: Santa Cruz; M. Nee 24092 (TEX); HM216772; HM216665; HM853855; HM853888; HM853919; HM853946; HM853973. *Rehdera penninervia* Standl. & Moldenke; Guatemala: El Peten; C.L. Lundell, Elias Contreras 19938 (TEX); HM216773; na. *Rehdera penninervia* Standl. & Moldenke; Guatemala: El Peten; M. Pena-Chocarro & N. Bonilla 1378 (MO); na; HM216666. *Rehdera trinervis* (Blake) Moldenke; Mexico: Campeche; E. Martínez S., D. Alvarez M., S. Ramírez A. 31706 (TEX); HM216774; HM216667. *Rehdera trinervis* (Blake) Moldenke; Mexico: Campeche; E. Martínez S. 30531 (TEX); na; na; HM853848; HM853882; HM853914; na; HM853966. *Rhaphithamnus spinosus* (Juss.) Moldenke; Cultivated: RBG Kew 128-83.01596; no voucher; L36409; FJ870056. *Rhaphithamnus venustus* B.L. Rob.; Chile: Juan Fernández Islands; T.F. Stuessy 11855 (OS); HM216775; HM216668; HM853842; HM853877; HM853908; HM853935; HM853962. *Stachytarpheta cayennensis* (Rich.) Vahl; Argentina: Corrientes; RGO 2004-113 (WTU); HM216776; HM216669; HM853853; HM853887; HM853918; HM853944; HM853971. *Stachytarpheta dichotoma* (Ruiz & Pav.) Vahl; USA: Hawaii; RGO 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; RGO 1996-68 (WTU); HM216778; HM216672. *Stachytarpheta mutabilis* (Jacq.) Vahl; Cultivated: Waimea Bot. Gard. 75C1444; RGO 1992-207 (WTU); HM216779; HM216673. *Tamonea boxiana* (Moldenke) R.A. Howard; USA: Puerto Rico, USA; RGO 2003-12 (WTU); HM216780; HM216674; HM853850; HM853884; HM853916; HM853942; HM853968. *Tamonea curassavica* (L.) Pers.; Cultivated: Germany; H. Rimpler 1917 (FB); HM216781; HM216675. *Verbena bonariensis* L.; Cultivated; RGO 2003-25 (WTU); HM216782; EF571518. *Verbena bracteata* Lag. & Rodr.; Cultivated; RGO 1992-131 (WTU); L36418; na. *Verbena bracteata* Lag. & Rodr.; USA: Arizona; Y.-W. Yuan 2005-11 (WTU); na; EF571526. *Verbena carnea* Medik. (= *Stylodon carneus* (Medik.) Moldenke); USA: Florida; Zomlefer 693 (WTU); HM216783; HM216676. *Verbena hastata* L.; Cultivated; RGO 2003-155 (WTU); HM216784; EF571531. *Verbena intermedia* Gillies & Hook.; Argentina: Entre Ríos; RGO 2004-106 (WTU); HM216785; EF571522. *Verbena litoralis* Kunth; Argentina: Entre Ríos; RGO 2004-105 (WTU); HM216786; EF571520. *Verbena litoralis* Vahl; USA: Puerto Rico; RGO 2003-9 (WTU); HM216787; HM216677. *Verbena montevidensis* Spreng.; Argentina: Corrientes; RGO 2004-112 (WTU); HM216788; EF571521. *Verbena officinalis* L.; Cultivated; RGO 98-55 (WTU); HM216789; EF571525. *Verbena officinalis* L.; Cultivated; RGO 2003-156 (WTU); na; na; HM853833; HM853866; HM853899; na; HM853955. *Verbena rigida* Spreng.; Argentina: Corrientes; RGO 2004-111 (WTU); HM216790; EF571519. *Verbena urticifolia* L.; Cultivated; RGO 2003-157 (WTU); HM216791; EF571524. *Xeroaloysia ovatifolia* (Moldenke) Troncoso; Argentina: San Luis; RGO 2004-184 (WTU); HM216792; HM216678; HM853952; HM853829; HM853862; HM853895; HM853925. *Xolocotzia asperifolia* Miranda; Mexico: Chiapas; David Neill 5477 (MO); HM216793; HM216680. *Xolocotzia asperifolia* Miranda; Nicaragua: Matagalpa; W.D. Stevens 22332 (MO); HM216794; HM216679.
- OUTGROUPS.** *Bignonia capreolata* L.; Cultivated: RBG Kew 1980-3846; no voucher; FJ887855/DQ222566*; FJ870021. *Eccremocarpus scaber* Ruiz & Pav.; Cultivated: RBG Kew 1988-132; M.W. Chase 2999 (K); AF102630; FJ870030. *Jacaranda mimosifolia* D. Don; Brazil; L. Lohmann 369 (MO); EF105012; EF105070. *Callicarpa dichotoma* Rausch; Cultivated: Beal Bot. Gard.; Olmstead 88-012 (WTU); L36395; AF363665. *Lamium purpureum* L.; USA: Ohio; Wagstaff 88-031 (BHO); U78694; AF363664. *Paulownia tomentosa* (Thunb.) Steud.; Cultivated; Olmstead 88-008 (WTU); L36406; na. *Paulownia tomentosa* (Thunb.) Steud.; Cultivated; Erixon & Bremer 22 (UPS); na; AJ430926. *Barleria prionitis* L.; Cultivated: Uppsala Bot. Gard. 1977-3036 (UPS); U12653; na. *Barleria prionitis* L.; R. Scotland s.n.; na; AF063118. *Elytraria crenata* Vahl; R. Scotland s.n.; U12657; na. *Elytraria imbricata* (Vahl.) Pers.; USA: Arizona; McDade & Jenkins 1155 (ARIZ); na; AF061819. *Sesamum indicum* L.; Cultivated; no voucher; L36413; na. *Sesamum indicum* L.; Cultivated; P. Jenkins 97-141 (ARIZ); na; AF067067. *Schlegelia parviflora* (Oerst.) Monach.; Venezuela (Cultivated at MO); Gentry 14221 (MO); L36410; AJ608570. *Scrophularia californica* Cham. & Schldtl.; USA: California; C.W.

dePamphilis s.n. (PAC); L36411; na. *Scrophularia californica* Cham. & **Schldtl.**; E. Freeman unpubl.; na; AF118802. *Buddleja davidii* Franch.; Cultivated; Olmstead 88-007 (WTU); L36394; na. *Buddleja araucana* Phil.; Argentina: Neuquen; RGO 2007-94 (WTU); na; HM216599. *Myoporum mauritianum* A. DC.; Cultivated: RBG Kew 1984-4220; no voucher; L36403; AJ299257. *Nematanthus hirsutus* (Mart.) Wiehler; Cultivated; no voucher; L36404; na. *Nematanthus strigillosus* (Mart.) **H. E. Moore**; Cultivated: USBRG; J. Skog 7751 (US); na; AY047148.

Nyctanthes arbor-tristis L.; Cultivated: RBG Kew 099-86.00993; no voucher; U78708; na. *Nyctanthes arbor-tristis* L.; K. Dahlstrand s.n. (GB); na; AF231863. *Antirrhinum majus* L.; Cultivated; no voucher; L36413; na. *Antirrhinum majus* L.; Cultivated; Erixon & Bremer 10 (UPS); na; AJ430929. *Digitalis purpurea* L.; Cultivated; K.-J. Kim 13943 (YNUH); AF130150; na. *Digitalis purpurea* L.; Cultivated; E. Freeman unpubl.; na; AF034871. *Martynia annua* L.; USA: Arizona; P. Jenkins 97-149 (ARIZ); HM216755; HM216649.
