

Phylogenetic relationships in tribe Buddlejeae (Scrophulariaceae) based on multiple nuclear and plastid markers

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Buddlejeae comprise c. 108 species in five commonly accepted genera: *Buddleja*, *Chilianthus*, *Emorya*, *Gomphostigma* and *Nicodemia*. Conflicting generic and infrageneric level classifications based on morphology attest to a need to evaluate relationships and trait evolution in a molecular phylogenetic framework. We use multiple independent loci from the nuclear and plastid genomes and representative taxonomic sampling to infer phylogenetic relationships using maximum likelihood and Bayesian analyses with single-locus and concatenated data and Bayesian multispecies coalescent analyses. *Nicodemia* and *Gomphostigma* are resolved as monophyletic. *Chilianthus* is not monophyletic, with three species in one clade and *Buddleja glomerata* (= *Chilianthus lobulatus*) possibly separate. *Buddleja* is paraphyletic with respect to *Chilianthus*, *Emorya*, *Nicodemia* and, probably, *Gomphostigma*. We propose a new classification to reflect phylogenetic relationships in Buddlejeae. Only *Buddleja* is retained at the generic level. *Chilianthus*, *Nicodemia*, *Gomphostigma* and *Emorya* are combined with *Buddleja*, with a new name and new combination erected for the two *Emorya* spp., *Buddleja normaniae* and *B. rinconensis*. Sectional classification of *Buddleja* is revised, with two new monotypic sections being proposed, *Salviifoliae* and *Pulchellae*, and *Gomphostigma* being lowered to sectional rank. Reproductive morphological traits traditionally used to define genera, including stamen exertion, corolla shape and inflorescence type, were reconstructed on the phylogenetic tree and are inferred to have converged on similar states multiple times. Plesiomorphic trait states in Buddlejeae include capsular fruits, included stamens, white and tube-shaped corollas and paniculate inflorescences.

ADDITIONAL KEYWORDS: *Buddleja*–*Chilianthus*–chloroplast DNA–classification–*Emorya*–*Gomphostigma*–morphology – *Nicodemia* – PPR loci.

INTRODUCTION

Scrophulariaceae s.s. were first recognized as a distinct clade in the more broadly circumscribed and polyphyletic Scrophulariaceae s.l. by Olmstead & Reeves (1995) and were subsequently upheld in additional phylogenetic analyses of DNA markers (Oxelman, Backlund & Bremer, 1999; Kornhall, Heidari & Bremer, 2001; Olmstead *et al.*, 2001; Oxelman *et al.*, 2005; Rahmzadeh *et al.*, 2005). Scrophulariaceae s.l. were predominantly bilateral in corolla symmetry and cosmopolitan in distribution, including many charismatic

taxa of the northern temperate flora (e.g. *Antirrhinum* L., *Castilleja* Mutis ex L.f., *Digitalis* L., *Mimulus* L., *Penstemon* Schmidel, *Scrophularia* L., *Verbascum* L., *Veronica* L.), whereas Scrophulariaceae s.s. as currently circumscribed (Olmstead *et al.*, 2001; APG II, 2003; Tank *et al.*, 2006; APG IV, 2016) are composed mostly of taxa with radial or sub-radial corolla symmetry and distribution in the Southern Hemisphere. Phylogenetic studies of Scrophulariaceae s.s. identified eight tribes (Kornhall *et al.*, 2001; Kornhall & Bremer, 2004; Oxelman *et al.*, 2005), including Buddlejeae, which comprise c. 108 species and are one of only two tribes that have major radiations in the Northern and Southern Hemispheres (Tank *et al.*, 2006). Buddlejeae are typically shrubs or

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trees with opposite leaves and interpetiolar stipules, stellate, glandular trichomes and tetramerous, radially symmetrical flowers arranged in cymes. Buddlejaceae have a broad distribution, encompassing tropical, subtropical and warm-temperate areas of Africa, Asia and North and South America, and display wide morphological diversity, especially in the flower and inflorescence (Norman, 2000; Oxelman, Kornhall & Norman, 2004). Several species are known for their horticultural value [e.g. *Buddleja davidii* Franch. (butterfly bush), *B. alternifolia* Maxim., *B. globosa* Hopel], invasiveness following introductions outside their native range [e.g. *B. davidii* and *B. madagascariensis* Lam. (= *Nicodemia madagascariensis* (Lam.) R.Parker)] and use in traditional medicine [e.g. *B. officinalis* Maxim. (Chinese: mi meng hua), *B. coriacea* J.Rémy and *B. incana* Ruiz & Pav. (Quechua: kiswar)] (Li & Leeuwenberg, 1996; Norman, 2000; Tallent-Halsell & Watt, 2009).

Tribe Buddlejaceae as now recognized have had a complicated taxonomic history (see Norman, 2000, for a detailed review). They have been considered at various ranks as part of Scrophulariaceae (Bentham, 1835, 1846) or Loganiaceae (Bentham, 1857; Bentham & Hooker, 1876; Solereder, 1895; Leeuwenberg & Leenhouts, 1980) or separated as the family Buddlejaceae (Wilhelm, 1910; Wagenitz, 1964; Hutchinson, 1973; Takhtajan, 1980; Cronquist, 1981; Dahlgren, 1983; Thorne, 1983, 1992; Norman, 2000; Oxelman *et al.*, 2004). Molecular data from the plastid genome resolved the position of the group in Scrophulariaceae s.s. (Olmstead & Reeves, 1995; Oxelman *et al.*, 1999; Olmstead *et al.*, 2001), which supported earlier evidence of affinity from embryology (Wagenitz, 1964; Hakki, 1980), palynology (Punt & Leenhouts, 1967) and phytochemistry (Jensen, Nielsen & Dahlgren, 1975). Molecular phylogenetic studies also clarified the positions of several taxa that were once thought to be closely related and included in the group. *Androya* H.Perrier was transferred to tribe Myoporeae in Scrophulariaceae, *Nuxia* Lam. to Stilbaceae, *Polypremum* L. to Tetrachondraceae and *Peltanthera* Benth. and *Sanango* G.S.Bunting & J.A.Duke to or near Gesneriaceae (Oxelman *et al.*, 1999; Refulio-Rodriguez & Olmstead, 2014), leaving five genera, *Buddleja* L., *Chilianthus* Burch., *Nicodemia* Ten., *Gomphostigma* Trucz. and *Emorya* Torr., in Buddlejaceae (Oxelman *et al.*, 2004).

The majority of the species diversity and distributional area of the tribe is encompassed by *Buddleja*, which includes > 90 species distributed in Africa, Asia, North America and South America. Reproductive morphology in the genus is variable especially in corolla shape (short and cup-shaped to long and tubular), corolla colour (various shades of white, yellow, orange or purple) and architecture of the inflorescence in which cymes are arranged (paniculate, thyrsoid,

spiciform or capitate) (Leeuwenberg, 1979; Norman, 2000; Oxelman *et al.*, 2004). *Buddleja* was last comprehensively treated by Bentham (1846), who divided the genus based on differences in floral and inflorescence morphology. The Asian species were reclassified by Marquand (1930) and Li (1982), who erected infrageneric taxa based on phyllotaxy and floral traits. Leeuwenberg (1979) conducted a study of the African and Asian species and proposed a global classification based on reproductive morphology, in which most species were placed in a single section. Norman (2000) completed a monograph of the New World species and proposed 12 series based on morphology and ecogeography. A summary of generic and infrageneric classifications is presented in Table 1.

Four species in Buddlejaceae from southern Africa have been treated as members of *Buddleja* (Leeuwenberg, 1979) or the segregate genus *Chilianthus* (Bentham, 1846; Norman, 2000; Oxelman *et al.*, 2004). This group of species has been recognized because their floral morphology is distinguished by short, cup-shaped corollas, stamens with relatively long filaments that are partly or fully exerted and cymes in highly branched paniculate inflorescences. Some studies have suggested, however, that these morphological characters are neither constant in, nor exclusive, to these four species (Phillips, 1946; Leeuwenberg, 1979). Leeuwenberg (1979), who completed the most recent taxonomic study of African members of Buddlejaceae, recognized the group at the section level in *Buddleja*. Additionally, he removed one species, *B. loricata* Leeuwenberg, from this group because it has anthers with shorter filaments that are barely exerted from the corolla. Earlier studies suggested an affinity between *Chilianthus* and *Nuxia* due to similarities in floral and pollen morphology (Leeuwenberg, 1979; Punt, 1980). However, phylogenetic analyses of plastid DNA sequences showed that *Nuxia* is outside Scrophulariaceae (Oxelman *et al.*, 1999).

Eight species from Madagascar are distinct in having fleshy, indehiscent berry-like fruits instead of dry, dehiscent capsules as in all other members of Buddlejaceae. Although originally described in *Buddleja* and sometimes treated at an infrageneric rank there (Bentham, 1846; Leeuwenberg, 1979; Li, 1982; Norman, 2000), these species have also been segregated into the genus *Nicodemia* (Marquand, 1930; Oxelman *et al.*, 2004). A subset of these species was placed in another segregate genus *Adenoplea* Radlk. because they have four-celled rather than two-celled ovaries as found in the rest of Buddlejaceae. Another genus *Adenoplusia* Radlk. was erected because its members, which have all been combined with the species *Buddleja axillaris* Willd., have drupe-like fruits with a chartaceous endocarp (Bruce & Lewis, 1960; Leenhouts, 1962; Leeuwenberg, 1979).

Table 1. Selected generic and infrageneric classifications for Buddlejeae

	Bentham (1846)	Marquand (1930)	Leeuwenberg (1977, 1979)	Li (1982)	Norman (2000)	Oxelman et al. (2004)	Chau et al. (this study)
Genus <i>Buddleja</i>		Genus <i>Buddleja</i>	Genus <i>Buddleja</i>	Genus <i>Buddleja</i>	Genus <i>Buddleja</i>	Genus <i>Buddleja</i>	Genus <i>Buddleja</i>
Section <i>Lozada</i>	Series <i>Gynandrae</i> (As)	Section <i>Buddleja</i> (NW)	Section <i>Buddleja</i> (NW)	Subgenus <i>Buddleja</i>	Section <i>Buddleja</i>	Genus <i>Buddleja</i>	Section <i>Salviifoliae</i> (Af)
Subsection <i>Paniculatae</i> (NW)	Series <i>Alternifoliae</i> (As)	Section <i>Neemda</i> (Af, As, NW)	Section <i>Neemda</i> (Af, As, NW)	Section <i>Alternifoliae</i> (As)	Series <i>Thyrsoideae</i> (NW)	Section <i>Pulchellae</i> (Af)	Section <i>Pulchellae</i> (Af)
Subsection <i>Globosae</i> (NW)	Series <i>Curviflorae</i> (As)			Section <i>Neemda</i>	Series <i>Oblongae</i> (NW)		
Subsection <i>Verticillatae</i> (NW)	Series <i>Rectiflorae</i> (Af, As)			Series <i>Curviflorae</i> (As)	Series <i>Stachyoideae</i> (NW)		Section <i>Alternifoliae</i> (As)
Section <i>Neemda</i>				Series <i>Rectiflorae</i> (As)	Series <i>Globosae</i> (NW)		Section <i>Buddleja</i> (NW)
Subsection <i>Glomeratae</i> (Af, As, M, NW)					Series <i>Anchoenses</i> (NW)		
Subsection <i>Thyrsoideae</i> (Af, NW)					Series <i>Glomeratae</i> (NW)		
Subsection <i>Stachyoideae</i> (NW)					Series <i>Brachiatae</i> (NW)		
Subsection <i>Macrothyrsae</i> (Af, As, M)					Series <i>Lanatae</i> (NW)		
					Series <i>Scordioideae</i> (NW)		
					Series <i>Buddleja</i> (NW)		
					Series <i>Verticillatae</i> (NW)		
					Series <i>Cordatae</i> (NW)		

Table 1. *Continued*

	Bentham (1846)	Marquand (1930)	Leeuwenberg (1977, 1979)	Li (1982)	Norman (2000)	Oxelman <i>et al.</i> (2004)	Chau <i>et al.</i> (this study)
Subsection <i>Axilliflorae</i> (M)		Genus <i>Nicodemia</i>	Section <i>Nicodemia</i> (M)	Subgenus <i>Nicodemia</i> (M)	Section <i>Nicodemia</i> (M)	Genus <i>Nicodemia</i>	Section <i>Nicodemia</i> (Af, M)
Genus <i>Chilianthus</i>	-	-	Section <i>Chilianthus</i> (Af)	-	Genus <i>Chilianthus</i>	Genus <i>Chilianthus</i>	Section <i>Chilianthus</i> (Af)
Genus <i>Gomphostigma</i>	-	-	Genus <i>Gomphostigma</i>	-	-	Genus <i>Gomphostigma</i>	Section <i>Gomphostigma</i> (Af)
-	-	-	-	-	Genus <i>Emorya</i>	Genus <i>Emorya</i>	(part of Section <i>Buddleja</i>)

Groups corresponding to five previously accepted genera are shown on same row. Distribution is indicated for infrageneric groups (Af = continental Africa; As = Asia; M = Madagascar; NW = New World). For additional classification schemes, see Leeuwenberg & Leenhouts (1980).

Gomphostigma includes two species from southern Africa. They were first described as members of *Buddleja*, but were later segregated on the basis of their distinctive inflorescences, which are racemose rather than cymose, and flowers with corollas that are short and cup-shaped rather than tubular. Recent taxonomic treatments have kept this group distinct from *Buddleja* (Leeuwenberg, 1977; Oxelman *et al.*, 2004).

Emorya, with two species occurring in northern Mexico and the adjoining south-western United States, is distinct in its floral morphology from other *Buddlejeae* in North America. Their flowers have exerted stamens with long filaments and an exerted style and the corollas are tubular and much longer than those in all North American *Buddleja*. The corolla morphology in *Emorya* is similar to that in South American members of *Buddleja* series *Stachyoides* (Benth.) E.M. Norman. However, South American *Buddleja* spp. have stamens and styles that are included (Norman & Moore, 1968; Norman, 2000). Taxonomic treatments have always treated *Emorya* as distinct from *Buddleja* (Norman, 2000; Oxelman *et al.*, 2004).

Molecular phylogenetic studies including members of these five genera have shown that they form a well-supported clade in Scrophulariaceae (Oxelman *et al.*, 1999; Kornhall *et al.*, 2001; Kornhall & Bremer, 2004; Oxelman *et al.*, 2005). These studies have focused on higher-level relationships or other groups in the family and included at most one or two exemplars from each genus of *Buddlejeae*. Additionally, New World *Buddleja*, which is the most species-rich group in the tribe, has been represented by only a single species in one study (Kornhall & Bremer, 2004). It remains uncertain whether each of the five genera is monophyletic and what the pattern of relationships is among and within them. Moreover, all prior molecular data have come from the non-recombining plastid genome. Single gene trees may not accurately reflect species evolutionary history due to confounding factors, including incomplete lineage sorting, hybridization and introgression (Maddison, 1997). Single- and low-copy loci from the nuclear genome provide a source of independent data and are also often more quickly evolving (Sang, 2002; Small *et al.*, 2004), which may be more appropriate for studies at the level of species in *Buddlejeae*.

We present here the first molecular phylogenetic analysis of tribe *Buddlejeae* with broadly representative taxonomic sampling, including members of all recognized genera and extensive sampling of species in the large genus *Buddleja* from all parts of its range. We use sequence data from the nuclear ribosomal locus external transcribed spacer (ETS), three low-copy nuclear genes from the pentatricopeptide repeat (PPR) gene family (At1G31430/PPR24, At4G30825/PPR97, At5G39980/PPR123) and three plastid regions (*rpoA*,

trnD-trnT, *trnS-trnfM*). Our goals are to assess monophyly of the genera in Buddlejeae, evaluate relationships of major clades against current classifications, investigate the evolutionary history of morphological traits traditionally important in delimiting genera and establish a revised classification that reflects the phylogenetic trees.

MATERIAL AND METHODS

TAXON SAMPLING

Representative species from all genera and major areas of distribution were selected for this study. We follow the species names used in the most recent monographic works for the Old World and New World taxa (Leeuwenberg, 1977, 1979; Norman, 2000) and subsequent reports of newly described and resurrected species (Liu & Peng, 2004, 2006; Morales & González, 2007; Zhang *et al.*, 2014). The species that have been segregated into *Chilianthus* and *Nicodemia* are referred to by their name in *Buddleja*, as in the monograph by Leeuwenberg (1979), but their phylogenetic coherence and position will be a focus of our analyses.

Seventy-three out of 104 *Buddleja* spp. were sampled, including all four species sometimes treated as *Chilianthus* and six of eight species sometimes treated as *Nicodemia*. In *Buddleja*, we included all four species from Africa without synonyms in *Chilianthus* or *Nicodemia*, 20 of 24 species from Asia, 12 of 19 species from North America and 28 of 46 species from South America, including two subspecies of *B. elegans* Cham. & Schlttdl. All series and sections of Marquand (1930), Li (1982) and Leeuwenberg (1979) for Old World species and 11 of the 12 series proposed by Norman (2000) for New World species are represented. Both *Gomphostigma* spp. and one of two *Emorya* spp. were sampled. Six species were included as outgroups based on prior studies (Oxelman *et al.*, 1999, 2005; Kornhall *et al.*, 2001), including two taxa from the sister clade to Buddlejeae [*Oftia africana* (L.) Bocq. and *Phygelius capensis* E.Mey. ex Benth.], two more distant taxa in the Scrophulariaceae (*Scrophularia nodosa* L. and *Nemesia fruticans* Benth.) and two additional taxa in Lamiales (*Nuxia floribunda* Benth. in Stilbaceae and *Lantana depressa* Small in Verbenaceae). Voucher information and collection localities for all specimens are presented in Table A1.

MOLECULAR METHODS

Leaf tissue was sampled from specimens either as silica gel-preserved material from plants collected in the field or as fragments from herbarium specimens. Total DNA was extracted from leaf tissue using a modified CTAB procedure (Doyle & Doyle, 1987) and purified

by isopropanol precipitation. For some specimens from herbarium material, DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). For four specimens, DNA was directly obtained from the DNA banks at the Missouri Botanical Garden or the Royal Botanic Gardens, Kew (Table A1).

PCR amplification reactions for nuclear markers were performed in 25 µL volumes with 1 µL genomic DNA, 0.125 µL *Taq* DNA polymerase and final concentrations of 1× PCR buffer, 3 mM MgCl₂, 1 µg/µL bovine serum albumin, 0.25 mM dNTP mix and 0.25 µM each of the forward and reverse primers. Where amplification proved difficult, 1× TBT-PAR was included in the reaction mix (Samarakoon, Wang & Alford, 2013). Reactions were run in a MJ Research (Bio-Rad, Hercules, CA, USA) thermocycler with the following conditions: initial denaturation at 94 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1.5–2.5 min; and a final extension at 72 °C for 10 min. Four nuclear loci were targeted: the ETS region of ribosomal DNA and three PPR loci. The universal 18S-IGS (Baldwin & Markos, 1998) and Lamiales-specific ETS-B (Beardsley, Yen & Olmstead, 2003) primers were used to amplify ETS. Lamiales-specific primers were designed to amplify and sequence two PPR loci (Table A2). For locus At1G31430, hereafter called PPR24 according to its position in table 1 of Yuan *et al.* (2009), primers PPR24-140F and PPR24-1354R were used. For locus At4G30825 (PPR97), primers PPR97-781F and PPR97-1585R were used. For a third PPR locus, At5G39980 (PPR123), the Lamiidae-specific primers 550F and 1890R (Yuan *et al.*, 2010) were used. Three regions in the plastid genome were also targeted. Amplification reactions for plastid markers followed the protocols used in Yuan & Olmstead (2008). The *trnD-trnT* region was amplified with primers *trnD*^{GUCF} and *trnT*^{GGU}, the *trnS-trnfM* region with primers *trnS*^{UGA} and *trnfM*^{CAU} (Demesure, Sodji & Petit, 1995; Shaw *et al.*, 2005) and the *rpoA* region with primers RPOA2 and RPOA5 (Petersen & Seberg, 1997). Amplification products were cleaned using polyethylene glycol precipitation.

Sanger cycle sequencing was performed using the standard Applied Biosystems protocol with BigDye v3.1 and PCR or internal primers (Table A2). Sequencing reaction products for nuclear loci were purified by filtering through Sephadex G-50 columns or precipitation with sodium acetate and ethanol and then read on an Applied Biosystems 3130XL or 3730 Genetic Analyzer (Thermo Fisher Scientific, Grand Island, NY, USA). Plastid loci and some ETS sequencing reactions were performed by MacroGen Inc. using Applied Biosystems PRISM BigDye Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, South Korea). For most

of the length of each locus, at least two overlapping sequence fragments were generated to check for random sequencing errors. Sequence fragment data were edited and assembled into full sequences using Sequencher 4.7 (Gene Codes Corp.). Sites with multiple peaks were coded as ambiguities. All sequences have been deposited in GenBank (Table A1).

PHYLOGENETIC ANALYSES

For each locus, sequences were aligned with MAFFT v7 (Kato & Standley, 2013) using the default strategy and parameters (scoring matrix = 200PAM/K = 2; gap opening penalty = 1.53). Alignments were checked by eye and minor adjustments performed manually using Se-AL v2.0a11. A few plastid sequences (*B. blattaria* J.F.Macbr.: *trnS-trnfM*, *B. incana*: *trnS-trnfM*, *B. lanata* Benth.: *trnD-trnT*, *B. rufescens* Willd. ex Schultes & Schultes: *trnS-trnfM*) had regions that were difficult to align and these were deleted from the sequence.

Statistical analyses were used to reconstruct phylogenetic trees for each of the four nuclear loci, a concatenated plastid three-locus dataset and a concatenated nuclear and plastid seven-locus dataset. In the concatenated datasets, sequences from multiple accessions of the same species were combined in order to maximize the number of loci with sequence data for each species. Phylogenetic analyses with ETS sequences from all accessions were performed (Supporting Information, Fig. S2) and sequences were combined for a species only if there was no support for non-monophyly among accessions of that species. Although it has been suggested that composite taxa may give misleading results in phylogenetic analyses (Malia, Lipscomb & Allard, 2003), it has been demonstrated that their use can perform as well as or better than data matrices with more missing data, especially when there is evidence that combined taxa are monophyletic (Campbell & Lapointe, 2009).

The substitution model for each locus was chosen according to the Akaike information criterion (AIC) as calculated using jModeltest 2.1.4 (Guindon & Gascuel, 2003; Durriba *et al.*, 2012) with three substitution schemes. To reduce the problem of large sampling error, models that account for among-site rate variation using both a gamma distribution and proportion of invariable sites were excluded in favour of those that use only a gamma distribution (Sullivan, Swofford & Naylor, 1997). Concatenated datasets were partitioned by locus for analyses such that all evolutionary model parameters were unlinked.

Maximum likelihood analyses were performed in GARLI 2.0 (Zwickl, 2006; <http://garli.googlecode.com>). For the full search analyses, the generation termination condition was set at 20 000 and the score improvement threshold was set at 0.001. All other settings

were left at the default. Search runs were repeated until at least two replicates resulted in best-scoring trees with the same topology or 100 replicates were performed. For bootstrapping, 1000 replicates were performed with the generation termination condition decreased to 10 000 and the number of search replicates per bootstrap replicate set at 1.

Bayesian analyses were performed using MrBayes 3.2.1 or 3.2.3 (Ronquist *et al.*, 2012) on CIPRES Science Gateway (<http://www.phylo.org/index.php>). For each analysis, two runs with four chains each were performed. Analyses were run for 10 000 000 generations with a sampling frequency of 1000 for single-locus and concatenated plastid datasets and 30 000 000 generations with a sampling frequency of 3000 for the concatenated seven-locus dataset. Convergence was assessed by checking that the average standard deviation of split frequencies was < 0.05, the estimated sample size of parameters was > 200 as calculated in Tracer v1.5 (Rambaut & Drummond, 2009) and the plot of split frequencies showed high correlation as generated in AWTY (Wilgenbusch, Warren & Swofford, 2004). The initial 25% of trees sampled were discarded as burn-in. To evaluate the appropriateness of concatenating data from separate loci, the topologies of individual gene trees were visually examined for incongruences that are well supported [bootstrap percentage (BP) > 70% and posterior probability (PP) > 0.90].

Species tree estimation under the multispecies coalescent model was performed using *BEAST in BEAST v1.8.1 or v1.8.0 (Drummond *et al.*, 2012) on CIPRES, with data from all seven loci. Each of the four nuclear loci and the combined plastid dataset were treated as independent and set to have unlinked trees and clock models. In addition, all individual loci, including each of the three plastid loci, were set to have unlinked substitution models. The clock model for each locus was set as an uncorrelated lognormal relaxed clock with a mean having an exponential distribution with a mean of 10. The birth-death process was used as the species tree prior. Two runs were performed, each for 700 000 000 generations with a sampling frequency of 40 000. Convergence was assessed by evaluating the estimated sample size of parameters and checking for stationarity in the plot of log-likelihoods using Tracer v1.5. The initial 25% of trees was removed as burn-in and trees from both runs were combined before generating the maximum clade credibility tree with median node heights in TreeAnnotator v1.8.1.

TOPOLOGY TESTING

Topology tests were used to assess the monophyly of proposed genera as previously circumscribed. The maximum likelihood tree was inferred using GARLI 2.0 for the full concatenated dataset, with topological

constraints such that species traditionally placed in genera formed a clade. Six different constraints were tested: (1) *Chilianthus s.l.*, including *B. loricata* as monophyletic; (2) *Chilianthus s.s.*, excluding *B. loricata* as monophyletic; (3) *Buddleja s.l.*, including members of *Chilianthus s.l.* and *Nicodemia* as monophyletic; (4) *Buddleja s.s.*, excluding members of *Chilianthus s.l.* and *Nicodemia* as monophyletic; (5) *Buddleja* excluding only members of *Chilianthus s.l.* as monophyletic; and (6) *Buddleja* excluding only members of *Nicodemia* as monophyletic. All constrained maximum likelihood trees were compared with the unconstrained maximum likelihood tree by performing the Shimodaira–Hasegawa (SH) test in PAUP* using the REL method and 1000 bootstraps. Because the SH test is relatively conservative, the approximately unbiased (AU) test (Shimodaira, 2002) was also performed. TREE-PUZZLE (Schmidt *et al.*, 2002) was used to compute site-log-likelihood values under the HKY + G model, which were then used to perform the AU test in CONSEL (Shimodaira & Hasegawa, 2001).

MORPHOLOGICAL CHARACTER STATE RECONSTRUCTION

We investigated the evolution of reproductive characters that have been important in generic delimitation. For each species in Buddlejeae in our phylogenetic tree, traits were classified into categories based on species descriptions in taxonomic treatments (Leeuwenberg, 1977, 1979; Norman, 2000). For fruit type, fleshy fruits were coded as ‘berry’ and dry fruits were coded as ‘capsule’. For stamens, those that extend outside the corolla tube were coded as ‘exserted’ and those that are hidden inside the corolla tube were coded as ‘included’. Corolla shape could not be easily divided into categories because of continuous variation in this trait. The ratio of corolla tube length to corolla lobe length was compared to verbal descriptions from published treatments and a ratio of 1.8 was chosen as the dividing point between ‘cup-shaped’ (< 1.8) and ‘tube-shaped’ (> 1.8) corollas. Most corollas with a ratio < 1.8 are described as cup-shaped or funnellform in species descriptions and most with a ratio > 1.8 are described as tubular, cylindrical or salverform. For corolla colour, the colour of the majority of the corolla, generally including the lobes and outer tube, was classified as yellow, orange, purple or white. In many species, the corolla throat, or inner tube, has a different colour, which was not considered. For inflorescences, those with sessile flowers and peduncled cymes on a primary branch were considered ‘capitate’, those with sessile cymes and sessile flowers were considered ‘spiciform’, those with peduncled cymes and pedicellate flowers were considered ‘thyrsoid’, those with greater than one order of branching were considered ‘paniculate’ regardless of presence

or absence of peduncles and pedicels and those with single-flowered cymes in a raceme were considered ‘racemose’ (Table A3).

All taxa were coded as having a single state for each trait, although in rare cases another state occurs at low frequency. States of taxa outside Buddlejeae were coded as missing because outgroup taxa represent large clades that typically include large variation in trait states and sampling was not sufficient to be representative. Maximum likelihood analyses were conducted under the one-rate Mk1 model in Mesquite v.2.75 (Maddison & Maddison, 2015) using the majority-rule consensus tree from Bayesian analyses of the concatenated seven-locus dataset. Bayesian analyses were conducted in BayesTraits v2.0 (Pagel & Meade, 2014) using a restricted one-rate model and the posterior distribution of trees from Bayesian analyses of the concatenated seven-locus dataset, excluding 25% burn-in. The prior for the rate was set as an exponential distribution with a mean of 10 and analyses were run for 1 000 000 generations with sampling every 1000 generations. The probabilities of trait states were averaged over generations after a burn-in of 10%.

RESULTS

DATASET CHARACTERISTICS

The total concatenated aligned dataset consisted of 6235 bp for each of 83 taxa, including 77 taxa in Buddlejeae. Among the characters, 2289 were variable, of which 1144 were potentially parsimony-informative. Seventy-seven taxa had sequence data for at least four of the seven loci, with 60 of these having data for at least six loci. The remaining six species had data for one or three loci and were included to increase taxonomic breadth and comprehensiveness. Characteristics of individual loci are shown in Table 2. The substitution model chosen using the AIC criterion was GTR + G for all loci.

PHYLOGENETIC RECONSTRUCTIONS

Topologies from maximum likelihood and Bayesian reconstructions for a dataset were generally consistent, with differences only at poorly supported nodes. We considered nodes to be strongly supported if they received support values of BP ≥ 90% and PP ≥ 0.95 and moderately supported if they received support values of 70% ≤ BP < 90% or 0.90 ≤ PP < 0.95.

Individual gene trees are shown in Supporting Information, Figure S1A–E. All individual gene trees confirm Buddlejeae to be monophyletic with strong or moderate support. The two *Gomphostigma* spp. formed a clade with strong support in all gene trees. Species that have been placed in *Nicodemia* formed a

Table 2. Characteristics of individual locus datasets

Locus	Genome	Sequenced length range (bp)	Aligned length (bp)	Variable characters (% of aligned length)	Potentially parsimony-informative characters (% of aligned length)	Taxa with sequence data [% of all taxa ($n = 83$)]
ETS	Nuclear	321–449	468	271 (57.9%)	170 (36.3%)	83 (100%)
PPR24	Nuclear	959–1192	1192	583 (48.9%)	327 (27.4%)	70 (84.3%)
PPR97	Nuclear	556–778	778	334 (42.9%)	164 (21.1%)	64 (77.1%)
PPR123	Nuclear	535–1276	1279	494 (38.6%)	272 (21.3%)	72 (86.7%)
<i>trnD-trnT</i>	Plastid	590–856	897	154 (17.2%)	61 (6.8%)	76 (91.6%)
<i>trnS-trnfM</i>	Plastid	522–829	889	218 (24.5%)	58 (6.5%)	48 (57.8%)
<i>rpoA</i>	Plastid	673–697	732	235 (32.1%)	92 (12.6%)	78 (94%)

clade with strong support in the ETS and PPR24 trees. A clade with all the Asian *Buddleja* spp. was inferred in three of the five gene trees and had strong support in the plastid tree. Species in *Buddleja* s.s. or *Chilianthus* did not form monophyletic groups in any of the five gene trees. Topologies among gene trees were not completely congruent, but no strongly supported differences occurred at deeper nodes in Buddlejeae.

The seven-locus concatenated dataset yielded congruent trees from maximum likelihood and Bayesian analyses (Fig. 1). Buddlejeae received strong support as monophyletic, as did *Gomphostigma*. Members of *Nicodemia* also formed a clade, with strong support in the Bayesian analysis. *Chilianthus* spp. did not form a monophyletic group. *Buddleja saligna* Willd. (= *Chilianthus oleaceus* Burch.) and *B. loricata* (= *C. corrugatus* Benth.) had strong support as sister species and they together with *B. dysophylla* (Benth.) Radlk. (= *C. dysophyllus* Benth.) and *B. auriculata* Benth. formed a clade, but with low support. These four species were found in a larger clade with *Gomphostigma*, which excluded *B. glomerata* H.Wendl. (= *C. lobulatus* Benth.). In *Buddleja*, there are two large well-supported clades, one comprising species from the New World and one comprising species from Asia. *Buddleja* was inferred to be paraphyletic. *Buddleja salviifolia* (L.) Lam. was sister to the rest of Buddlejeae. *Emorya* was sister to the clade of New World *Buddleja*. The Asian *Buddleja* clade was part of a well-supported clade with *B. polystachya* Fresen. and *Nicodemia*. The backbone representing relationships among these major groups generally had low support, particularly in the maximum likelihood analysis.

The species tree from the coalescent-based *BEAST analyses (Fig. 2) had a topology similar to the phylogenetic trees from the concatenated seven-locus dataset. Strongly supported relationships inferred in all analyses include monophyletic Buddlejeae, *Gomphostigma* and Asian *Buddleja*. In the species tree analysis, a clade comprising all New World *Buddleja* and *Emorya* received strong support, as did a clade comprising

B. polystachya and *Nicodemia*; these clades also received strong support in the Bayesian analyses of concatenated data. Topological differences were at weakly supported nodes. In the species tree analyses, *Gomphostigma* was sister to the rest of Buddlejeae, but with weak support.

TOPOLOGY TESTS

SH tests were not significant when *Chilianthus* was constrained to be monophyletic in either its narrow ($P = 0.14$) or broad ($P = 0.29$) circumscriptions. AU tests rejected the monophyly of *Chilianthus* s.s. ($P < 0.01$), but when *B. loricata* is included, the group could marginally not be rejected ($P = 0.06$). SH tests were significant when *Buddleja* s.s. ($P = 0.00$) or *Buddleja* without *Nicodemia* (0.03) were constrained, but not when *Buddleja* s.l. (0.43) or *Buddleja* without *Chilianthus* ($P = 0.07$) were constrained. In AU tests, monophyly of *Buddleja* in all of its narrower circumscriptions was rejected ($P < 0.05$), but the monophyly of *Buddleja* s.l. could not be rejected ($P = 0.15$).

MORPHOLOGICAL CHARACTER STATE RECONSTRUCTION

Maximum likelihood and Bayesian analyses generally agreed on the highest-probability states for the nodes representing the most recent common ancestors of major clades (Table 3, Fig. 3). The most recent common ancestor of Buddlejeae was inferred to have capsular fruits, included stamens, tube-shaped, white corollas and paniculate inflorescences. The most recent common ancestor of *Nicodemia* had berries and represented the only transition to fleshy fruits. Exserted stamens and cup-shaped corollas evolved multiple times, possibly twice in African taxa with one reversal and at least once in the New World clade. Corolla colour transitioned many times: to yellow in the most recent common ancestor of the New World species; to purple in the most recent common ancestor of the

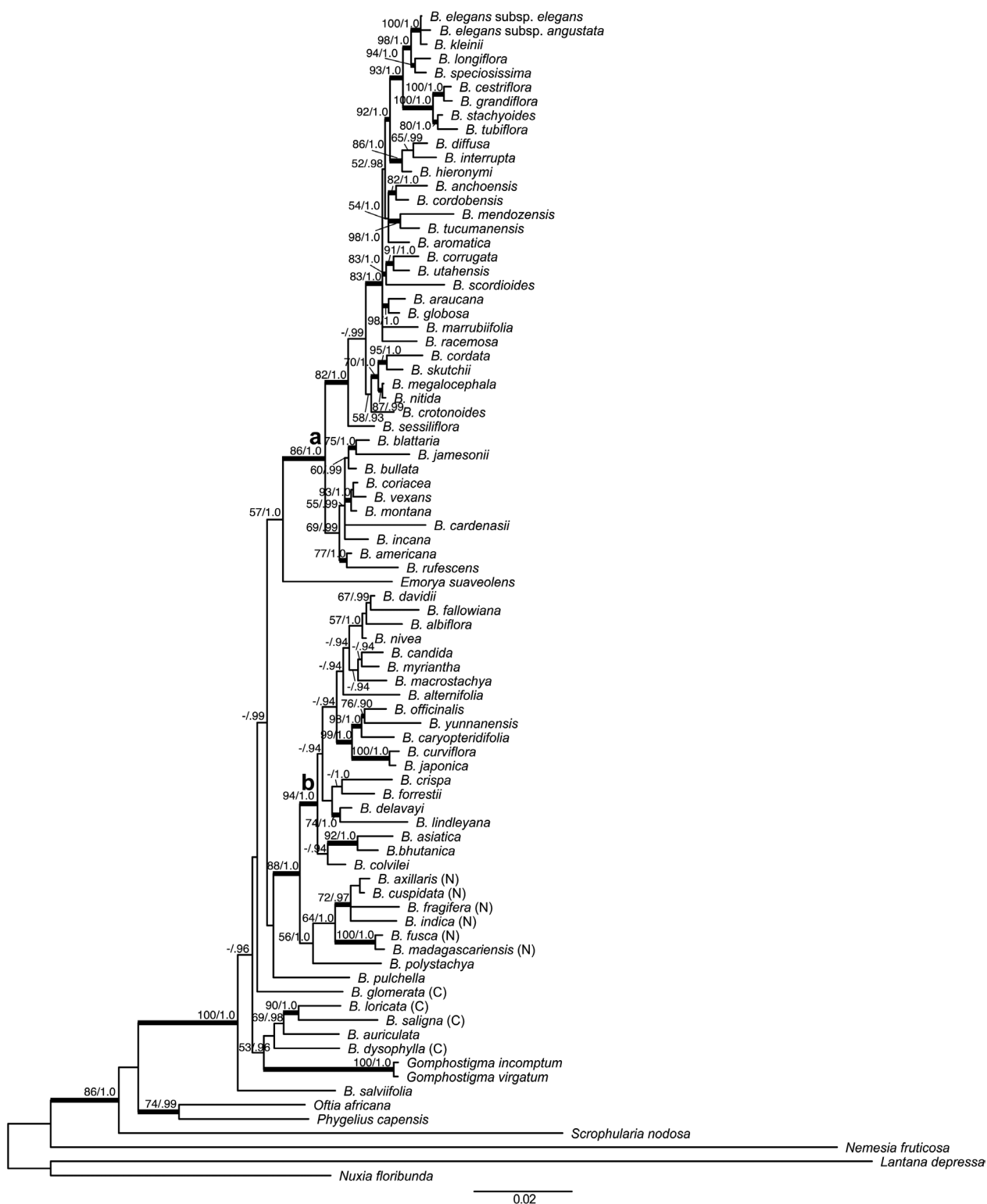


Figure 1. Majority-rule consensus phylogram from Bayesian analysis of concatenated seven-locus dataset. Values at nodes indicate support: maximum likelihood bootstrap percentage (BP)/Bayesian posterior probability (PP), if > 50% BP or 0.5 PP. Nodes with > 70% BP and 0.9 PP support are highlighted with thicker branches. Letter after species name indicates species that has also been considered a member of *Chilianthus* (C) or *Nicodemia* (N). Two nodes are marked: (a) clade of New World *Buddleja* spp. and (b) clade of Asian *Buddleja* spp.

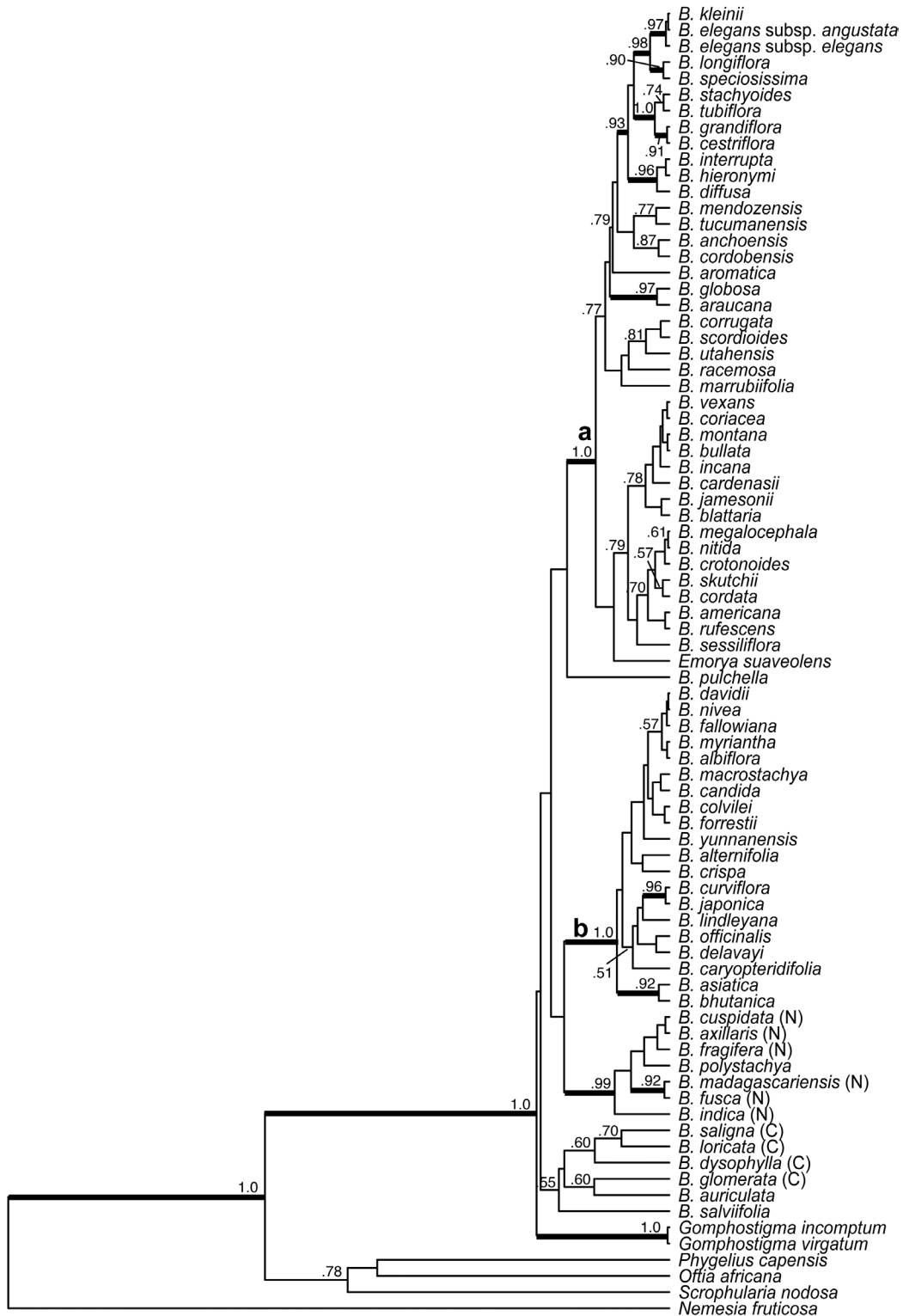


Figure 2. Maximum clade credibility tree from Bayesian multispecies coalescent analyses (*BEAST). Values at nodes indicate posterior probability (PP) support, if > 0.5. Nodes with > 0.9 PP support are highlighted with thicker branches. Letter after species name indicates species that has also been considered a member of *Chilianthus* (C) or *Nicodemia* (N). Two nodes are marked: (a) clade of New World *Buddleja* spp. and *Emorya suaveolens* and (b) clade of Asian *Buddleja* spp. Outgroups outside Scrophulariaceae are not shown.

Table 3. Probabilities of trait states at nodes corresponding to numbers in Figure 3

Node	Fruit type (berry/capsule)	Stamen exertion (exserted/included)	Corolla shape (cup-shaped/tube-shaped)	Corolla colour (yellow/orange/purple/white)	Inflorescence (capitate/spiciform/thyrsoid/paniculate/racemose)
1 – Buddlejeae	0/1 (0/1)	0.44/ 0.56 (0.31/ 0.69)	0.32/ 0.68 (0.30/ 0.70)	0.17/0.07/0.08/ 0.68 (0.15/0.06/0.06/ 0.72)	0.02/0.02/0.03/ 0.92 /0.02 (0.02/0.02/0.03/ 0.91 /0.02)
2	0/1 (0/1)	0.51 /0.49 (0.69 /0.31)	0.36/ 0.64 (0.54/ 0.46)	0.18/0.04/0.05/ 0.72 (0.27/0.06/0.07/ 0.60)	0/0/0.01/ 0.98 /0 (0.01/0.01/0.04/ 0.93 /0.01)
3	0/1 (0/1)	0.49/ 0.51 (0.41/ 0.59)	0.32/ 0.68 (0.29/ 0.71)	0.25/0.05/0.07/ 0.63 (0.50 /0.10/0.12/0.28)	0/0/0.02/ 0.97 /0 (0.02/0.03/0.16/ 0.77 /0.02)
4	0/1 (0/1)	0.14/ 0.86 (0.01/ 0.99)	0.08/ 0.92 (0.01/ 0.99)	0.29/0.07/0.10/ 0.53 (0.43 /0.18/0.22/0.17)	0/0/0.05/ 0.94 /0 (0.03/0.05/ 0.46 /0.42/0.03)
5 – section <i>Buddleja</i>	0/1 (0/1)	0.14/ 0.86 (0.28/ 0.72)	0.06/ 0.94 (0.11/ 0.89)	0.48 /0.10/0.09/0.33 (0.75 /0.10/0.08/0.07)	0.01/0.01/0.06/ 0.90 /0.01 (0.09/0.10/0.20/ 0.53 /0.08)
6	0/1 (0/1)	0.82 /0.18 (0.98 /0.02)	0.58 /42 (0.92 /0.08)	0.09/0.03/0.03/ 0.85 (0.04/0.03/0.03/ 0.89)	0/0/0.01/ 0.98 /0.01 (0.03/0.03/0.03/ 0.83 /0.06)
7 – section <i>Chilianthus</i>	0/1 (0/1)	0.87 /0.13 (0.97 /0.03)	0.67 /0.33 (0.90 /0.10)	0.04/0.01/0.02/ 0.93 (0.02/0.02//0.02/ 0.93)	0/0/0/ 0.99 /0 (0.01/0.01/0.01/ 0.95 /0.01)
8	0/1 (0/1)	0.88 /0.12 (0.74 /0.26)	0.68 /0.32 (0.68 /0.32)	0.02/0.01/0.01/ 0.96 (0.03/0.03/0.03/ 0.91)	0/0/0/1/0 (0.02/0.02/0.02/ 0.93 /0.02)
9	0/1 (0/1)	0.97 /0.03 (0.99 /0.01)	0.85 /0.15 (0.96 /0.04)	0.02/0.01/0.01/ 0.96 (0.05/0.05/0.05/ 0.85)	0/0/0/ 0.99 /0 (0.04/0.04/0.04/ 0.86 /0.04)
10 – section <i>Gomphostigma</i>	0/1 (0/1)	1/0 (1/0)	1/0 (1/0)	0/0/0/ 1.0 (0/0/0/ 1.0)	0/0/0/0/1 (0/0/0/0/1)
11	0/1 (0/1)	0.04/ 0.96 (0/1)	0.05/ 0.95 (0.02/ 0.98)	0.26/0.09/0.14/ 0.51 (0.20/0.23/ 0.33 /0.25)	0.01/0.01/0.10/ 0.88 /0.01 (0.05/0.07/ 0.55 /0.28/0.05)
12	0/1 (0.03/ 0.97)	0/1 (0/1)	0/1 (0.01/ 0.99)	0.19/0.19/ 0.43 /0.19 (0.08/0.32/ 0.54 /0.05)	0.01/0.03/ 0.82 /0.13/0.01 (0.01/0.05/ 0.91 /0.02/0.01)
13 – section <i>Alternifoliae</i>	0/1 (0/1)	0/1 (0/1)	0/1 (0/1)	0.01/0.01/ 0.95 /0.03 (0/0/ 0.98 /0.01)	0/0.01/ 0.92 /0.06/0 (0/0.03/ 0.91 /0.05/0)
14 – section <i>Nicodemia</i>	0.01/ 0.99 (0.74 /0.26)	0/1 (0/1)	0/1 (0.02/ 0.98)	0.24/ 0.37 /0.25/0.14 (0.16/ 0.73 /0.05/0.07)	0.01/0.07/ 0.84 /0.08/0.01 (0.06/0.28/ 0.56 /0.05/0.05)
15	1/0 (1/0)	0/1 (0/1)	0/1 (0.01/ 0.99)	0.38/ 0.43 /0.09/0.09 (0.34/ 0.53 /0.04/0.08)	0.01/0.03/ 0.94 /0.01/0 (0.03/0.04/ 0.89 /0.01/0.01)

Nodes representing most recent common ancestors of major clades in revised classification are indicated. The first set of numbers are from maximum likelihood analyses under an equal rates model. The second set of numbers, in parentheses, are averaged posterior probabilities from Bayesian analyses. Highest probabilities are highlighted in bold.

Asian *Buddleja* clade, *B. polystachya* and *Nicodemia*; and to orange in the most recent common ancestor of *B. polystachya* and *Nicodemia*. Inflorescence type has also been evolutionarily labile. The most recent common ancestor of *Gomphostigma* evolved racemose inflorescences and the most recent common ancestor of the Asian *Buddleja* clade, *B. polystachya* and *Nicodemia* probably evolved thyrsoid inflorescences.

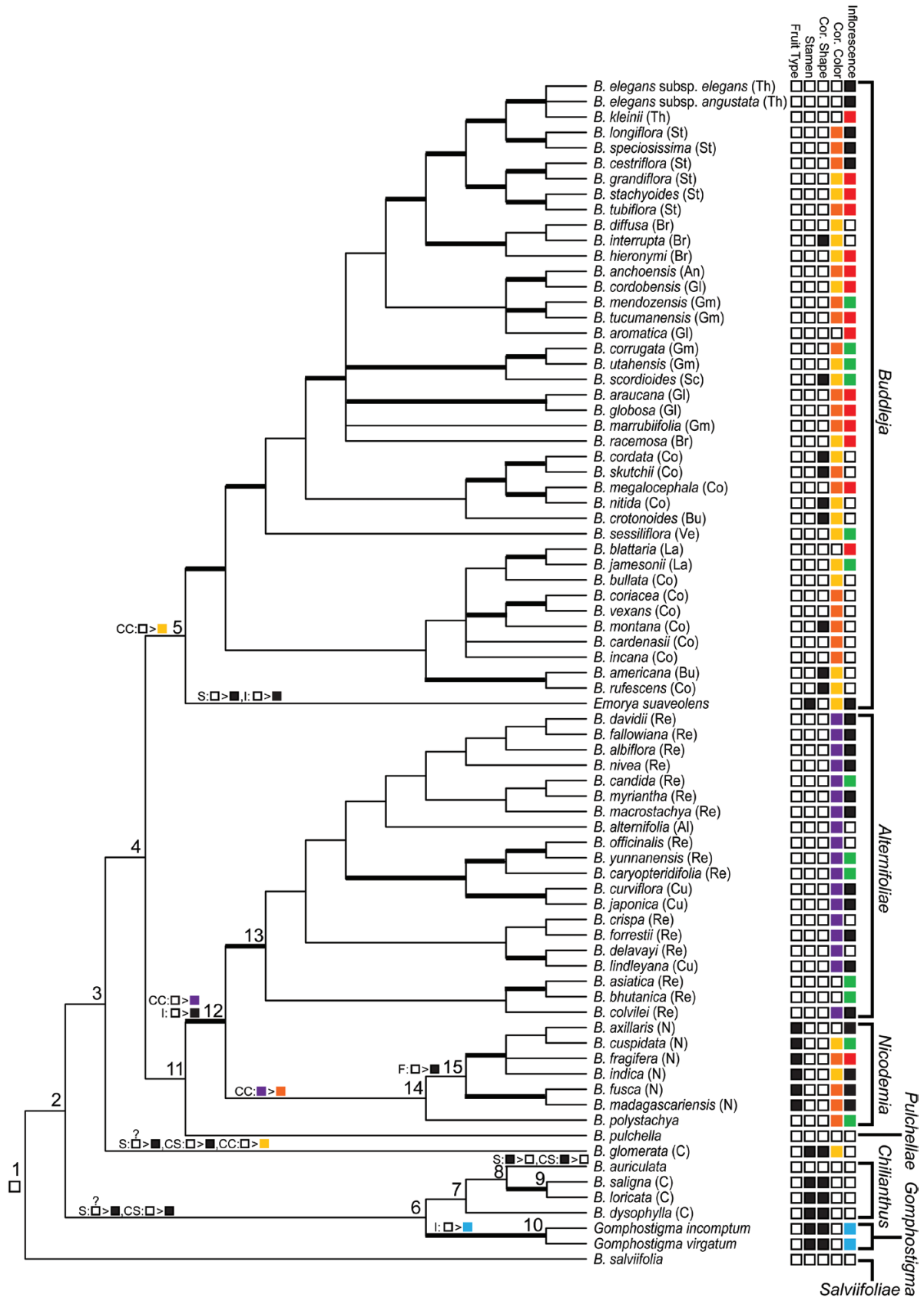
DISCUSSION

We have inferred the first molecular phylogenetic hypotheses of species relationships in tribe Buddlejeae with extensive sampling encompassing > 70% of the species diversity in the tribe. Members

of Buddlejeae form a strongly supported clade in Scrophulariaceae in all analyses of nuclear and plastid sequence data, corroborating results from previous studies of the tribe and family using plastid sequences (Olmstead & Reeves, 1995; Oxelman et al., 1999, 2005; Kornhall et al., 2001). Our data from the nuclear genome also reject a close relationship between *Nuxia* and *Chilianthus* in Buddlejeae, consistent with previous analyses of plastid data (Oxelman et al., 1999, 2005).

GENERIC CIRCUMSCRIPTION AND RELATIONSHIPS

Buddleja, in any of its previous circumscriptions, is paraphyletic. There is strong evidence from both concatenated and species tree analyses that *Emorya* and



Nicodemia are derived from within *Buddleja*. New World *Buddleja* spp. are more closely related to *Emorya* than they are to the Asian or African species. *Buddleja polystachya* is more closely related to *Nicodemia* than to other *Buddleja* spp. Reconstructions from both analyses suggest that *Chilianthus* is also derived from within *Buddleja*, though with lower support; and in the concatenated analyses, *Buddleja* is also paraphyletic with respect to *Gomphostigma*. *Buddleja auriculata* is inferred to be more closely related to *Chilianthus* and, in the concatenated analyses also to *Gomphostigma*, than to other *Buddleja* spp. Topology tests rejected the monophyly of *Buddleja* when it excluded the species in either or both *Chilianthus* and *Nicodemia*, but could not reject a more inclusive clade comprising members of all three genera, leaving only *Emorya* and *Gomphostigma* outside the group. However, with strong support across phylogenetic analyses for the close relationship between New World *Buddleja* and *Emorya* and some support for a close relationship among *B. auriculata*, *Chilianthus* and *Gomphostigma*, we believe there is sufficient evidence to assert that *Buddleja* is not monophyletic even in this broadest circumscription.

The monophyly of *Chilianthus* was not supported in our analyses. Three species in the group, *B. dysophylla*, *B. loricata* and *B. saligna*, were found in a clade (hereafter, called core *Chilianthus*), which also included *B. auriculata* in analyses with concatenated data. A sister species relationship between *B. loricata* and *B. saligna* was recovered in both concatenated and species tree analyses, with strong support in the concatenated analyses. The fourth member of the *Chilianthus* group, *B. glomerata*, was consistently outside of this clade, but its precise phylogenetic position is equivocal. In the species tree analysis, *B. glomerata* together with *B. auriculata* is sister to core *Chilianthus*, whereas in the concatenated data analyses, it is more distantly related. Topology tests

indicated that a monophyletic *Chilianthus* including all four species could not be rejected, but only marginally. Leeuwenberg (1979) removed *B. loricata* from the *Chilianthus* group because its stamens have shorter filaments and are barely exerted. A clade comprising the remaining three species in *Chilianthus*, *B. dysophylla*, *B. glomerata* and *B. saligna* was never recovered and topology tests rejected the monophyly of this group. The three species in core *Chilianthus* share several traits besides the typical *Chilianthus* floral morphology of short, cup-shaped corollas, long, exerted stamens and paniculate inflorescences. They also have white or cream corollas with an orange or maroon throat, pubescence on the inside of the corolla and a reticulate seed coat. *Buddleja glomerata* has the typical *Chilianthus* floral morphology, but has yellow corollas, is glabrous inside the corolla tube and has seeds with a smooth coat. *Buddleja auriculata*, which was found to be closely related to core *Chilianthus* in both analyses, is similar morphologically in having white corollas with an orange throat, pubescence inside the corolla tube and a reticulate seed coat, but the corolla shape is long and tubular and the stamens are included (Leeuwenberg, 1979). *Gomphostigma* was resolved to be closely related to core *Chilianthus* in the concatenated analyses, but without strong support. Both *Gomphostigma* and *Chilianthus* have short corolla tubes and exerted stamens, but *Gomphostigma* is distinct in having racemose inflorescences and corollas that are pure white (Leeuwenberg, 1977; Oxelman et al., 2004). Relationships among core *Chilianthus*, *B. glomerata*, *B. auriculata* and *Gomphostigma* were poorly supported and inconsistent in our analyses and additional data will be required to fully resolve their history.

Nicodemia spp. formed a clade in the phylogenetic trees inferred from concatenated data, with strong support in the Bayesian analyses. They are unique in

Figure 3. Majority-rule consensus cladogram from Bayesian analysis of concatenated seven-locus dataset. Nodes with > 70% bootstrap percentage and 0.9 posterior probability support are highlighted with thicker branches. Single letter after species name indicates species that has also been considered a member of *Chilianthus* (C) or *Nicodemia* (N). Two-letter codes after species name indicate infrageneric classification in *Buddleja*. For New World species, this follows Norman (2000): series *Anchoenses* (An), *Brachiatae* (Br), *Buddleja* (Bu), *Cordatae* (Co), *Globosae* (Gl), *Glomeratae* (Gm), *Lanatae* (La), *Scordioides* (Sc), *Stachyoides* (St), *Thyrsooides* (Th), *Verticillatae* (Ve). For Asian species, this follows Marquand (1930): series *Alternifoliae* (Al), *Curviflorae* (Cu), *Rectiflorae* (Re). Circumscription of sections in revised classification of *Buddleja* shown at far right. Coloured boxes indicate trait states of taxa. First column from left – fruit type: capsule (white) or berry (black); second column – stamen exertion: included (white) or exerted (black); third column – corolla shape: tube-shaped (white) or cup-shaped (black); fourth column – corolla colour corresponds to box colour: white, yellow, orange or purple; fifth column – inflorescence type: paniculate (white), thyrsoid (black), spiciform (green), capitata (red) or racemose (blue). Inferred ancestral states of Buddlejeae indicated by white box at root: capsule, included stamens, tube-shaped, white corolla and paniculate inflorescence. Major transitions between states are indicated above branches where inferred (F = fruit type, S = stamen exertion, CS = corolla shape, CC = corolla colour, I = inflorescence). Question mark (?) above transition indicates equivocal reconstruction. Transitions within sections *Buddleja*, *Alternifoliae* and *Nicodemia* are generally not indicated. Numbers at nodes correspond to those in Table 3. Outgroups outside Buddlejeae are not shown.

Buddlejeae in having indehiscent fleshy fruits, usually considered berries, and they share a main distribution in Madagascar with some species also found in surrounding islands and eastern Africa. *Buddleja polystachya*, a species from eastern Africa and the Arabian peninsula not previously assigned to *Nicodemia*, was resolved to be closely related to *Nicodemia* in all analyses. It is sister to *Nicodemia* in the concatenated analyses and is nested in *Nicodemia* in the species tree analyses. *Buddleja polystachya* shares a yellow to orange corolla with many *Nicodemia* spp. and they all have thyrsoid inflorescences, which differ from the paniculate inflorescences found in the basal grade of African Buddlejeae. The fruits of *B. polystachya* may represent an intermediate condition between the dry, septicidally dehiscent capsules of most Buddlejeae and the fleshy, indehiscent berries in *Nicodemia*; its dry fruits are partially indehiscent, with valves described as 'not torn' (Leeuwenberg, 1979). Some members of *Nicodemia* have at times been placed in other segregate genera. *Adenoplea* is differentiated by its four-celled ovaries, as opposed to the two-celled ovaries found in the rest of Buddlejeae and most of Scrophulariaceae (Leenhouts, 1962; Leeuwenberg, 1979). The two species we sampled with four-celled ovaries, *B. fusca* Baker and *B. madagascariensis*, consistently formed a well-supported clade. The two other species with this trait, *B. acuminata* Poir. and *B. sphaerocalyx* Baker, need to be sampled to determine their phylogenetic position. *Adenoplusia* is distinct in having drupe-like fruits with a chartaceous endocarp (Bruce & Lewis, 1960). All of its species have been combined with *B. axillaris* Willd., which is in the *Nicodemia* clade.

The two *Gomphostigma* spp. received strong support as sister taxa in all analyses. Both species are from southern Africa and share a distinct suite of morphological traits, including racemose inflorescences, cup-shaped corollas and exserted stamens (Oxelmann et al., 2004). *Gomphostigma* is part of a basal grade of African members of Buddlejeae, although its exact position is not well supported. In the concatenated analyses, it is sister to a clade consisting of core *Chilianthus* and *B. auriculata*, whereas in the species tree analysis, it forms the sister group to the rest of the tribe.

Only one of two *Emorya* spp. was sampled in this study, so the monophyly of this group could not be assessed. Both species are distributed in north-central Mexico, but the unsampled species *E. rinconensis* Mayfield is known from only a single locality in Coahuila state. The two species share several traits, including long-tubular corollas, exserted styles and exserted stamens with long filaments, that suggest a close relationship, but there are also notable differences. Inflorescences are thyrsoid in *E. suaveolens*

Torr. but racemose in *E. rinconensis* and pollen is tetracolporate in *E. suaveolens* but tricolporate in *E. rinconensis* (Mayfield, 1999). *Emorya suaveolens* forms a clade with New World *Buddleja* spp. with strong support. In the concatenated analyses, *Emorya* is sister to all New World *Buddleja*, whereas in the species tree analysis, it is sister to one of two main New World clades. Despite noted similarities in floral morphology, including a long corolla tube, between *Emorya* and members of the South American *Buddleja* series *Stachyoides* (Norman, 2000), a close relationship between these two groups was not found.

INFRAGENERIC RELATIONSHIPS IN *BUDDELEJA*

Relationships among *Buddleja* spp. show strong geographical signal, particularly at the continental level. Based on our results, infrageneric classification schemes in *Buddleja* (Table 1) that ignore geographical distribution and group species from separate continents in the same taxon do not reflect evolutionary relationships. Most systematic studies in *Buddleja* have been regionally focused and the composition of their proposed infrageneric taxa has been limited to species from a single region. However, the classifications of Bentham (1846), Marquand (1930) and Leeuwenberg (1979) included several infrageneric groups with distributions spanning multiple continents, which are not supported by our results (e.g. section *Neemda* Benth., subsection *Glomeratae* Benth., subsection *Thyrsoideae* Benth., subsection *Macrothrysa* Benth., series *Rectiflorae* Marquand and section *Neemda sensu* Leeuwenberg).

Southern African members of Buddlejeae, including *B. salviifolia*, *B. auriculata*, *Chilianthus* and *Gomphostigma*, make up a basal grade. *Buddleja salviifolia* is resolved as sister to all other species of Buddlejeae in the concatenated analyses, whereas it is in a clade with *B. auriculata* and *Chilianthus* in the species tree analyses. The remaining species in Buddlejeae are found in two major clades. One of them comprises the rest of the Old World species and forms two groups: a clade with all the Asian *Buddleja* and a clade with *Nicodemia* and *B. polystachya*, species from Madagascar and eastern Africa. The other major clade consists of all the New World species. The position of *B. pulchella* N.E.Br. from southern and eastern Africa is not well supported, but it may be sister to one of these two major clades. The relationships of *B. auriculata* with *Chilianthus* and *Gomphostigma* and of *B. polystachya* with *Nicodemia* are discussed in the preceding section on generic relationships.

Bentham (1846), Marquand (1930) and Leeuwenberg (1979) placed Asian *Buddleja* spp. in groups with

species from Africa and sometimes Madagascar and the New World, for example subsection *Glomeratae* Benth., subsection *Macrothrysa* Benth., series *Rectiflorae* Marquand and section *Neemda sensu* Leeuwenberg, none of which was supported by our analyses. Marquand (1930) focused mostly on Asian *Buddleja* and he proposed an infrageneric classification, which was generally followed by Li (1982), based on differences in leaf arrangement and floral morphology. *Buddleja alternifolia*, which is unique in the genus in having alternate leaves, is the only currently accepted species in section *Alternifoliae* Kränzl. The remaining species are considered synonyms of *B. alternifolia* or *B. asiatica* Lour., both of which are in the Asian *Buddleja* clade. (Although the type of section *Alternifoliae* is *B. amentacea* Kränzl., synonymized with *B. asiatica*, this has not always been recognized by previous taxonomists when circumscribing the group). Series *Curviflorae* Marquand, which is distinguished by curved corolla tubes, was partly supported by our phylogenetic reconstructions. *Buddleja curviflora* Hook. & Arn. and *B. japonica* Hemsl. are strongly supported as sister species in concatenated and species tree analyses, but the position of the third species, *B. lindleyana* Fortune, is uncertain. In the species tree analyses, it forms a clade with the other two species, but in the concatenated analyses, they are not closely related. The varying position of *B. lindleyana* in the different gene trees suggests that introgression or retention of ancestral polymorphism may be a factor (Maddison, 1997). Series *Rectiflorae*, which includes the majority of the Asian species and is characterized by straight corolla tubes, is paraphyletic with respect to *Alternifoliae* and *Curviflorae* (Fig. 3).

For New World *Buddleja*, the classification of Bentham (1846) included several groups, for example, subsection *Paniculatae* Benth., subsection *Globosae* Benth., subsection *Verticillatae* Benth., subsection *Stachyoides* Benth., none of which was supported as monophyletic in our phylogenetic analyses. The most recent and comprehensive study of New World *Buddleja* by Norman (2000) included 12 series based on morphology and ecogeography (Fig. 3). Our study included representatives from all series, except the monotypic *Oblongae* E.M.Norman. We sampled multiple species for each included series, except *Scordioides* E.M.Norman, *Verticillatae* (Benth.) E.M.Norman and the monotypic *Anchoenses* E.M.Norman, which enabled us to begin investigating the monophyly and relationships among these infrageneric groups.

The monophyly of series *Thyrsoides* (Benth.) E.M.Norman, including *B. elegans* and *B. kleinii* E.M.Norman & L.B.Sm., was supported by the phylogenetic analyses. Most of the species in series *Stachyoides* (Benth.) E.M.Norman also formed a well-supported clade. However, *B. longiflora* Brade and *B.*

speciosissima Taub from *Stachyoides* are more closely related to series *Thyrsoides*, to which they form the sister group. These two species differ from the rest of series *Stachyoides*, but is similar to series *Thyrsoides*, in having subcoriaceous rather than membranaceous leaves and pedicellate rather than sessile flowers (Norman, 2000). Series *Thyrsoides* and *Stachyoides* are most closely related to each other and together they are sister to series *Brachiatae* E.M.Norman. These three series are mainly South American in distribution, occurring in south-eastern Brazil and the Andes (Norman, 2000). Species in *Brachiatae* form a strongly supported clade when *B. racemosa* Torr., the only North American species in the group, is excluded.

Series *Glomeratae* (Benth.) E.M.Norman is inferred to be polyphyletic. *Buddleja mendozensis* Gillies ex Benth. and *B. tucumanensis* Griseb., from Argentina and Bolivia, are sister species, but they are not closely related to the other members of series *Glomeratae*, which are mostly North American. *Buddleja mendozensis* and *B. tucumanensis* are more closely related to *B. anchoensis* Kuntze from series *Anchoenses* and *Buddleja aromatica* J.Rémy and *B. cordobensis* Griseb. from series *Globosae* (Benth.) E.M.Norman. These five South American species share similar seed morphology and sessile flowers (Norman, 2000). The other two species in series *Globosae*, *B. araucana* Phil. and *B. globosa* from Chile and Argentina, form a clade with strong support. North American members of series *Glomeratae*, *B. corrugata* M.E.Jones and *B. utahensis* Coville, are more closely related to the North American species *B. scordioides* Kunth in series *Scordioides*.

Series *Cordatae* E.M.Norman is paraphyletic with respect to series *Buddleja*, *Lanatae* E.M.Norman and *Verticillatae*. Members of these four series fall into two clades according to geographical distribution. A North American clade includes *B. cordata* Kunth, *B. megalcephala* Donn.Sm., *B. nitida* Benth. and *B. skutchii* C.V.Morton from *Cordatae*, *B. crotonoides* A.Gray from *Buddleja* and, in the species tree analyses, *B. sessiliflora* Kunth from *Verticillatae*. The other clade is South American and contains *B. cardenasii* Standl. ex E.M.Norman, *B. coriacea*, *B. incana*, *B. montana* Britton and *B. vexans* Kraenzl. & Loes. ex E.M.Norman from *Cordatae* and *B. blattaria* and *B. jamesonii* Benth. from series *Lanatae*. *Buddleja americana* L., the range of which spans North and South America, and *B. rufescens* from Peru are sister species, but their phylogenetic position is equivocal. They fall with the South American clade in the concatenated analysis and with the North American clade in the species tree analysis. The distant relationship between *B. americana* and *B. crotonoides* indicates that series *Buddleja* is polyphyletic. The sampled species in series *Lanatae* form a strongly supported clade. All species from series *Buddleja*, *Cordatae* and *Verticillatae* for which ploidy

has been determined are polyploid (Norman, 2000). There are no published chromosome counts for any species in series *Lanatae*, but we predict based on these relationships that they are also polyploid.

TRAIT EVOLUTION

The evolution of morphological traits traditionally used to characterize genera in Buddlejaceae was investigated (Fig. 3). For *Nicodemia*, fleshy berries remain a synapomorphy and useful distinguishing character. Fruit type evolved once from dry capsules to fleshy berries in the most recent common ancestor of this group.

The other reproductive traits traditionally used to delimit genera have been evolutionarily labile and evolved independently to similar states multiple times. Although traditionally used to distinguish *Buddleja* from other genera, included stamens and tube-shaped corollas are inferred to be symplesiomorphic in Buddlejaceae. Exserted stamens and cup-shaped corollas evolved at least once in the African species. They may have evolved independently in the ancestor of *B. glomerata* and in the ancestor of a clade comprising core *Chilianthus*, *Gomphostigma* and *B. auriculata*, with reversals occurring in *B. auriculata*. Alternatively, exserted stamens and cup-shaped corollas may have evolved in an earlier ancestor of core *Chilianthus*, *Gomphostigma*, *B. auriculata* and *B. glomerata*, with reversals occurring in *B. auriculata* and in the ancestor of *Nicodemia* and the Asian and New World *Buddleja*. Support is low for some relationships among the African species, including on the backbone of the tree, and trait states at several of these nodes are equivocal. Phylogenetic analyses with more data to increase resolution and support for the relationships among these groups are needed to fully understand the evolution of these traits. Additionally, stamen exsertion and corolla shape underwent independent transitions in the New World clade. Exserted stamens evolved once in *Emorya* and cup-shaped corollas evolved multiple times in New World *Buddleja*.

Ancestral white corolla colour was retained in the basal African grade, including *B. salviifolia*, *B. auriculata*, core *Chilianthus* and *Gomphostigma*. Corolla colour evolved from white to purple in the most recent common ancestor of *Nicodemia*, *B. polystachya* and Asian *Buddleja* and then transitioned to orange in the most recent common ancestor of *Nicodemia* and *B. polystachya*. In *Nicodemia*, transitions to white and yellow also occurred and in Asian *Buddleja*, the purple corolla colour was mostly retained, with a single reversal back to white. In the ancestor of *Emorya* and New World *Buddleja*, corolla colour evolved from white to yellow. Yellow corollas were retained in many New World *Buddleja*, but

there have also been multiple transitions to white and orange. Yellow corollas evolved independently in *B. glomerata*.

In Buddlejaceae, the ancestral inflorescence form was the highly branched paniculate type and reductions in branching occurred multiple times. In *Gomphostigma*, the inflorescence was reduced to a racemose form; that inflorescence type, cup-shaped corollas and exserted stamens remain a useful suite of characters for recognizing the clade. Paniculate inflorescences were retained in the rest of the basal African grade and in the ancestor of *Emorya* and New World *Buddleja*. In the New World clade, multiple independent reductions in branching and loss of peduncles and/or pedicels produced a range of thyrsoid, capitate, spicate and racemose inflorescences. In the ancestor of *Nicodemia*, *B. polystachya* and Asian *Buddleja*, inflorescence form was reduced to the thyrsoid type. Further reductions of pedicels and/or peduncles resulted in spicate or capitate inflorescences in some species and reversals to paniculate inflorescences also occurred in Asian *Buddleja*.

CLASSIFICATION

We present here a revised classification for Buddlejaceae reflecting our phylogenetic results. Our general philosophy is to name supported monophyletic groups in order to facilitate communication and understanding of relationships. Although relationships among named clades are not all strongly supported, they are exclusive of other clades and represent distinct lineages.

Only the genus *Buddleja* is maintained and its circumscription is expanded. Evidence shows that *Buddleja* as previously circumscribed is paraphyletic. Despite rendering *Buddleja* redundant with Buddlejaceae, we take this conservative approach to the taxonomy because Buddlejaceae is clearly monophyletic and uncertainty in some relationships between *Buddleja* and other small lineages (e.g. *Gomphostigma*) precludes accepting previously recognized segregate genera. All species in *Chilianthus*, *Gomphostigma*, *Nicodemia* and *Emorya* are combined with *Buddleja*. Species in *Chilianthus*, *Gomphostigma* and *Nicodemia* already have synonyms in *Buddleja*, but two new names are proposed for the species in *Emorya*.

Seven groups of species consistently obtained in analyses are recognized at the sectional rank in *Buddleja* (Fig. 3). Two new monotypic sections are recognized for *B. salviifolia* and *B. pulchella*. *Gomphostigma* is lowered from the genus to sectional rank. Section *Chilianthus* comprises *B. dysophylla*, *B. loricata*, *B. saligna* and *B. auriculata*. The position of *B. glomerata* remains equivocal and may be included in this section if additional evidence supports this relationship. Section *Nicodemia* is expanded to include *B. polystachya* in addition to the eight species traditionally

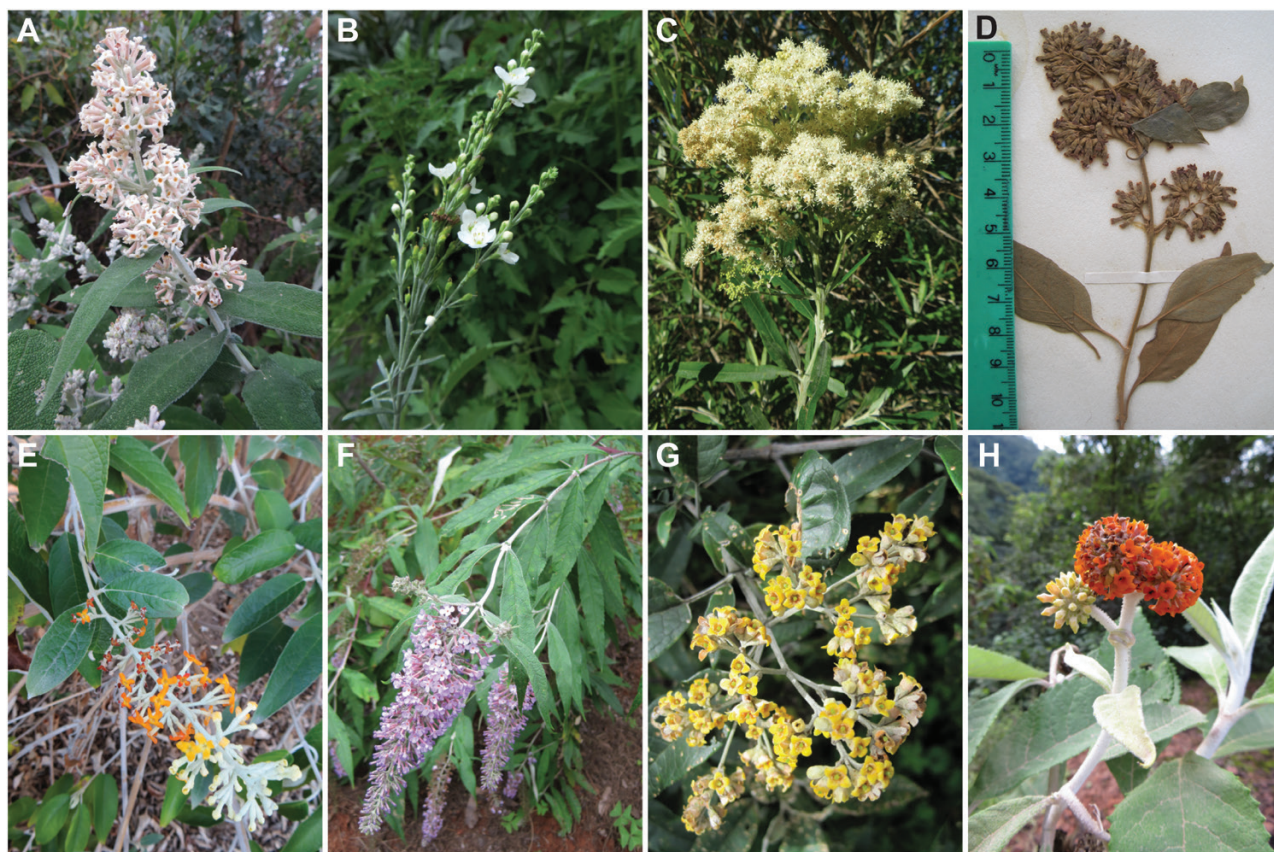


Figure 4. Representatives of seven sections of *Buddleja* in revised classification of Buddlejeae. (A) *Buddleja salviifolia*, section *Salviifoliae*, (B) *Buddleja virgata*, section *Gomphostigma*, (C) *Buddleja saligna*, section *Chilianthus*, (D) *Buddleja pulchella*, section *Pulchellae*, (E) *Buddleja madagascariensis*, section *Nicodemia*, (F) *Buddleja davidii*, section *Alternifoliae*, (G) *Buddleja nitida*, section *Buddleja*, (H) *Buddleja anchoensis*, section *Buddleja*. All photographs by J.H. Chau.

in the group. Section *Alternifoliae* is expanded to include all species of *Buddleja* distributed in Asia. All species found in North and South America are placed in section *Buddleja*, including members of *Emorya*. Circumscriptions and species names in revised classification are listed in Table A4 and select representatives of sections are shown in Figure 4.

I. *Buddleja* L., Sp. Pl. 1: 112. 1753. Type: *Buddleja americana* L.

Inflorescence paniculate, thyrsoid, capitate, spicate or racemose. Flowers with tube- or cup-shaped corollas and included or exserted stamens. Ovary two- or four-celled. Fruit a capsule or berry. Distribution: Africa, Madagascar, Asia, North America, South America.

1. Section *Salviifoliae* J.H.Chau, **sect. nov.** Type: *Buddleja salviifolia* (L.) Lam.

Inflorescence paniculate. Corolla white or lilac to purple, with deep orange throat; tube-shaped. Stamens included. Ovary two-celled. Fruit a capsule. Distribution: southern and eastern Africa.

2. Section *Gomphostigma* (Turcz.) J.H.Chau, **stat. nov.** Basionym: *Gomphostigma* Turcz., Bull. Soc. Nat. Mosc. 16: 53. 1843. Type: *Gomphostigma scoparioides* Turcz. = *Buddleja virgata* L.f.

Inflorescence racemose. Corolla white, cup-shaped. Stamens exserted. Ovary two-celled. Fruit a capsule. Distribution: southern Africa.

3. Section *Chilianthus* (Burch.) Leeuwenberg, Meded. Landbouwhogeschool Wageningen 79 (6): 7. 1979. Type: *Chilianthus oleaceus* Burch. = *Buddleja saligna* Willd.

Inflorescence paniculate. Corolla white or cream, with orange or mauve throat; cup- or tube-shaped. Stamens exserted or included. Ovary two-celled. Fruit a capsule. Distribution: southern Africa.

4. Section *Pulchellae* J.H.Chau, **sect. nov.** Type: *Buddleja pulchella* N.E.Br.

Inflorescence paniculate. Corolla white, yellow or pale orange, with yellow or orange throat; tube-shaped. Stamens included. Ovary two-celled. Fruit a capsule. Distribution: southern and eastern Africa.

5. Section *Nicodemia* (Tenore) Leeuwenberg, Meded. Landbouwhogeschool Wageningen 79 (6): 9. 1979. Type: *Nicodemia diversifolia* (Vahl) Tenore = *Buddleja indica* Lam.
Inflorescence thyrsoïd, capitate or spicate. Corolla white, yellow or orange; tube-shaped. Stamens included. Ovary two- or four-celled. Fruit a berry or capsule. Distribution: Madagascar, eastern Africa, Arabian Peninsula.
6. Section *Alternifoliae* Kränzl., Bull. Jard. Imp. Bot. Petersb. 8 (4): 89. 1913. Type: *Buddleja amentacea* Kränzl. = *Buddleja asiatica* Lour.
Inflorescence thyrsoïd, spicate or paniculate. Corolla purple or white, often with orange throat; tube-shaped. Stamens included. Ovary two-celled. Fruit a capsule. Distribution: Asia.
7. Section *Buddleja*. Type: *Buddleja americana* L.
Inflorescence paniculate, thyrsoïd, capitate, spicate or racemose. Corolla white, yellow or orange; tube- or cup-shaped. Stamens included or exerted. Ovary two-celled. Fruit a capsule. Distribution: North and South America.
- i. ***Buddleja normaniae*** J.H.Chau, **nom. nov.**
Basionym: *Emorya suaveolens* Torr., Rep. U.S. Mex. bound. 2(1): 121 t. 36. 1859.
The epithet recognizes the work of Eliane Norman in the study of Buddlejaceae, especially its New World members.
- ii. ***Buddleja rinconensis*** (Mayfield) J.H.Chau, **comb. nov.**
Basionym: *Emorya rinconensis* Mayfield, Sida 18: 693–699. 1999.

CONCLUSIONS

Buddlejeae are among the larger and most broadly distributed tribes in Scrophulariaceae. We present the first phylogenetic reconstruction of relationships in the tribe based on multiple independent genetic markers and with extensive and representative taxonomic sampling. We show that *Buddleja* is paraphyletic with respect to *Chilianthus*, *Nicodemia*, *Emorya* and probably *Gomphostigma* and the traits used to distinguish *Buddleja*, namely flowers with included stamens and capsular fruits, are plesiomorphic. Additional data and analyses will be required to definitively resolve some relationships that remain poorly supported and their implications for patterns in trait evolution, including among some of the African taxa and in the Asian and New World clades. Extensive polyploidy in the Asian and New World clades complicates analyses due to uncertainties in orthology assessment and separation of copy sequences through cloning or next-generation sequencing methods will be necessary. Our revised classification clarifies evolutionary relationships in

Buddlejeae and can serve as a framework for future investigations on evolution in this diverse group.

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APPENDIX

Table A1. Specimens included in study, with collection locality, voucher information and GenBank accession numbers for sequences

Taxon	Collection locality	Voucher	ETS	PPR24	PPR97	PPR123	<i>rpoA</i>	<i>trnD-trnT</i>	<i>trnS-trnFM</i>
<i>Buddleja albiflora</i> Hemsl. (A)	Cultivated, USA: Arnold Arboretum (acc. #: 13-92-A)	<i>J. Chau 260</i> (WTU, A)	KX827818	KX827926	KX827996	KX833264	–	–	–
<i>Buddleja albiflora</i> Hemsl. (B)*	China: Hubei	<i>1980 Sino-Amer. Exped. 257</i> (UC)	KX827819	–	–	–	KX856095	–	–
<i>Buddleja alternifolia</i> Maxim. (A)	Cultivated, USA: University of Washington Botanic Gardens	<i>R. Olmstead 2010-50</i> (WTU)	KX827820	KX827927	KX827997	KX833265	–	–	–
<i>Buddleja alternifolia</i> Maxim. (B)	China: Tibet	<i>G. Chen 070802</i> [(KUN)]	KX827821	–	–	–	KX856096	KX828060	KX855287
<i>Buddleja americana</i> L.	Peru: La Libertad	<i>J. Chau 97</i> (HAO)	KX827822	KX827928	KX827998	KX833266	KX856097	KX828061	KX855288
<i>Buddleja anchoensis</i> Kuntze (A)	Bolivia: Tarija	<i>J. Chau 224</i> (WTU, LPB)	KX827823	KX827929	KX827999	KX833267	–	–	KX855289
<i>Buddleja anchoensis</i> Kuntze (B)*	Bolivia: Santa Cruz	<i>M. Nee 53158</i> (LPB)	KX827824	–	–	–	KX856098	KX828062	–
<i>Buddleja araucana</i> Phil. (A)	Argentina: Neuquén	<i>R. Olmstead 2007-94</i> (WTU)	KX827825	KX827930	–	KX833268	–	–	–
<i>Buddleja araucana</i> Phil. (B)*	Argentina: Rio Negro	<i>C. Calvino 743</i> (SI)	KX827826	–	–	–	KX856099	KX828063	KX855290
<i>Buddleja aromatica</i> J.Rémy (A)	Bolivia: La Paz	<i>J. Chau 206</i> (WTU, LPB)	KX827827	KX827931	KX828000	KX833269	–	–	KX855291
<i>Buddleja aromatica</i> J.Rémy (B)*	Bolivia: La Paz	<i>J. Solomon 13053</i> (CAS)	KX827828	–	–	–	KX856100	KX828064	–
<i>Buddleja asiatica</i> Lour. (A)	China: Yunnan	<i>J. Chau 157</i> (WTU)	KX827829	KX827932	KX828001	KX833270	–	–	–
<i>Buddleja asiatica</i> Lour. (B)	China: Yunnan	<i>G. Chen 015</i> (KUN)	KX827830	–	–	–	KX856101	KX828065	KX855292
<i>Buddleja auriculata</i> Benth.	South Africa: Eastern Cape	<i>J. Chau 246</i> (WTU, GRA)	KX827831	KX827933	KX828002	KX833271	KX856102	KX828066	KX855293
<i>Buddleja axillaris</i> Willd. [<i>Nicodemia</i>]*	Madagascar: Atsinanana	<i>B. Lewis & S. Razafim andimbison 687</i> (MO)	KX827832	–	–	–	–	–	–
<i>Buddleja bhutanica</i> Yamazaki*	Bhutan	<i>B. Barthol omew 3904</i> (CAS)	KX827833	–	–	–	KX856103	KX828067	–
<i>Buddleja blattaria</i> J.F.Macbr.	Peru: Cajamarca	<i>J. Chau 101</i> (HAO)	KX827834	KX827934	KX828003	KX833272	KX856104	KX828068	KX855294
<i>Buddleja bullata</i> Kunth	Peru: Cajamarca	<i>J. Chau 98</i> (HAO)	KX827835	KX827935	KX828004	KX833273	KX856105	KX828069	–
<i>Buddleja candida</i> Dunn	China: Tibet	<i>G. Chen 070817</i> (KUN)	KX827836	–	–	–	KX856106	KX828070	KX855295
<i>Buddleja cardenasii</i> Standl. ex E.M.Norman (A)	Bolivia: La Paz	<i>J. Chau 196</i> (WTU, LPB)	KX827837	KX827936	KX828005	KX833274	–	–	KX855296
<i>Buddleja cardenasii</i> Standl. ex E.M.Norman (B)*	Bolivia: Cochabamba	<i>S. Beck 14418</i> (LPB)	KX827838	–	–	–	KX856107	KX828071	–

Table A1. *Continued*

Taxon	Collection locality	Voucher	ETS	PPR24	PPR97	PPR123	<i>rpoA</i>	<i>trnD-trnT</i>	<i>trnS-trnfM</i>
<i>Buddleja caryopteridifolia</i> W.W.Sm. (A)	China: Yunnan	<i>J. Chau 171</i> (WTU)	KX827839	KX827941	KX828009	KX833279	–	–	KX855297
<i>Buddleja caryopteridifolia</i> W.W.Sm. (B)*	China: Sichuan	<i>D. Boufford et al. 29045</i> (CAS)	KX827840	–	–	–	KX856108	KX828072	–
<i>Buddleja cestri-flora</i> Cham.	Brazil: Santa Catarina	<i>R. Olmstead 2010-213</i> (WTU, ICN)	KX827841	KX827937	–	KX833275	KX856109	KX828073	–
<i>Buddleja colvilei</i> Hook.f.	Cultivated, USA: San Francisco Botanical Garden (acc. #: XY-1801)	<i>J. Chau 42</i> (WTU)	KX827842	KX827938	KX828006	KX833276	KX856110	KX828074	–
<i>Buddleja cordata</i> Kunth	Cultivated, USA: Leu Gardens	<i>E. Norman s.n.</i> (FTU)	KX827843	–	–	–	KX856111	KX828075	KX855298
<i>Buddleja cordobensis</i> Griseb.*	Argentina: Córdoba	<i>F. Zuloaga 11302</i> (SI)	KX827844	–	–	–	KX856112	KX828076	KX855299
<i>Buddleja coriacea</i> J.Rémy (A)	Peru: Cajamarca	<i>J. Chau 110</i> (HAO)	KX827845	KX827939	KX828007	KX833277	KX856113	KX828077	–
<i>Buddleja coriacea</i> J.Rémy (B)*	Bolivia: La Paz	<i>E. Urtubey 498</i> (SI)	KX827846	–	–	–	–	–	KX855300
<i>Buddleja corrugata</i> M.E.Jones*	Mexico: Baja California Sur	<i>A. Carter & R. Moran 5330</i> (UC)	KX827847	–	–	–	KX856114	KX828078	–
<i>Buddleja crispa</i> Benth. (A)	China: Yunnan	<i>J. Chau 170</i> (WTU)	KX827848	KX827940	KX828008	KX833278	–	–	–
<i>Buddleja crispa</i> Benth. (B)	China: Yunnan	<i>G. Chen 070818</i> (KUN)	KX827849	–	–	–	KX856115	KX828079	KX855301
<i>Buddleja crotonoides</i> A.Gray+	Nicaragua: Madriz	<i>W. Stevens et al. 29357</i> (MO)	KX827850	KX827942	KX828010	KX833280	KX856116	KX828080	–
<i>Buddleja curviflora</i> Hook. & Arn.	Cultivated, USA: University of Washington Botanic Gardens (acc. #: 38-94)	<i>R. Olmstead 2010-49</i> (WTU)	KX827851	KX827943	–	KX833281	KX856117	KX828081	–
<i>Buddleja cuspidata</i> Baker [Nicodemia]*	Madagascar: Sava	<i>C. Rakotovoao et al. 3263</i> (MO)	KX827852	–	–	–	–	–	–
<i>Buddleja davidii</i> Franch. (A)	Cultivated, China: Kunming Botanical Garden	<i>J. Chau 177</i> (WTU)	KX827853	KX827944	KX828011	–	–	–	–
<i>Buddleja davidii</i> Franch. (B)	China: Yunnan	<i>W. Sun 019</i> (KUN)	KX827854	–	–	–	KX856118	KX828082	KX855302
<i>Buddleja davidii</i> Franch. (C)	Cultivated, USA: University of Colorado	<i>R. Olmstead 92-192</i> (WTU)	KX827855	–	–	KX833282	–	–	–
<i>Buddleja delavayi</i> L.F.Gagnep.	China: Yunnan	<i>J. Chau 165</i> (WTU)	KX827856	KX827945	KX828012	KX833283	KX856119	KX828083	KX855303
<i>Buddleja diffusa</i> Ruiz & Pav.*	Bolivia: La Paz	<i>R. Seidel et al. 1314</i> (LPB)	KX827857	–	–	–	KX856120	KX828084	–
<i>Buddleja dysophylla</i> (Benth.) Radlk. [Chilianthus dysophyllus Benth.]	South Africa: Eastern Cape	<i>J. Chau 233</i> (WTU, LPB)	KX827858	KX827946	KX828013	KX833284	KX856121	KX828085	KX855304

Table A1. Continued

Taxon	Collection locality	Voucher	ETS	PPR24	PPR97	PPR123	<i>rpoA</i>	<i>trnD-trnT</i>	<i>trnS-trnM</i>
<i>Buddleja elegans</i> Cham. & Schltdl. subsp. <i>elegans</i> (A)	Brazil: Rio Grande do Sul	<i>R. Olmstead</i> 2010-214 (ICN)	KX827860	KX827947	–	KX833285	–	–	–
<i>Buddleja elegans</i> Cham. & Schltdl. subsp. <i>elegans</i> (B)	Brazil: Rio Grande do Sul	<i>R. Olmstead</i> 2010-210 (WTU, ICN)	KX827861	–	–	–	KX856122	KX828086	–
<i>Buddleja elegans</i> Cham. & Schltdl. subsp. <i>angustata</i> (Benth.) E.M.Norman	Brazil: Rio Grande do Sul	<i>V. Thode et al. 399</i> (ICN)	KX827859	KX827982	KX828047	KX833320	KX856159	KX828122	KX855323
<i>Buddleja fallowiana</i> Balf.f. & W.W.Smith (A)	China: Yunnan	<i>J. Chau 166</i> (WTU)	KX827862	KX827948	KX828014	KX833286	–	–	–
<i>Buddleja fallowiana</i> Balf.f. & W.W.Smith (B)	China: Yunnan	<i>G. Chen 059</i> (KUN)	KX827863	–	–	–	KX856123	KX828087	KX855305
<i>Buddleja forrestii</i> Diels (A)	China: Yunnan	<i>J. Chau 161</i> (WTU)	KX827864	KX827949	–	–	KX856124	KX828088	KX855306
<i>Buddleja forrestii</i> Diels (B)	Cultivated, USA: University of California Botanical Garden (acc. #: 91.0429)	<i>R. Welch s.n.</i> (UC)	KX827865	–	KX828015	KX833287	–	–	–
<i>Buddleja fragifera</i> Leeuwenb. [<i>Nicodemia</i>]*	Madagascar: Atsimo- Andrefana	<i>P. Phillipson 3007</i> (MO)	KX827866	–	–	KX827817	KX856125	KX828089	–
<i>Buddleja fusca</i> Baker [<i>Nicodemia</i>]*	Madagascar: Vakinan karatra	<i>P. Phillipson et al.</i> 5634 (MO)	KX827867	KX827950	–	KX833288	KX856126	KX828090	–
<i>Buddleja globosa</i> Hope	Cultivated, USA: University of Washington Botanic Gardens	<i>R. Olmstead</i> 2010-46 (WTU)	KX827868	KX827951	KX828016	KX833289	KX856127	KX828091	KX855307
<i>Buddleja glomerata</i> H.Wendl. [<i>Chilianthus lobulatus</i> Benth.]	South Africa: Eastern Cape	<i>J. Chau 254</i> (WTU, GRA)	KX827869	KX827952	KX828017	KX833290	KX856128	KX828092	KX855308
<i>Buddleja grandiflora</i> Cham. & Schltdl.	Brazil: Rio Grande do Sul	<i>R. Olmstead</i> 2010-207 (WTU, ICN)	KX827870	KX827953	–	KX833291	KX856129	KX828093	–
<i>Buddleja hieronymi</i> R.E.Fr. (A)	Bolivia: Tarija	<i>J. Chau 225</i> (WTU, LPB)	KX827871	KX827954	KX828018	–	KX856130	KX828094	KX855309
<i>Buddleja hieronymi</i> R.E.Fr. (B)	Argentina: Jujuy	<i>R. Olmstead</i> 2007-59 (WTU)	KX827872	–	–	KX833292	–	–	–
<i>Buddleja incana</i> Ruiz & Pav.	Peru: Cajamarca	<i>J. Chau 111</i> (HAO)	KX827873	KX827955	KX828019	KX833293	KX856131	KX828095	KX855310
<i>Buddleja indica</i> Lam. [<i>Nicodemia diversifolia</i> (Vahl) Ten.]+	Madagascar: Atsinanana	<i>J. Rabenan toandro</i> 1234 (MO)	KX827874	KX827956	KX828020	KX833294	KX856132	KX828096	–

Table A1. *Continued*

Taxon	Collection locality	Voucher	ETS	PPR24	PPR97	PPR123	<i>rpoA</i>	<i>trnD-trnT</i>	<i>trnS-trnfM</i>
<i>Buddleja interrupta</i> Kunth	Peru: Cajamarca	<i>J. Chau 117</i> (HAO)	KX827875	KX827957	KX828021	KX833295	KX856133	KX828097	KX855311
<i>Buddleja jamesonii</i> Benth.*	Ecuador: Azuay	<i>P. Jorgensen</i> 92920 (MO)	KX827876	–	–	–	KX856134	KX828098	–
<i>Buddleja japonica</i> Hemsl.	Cultivated, USA: Arnold Arboretum (acc. #: 7-92-B)	<i>J. Wood</i> 124-2014 (A)	KX827877	KX827958	KX828022	KX833296	–	–	–
<i>Buddleja kleinii</i> E.M.Norman & L.B.Sm.	Brazil: Santa Catarina	<i>R. Olmstead</i> 2010-220 (WTU, ICN)	KX827878	KX827959	KX828023	KX833297	KX856135	KX828099	–
<i>Buddleja lindleyana</i> Fortune (A)	China: Hubei	<i>G. Chen 053</i> (KUN)	KX827879	–	KX828024	KX833298	KX856136	KX828100	KX855312
<i>Buddleja lindleyana</i> Fortune (B)	Cultivated, USA: R. Olmstead garden	<i>R. Olmstead</i> 2009-51 (WTU)	KX827880	KX827960	–	–	–	–	–
<i>Buddleja longiflora</i> Brade	Cultivated, USA: University of Washington Biology greenhouse	<i>J. Chau 308</i> (WTU)	KX827881	KX827961	KX828025	KX833299	–	–	–
<i>Buddleja loricata</i> Leeuwenberg [<i>Chilianthus corrugatus</i> Benth.]	Cultivated, USA: University of California Botanical Garden (acc. #: 2006.0671)	<i>R. Welch s.n.</i> (UC)	KX827882	KX827962	KX828026	KX833300	KX856137	KX828101	–
<i>Buddleja macrostachya</i> Benth. (A)	China: Yunnan	<i>J. Chau 159</i> (WTU)	KX827883	KX827963	KX828027	–	–	–	–
<i>Buddleja macrostachya</i> Benth. (B)	China: Yunnan	<i>G. Chen 045</i> (KUN)	KX827884	–	–	KX833333	KX856138	KX828102	KX855313
<i>Buddleja madagascariensis</i> Lam. [<i>Nicodemia madagascariensis</i> (Lam.) R.Parker]	Cultivated, USA: Los Angeles County Arboretum (acc. #: 20050221)	<i>J. Chau 256</i> (WTU)	KX827885	KX827964	KX828028	KX833301	KX856139	KX828103	KX855314
<i>Buddleja marrubifolia</i> Benth.	Cultivated, USA: University of California- Davis Arboretum (acc. #: A85.0360)	<i>J. Chau 40</i> (WTU)	KX827886	KX827965	KX828029	KX833302	KX856140	KX828104	–
<i>Buddleja megalcephala</i> Donn. Sm.+	Guatemala: Huehue tenango	<i>M. Christe nhusz et al.</i> 5266 (MO)	KX827887	–	KX828030	KX833303	KX856141	KX828105	–
<i>Buddleja mendozaensis</i> Gillies ex Benth.*	Argentina: Catamarca	<i>F. Zuloaga 12016</i> (SI)	KX827888	–	–	–	KX856142	KX828106	KX855315
<i>Buddleja montana</i> Britton	Bolivia: La Paz	<i>J. Chau 186</i> (WTU, LPB)	KX827889	KX827966	KX828031	KX833304	KX856143	KX828107	KX855316
<i>Buddleja myriantha</i> Diels (A)	China: Yunnan	<i>J. Chau 158</i> (WTU)	KX827890	KX827967	KX828032	KX833305	–	–	–
<i>Buddleja myriantha</i> Diels (B)	China: Yunnan	<i>W. Sun 033</i> (KUN)	KX827891	–	–	–	KX856144	KX828108	KX855317
<i>Buddleja nitida</i> Benth. (A)	Costa Rica: Cartago	<i>J. Chau 150</i> (WTU)	KX827892	KX827968	KX828033	–	–	–	–

Table A1. Continued

Taxon	Collection locality	Voucher	ETS	PPR24	PPR97	PPR123	<i>rpoA</i>	<i>trnD-trnT</i>	<i>trnS-trnfM</i>
<i>Buddleja nitida</i> Benth. (B)	Cultivated, USA: University of California Botanical Garden (acc. #: 87.0253)	<i>M. Grayum 8188</i> (CR)	KX827893	–	–	KX833306	KX856145	KX828109	–
<i>Buddleja nivea</i> Duthie	Cultivated, USA: University of Washington Botanic Gardens (acc. #: 396-61*A)	<i>R. Olmstead</i> 2010-47 (WTU)	KX827894	KX827969	KX828034	KX833307	KX856146	KX828110	–
<i>Buddleja</i> <i>officinalis</i> Maxim. (A)	China: Yunnan	<i>J. Chau 179</i> (WTU)	KX827895	KX827970	KX828035	KX833308	–	–	–
<i>Buddleja</i> <i>officinalis</i> Maxim. (B)	China: Yunnan	<i>G. Chen 012</i> (KUN)	KX827896	–	–	–	KX856147	KX828111	KX855318
<i>Buddleja poly-</i> <i>stachya</i> Fresen.*	Tanzania: Arusha	<i>G. Simon 308</i> (MO)	KX827897	KX827971	KX828036	KX833309	KX856148	KX828112	–
<i>Buddleja pulch-</i> <i>ella</i> N.E.Br.*	South Africa: KwaZulu- Natal	<i>I. Nanni 319</i> (NBG)	KX827898	KX827972	KX828037	KX833310	KX856149	–	–
<i>Buddleja rac-</i> <i>emosa</i> Torr.*	USA: Texas	<i>G. Webster &</i> <i>B. Westlund</i> 32714 (DAV)	KX827899	KX827973	KX828038	KX833311	KX856150	KX828113	KX855319
<i>Buddleja rufes-</i> <i>cens</i> Willd. ex Schultes & Schultes	Peru: Cajamarca	<i>J. Chau 99</i> (HAO)	KX827900	KX827974	KX828039	KX833312	KX856151	KX828114	KX855320
<i>Buddleja</i> <i>saligna</i> Willd. [<i>Chilianthus</i> <i>oleaceus</i> Burch.]	South Africa: Western Cape	<i>R. Olmstead</i> 99-20	KX827901	KX827975	KX828040	KX833313	KX856152	KX828115	–
<i>Buddleja salvii-</i> <i>folia</i> (L.) Lam.	Cultivated, USA: San Francisco Botanical Garden (acc. #: XY-1999)	<i>J. Chau 43</i> (WTU)	KX827902	KX827976	KX828041	KX833314	KX856153	KX828116	–
<i>Buddleja scordi-</i> <i>oides</i> Kunth (A)*	Mexico: Sonora	<i>T. Van Devender</i> 2007-744 (CAS)	KX827903	–	–	–	KX856154	KX828117	–
<i>Buddleja scordi-</i> <i>oides</i> Kunth (B)	Mexico: Coahuila	<i>M. Moore 2560</i> (WTU)	KX827904	KX827977	KX828042	KX833315	–	–	–
<i>Buddleja sessili-</i> <i>flora</i> Kunth*	USA: Texas	<i>G. Webster 31455</i> (DAV)	KX827905	KX827978	KX828043	KX833316	KX856155	KX828118	–
<i>Buddleja skutchii</i> C.V.Morton	Costa Rica: San José	<i>J. Chau 152</i> (WTU)	KX827906	KX827979	KX828044	KX833317	KX856156	KX828119	KX855321
<i>Buddleja specios-</i> <i>issima</i> Taub. (A)	Brazil: Rio de Janeiro	<i>F. Salimena 2980</i> (CESJ)	KX827907	–	–	KX833318	KX856157	KX828120	–
<i>Buddleja specios-</i> <i>issima</i> Taub. (B)	Cultivated, USA: University of Washington Biology greenhouse	<i>J. Chau 259</i> (WTU)	KX827908	KX827980	KX828045	–	–	–	–
<i>Buddleja stachy-</i> <i>oides</i> Cham. & Schtdl. (A)	Brazil: Minas Gerais	<i>F. Salimena 2947</i> (CESJ)	KX827909	KX827981	KX828046	KX833319	–	–	–

Table A1. *Continued*

Taxon	Collection locality	Voucher	ETS	PPR24	PPR97	PPR123	<i>rpoA</i>	<i>trnD-trnT</i>	<i>trnS-trnfM</i>
<i>Buddleja stachyoides</i> Cham. & Schlttdl. (B)*	Argentina: Jujuy	<i>F. Zuloaga 11630</i> (SI)	KX827910	–	–	–	KX856158	KX828121	KX855322
<i>Buddleja tubiflora</i> Benth.	Cultivated, USA: E. Norman garden	<i>Norman s.n.</i> (WTU)	KX827911	KX827983	KX828048	KX833321	KX856160	KX828123	KX855324
<i>Buddleja tucumanensis</i> Griseb.	Bolivia: Chuquisaca	<i>J. Chau 212</i> (WTU, LPB)	KX827912	KX827984	KX828049	KX833322	KX856161	KX828124	KX855325
<i>Buddleja utahensis</i> Coville	Cultivated, USA: Rancho Santa Ana Botanic Garden (acc. #: 17353)	<i>J. Chau 39</i> (WTU)	KX827913	KX827985	KX828050	KX833323	KX856162	KX828125	–
<i>Buddleja vexans</i> Kraenzl. & Loes. ex E.M.Norman	Peru: Huancavelica	<i>J. Chau 136</i> (HAO)	KX827914	–	–	–	KX856163	KX828126	KX855326
<i>Buddleja yunnanensis</i> L.F.Gagnep. (A)	Cultivated, China: Kunming Botanical Garden	<i>J. Chau 178</i> (WTU)	KX827915	KX827986	KX828051	–	–	–	–
<i>Buddleja yunnanensis</i> L.F.Gagnep. (B)	China: Yunnan	<i>W. Sun 028</i> (KUN)	KX827916	–	–	KX833324	KX856164	KX828127	KX855327
<i>Emorya suaveolens</i> Torr.*	Mexico: Coahuila	<i>D. Riskind 23860</i> (TEX)	KX827917	KX827987	KX828052	KX833325	KX856165	KX828128	–
<i>Gomphostigma incomptum</i> (L.f.) N.E.Br.+	South Africa: Northern Cape	<i>P. Goldblatt & L. Porter 12664</i> (NBG)	KX827918	KX827988	KX828053	KX833326	KX856166	KX828129	KX855328
<i>Gomphostigma virgatum</i> (L.f.) Baill.	Cultivated, USA: University of California-Davis Arboretum (acc. #: M06.9257)	<i>J. Chau 180</i> (WTU)	KX827919	KX827989	KX828054	KX833327	KX856167	KX828130	KX855329
<i>Oftia africana</i> (L.) Bocq.	South Africa: Western Cape	–	KX827920	KX827990	KX828055	KX833328	KX856168	KX828131	KX855330
<i>Phygelius capensis</i> E.Mey. ex Benth.	Cultivated, USA: R. Olmstead garden	<i>R. Olmstead 07-153</i> (WTU)	KX827921	KX827991	KX828056	KX833329	KX856169	KX828132	KX855331
<i>Scrophularia nodosa</i> L.	Cultivated, USA: University of Washington Medicinal Herb Garden	<i>J. Chau 228</i> (WTU)	KX827922	KX827992	–	KX827816	KX856170	KX828133	KX855332
<i>Nemesia fruticans</i> Benth.	Cultivated, USA: R. Olmstead garden	<i>R. Olmstead 07-107</i> (WTU)	KX827923	KX827993	KX828057	KX833330	–	–	–
<i>Nuxia floribunda</i> Benth.	Cultivated, USA: Los Angeles County Arboretum	<i>J. Chau 258</i> (WTU)	KX827924	KX827994	KX828058	KX833331	KX856171	KX828134	KX855333
<i>Lantana depressa</i> Small	Cultivated, USA: Fairchild Tropical Botanic Garden	<i>P. Lu-Irving 12-1</i> (WTU)	KX827925	KX827995	KX828059	KX833332	KX856172	KX828135	KX855334

Specimens with DNA extracted from herbarium specimen tissue indicated with asterisk (*). Specimens with DNA from DNA banks indicated with a cross (+). All other specimens have DNA extracted from silica-preserved leaf tissue. For species with multiple specimens, concatenated dataset always included ETS sequence from specimen (A). For species that have been included in *Buddleja* and *Chilianthus* or *Nicodemia*, accepted names in both genera are listed where available.

Table A2. Sequences of new primers used in this study

Name	Sequence (5'→3')
PPR24-140F	CACGTACCCKTTTGTGKTTTAAGGC
PPR24-1354R	ACTMAGCAAAGCACCRATAAAGTGG
PPR24-310F-Bud	GATGAGGCTACRGTGTTAGTAC
PPR24-600R-Bud	GATACCATAMTTGTCCAACAAATAACATTCTT
PPR24-950F-Bud	CTTACAGGRTGTGCYCAATTAGG
PPR24-970R	TCTAAGMAACCACATTTTGCRTACAT
PPR97-781F	CTTGTRGATTTGGGTGCWARGTGGTT
PPR97-1585R	TTTTTACATAAGCWGTYACAAGAAT
PPR97-1165F	AACACAATGATCACTGGAYATGGGA
PPR97-1351R	AAGTTTGAYGAATTRGGCTTAAA
PPR123-820F	ATGATTAAYGTGTTTGGAAAGGC
PPR123-1370F-Bud	GGAAAGTTAGATCGTGCAGC
PPR123-1500R-Bud	GAGCAACCAAACCAGCCCTCTC

Table A3. Trait states for taxa included in study as determined from published species descriptions in monographs and floras

Taxon	Fruit type	Stamen	Corolla shape	Corolla colour	Inflorescence type
<i>Buddleja albiflora</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja alternifolia</i>	Capsule	Included	Tube-shaped	Purple	Paniculate
<i>Buddleja americana</i>	Capsule	Included	Cup-shaped	Yellow	Paniculate
<i>Buddleja anchoensis</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja araucana</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja aromatica</i>	Capsule	Included	Tube-shaped	White	Capitate
<i>Buddleja asiatica</i>	Capsule	Included	Tube-shaped	White	Spiciform
<i>Buddleja auriculata</i>	Capsule	Included	Tube-shaped	White	Paniculate
<i>Buddleja axillaris</i>	Berry	Included	Tube-shaped	White	Thyrsoid
<i>Buddleja bhutanica</i>	Capsule	Included	Tube-shaped	White	Spiciform
<i>Buddleja blattaria</i>	Capsule	Included	Tube-shaped	White	Capitate
<i>Buddleja bullata</i>	Capsule	Included	Tube-shaped	Yellow	Paniculate
<i>Buddleja candida</i>	Capsule	Included	Tube-shaped	Purple	Spiciform
<i>Buddleja cardenasii</i>	Capsule	Included	Tube-shaped	Orange	Paniculate
<i>Buddleja caryopteridifolia</i>	Capsule	Included	Tube-shaped	Purple	Spiciform
<i>Buddleja cestriflora</i>	Capsule	Included	Tube-shaped	Orange	Thyrsoid
<i>Buddleja colvilei</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja cordata</i>	Capsule	Included	Cup-shaped	Yellow	Paniculate
<i>Buddleja cordobensis</i>	Capsule	Included	Tube-shaped	Yellow	Capitate
<i>Buddleja coriacea</i>	Capsule	Included	Tube-shaped	Orange	Paniculate
<i>Buddleja corrugata</i>	Capsule	Included	Tube-shaped	Orange	Spiciform
<i>Buddleja crispa</i>	Capsule	Included	Tube-shaped	Purple	Paniculate
<i>Buddleja crotonoides</i>	Capsule	Included	Cup-shaped	Yellow	Paniculate
<i>Buddleja curviflora</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja cuspidata</i>	Berry	Included	Tube-shaped	Yellow	Spiciform
<i>Buddleja davidii</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja delavayi</i>	Capsule	Included	Tube-shaped	Purple	Paniculate
<i>Buddleja diffusa</i>	Capsule	Included	Tube-shaped	Yellow	Paniculate
<i>Buddleja dysophylla</i>	Capsule	Exserted	Cup-shaped	White	Paniculate
<i>Buddleja elegans</i> subsp. <i>angustata</i>	Capsule	Included	Tube-shaped	White	Thyrsoid
<i>Buddleja elegans</i> subsp. <i>elegans</i>	Capsule	Included	Tube-shaped	White	Thyrsoid
<i>Buddleja fallowiana</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid

Table A3. *Continued*

Taxon	Fruit type	Stamen	Corolla shape	Corolla colour	Inflorescence type
<i>Buddleja forrestii</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja fragifera</i>	Berry	Included	Tube-shaped	Orange	Capitate
<i>Buddleja fusca</i>	Berry	Included	Tube-shaped	Orange	Thyrsoid
<i>Buddleja globosa</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja glomerata</i>	Capsule	Exserted	Cup-shaped	Yellow	Paniculate
<i>Buddleja grandiflora</i>	Capsule	Included	Tube-shaped	Yellow	Capitate
<i>Buddleja hieronymi</i>	Capsule	Included	Tube-shaped	Yellow	Capitate
<i>Buddleja incana</i>	Capsule	Included	Tube-shaped	Orange	Paniculate
<i>Buddleja indica</i>	Berry	Included	Tube-shaped	Yellow	Thyrsoid
<i>Buddleja interrupta</i>	Capsule	Included	Cup-shaped	Yellow	Paniculate
<i>Buddleja jamesonii</i>	Capsule	Included	Tube-shaped	Yellow	Spiciform
<i>Buddleja japonica</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja kleinii</i>	Capsule	Included	Tube-shaped	White	Capitate
<i>Buddleja lindleyana</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja longiflora</i>	Capsule	Included	Tube-shaped	Orange	Thyrsoid
<i>Buddleja loricata</i>	Capsule	Exserted	Cup-shaped	White	Paniculate
<i>Buddleja macrostachya</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja madagascariensis</i>	Berry	Included	Tube-shaped	Orange	Thyrsoid
<i>Buddleja marrubiiifolia</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja megalcephala</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja mendozensis</i>	Capsule	Included	Tube-shaped	Orange	Spiciform
<i>Buddleja montana</i>	Capsule	Included	Cup-shaped	Orange	Paniculate
<i>Buddleja myriantha</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja nitida</i>	Capsule	Included	Cup-shaped	Yellow	Paniculate
<i>Buddleja nivea</i>	Capsule	Included	Tube-shaped	Purple	Thyrsoid
<i>Buddleja officinalis</i>	Capsule	Included	Tube-shaped	Purple	Paniculate
<i>Buddleja polystachya</i>	Capsule	Included	Tube-shaped	Orange	Spiciform
<i>Buddleja pulchella</i>	Capsule	Included	Tube-shaped	White	Paniculate
<i>Buddleja racemosa</i>	Capsule	Included	Tube-shaped	Yellow	Capitate
<i>Buddleja rufescens</i>	Capsule	Included	Cup-shaped	Yellow	Paniculate
<i>Buddleja saligna</i>	Capsule	Exserted	Cup-shaped	White	Paniculate
<i>Buddleja salviifolia</i>	Capsule	Included	Tube-shaped	White	Paniculate
<i>Buddleja scordioides</i>	Capsule	Included	Cup-shaped	Yellow	Spiciform
<i>Buddleja sessiliflora</i>	Capsule	Included	Tube-shaped	Yellow	Spiciform
<i>Buddleja skutchii</i>	Capsule	Included	Cup-shaped	Orange	Paniculate
<i>Buddleja speciosissima</i>	Capsule	Included	Tube-shaped	Orange	Thyrsoid
<i>Buddleja stachyoides</i>	Capsule	Included	Tube-shaped	Yellow	Capitate
<i>Buddleja tubiflora</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja tucumanensis</i>	Capsule	Included	Tube-shaped	Orange	Capitate
<i>Buddleja utahensis</i>	Capsule	Included	Tube-shaped	Yellow	Spiciform
<i>Buddleja vexans</i>	Capsule	Included	Tube-shaped	Orange	Paniculate
<i>Buddleja yunnanensis</i>	Capsule	Included	Tube-shaped	Purple	Spiciform
<i>Emorya suaveolens</i>	Capsule	Exserted	Tube-shaped	Yellow	Thyrsoid
<i>Gomphostigma incomptum</i>	Capsule	Exserted	Cup-shaped	White	Racemose
<i>Gomphostigma virgatum</i>	Capsule	Exserted	Cup-shaped	White	Racemose

For fruit type, fleshy fruits are coded as 'berry' and dry fruits are coded as 'capsule'. For stamens, those that extended outside the corolla tube are coded as 'exserted' and those that are hidden inside the corolla tube are coded as 'included'. For corolla shape, those with a corolla tube length to lobe length ratio < 1.8 are coded as 'cup-shaped' and those with a ratio > 1.8 are coded as 'tube-shaped'. For corolla colour, the colour of the majority of the corolla is considered. In many species, the throat has a different colour which is not considered. For inflorescence type, those with peduncled cymes and sessile flowers are considered 'capitate', those with sessile cymes and sessile flowers are considered 'spiciform', those with pedunculate cymes and pedicellate flowers are considered 'thyrsoid', those with more than one order of branching are considered 'paniculate' and those with single-flowered cymes in a raceme are considered 'racemose'. All taxa were coded as having a single state for each trait, although in some cases polymorphism exists.

Table A4. Revised classification and list of species in Buddlejeae

Genus *Buddleja* L. [108]
 Section *Salviifoliae* J.H.Chau [1]
B. salviifolia (L.) Lam.
 Section *Gomphostigma* (Turcz.) J.H.Chau [2]
B. incompta L.f.
B. virgata L.f.
 Section *Chilianthus* (Burch.) Leeuwenberg [4]
B. auriculata Benth.
B. dysophylla (Benth.) Radlk.
B. loricata Leeuwenberg
B. saligna Willd.
 Section *Pulchellae* J.H.Chau [1]
B. pulchella N.E.Br.
 Section *Nicodemia* (Ten.) Leeuwenberg [9]
B. acuminata Poir.
B. axillaris Willd.
B. cuspidata Baker
B. indica Lam.
B. fragifera Leeuwenberg
B. fusca Baker
B. madagascariensis Lam.
B. polystachya Fresen.
B. sphaerocalyx Baker
 Section *Alternifoliae* Kränzl. [24]
B. albiflora Hemsl.
B. alternifolia Maxim.
B. asiatica Lour.
B. bhutanica Yamazaki
B. brachystachya Diels
B. candida Dunn
B. caryopteridifolia W.W.Sm.
B. colvillei Hook.f.
B. crispa Benth.
B. davidii Franch.
B. delavayi L.F.Gagnep.
B. fallowiana Balf.f. & W.W.Smith
B. forrestii Diels
B. japonica Hemsl.
B. jinsixiaensis R.B.Zhu
B. lindleyana Fortune
B. macrostachya Benth.
B. microstachya E.D.Liu & H.Peng
B. myriantha Diels
B. nivea Duthie
B. officinalis Maxim.
B. paniculata Wall.
B. subcapitata E.D.Liu & H.Peng
B. yunnanensis L.F.Gagnep.
 Section *Buddleja* [66]
B. americana L.
B. anchoensis Kuntze
B. araucana Phil.
B. aromatica J.Rémy

Table A4. *Continued*

B. blattaria J.F.Macbr.
B. brachiata Cham. & Schltdl.
B. bullata Kunth
B. cardenasii Standl. ex E.M.Norman
B. cestriflora Cham.
B. chapalana B.L.Rob.
B. chenopodiifolia Kraenzl.
B. cordobensis Griseb.
B. cordata Kunth
B. coriacea J.Rémy
B. corrugata M.E.Jones
B. crotonoides A.Gray
B. cuneata Cham.
B. diffusa Ruiz & Pav.
B. domingensis Urb.
B. elegans Cham. & Schltdl.
B. euryphylla Standl. & Steyerl.
B. filibracteolata J.A.González & J.F.Morales
B. globosa Hope
B. grandiflora Cham. & Schltdl.
B. hatschbachii E.M.Norman & L.B.Sm.
B. hieronymi R.E.Fr.
B. ibarrensii E.M.Norman
B. incana Ruiz & Pav.
B. interrupta Kunth
B. iresinoides (Griseb.) Hosseus
B. jamesonii Benth.
B. kleinii E.M.Norman & L.B.Sm.
B. lanata Benth.
B. lojensis E.M.Norman
B. longiflora Brade
B. longifolia Kunth
B. marrubiiifolia Benth.
B. megalcephala Donn.Sm.
B. mendozensis Gillies ex Benth.
B. misionum Kraenzl.
B. montana Britton
B. multiceps Kraenzl.
B. nitida Benth.
B. normaniae J.H.Chau
B. oblonga Benth.
B. parviflora Kunth
B. perfoliata Kunth
B. pichinchensis Kunth
B. polycephala Kunth
B. racemosa Torr.
B. ramboi L.B.Sm.
B. rinconensis (Mayfield) J.H.Chau
B. rufescens Willd. ex Schultes & Schultes
B. scordioides Kunth
B. sessiliflora Kunth
B. simplex Kraenzl.
B. skutchii C.V.Morton
B. soratae Kraenzl.

Table A4. *Continued*

<i>B. speciosissima</i> Taub.
<i>B. stachyoides</i> Cham. & Schltdl.
<i>B. suaveolens</i> Kunth & Bouché
<i>B. thyrsoides</i> Lam.
<i>B. tubiflora</i> Benth.
<i>B. tucumanensis</i> Griseb.
<i>B. utahensis</i> Coville
<i>B. vexans</i> Kraenzl. & Loes. ex E.M.Norman
<i>Incertae sedis</i>
<i>B. glomerata</i> H.Wendl.

Number of species in each taxon indicated in brackets.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Majority-rule consensus phylograms from Bayesian analyses of individual locus datasets, excluding 25% burn-in. Values at nodes indicate support: maximum likelihood bootstrap percentage (BP)/Bayesian posterior probability (PP), if > 50% BP or 0.5 PP. Nodes with > 70% BP and 0.9 PP support are highlighted with thicker branches. Letter after species name indicates species that has also been considered a member of *Chilianthus* (C) or *Nicodemia* (N). (A) ETS, (B) PPR24, (C) PPR97, (D) PPR123, (E) plastid, consisting of partitioned concatenated dataset with *trnD-trnT*, *trnS-trnfM* and *rpoA*.

Figure S2. Majority-rule consensus tree from Bayesian analysis of ETS dataset with expanded specimen sampling, excluding 25% burn-in. Values at nodes indicate support: maximum likelihood bootstrap percentage (BP)/Bayesian posterior probability (PP), if > 70% BP or 0.9 PP. Nodes with > 70% BP and 0.9 PP support are highlighted with thicker branches.