

2013

## Time-Calibrated Phylogenies of Hummingbirds and Hummingbird-Pollinated Plants Reject a Hypothesis of Diffuse Co-Evolution

Erin A. Tripp

*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder*

Lucinda A. McDade

*Rancho Santa Ana Botanic Garden, Claremont, California*

Follow this and additional works at: <https://scholarship.claremont.edu/aliso>

---

### Recommended Citation

Tripp, Erin A. and McDade, Lucinda A. (2013) "Time-Calibrated Phylogenies of Hummingbirds and Hummingbird-Pollinated Plants Reject a Hypothesis of Diffuse Co-Evolution," *Aliso: A Journal of Systematic and Floristic Botany*. Vol. 31: Iss. 2, Article 5.

Available at: <https://scholarship.claremont.edu/aliso/vol31/iss2/5>

## TIME-CALIBRATED PHYLOGENIES OF HUMMINGBIRDS AND HUMMINGBIRD-POLLINATED PLANTS REJECT A HYPOTHESIS OF DIFFUSE CO-EVOLUTION

ERIN A. TRIPP<sup>1,3</sup> AND LUCINDA A. MCDADE<sup>2</sup>

<sup>1</sup>University of Colorado, Boulder, Museum of Natural History and Department of Ecology and Evolutionary Biology, UCB 334, Boulder, Colorado 80309

<sup>2</sup>Rancho Santa Ana Botanic Garden, 1500 North College Avenue, Claremont, California 91711

<sup>3</sup>Corresponding author (erin.tripp@colorado.edu)

### ABSTRACT

Neotropical ecosystems house levels of species diversity that are unmatched by any other region on Earth. One hypothesis to explain this celebrated diversity invokes a model of biotic interactions in which interspecific interactions drive diversification of two (or more) lineages. When the impact of the interaction on diversification is reciprocal, diversification of the lineages should be contemporaneous. Although past studies have provided evidence needed to test alternative models of diversification such as those involving abiotic factors (e.g., Andean uplift, shifting climatological regimes), tests of the biotic model have been stymied by lack of evolutionary time scale for symbiotic partners. In this study, we infer timescales for diversification of hummingbirds and a species-rich plant lineage that is ~50% hummingbird pollinated, *Ruellia* (Acanthaceae). Results demonstrate that hummingbirds originated about 20 million years before New World *Ruellia* and that all but one major hummingbird clade was extant before the plant group originated. Thus, the classic model of “diffuse co-evolution” between hummingbirds and this group of plants is rejected by our data. However, together with the observation that the Neotropical clade of *Ruellia* (~350 species) is far more species rich than its Old World sister group (~75 species), our results are consistent with the hypothesis that plant diversification in the Neotropics has been facilitated in part by a pre-existing diversity of hummingbirds. This hypothesis may find support in other lineages of Neotropical plants that similarly exhibit asymmetrical partitioning of species diversity in the Paleo- vs. Neotropics.

Key words: Acanthaceae, diffuse co-evolution, divergence time, diversification, hummingbirds, Neotropics, time-calibrated phylogeny, Trochilidae.

### INTRODUCTION

The Neotropics are home to more species than any other terrestrial region of the world, thus serving as a crucial reservoir for life on Earth as well as a natural laboratory for understanding biological diversification (Raven 1976; Gentry 1982; Wilson 1988). One of the longest running debates in ecology and evolutionary biology is that of causes of the latitudinal gradient in species diversity (e.g., time and/or area models, speciation and/or extinction rate models; Wallace 1878; Haffer 1969; van der Hammen 1974; Stebbins 1974; Simpson 1980; Gentry 1982; Prance 1982; Burnham & Graham 1999; Fine et al. 2005; Mittelbach et al. 2007; Antonelli et al. 2009; Pennington et al. 2010; Hoorn et al. 2010; Rull 2011; see Hughes et al. 2013 and other papers in special issue of *Bot. J. Linn. Soc.*). Mittelbach et al. (2007; Table 1 therein) organized ideas about causes of the latitudinal gradient into testable hypotheses based on biological processes, such as speciation and extinction in the context of geological time and geographical space. Among these ideas, the biotic interactions hypothesis posits that “interrelationships between competing and symbiotic species become the paramount adaptive problem” (Dobzhansky 1950: 220–221) that drives speciation to a much higher degree in tropical than temperate environments.

In discussing the biotic interactions hypothesis, Mittelbach et al. (2007) focused on niche dimensions added by such interactions and the relative strength of selection from biotic interactions as testable predictions. The authors concluded that this hypothesis remains little explored. We test here a

prediction of the biotic interactions hypothesis that applies when closely interacting symbionts are involved: ecological interactions between the interactors should drive adaptive diversification of both lineages of interactors to yield a pattern of diffuse co-evolution (Ehrlich and Raven 1964). If diffuse co-evolution occurred, then we expect diversification between the interacting lineages to have been more or less contemporaneous, a prediction that is testable with time-calibrated phylogenies. The scarcity of studies that have as yet explored the diffuse co-evolution hypothesis may be attributable to a paucity of such phylogenies for species-rich clades of not just one but two or more lineages of interacting organisms. Here, we explore one conspicuous class of biotic interactions that epitomizes the importance of ecological interactions in the Neotropics: that between plants and their animal pollinators (Ehrlich and Raven 1964; Stebbins 1970).

Hummingbirds (Trochilidae) are obligate pollinators of thousands of New World angiosperms. Both micro- and macroevolutionary studies involving hummingbirds and the plants they pollinate have revealed striking findings that suggest major roles for these birds in plant diversification: (1) single-locus mutations that lead to major changes in floral phenotype and animal visitation, thus facilitating rapid adaptive divergence (Bradshaw and Schemske 2003); (2) cases of intricate matching of morphologies of hummingbird bills and flowers (Lindberg and Olesen 2001; Temeles and Kress 2003; Temeles et al. 2013); (3) specialization onto hummingbirds as a primary mechanism for reproductive isolation in plants, thus facilitating plant speciation (Kay and Schemske

Table 1. Fossils of Acanthaceae used in this study, arranged phylogenetically. # refers to fossil number from a list of all 51 reports that we examined for this study (see Tripp and McDade, in press, for expanded information and discussion of fossil reports; criteria for acceptance included reliable identification and dating, and phylogenetic utility). Figure 1 shows nodes calibrated by these fossils. Calibration priors (lognormal mean, standard deviation, and zero offsets) are provided in Table 2.

Taxon	#	Provenance	Geologic period	Age (mya)	Fossil synapomorphy(ies)	Reference
<i>Avicennia</i> ( <i>Avicennia</i> lineage)	1	Spain	Middle Bartonian	39.4-38.3	<i>Avicennia</i> is monophyletic (Schwarzbach and McDade 2002) and its species share unique pollen features: spheroidal and tricolporate with longitudinally elongated ora and reticulate surface ornamentation with broad muri and small lumina (Borg and Schönenberger 2011); fossil #1 shares these traits and thus was used to constrain the MRCA of the 2 sampled spp. of <i>Avicennia</i> .	Cavagnetto and Anadón (1996)
<i>Acanthus</i> (Acantheae)	11	England	Lower to Middle Oligocene	33.7-28.75	Plants of <i>Acanthus</i> produce seeds with distinctly rugose surface sculpturing (Tomlinson 1986; McDade et al. 2005) exactly like that of this fossil; thus, fossil #11 was used to constrain the MRCA of the lineage containing <i>Acanthus semii</i> .	Reid and Chandler (1926)
<i>Hulemactanthus</i> (Barlerieae)	15	Nigeria	Miocene	23.8-5.3	<i>Hulemactanthus</i> pollen is prolate, tricolporate with broadened colpi, and has coarsely reticulate exine sculpturing (Scotland and Vollesen 2000); we agree with Raj's (1961) assignment of fossil #15 to this genus. Phylogenetic placement of <i>Hulemactanthus</i> within Barlerieae remains unknown (see McDade et al. 2008); thus we constrained the MRCA of all Barlerieae with this fossil.	Kuyt et al. (1955)
NW <i>Justicia</i> (Justicieae)	32	Brazil	Lower Miocene	23.8-14.6	This fossil is dicolporate with distinctive areoles that surround the germinal apertures, traits thus far known only from Justiceae (Graham 1988).	Regali et al. (1974)
Pseudocolpate Ruellieae	36	Senegal	Upper Miocene	14.6-5.3	Fossil attributable to clade of Ruellieae with pseudocolpate pollen (Tripp et al. 2013); used to constrain the MRCA of the 6 taxa herein sampled that are in that clade (but excluding Trichantherinae, which have distinctively differing pseudocolpal arrangements).	Medus (1975)
NW pseudocolpate Ruellieae	51	Haiti	Miocene-Pliocene	23.8-1.8	Fossil distinctly pseudocolpate with sexine lips surrounding germinal apertures; confidently assigned to the clade of Ruellieae that shares these synapomorphies (Tripp et al. 2013). Because of its geographic source (Haiti), it is likely of a taxon with affinities to modern <i>Dyschoriste</i> (formerly <i>Apasahus</i> ; see Tripp et al. 2013).	Graham (1990)
Trichantherinae (Ruellieae)	43	Trinidad	Tertiary	65.0-1.8	Pollen of Trichantherinae (except <i>Louleridium</i> ; Tripp et al. 2013) is arguably the most distinctive of any Acanthaceae and among the most distinctive across angiosperms. The synapomorphic bands of pseudocolpi arranged in opposing directions and apertures with sexine lips indicate that this palynomorph can be used to constrain the MRCA of Trichantherinae.	Graham (1976)

Table 2. Taxon sets (TS #) calibrated by age, and calibration priors used for each plant fossil. As in Table 1, # refers to fossil number from a list of all 51 reports that we examined for this study (Table 1 in Tripp and McDade [in press]). Figure 1 shows nodes calibrated by these fossils.

Taxon set	#	Taxa constrained	Age	Zero offset	Log (Stdev)	Mean	5% quantile	95% quantile
TS 1	N/A	All Acanthaceae monophyletic	None designated (used tree prior)	N/A	N/A	N/A	N/A	N/A
TS 2	#1	<i>Avicennia</i> (2 species)	Mid Bartonian (~39.4–38.3 mya)	38.3	0.6	0.5	38.46	39.42
TS 3	#11	<i>Acanthopsis</i> + <i>Acanthus</i>	Early-Mid Oligocene (~33.7–28.8 mya)	28.8	1.1	1.5	28.9	33.8
TS 4	#15	Barlerieae (2 taxa)	Miocene (~23.8–5.3 mya)	5.3	1.1	5.5	5.8	23.6
TS 5	#32	Justicieae ( <i>Dicliptera</i> + <i>Rhinacanthus</i> )	Lower Miocene (~23.8–14.6 mya)	14.6	1.3	2.5	14.7	23.7
TS 6	#36	pseudocolpate Ruellieae excluding Trichantherinae (6 taxa)	Upper Miocene (~14.55–5.3 mya)	5.3	1.4	2.5	5.4	14.7
TS 7	#51	Petalidiinae + Mimulopsiinae (5 taxa)	Mio-Pliocene (~23.8–1.8 mya)	1.8	1.3	6	2.1	23.7
TS 8	#43	Trichantherinae excluding <i>Louleridium</i> (3 taxa)	10–12 mya	10	0.5	1	10.4	12.0

2003; Kay 2006); (4) numerous independent evolutionary origins of hummingbird pollination across angiosperms (Van der Niet and Johnson 2012), suggesting an adaptive value; (5) the heterodox discovery that specialized pollination by hummingbirds is not an evolutionary dead-end, but rather precedes further evolution of pollination systems (McDade 1992; Tripp and Manos 2008); and (6) a positive correlation between species richness of plant lineages and the presence of hummingbird pollination (Schmidt-Lebuhn et al. 2007; although data support an Old World origin for these birds [see below], they are no longer extant there).

Correlation between hummingbird and flowering plant diversity has been predicted additionally by earlier authors (Stebbins 1970; Bleiweiss 1998), but studies that dually examine evolutionary divergence times in these two groups are lacking. We tested the hypothesis that diffuse co-evolution has occurred between hummingbirds and a lineage of plants that is ~50% hummingbird-pollinated, *Ruellia* Juss. (Acanthaceae). Both hummingbird and plant groups contain hundreds of species, thus making them ideal for exploration of the biotic interactions hypothesis in a comparative framework. Largely contemporaneous radiations of the two lineages would provide support for the diffuse co-evolution hypothesis; in contrast, substantial temporal gaps between the diversification of hummingbirds and that of the plants would refute the hypothesis.

#### METHODS

##### Plant Dataset

The genus *Ruellia* contains ~350 species, of which ~275 occur in the New World (Tripp and Manos 2008). In this study, we sampled 172 species of *Ruellia* (26 Old World [OW] + 146 New World [NW]), representing all major clades of extant species (McGuire et al. 2007; Tripp 2007). Thirty-six outgroups that span the phylogenetic diversity of Acanthaceae (cf. McDade et al. 2008) were also included, for a total of 209 sampled plant species. To reconstruct phylogeny, we used concatenated sequence data from five regions: two nuclear (*Eif3E*, ITS + 5.8S) and three chloroplast (*psbA-trnH*, *trnG-*

*trnR*, *trnG-trnS*). DNA sequence data assembled for this study (Appendix 1) were generated during our prior work on phylogenetic relationships among Acanthaceae using methods reported in Tripp and McDade (in press) and Tripp and Fatimah (2012). Sequences were aligned by eye in MacClade (Maddison and Maddison 2000). Data from all loci were manually concatenated following observation of congruent relationships derived from individual partitions (data not shown). Following conservative criteria for assessing sequence homology, 4558 of the original 5191 characters that could be unambiguously aligned were retained for analysis. For *Eif3E* and *psbA-trnH*, only sequence data from Ruellieae (Tripp et al. 2013) were included in analyses due to hypervariability of these loci across all Acanthaceae (data not shown). A GTR +  $\gamma$  + I model of sequence evolution with six gamma categories was applied to all five partitions because the complexity of this model can account for the behavior of any one of the individual partitions (Rogers 2001).

To estimate divergence times, we used minimum ages of seven Acanthaceae fossils (Table 1) as priors to calibrate our phylogeny. As reported by Tripp and McDade (in press), these fossils were scrutinized for validity and utility by rigorous study of reports for 51 total fossils (see Table 1; additional data on fossil reports presented in Tripp and McDade [in press]). Only fossils that we accepted as both reliably identified and reliably aged were used in the present study. Our assessment of affinities to extant lineages is based on extensive knowledge of Acanthaceae systematics and the structural traits that are congruent with phylogenetic relationships (e.g., McDade et al. 2000a,b; Scotland and Vollesen 2000; Schwarzbach and McDade 2002; McDade et al. 2005; Tripp 2007; Daniel et al. 2008; McDade et al. 2008; Tripp et al. 2009; McDade et al. 2012; Tripp et al. 2013). Fossils constrained the most recent common ancestor (MRCA) of the taxon to which the fossil could be unambiguously attributed. We applied zero offsets to reflect fossil minimum ages and then adjusted standard deviations such that 95% of the highest posterior density (HPD) intervals fell within the range encompassed by geologic ages to which fossils were attributed (Table 2).

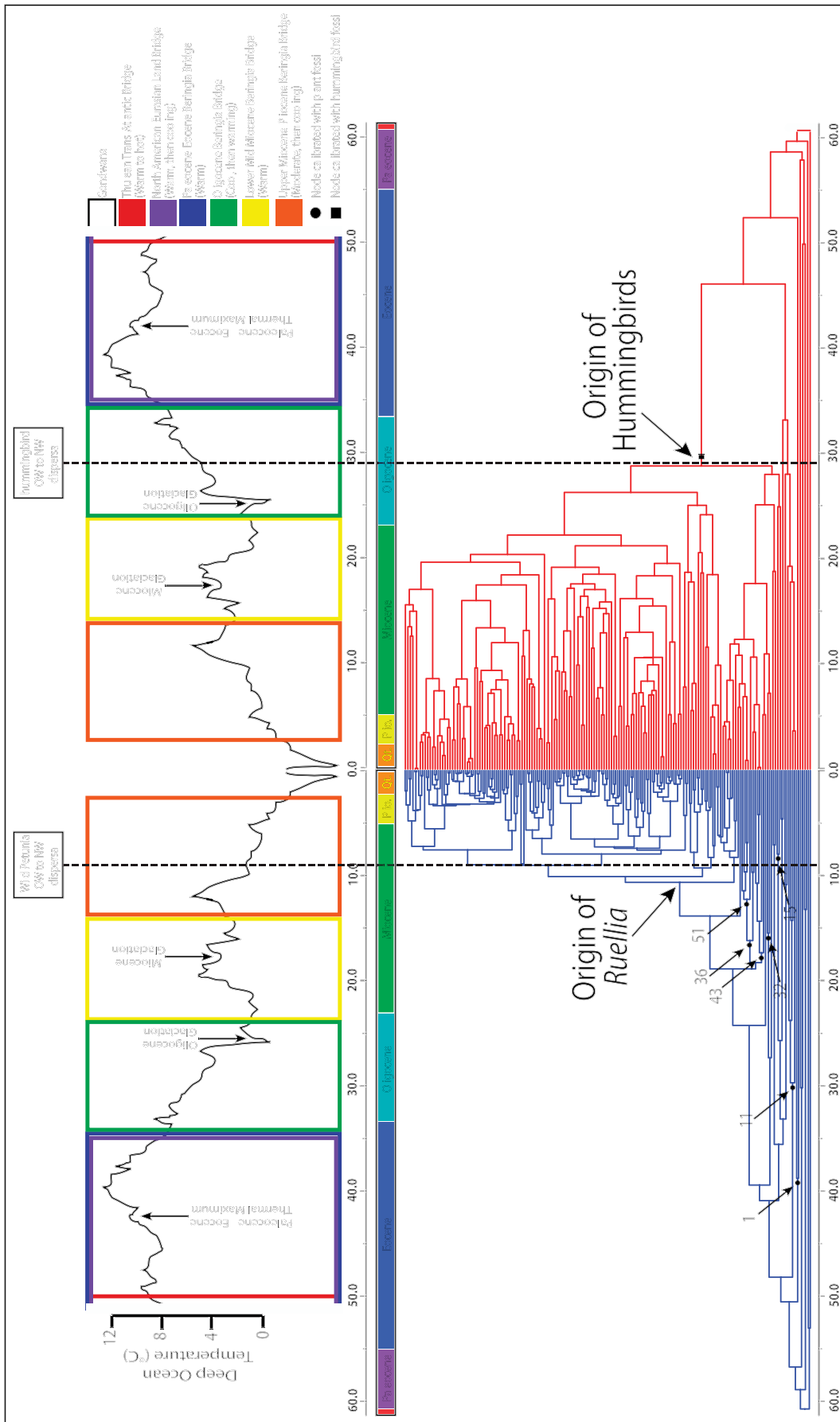


Fig. 1. Divergence times in hummingbirds and *Ruellia* in the context of major climatic and geological episodes in Earth history since the Early Eocene (key in center left and labeled arrows on climatological record). Approximate delimitation of climatic events summarized from International Panel on Climate Change (2007). Reconstruction of deep ocean temperatures (as a proxy for global temperature) derived from oxygen isotopes and corrected for variation in global ice volume (from International Panel on Climate Change 2007; see Fig. 6.1 therein), to provide climatological context for clade histories. Filled circles indicate seven plant fossil calibrations, these identified by number (see Table 1); filled square indicates the hummingbird fossil calibration. Posterior probabilities for major clades and 95% HPDs given in Table 3.

### Hummingbird Dataset

The family Trochilidae contains ~330 species, all extant members of which occur in the New World. In this study, we used the DNA matrix of McGuire et al. (2007), which includes 151 hummingbird species and 12 outgroups that span a range of phylogenetic diversity of Apodiformes (hummingbirds and swifts) and Caprimulgiformes (nightjars and relatives). This matrix includes concatenated sequence data from five regions: two nuclear (*BFib*, *AKI*) and three mitochondrial (*ND2*, *ND4*, flanking tRNAs), and is 4122 bp in length after alignment. In a rigorous exploration of model selection and performance using the above-mentioned hummingbird dataset, McGuire et al. (2007) reported that various partitioning strategies returned highly similar topologies with respect to hummingbird relationships and branch lengths. As such, we chose to apply a GTR +  $\gamma$  + I model of sequence evolution with six gamma categories to all five partitions because the complexity of this model accommodates simpler models whereas the reverse is not true (Rogers 2001).

To estimate divergence times, we used the only reports of fossil hummingbirds to date to calibrate the phylogeny (Mayr 2004, 2007). Mayr convincingly argued that these northern European fossils, which he named *Eutrochilus inexpectus* Mayr, represent the sister taxon to crown Trochilidae, i.e., the clade that includes extant hummingbirds. Mayr dated these OW hummingbird fossils to the Rupelian (i.e., 33.9–28.4 million years ago [mya]; International Commission on Stratigraphy 2013). We applied a zero offset of 28.4 to this prior and adjusted the standard deviation such that 95% of the highest posterior density (HPD) interval fell within the range encompassed by this geologic age (mean = 1.0; standard deviation = 1.9).

### Divergence Time Estimations

We used BEAST (Drummond and Rambaut 2007) to simultaneously estimate phylogeny and divergence times within *Ruellia* and within hummingbirds, using the above matrices, each of which sampled about 50% of species diversity (*Ruellia*: 172 of 350 species, ~50%; Trochilidae: 151 of 331 species, ~46%). Rate heterogeneity across branches was permitted via implementation of a relaxed molecular clock and the uncorrelated lognormal distribution was implemented (Drummond et al. 2006). We used a uniform prior for UCLD means for each data partition, with an initial value of 1.0, an upper value of 100, and a lower value of 0.0. A Yule Process speciation model was specified for the tree priors (Gernhard 2008), and a random starting tree was implemented. Fossil calibrations were input as lognormal probability distributions (Ho and Phillips 2009).

Tracer vers. 1.5 (Rambaut and Drummond 2007), Log-Combiner v1.7.3, and TreeAnnotator vers. 1.7.0 were used to ensure sufficient sampling of posterior distributions, to thin sampled trees, and to construct maximum clade credibility trees keeping target age “heights.” Chains were run for 50 (plant) or 35 (bird) million generations, depending on when stable effective sample sizes (ESS values) were achieved. We also ran BEAST analyses sampling only from the prior (i.e., alignment-free) to explore effects of the remaining priors on the posterior distribution.

## RESULTS

Our alignment-free BEAST analyses (sampling only from the prior) produced extremely low posterior probabilities and unreasonable taxon assemblages, indicating that our molecular matrices contained ample phylogenetic signal for tree reconstruction. The relationships recovered in our analysis of the plant dataset as well as the hummingbird dataset reflect relationships recovered in prior phylogenetic study of these two groups (*Ruellia*: Tripp 2007 and Tripp and Manos 2008; hummingbirds: McGuire et al. 2007).

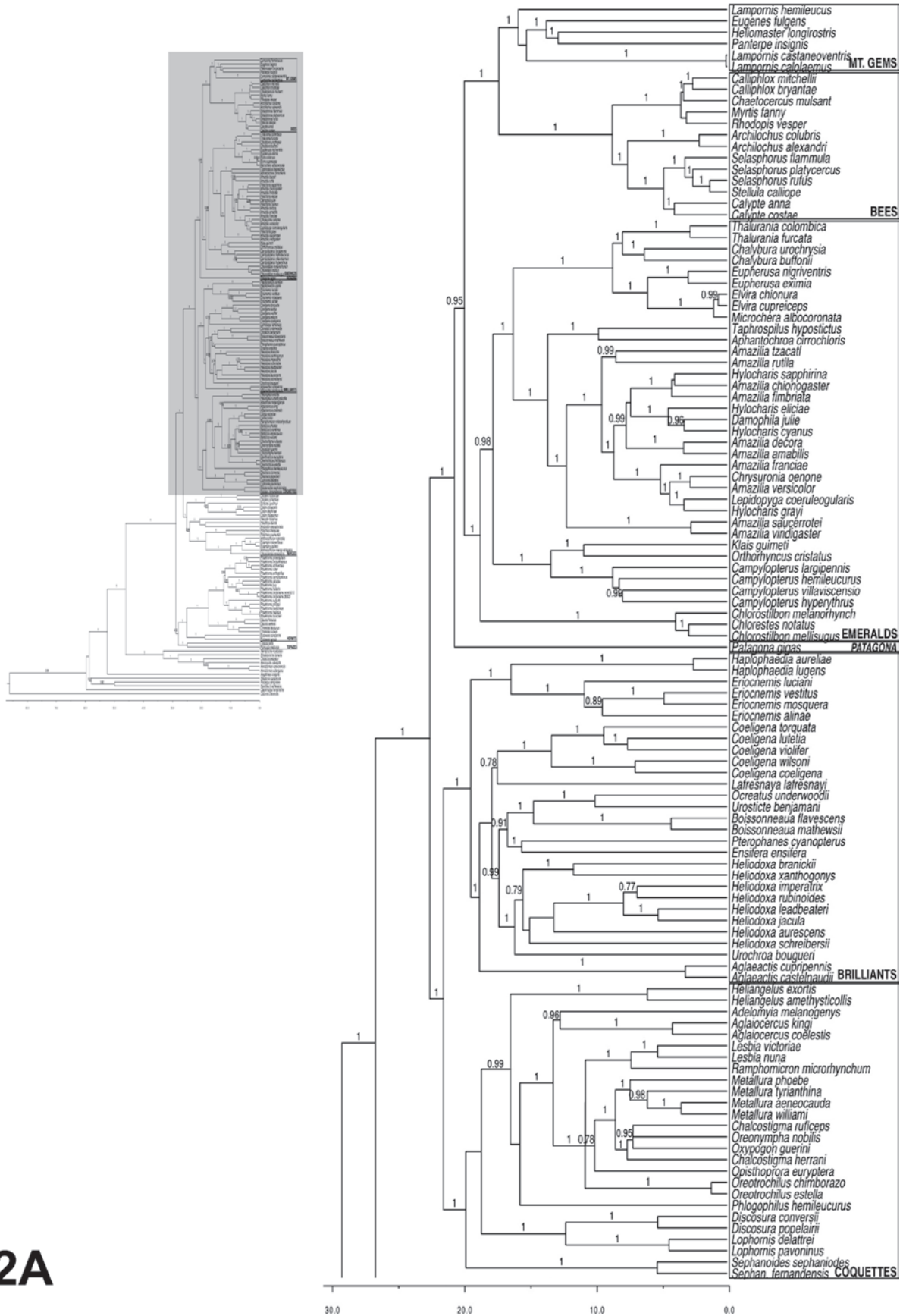
### Divergence Times

Results are inconsistent with a hypothesis of diffuse co-evolution between hummingbirds and *Ruellia* (Fig. 1): hummingbirds evolved in the middle Oligocene (28.8 mya, 95% HPD = 28.4 to 29.9 mya) and diversified primarily in the middle to late Miocene (Fig. 1, 2; Table 3) whereas New World *Ruellia* originated in the Late Miocene (9.0 mya, 95% HPD = 8.3 to 13.5 mya) and diversified primarily in the Pliocene to Quaternary, with all major clades originating more recently than 7 mya (Fig. 1, 3; Table 3). Table 3 reports the means, 95% HPD intervals, and posterior probabilities of divergence times for all major clades of hummingbirds and *Ruellia*. Our dates for hummingbird diversification largely corroborate dates estimated by Bleiweiss (1998) using DNA hybridization data from 28 hummingbirds and fossils from sister groups to construct a timescale. One exception is the estimated age for the split between hermits and non-hermits, which was 28.8 mya (95% HPD = 28.4 to 30.0 mya) in our study but 17 mya in Bleiweiss’s study. All but two major lineages of hummingbirds are estimated to have been extant by approximately 19 mya. The clade of Mountain gems (mean crown group age = 15.6 mya, 95% HPD = 17.0–13.8) and its sister group, Bee hummingbirds (mean crown group age = 8.6 mya, 95% HPD = 9.7–7.2), is estimated to be somewhat younger (mean = 17.1 mya, 95% HPD = 18.4–15.1).

→

Fig. 2A–B. Maximum Clade Credibility Tree (as in Fig. 1) of hummingbirds, showing taxon labels and clade names (McGuire et al. 2007; Table 3) as well as branch posterior probabilities. The tree is split into two parts displayed on sequential pages.—A. Upper part.—B. Lower part. A thumbnail of the complete tree is provided alongside each partial tree.

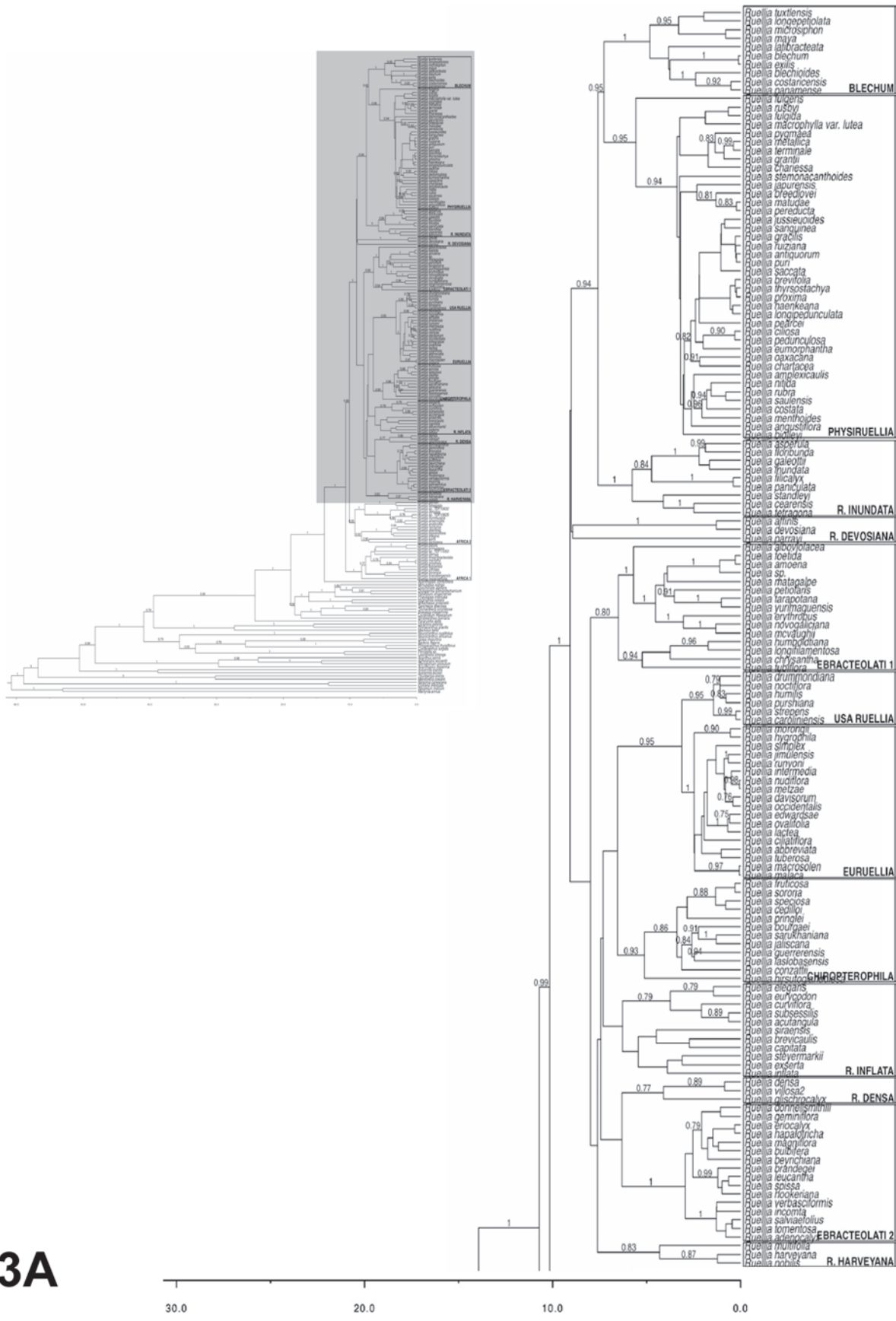
Fig. 3A–B. Maximum Clade Credibility Tree (as in Fig. 1) of *Ruellia*, showing taxon labels and clade names (Tripp 2007; Table 3), as well as branch posterior probabilities. The tree is shown in two parts on sequential pages.—A. Upper part.—B. Lower part. A thumbnail of the complete tree is provided alongside each partial tree.



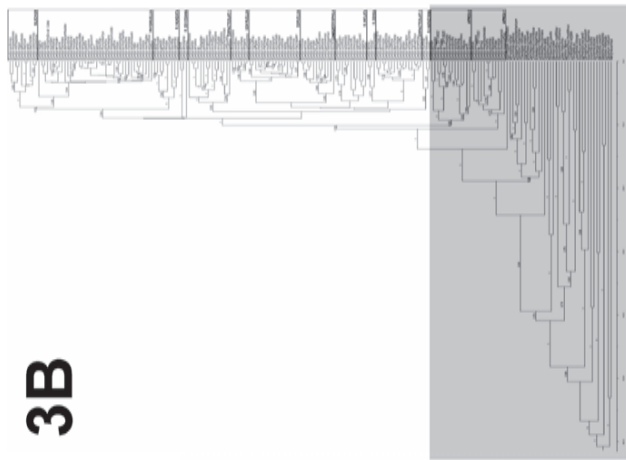
2A







3A



3B

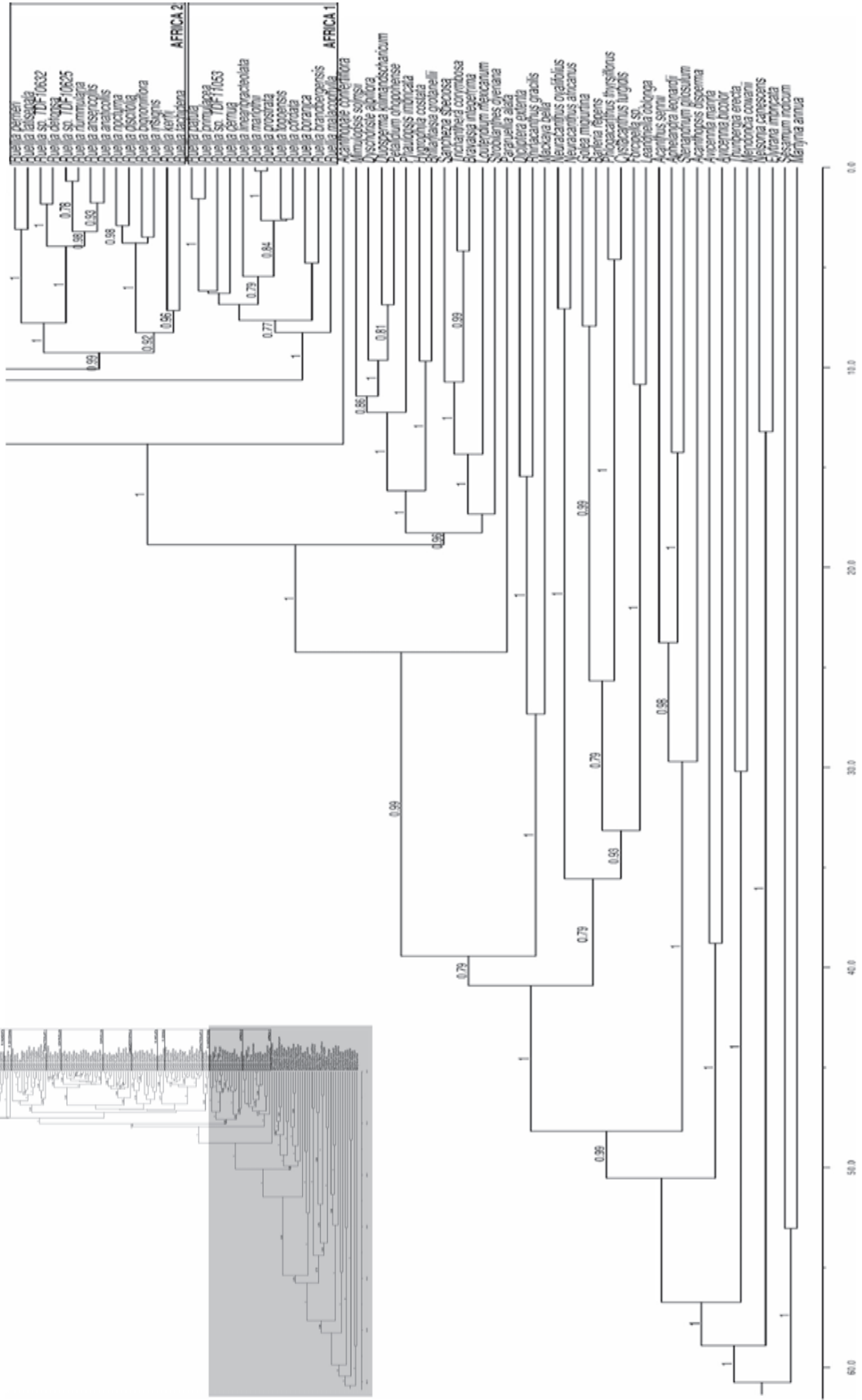


Fig. 3A-B. Continued.

Table 3. Posterior probabilities, means, and 95% HPD intervals from fossil-calibrated BEAST analyses for major lineages of hummingbirds (above, lineages follow McGuire et al. 2007) and of *Ruellia* (below, lineages follow Tripp 2007). NA = 95% HPD not reported because posterior probability for lineage <0.50. See Fig. 2 and 3 for bird and plant clades, respectively.

Lineage	Posterior probability	Mean age (mya)	95% HPD
<b>HUMMINGBIRDS</b>			
Trochilidae	1	28.8	30.0–38.4
Topazes	1	25.0	27.3–21.6
Hermits	1	25.1	27.4–23.7
Mangoes	1	25.1	26.4–22.8
Andean Clade (Brilliant + Coquettes)	1	21.2	22.1–18.6
Brilliant	1	19.2	19.5–16.6
Coquettes	1	19.6	20.7–17.1
(Patagona-Emeralds – (Mt. Gems + Bees))	1	20.4	21.6–18.4
(Emeralds – (Mt. Gems + Bees))	0.95	19.7	20.6–18.6
Emeralds	1	18.5	19.1–16.0
Mt. Gems + Bees	1	17.1	18.4–15.1
Mt. Gems	1	15.6	17.0–13.8
Bees	1	8.6	9.7–7.2
<b>RUELLIA (Acanthaceae)</b>			
<i>Ruellia</i>	1	10.6	16.9–10.5
Africa Clade 1	1	8.2	12.9–7.0
Africa Clade 2	0.99	9.3	14.2–8.7
New World <i>Ruellia</i>	1	10.1	13.5–8.3
Ebracteolati 1	0.80	2.9	4.6–2.3
Ebracteolati 2	1	6.5	10.4–5.5
<i>R. densa</i> clade	0.77	4.1	5.7–1.6
<i>Chiropterophila</i>	0.93	5.1	7.2–3.4
<i>R. harveyana</i> clade	0.83	4.3	6.9–2.0
<i>R. inflata</i> clade	0.45	6.2	NA
<i>Euruellia</i>	1	3.1	4.7–2.2
USA <i>Ruellia</i>	0.95	1.4	3.2–1.0
<i>R. inundata</i> clade	1	5.7	9.5–5.4
<i>R. devosiana</i> clade	0.10	8.9	NA
<i>Physiruellia</i> clade	0.95	5.5	8.3–4.5
<i>Blechum</i> clade	1	4.8	8.3–4.5

#### DISCUSSION

Our results indicate that the arrival of hummingbirds into the Neotropics predated that of *Ruellia* by nearly 20 million years; further, all but one clade of hummingbirds was in place well before the arrival of *Ruellia*. Although our analyses reject a scenario of contemporaneous diffuse co-evolution, they do not refute a hypothesis of pollinator-driven diversification of the plants via mechanisms such as floral isolation (Grant 1992) and pollinator discrimination (Schemske and Bradshaw 1999). In contrast, we do not find evidence that *Ruellia* contributed to diversification of hummingbirds. Diversification of hummingbirds in the middle to late Miocene, prior to the establishment of *Ruellia* in the New World, is likely explained by multiple factors, both abiotic (Fine et al. 2005; Antonelli et al. 2009) and biotic. Over this time span, the Americas were geologically and climatologically dynamic, resulting in a changing mosaic of habitats across latitudinal, elevational, and rainfall gradients over geological time and space (Hoorn et al. 2010). There is also ample evidence for correspondingly dynamic changes in floristic composition across these Neotropical habitats (Hoorn et al. 2010; Jaramillo et al. 2006), leaving little doubt that hummingbird-pollinated plants that pre-date the arrival of *Ruellia* provided important resources for diversifying hummingbirds.

The remarkable species richness of flowering plants present in the Neotropics has been assembled in the context of these

same historical factors and multiple kinds of biotic interactions. As well sampled and resolved phylogenies become available for clades that include Neotropical plants, the phylogenetic patterns that underlie species richness can be understood. A pattern emerging from our work and that of several colleagues is one of marked asymmetries in species diversity between richer New World compared to Old World sister clades (McDade et al. 2000a; McDade et al. 2005; Hansen et al. 2006; Kiel et al. 2006; Tripp 2007; Borg et al. 2008; Daniel et al. 2008; McDade et al. 2008; Tripp and Manos 2008; David and Anderson 2010; McDade et al. 2012; Yang et al. 2012; Drew and Sytsma 2013; Tripp et al. 2013) suggesting that high Neotropical diversity is an evolutionary phenomenon as well as an ecological one.

This study corroborates a growing consensus (e.g., Hughes et al. 2013) that full explanation of high Neotropical diversity will require synthesis of multiple ideas regarding sources of diversity, including both abiotic and biotic axes of speciation, as well as phylogenetic studies of lineages that occur in the New World. We anticipate contributing to this synthesis by expanding sampling of New World *Ruellia* to study phylogenetically patterned correlates of diversity in a spatio-temporal context.

Dating clades of organisms that are key actors in major symbioses such as pollinators and the plants they pollinate is of broad interest to biologists and others, owing to the implications that such results may have for understanding

processes and patterns of evolution. Moreover, understanding the context for diversification may inform conservation efforts, which are particularly challenging when dealing with obligate partners (e.g., co-extinctions; Koh et al. 2004). As well-sampled species phylogenies from diverse tropical clades continue to accumulate, we will have capacity to more fully explore biotic factors that have been hypothesized to facilitate diversification. In turn, we will have enhanced capacity to more comprehensively reconstruct the temporal context of the individual evolutionary histories that, together, have resulted in one of the most celebrated yet endangered biotas on Earth.

## ACKNOWLEDGMENTS

We thank Gerald Mayr for discussions on hummingbird fossils herein utilized; Mark Chase, Kyle Dexter, and John Lundberg for comments on an earlier draft of the manuscript; and Peter Stevens for useful conversation of data herein presented. Noravit Chumchim and Amanda Fisher provided assistance in finalizing the paper. All data presented in this paper are archived in GenBank (Appendix 1) and Dryad. This research was supported by NSF-DEB Award 0919594 to EAT and LAM. EAT collected data, conducted analyses and wrote the paper. LAM collected data and wrote the paper.

## LITERATURE CITED

- ANTONELLI, A., J. A. A. NYLANDER, C. PERSSON, AND I. SANMARTÍN. 2009. Tracing the impact of the Andean uplift on neotropical plant evolution. *Proc. Natl. Acad. Sci. U.S.A.* **106**: 9749–9754.
- AND I. SANMARTÍN. 2011. Why are there so many plant species in the Neotropics? *Taxon* **60**: 403–414.
- BLEIWEISS, R. 1998. Tempo and mode of hummingbird evolution. *Biol. J. Linn. Soc.* **65**: 63–76.
- BORG, A. J. AND J. SCHÖNENBERGER. 2011. Comparative floral development and structure of the black mangrove genus *Avicennia* L. and related taxa in Acanthaceae. *Int. J. Pl. Sci.* **172**: 330–344.
- , L. A. McDADE, AND J. SCHÖNENBERGER. 2008. Molecular phylogenetics and morphological evolution of Thunbergioideae (Acanthaceae). *Taxon* **57**: 1–12.
- BRADSHAW, H. D. AND D. W. SCHEMSKE. 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**: 176–178.
- BURNHAM, R. J. AND A. GRAHAM. 1999. The history of Neotropical vegetation: new developments and status. *Ann. Missouri Bot. Gard.* **86**: 546–589.
- CAVAGNETTO, C. AND P. ANADÓN. 1996. Preliminary palynological data on floristic and climatic changes during the Middle Eocene-Early Oligocene of the eastern Ebro Basin, northeast Spain. *Rev. Palaeobot. Palynol.* **92**: 281–305.
- DANIEL, T. F., L. A. McDADE, M. MANKTELOW, AND C. A. KIEL. 2008. The “*Tetramerium* Lineage” (Acanthaceae: Acanthoideae: Justiceae): delimitation and intra-lineage relationships based on cp and nrITS sequence data. *Syst. Bot.* **33**: 416–436.
- DAVIS, C. C. AND W. R. ANDERSON. 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *Amer. J. Bot.* **97**: 2031–2048.
- DOBZHANSKY, T. 1950. Evolution in the tropics. *Amer. Sci.* **38**: 208–221.
- DREW, B. T. AND K. J. SYTSMAN. 2013. The South American radiation of *Lepechinia* (Lamiaceae): phylogenetics, divergence times and evolution of dioecy. *Bot. J. Linn. Soc.* **171**: 171–190.
- DRUMMOND, A. J. AND A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *B. M. C. Evol. Biol.* **7**: 214.
- , S. Y. W. HO, M. J. PHILLIPS, AND A. RAMBAUT. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* **4**: 699–710.
- EHRlich, P. R. AND P. H. RAVEN. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**: 586–608.
- FINE, P. V. A., D. C. DALY, G. VILLA MUÑOZ, G. MESONES, AND K. M. CAMERON. 2005. The contribution of edaphic heterogeneity to the evolution and diversity of Burseraceae trees in the western Amazon. *Evolution* **59**: 1464–1478.
- GENTRY, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Ann. Missouri Bot. Gard.* **69**: 557–593.
- GERNHARD, T. 2008. The conditioned reconstructed process. *J. Theor. Biol.* **253**: 769–778.
- GRAHAM, A. 1976. Studies in neotropical paleobotany. II. The Miocene communities of Veracruz, Mexico. *Ann. Missouri Bot. Gard.* **63**: 787–842.
- . 1990. A late Tertiary microfossil flora from the Republic of Haiti. *Amer. J. Bot.* **77**: 911–926.
- GRAHAM, V. A. W. 1988. Delimitation and infra-generic classification of *Justicia* (Acanthaceae). *Kew Bull.* **43**: 551–624.
- GRANT, V. 1992. Floral isolation between ornithophilous and sphingophilous species of *Ipomopsis* and *Aquilegia*. *Proc. Natl. Acad. Sci. U.S.A.* **89**: 11828–11831.
- HAFFER, J. 1969. Speciation in Amazonian forest birds. *Science* **165**: 131–137.
- HANSEN, K. A., L. E. GILBERT, B. B. SIMPSON, S. R. DOWNIE, A. C. CERVI, AND R. K. JANSEN. 2006. Phylogenetic relationships and chromosome number evolution in *Passiflora*. *Syst. Bot.* **31**: 138–150.
- HO, S. Y. W. AND M. J. PHILLIPS. 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Syst. Biol.* **58**: 367–380.
- HOORN, C., F. P. WESSELINGH, H. TER STEEGE, M. A. BERMUDEZ, A. MORA, J. SEVINK, I. SANMARTÍN, A. SANCHEZ-MESEGUER, C. L. ANDERSON, J. P. FIGUEIREDO, C. JARAMILLO, D. RIFF, F. R. NEGRI, H. HOOGHIEMSTRA, J. LUNDBERG, T. STADLER, T. SÄRKINEN, AND A. ANTONELLI. 2010. Amazonia through time Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330**: 927–931.
- HUGHES, C. E., R. T. PENNINGTON, AND A. ANTONELLI. 2013. Neotropical plant evolution: assembling the big picture. *Bot. J. Linn. Soc.* **171**: 1–18.
- INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE. WORLD METEOROLOGICAL ORGANIZATION AND THE UNITED NATIONS ENVIRONMENT PROGRAMME. 2007. Fourth Assessment Report: Climate Change (AR4): Fig. 6.1 (Geneva). <http://www.ipcc.ch/organization/organization.shtml>.
- INTERNATIONAL COMMISSION ON STRATIGRAPHY. 2013. International Chronostratigraphic Chart, vers. 2013/01. <http://www.stratigraphy.org/index.php/lcs-chart-timescale> (13 Aug 2013).
- JARAMILLO, C., M. J. RUEDA, AND G. MORA. 2006. Cenozoic plant diversity in the Neotropics. *Science* **311**: 1893–1896.
- KAY, K. M. AND D. W. SCHEMSKE. 2003. Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus* (Costaceae). *Biotropica* **35**: 198–207.
- . 2006. Reproductive isolation between two closely related hummingbird-pollinated Neotropical gingers. *Evolution* **60**: 538–552.
- KIEL, C. A., L. A. McDADE, T. F. DANIEL, AND D. CHAMPLUVIER. 2006. Phylogenetic delimitation of *Isoglossinae* (Acanthaceae: Justiceae) and relationships among constituent genera. *Taxon* **55**: 683–694.
- KOH, L. P., R. R. DUNN, N. S. SODHI, R. K. COLWELL, H. C. PROCTOR, AND V. S. SMITH. 2004. Species coextinctions and the biodiversity crisis. *Science* **305**: 1632–1634.
- KUHL, O. S., J. MULLER, AND H. T. WATERBOLK. 1955. The application of palynology to oil geology with special reference to western Venezuela. *Geologie en Mijnbouw* **17**: 49–76.

- LINDBERG, A. B. AND J. M. OLESEN. 2001. The fragility of extreme specialization: *Passiflora mixta* and its pollinating hummingbird *Ensifera ensifera*. *J. Trop. Ecol.* **17**: 323–329.
- MADDISON, D. R. AND W. P. MADDISON. 2000. MacClade 4: analysis of phylogeny and character evolution, vers. 4.0. Sinauer Associates, Sunderland, Massachusetts.
- MAYR, G. 2004. Old World fossil record of modern-type hummingbirds. *Science* **304**: 861.
- . 2007. New specimens of the early Oligocene Old World hummingbird *Eurotrochilus inexpectatus*. *Journal of Ornithology* **148**: 105–111.
- MCDADE, L. A. 1992. Pollinator relationships, biogeography, and phylogenetics. *BioScience* **42**: 21–26.
- , T. F. DANIEL, AND C. A. KIEL. 2008. Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales). *Amer. J. Bot.* **95**: 1136–1152.
- , ———, S. E. MASTA, AND K. M. RILEY. 2000a. Phylogenetic relationships within the tribe Justicieae (Acanthaceae): evidence from molecular sequences, morphology, and cytology. *Ann. Missouri Bot. Gard.* **87**: 435–458.
- , ———, C. A. KIEL, AND A. J. BORG. 2012. Phylogenetic placement, delimitation, and relationships among genera of the enigmatic Nelsonioideae (Lamiales: Acanthaceae). *Taxon* **61**: 637–651.
- , C. A. KIEL, T. F. DANIEL, AND K. VOLLESEN. 2005. Phylogenetic relationships among Acantheae (Acanthaceae): major lineages present contrasting patterns of molecular evolution and morphological differentiation. *Syst. Bot.* **30**: 834–862.
- , S. E. MASTA, M. L. MOODY, AND E. WATERS. 2000b. Phylogenetic relationships among Acanthaceae: evidence from two genomes. *Syst. Bot.* **25**: 106–121.
- MCGUIRE, J. A., C. C. WITT, D. L. ALTSHULER, AND J. V. REMSEN. 2007. Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. *Syst. Biol.* **56**: 837–856.
- MEDUS, J. 1975. Palynologie de sédiments tertiaires du Sénégal méridional. *Pollen et Spores* **17**: 545–601.
- MITTELBACH, G. G., D. W. SCHEMSKE, H. V. CORNELL, A. P. ALLEN, J. M. BROWN, M. B. BUSH, S. P. HARRISON, A. H. HURLBERT, N. KNOWLTON, H. A. LESSIOS, C. M. MCCAIN, A. R. MCCUNE, L. A. MCDADE, M. A. MCPEEK, T. J. NEAR, T. D. PRICE, R. E. RICKLEFS, K. ROY, D. F. SAX, D. SCHLUTER, J. M. SOBEL, AND M. TURELLI. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol. Letters* **10**: 315–331.
- PENNINGTON, R. T., M. LAVIN, T. SÄRKINEN, G. P. LEWIS, B. B. KLITGAARD, AND C. E. HUGHES. 2010. Contrasting plant diversification histories within the Andean biodiversity hotspot. *Proc. Natl. Acad. Sci. U.S.A.* **107**: 13783–13787.
- PRANCE, G. T. 1982. Biological diversification in the tropics. Plenum Press, New York.
- PYBUS, O. AND P. H. HARVEY. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* **267**: 2267–2272.
- RAJ, B. 1961. Pollen morphological studies in the Acanthaceae. *Grana Palynol.* **3**: 3–108.
- AND A. J. DRUMMOND. 2007. Tracer vers. 1.4. <http://beast.bio.ed.ac.uk/Tracer>.
- RAVEN, P. H. 1976. Ethics and attitudes, pp. 155–181. In J. Simmons, R. Beyer, P. Brandham, G. Lucas, and V. Parry [eds.], Conservation of threatened plants. Plenum Press, New York.
- REGALI, M. S. P., N. UESUGUI, AND A. S. SANTOS. 1974. Palinologia dos sedimentos meso-cenozoicos do Brasil (I). *Boletim Técnico da Petrobras* **17**: 177–191.
- REID, E. M. AND M. E. J. CHANDLER. 1926. Catalogue of Cainozoic plants in the Department of Geology, vol. I. The Bembridge Flora. British Museum (Natural History), London, UK. (cited in Martínez-Millán, M. 2010. Fossil record and age of the Asteridae. *Bot. Rev. (Lancaster)* **76**: 83–927.
- ROGERS, J. S. 2001. Maximum likelihood estimation of phylogenetic trees is consistent when substitution rates vary according to the invariable sites plus gamma distribution. *Syst. Biol.* **50**: 713–722.
- RULL, V. 2011. Neotropical biodiversity: timing and potential drivers. *Trends Ecol. Evol.* **26**: 508–513.
- SCHEMSKE, D. W. AND H. D. BRADSHAW, JR. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc. Natl. Acad. Sci. U.S.A.* **96**: 11910–11915.
- SCHMIDT-LEBUHN, A. N., M. KESSLER, AND I. HENSEN. 2007. Hummingbirds as drivers of plant speciation. *Trends Pl. Sci.* **12**: 329–331.
- SCHWARZBACH, A. E. AND L. A. MCDADE. 2002. Phylogenetic relationships of the mangrove family Avicenniaceae based on chloroplast and nuclear ribosomal DNA sequences. *Syst. Bot.* **27**: 84–98.
- SCOTLAND, R. W. AND K. VOLLESEN. 2000. Classification of Acanthaceae. *Kew Bull.* **55**: 513–589.
- SIMPSON, G. G. 1980. Splendid isolation: the curious history of South American mammals. Yale Univ Press, New Haven, Connecticut.
- STEBBINS, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I. Pollination mechanisms. *Ann. Rev. Ecol. Syst.* **1**: 307–326.
- . 1974. Flowering plants: evolution above the species level. Arnold Press, London, UK.
- TEMELES, E. J. AND W. J. KRESS. 2003. Adaptation in a plant-hummingbird association. *Science* **300**: 630–633.
- , Y. J. RAH, J. ANDICOECHEA, K. L. BYANOVA, G. S. J. GILLER, S. B. STOLK, AND W. J. KRESS. 2013. Pollinator-mediated selection in a specialized hummingbird-*Heliconia* system in the Eastern Caribbean. *J. Evol. Biol.* **26**: 347–356.
- TOMLINSON, P. B. 1986. The botany of mangroves. Cambridge University Press, Cambridge.
- TRIPP, E. A. 2007. Evolutionary relationships within the species-rich genus *Ruellia* (Acanthaceae). *Syst. Bot.* **32**: 628–649.
- AND S. FATIMAH. 2012. Comparative anatomy, morphology, molecular phylogenetics of the African genus *Satanocrater* (Acanthaceae). *Amer. J. Bot.* **99**: 967–982.
- AND P. S. MANOS. 2008. Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruellia* (Acanthaceae). *Evolution* **62**: 1712–1737.
- AND L. A. MCDADE. In press. Rich fossil record of Acanthaceae yields calibrated phylogeny and evidence for marked biases in timing and directionality of intercontinental disjunctions. *Syst. Biol.*
- , T. F. DANIEL, S. FATIMAH, AND L. A. MCDADE. 2013. Phylogenetic relationships within Ruellieae (Acanthaceae) and a revised classification. *Int. J. Pl. Sci.* **174**: 97–137.
- , ———, J. C. LENDEMER, AND L. A. MCDADE. 2009. New molecular and morphological insights prompt transfer of *Blechnum* to *Ruellia* (Acanthaceae). *Taxon* **58**: 893–906.
- VAN DER HAMMEN, T. 1974. A palynological systematic nomenclature. *Bol. Geol.* **4**: 63–101.
- VAN DER NIET, T. AND S. D. JOHNSON. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends Ecol. Evol.* **27**: 353–361.
- WALLACE, A. R. 1878. Tropical nature, and other essays. Macmillan & Co., London, UK.
- WILSON, E. O. 1988. Biodiversity. National Academy Press, Washington, D.C.
- YANG, Y., R. RIINA, J. J. MORAWETZ, T. HAEVERMANS, X. AUBRIOT, AND P. E. BERRY. 2012. Molecular phylogenetics and classification of *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). *Taxon* **61**: 764–789.

## APPENDIX 1

Voucher information and Genbank numbers (*trnG-trnS*, *trnG-trnR*, *psbA-trnH*, *ITS*, *Eif3E*; – = sequence not obtained) for plant accessions used in this study, arranged phylogenetically. The hummingbird dataset is unmodified from McGuire et al. (2007) and thus accession numbers can be found in Appendix 1 of that study.

**Outgroups:** *Martynia annua* L.: EU528979, KC118389, –, AF169854, –, Mexico, *Jenkins 97-149* (ARIZ); *Sesamum indicum* L.: EU528998, JQ781019, EU531713, AF169853, –, cultivated, Mexico, *Jenkins 97-141* (ARIZ). **Nelsonioideae:** *Elytraria imbricata* (Vahl) Pers.: EU528957, KC118367, –, AF169852, –, Arizona, USA, *McDade & Jenkins 1155* (ARIZ); *Nelsonia canescens* (Lam.) Spreng.: EU528985, –, JQ691822, –, Panama, *Daniel et al. 5452* (CAS). **Avicennia lineage:** *Avicennia bicolor* Standl.: EU528943, JQ780995, –, EU528877, –, Costa Rica, *Borg 10* (S); *Avicennia marina* (Forssk.) Vierh.: EU528945, KC118340, –, EU528879, –, Australia, *Schwarzbach 97-A1* (KE). **Thunbergioideae:** *Mendoncia cowanii* (S.Moore) Benoist: EU528981, –, EU528899, –, Madagascar, *Hearn Mad-3* (PH); *Thunbergia erecta* (Benth.) T.Anderson: EU529001, –, AF169851, –, cultivated (Missouri Botanical Garden), *Missouri 802421* (MO). **Acantheae:** *Acanthus sennii* Chiov.: EU528941, KC118335, –, DQ028415, –, Ethiopia, *Friis et al. 7006* (C); *Acanthopsis disperma* Nees: DQ059218, –, –, –, South Africa, *Balkwill et al. 11780* (J); *Aphelandra leonardii* McDade: KC118338, KC118338, –, AF169761, –, Panama, *McDade 310* (DUKE); *Stenandrium pilosulum* (S.F.Blake) T.F.Daniel: DQ059270, KC118447 (*Van Devender & Reina 97-454* [ARIZ]), –, AF169758, –, Mexico, *Van Devender & Reina 97-434* (ARIZ). **Barlerieae:** *Barleria repens* Nees: EU528947, –, AF169750, –, cultivated (Missouri Botanical Garden), *Missouri 97003* (MO); *Golaea migiurtina* Chiov.: EU528959, KC118370, –, –, Somalia, *Thulin et al. 10665* (UPS). **Andrographideae:** *Cystacanthus turgidus* G.Nicholson: EU528954, JQ781001, –, –, cultivated (Royal Botanic Gardens Kew), *1996-479* (K); *Phlogacanthus thyrsoiflorus* Nees: EU528993, KC118401, –, EU528907, –, India, *Lindburg 200* (DAV). **Whitfieldieae:** *Forcipella* sp. Baill.: EU528958, KC118368, –, EU528887, –, Madagascar, *Daniel et al. 10432* (CAS); *Leandriella oblonga* Benoist: EU528970, –, –, EU528893, –, Madagascar, *DuPuy et al. MB767* (P). **Neuracanthus lineage:** *Neuracanthus africanus* T.Anderson ex S.Moore: EU528986, KC118390, –, EU528900, –, South Africa, *McDade et al. 1258* (J); *Neuracanthus ovalifolius* (Fiori) Bidgood & Brummitt: EU528988, KC118391, –, EU528902, –, Somalia, *Friis et al. 5032* (K). **Justiceae:** *DiCLIPTERA extenta* S.Moore: EU528955, –, –, KC420541, –, cultivated (private garden, South Africa), *McDade 1306* (J); *Mackaya bella* Harv.: EU528979, –, AF289796, –, cultivated (Strybing Arboretum, San Francisco), *Daniel s.n.* (CAS); *Rhinacanthus gracilis* Klotzsch: EU528995, JQ781009, –, AF289766, –, cultivated (San Francisco Conservatory of Flowers), *Daniel s.n.* (CAS). **Ruellieae:** *Acanthopale confertiflora* (Lindau) C.B.Clark: JQ7801022, EF214651, JQ781035, EF214470, JQ763413, Madagascar, *Phillipson 2117* (MO); *Bravaisia integerrima* (Spreng.) Standl.: –, EF214603, –, EF214413, –, Costa Rica, cultivated (Fairchild Tropical Botanic Garden); *Brilliantaisia grotanellii* Pic.Serm.: JQ7801023, JQ780997, JQ7801036, JX443752, JQ763418, Ethiopia, *Tripp & Ensermu 924* (RSA); *Duosperma kilimandscharicum* (Lindau) Dayton: JQ7801025, EF214605, JQ7801037, EF214415, JQ763415, Tanzania, *Kindeketa et al. 1526* (MO); *Dyschoriste albiflora* Lindau: GQ995605, EF214606, GQ995666, EF214416, –, Zambia, *Luwika et al. 580* (MO); *Hygrophila costata* Nees: –, EF214419, KF945489, EF214608, JX443721, *Daniel & Pilz 9592* (MO); *Louteridium mexicanum* (Baill.) Standl.: JX444021, EF214611, GQ995626,

EF214422, –, Oaxaca, *Manriquez et al. 3758* (MO); *Mimulopsis solmsii* Schweinf.: KF945573, EF214616, –, EF214427, KF945625, Uganda, *ATBP 530* (MO); *Pararuellia alata* H.P.Tsui: –, KC118394, KC420650, JX443788, JX443726, China, *Zhiduan 960432* (MO); *Petalidium ohopohense* P.G.Mey.: JX444032, JX443954, –, JX443791, KF945626, Namibia, *Tripp and Dexter 849* (RSA); *Phaulopsis imbricata* Sweet: JX444034, EF214619, KC420651, EF214430, JX443729, Tanzania, *Bidgood et al. 4589* (MO); *Sanchezia speciosa* Leonard: EU528997, EU431005, –, AF169835, JX443735, cultivated (Duke greenhouses), *McDade 1180* (ARIZ); *Strobilanthes dyeriana* Mast.: JX444046, JX443973, JX443892, EU528908, JX443739, cultivated (DUKE greenhouses), *McDade 1328* (RSA-POM); *Trichanthera corymbosa* Leonard: –, JX443979, JX443897, JX443814, JX443743, Venezuela, *Tripp & Lujan 520* (RSA); *Ruellia abbreviata* D.N.Gibson: –, –, KF945490, –, –, Mexico, *Tripp 171* (DUKE); *Ruellia acutangula* Nees: KF945574, EF214625, KF945491, EF214436, –, Brazil, *Lombardi 2005* (US); *Ruellia adenocalyx* Lindau: EU431012, EF214627, KF945492, EF214438, KF945627, Brazil, *Aparecida et al. 5047* (US); *Ruellia affinis* Lindau: EU431013, EF214628, KF945493, EF214439, KF945628, Brazil, *Carvalho et al. 6006* (US); *Ruellia alboviolacea* Lindau: EU431014, EF214629, –, EF214440, KF945629, *Hinton 11018* (US); *Ruellia amoena* Sessé & Moc.: EU431015, EF214631, KF945494, EF214441, KF945630, Michoacán, *Koch & Fryxell 83210* (US); *Ruellia amplexicaulis* Lindau: –, EF214630, KF945495, EF214442, KF945631, Brazil, *Kral & Wanderly 75013* (US); *Ruellia anaticollis* Benoist: KF945575, EF214632, KF945496, EF214443, –, Madagascar, *Sussman 101* (MO); *Ruellia angustiflora* (Nees) Lindau ex Rambo: –, EF214633, KF945497, EF214445, –, Argentina, *Arbo et al. 5922* (US); *Ruellia ansericollis* Benoist: GQ995606, EF214635, –, EF214447, KF945632, Madagascar, *Phillipson 3069* (MO); *Ruellia antiquorum* Washh. & J.R.I.Wood: –, –, –, Bolivia, *Wood 8630B* (US); *Ruellia asperula* (Mart. & Nees) Lindau: KF945576, EF214636, KF945498, F214448, –, Brazil, *Agra et al. 4777* (MO); *Ruellia beyrichiana* Lindau: EU431016, EF214637, KF945499, EF214449, KF945633, Brazil, *Gottsberger 30983* (US); *Ruellia bignoniiflora* S.Moore: EU431052, EF214696, KF945500, EF214539, KF945634, Kenya, Napper s.n. 3 Jan 1969; *Ruellia biolleyi* Lindau: –, –, –, EF214451, KF945635, Costa Rica, *Tripp 134* (DUKE); *Ruellia blechioides* Sw.: –, –, KF945501, EU812548, –, Jamaica, *Proctor 37835* (MO); *Ruellia blechum* L.: JX444039, EF214601, JX443882, EF214412, –, Vera Cruz, *Sianca-Colin 1914* (MO); *Ruellia boranica* Ensermu: –, –, –, KF945455, –, Ethiopia, *Tripp & Ensermu 903* (RSA); *Ruellia bourgaei* Hemsl.: KF945577, KF945552, GQ995637, EF214453, –, Jalisco, *Iltis et al. 28799* (US); *Ruellia brandbergensis* Kers: –, –, KF945502, KF945456, –, Namibia, *Norderostam 2562* (S); *Ruellia brandegei* Leonard, *ined.*: –, –, –, KF945457, –, Sinaloa, *H.S. Gentry 5122* (US); *Ruellia breedlovei* T.F.Daniel: EU431018, –, KF945503, EF214454, –, Chiapas, *Neill 5560* (MO); *Ruellia brevicaulis* Baker: –, –, –, KF945504, –, –, Brazil, *Poliquesi et al. 514* (US); *Ruellia brevifolia* (Pohl) C.Ezcurra: –, EF214641, KF945505, EF214456, –, cultivated (Duke greenhouses); *Ruellia bulbifera* Lindau: EU431020, EF214644, KF945506, EF214459, –, Argentina, *Wasshausen et al. 1994* (US); *Ruellia capitata* Buch.-Ham. ex D.Don: –, –, KF945507, –, –, Brazil, *Fonseca et al. 431* (US); *Ruellia carolinensis* (J.F.Gmel.) Steud.: –, EF214645, –, EF214460, –, *Wilbur 66082* (DUKE); *Ruellia cearensis* Lindau: –, KF945553, –, KF945458, –, Brazil, *Silva et al. 64* (US); *Ruellia cedilloi* Ramamoorthy: –, –, GQ995642, GQ005589, –, Mexico, *Cedillo & Torres 1425* (CAS); *Ruellia cernua* Roxb.: KF945578, KF945554, –, KF945459, –, Nepal, collector unknown, s.n.; *Ruellia chariessa* Leonard: –, –, KF945508, –, –, Panama, *Nevers et al. 8255* (US); *Ruellia chartacea* (T.Anderson) Wassh.: EU431021, EF214646, KF945509, EF214461, –, Ecuador, cultivated

- (Duke greenhouses); *Ruellia chrysantha* Mildbr.: -, KF945555, -, KF945460, -, Venezuela, *Pittier 11774* (US); *Ruellia ciliatiflora* Hook.: EU431022, EF214648, GQ995627, EF214463, -, Bolivia, *Wood 10383* (US); *Ruellia ciliosa* Pursh: -, -, KF945510, -, -, North Carolina, *Wilbur 44059* (DUKE); *Ruellia cortantii* Standl.: EU431023, -, GQ995644, EF214467, -, Oaxaca, *Martinez 1770* (US); *Ruellia cordata* Thunb.: -, KF945556, -, EF214468, -, South Africa, *McDade 1236* (PH); *Ruellia costaricensis* (Oerst.) E.A.Tripp & McDade: KF945579, -, JX443883, EU812551, -, Costa Rica, *Daniel 6342 et al.* (DUKE); *Ruellia costata* Lindau: -, KF945557, KF945511, EF214469, KF945636, Brazil, *Silva 363* (US); *Ruellia curviflora* Nees & Mart.: -, -, KF945512, -, -, Brazil, *Hatschbach 51380 & Cervi* (MO); *Ruellia davisorum* Tharp & F.A.Barkley: -, -, -, KF945461, -, Texas, *Tharp 47456* (DUKE); *Ruellia densa* Hieron.: -, KF945558, -, EF214471, KF945637, Brazil, *Tameirao & Franca 81* (US); *Ruellia detonsa* Benoist: GQ995607, GQ995615, -, EF214472, -, Madagascar, *Miller & Randrianasolo 6159* (MO); *Ruellia devosiana* Makoy ex E.Murr.: KF945580, EF214653, KF945513, EF214474, -, Brazil, cultivated (Duke greenhouses); *Ruellia discifolia* Oliv.: -, -, -, EF214475, -, Ethiopia, *Boulos 10439* (MO); *Ruellia donnell-smithii* Leonard: EU431025, KF945559, -, KF945462, -, Ventura 925 (DUKE); *Ruellia drummondiana* (Nees) A.Gray: -, -, -, EF214479, KF945638, Texas, *York 46274* (DUKE); *Ruellia edwardsae* Tharp & F.A.Barkley: -, EF214656, KF945514, EF214481, -, Guanajuato, *Genelle & Fleming 843* (MO); *Ruellia elegans* Poir.: -, EF214658, -, EF214483, KF945639, Brazil, cultivated (Duke greenhouses); *Ruellia eriocalyx* Glaz.: EU431027, EF214660, KF945515, EF214485, KF945640, Brazil, *Oliveira & Alvarenga 130* (US); *Ruellia erythropus* (Nees) Lindau: -, EF214661, -, EF214486, -, Argentina, *Cristobal et al. 2282* (MO); *Ruellia eumorphantha* Lindau: EU431028, EF214662, GQ995629, EF214487, -, Oaxaca, *Acosta 1172* (MO); *Ruellia eurycodon* Lindau: EU431029, EF214664, -, EF214489, KF945641, Brazil, *Costa 38.768* (US); *Ruellia exilis* McDade & E.A.Tripp: -, -, -, KF945463, -, Costa Rica, *Quesada 287*; *Ruellia exserta* Wassh. & J.R.I.Wood: -, EF214665, KF945516, EF214490, -, Brazil, *Nave et al. 1581* (US); *Ruellia filicalyx* Lindau: -, KF945560, KF945517, KF945464, -, Bolivia, *Israel G. Vargas & E. Tapia 1042* (US); *Ruellia floribunda* Hook.: EU431030, EF214667, GQ995630, EF214492, -, Ecuador, *Bonitaz & Cornejo 3612* (US); *Ruellia foetida* Willd.: EU431031, EF214668, GQ995631, EF214493, -, Jalisco, *Daniel 2081* (DUKE); *Ruellia fruticosa* Sessé & Moc.: -, -, -, GQ995591, -, Guerrero, *Rzedowski 30507* (MICH); *Ruellia fulgens* (Bremek.) E.A. Tripp: -, JX443961, JX443884, EF214432, JX443732, French Guiana, *Prance et al. 30665* (US); *Ruellia fulgida* Andrews: EU431032, EF214671, KF945518, EU431001, -, Venezuela, *Machado 9* (DUKE); *Ruellia galeottii* Leonard: -, EF214673, -, EF214497, -, Oaxaca, *Tripp & Dexter 159* (DUKE); *Ruellia geminiflora* Kunth: -, EF214674, -, EF214498, KF945642, Chiapas, *Tripp & Dexter 160* (DUKE); *Ruellia glischrocalyx* Lindau: -, KF945465, -, -, -, Peru, *Salick et al. 7608*; *Ruellia gracilis* Rusby: EU431033, -, KF945519, EF214499, -, Bolivia, *Daly et al. 6633* (US); *Ruellia grantii* Leonard: EU431034, EU431006, KF945520, EF214500, -, Colombia, *Wood 5221* (US); *Ruellia guerrerensis* T.F.Daniel: -, KF959827, GQ995651, GQ995592, -, Mexico, *Hinton et al. 11296* (US); *Ruellia haenkeana* (Nees) Wassh.: EU431035, KF945561, KF945521, EF214501, -, Bolivia, *Smith & Smith 13087* (MO); *Ruellia hapalotricha* Lindau: -, -, -, EF214503, -, Brazil, *Macedo 5859* (US); *Ruellia harveyana* Stapf: -, KF945562, -, KF945466, -, Vera Cruz, *Vasquez 434* (MO); *Ruellia hirsutoglandulosa* (Oerst.) Hemsl.: -, -, -, EF214505, KF945643, Puebla, *Chiang et al. 2023* (MO); *Ruellia hookeriana* (Nees) Hemsl.: EU431037, KF945563, -, KF945467, -, Chiapas, *Breedlove & Daniel 71041* (US); *Ruellia humboldtiana* (Nees) Lindau: -, -, -, EF214507, -, Venezuela, *McDade & Lundberg 914* (DUKE); *Ruellia humilis* Nutt.: EU431038, EF214678, GQ995632, EF214508, -, Pennsylvania, *Tripp 14* (PH); *Ruellia hygrophila* Mart.: EU431039, EF214679, -, EF214509, -, Misiones, cultivated (Duke greenhouses); *Ruellia incomta* Lindau: EU431040, -, -, EF214510, -, Brazil, *Mori et al. 16639* (MO); *Ruellia inflata* Rich.: -, -, -, EF214512, -, Bolivia, *Gentry & Perry 77992* (MO); *Ruellia insignis* Balf.f.: EU431041, EF214680, JX443885, EF2145113, -, *Smith 566* (K); *Ruellia intermedia* Leonard: -, -, -, KF945468, -, Tamaulipas, *Banuet & Viveros 217* (US); *Ruellia inundata* Kunth: -, -, KF945522, EF214515, -, Honduras, *Daniel 9465* (US); *Ruellia jaliscana* Standl.: GQ995608, GQ995616, GQ995653, GQ995593, -, Jalisco, *Tripp & Tripp 178* (DUKE); *Ruellia japurensis* Mart.: -, -, -, KF945469, -, Brazil, *Berg & Henderson 681* (NY); *Ruellia jimulensis* Villarreal: EU431043, EF214681, KF945523, EF214518, -, Durango, *Henrickson & Bekey 18504* (US); *Ruellia jussieuoides* Schtdl. & Cham.: -, JQ043327, -, JQ043326, -, Costa Rica, *Valerio 83* (DUKE); *Ruellia kerrii* Craib: -, -, KF945524, KF945470, -, Thailand, *Beusekom et al. 3915* (L); *Ruellia lactea* Cav.: -, EU431007, -, EF214520, -, *Correll & Johnston 20148* (US); *Ruellia laslobasensis* E.A.Tripp: -, GQ995620, GQ995667, GQ995598, -, Tripp et al. s.n.; *Ruellia latibracteata* D.N.Gibson: -, -, -, EU812553, -, Nicaragua, *Moreno 19977* (MO); *Ruellia latisepala* Benoist: -, -, -, EF214521, -, Madagascar, *Randrianaivo et al. 324* (MO); *Ruellia leucantha* Nees: EU431044, EF214684, KF945525, EF214522, KF945644, Baja California Sur (Mexico), *Carter & Heckard et al. 5896* (US); *Ruellia linearibracteolata* Lindau: -, EF214685, KF945526, EF214523, KF945645, Ethiopia, *Gilbert 7544* (MO); *Ruellia longepetiolata* (Oerst.) Hemsl.: -, -, -, EF214524, KF945646, Mexico, cultivated (Duke greenhouses); *Ruellia longifilamentosa* Lindau: -, -, -, EF214525, -, Colombia, *Gamboja 307* (US); *Ruellia longipedunculata* Lindau: EU431045, EF214686, GQ995633, EF214526, JQ763412, Bolivia, *Wood 13750* (US); *Ruellia macrophylla* var. *lutea* Leonard: EU431046, EF214689, KF945527, EF214528, KF945647, Venezuela, *Simmons 243* (MO); *Ruellia macrosolen* Lillo: -, -, -, EF214529, -, Argentina, *Krapovickas & Cristobal 46267* (US); *Ruellia magniflora* C.Ezcurra: EU431047, EF214687, KF945528, EF214530, -, Argentina, *Wasshausen et al. 2000* (US); *Ruellia malaca* Leonard: EU431048, -, -, EF214531, -, Venezuela, *Stergios & Delgado 13487* (MO); *Ruellia malacophylla* C.B.Clark: -, -, -, EF214532, KF945648, S. Africa, *Balkwill & Cadman 3384* (MO); *Ruellia marlothii* Engl.: -, -, -, KF959828, -, Namibia, *Tripp & Dexter 881* (RSA); *Ruellia matagalpe* Lindau: EU431049, EF214690, -, EF214533, -, Belize, *Rees et al. 182* (MO); *Ruellia matudae* Leonard: KF945581, EF214693, KF945529, KF945471, KF945649, Chiapas, *Breedlove & Bourell 67437* (US); *Ruellia maya* T.F.Daniel: EU431051, EF214693, -, EF214536, KF945650, Chiapas, *Tripp & Dexter 157* (DUKE); *Ruellia maya-2* T.F.Daniel: -, KF945564, -, -, -, Guatemala, *Steyermark 44688* (US); *Ruellia mcvaughii* T.F.Daniel: -, EF214694, KF945530, EF214537, -, Jalisco, *Panero et al. 5601* (MO); *Ruellia menthoides* (Nees) Hiern: EU431053, EF214698, -, EF214541, -, Bolivia, *Foster et al. 110* (MO); *Ruellia metallica* Leonard: EU431054, -, KF945531, EU431003, -, Costa Rica, *Tripp & Salazar-Amoretti 148* (DUKE); *Ruellia metzcae* Tharp: -, EF214699, -, EF214542, -, Texas, *Tharp 46054* (DUKE); *Ruellia morongii* Britton: -, EF214700, -, EF214543, -, Paraguay, *Zardini & Velazquez 24875* (MO); *Ruellia multifolia* (Nees) Lindau: -, EF214701, -, EF214544, -, Paraguay, *Zardini 8699* (MO); *Ruellia nitida* (Nees) Wassh. & J.R.I.Wood: EU431055, EF214702, -, EF214545, -, Bolivia, *Wood 16518* (US); *Ruellia nobilis* (S.Moore) Lindau: -, -, -, EF214546, -, Bolivia, *Vargas et al. 3343* (US); *Ruellia noctiflora* (Nees) A.Gray: -, KF945565, -, KF945472, -, Florida, *Tripp & Deregibus 257*; *Ruellia nocturna* Hedrén: -, -, -,

- KF945473, -, Somalia, *Thulin & Dahir 6545* (UPS); *Ruellia novogaliciana* T.F.Daniel: EU431056, EF214703, -, EF214547, -, Michoacán, *Hinton et al. 12954* (US); *Ruellia nudiflora* (Engelm. & A.Gray) Urb.: EU431057, -, -, EF214548, -, Texas, *Whitson & Whitson 814* (DUKE); *Ruellia nummularia* Benoist: -, -, KF945532, -, -, *Rakotomalaza 574* (BR); *Ruellia oaxacana* Leonard: -, EF214705, KF945533, EF214551, -, Michoacán, *Hinton 15831* (US); *Ruellia occidentalis* (A.Gray) Tharp & F.A.Barkley: -, -, -, KF945474, -, Texas, *Tharp & Harvard 49334* (DUKE); *Ruellia ovalifolia* (Oerst.) Hemsl.: -, -, -, KF945475, -, collector unknown, s.n. (US); *Ruellia panamensis* (Lindau) E.A.Tripp: -, -, -, KF945476, -, Panama, *Churchill & de Nevers 4458* (DUKE); *Ruellia paniculata* L.: GQ995609, -, GQ995634, EF214552, KF945651, Costa Rica, *Tripp & Deinert 122* (DUKE); *Ruellia parryi* A.Gray: -, -, -, KF945477, -, Chihuahua, *Chiang et al. 9745* (MO); *Ruellia patula* Jacq.: KF945582, EF214706, -, EF214555, KF945652, Tanzania, *Sallu 140* (MO); *Ruellia pearcei* Rusby: EU431059, EF214708, -, EF214557, KF945653, Bolivia, *Wasshausen & Wood 2139* (US); *Ruellia pedunculosa* (Nees) B.D.Jacks. & Hook.f.: EU431060, EF214712, KF945534, EF214561, KF945654, Peru, *Sanchez & Dillon 10194* (US); *Ruellia pereducta* Standl. ex Lundell: -, -, KF945535, -, -, Guatemala, *Daniel & Veliz 11354* (CAS); *Ruellia perrieri* Benoist: JX444037, JX443957, JX443879, JX443796, -, Madagascar, *Phillipson et al. 3432* (K); *Ruellia petiolaris* (Nees) T.F.Daniel: EU431062, EF214711, GQ995635, EF214560, -, Guerrero, *Daniel & Bartholomew 4930* (US); *Ruellia primulacea* F.Muell. ex Benth.: -, JX443962, JX443886, JX443800, -, Australia, *Scarth-Johnson 85* (K); *Ruellia pringlei* Fernald: KF945583, GQ995622, GQ995658, GQ995600, -, Oaxaca, *Tripp 186* (DUKE); *Ruellia prostrata* Poir.: -, -, -, KF945478, -, Ethiopia, *Tripp & Ensermu 894* (RSA); *Ruellia proxima* Lindau: -, -, -, KF945479, -, Peru, *Michelangeli 471* (US); *Ruellia puri* Mart. ex Nees: -, -, -, JQ043329, -, Peru, *A. Gentry et al. 23667* (MO); *Ruellia purshiana* Fernald: EU431064, EF214717, -, EF214566, -, Georgia, *Eyles 695* (DUKE); *Ruellia pygmaea* Donn.Sm.: -, -, -, EF214567, -, Guatemala, *Contreras 11429* (US); *Ruellia rubra* Aubl.: EU431065, EF214718, KF945538, EF214569, -, French Guiana, *Feuillet et al. 10300* (US); *Ruellia ruiziana* (Nees) Lindau: EU431066, -, KF945539, EF214570, KF945655, Peru, *Foster 8502* (MO); *Ruellia runyoni* Tharp & F.A.Barkley: EU431067, EF214719, -, EF214571, -, Texas, *Tharp & Brown 3358* (DUKE); *Ruellia rusbyi* Leonard: EU431042, -, KF945540, EF214516, KF945656, *Wood 4757* (US); *Ruellia saccata* Schmidt-Leb. & E.A.Tripp: -, JQ043334, KF945541, JQ043333, -, Bolivia, *Schmidt-Lebuhn 60* (GOET); *Ruellia salviifolius* (Nees) Profice: EU431068, EF214720, -, EF214572, -, *Hatschbach et al. 70655* (US); *Ruellia sanguinea* Griseb.: -, EF214721, -, EF214574, -, Bolivia, *Wood 1241* (US); *Ruellia sarukhaniana* Ramamoorthy: KF945584, KF945566, KF945542, JX443801, JX443733, Mexico, *Tripp 1230* (RSA); *Ruellia saulensis* Wassh.: -, EF214722, -, EF214575, -, French Guiana, *Granville et al. 14887* (US); *Ruellia simplex* C.Wright: EU431019, EF214643, KF945543, EF214458, -, Central America, cultivated (Duke greenhouses); *Ruellia sirraensis* Wassh.: -, KF945567, KF945544, KF945480, -, Peru, *Graham 2223* (US); *Ruellia sororia* Standl.: KF945585, KF945568, KF945545, KF945481, KF945657, Mexico, *Tripp 1206* (RSA); *Ruellia speciosa* (Nees ex A.DC.) Lindau: EU431069, EF214723, GQ995663, EF214576, -, D.F., *Lyonnet 747* (US); *Ruellia spissa* Leonard: -, EF214724, -, EF214577, KF945659, D.F., *Hinton 1068* (US); *Ruellia standleyi* Leonard: -, KF945571, KF945550, EF214580, KF945660, Costa Rica, *Tripp 147 & Salazar-Amoretti* (DUKE); *Ruellia stemonacanthoides* (Oerst.) Hemsl.: EU431070, EF214727, -, EF214583, -, Costa Rica, *Tripp 151 & Salazar-Amoretti* (DUKE); *Ruellia steyermarkii* Wassh.: -, EF214726, -, EF214582, -, Venezuela, *Steyermark 89113* (US); *Ruellia strepens* L.: EU431071, EF214728, -, EF214585, -, Pennsylvania, *Tripp 25* (PH); *Ruellia subsessilis* Lindau: EU431072, EF214729, -, EF214586, -, Brazil, *Vasconcelos s.n.* (US); *Ruellia tachiadena* (Heine & A.Raynal) E.A.Tripp: JX443989, -, KF945551, JX443750, JX443715, *Daniel 11024*; *Ruellia tarapotana* Lindau: KF945589, KF945572, -, KF945486, -, Peru, *Nunez et al. 9311* (MO); *Ruellia terminale* (Nees) Wassh.: EU431074, EF214730, -, EF214588, -, Ecuador, *Clark 3034* (US); *Ruellia tetragona* Link: -, -, -, KF945487, -, Brazil, *Smith et al. 6657* (US); *Ruellia thyrsoctachya* Lindau: -, -, -, SJQ043341, -, Bolivia, *Killen et al. 2906* (MO); *Ruellia togoensis* (Lindau) Heine: -, -, -, KF945488, -, *ST Manktelow et al. 111* (UPS); *Ruellia tomentosa* (Nees) Lindau: EU431075, -, -, EF214589, -, Brazil, *Aparecida et al. 3821* (US); *Ruellia tuberosa* L.: EU431076, EF214732, -, EF214592, -, Guyana, *Jansen-Jacobs et al. 3869* (US); *Ruellia tubiflora* Kunth: -, EF214731, -, EF214591, -, Costa Rica, *Daniel et al. 6343* (US); *Ruellia tuxtensis* Ramamoorthy & Hornelas: U431077, -, -, EF214596, -, Vera Cruz, *Manriquez et al. 2366* (US); *Ruellia verbasciformis* (Nees) C.Ezcurra & Zappi: EU431078, -, -, EF214597, KF945661, Brazil, *Mendonca 3519 et al.* (US); *Ruellia villosa* Lindau: -, -, -, EF214599, KF945662, Brazil, *Lombardi 831* (US); *Ruellia yurimaguensis* Lindau: KF945590, -, -, EF214600, KF945663, Bolivia, *Wood 15005* (US); *Ruellia* sp17: -, KF945569, KF945546, KF945482, KF945658, Mexico, collector unknown; *Ruellia* sp21: KF945586, -, KF945547, KF945483, -, Madagascar, *Daniel 11053*; *Ruellia* sp22: KF945587, KF945570, KF945548, KF945484, -, Madagascar, *Daniel 10632*; *Ruellia* sp24: KF945588, -, KF945549, KF945485, -, Madagascar, *Daniel 10625*.