

Artificial Hybridization between U.S. Native *Ruellia caroliniensis* and Invasive *Ruellia simplex*: Crossability, Morphological Diagnosis, and Molecular Characterization

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Abstract. The potential for natural hybridization to occur between non-native, invasive species and closely related native species is of interest to biologists, conservationists, and land managers, particularly in regions such as the southeastern United States where numerous non-native species have become serious environmental pests. To explore this potential between the invasive plant species *Ruellia simplex* and the closely related, sympatric *Ruellia caroliniensis*, we conducted a study of reproductive crossability and hybrid viability. Results indicate that the production of interspecific hybrids is possible, but only in one direction (i.e., with *R. caroliniensis* as the maternal parent). Artificial hybrids were weak, slow-growing, and sterile. These data suggest that it is unlikely that *R. caroliniensis* × *R. simplex* hybrids could invade the gene pool of native *R. caroliniensis*. We also characterized hybrids at the molecular level by sequencing parents plus F₁ progeny for the nuclear ribosomal internal transcribed spacer (ITS) + 5.8S region. All hybrid genotypes formed a strongly supported clade with the maternal parent, *Ruellia caroliniensis*. Within this clade, hybrid individuals were not differentiable from maternal genotypes. We then examined general plant morphology of hybrid individuals and the two parents. Unlike results from the molecular characterization, there was a strong signal of hybrid intermediacy from this morphological work. We conclude that morphology but not molecular sequence data (from nrITS) can be used to distinguish the two parents and their F₁ hybrids.

There are ≈350 species of *Ruellia* (Acanthaceae) that are perennial herbs, subshrubs, or shrubs with mostly tropical and subtropical distributions (Tripp and McDade, 2014). A chromosome number of $2n = 34$ appears to be widespread in this large and variable genus (Daniel et al., 1984, 1990; Daniel and Chuang, 1993). Twenty-four species of *Ruellia* have been described as found in the continental United States, Hawaii, Puerto Rico, and the Virgin Islands, five of which are native to Florida: *R. caroliniensis*, *R. ciliosa*, *R. noctiflora*, *R. pedunculata* subsp.

pinetorum, and *R. succulenta*. Additionally, three non-native species are naturalized in the state: *R. blechum*, *R. ciliatiflora*, and *R. simplex* (Wunderlin and Hansen, 2014). Two of these—one native (*R. caroliniensis*; $2n = 34$, Long, 1976) and one non-native (*R. simplex*; $2n = 34$, Piovano and Bernadello, 1991)—are the focus of the present study.

Ruellia caroliniensis (J.F. Gmel.) Steud., also known as “Carolina Wild Petunia,” is native to 18 U.S. states, from North Carolina to Texas, reaching as far north as Illinois and Indiana. It is now considered rare in Ohio (Biota of North America Program, 2010) and extirpated in Pennsylvania (Tripp, 2004). In Florida, it occurs primarily in native woodlands, and plants are known as strong growers under adverse conditions (Gilman and Landrum, 1999). In the 1970s, Robert Long conducted detailed studies on floral polymorphisms, breeding systems (Long,

1971), and variation in natural populations (Long, 1974) of *R. caroliniensis* as well as artificial hybridization between this taxon and *R. geminiflora* (Long, 1976).

There has been no shortage of names that have been used to refer to a widespread and morphologically highly variable taxon here recognized as *Ruellia simplex* Wright (“Britton’s Petunia,” “Mexican Petunia,” or “Mexican Bluebell”). Scientific names for this plant that have been used throughout the botanical and horticultural literature include *Ruellia brittoniana* Leonard, *R. coerulea* Morong, *R. malacosperma* Greenm., and *R. tweediana* Griseb. The extremely complex taxonomic and nomenclatural history of these names has been discussed by several authors, most recently by Ezcurra and Daniel (2007) who reduced the aforementioned names to synonyms of the oldest name and thus that with priority, *Ruellia simplex*.

Ruellia simplex is found in sunny areas on periodically inundated soils in Mexico, the Antilles, and central–western South America (Ezcurra and Daniel, 2007). This species was introduced to Florida sometime before 1940 (Hupp et al., 2009) and is now a very popular landscape plant in the southeastern United States as a result of its copious flowering and low maintenance requirements (Gilman, 1999). Since its introduction to United States, it has naturalized in disturbed uplands and wetlands of seven continental U.S. states (from North Carolina west to Texas) in addition to the Virgin Islands, Puerto Rico, and Hawaii (U.S. Department of Agriculture, NRCS, 2014). In Florida, *R. simplex* has formed naturalized populations in 29 counties throughout the state (Wunderlin and Hansen, 2014). Of particular concern is that the species has been recorded in 21 designated conservation areas in south Florida (Institute for Regional Conservation, 2014). Since 2001, the Florida Exotic Pest Plant Council has considered Mexican petunia as a Category I invasive plant, which describes “plants that are altering native plant communities by displacing native species, changing community structures or ecological functions, or hybridizing with natives” (Florida Exotic Pest Plant Council, 2013).

Ruellia caroliniensis and *R. simplex* have sympatric distributions in numerous areas of the southeastern United States, and both have broad habitat affinities and are equally likely to occur in wetlands or non-wetlands. However, to date, there are no reports of populations of hybrid origin. Extensive artificial hybridizations among 25 different species of *Ruellia* were conducted at the University of South Florida by Long (1975). Among these, *R. simplex* (as *R. brittoniana*) and three varieties of *R. caroliniensis* were included in crossing studies. Only a cross between *R. simplex* and *R. caroliniensis* var. *succulenta* (direction unknown) was attempted; this cross produced viable seeds; however, no details on the F₁ hybrids obtained were provided in this article.

The purpose of the present study was to conduct artificial crosses between *Ruellia*

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Table 1. Hybridizations between *Ruellia caroliniensis* × *R. simplex*

Female parent	Male parent	No. crosses	Fruits	Normal (abnormal) seeds	Seeds germinated
car1	sim1	20	12	40(5)	17
car1	sim2	20	11	33(11)	8
car2	sim1	20	7	15(7)	8
car2	sim2	20	5	18(1)	5
car3	sim1	0	—	—	—
car3	sim2	20	5	22(2)	7
Total		100	40	128(26)	45



Fig. 1. *Ruellia caroliniensis* × *R. simplex* hybrid plant in a 15-cm pot in a research greenhouse in Gainesville, FL. See Table 2 and Appendix 1 for morphological details.



Fig. 2. Flower, leaf, and immature fruit of *Ruellia caroliniensis* (top left), *R. simplex* (top right), and *R. caroliniensis* × *R. simplex* hybrid (bottom; lacking fruit because all hybrids were sterile).

simplex and *R. caroliniensis* to test whether the two taxa could be artificially hybridized. Hybrids obtained were analyzed both morphologically and molecularly. Results from this investigation should be of interest to conservation biologists and botanists of the southeastern United States because they contribute new knowledge regarding potential natural hybridization between native and invasive species of *Ruellia*.

Materials and Methods

Plant material. One accessions and one cultivar of *Ruellia simplex* (as described in Wilson and Mecca, 2003) were included in this study: wild-type purple-flowered *R. simplex* (sim1, wild collected in Tallahassee, FL) and pink-flowered *R. simplex* ‘Chi Chi’ (sim2, cultivar from Boynton Botanicals, Palm Beach, FL). Additionally,

three different accessions of *R. caroliniensis* were included: car1 (wild collected in Fort Pierce, FL), car2 (wild collected in Alachua, FL), and car3 (from Superior Trees Inc., Lee, FL).

Hybridizations. All plants were propagated by cuttings from stock plants and were cared for in greenhouses at the University of Florida, Gainesville. Cuttings were grown in 128-cell cutting trays with Fafard 2P mix (60% Canadian peatmoss, 40% perlite; Concord Fafard Inc., Agawam, MA) and placed under mist in a research greenhouse for 2 weeks. After 5 to 6 weeks, rooted cuttings were transplanted individually into 15-cm Kord Traditional Standard pots with Fafard 2P mix and placed on raised benches in a polyethylene greenhouse. Plants were watered as needed with 150 ppm nitrogen using Peters liquid fertilizer (20N–10P₂O₅–20K₂O; Everris™, Charleston, SC). Hybridizations were conducted between March and May 2008 in greenhouses isolated from potential pollinators. Fully expanded flower buds (where the anthers had not dehisced pollen) on the maternal parent plants were emasculated by removing the corolla and attached anthers. Immediately afterward, the stigmas of a maternal plant were hand-pollinated using pollen from anthers of the paternal plant. The pollinated flower was then tagged with a colored plastic string. When the fruit developed, it was enclosed with an empty tea bag secured with a paper clip to prevent loss of seeds during fruit dehiscence (fruits of Acanthaceae have explosive dehiscence).

Progeny analyses. For each cross in which a fruit developed, the total number of seeds per capsule was counted. Immature or damaged seeds were separated from mature, apparently viable seeds. In Sept. 2008, normal seeds were sown ≈1 to 2 cm deep in 20-row seeder trays (Landmark Plastics, Akron, OH) using pre-wetted Fafard 2P mix. Seed trays were placed in a polycarbonate mist house (with 30% light irradiance) and received misting from 0800 HR to 1800 HR (5 s/30 min). Temperature was maintained between 18 and 24 °C. After 10 and 15 d, seedlings were transplanted into 15-cm pots and maintained in a greenhouse. Plants were grown to reproductive maturity (i.e., flowering, from which herbarium vouchers were taken for subsequent morphological and molecular study).

Morphological diagnosis. Plants of artificial hybrid origin were studied using light

microscopy, and standard taxonomic descriptions were prepared. Plants of car1, car2, car3, sim1, sim2, and wild-collected *Ruellia caroliniensis* and *R. simplex* of miscellaneous geographic origin were studied and described taxonomically for comparison with hybrids (Appendix 1).

Analysis of the nuclear ribosomal ITS region. To determine whether *Ruellia caroliniensis*, *R. simplex*, and experimental hybrids could be differentiated genetically, data from the nrITS region were generated and analyzed. The ITS region is among the most used nuclear markers in plant molecular systematics and can be especially useful for species level analyses (Feliner and Roselló, 2007, and references within). The locus ITS is biparentally inherited and its data have been used successfully for resolving species relationships within *Ruellia* (Tripp, 2007; Tripp et al., 2009; Tripp and Manos, 2008).

Taxon sampling. Sequences from a total of 57 individuals (listed in Appendix 2) were included in phylogenetic analyses. These include: four *R. caroliniensis*; four *R. simplex*; 29 *Ruellia caroliniensis* (maternal) × *R. simplex* (paternal) hybrids [five samples did not amplify during polymerase chain reaction (PCR)]; and 20 other closely related species in the genus, based on Tripp (2007). Of the four *R. caroliniensis* and four *R. simplex* accessions analyzed, three of *R. caroliniensis* (car1, car2, car3) and two of the *R. simplex* (sim1, sim2) were derived from the same plants used in artificial hybridization (see previously). Samples that we suspected may have represented apomictic events within *R. caroliniensis* (n = 11) were excluded from this data set. Crosses in the reverse direction, i.e., *R. simplex* (maternal) × *R. caroliniensis* (paternal), were unsuccessful. Of these 57 sequences, 35 were newly generated for this study (all four of the *R. caroliniensis*, all 29 *R. caroliniensis* maternal hybrids, and two of the four *R. simplex*). The Old World species *Ruellia insignis* was used as the outgroup based on prior phylogenetic work (Tripp, 2007).

DNA extraction and amplification. Total genomic DNA was extracted from silica gel-dried leaf material using a modification of the CTAB method (Doyle and Doyle, 1987). The nrITS + 5.8S region was amplified using primers ITS4 and ITS9 and reaction conditions as described in Tripp (2007). PCR products were cleaned using Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, MA). All 34 newly contributed sequences were bidirectionally sequenced on an Applied Biosystems 3130x Automated Genetic Analyzer at Rancho Santa Ana Botanic Garden in Claremont, CA. All sequence contigs were assembled and proofread using Sequencher Version 5.8 (Gene Codes Corp., Ann Arbor, MI).

During the sequence assembly and proofing process in Sequencher, we were especially attentive to possible effects of the artificial hybridizations on nucleic acid composition

genotype for the nrITS region (unlike that documented in other studies on hybrid nrITS types, e.g., see Koch et al., 2003). It is possible that rapid homogenization to one of two types of ITS present in hybrid offspring contributed to our finding of only one copy (Buckler et al., 1997; Koch and Al-Shehbaz, 2000). Cumulatively, morphological and molecular data presented in this study suggest that putative natural hybrids recovered in the field will not be distinguishable molecularly using the nrITS region but can likely be distinguished morphologically (Table 2; Appendix 1).

Ecological implications. A previous study comparing growth and development of *R. caroliniensis* and *R. simplex* established that under wet conditions in laboratory experiments, *R. simplex* exhibited several traits that favor efficient use of resources and high growth rates. Thus, under typical wetland conditions in parts of southern Florida, *R. simplex* might be expected to outgrow and outcompete native *R. caroliniensis*, especially if the supply of nutrients is limited (Wilson et al., 2004).

Detailed studies performed in the 1970s in south Florida indicated that *R. caroliniensis* produces six different floral morphs, which give the plant a nearly balanced breeding system of allogamous and autogamous reproduction. Essentially, it is seasonally cleistogamous in that open, chasmogamic flowers, chiefly cross-pollinated and incompletely dichogamous, are generally produced early in the growing season from May to July, and cleistogamic flowers, closed and self-pollinated, are typically formed in the late summer and fall (Long, 1971). We conclude that in regions where both *R. caroliniensis* and *R. simplex* occur sympatrically, interspecific hybridization may occur but likely would take place only early in the growing season.

Our study indicates that production of interspecific hybrids is possible, but only in the *R. caroliniensis* (maternal) × *R. simplex* (paternal) direction. These hybrids were very weak and slow-growing and, if formed naturally, may possibly be outcompeted by other species. Moreover, all F₁ hybrids were sterile and thus incapable of selfing or backcrossing to either parental species. In sum, these data suggest very low likelihood that interspecific *R. caroliniensis* × *R. simplex* hybrids could invade the gene pool of native *R. caroliniensis*.

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Appendix 1

Ruellia caroliniensis (J.F. Gmelin) Steudel

Native to the United States from New Jersey to Ohio, Indiana, south to Florida, and west to Texas; common in dry to moist forests, woodlands, and woodland borders; n.v. Carolina Petunia, Common Wild Petunia, $2n = 34$ (Long, 1976).

Herbs to 0.75 m, stems erect, quadrangular with short, eglandular trichomes (these sometimes restricted to two opposing stem surfaces). *Leaves* elliptical to ovate (rarely obovate), petioles of mature leaves 4 to 7 mm long, pubescence-like stems to glabrous, laminae (18–)31 to 66 × (7–)11 to 23 mm, 1.9 to 3.7(–4.2) times longer than wide, apices acute to short attenuate (rarely rounded), bases acute to attenuate, margins of leaves entire to irregularly crenate–dentate, abaxial surfaces with scattered, eglandular trichomes, these sometimes only along veins, cystoliths not visible, adaxial surfaces like abaxial surfaces except cystoliths highly conspicuous. *Inflorescences* of axillary and terminal, congested dichasia, inflorescence sessile on peduncles 1 to 2 mm long, paired bracts subtending entire inflorescence elliptical to broadly

lanceolate, 16 to 25 × 3.5 to 9 mm, pubescence and cystoliths like leaves, sessile, patelliform glands inconspicuous on both surfaces (drying pale red), paired bracteoles subtending each dichasium, elliptical, 10 to 13 × 1.5 to 3 mm, pubescence like leaves adaxially with mixed eglandular and glandular trichomes below (the latter especially along margins), cystoliths not apparent on either surface, sessile, patelliform glands conspicuous abaxially. *Flowers* sessile, (28–)57.5 to 67 mm long. *Calyx* (in flower or fruit) 11 to 17 mm long, lobes linear, (11)17 to 21 × ≈1 mm long, sparsely villous to woolly with eglandular and glandular trichomes. *Corollas* purple, infundibular, pubescent with eglandular trichomes externally, glabrous (distal portions) to pubescent (proximal portions) with eglandular trichomes internally, narrow unexpanded portion of tube (13–)25 to 29 × (1–)1.5 to 2.5 mm, expanded portion of tube (throat) (8.5–)17.5 to 20 × (7–)12 to 14 mm, lobes (6.5–)15 to 17 × (5.5–)14 to 15 mm. *Stamens* inserted, weakly didynamous, filaments ≈21 to 39 (shorter) and 22 to 40 (longer) mm long, lower half pubescent with eglandular, lax trichomes, upper half glabrous, fused filament sheath (“curtain”) enclosing approximately one-fourth of unexpanded portion of tube, anthers 2 to 3.5 mm long, rounded at base. *Styles* (21–)37 to 47 mm long with antrorse, eglandular trichomes to 0.3 mm long, becoming glabrous toward apex, stigmas bifid, lobes approximately equal in length, 3 to 4 mm long, stigmas persistent in fruit. *Capsules* weakly clavate, 11 to 13 × 4 mm, sterile portions 2 mm long, walls ≈0.2 mm thick, glabrous. *Seeds* to eight per capsule, orbicular, 2.5 to 3 mm in diameter with dense, hygroscopic trichomes covering the entire surface.

***Ruellia simplex* Wright**

Native range uncertain but probably Mexico and Caribbean through South America, introduced into the United States; commonly planted in coastal plain, naturalized in disturbed areas; n.v. Mexican Bluebell, $2n = 34$ (Piovano and Bernadello, 1991); synonyms: = *R. brittoniana* Leonard, = *R. coerulea* Morong, = *R. malacosperma* Greenman = *R. tweediana* Grisebach, see Ezcurra and Daniel (2007) for full citation.

Herbs to 1.25 m, stems erect, weakly quadrangular, glabrous but with short, dense tufts of eglandular trichomes at nodes. *Leaves* narrowly elliptical or linear, petioles of mature leaves 13 to 22 mm long, glabrous or with few, villous eglandular trichomes, laminae 135 to 225 × 6 to 20 mm, 10 to 22.5 times longer than wide, apices very narrowly acute, bases attenuate, margins of leaves entire or with occasional irregular tooth, abaxial surfaces with glabrous or with few, eglandular trichomes, cystoliths inconspicuous and concentrated along veins, sessile patelliform glands present but inconspicuous (becoming conspicuous on younger leaves), drying pale red, adaxial surfaces glabrous, cystoliths and sessile patelliform glands highly conspicuous. *Inflorescences* of axillary, expanded

dichasia, strongly pedunculate, peduncles to 160 mm long, slightly winged, pubescence-like stems, cystoliths and sessile patelliform glands present, paired bracts subtending entire inflorescence linear, 14 to 21 × 1 to 1.5(–2) mm, abaxial surfaces glabrous, cystoliths not apparent, sessile patelliform glands conspicuous, adaxial surfaces glabrous, cystoliths and sessile patelliform glands conspicuous, apices rounded, margins hyaline, hyaline portions ≈0.1 mm wide, paired bracteoles subtending each dichasium linear, 4 to 14 × 1 mm, abaxial surfaces with occasional eglandular trichomes but otherwise glabrous, cystoliths not apparent, sessile patelliform glands conspicuous, adaxial surfaces glabrous, cystoliths and glands conspicuous. *Flowers* pedicellate, pedicels to 34 mm long with short glandular trichomes, sessile patelliform glands conspicuous. *Calyx* (in flower or fruit) 10 to 17 mm long, lobes linear to narrowly lanceolate, 8.5 to 16 × 1 to 2 mm, glabrous or with sparse, eglandular trichomes. *Corollas* purple, infundibular, pubescent with eglandular (and few glandular) trichomes externally, glabrous internally, unexpanded portion of tube 12 to 14 × 3 to 4 mm, expanded portion of tube (throat) 21 to 25 × 16 to 18, lobes 16 to 21 × 14 to 20 mm. *Stamens* inserted, strongly didynamous, filaments ≈18 (shorter) and 22 (longer) mm long, glabrous, fused filament sheath (“curtain”) enclosing approximately one-third of unexpanded portion of tube, anthers ≈3 to 3.5 mm long, rounded at base. *Styles* 20 to 25 mm long, mostly glabrous but sometimes with sparse trichomes, stigmas bifid but dorsal lobe completely reduced, ventral lobe ≈1.5 to 2 mm long, stigmas not persistent in fruit. *Capsules* elliptical, (21–)25 to 32 × 4 to 6 mm, sterile portions 3 to 5 mm long, glabrous or with a few, glandular trichomes apically, walls ≈0.2 mm thick. *Seeds* to 24 per capsule, 2 to 3 mm in diameter with dense, hygroscopic trichomes covering entire surface.

Ruellia caroliniensis* × *simplex

No natural hybrids yet reported.

Herbs to 0.2 m, stems erect, terete to weakly quadrangular with sparse eglandular trichomes and short, dense tufts of eglandular trichomes at nodes. *Leaves* elliptical, petioles of mature leaves 3 to 9.5 mm long, glabrous, laminae 26 to 73 × 7 to 15 mm, 2.9 to 6.8 times longer than wide, apices long-acute, occasionally acuminate, bases attenuate to acute, margins finely and irregularly dentate to entire, abaxial surfaces glabrous, cystoliths and sessile patelliform glands inconspicuous, adaxial surfaces glabrous, cystoliths conspicuous, sessile patelliform glands not apparent. *Inflorescences* of axillary and terminal, partially expanded dichasia, pedunculate with peduncles to 15 mm long, slightly winged, mostly glabrous, cystoliths and sessile patelliform glands apparent, paired bracts subtending entire inflorescence narrowly elliptical or narrowly lanceolate, 12 to 21 × 1.5 to 4 mm, both

surfaces glabrous or with hairs along margins and with sessile patelliform glands but cystoliths not apparent, apices acute, margins hyaline, hyaline portions ≈0.1 mm wide, paired bracteoles surrounding each dichasium 3 to 13 × 0.5 to 1.5 mm, otherwise like bracts. *Flowers* chasmogamous and cleistogamous, pedicellate, pedicels to 6 mm long, glabrous, cystoliths apparent but sessile patelliform glands not. *Calyx* (in flower or fruit) 8 to 19.5 mm long, lobes linear, 6.5 to 18 × 1 mm pubescent with eglandular trichomes. *Corollas* of chasmogamous flowers purple, infundibular, pubescent with eglandular trichomes externally, glabrous internally, unexpanded portion of tube 7(–17) × 2 to 3 mm, expanded portion of tube 6.5 to 9(–19) × 3 to 5(–12) mm, lobes 5.5 to 7(–17.5) × 5 to 8(–15.5) mm. *Stamens* inserted, didynamous, filaments ≈10 (shorter) and 11 (longer) mm long, glabrous, fused filament sheath (“curtain”) enclosing nearly all of unexpanded portion of tube, anthers ≈2 mm long, rounded at base. *Styles* 8 to 10 mm long, with antrorse, eglandular trichomes to 0.2 mm long, becoming glabrous toward apex, stigmas bifid, ventral lobe 1.2 to 1.5 mm long, dorsal lobe reduced to one-third the length of ventral lobe. *Capsules* not seen (not produced by hybrids).

Appendix 2

Specimen vouchers used in this study and their associated Genbank numbers

Ruellia bourgaei Hemsl.—Tripp & Tripp 181 (DUKE), Mexico [GQ995586]; *Ruellia caroliniensis* (J.F. Gmel.) Steud.—living collection, University of Florida Greenhouse, [KM083710]; *Ruellia caroliniensis* (J.F. Gmel.) Steud. [car1]—living collection, University of Florida Greenhouse [KM083711]; *Ruellia caroliniensis* (J.F. Gmel.) Steud. [car2]—living collection, University of Florida Greenhouse [KM083712]; *Ruellia caroliniensis* (J.F. Gmel.) Steud. [car3]—living collection, University of Florida Greenhouse, [KM083713]; *Ruellia caroliniensis* (J.F. Gmel.) Steud. × *Ruellia simplex* Wright [29 artificial hybrids]—living collections, University of Florida Greenhouse [KM083714 through KM083742]; *Ruellia ciliatiflora* Hook.—Wood 10383 (US), Bolivia [EF214463]; *Ruellia donnell-smithii* Leonard—Tripp & Dexter 158 (DUKE), Mexico [EF214478]; *Ruellia drummondiana* (Nees) A. Gray—York 46274 (DUKE), Texas [EF214479]; *Ruellia galeottii* Leonard—Tripp & Dexter 159 (DUKE), Mexico [EF214497]; *Ruellia humilis* Nutt.—Tripp 14 (PH), Pennsylvania [EF214508]; *Ruellia insignis* Balf. f.—cult. RSABG greenhouses (source: Kew), collected from Socotra [EF214513]; *Ruellia lactea* Cav.—Tripp & Acosta 164 (DUKE), Mexico [KM083706]; *Ruellia macrosolen* Lillo ex Ezcurra—Krapovickas & Cristobal 46267 (US), Paraguay [EF214529]; *Ruellia malaca* Leonard—Stergios & Delgado 13487 (MO), Venezuela [EF214531]; *Ruellia metallica* Leonard—Tripp & Salazar-Amoretti 148 (DUKE), Costa Rica [EU431003]; *Ruellia*

metzae Tharp—Tharp 46054 (DUKE), Texas [EF214542]; *Ruellia morongii* Britton—Zardini & Velazquez 24875 (MO) [EF214543]; *Ruellia noctiflora* A. Gray—Tripp & Deregibus 257 (DUKE), Florida [KF945472]; *Ruellia nudiflora* (Engelm. & A. Gray) Urb.—Whitson & Whitson 814 (DUKE), Texas [EF214548]; *Ruellia purshiana* Fernald—Eyles 695 (DUKE), Georgia [EF214566]; *Ruellia simplex* Wright—Hahn 1859 (MO), Paraguay [EF214466]; *Ruellia simplex* Wright—cult. DUKE greenhouses (source: Austin, TX) [KM083707]; *Ruellia simplex* Wright [sim1]—living collection, University of Florida Greenhouse [KM083709]; *Ruellia simplex* Wright [sim2]—living collection, University of Florida Greenhouse [KM083708]; *Ruellia steyermarkii* Wassh.—Steyermark 89113 (US), Venezuela [EF214582]; *Ruellia strepens* L.—Tripp 25 (PH), Pennsylvania [EF214585]; *Ruellia tuberosa* L.—Jansen-Jacobs et al., 3869 (US), Guyana [EF214592]; *Ruellia tubiflora* Kunth—Tripp & McDade 131 (DUKE), Costa Rica [EF214590].