PROCEEDINGS OF THE CALIFORNIA ACADEMY OF SCIENCES

Series 4, Volume 60, No. 5, pp. 53–68, 8 figs., 2 appendices., 2 tables May 7, 2009

Molecular Phylogeny of Nelsonioideae (Acanthaceae) and Phylogeography of *Elytraria*

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Nelsonioideae comprise the basal lineage among clades of Acanthaceae. Species in this subfamily are characterized morphologically by several symplesiomorphies and at least one synapomorphy (descending-cochlear aestivation pattern of the corolla lobes). Phylogenetic relationships were studied among five of the seven genera in the subfamily: *Anisosepalum*, *Elytraria, Nelsonia*, *Ophiorrhiziphyllon*, and *Staurogyne*. DNA sequence data from two genic regions (chloroplast *trnS-G* and nrITS) of 22 collections were used to generate phylogenetic trees using parsimony, maximum likelihood, and Bayesian methods of cladistic analysis. Combined molecular analyses all strongly support *Elytraria* as monophyletic, recover a clade consisting of the morphologically similar genera ((*Ophiorrhiziphyllon* + *Staurogyne*) *Anisosepalum*), and are ambiguous with respect to the topologic position of *Nelsonia*. Nelsonioideae include about 170 species that occur primarily in tropical regions of both the Old World and the New World. Phylogeographic analyses of 11 species of the widely distributed *Elytraria* using Fitch optimization and dispersal-vicariance analysis (DIVA) support an African origin for the genus with a single dispersal event to the New World (likely by rafting of seeds or entire plants) followed by radiation and speciation there.

Acanthaceae contain about 220 genera (Scotland & Vollesen 2000) and some 4,000 species (McDade et al. 2008) that occur primarily in tropical and subtropical regions worldwide. Four morphologically distinctive subfamilies were recognized by Lindau (1895): Nelsonioideae, Mendoncioideae, Thunbergioideae, and Acanthoideae. Recent molecular studies have confirmed monophyly for each of these, but suggest that Mendoncioideae and Thunbergioideae might be better treated as sister taxa of a single subfamily, Thunbergioideae (McDade et al. 2008; Borg et al. 2008). Phylogenetic relationships based on a combination of nuclear and chloroplast DNA sequence data have been studied in numerous genera in each of these subfamilies except Nelsonioideae (e.g., Hedrén et al. 1995; Scotland et al. 1995; McDade et al. 2000, 2005, 2008; Manktelow et al. 2001; Kiel et al. 2006; Daniel et al. 2008; Borg et al. 2008). Based on a limited sampling of genera, Nelsonioideae have been shown to be basal to all other lineages of Acanthaceae (e.g., Scotland et al. 1995; McDade et al. 2000, 2008). Studies of phylogenetic relationships and biogeographic patterns based on phylogenetic trees (phylogeography) among genera of this subfamily are particularly desirable to better understand aspects of morphological and molecular evolution in the family and among related lineages of Lamiales.

Nelsonioideae include about 170 species in seven genera that occur primarily in tropical

TABLE 1. Genera, species richness, and geographic distribution of genera of Nelsonioideae. Total number of species per genus is followed in parentheses by number sampled here.

Genera	*No. Species (no. sampled)*	*Geographic Distribution*
Anisosepalum E. Hossain	3 (1)	tropical Africa
Elytraria Michx.	22 (11)	tropical Africa, Madagascar, Indian subcontinent, New World
Gynocraterium Bremek.	1 (0)	northern South America
Nelsonia R. Br.	1—5 (1)	tropical Africa, southern Asia, Australia, New World (native?)
Ophiorrhiziphyllon Kurz	1 (1)	southeastern Asia
Saintpauliopsis Staner	1 (0)	tropical Africa
Staurogyne Wall.	~140 (1)	Africa, Asia, northern Australia, New World

regions of both the Old World (OW) and the New World (NW) (Table 1). The subfamily is characterized by the following morphological traits: lack of cystoliths (symplesiomorphy), lack of retinacula (symplesiomorphy), relatively large numbers of ovules (symplesiomorphy), and descending-cochlear aestivation pattern of the corolla lobes (synapomorphy). Although usually treated within Acanthaceae, this assemblage of genera has also been treated as a tribe of Scrophulariaceae (Bremekamp 1953, 1965) and as a distinct family (Sreemadhavan 1977; Lu 1990).

In this study, we present putative phylogenetic relationships among five of the seven genera of nelsonioids based on sequence data from the internal transcribed spacer region of the nuclear ribosomal repeat (nrITS) and the non-coding chloroplast *trnS-G* intergenic spacer. In addition, sampling within the second largest and most widely distributed genus of the subfamily, *Elytraria*, was more extensive so that phylogenetic and biogeographic relationships of species in this genus might begin to be assessed. Specifically, our goals include: 1) proposing generic relationships within the subfamily, 2) testing monophyly for this sampling of *Elytraria*, 3) proposing specific relationships within *Elytraria*, 4) establishing an area of origin for *Elytraria*, and 5) providing additional biogeographic insights based on phylogeographic analyses.

MATERIALS AND METHODS

TAXON SAMPLING.— Full or partial sequence data of 22 collections, representing five of the seven genera of Nelsonioideae and 11 of the 22 species of *Elytraria*, were obtained (Appendix 1). We were unable to obtain sequence data for samples of *Gynocraterium*, *Saintpauliopsis*, and *E. tuberosa*; samples of the other eight species of *Elytraria* were unavailable. Indeed, many species of *Elytraria* remain poorly collected due to their limited distributions. For example, *E. klugii* is known only by the type collection from Peru. Because one of our goals was to elucidate major biogeographic relationships in *Elytraria*, and because several species are poorly known or collected, our taxon sampling for phylogeographic analyses sometimes included only one representative from a geographic area (e.g., only one of the six species endemic to Cuba was sampled). *Aphelandra maculata* (Acanthoideae) was used as an outgroup; its sequence data were obtained from GenBank. Appendix 1 lists voucher data for each taxon sequenced including: taxon name, GenBank accession number, source, collection data, and type of material (herbarium specimen or fresh sample dried in silica).

MOLECULAR METHODS.— Total genomic DNA was extracted from herbarium samples up to 80 years old or from fresh plant material dried in silica gel by using DNEasy kits (Qiagen Inc.,

Valencia, CA) following the manufacturer's instructions. The nrITS and *trnS-G* regions were chosen for sequencing because of previous success and significant phylogenetic signal for those loci in other species of Acanthaceae (McDade et al. 2005).

DNA was amplified in 25-µl reactions. The nrITS region was amplified by using a combination of the universal primers its4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and its5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) (White et al. 1990) and the plant-specific primers C26A (5′-GTT TCT TTT CGT CCG CT-3′) and N-nc18S10 (5′-AGG AGA AGT CGT AAC AAG-3′) (Wen & Zimmer 1996). The its4, its5 PCR reaction contained 2.5 µl 10X buffer, 0.438µl 10 mM dNTPs, 0.625 µl 50 mM MgCl2, 0.25 units of Biolase *Taq* polymerase (Bioline, Randolph, MA), and 0.54 µl of each primer (25mM). The PCR program consisted of a denaturing step for 6 minutes at 94°C, 35 amplification cycles of 60s at 94°C, 60s at 50–56°C, 80s at 72°C, and a final extension step of 6 minutes at 72°C in a Biorad MyCycler thermal cycler (Biorad, Hercules, CA). The C26A, N-nc18S10 PCR reaction contained 2.5–3.0 µl 10X buffer, 0.438–1.0 µl 10 mM dNTPs, 0.625–0.75 µl 50 mM MgCl2, 0.25 units of Biolase *Taq* polymerase, and 0.54 µl of each primer (25mM). The PCR program consisted of a denaturing step for 10 minutes at 94°C, 35 amplification cycles of 60s at 94°C, 60–80s at 50°C, 80s at 72°C, and a final extension step of 10 minutes at 72°C in a Biorad MyCycler thermal cycler. The *trnS-G* spacer was amplified by using primers trnS-GCU (5′-GCC GCT TTA GTC CAC TCA GC-3′) and trnG-UCC (5′-GAA CGA ATC ACA CTT TTA CCA C) (Hamilton 1999). The *trnS-G*CU, trnG-UCC PCR reaction contained 2.5 µl 10X buffer, 0.438 µl 10 mM dNTPs, 0.625 µl 50 mM MgCl2, 0.25 units of Biolase *Taq* polymerase, and 0.54 µl of each primer (25mM). The PCR program consisted of a denaturing step for 4 minutes at 94°C, 35 amplification cycles of 45s at 94°C, 60s at 57°C, 80s at 72°C, and a final extension step of 6 minutes at 72°C in a Biorad MyCycler thermal cycler.

PCR products were verified prior to sequencing by using agarose gel electrophoresis; they were cleaned with ExoSAP (Exonuclease I and Shrimp Alkaline Phosphatase). The PCR product was prepared for sequencing by using the primers from PCR amplification and BigDye fluorescent dye-terminator reagent mix (Perkin-Elmer, Foster City, CA). Sequence reaction products were purified with Sephadex G-50 columns (GE Healthcare, Piscataway, NJ) to remove unincorporated dye terminators. Sequences were generated on an ABI 3100 automated sequencer (Applied Biosystems, Inc., Foster City, CA) in the Osher Molecular Lab at the Center for Comparative Genomics at the California Academy of Sciences.

Sequence alignment.— Sequences were manually edited in Sequencher 4.7 (Gene Codes Corp., Inc., Ann Arbor, MI). Rough alignments were performed in Sequencher 4.7 and ClustalX 1.83 (Thompson et al. 1997) and sequences were further aligned by eye in MacClade 4.08 (Maddison & Maddison 2005). The nrITS matrix was trimmed to include only the two spacers, ITS1 and ITS2, and the 5.8S RNA region. Sequences of nrITS could not be obtained for seven (about one-third) of the taxa, possibly due to the low quality of DNA in some of the herbarium specimens. The *trnS-G* sequence data for one taxon were not obtained, likely for the same reason.

Data congruence.— Congruence of the two data sets (nrITS and *trnS-G*) was tested using the partition homogeneity test (Farris et al. 1994, 1995) as implemented by PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). To test for congruence, the two data sets were reduced to 14 taxa, i.e., they included only those taxa for which sequences of both regions were obtained. Parameters were set to 100 replicates and 5,000 maximum trees. The test found the two data sets to be congruent ($p = 0.12$), and further phylogenetic analyses were conducted for each region separately and in a combined data set.

Phylogenetic analyses.— Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference tests were conducted to create phylogenetic trees. Clade support was calculat-

ed by using bootstrap methods (Felsenstein 1985) for MP trees and Bayesian Posterior Probabilities (BPP) for ML and Bayesian trees.

MP analyses were performed in PAUP* by using branch and bound searches for single regions and a heuristic search for the combined data set. Characters were equally weighted and unordered, with gaps treated as missing data. Heuristic searches were run using 5,000 random replicates and TBR branch swapping. Bootstrap analyses for single region data sets were run using 1,000 replicates, TBR branch swapping, 10,000 max trees (5,000 in the combined analysis), and 10 random addition sequences per bootstrap replicate. Clades with a bootstrap value of > 90% were considered to have strong support, 80–90% moderate support, and < 80% weak support.

For the ML and Bayesian analyses, we used MrModeltest 2.2 (Nylander 2004) to find the best-fit model of nucleotide substitution. MrModeltest uses four different hierarchies to calculate the likelihood ratio between models (Posada & Crandall 2001). We then conducted the ML analyses in PAUP*, starting with a neighbor joining tree and using the parameters in the best-fit model chosen by the hierarchical likelihood test. Bayesian analyses were performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). We used a separate best-fit model to represent each DNA region, then combined the two data sets, and implemented both models by partitioning the data. Two Markov chain Monte Carlo (MCMC) runs were performed, each with one cold chain and three heated chains for 5,000,000 generations, saving a tree every 1,000 generations. Likelihood scores were determined to be stationary at 345,000 generations (the "burn-in" point), and data prior to this point were discarded. Clades with Bayesian Posterior Probabilities > 95% were considered to have strong support, 90–95% moderate support, and < 90% weak support.

Constraint analysis.— An alternative phylogenetic hypothesis was evaluated using MacClade to create a tree that constrained the clade of interest. This tree was loaded into PAUP* as a constraint tree, where PAUP* used the maximum parsimony search criteria described above to find the shortest tree consistent with the new tree topology. The difference in length between the original and constrained trees provides an indication of the parsimony cost (additional steps) involved in accepting the alternative hypothesis.

Phylogeographic analyses.— Ancestral areas for *Elytraria* were located using both Fitch optimization (Maddison et al. 1992) and dispersal-vicariance analysis (DIVA 1.1; Ronquist 1996, 1997). Fitch optimization is run in PAUP* and codes geographic area as a single multistate character that is treated as unordered. DIVA codes for presence/absence and uses each geographic area as a separate character. DIVA requires a fully resolved tree. For both of these analyses, a pruned version of the ML phylogeny shown in Figure 6 was used. To simplify the dataset, we included a single branch per species. Each taxon used in the analysis (with the exception of *E. imbricata*) was assigned to one of six geographic areas: Africa, Madagascar, Greater Antilles, southeastern USA, South America, and western USA/Mexico/Central America. These areas were chosen because most species of *Elytraria* are restricted to just one of them. *Elytraria imbricata* is the only species present in two areas, western USA/Mexico/Central and South America. Fitch optimization assumes dispersal as the only mode of speciation. DIVA finds the optimal ancestral area by assuming vicariance and penalizing for dispersal and extinction events. In DIVA, vicariance and duplication events carry a cost of zero, whereas dispersal and extinction events each carry a cost of one. Optimizations try to minimize the cost. For the DIVA analysis, we constrained the number of ancestral areas to include no more than two using the "maxareas" option. The number of maxareas was chosen on the basis of the maximum number of areas occupied by an extant species (Mansion & Struwe 2004; Oberprieler 2005).

Results

Sequence data.— Table 2 summarizes the sequence data characteristics for each genic region as well as the combined data set, and includes number of OTUs sequenced, aligned length, % GC content base frequencies, proportion of variable sites, proportion of parsimony informative sites, range of pairwise distances (for all taxa and for *Elytraria* only), MP analysis tree parameters, and each of the models chosen by MrModeltest and used in the ML and Bayesian analyses.

Phylogenetic analyses.— MP analysis of the *trnS-G* data set resulted in two equally most parsimonious trees. A strict consensus of these trees is moderately well resolved (Fig. 1). There is moderate to strong support for six clades: *Elytraria* (Bootstrap Support, BS = 97%), *E. imbricata* (BS = 88%), *E. imbricata* + *E. mexicana* (BS = 95%), *E. bromoides* (BS = 90%), *E. nodosa* (Madagascar) + *E. acaulis* (Malawi) (BS = 90%), and *Anisosepalum* + *Staurogyne* (BS = 98%). The relationships of other species within *Elytraria* and the relationships of the other genera of Nelsonioideae are poorly supported or are represented by polytomies. The *trnS-G* ML analysis yielded a similar tree (Fig. 2). The ML tree shows strong support for monophyly of *Elytraria* (BPP = 97%),

Table 2. Summary of sequence data characteristics for nrITS region, *trnS-G* spacer, and combined dataset. Search method used in maximum parsimony analyses: *branch and bound, **heuristic.

	nrITS	*trnS-G*	*combined dataset*
Number of OTUs	15	21	22
Aligned length	612	704	1316
% CG content	64.10%	30.00%	45.86%
Base frequencies			
A	0.194	0.385	0.296
C	0.33	0.136	0.226
G	0.311	0.164	0.232
T	0.165	0.315	0.245
Variable sites (proportion)	340 (0.556)	224 (0.318)	564 (0.429)
Parsimony informative sites (proportion)	163 (0.266)	72 (0.102)	235 (0.179)
Pairwise distances, all taxa (range, %)	0.2—33.8%	0.0—23.2%	0.1—25.5%
Pairwise distances, *Elytraria* only (range, %)	0.2—11.4%	0.0—3.7%	0.1—9.5%
Maximum parsimony analyses			
Number of most parsimonious trees found	1*	2*	20**
Length (all characters)	597	283	900
Length (parsimony informative characters)	383	118	512
Consistency index (parsimony informative characters)	0.674	0.678	0.668
Retention index (parsimony informative characters)	0.642	0.796	0.688
Maximum likelihood analyses			
Model chosen by MrModelTest	K80+G	HKY85+G	GTR+G
Bayesian analyses			
Model chosen by MrModelTest			HKY85+G

E. imbricata (BPP = 100%), *E. imbricata* + *E. mexicana* (BPP = 100%), *E. bromoides* (BPP = 100%), a "Greater Antilles" clade consisting of species of *Elytraria* from Cuba (*E. planifolia*) and Haiti (*E. prolifera*) (BPP = 95%), a "Gulf of Mexico" clade consisting of (*E. caroliniensis* + *E. macrophylla*) + "Greater Antilles" clade (BPP = 100%), *E. nodosa* (Madagascar) + *E. acaulis* (Malawi) (BPP = 100%), *E. nodosa* + two eastern African species (BPP = 97%), and *Anisosepalum* + *Staurogyne* (BPP = 100%). Strong support for a clade of African/Malagasy + "Gulf of Mexico" species of *Elytraria* (BPP = 97%) was recovered in this tree, but not in any other analysis. Bayesian analysis yielded a tree (not shown) almost identical to the ML tree, but with *E. marginata* sister to the "Gulf of Mexico" clade (BPP = 85%) instead of to the eastern African species of *Elytraria*.

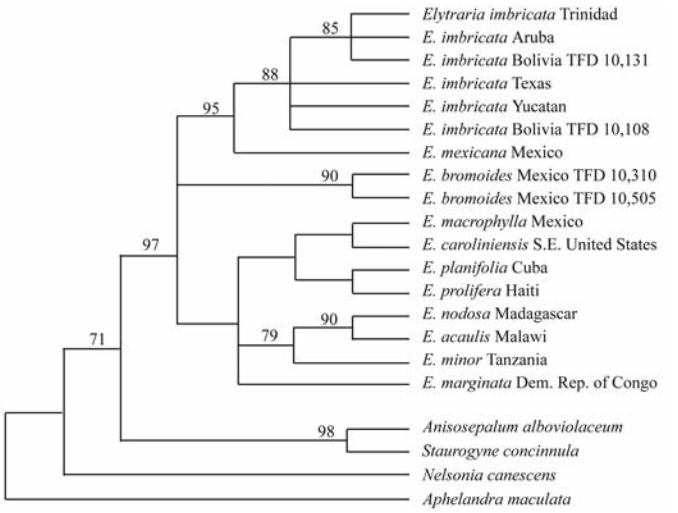

FIGURE 1. Strict consensus of two most parsimonious trees from a *trnS-G* branch and bound analysis. Values at nodes indicate bootstrap support > 70%. L = 597 (all characters), CI = 0.674, RI = 0.642.

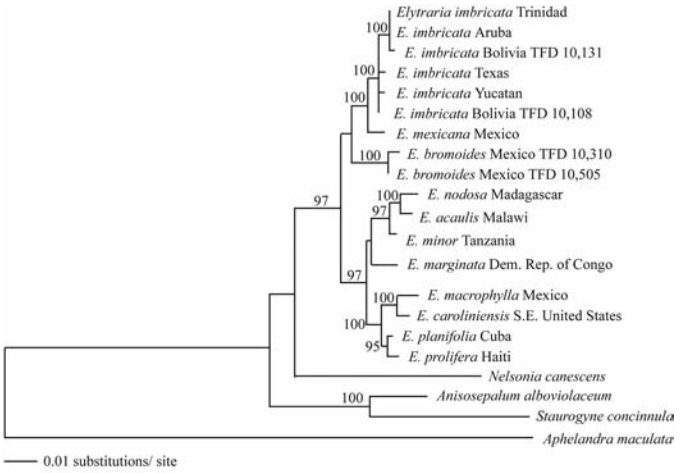

FIGURE 2. Maximum likelihood tree based on a *trnS-G* analysis using the K80+G model of evolution. Values at nodes indicate Bayesian posterior probabilities > 90%.

MP analysis of the nrITS data set resulted in a single, moderately well resolved tree (Fig. 3). There is strong support for monophyly of *Elytraria* (BS = 99%), the "Greater Antilles" clade (BS = 96%), *E. nodosa* + *E. acaulis* (BS = 100%), and *Ophiorrhiziphyllon* + *Staurogyne* (BS = 100%). All NW *Elytraria* form a clade sister to the eastern African/Malagasy clade (BS = 99%). The ML tree (Fig. 4) shows strong support for *Elytraria* (BPP = 100%), *E. imbricata* + *E. mexicana* (BPP = 100%), *E. nodosa* (Madagascar) + *E. acaulis* (Malawi) (BPP = 100%), NW *Elytraria* as sister to the eastern African/Malagasy clade (BPP = 100%), and *Ophiorrhiziphyllon* + *Staurogyne* (BPP = 99%). The Bayesian (not shown) and ML trees were nearly identical, except that *E. bromoides* formed an unsupported clade together with *E. planifolia* and *E. prolifera* in the former tree.

MP analysis of the combined nrITS + *trnS-G* data set resulted in 20 equally most parsimonious trees. A strict consensus of these trees (Fig. 5) is similar to the nrITS MP tree (Fig. 3), but less well

resolved due to multiple collapsed branches. This tree shows moderate to strong BS support for eight clades: *Elytraria* (BS = 99%), *E. imbricata* + *E. mexicana* (BS = 98%), *E. bromoides* (BS = 87%), the "Greater Antilles" clade (BS = 98%), *E. nodosa* + *E. acaulis* (BS = 92%), *E. nodosa* + two eastern African species (BS = 80%), *Ophiorrhiziphyllon* + *Staurogyne* (BS = 100%), and *Ophiorrhiziphyllon* + *Staurogyne* + *Anisosepalum* (BS = 84%). The combined ML tree (Fig. 6) is identical to the Bayesian tree (not shown), except for the placement of *Nelsonia*, which has no BPP support. The ML tree shows strong BPP support for all eight clades supported in the MP analysis: *Elytraria* (BPP = 100%), *E. imbricata* + *E. mexicana* (BPP = 100%), *E. bromoides* (BPP = 100%), the "Greater Antilles" clade (BPP = 100%), *E. nodosa* + *E. acaulis* (BPP = 100%), *E. nodosa* + two eastern African species (BPP = 97%), *Ophiorrhiziphyllon* + *Staurogyne* (BPP = 100%), and (*Ophiorrhiziphyllon* + *Staurogyne*) *Anisosepalum* (BPP = 100%). BPP values also add strong support for the "Gulf of Mexico" clade (BPP = 99%), an eastern African/Malagasy clade (BPP = 97%), NW *Elytraria* (BPP = 98%), and NW *Elytraria* + western/central African *Elytraria* (BPP = 99%). The eastern African/Malagasy clade is basal in the genus.

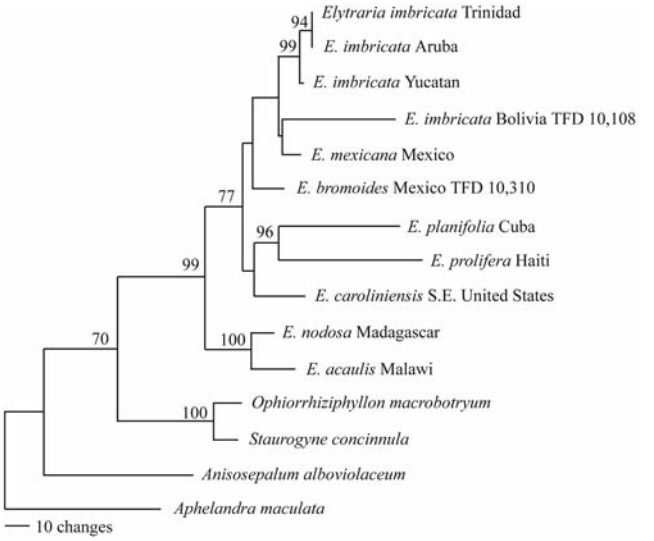

FIGURE 3. Single most parsimonious tree resulting from a nrITS branch and bound analysis. Values at nodes indicate bootstrap support > 70%. L = 283 (all characters), CI = 0.678, RI = 0.796.

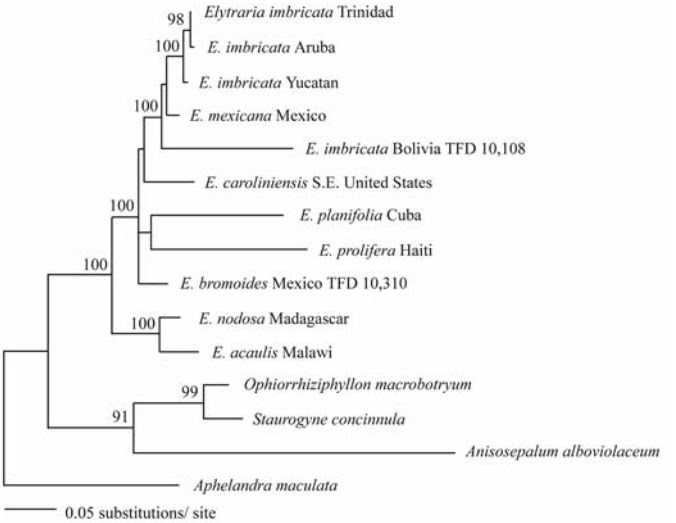

FIGURE 4. Maximum likelihood tree based on a nrITS analysis using the HKY+G model of evolution. Values at nodes indicate Bayesian posterior probabilities > 90%.

PHYLOGEOGRAPHIC ANALYSES.— Fitch parsimony optimization resulted in an optimization tree (Fig. 7) of five steps (dispersals), with the basal node ancestral area being scored unambiguously as Africa. A single trans-Atlantic dispersal event occurred from Africa to western USA/Mexico/Central America, and from there dispersals occurred to the southeastern USA, the Greater Antilles, and South America. There was also a dispersal event from Africa to Madagascar.

The DIVA reconstruction (Fig. 8) yielded two alternative ancestral areas for the basal node. One scenario identifies an ancestral area of Africa with a subsequent dispersal event to western USA/Mexico/Central America. The other scenario identifies a vicariance event with an ancestral area of Africa and western USA/Mexico/Central America. DIVA identified five dispersal events, four to five vicariance events, and five to six duplication events, with the number of vicariance and duplication events depending on the ancestral area of the basal node.

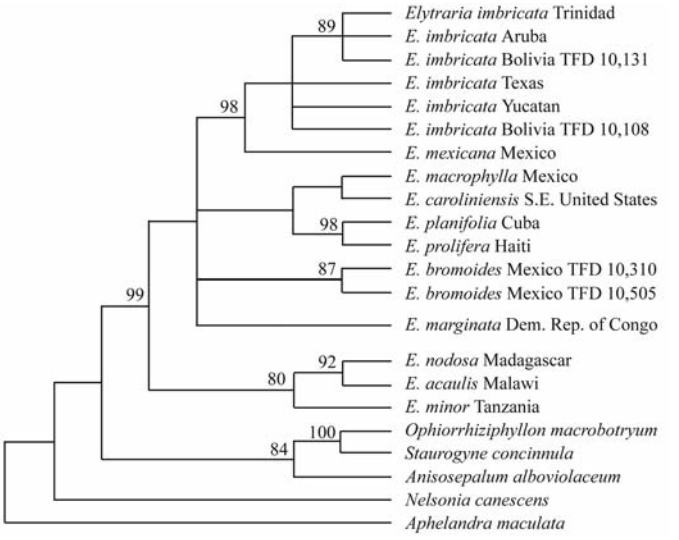

FIGURE 5. Strict consensus of 20 most parsimonious trees from a combined *trnS-G* and nrITS heuristic analysis. Values at nodes indicate bootstrap support > 70%. L = 900 (all characters), CI = 0.668, RI = 0.688.

DISCUSSION

Molecular Phylogenetics

RELATIONSHIPS OF GENERA OF NELSONIOIDEAE.— From our analyses of samples from five of the seven genera of Nelsonioideae, a lineage consisting of *Staurogyne*, *Ophiorrhiziphyllon*, and *Anisosepalum* is recovered in both combined analyses (e.g., Fig. 6, BPP = 100%). Hossain (1971) hypothesized that based on morphological characters (e.g., calyx five-partite), these three "staurogynoid" genera (plus *Gynocraterium* and *Saintpauliopsis*, neither of which were included in our analyses) are more closely related to each other than to *Elytraria* and *Nelsonia* (both of which have the calyx four-partite with the posterior lobe incompletely fused). Indeed, several species of *Anisosepalum* were originally described in *Staurogyne*, and *Ophiorrhiziphyllon* differs from *Staurogyn*e in rather minor characters (e.g., smaller connective between anther thecae, stamens long-exserted vs. included or slightly exserted in *Staurogyne*, and thecae elongate vs. subglobose to globose in *Staurogyne*).

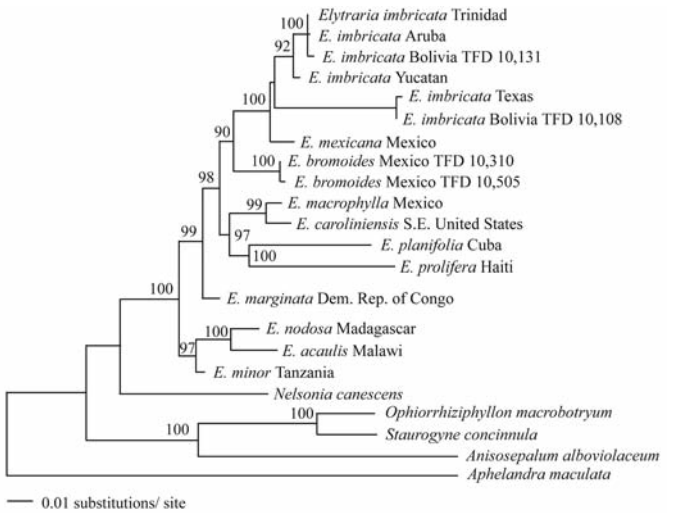

FIGURE 6. Maximum likelihood tree based on a combined *trnS-G* and nrITS analysis using the GTR+G model of evolution. Values at nodes indicate Bayesian posterior probabilities > 90%.

Additional sampling should reveal whether *Staurogyne* should be treated more broadly so as to include some or all of the four other genera either known or hypothesized to be closely related to it.

Hossain (1971) indicated that *Nelsonia* and *Elytraria* were morphologically distinct from the "staurogynoid" genera of Nelsonioideae. Our analyses usually place *Nelsonia* separate from either *Elytraria* or the "staurogynoid" genera, but are unable to establish its topologic position relative to these other nelsonioid clades. Morphologically, *Nelsonia* shares some characters with *Elytraria* (e.g., inflorescences of dense spikes bearing alternate to spirally arranged bracts), other characters with the "staurogynoid" genera (e.g., hooklike projections on the seeds), and some characters unique to the subfamily (e.g., absence of bracteoles). Divergent taxonomies of *Nelsonia* have been proposed with the number of species varying from one (Hossain 1984) to five (Vollesen 1994). Additional sampling will enable testing for monophyly of that genus, infrageneric relationships, and its placement within Nelsonioideae.

MONOPHYLY OF *ELYTRARIA*.— All phylogenetic analyses strongly support monophyly of *Elytraria* (BS = 97-99%, BPP = 97-100%). On the basis of morphological characters, *Elytraria* has been recognized for more than 150 years (Nees 1847) as a cohesive taxon that occurs on multiple continents in both the OW and the

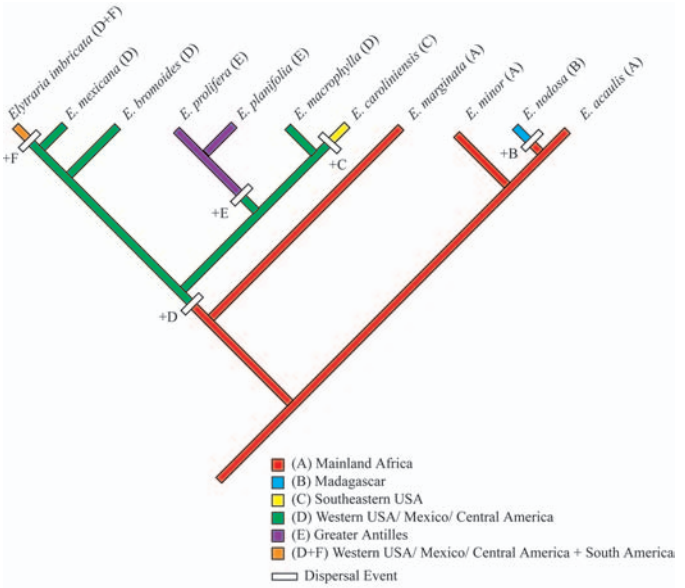

FIGURE 7. Fitch optimization tree showing five dispersal events. Area designations (in parentheses): (A) Mainland Africa, (B) Madagascar, (C) Southeastern USA, (D) Western USA/Mexico/Central America, (E) Greater Antilles, (F) South America.

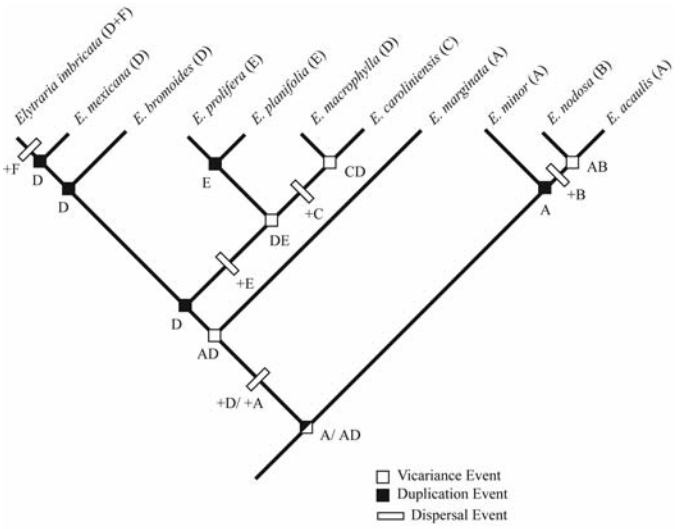

FIGURE 8. Ancestral area reconstruction based on DIVA, using a pruned tree from the ML analysis of the combined dataset. DIVA set to maxareas = 2. Area designations (in parentheses): (A) Mainland Africa, (B) Madagascar, (C) Southeastern USA, (D) Western USA/Mexico/Central America, (E) Greater Antilles, (F) South America. Ancestral areas separated by a slash represent equally parsimonious reconstructions. Results include 4—5 vicariance events (allopatric speciation), 5—6 duplication events (sympatric speciation), and 5 dispersal events, depending on basal node reconstruction.

NW. These characters include: 1) inflorescence peduncles (scapes) covered with alternate to spirally arranged scales (probable synapomophy), 2) four-partite calyx with anterior lobe bifid to bilobed (symplesiomorphy; shared with *Nelsonia*), 3) seeds lacking minute hooklike projections (probable symplesiomorphy; shared with *Anisosepalum*), and 4) touch-sensitive stigma (probable synapomorphy). Molecular data now confirm the taxonomic delimitation of *Elytraria*, and show that the morphological characters coincide with the phylogeny recovered in our analyses.

Infrageneric relationships of *Elytraria* and monophyly of *E. imbricata*.— *Elytraria* consists of 22 species that occur in tropical, subtropical, and temperate regions (Appendix 2). Concentrations of species are found in tropical Africa (six species; Dokosi 1979), Cuba (6 species; Borhidi and Muñiz 1977; Dietrich 1982), and Mexico (4 species; Daniel and Acosta C. 2003). Hossain (1971, 1972) divided *Elytraria* into two subgenera. Subgenus *Tetrandra* consists of the two species (*E. madagascariensis* and *E. nodosa*) endemic to Madagascar; subgenus *Elytraria* includes all of the other taxa. Morphologically, the Malagasy species are unique in *Elytraria* by having four (vs. two) fertile stamens and an anther connective with (vs. without) an apical appendage. Comparing morphological and anatomical characters of these two species to others of *Elytraria*, as well as to other nelsonioid genera, Hossain (1971, 1972) hypothesized that many of the characteristics of subgenus *Tetrandra* are primitive within *Elytraria*. Specifically, he noted the probable reduction of stamens from four to two in the genus. Among extant nelsonioids, androecial elements consist of two fertile stamens and no staminodes (*Nelsonia* and some *Elytraria*), two fertile stamens and one or more staminodes (*Ophiorrhiziphyllon*, most *Elytraria*, and some *Staurogyne*), and four fertile stamens with zero or one staminode (*Anisosepalum*, *Gynocraterium*, *Saintpauliopsis*, most *Staurogyne*, and the two Malagasy *Elytraria*). Combined molecular phylogenetic analyses place the Malagasy endemic, *E. nodosa*, in a moderately or strongly supported clade together with two species from eastern Africa (Fig. 5, BS = 80%; Fig. 6, BPP = 97%), at the base of the genus. On the basis of this topology, Hossain's hypothesis that many characters of subgenus *Tetrandra* are primitive (specifically the number of stamens) is not supported. Also, topologies of the trees obtained in this study do not support recognition of two monophyletic subgenera as treated by Hossain. However, based on morphological characters, the two Malagasy endemics would indeed appear to represent closely related species worthy of infrageneric recognition.

The combined ML analysis (Fig. 6) reveals that the OW species of *Elytraria* do not form a monophyletic group. Indeed, the central to western African species *E. marginata* is strongly supported as sister to the NW clade of the genus in this analysis (Fig. 6, BPP = 99%). In both MP and ML analyses, a clade consisting of the eastern African species and *E. nodosa* from Madagascar are supported as monophyletic (Fig. 5, BS = 80%; Fig. 6, BPP = 97%) and strongly supported as sister to NW *Elytraria* + *E. marginata* (Fig. 5, BS = 99%; Fig. 6, BPP = 100%). Constraining OW *Elytraria* to monophyly by altering the placement of *E. marginata* in our combined MP tree (Fig. 5) requires only two additional steps (L=900 vs. 902). The constraint analysis suggests that monophyly of OW *Elytraria* cannot be rejected by our data. The taxonomy of OW species has yet to be fully resolved and our samples do not include any plants from Asia (i.e., *E. acaulis* from India and Sri Lanka).

In the combined ML analysis, the NW species of *Elytraria* form a strongly supported clade (Fig. 6, BPP = 98%), but this clade is not supported in the MP analyses. The topologic positions of three oft recovered NW lineages: *E. imbricata* + *E. mexicana*, *E. bromoides*, and the "Gulf of Mexico" clade, vary in each of the analyses. Moderate to strong support for a monophyletic *E. imbricata* is shown only in the *trnS-G* phylogenies (Fig. 1, BS = 88%; Fig. 2, BPP = 100%), which have *E. mexicana* as sister to *E. imbricata*. When the nrITS data is added, a monophyletic *E. imbricata* either has no support (Figs. 5, 6), or *E. mexicana* is placed within *E. imbricata* (Figs. 3, 4). *Ely-*

traria mexicana is known only from semi-arid regions of central and western Mexico (Daniel & Acosta C. 2003), within the range of *E. imbricata*. Morphologically, these species appear to be very similar; they differ primarily in characters of the bracteal apex (acuminate-spinose in *E. mexicana* vs. prominently 3-parted, at least among distal bracts, in *E. imbricata*), bracteal pubescence (abaxially villous in *E. mexicana* vs. abaxially usually glabrous in *E. imbricata*), and color of the corolla limb (whitish with dark purple markings on the lobes of the upper lip in *E. mexicana* vs. blue with white and yellow markings on the lower lip or rarely entirely white in *E. imbricata*). The morphological distinctions and taxonomic status of these two species are currently being reevaluated; however, our results substantiate a close relationship between them.

Phylogeography

Distributions of Nelsonioideae.— Genera of Nelsonioideae exhibit diverse patterns of present-day distribution (Table 1). The largest genus, *Staurogyne*, has concentrations of species in South America, Africa, and Asia; a few species also occur in southern North America and northern Australia. Close relatives of *Staurogyne*, both known (*Anisosepalum* and *Ophiorrhiziphyllon*) and suspected (*Gynocraterium* and *Saintpauliopsis*), are restricted to areas with significant concentrations of species of that genus. *Nelsonia* is pantropical with native occurrences in Africa, Madagascar, Asia, Australia, and possibly the NW (Hossain 1971; Vollesen 1994). *Elytraria* appears to be particularly widespread in both the OW and NW tropics, with several NW species either extending their ranges into (e.g., *E. bromoides*, *E. imbricata*) or endemic to (e.g., *E. caroliniensis*) temperate regions.

Area of origin and dispersal of Elytraria.— DIVA provides two alternative ancestral areas, either Africa or Africa and western USA/Mexico/Central America. Fitch optimization results in an ancestral area of Africa, with a single dispersal event to the New World, from western or central Africa across the Atlantic Ocean. Because both analyses include an optimization of Africa at the ancestral node of *Elytraria*, most evidence supports Africa as the area of origin for this genus. Because available evidence and analyses to date (see McDade et al. 2005, 2008) establish that Acanthaceae evolved subsequent to the breakup of the Gondwanan landmass, dispersal is the most appropriate explanation for intercontinental disjunctions. Most recent phylogenetic studies on other clades of Acanthaceae have revealed an OW origin followed by a single dispersal event to the NW with subsequent diversification there (i.e., Acantheae, McDade et al. 2005; Isoglossinae, Kiel et al. 2006; *Tetramerium* lineage of Justicieae, Daniel et al. 2008; Thunbergioideae, Borg et al. 2008). Our results suggest a similar scenario for *Elytraria* as well.

Renner (2004) studied the mechanisms for plant dispersal across the tropical Atlantic. Wind, birds, water, and floating rafts of vegetation are all possible mechanisms of trans-oceanic dispersal. Strong east-to-west surface winds rarely make it across the entire central Atlantic, although Saharan dust does occasionally make it to the New World (Renner 2004). Seeds of *Elytraria* are small (between 0.3 and 1.0 mm in diameter), but lack attributes (e.g., wings) that might make them candidates for wind dispersal. Seeds of *Elytraria* may have been transported across the ocean by sticking to mud on the feet of birds. Today there are no avian species that have tropical trans-Atlantic migration routes (Renner 2004), though a few sea birds are carried from West Africa across the Atlantic to North America by tropical storms (Rappole et al. 2000). Bird transportation remains a possible, though unlikely, mode of trans-Atlantic dispersal for *Elytraria*.

Oceanic surface water currents influence the last two modes of dispersal under consideration: water and rafting. Today's patterns of oceanic surface currents have changed little since the end of the Late Cretaceous (Parrish 1993). Currents in the northern South Atlantic are primarily deter-

mined by the placement of the continents, and modern day ocean circulation patterns are thought to have arisen shortly after the formation of the South Atlantic (Parrish 1993). Today, currents are predominantly east-to-west, with a single west-to-east current that originates well offshore (Renner 2004).

The small size and unprotected coating of seeds of *Elytraria* render them unlikely to survive in salt water for prolonged periods of time. A raft of vegetation carrying seeds or entire plants provides the best explanation for the present day distribution of *Elytraria* in both the OW and the NW. Equatorial currents can transport large mats of vegetation coming out of the mouths of the Niger and Congo Rivers across the Atlantic Ocean in less than two weeks (Renner 2004; Measey et al. 2007). There is evidence suggesting that east-to-west trans-Atlantic rafting events may account for dispersal in multiple plant and animal groups (Carranza et al. 2000; Cronn and Wendel 2004; Vidal et al. 2008).

While the amphi-Atlantic distribution of *Elytraria* can be explained by a single oceanic dispersal event, several shorter dispersals must also have taken place to account for its multiple insular occurrences. Two species of *Elytraria*, *E. nodosa* and *E. madagascariensis*, are endemic to the island of Madagascar. These species share several morphological characters not found in any other species of *Elytraria* (see above), and can be assumed to be sister species based on these putative synapomorphies. All molecular phylogenetic analyses strongly support placing *E. nodosa* sister to *E. acaulis* from eastern Africa (e.g., Fig. 5, BS = 92%; Fig. 6, BPP = 100%), suggesting a single dispersal event to the island from eastern Africa. Madagascar lies 400 km off the eastern coast of Africa and has maintained this position relative to the mainland for the last 120–130 my (Rabinowitz & Woods 2006). Rabinowitz & Woods (2006) found that the assumption of previous studies supporting the exposure of dry land in the Mozambique Channel, connecting Madagascar to the mainland, to be incorrect. Rather, they found that it is unlikely there has been any land exposure since before the middle Eocene (50 mya), when the first mammals migrated to Madagascar. Rabinowitz & Woods (2006) hypothesized that the early mammals probably arrived by swimming or by floating on vegetation mats, the most likely method for dispersal of *Elytraria* there as well.

Island dispersal has also occurred in the Gulf of Guinea, where the widespread West African *Elytraria marginata* is found on the islands of São Tomé and Príncipe. These oceanic islands are part of the Cameroon Line of volcanoes and lie over 200 km from the mainland. With ages of at least 13 and 31 million years (Lee et al. 1994), respectively, São Tomé and Príncipe have never been connected to continental Africa (Jones 1994). Measey et al. (2007) propose that the colonization of São Tomé by amphibians was via rafting events. Major sea surface currents in the Gulf of Guinea would carry any rafts of debris from the Niger and Congo rivers directly towards São Tomé, making rafting a plausible explanation for the dispersal of *Elytraria* to these islands.

In the Caribbean Sea, *Elytraria* occurs on several islands. In some cases, dispersals have led to the formation of new species (e.g., Cuba and Hispaniola); in other cases, insular occurrences of *E. imbricata* likely result from recent colonizations or persistence of the species on islands previously connected to the American mainland during periods of lower sea levels (e.g., Trinidad, Tobago, Bonaire, Curaçao, Isla San Andrés).

Future Studies

These preliminary findings address phylogenetic relationships of several constituent genera of Nelsonioideae, relationships of species in *Elytraria*, and biogeographic patterns based on the phylogenies recovered. Relationships of the seven genera of Nelsonioideae remain unresolved due to weak or no support in the lower branches of the trees and absence of data for some genera

(*Gynocraterium* and *Saintpauliopsis*). Future studies on Nelsonioideae will include additional field and herbarium work in order to obtain collections of more taxa (i.e., species of *Elytraria* and other genera of Nelsonioideae), and will add sequences from additional genic regions. These additions should result in more comprehensive and better resolved trees that can further address taxonomic composition and monophyly of the subfamily, whether "staurogynoid" genera should be treated in a broader *Staurogyne*, the infrageneric classification of *Staurogyne* proposed by Hossain (1971), and phylogeography of the entire subfamily. Phylogeography of this basal lineage of Acanthaceae should be particularly revealing because the distribution patterns of extant genera common to both the OW and the NW (*Elytraria*, *Nelsonia*, and *Staurogyne*) suggest a more dynamic history of intercontinental dispersals than is known for other lineages of Acanthaceae studied to date.

Acknowledgments

This study represents a portion of a Master of Science thesis from San Francisco State University by the first author. Molecular sequencing was supported by the SFSU Biology Department IRA Research Grant. Wenk's travel to meetings was funded by the SFSU Graduate Student Council in Biology Travel Grants and the Department of Botany at the California Academy of Sciences. Daniel's studies of *Elytraria* were supported, in part, by a grant from the National Science Foundation (DEB-0743273). We thank A. Carmichael, F. Cipriano, B. Cruz, K. Deiner, M. Flannery, P. Fritsch, W.B. Simison, and G. Spicer for help with molecular sequencing and phylogenetic analyses. Also, we thank F. Almeda, D. Champluvier, J.L. Clark, and L. McDade for providing DNA samples. We are grateful to the following herbaria for allowing us to borrow specimens for morphological studies and/or DNA sampling: A, BR, CAS, DS, F, GH, MO, NY, P, S, UC, and US.

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Appendix I

Taxa, GenBank accession numbers (trnS-G, nrITS; — = sequence not obtained), sources of plant materials from which DNA was extracted for sequencing, and type of material used for sequencing (herbarium specimen = H vs. silica-dried sample = S). Cultivated plants include native range in parentheses. Abbreviations for herbaria follow Holmgren et al. (1990). Taxa are listed in alphabetical order by genus and species.

Anisosepalum alboviolaceum (R. Ben.) E. Hossain; FJ868921, FJ868907; Champluvier 5,295 (CAS); Mbomo, Congo; H. Aphelandra maculata (Tafalla ex Nees) Voss; DQ059281, DQ028451, sequences downloaded from GenBank. Elytraria acaulis Lindau; FJ868940, FJ868920; Goyoler & Paton 3,520 (BR); Malawi; H. Elytraria bromoides Oerst.; FJ868929, FJ868914; T.F. Daniel 10,310 (CAS); Yucatán, Mexico; S. Elytraria bromoides Oerst.; FJ868930, —; T.F. Daniel 10,505 (CAS); Yucatán, Mexico; S. Elytraria caroliniensis (J.F. Gmel.) Pers.; FJ868936, FJ868918; 19712929-00cv, cultivated at BR (southeastern United States); S. Elytraria imbricata (Vahl) Pers.; FJ868928, FJ868913; T.F. Daniel 10,108 (CAS); Santa Cruz, Bolivia; S. Elytraria imbricata (Vahl) Pers.; FJ868927, —; T.F. Daniel 10,131 (CAS); Santa Cruz, Bolivia; S. Elytraria imbricata (Vahl) Pers.; FJ868924, FJ868910; T.F. Daniel 10,501 (CAS); Yucatán, Mexico; S. Elytraria imbricata (Vahl) Pers.; FJ868925, FJ868911; T.F. Daniel s.n. (CAS); Trinidad; S. Elytraria imbricata (Vahl) Pers.; FJ868923, —; E.J. Lott 5,175 (CAS); Texas, United States; H. Elytraria imbricata (Vahl) Pers.; FJ898626, FJ868912; Van Proosij et al. 719 (NY); Paradera, Aruba; H. Elytraria macrophylla Leonard; FJ868933, —; Rzedowski 43,302 (CAS); Querétaro, Mexico; H. Elytraria marginata Vahl; FJ868937, —; 19860332cv, cultivated at BR (Democratic Republic of Congo); S. Elytraria mexicana Fryxell & S.D. Koch; FJ868931, FJ868915; T.F. Daniel 5,284 (CAS); Colima, Mexico; H. Elytraria minor O.B. Dokosi; FJ868938, —; Faulkner 1,783A (BR); Tanzania; H. Elytraria nodosa E. Hossain; FJ868939, FJ868919; T.F. Daniel et al. 10,452 (CAS); Antsiranana, Madagascar; S. Elytraria planifolia Leonard; FJ868934, FJ868916; R.A. Howard et al. 341 (NY); Villa Clara Province, Cuba; H. Elytraria prolifera Leonard; FJ868935, FJ868917; Plantae Indiae Occidentalis 8,877 (NY); Ile La Gunave, Haiti; H. Nelsonia canescens (Lam.) Spreng.; FJ868932, —; T.F. Daniel et al. 5,452cv, cultivated at the San Francisco Conservatory of Flowers (Panama); S. Ophiorrhiziphyllon macrobotryum Kurz; —, FJ868908; J.F. Maxwell 91-213 (CAS); Mae Hong Song, Thailand; H. Staurogyne concinnula (Hance) O. Kuntze; FJ868922, FJ868909; Bartholomew & Boufford 6,215 (CAS); Taipei Hsien, Taiwan; H. Elytraria acaulis Lindau: tropical and southern Africa, Indian subcontinent

Appendix II

Species of *Elytraria* and their native distributions.

Elytraria bissei H. Dietr.: Cuba
Elytraria bromoides Oerst.: North America, northern Central America
Elytraria caroliniensis (Gmel.) Pers.: southeastern USA
Elytraria cubana Alain: Cuba
Elytraria filicaulis Borhidi & O. Muniz: Cuba
Elytraria imbricata (Vahl) Pers.: North America, Central America, South America
Elytraria ivorensis Dokosi: tropical western Africa
Elytraria klugii Leonard: Peru
Elytraria lyrata Vahl: tropical Africa
Elytraria macrophylla Leonard: Mexico
Elytraria madagascariensis (R. Ben.) E. Hossain: Madagascar
Elytraria marginata Vahl: tropical western and central Africa
Elytraria maritima J.K. Morton: tropical western Africa
Elytraria mexicana Fryxell & S.D. Koch: Mexico
Elytraria minor Dokosi: tropical eastern Africa
Elytraria nodosa E. Hossain: Madagascar
Elytraria planifolia Leonard: Cuba
Elytraria prolifera Leonard: Haiti
Elytraria shaferi (P. Wilson) Leonard: Cuba
Elytraria spathulifolia Borhidi & O. Muniz: Cuba
Elytraria tuberosa Leonard: Ecuador

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