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PHYLOGENY OF *DYSCHORISTE* (ACANTHACEAE)

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ABSTRACT

The pantropical and poorly known genus *Dyschoriste* (Acanthaceae) is sister to *Strobilanthes* within subtribe Petalidiinae. The present study included 38 accessions of 28 species as sources of DNA data for one nuclear (nrITS) and four chloroplast (intergenic spacers: *psbA-trnH*, *trnS-trnG*, *ndhF-rpl32*, *rpl32-trnL*^(uag)) regions to provide an estimate of the phylogeny of the genus. We found that *Dyschoriste* is strongly supported as monophyletic inclusive of *Apassalus*, *Chaetacanthus*, and *Sautiera*. Within *Dyschoriste*, three geographically cohesive lineages were recovered with moderate to strong support: a mainland African clade, a Caribbean and southeastern United States clade, and a South and Central America clade. A third New World clade composed of accessions from the south central through southwestern US to Mexico is weakly supported and corresponds to the *D. linearis* species complex recognized by previous researchers (six of the ten taxa putatively part of this complex were sampled). A second Old World clade unites taxa from across the Old World tropics (mainland Africa, Madagascar and southeast Asia). Some aspects of relationships among these main clades were unresolved or not strongly supported, and two Old World taxa, south Asian *D. dalzellii* and the wide-ranging *D. nagchana*, were not placed with confidence in any of these clades. The simplest explanation for the current distribution of the genus is that there was a single dispersal event of *Dyschoriste* from the Old to the New World, with a subsequent radiation in the New World.

Key words: Acanthaceae, *Apassalus*, *Chaetacanthus*, *Dyschoriste*, *Dyschoriste linearis* species complex, *Sautiera*.

INTRODUCTION

Dyschoriste Nees is a genus of Acanthaceae with ca. 80 species that are distributed in the tropics and subtropics of the Americas, Africa, and Asia (Fig. 1). The genus as a whole is thus pantropical, with slightly more than half of the described species (ca. 45) in the New World (NW). Within the Americas, the genus is distributed across the southern United States (US; ca. 7 species), throughout the Caribbean (ca. 3 species), Mexico (ca. 20 species), Central America (ca. 5 species), and South America (ca. 20 species). Old World (OW) species are found in Africa (ca. 20 species), Madagascar (ca. 8 species), and southwest (SW) to southeast (SE) Asia (ca. 7 described plus at least 3 undescribed species).

Dyschoriste is composed of herbaceous perennials or shrubs with opposite, entire (rarely crenulate) leaves. The dichasial inflorescences develop in leaf axils and are sometimes congested in a spicate or capitate thyrse; more rarely, flowers are solitary in leaf axils. The flowers are bracteate and have a five-lobed calyx and corolla with left-contort aestivation. The purple, white, or rarely red, corollas are sympetalous and may have weakly or strongly differentiated upper and lower lips. Stamens are didynamous with an appendage on each theca (lost in some taxa); pollen is triaperturate with sexine lips and 0–48 pseudocolpi (Furness

1995; Tripp et al. 2013: Fig. 14 BB). The style is unequally bilobed and persistent. Four (sometimes two) seeds from four ovules are matured per capsule; these are covered in hygroscopic trichomes.

Some *Dyschoriste* are difficult to identify to species because they seem to have few distinguishing characters. Perhaps reflective of this lack of clear morphological patterns, infrageneric taxa have not been designated. There is no comprehensive monograph for the genus, although Kobuski (1928b) published a monograph of American *Dyschoriste*, recording 40 NW species. *Dyschoriste* has also been included in many floristic and taxonomic treatments in the OW and NW (Long 1970; Daniel 1984, 1995, 2013; Wasshausen 1998; Henrickson 1999; Daniel and Acosta 2003; Vollesen 2008). New species of *Dyschoriste* have recently been described and species delineations continue to be investigated (Gentry 1948; Raizada and Bennet 1983; Ramamoorthy and Wasshausen 1985; Daniel 1990, 1996; Wasshausen and Wood 2003; Thulin 2005; Malombe et al. 2006; Tripp et al. 2013).

Although *Dyschoriste* is one of the largest genera in Ruellieae (Tripp et al. 2013), its morphological diversity and geographic range have not yet been densely sampled in a molecular phylogenetic study. The only phylogenetic study to focus on *Dyschoriste* analyzed nrITS and *trnG-trnR* sequences from 11 species in the US, Mexico, and the Caribbean, and eight species from other areas (24% of the species in the genus; Andrews 2009, unpublished thesis, Eberhard Karls University, Tübingen, Germany). Andrews (2009) found strong support for monophyly of the genus, but there was little to no support for relationships within the genus. Five species of *Dyschoriste* (6% of the species in the



Fig. 1. Map showing the pantropical distribution of the genus *Dyschoriste* (Acanthaceae).

genus) were sampled in a recent phylogenetic study of Ruellieae (Tripp et al. 2013); these were placed within subtribe Petalidiinae of Ruellieae, together with *Strobilanthis* S. Moore, *Duosperma* Dayton, *Petalidium* Nees, *Phaulopsis* Willd., and *Ruelliopsis* C.B. Clarke. Plants of Petalidiinae tend to have anthers with basal appendages (i.e., *Duosperma*, *Dyschoriste*, *Strobilanthis*), four or fewer ovules per ovary, and seeds with hygroscopic trichomes (Tripp et al. 2013). Species of *Dyschoriste* can be distinguished from other Petalidiinae by a combination of characters (see Fig. 2 A–C) including proximally fused setaceous calyx lobes with hyaline tissue connecting the lobes and four stamens (reduced to two in a few species) with a conspicuous appendage at the base of each theca (lost in some OW and NW taxa). Tripp et al. (2013) stated that anther appendages are also found in other subtribes of Ruellieae (i.e., Trichantherinae, Mimulopsinae) and in genera that are of uncertain placement in Ruellieae (i.e., *Diceratotheca*, *Echinacanthus*, *Sinoacanthus*, *Stenothyrsus*). *Strobilanthis* was strongly supported as sister to *Dyschoriste*, *Apassalus* Kobuski, and *Sautiera* Decne. in Tripp et al.'s (2013) study. Plants of these genera have four stamens with basal thecal appendages (minute in *Strobilanthis*), and four-seeded capsules. *Strobilanthis* can be readily differentiated from the other three genera by calyx lobes that are free to the base (versus at least partially fused in *Dyschoriste*, *Apassalus*, and *Sautiera*).

Tripp et al.'s (2013) study of Ruellieae found that *Dyschoriste* was paraphyletic to *Apassalus* with weak support and that *Sautiera* was strongly supported as sister to these genera together. Because *Sautiera* and *Apassalus* also have morphological affinities to *Dyschoriste*, the authors transferred these genera to *Dyschoriste*. *Apassalus* was originally described by Kobuski (1928a) based on the absence of anther appendages common in *Dyschoriste*. Decaisne (1834) described *Sautiera* as a monotypic genus only two years after Nees described *Dyschoriste* (in 1832), based mainly on the presence of strongly bilabiate corollas in *Sautiera*. Tripp et al. (2013) found that, although the monophyly of *Dyschoriste*, including *Apassalus*, was strongly supported, a Shimodaira-Hasegawa test failed to reject the alternative of a monophyletic *Dyschoriste* that excludes *Apassalus*. More sequence data and denser

taxon sampling are necessary to clarify relationships among *Dyschoriste*, *Apassalus*, and *Sautiera*.

In addition to *Apassalus* and *Sautiera*, some authors have treated *Chaetacanthus* Nees as part of *Dyschoriste* (Kuntze 1981; Scotland and Vollesen 2000; Balkwill 2012). *Chaetacanthus* is a South African genus of ca. four species that has at times been treated as distinct from *Dyschoriste* (Clarke 1901; Dyer and Milne-Redhead 1934) based on the presence of two (*Chaetacanthus*) versus four stamens (*Dyschoriste*). Although most species of *Dyschoriste* have four fertile stamens, some species occasionally have two fertile stamens and two staminodes (Vollesen 2008; Balkwill 2012). *Chaetacanthus* pollen morphology is similar to *Dyschoriste* (Furness 1995), and other macromorphological traits that are key characters for *Dyschoriste* are also present in *Chaetacanthus* (e.g., anther appendages and calyx lobes with hyaline tissue). For these reasons, Balkwill (2012) provided new combinations for all species of *Chaetacanthus* in *Dyschoriste*. The present study samples *Chaetacanthus* for the first time in a molecular phylogenetic analysis with the goal of testing Balkwill's (2012) taxonomic treatment of these plants.

Several taxa of *Dyschoriste* from the south central and SW US (i.e., Oklahoma, Texas, New Mexico, Arizona) and Mexico seem to intergrade and have been collectively referred to as the *D. linearis* species complex. Since 1928, multiple taxonomic concepts have been proposed for the *D. linearis* complex (Kobuski 1928b; Henrickson 1999; Turner 2003; Daniel 2013), but these concepts have generally been limited in their geographic scope. At various times, the *D. linearis* species complex has been hypothesized to include *D. cinerascens*, *D. crenulata* Kobuski, *D. decumbens*, *D. linearis* var. *linearis*, *D. linearis* var. *sanpatriciensis* Henr., *D. schiedeana* var. *schiedeana*, and *D. schiedeana* var. *prostrata*. Additionally, some Mexican populations of *D. decumbens* show morphological affinities to *D. microphylla* (Daniel and Acosta 2003). Henrickson (1999) also included *D. greenmanii* Kobuski and *D. poliodes* Leonard & Gentry in the complex. Other species of *Dyschoriste* in the US (e.g., *D. angusta*, *D. humistrata*, *D. oblongifolia*) or Mexico (e.g., *D. angustifolia*, *D. hirsutis-*

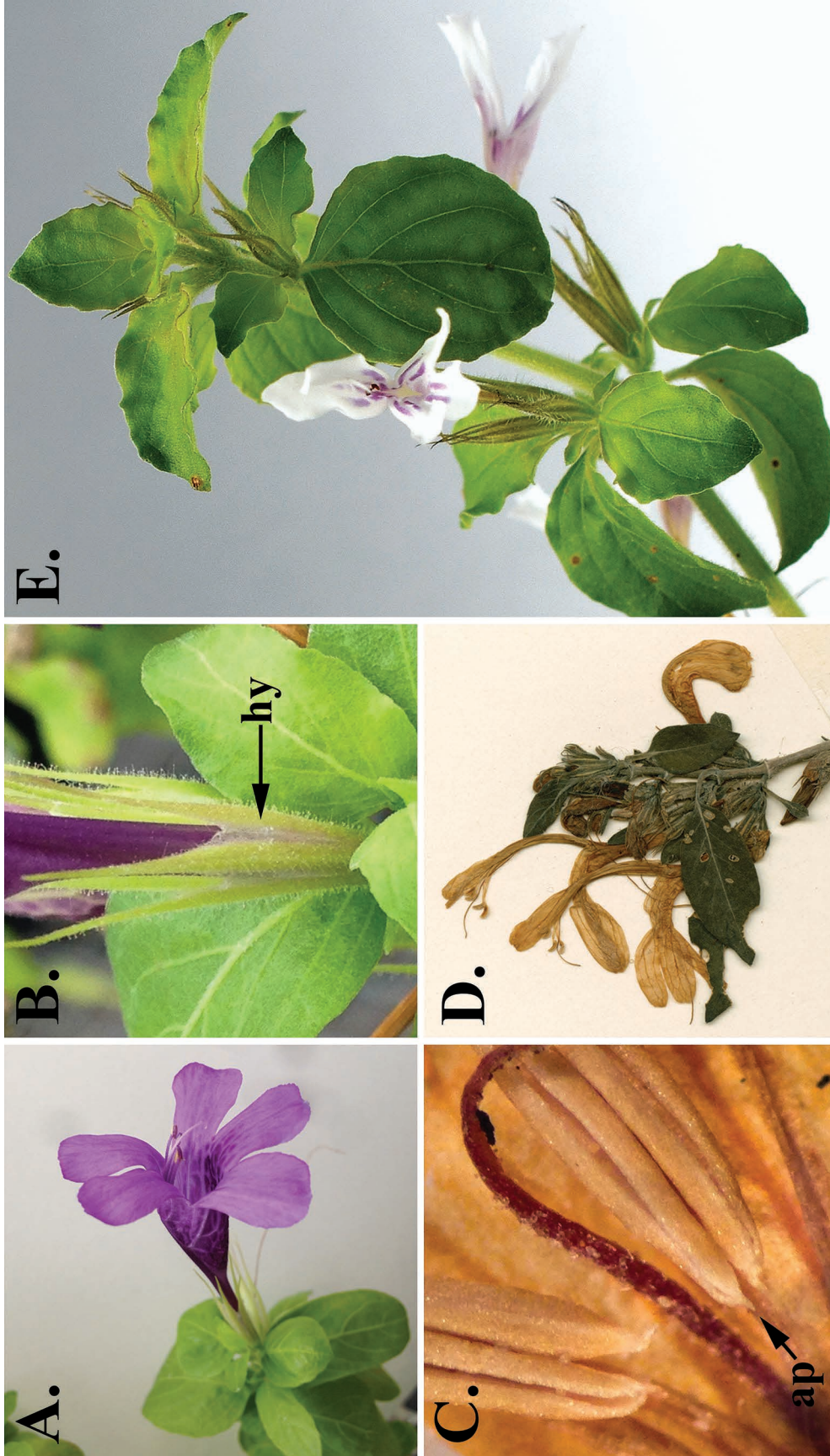


Fig. 2A-E. Morphology of *Dyschoriste*.—A-B. *Dyschoriste thunbergiflora* (Tripp & Ly 937 [RSA-POM], RSABG greenhouses).—A. Side view of flower.—B. Calyx with hyaline tissue (hy; labeled with arrow).—C. *Dyschoriste erecta* C.B. Clarke (Daniel et al. 9378 [US], South Africa) showing anther appendages (ap; labeled with arrow).—D. *Dyschoriste tinctorum* (syn. *Sautiera tinctorum*) (R. Cinatti 302 [L], Timor).—E. *Dyschoriste setigera* (syn. *Chaetacanthus setiger*) (Daniel et al. 9321 [CAS], South Africa). Photograph of *D. setigera* in the RSABG greenhouses by Hester Bell.

sim, *D. mcvaughii* T.F. Daniel, *D. salutensis* Fernald) do not appear to be part of the *D. linearis* species complex. Evolutionary relationships among the ten taxa hypothesized to make up the *D. linearis* species complex and between these and other *Dyschoriste* remain unclear and phylogenetic approaches are needed to clarify relationships.

This is the first phylogenetic study focused on *Dyschoriste* with the objective of testing previous hypotheses of relationships between *Dyschoriste* and close relatives in Petalidiinae, and estimating relationships within the genus. We sampled a total of 38 accessions of *Dyschoriste* s.l. (including two *Apassalus*, three *Chaetacanthus*, and one *Sautiera*), with emphasis on the NW taxa and the *D. linearis* complex in particular. To address biogeographic patterns, the 38 sampled accessions included ten taxa from Africa and three accessions from Asia. Our goals were (1) to further refine the placement of *Dyschoriste* in Petalidiinae; (2) to test the monophyly of *Dyschoriste* s.l., including recently synonymized genera (*Apassalus*, *Chaetacanthus*, *Sautiera*); (3) to test the composition of the *D. linearis* species complex by sampling six of ten taxa potentially in the complex along with 12 other NW taxa; and (4) to assess whether there is a geographic signal to evolutionary relationships in *Dyschoriste* at the continent level.

MATERIALS AND METHODS

Taxon Sampling

To understand relationships among *Dyschoriste*, *Apassalus*, *Sautiera*, and *Chaetacanthus*, a total of 38 accessions were sampled; these represent 28 species (35%) of the currently recognized species of *Dyschoriste* s.l. and included two accessions of *Apassalus*, one of *Sautiera*, and three of *Chaetacanthus*. Samples representing all five of the other genera in subtribe Petalidiinae were used as outgroups: *Duosperma*, *Petalidium*, *Phaulopsis*, *Ruellioopsis*, and *Strobilanthis*. Within the *D. linearis* complex, we sampled ten accessions representing six taxa to test whether the complex is monophyletic and to estimate relationships among these taxa. Finally, to examine biogeographic patterns, samples from across the entire distribution of the genus were included (Appendix 1).

Molecular Methods

Silica-gel dried leaves were used for DNA extraction whenever possible, but herbarium specimens were also sampled with permission (BCU, CAS, J, MO, NY, RSA-POM, US). In a few cases, DNA sequences were downloaded from Genbank (Appendix 1). Total genomic DNA was extracted and cleaned using DNeasy Plant Mini Kits (Qiagen, Valencia, CA) following the manufacturer's instructions, or via a modified CTAB protocol (Doyle and Doyle 1987).

Seven chloroplast (cp) markers (*rps16* intron; *ndhF-rpl32*, *rpl32-trnL^(uag)*, *trnL-trnF*, *trnL-trnT*, *trnS-trnG*, *psbA-trnH* intergenic spacers) were screened for six to eight *Dyschoriste* species in an effort to identify regions with sufficient variation for phylogenetic analysis. We also assembled a *trnG-trnR* dataset from Genbank. All of these loci have been used successfully in other studies of Acanthaceae (McDade et al. 2000, 2005; Kiel et al. 2006; Tripp 2007; Daniel et al. 2008; Tripp et al. 2013; Kiel and McDade 2014). In addition to the

cp loci, we amplified nrITS (ITS1, 5.8S, ITS2) and a fragment of the nuclear-encoded, chloroplast-expressed glutamine synthetase gene (*nepGS*), following the methods of Emshwiller and Doyle (1999) and Kiel et al. (2014). Four cp regions were identified as likely to be sufficiently variable for use within *Dyschoriste*: *psbA-trnH*, *trnS-trnG*, *ndhF-rpl32*, and *rpl32-trnL^(uag)*. As for the nuclear loci, both *nepGS* and nrITS were highly variable, but it was difficult to PCR-amplify *nepGS* (< 30% success). There was gel electrophoresis evidence that two copies of *nepGS* were amplified such that cloning would be necessary to obtain data for this region. Therefore, nrITS was the only nuclear locus sampled in this study.

Amplification of cp and nuclear regions used Go-Taq FlexiTM DNA polymerase (Promega, Madison, WI), except that nrITS required Phusion High-Fidelity DNA polymerase for a few recalcitrant accessions (Thermo Scientific Inc., Waltham, MA). The chloroplast intergenic spacer regions were amplified following the thermocycler conditions for *trnS-trnG* given in McDade et al. (2005). The *ndhF-rpl32* and *rpl32-trnL^(uag)* regions were amplified using primers 5' *ndhF* + 3' *rpl32-R* and 5' *rpl32-F* + 3' *trnL^(uag)*, respectively (Shaw et al. 2007). Some accessions of *Dyschoriste* were difficult to amplify and so we designed a new *Dyschoriste*-specific primer pair: *rpl32-R-Dys* (5'-CCARTGCCCTTYTITTTCCAA-3') and *rpl32-F-Dys* (5'-CAGTTCCAAAAAACGCATTT C-3') to replace *rpl32-R* and *rpl32-F*, respectively. The *trnS-trnG* and *psbA-trnH* regions were amplified using primers 5' *trnS* + 3' *trnG* (Hamilton 1999) and 5' *psbA-F* + 3' *trnH-R*, respectively (Sang et al. [1997], with modifications as in Tripp [2010]). The nrITS region was successfully amplified in two parts (ITS1 and ITS2) using ITS-A + ITS-C and ITS-E + ITS-B (Blattner 1999) as internal and external primer pairs after many other primers and primer combinations were trialed. For some accessions that were difficult to amplify, touchdown PCR (Don et al. 1991) was used, following Andrews (2009).

Amplified PCR products were purified using PEG precipitation. DNA sequences were generated using an ABI 3100 Genetic Analyzer (Applied Biosciences/Fisher Scientific, Waltham, MA) at Rancho Santa Ana Botanic Garden. Both forward and reverse strands were sequenced in order to reconcile base calls.

Alignment and Analysis

All DNA sequences were assembled, edited, aligned, and concatenated using Geneious 7.0.6 (Biomatters, Auckland, New Zealand). Sequences were aligned using the Geneious alignment plugin, followed by manual editing. Portions of the sequence data that could not be confidently aligned were removed from analyses. Alignments were exported as NEXUS files and indels from all regions were coded as binary characters following the simple indel coding method (Kelchner 2000; Simmons and Ochoterena 2000).

Models of evolution were selected using the Akaike Information Criterion (AIC) in jModelTest 2.0.6 (Guindon and Gascuel 2003; Darriba et al. 2012). The cp and nrITS datasets were evaluated separately and GTR+I+G was selected as the best model for both datasets.

Six data matrices (cp, nuclear, cp + nuclear; with and without coded indels) were analyzed under the optimality criterion of maximum likelihood (ML; Felsenstein 1981) using

Table 1. Summary of five loci used in this study.

	<i>psbA-trnH</i>	<i>trnS-trnG</i>	<i>ndhF-rpl32</i>	<i>rpl32-trnL</i> ^(uag)	nrITS
Aligned length	604	959	745	702	786
Variable characters (%)	95 (15.7%)	109 (11.4%)	80 (10.7%)	73 (10.4%)	164 (20.9%)
Parsimony-informative characters (%)	38 (6.3%)	34 (3.6%)	29 (3.9%)	34 (4.8%)	83 (10.6%)
Parsimony-informative coded indels	5	9	3	5	1

GARLI 2.01 (Zwickl 2006). Bootstrap values (BS; Felsenstein 1985) were used to indicate strength of clade support and were calculated using PAUP*4.0a136 (Swofford 2003).

The six datasets were also analyzed in a Bayesian inference (BI) framework using MrBayes 3.2.2 (Ronquist et al. 2012). A partitioned GTR+I+G model was used to estimate parameters for the cp and nuclear datasets and a one-rate model was used for the indel partition. Two simultaneous runs of four Monte Carlo Markov chains were analyzed for 10 million generations for the combined cp + nuclear dataset and 5 million generations for individual cp and nuclear datasets with trees saved every 1000 generations. The burn-in was set to 25%, and a 50% majority rule consensus tree was calculated with posterior probability values (PP).

Dataset Congruence

The cp and nuclear datasets were tested for congruence using the Incongruence Length Difference test (ILD; Farris et al. 1994) implemented in PAUP*4.0a136 as the partition homogeneity test with 1000 replicates and 10 random additions. The ILD test has been shown to be overly conservative (Barker and Lutzoni 2002; Darlu and Lecointre 2002) and so we also looked for evidence of strongly supported conflict between the cp and nuclear datasets in the ML bootstrap and BI consensus tree topologies for each dataset.

Hypothesis Testing

We tested 15 alternative hypotheses to the results of our ML phylogeny estimate:

- (H₁) *Dyschoriste*, *Apassalus*, and *Sautiera* are together monophyletic, exclusive of *Chaetacanthus*;
- (H₂) *Dyschoriste*, *Sautiera*, and *Chaetacanthus* are together monophyletic, exclusive of *Apassalus*;
- (H₃) *Dyschoriste*, *Apassalus*, and *Chaetacanthus* are together monophyletic, exclusive of *Sautiera*;
- (H₄) two sampled species of *Apassalus* are sister;
- (H₅) three sampled species of *Chaetacanthus* are together monophyletic;
- (H₆) NW species are monophyletic;
- (H₇) Caribbean + SE US, south central + SW US to Mexico species are together monophyletic;
- (H₈) Asian species (including *D. nagchana*) are monophyletic;
- (H₉) south + SE Asian species are monophyletic (excluding *D. nagchana*, the range of which extends to mainland Africa);
- (H₁₀) OW *D. nagchana* + *D. dalzellii* are sister (both are OW but closely related to NW species);
- (H₁₁) *D. gracilicaulis*, *D. mutica*, *D. sp. 681*, *D. tinctorum* and *D. nagchana* are together monophyletic (the

geographically heterogeneous clade and the Asian and African species, *D. nagchana*);

- (H₁₂) *D. gracilicaulis*, *D. mutica*, *D. sp. 681*, *D. tinctorum* and *D. dalzellii* are together monophyletic (the geographically heterogeneous clade and the Asian species, *D. dalzellii*);
- (H₁₃) mainland African species are monophyletic (including *D. nagchana*);
- (H₁₄) mainland African species are monophyletic (excluding *D. nagchana*);
- (H₁₅) south central + SW US to Mexico clade is monophyletic, exclusive of *D. angustifolia*.

We constructed constraint trees in Mesquite 2.75 (Maddison and Maddison 2011) and estimated constrained ML trees in RAxML (Stamatakis 2014) under the same model as for the unconstrained ML analysis. The constrained ML trees were independently compared to the unconstrained ML trees using a Shimodaira-Hasegawa test (SH; Shimodaira and Hasegawa 1999) in RAxML vers. 8 (Stamatakis 2014).

RESULTS

Dataset Congruence

The combined cp and nuclear alignment of 43 accessions consisted of 3796 bases with 218 parsimony-informative sites, 23 coded indels, and 7.3% missing data (Table 1). Notably, *Dyschoriste* [*Sautiera*] *tinctorum* was only sampled with a *trnS-trnG* sequence generated by Tripp et al. (2013). An approximately 97-base section of ITS1 was excluded from analyses because the alignment was ambiguous. The ILD test found that the cp and nuclear datasets were incongruent ($p < 0.05$), but the only strongly supported conflict in the ML and BI trees involved placement of *D. repens* (Fig. 3); this taxon was removed from subsequent analyses.

The ML and consensus BI trees, whether with or without coded indels, resulted in similar topologies, but the BI tree was more resolved than the ML tree. The only well-supported difference between the BI and ML trees was that *D. dalzellii* was sister to the NW lineage with strong support (PP ≥ 0.95) in the BI tree (Fig. 3) whereas the placement of this taxon was unresolved within *Dyschoriste* in the ML tree (Fig. 4). We focus on the results of the BI analysis from the combined cp and nuclear data with coded indels below, noting differences with the ML tree when relevant (Fig. 3).

Phylogenetic Relationships

Strobilanthisopsis is strongly supported (PP ≥ 0.95 or BS $\geq 70\%$) as sister to *Ruelliaopsis* in the BI and ML trees, and *Phaulopsis* is strongly supported as sister to *Petalidium* + *Duosperma* (Fig. 4). *Dyschoriste*, *Apassalus*, *Sautiera*, and *Chaetacanthus* form a monophyletic group with strong support

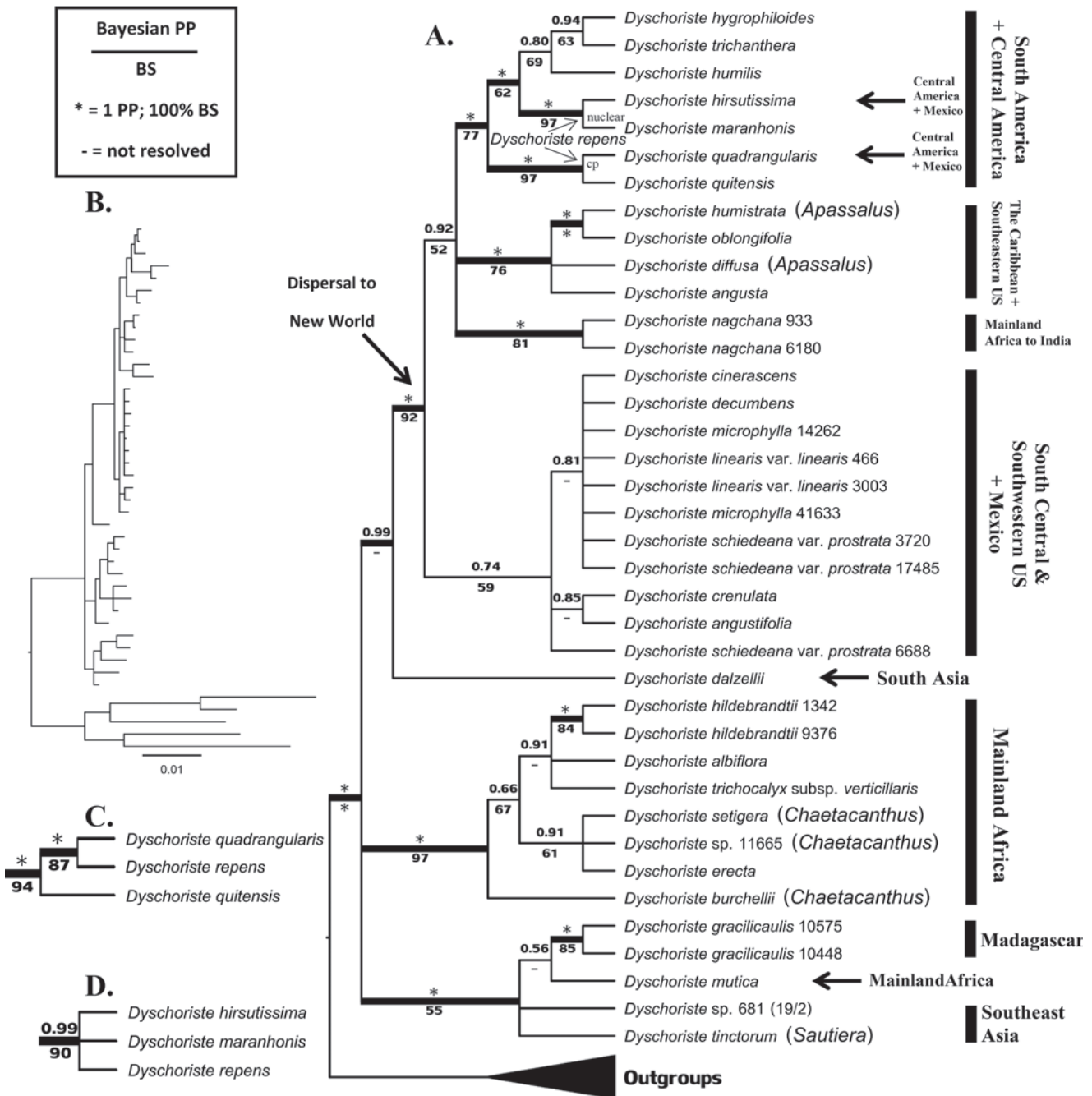


Fig. 3A–D. Results of the Bayesian analysis.—A. Bayesian inference consensus tree estimated with the combined cp + nuclear + coded indels dataset. Thick branches are strongly supported (PP \geq 95 and/or ML BS \geq 70%). Previous generic names are in brackets.—B. BI phylogram from the combined cp + nuclear dataset with coded indels.—C. Placement of *D. repens* by the cp dataset.—D. Placement of *D. repens* by the nuclear dataset. We inferred that dispersal to the NW occurred on the branch leading to the most recent common ancestor of the NW *Dyschoriste* species. This is the simplest explanation for the current distribution of species and would require at least one dispersal back to Africa (*D. nagchana*). There are other possible dispersal scenarios, such as dispersal to the NW by the ancestor of the south central and southwestern US and Mexico lineage and another dispersal to the NW in the ancestor of the other NW clade after *D. nagchana* diverged.

(1 PP; 100% BS). Our data reject exclusion of *Chaetacanthus* and *Apassalus* from this clade but cannot reject exclusion of *Sautiera* (H_1 , H_2 , H_3 , respectively, Table 2).

The sampled accessions formerly treated as *Apassalus* and *Chaetacanthus* are nested deeply among accessions of *Dyschoriste*. Neither of these former genera is monophyletic and our data reject monophyly of *Apassalus* but not of *Chaetacanthus* (H_4 , H_5 , respectively; Table 2). *Dyschoriste tinctorum* (former-

ly *Sautiera*) is placed near the base of *Dyschoriste* but it does not resolve as sister to the rest of *Dyschoriste*. However, as noted above, monophyly of *Dyschoriste*, *Apassalus*, and *Chaetacanthus* exclusive of *Sautiera* could not be rejected by our data (H_2 , Table 2).

Within *Dyschoriste*, there are three major clades (BI tree, Fig. 3) or three clades and a single species (ML tree, Fig. 4) that are unresolved along the backbone of the tree. South

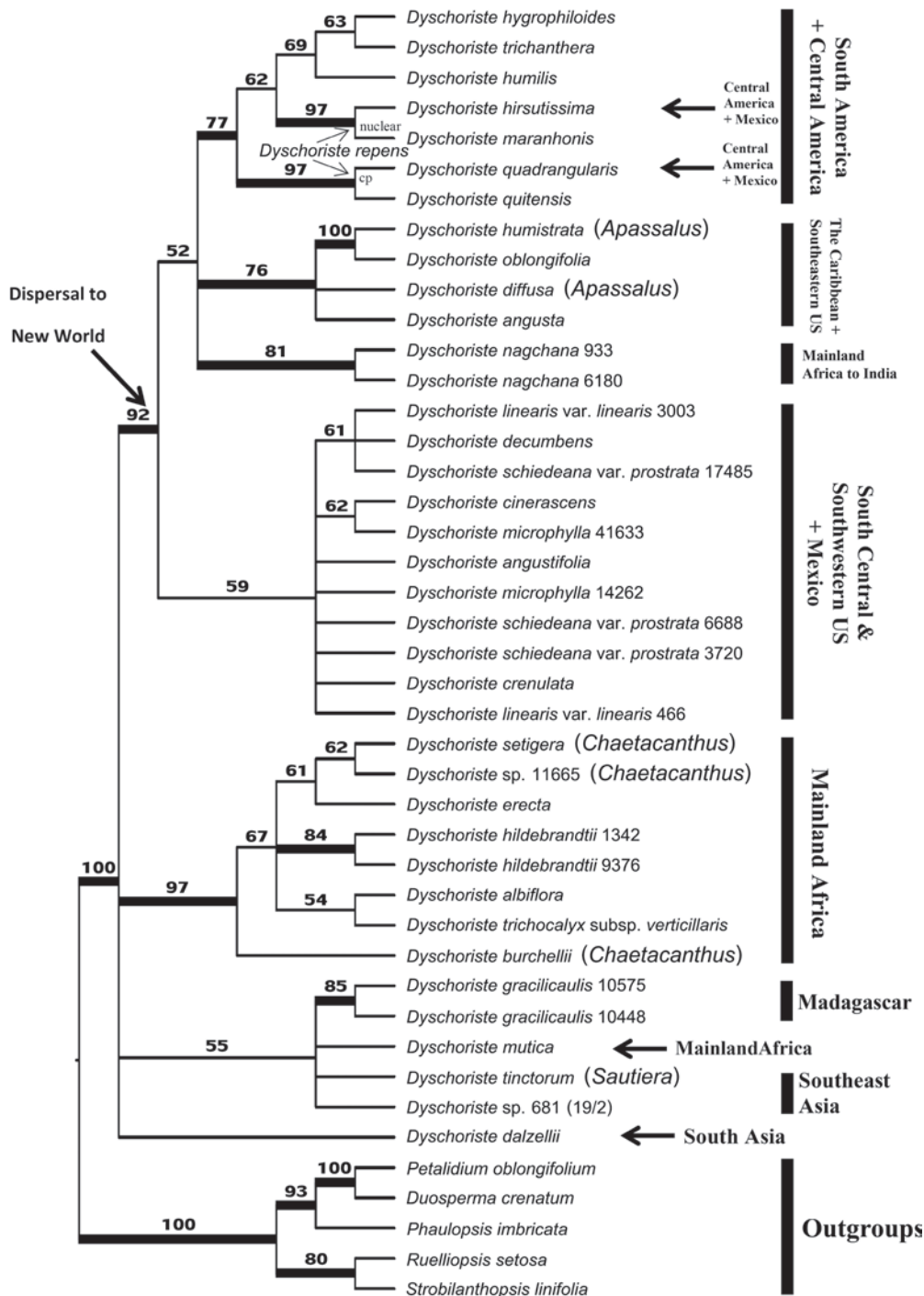


Fig. 4. Maximum likelihood 50% majority rule consensus cladogram from GARLI analyses of combined cp + nuclear dataset with coded indels. Thick branches are strongly supported (BS ≥ 70%). Previous generic names are in brackets. The placement of *D. repens* in the separate ML analyses of the cp versus nuclear datasets was congruent with placements in the BI trees from those datasets shown in Fig. 3C, D.

Asian *D. dalzellii* is unresolved within *Dyschoriste* in the ML tree (Fig. 4), but the BI analysis strongly supports it as sister to the New World clade (0.99 PP; Fig. 3). Two major clades are strongly supported in both the BI and ML trees, one comprised of seven of nine sampled mainland African species (1 PP; 97% BS) and the other of NW species plus the wide-ranging *D. nagchana* (mainland Africa to India; 1 PP; 92%

BS). Regarding the latter, our data cannot reject monophyly of the NW species exclusive of *D. nagchana* (H_6 , Table 2). Three lineages within the NW clade were resolved: (1) a weakly supported south central + SW US to Mexican lineage (0.74 PP; 59% BS); (2) a Caribbean + SE US lineage (1 PP; 76% BS); and (3) a mainly South + Central American lineage (1 PP; 77% BS). The south central + SW US to Mexican lineage and the

Table 2. Results of hypothesis testing.

	Hypothesis	-lnL unconstrained	-lnL constrained	-lnL difference	Reject? (<0.05)
H_1	<i>Dyschoriste</i> , <i>Apassalus</i> , and <i>Sautiera</i> are together monophyletic, exclusive of <i>Chaetacanthus</i>	-10100.323049	-10137.577044	-37.253995	Yes**
H_2	<i>Dyschoriste</i> , <i>Sautiera</i> , and <i>Chaetacanthus</i> are together monophyletic, exclusive of <i>Apassalus</i>	-10100.323049	-10160.734296	-60.411246	Yes**
H_3	<i>Dyschoriste</i> , <i>Apassalus</i> , and <i>Chaetacanthus</i> are together monophyletic, exclusive of <i>Sautiera</i>	-10100.323049	-10105.470606	-5.147557	No
H_4	Sampled species of <i>Apassalus</i> (2) are sister	-10100.323049	-10124.470843	-24.147793	Yes*
H_5	<i>Chaetacanthus</i> (3) is monophyletic	-10100.323049	-10109.531223	-9.208174	No
H_6	NW species are monophyletic	-10100.323049	-10104.051779	-3.728730	No
H_7	Caribbean + southeastern US and south central + southwest US to Mexico species are monophyletic	-10100.323049	-10105.004912	-4.681863	No
H_8	Asian species are monophyletic, inclusive of <i>D. nagchana</i>	-10100.323049	-10164.450414	-64.127365	Yes**
H_9	Asian species are monophyletic, exclusive of <i>D. nagchana</i>	-10100.323049	-10111.567071	-11.244022	No
H_{10}	<i>D. nagchana</i> + south Asian <i>D. dalzellii</i> are sister	-10100.323049	-10129.329590	-29.006541	Yes*
H_{11}	Geographically heterogeneous clade (<i>D. gracilicaulis</i> , <i>D. mutica</i> , <i>D. sp.</i> 681, <i>D. tinctorum</i>) + <i>D. nagchana</i> are together monophyletic	-10100.323049	-10162.731014	-62.407964	Yes**
H_{12}	Geographically heterogeneous clade (<i>D. gracilicaulis</i> , <i>D. mutica</i> , <i>D. sp.</i> 681, <i>D. tinctorum</i>) + <i>D. dalzellii</i> are together monophyletic	-10100.323049	-10106.665837	-6.342788	No
H_{13}	Mainland African species are monophyletic, inclusive of <i>D. nagchana</i>	-10100.323049	-10181.655287	-81.332238	Yes**
H_{14}	Mainland African species are monophyletic, exclusive of <i>D. nagchana</i>	-10100.323049	-10120.390019	-20.066970	No
H_{15}	South central and southwest US + Mexico clade is monophyletic, exclusive of <i>D. angustifolia</i>	-10100.323049	-10104.071575	-3.748526	No

* reject at 5% level. ** reject at 1% level.

Caribbean + SE US lineage are not sister in the BI (Fig. 3) or ML trees (Fig. 4), but our data cannot reject the hypothesis that the clades are sister (H_7 , Table 2).

One of the three unresolved clades along the backbone is strongly supported only in the BI tree (1 PP; 55% BS). This geographically heterogeneous clade contains the SE Asian *D. tinctorum* (formerly *Sautiera*), an undescribed species from SE Asia (*D. sp.* 681), the African *D. mutica*, and the Malagasy *D. gracilicaulis*.

The Asian *Dyschoriste* species are not monophyletic as the four sampled species resolved in three distant clades. Two Asian species are part of the geographically heterogeneous clades just described, whereas the South Asian *D. dalzellii* is sister to the NW lineage in the BI estimate (Fig. 3). The distribution of *D. nagchana* ranges from Africa to India, but it resolved in the NW clade instead of being closely related to the other OW taxa. Monophyly of Asian species including *D. nagchana* was strongly rejected by our data (H_8 , Table 2), but monophyly of Asian species exclusive of *D. nagchana* could not be rejected (H_9 , Table 2). In contrast, our data reject a sister relationship between the two phylogenetically isolated Asian species, *D. nagchana* and *D. dalzellii* (H_{10} , Table 2). Inclusion of *D. nagchana* in the geographically heterogeneous clade that includes *D. tinctorum*, *D. sp.* 681, *D. mutica*, and *D. gracilicaulis* was rejected by our data (H_{11} , Table 2), but monophyly of the geographically heterogeneous clade + *D. dalzellii* could not be rejected (H_{12} , Table 2).

The mainland African species are also not monophyletic. *Dyschoriste mutica* and *D. nagchana* are distantly related to the mainland African lineage and our data reject monophyly of the mainland African clade + *D. nagchana* (H_{13} , Table 2), but could not reject monophyly of the mainland African clade + *D. mutica* (H_{14} , Table 2).

The six sampled accessions hypothesized to be part of the *D. linearis* complex form a weakly supported and poorly

resolved clade that is sister to the other NW clades and includes *D. cinerascens*, *D. crenulata*, *D. decumbens*, *D. linearis* var. *linearis*, *D. microphylla*, *D. schiedeana* var. *prostrata*, and *D. angustifolia*. Our data could not reject monophyly of the first six taxa just listed, with the last, *D. angustifolia*, sister to the complex (H_{15} , Table 2).

DISCUSSION

We estimated the phylogeny of the large, pantropical genus *Dyschoriste* by sampling approximately 35% of the species in the genus plus five representative outgroups. An accession of *D. repens* from Venezuela was originally included in the analysis, but its placement in the NW clade conflicted in initial analyses of cp and nrITS data sets. Chloroplast data placed *D. repens* sister to *D. quadrangularis* (Fig. 3C), whereas nrITS placed *D. repens* in a polytomy with *D. maranhonis* and *D. hirsutissima* (Fig. 3D). These results suggest a hybrid origin for *D. repens*, a hypothesis that warrants further testing.

Our study provides evidence that *Dyschoriste* is monophyletic and includes *Apassalus*, *Sautiera*, and *Chaetacanthus* (Fig. 3). We found strong support for relationships among the other genera in Petalidiinae (Fig. 4) but, except for the sister relationship between *Duosperma* and *Petalidium*, these relationships are in conflict with the topology found by Tripp et al. (2013). This conflict may be caused by our relatively sparse sampling from these outgroup genera in comparison to Tripp et al.'s (2013) more comprehensive sample. Our datasets were also not identical in terms of loci sampled (both studies: nrITS, *psbA-trnH*, *trnS-trnG*; Tripp et al. (2013): *trnG-trnR*, *Eif3E*; present study: *ndhF-rpl32*, *rpl32-trnL*). Future studies on Petalidiinae should focus on testing relationships among these genera.

The inclusion of *Apassalus*, *Sautiera*, and *Chaetacanthus* in *Dyschoriste* suggests that there is no phylogenetic basis for

recognizing any of these three segregate genera in support of the opinions of others regarding *Chaetacanthus* (Kuntze 1891; Scotland and Vollesen 2000; Balkwill 2012), as well as the taxonomic changes made by Tripp et al. (2013). Tripp et al. (2013) found that *Apassalus* is nested within *Dyschoriste* with strong support and proposed new nomenclatural combinations to transfer the four species of *Apassalus* into *Dyschoriste*. Interestingly, *D. humistrata* and *D. diffusa*, formerly treated as *Apassalus*, are not sister taxa in our tree (Fig. 3) although they are closely related in the Caribbean + SE US clade. An SH test rejected a tree that constrains them to monophyly. These two species, along with the Caribbean species *D. cubensis* Urb. (not sampled), had been treated in *Dyschoriste* until Kobuski (1928a) moved them to his newly described genus *Apassalus* based on lack of basal appendages on the anthers. Several authors have not accepted *Apassalus* (Long 1970; Wasshausen 1998; Greuter and Rodríguez 2010) because presence of basal appendages is homoplasious across *Dyschoriste* (e.g., appendages are also absent in *D. mutica* [S. Moore] C.B. Clarke [not sensu C.B. Clarke in Fl. Cap. 5: 16 (1901) as noted in Fl. Trop. E. Africa, Acanthaceae part 1: 188 (2008)] and sometimes in *D. mcvaughii* [Daniel 1990]).

Tripp et al. (2013) also transferred the Timor-endemic monotypic genus *Sautiera* (Fig. 2D) to *Dyschoriste* as *D. tinctorum* because they found it was sister to the five sampled members of *Dyschoriste* and morphologically consistent with the description of *Dyschoriste*. We were only able to include Tripp et al.'s (2013) *trnS-G* sequence of *D. tinctorum* in our dataset and we found that *D. tinctorum* is part of the geographically heterogeneous clade that also includes the unidentified SE Asian accession *D. sp. 681*, *D. mutica*, and *D. gracilicaulis* (Fig. 3). However, an SH test indicates that our data could not reject the monophyly of *Dyschoriste*, *Apassalus*, and *Chaetacanthus*, exclusive of *D. tinctorum*, probably due to missing sequence data for four of the five loci for this last taxon. Additional molecular data from *D. tinctorum* and inclusion of more accessions of *Dyschoriste* species from SE Asia and Madagascar may further refine relationships among *D. tinctorum* and other *Dyschoriste*, but our increased species sampling in OW *Dyschoriste* bolsters the argument of Tripp et al. (2013) that *Sautiera* should be included in *Dyschoriste*.

Species formerly in *Chaetacanthus*, including *D. setigera*, *D. burchellii*, and an undescribed species identified as *Chaetacanthus* sp. by Balkwill (Balkwill et al. 11665) resolved in a clade of mainland African *Dyschoriste* species. In the BI and ML trees (Fig. 3, 4), *D. burchellii* is sister to the rest of the mainland African lineage, whereas the other two *Chaetacanthus* accessions plus *D. erecta* C.B. Clarke (not *D. erecta* [Burm. f.] Kuntze from South Africa) form a weakly supported polytomy (0.91 PP; 61% BS). An SH test could not reject an alternative hypothesis of a monophyletic *Chaetacanthus*. Clarke (1901) and Dyer and Milne-Redhead (1934) distinguished *Chaetacanthus* from *Dyschoriste* on the basis of its two fertile stamens + two staminodes (vs. four fertile stamens). We expected *Chaetacanthus* to be monophyletic because these androecial traits plus > 8 pseudoapertures on the pollen mesocolpium (vs. < 8 in "typical" *Dyschoriste*) are potentially synapomorphic characters (Furness 1995); also species of *Chaetacanthus* are restricted to southern Africa. However, these characters seem to be homoplasious. Furness (1995) noted that, like *Chaetacanthus*, at least one specimen of

D. erecta had 8–13 pseudoapertures on each pollen mesocolpium, in contrast to < 8 pseudoapertures found in other specimens of *D. erecta*. Additionally, several other mainland African *Dyschoriste* species (*D. nagchana*, *D. radicans*) typically have four stamens, but occasional specimens have two fertile stamens and two staminodes, as occurs in *Chaetacanthus* (Vollesen 2008).

With the addition of *Apassalus*, *Chaetacanthus*, and *Sautiera*, the description of *Dyschoriste* does not substantially change, although pollen should now be described as 3(4)-colporate with sexine lips and 0–48 pseudocolpi (Scotland 1993; Furness 1995; Palacios-Chavez 1996; Daniel 1998; Greuter and Rodríguez 2010; Tripp et al. 2013; Al-Hakimi and Latiff 2015). Pseudocolpi are rarely relatively short or absent in some species of *Dyschoriste*, including those formerly placed in *Sautiera* and *Chaetacanthus* (Scotland 1993; Furness 1995). The basic chromosome number of the genus is most likely $x = 15$, as many NW species (Grant 1955; Daniel et al. 1990; Piovano and Bernardello 1991; Daniel 2000) are $n = 15$, and $n = 30$ has been recorded for the Asian *D. depressa* Nees (Saggio and Bir 1982) and the Mexican *D. hirsutissima* (Nees) Kuntze (Daniel et al. 1990).

The unresolved backbone of *Dyschoriste* includes two clades that generally correspond to the species' geographic distributions (African and NW clades), as well as a third, geographically heterogeneous, OW clade, and the south Asian *D. dalzellii* (resolved as sister to the NW clade by BI). The geographically heterogeneous clade includes two SE Asian species (*D. tinctorum* and *D. sp. 681*), the mainland African *D. mutica*, and the Malagasy *D. gracilicaulis*. The four sampled Asian *Dyschoriste* species (*D. tinctorum*, *D. sp. 681*, *D. dalzellii*, *D. nagchana*) thus do not form a clade and an SH test rejects their monophyly, but an SH test cannot reject monophyly when the wide-ranging *D. nagchana* (Africa to south Asia) is excluded. We also found that an SH test could not reject inclusion of the south Asian *D. dalzellii* in the geographically heterogeneous clade with *D. gracilicaulis*, *D. mutica*, *D. sp. 681* and *D. tinctorum*. The mainland African species of *Dyschoriste*, except *D. mutica* (placed in the heterogeneous OW clade) and *D. nagchana* (nested within the NW clade), are monophyletic. An SH test rejects the monophyly of African species inclusive of these last two species, but monophyly is not rejected if *D. nagchana* is excluded. The alternative hypotheses that *D. nagchana* is sister to the Indian species *D. dalzellii* or that *D. nagchana* is sister to the Asian species were also rejected by our data. Taken together, these results for the two species whose ranges include south Asia suggest that *D. dalzellii* may be of SE Asian origin, but do not shed light on the place of origin of *D. nagchana*. Especially as both of our accessions of this last taxon were from Africa, further study with greater intraspecific sampling is warranted.

There are three main lineages within the NW: (1) a south central + SW US to Mexican lineage, (2) a South + Central American lineage (including *D. hirsutissima* and *D. quadrangularis* which are widely distributed in Central America and Mexico), and (3) a Caribbean + SE US lineage. The discontinuous distribution of *Dyschoriste* in the US (no plants have been found in the Mississippi Valley) is reflected in the placement of these species in two distinct clades; however, our data could not reject a sister relationship for these clades.

The clade from the south central and SW US and Mexico (the *D. linearis* species complex) is weakly supported (Fig. 3; 0.74 PP; 59% BS). Poor resolution in this group may be the result of low sequence variation among these taxa in our dataset, as indicated by extremely short branches in the BI phylogram (Fig. 3B) and it suggests ongoing introgression or that these are recently diverged taxa still undergoing lineage sorting. The *D. linearis* complex, inclusive of *D. cinerascens* and *D. microphylla*, forms a polytomy that corresponds well to previous hypotheses about the members of the species complex (Kobuski 1928b; Henrickson 1999; Daniel and Acosta 2003; Turner 2003; Daniel 2013). Our data confirm that four sampled US and Mexican species (*D. angusta*, *D. hirsutissima*, *D. humistrata*, *D. oblongifolia*) are not in the *D. linearis* species complex. Although it has never been hypothesized to be a member of the complex, *D. angustifolia* is embedded within the *D. linearis* species complex in the BI and ML trees, but our data cannot reject that it is sister to the complex. *Dyschoriste angustifolia* occurs in central Mexico and is unusual among *Dyschoriste* species in having red corollas with a relatively long floral tube that are likely adaptations to hummingbird pollination. In contrast, most species of *Dyschoriste* have blue or purple corollas and are thought to be bee or butterfly pollinated. Although *D. angustifolia* appears to have undergone a dramatic morphological shift compared to other members of the *D. linearis* species complex, floral morphology in other lineages of Acanthaceae has been shown to be especially labile in response to pollinator selection; e.g., the shift from insect to hummingbird pollination and corresponding morphological change has occurred at least eight times in the *Tetramerium* lineage (Daniel et al. 2008).

Taxa in the *D. linearis* species complex can be difficult to differentiate and taxonomic treatments have used a combination of habit, pubescence, leaf margin, leaf shape, leaf size, calyx size, flower size, and geographic distribution (Kobuski 1928b; Daniel 1984; Henrickson 1999; Daniel 2013). Some plants in the *D. linearis* species complex exhibit intermediate morphologies in locations where multiple taxa are sympatric. For example, plants in west Texas and SE New Mexico are intermediate between *D. cinerascens* and either *D. linearis* var. *linearis* or *D. decumbens* in terms of plant habit and pubescence (Daniel 1984; Henrickson 1999; Daniel 2013). From central Durango to San Luis Potosí and south into Guanajuato, Aguascalientes, Hidalgo, Puebla and Oaxaca, there is a broad morphological transition zone between *D. schiedeana* var. *decumbens*, *D. schiedeana* var. *schiedeana*, and *D. schiedeana* var. *prostrata* (Henrickson 1999). Additionally, Henrickson (1999) noted that one specimen of *D. greenmanii* Kobuski from Tamaulipas is pubescent throughout and has multiple flowers per node, whereas *D. greenmanii* is typically nearly glabrous with one flower per node. Henrickson (1999) suggested that these unusual characters may have been the result of introgression with *D. schiedeana* var. *schiedeana*. Finally, some populations of *D. decumbens*, with a more southerly distribution in Mexico, have affinities to *D. microphylla* (i.e., trichomes, leaf size; Daniel and Acosta 2003). Based on their close but largely unresolved relationships and the presence of intermediate characters as noted above, it is likely that hybridization and introgression occur between taxa in this clade and it seems appropriate to consider them members of a species complex.

Although we used five loci, we could not resolve relationships among these plants and further clarification of their relationships may be aided by morphometric studies and population-level molecular data.

Dyschoriste is pantropical (Fig. 1) with 45 currently recognized species in the Americas and 35 in Africa and Asia. The basal polytomy among the main clades of *Dyschoriste* restricts our ability to make inferences about where *Dyschoriste* originated, other than that the group is likely OW in origin. Our results (Fig. 3) suggest that *Dyschoriste* dispersed a single time from the OW to the NW and that the ancestor of *D. nagchana* may have dispersed back from the NW to the OW. However, our data could not reject that *D. nagchana* is sister to a monophyletic NW clade. On the other hand, our data reject hypotheses of a close relationship between *D. nagchana* and other OW plants, which would be expected if it represents a reverse dispersal from NW to OW. Clearly this hypothesis merits testing with additional data. A time-calibrated phylogeny of Acanthaceae (Tripp and McDade 2014) estimated that *Dyschoriste* diverged approximately 14.4 Mya (11.2–17.7 Mya, 95% highest posterior probability density). In the same paper, Tripp and McDade (2014) suggested that Acanthaceae may have dispersed from the OW to the NW via long-distance dispersal or the Beringian land bridge, but they argue that the former is a more reasonable hypothesis. If our estimate of the species phylogeny (Fig. 3) is accurate, then *Dyschoriste* species have been able to disperse across oceanic barriers from the OW to NW (and potentially a return to the OW by the ancestor of *D. nagchana*), throughout the Antilles, and to Madagascar. Denser sampling of Malagasy and south Asian species will be necessary to understand biogeographic patterns in the OW, as well as to make inferences about the number of intercontinental dispersal events that have occurred in the history of this lineage.

The results of our study indicate that there are some well-supported subgeneric relationships within *Dyschoriste* and these clades generally contain species from the same continent. Other aspects of our results, such as the relationships among clades and the placement of some OW species, may be improved by additional taxon sampling and additional molecular data. Our knowledge of the NW lineages would be improved by resolving relationships among *D. nagchana* and the NW clades, as well as relationships in the *D. linearis* species complex to better understand the pattern of evolution in *Dyschoriste* in Mexico and the US. In particular, resolution of the *D. linearis* species complex will likely require population-level work.

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APPENDIX 1

Voucher of accessions included in this study and Genbank numbers (*psbA-trnH*, *trnS-trnG*, *ndhF-rpl32*, *rpl32-trnL*^(uag), nrITS, - = sequence not obtained). Taxa are ordered alphabetically from outgroups to the ingroup (*Apassalus* to *Sautiera*).

OUTGROUP

Duosperma crenatum P.G. Mey., South Africa, McDade et al. 1280 (RSA); KR230810, KR230851, KR230892, KR230932, KR230973. *Petalidium oblongifolium* C.B. Clarke, South Africa, Daniel et al. 9374 (CAS); KR230811, KR230852, KR230893, KR230933, KR230974. *Phaulopsis imbricata* (Forssk.) Sweet, Ethiopia, Tripp & Kelbessa 917 (RSA); KR230812, KR230853, KR230894, KR230934, KR230975. *Ruelliospis setosa* C.B. Clarke, Namibia, Tripp & Dexter 4128 (RSA); KR230813, KR230854, KR230895, KR230935, KR230976. *Strobilanthis linifolia* (T. Anderson ex C.B. Clarke) Milne-Redh., Zambia, Smith 632 (MO); JX443894, JX444048, -, -, JX443810.

INGROUP

Apassalus: *Dyschoriste diffusa* (Nees) Urb., Dominican Republic, Garcia et al. 4412 (US); KR230814, KR230855, KR230896, KR230936, -. *Dyschoriste humistrata* (Michx.) Kuntze, Florida, Anderson 11026 (RSA); KR230815, KR230856, KR230897, KR230937, KR230977.

Chaetacanthus: *Dyschoriste* sp., South Africa, Balkwill et al. 11665 (J); KR230816, KR230857, KR230898, KR230938, KR230978. *Dyschoriste burchellii* (Nees) Kuntze, South Africa, Daniel et al. 9335 (CAS); KR230817, KR230858, KR230899, KR230939, KR230979. *Dyschoriste setigera* (Pers.) J.C. Manning & Goldblatt, South Africa, Daniel 9321 (CAS); KR230818, KR230859, KR230900, KR230940, KR230980.

Dyschoriste: *Dyschoriste* sp., Thailand, Herb. Tr. 681 (19/2) (BCU); KR230819, KR230860, KR230901, KR230941, KR230981. *Dyschoriste albiflora* Lindau, Zambia, Luwiika et al. 580 (MO); KR230820, KR230861, KR230902, KR230942, KR230982. *Dyschoriste angusta* (A. Gray) Small, Florida, Herndon 2582 (RSA); KR230821, KR230862, KR230903, KR230943, KR230983. *Dyschoriste angustifolia* (Hemsl.) Kuntze, Mexico, Phillips et al. 1028 (CAS); KR230822, KR230863, KR230904, KR230944, KR230984. *Dyschoriste cinerascens* (Henr. & Hilsenb.) T.F. Daniel, Texas, Daniel 114 (CAS); KR230823, KR230864, -, KR230945, KR230985. *Dyschoriste crenulata* Kobuski, N/A, Johnston 4382 (TEX); -, -, -, FJ605209. *Dyschoriste dalzellii* (T. Anderson) Kuntze, India, H. S. 5650 (RSA); KR230824, KR230865, KR230905, KR230946, -. *Dyschoriste decumbens* (A. Gray) Kuntze, Arizona, Makings & Setaro 3800 (RSA); KR230825, KR230866, KR230906, KR230947, KR230986. *Dyschoriste erecta* C.B. Clarke, South Africa, Daniel et al. 9378 (US); KR230826, KR230867, KR230907, KR230948, -. *Dyschoriste gracilicaulis* (Benoist) Benoist, Madagascar, Daniel 10448 (CAS); KR230827, KR230868, KR230908, KR230949, KR230987. *Dyschoriste gracilicaulis* (Benoist) Benoist, Madagascar, Daniel & Ranarivelo 10575 (CAS); KR230828, KR230869, KR230909, KR230950, KR230988. *Dyschoriste hildebrandtii* (S. Moore) S. Moore, South Africa, Daniel et al. 9376 (US); KR230829, KR230870, KR230910, KR230951, KR230989. *Dyschoriste hildebrandtii* (S. Moore) S. Moore, Southern Rhodesia [Zimbabwe], Plawes 1342 (NY); KR230830, KR230871, KR230911, KR230952, KR230990. *Dyschoriste hirsutissima* (Nees) Kuntze, Mexico, Daniel et al. 8559 (CAS); KR230831, KR230872, KR230912, KR230953, KR230991. *Dyschoriste humilis* Lindau, Argentina, Burkart 22.309 (RSA); KR230832, KR230873, KR230913, KR230954, KR230992. *Dyschoriste hygrophiloides* (Nees) Kuntze, Bolivia, Wood 9471 (US); KR230833, KR230874, KR230914, KR230955, KR230993. *Dyschoriste linearis* (Torr. & A. Gray) Kuntze, Texas, Flyr 466 (CAS); KR230834, KR230875, KR230915, KR230956, KR230994. *Dyschoriste linearis* (Torr. & A. Gray) Kuntze, Texas, Hutchins 3003 (RSA); KR230835, KR230876, KR230916, KR230957, -. *Dyschoriste maranthonis* Kuntze, Brazil, Hatschbach & Guimarães 45130 (US); KR230836, KR230877, KR230917, KR230958, KR230995. *Dyschoriste microphylla* Kuntze, Mexico, Rzedowski 41633 (CAS); KR230837, KR230878, KR230918, KR230959, -. *Dyschoriste microphylla* Kuntze, Mexico, Zamudio & Beltrán R. 14262 (CAS); KR230838, KR230879, KR230919, KR230960, KR230996. *Dyschoriste*

- mutica* (S. Moore) C.B. Clarke, Tanzania, *Biggood et al. 2092* (CAS); KR230839, KR230880, KR230920, KR230961, KR230997. *Dyschoriste nagchana* (Nees) Bennet, Burkina Faso, *Madsen 6180* (NY); KR230840, KR230881, KR230921, KR230962, KR230998. *Dyschoriste nagchana* (Nees) Bennet, Ethiopia, *Tripp & Kelbessa 933* (RSA); KR230841, KR230882, KR230922, KR230963, KR230999. *Dyschoriste oblongifolia* (Michx.) Kuntze, Florida, *Daniel 11763* (CAS); KR230842, KR230883, KR230923, KR230964, KR231000. *Dyschoriste quadrangularis* (Oerst.) Kuntze, Honduras, *Daniel 9551* (CAS); KR230843, KR230884, KR230924, KR230965, KR231001. *Dyschoriste quitensis* (Kunth) Kuntze, Peru, *Jenkins 00-156* (CAS); KR230844, KR230885, KR230925, KR230966, KR231002. *Dyschoriste repens* (Nees) Kuntze, Venezuela, *Tripp & Lujan 516* (CAS); KR230845, KR230886, KR230926, KR230967, KR231003. *Dyschoriste schiedeana* var. *prostrata* Henr., Arizona, *Daniel & Baker 3720* (CAS); KR230846, KR230887, KR230927, KR230968, KR231004. *Dyschoriste schiedeana* var. *prostrata* Henr., Mexico, *Henrickson 6688* (RSA); KR230847, KR230888, KR230928, KR230969, KR231005. *Dyschoriste schiedeana* var. *prostrata* Henr., Mexico, *Henrickson & Lee 17485* (RSA); KR230848, KR230889, KR230929, KR230970, KR231006. *Dyschoriste trichanthera* Kobuski, Bolivia, *Wood et al. 13154* (CAS); KR230849, KR230890, KR230930, KR230971, KR231007. *Dyschoriste trichocalyx* subsp. *verticillaris* (C.B. Clarke) Vollesen, Tanzania, *Faden et al. 961375* (US); KR230850, KR230891, KR230931, KR230972, KR231008.
- Sautiera: Dyschoriste tinctorum* (Decaisne) E. Tripp & T.F. Daniel, Timor, *Schmutz 2939* (L); -, JX444043, -, -, -.