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Resolving incongruence: Species of hybrid origin in *Columnea* (Gesneriaceae)

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ABSTRACT

Speciation by hybridization has long been recognized among plants and includes both homoploid and allopolyploid speciation. The numbers of presumed hybrid species averages close to 11% and tends to be concentrated in a subset of angiosperm families. Recent advances in molecular methods have verified species of hybrid origin that had been presumed on the basis of morphology and have identified species that were not initially considered hybrids. Identifying species of hybrid origin is often a challenge and typically based on intermediate morphology, or discrepancies between molecular datasets. Discrepancies between data partitions may result from several factors including poor support, incomplete lineage sorting, or hybridization. A phylogenetic analysis of species in *Columnea* (Gesneriaceae) indicated significant incongruencies between the cpDNA and nrDNA datasets. Tests that examined whether one or both of the datasets had the phylogenetic signal to reject the topology of the alternate dataset (Shimodaira and Hasegawa [SH] and approximately unbiased [AU] tests) indicated significant differences between the topologies. Splitstree analyses also showed that there was support for the placement of the discrepant taxa in both datasets and that the combined data placed the putative hybrid species in an intermediate position between the two datasets. The genealogical sorting index (GSI) implied that coalescence in nrDNA had occurred in all species where more than a single individual had been sampled, but the GSI value was lower for the cpDNA of most of the putative hybrids, implying that these regions have not yet coalesced in these lineages despite being haploid. The JML test that evaluates simulated species pairwise distances against observed distances also implies that observed nrDNA data generate shorter distances than simulated data, implying hybridization. It is most likely that *C. gigantifolia*, *C. rubriacuta*, and *C.* sp. nov. represent a lineage from a hybrid ancestor, but *C. moorei* may be a more recent hybrid and may still be undergoing hybridization with sympatric species.

1. Introduction

The process of speciation has long been a focus of evolutionary and systematic biology (Darwin, 1859; Mayr, 1942; Levin, 1978, 2000; Coyne and Orr, 2004; Lexer and Widmer, 2008; Givnish, 2010, 2015; Sochar et al., 2015; Kadereit, 2015; Arnold, 2016). Allopatric speciation is a well documented process and often is divided into vicariant speciation where landscapes change to isolate populations from each other (Pereira and Baker, 2004; Struwe et al., 2009; Bentley et al., 2014), or isolation by dispersal and founder effects (Glor et al., 2005; Smith et al., 2014; Schulte et al., 2015). More rapid means of generating species include hybridization either through homoploid or polyploid means and generally is estimated to be responsible for 11% of all species (Stebbins, 1959; Rieseberg et al., 1996a; Rieseberg, 1997, 2006; Wendel and Cronn, 2003; Soltis and Soltis, 2009; Abbott et al., 2013). Detecting hybrids is not always a simple task. Intermediacy in morphology has long been a staple for detecting hybrids, but it is now clear that hybrids may possess transitive morphologies: character states that are unknown in the parental species (Rieseberg and Ellstrand, 1993). For example, *Castilleja christii* N. H. Holmgren was recently documented to be a homoploid hybrid species (Clay et al., 2012), but was never suspected to be of hybrid origin. In

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large part the lack of suspicion stemmed from *C. christii* having primarily yellow bracts in contrast to the crimson and scarlet bracts of the parental species, *C. miniata* and *C. linariifolia*. *Castilleja christii* was shown to be a hybrid only when direct sequencing of low copy nuclear genes demonstrated multiple peaks in chromatograms that corresponded to the two separate peaks that were present in the parental copies of the homologous gene (Clay et al., 2012).

Modern means of detecting hybrids often occur when a species is placed in different clades when chloroplast and nuclear DNA data are analyzed independently (Smith and Sytsma, 1990; Rieseberg and Soltis, 1991; Rieseberg et al., 1996a or b; Baum et al., 1998; Wendel and Doyle, 1998; Linder and Rieseberg, 2004; Howarth and Baum, 2005; Friar et al., 2008; Rothfels et al., 2015; Walker et al., 2015). Discrepancies between data partitions have posed challenges to phylogenetic analyses since systematists started comparing more than a single dataset (Kluge, 1989; Smith and Sytsma, 1994; Mason-Gamer and Kellogg, 1996; Smith, 2000). Incongruences can occur from a multitude of causes and the greatest challenge is to resolve the source of the incongruency. One possibility is that the incongruence is the result of poor support in one or more of the partitions (Farris et al., 1994; Seelanan et al., 1997; Morrison, 2009). As a result, random noise and homoplasy may have as much influence on the resulting topology as the phylogenetic signal in the data. Such discrepancies are largely overcome with more data (sometimes by combining several datasets with weak, but essentially congruent signal (Smith, 2000) or data that has a higher proportion of phylogenetically informative characters (Small et al., 1998). In other cases the datasets may each strongly support conflicting relationships. In such cases, the challenge is to discover the biological explanation for the incongruency and requires knowledge beyond what is recovered in the phylogenetic analyses. In cases where there is potential for paralogy such as low copy nuclear genes, the inclusion of non-orthologous loci for some taxa will generate incongruency (Rokas et al., 2003). In these cases removing the paralogs may provide a simple answer as long as resolving which sequences are the ones in conflict can be identified (Schulte et al., 2015). In other cases, incongruencies can occur with loci that are either haploid (mitochondrial or chloroplast DNA regions) or are considered to be essentially single copy as the result of concerted evolution such as nuclear ribosomal RNA regions (Álvarez and Wendel, 2003; Feliner and Rosselló, 2007). Here, the challenge is to tease out whether the incongruency is the result of incomplete lineage sorting (Avise et al., 1983; Pamilo and Nei, 1988; Doyle, 1992; Maddison, 1997; Rosenberg, 2002, 2003), or hybridization (Alice et al., 2001; Martinsen et al., 2001; Lumaret and Jabbour-Zahab, 2009; Pirie et al., 2009; Jabaily and Sytsma, 2010) because the patterns can be similar (Holder et al., 2001).

Resolving between incomplete lineage sorting and hybridization is not a trivial task and in many studies where discrepancies are detected, authors often resort to ad hoc explanations about the potential geographic overlap that may bias or preclude hybridization, and current population size or time since divergence from a common ancestor to bias or preclude incomplete lineage sorting (Wendel and Doyle, 1998; Comes and Abbott, 2001; Jabaily and Sytsma, 2010). A growing number of studies to date are attempting to analyze data to discriminate between these factors (Buckley et al., 2006; Kubatko and Degnan, 2007; Holland et al., 2008; Maureira-Butler et al., 2008; Joly et al., 2009; Pirie et al., 2009; Polihronakis, 2009; Willyard et al., 2009; Pelser et al., 2010; de Viliers et al., 2013; Yu et al., 2011, 2013; Kuppler et al., 2015).

In the present study, chloroplast DNA and nuclear ribosomal DNA gave supported conflicting placement for four species in *Columnea* (Gesneriaceae). Neither of these is likely to include paralogs although it should be noted that evidence for incomplete concerted evolution in nrDNA has been documented in *Aeschynanthus* (Denduangboripant and Cronk, 2000). Therefore we evaluate whether incomplete lineage sorting or hybridization may be most prevalent. We examined our data using several alternative analytical approaches including using Shimodaira and Hasegawa (SH; Shimodaira and Hasegawa, 1999) and approximately unbiased (AU; Shimodaira, 2002) tests and two coalescent approaches. The first used the genealogical sorting index (GSI, Cummings et al., 2008) that evaluates whether a pre-defined clade is monophyletic even when it may not be recovered as monophyletic in standard phylogenetic analyses. Clades that are monophyletic are more likely to have achieved coalescence and therefore are less likely to reflect incomplete lineage sorting (Palumbi et al., 2001; Hedrick, 2007; de Viliers et al., 2013).

Joly et al. (2009) and Joly (2012) developed the software JML to detect whether intraspecific variation in a gene that produces a discrepant relationship relative to another gene is likely the result of incomplete coalescence. If incomplete lineage sorting can be eliminated, the probability that the incongruency is the result of hybridization is increased. The software uses a posterior distribution of species trees, simulates gene trees and DNA sequences, then calculates the minimum distance between simulated sequences for all pairs of species. These are then compared to the empirical data. If the observed distances are smaller than the simulated distances, then hybridization is a better explanation to account for the more recent common ancestry of the sequences than incomplete lineage sorting.

The taxonomic focus of the present study is the genus *Columnea* (Gesneriaceae). *Columnea* is a genus of over 209 species that has been the focus of several recent phylogenetic investigations to re-evaluate the subgeneric classification system and to explore character state evolution (Smith et al., 2013; Schulte et al., 2014, 2015). Preliminary analyses that included two individuals of *C. rubriacuta* (Wiehler) L.P. Kvist & L.E. Skog, and one cultivated individual of *C. moorei* C.V. Morton, had indicated these species were incongruently placed in the phylogeny using cpDNA or nrDNA. They were excluded from the analyses pending increased sampling or further analyses. Collections made in Colombia in 2013 afforded increased sampling of populations and individuals of *C. rubriacuta* and these additional samples resulted in similar incongruence that had been seen with the two previously sampled individuals. Additionally, previously unsampled *C. gigantifolia* L.P. Kvist & L.E. Skog and an undescribed species from Colombia showed a similar pattern of incongruence to that of *C. rubriacuta*. Interspecific hybridization is well documented in *Columnea* (Moore, 1954; Lee and Sherk, 1963; Sherk and Lee, 1967; Morley, 1971, 1975, 1976; Saylor, 1971; Byrne and Morley, 1976; Wiehler, 1976, 1983; Smith, 1991, 1994) and naturally occurring hybrids have been speculated on, but to date, have only been documented with morphological and cytological data between the Jamaican species *C. urbanii* W.T. Stearn and *C. rutilans* Swartz (Morley, 1971) and could not reject a hybrid origin of *C. querceti* Oerst. (Byrne and Morley, 1976). Here we examine the potential hybrid origin of *C. rubriacuta, C. gigantifolia, C.* sp. nov., and *C. moorei* using phylogenetic analyses of DNA sequences and coalescent analyses to assess the degree to which the different DNA sequence partitions have achieved coalescence within each of the species.

2. Materials and methods

2.1. Taxon sampling, DNA extraction, amplification, and alignment

A complete list of samples, voucher specimens, and GenBank numbers for all sequences used in all analyses is in Appendix A. The majority of species and accessions sampled is based on earlier phylogenetic analyses of the genus. Collections of *C. gigantifolia* and *C.* sp. nov. were opportunistic collections made in 2013 in

Colombia. All available accessions of *C. rubriacuta* were sampled. These included numerous collections made in Colombia in 2013 and all available collections made by J. L. Clark including one accession of *Columnea albovinosa* (Freiberg) J.L. Clark & L.E. Skog that is often annotated as a heterotypic synonym of *C. rubriacuta*. *Columnea moorei* was sampled using one cultivated specimen and a recently collected sample from the wild. The recent collection of *C. moorei* from the wild also afforded the opportunity to collect, and include all other species found in sympatry with *C. moorei* in Panama.

DNA was extracted from silica-dried leaf material using Qiagen DNeasy plant mini kits (Valencia, CA) according to manufacturer's instructions. The outgroup included four species of *Glossoloma* Hanst. that has been identified as the sister genus to *Columnea* (Clark et al., 2012). Our sampling relied on species that were sampled in previous studies (Smith et al., 2013; Schulte et al., 2014, 2015) but reduced the number of individuals for species that had previously been recovered as monophyletic. We also included *C. gigantifolia* (two individuals), *C.* sp. nov. (three individuals), *C. rubriacuta* (29 individuals), and *C. moorei* (two individuals) (Fig. 1). All samples were collected in the wild with the exception of one accession of *C. moorei* (*JLC 11307*) that was made from cultivated material., These latter four species were included because preliminary analyses had indicated discrepancies in their phylogenetic relationships between cpDNA and nrDNA.

The following five cpDNA gene regions were chosen: *trnQ-rps16* spacer (Shaw et al., 2007), *rpl32-trnL$_{UAG}$* spacer (Shaw et al., 2007), *rps16* intron (Oxelman et al., 1997), *trnS-G* spacer (Hamilton, 1999), and *trnH-psbA* spacer (Clark et al., 2006). The cpDNA gene regions were treated as a single partition because they are inherited as a single non-recombining unit. The two nuclear DNA gene regions, ITS (Baldwin et al., 1995) and ETS (Baldwin and Markos, 1998) were treated as a single partition. These datasets were analyzed separately as the cpDNA and nrDNA data, respectively, and as a concatenated dataset that included all regions for all samples. We were investigating the potential for hybrids in this study, therefore we also included analyses that had all cpDNA or all nrDNA for the individuals that were discrepant between the two datasets, but complete data for the remaining taxa. These are referred to as comb + cpDNA and comb + nrDNA, respectively herein.

Double-stranded DNA was amplified via PCR, following the methods of Smith et al. (1997). Sequences were obtained either through the methods of Smith et al. (2004) or through Genewiz (South Plainfield, NJ), chromatograms were viewed and sequences edited and aligned by hand in PhyDE (Müller et al., 2005).

2.2. Test of incongruence

The partition homogeneity test (PHT: Farris et al., 1994) was performed as implemented in PAUP* v4.0 b10 (Swofford, 2002) with 10,000 bootstrap replicates (using a heuristic search, simple addition, and no branch swapping). As an additional measure of congruence among partitions, bootstrap analyses were performed on each partition separately to assess areas of conflict and to determine if any conflict was strongly supported (>70% support; Seelanan et al., 1997). Specific sequences that could be individually identified as incongruent with other partitions were identified as potentially from hybrid species. The PHT was also performed using the same parameters on a dataset that excluded the putative hybrid taxa.

2.3. Phylogenetic analyses

Phylogenetic trees were estimated using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) for all datasets. Maximum parsimony analyses were performed using PRAP2 (Müller, 2004; using the default settings but uploading the nexus file for each dataset) in conjunction with PAUP* v4.0 b10 (Swofford, 2002). Bootstrap support (BS; Felsenstein, 1985) was estimated with 1000 heuristic replicates using PRAP2 (Müller, 2004). Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analysis were given by the consistency index (CI; Kluge and Farris, 1969), retention index (RI; Farris, 1989), and the resulting rescaled consistency index (RC; Farris, 1989). Maximum likelihood and ML bootstrapping was investigated using RaxML-HPC2 (Stamatakis, 2006; Stamatakis et al., 2008) on the CIPRES portal (Miller et al., 2010) using the defaults, but allowing bootstrap replicates to be terminated automatically.

Bayesian inference analyses were performed using optimal substitution models suggested by jModeltest 3.6 (Posada, 2008). The Akaike information criterion (AIC), which allows non-nested models to be evaluated, was used as a selection criterion (Posada and Buckley, 2004) for all datasets. All analyses were run with four chains, for ten million generations. Convergence was determined by viewing in Tracer v1.3 (Rambaut and Drummond, 2005), and a burnin of 50,000 generations was discarded prior to sampling the posterior distribution for all BI analyses. All BI analyses were repeated twice to ensure that parameter estimates converged to similar values. The separate runs were compared using the online version of Are We There Yet (AWTY; Nylander et al., 2008) as a means of determining if the separate chains approximated the same target distribution.

2.4. Estimating potential for hybridization

Initially we viewed the cpDNA, nrDNA and combined datasets using Splitstree (Huson, 1998; Morrison, 2009) to get an estimate of the support that was present in each data set. Network relationships for each of the datasets (cpDNA, nrDNA, concatenated) were examined in Splitstree using neighbornet optimization.

The SH and AU tests were performed with the same constraints. *Columnea gigantifolia*, *C.* sp. nov., *C. rubriacuta*, and *C. moorei* were all discrepant in their phylogenetic relationships between cpDNA and nrDNA. The placement of each of the species was constrained to the topology of one of the partitions and then tested against the data of the other with no other changes made in the topology of the tree. For example, the nrDNA tree was used, except *Columnea moorei* was constrained to be part of the section *Columnea* clade, where it was recovered from cpDNA data and then this topology was tested against the recovered nrDNA topology and SH (Shimodaira and Hasegawa, 1999) and AU (Shimodaira, 2002) tests were conducted. This was done for each of the four species separately and testing all topology/data combinations as well as constraining *C.* sp. nov., *C. gigantifolia*, and *C. rubriacuta* to be a single clade, and all four species to the topology of the other dataset. The SH tests were conducted in PAUP* using full optimization and 1000 replicates. The AU tests were conducted in Consel with the site likelihood values generated from PAUP*.

The GSI (Cummings et al., 2008) was run on both the nrDNA and cpDNA datasets separately as well as a concatenated dataset that either included missing values for the four discrepantly placed species for nrDNA (comb + cpDNA) or cpDNA (comb + nrDNA). *Columnea gigantifolia*, *C.* sp. nov., *C. rubriacuta*, *C. moorei, C. citriflora, C. dissimilis, C. strigosa*, and *C. minutiflora* were each tested in terms of monophyly. *Columnea citriflora, C. dissimilis, C. strigosa* and *C. minutiflora* were tested because we had multiple accessions and they serve as a means of evaluating the GSI results when a species is already expected to be monophyletic. An additional analysis was run that constrained *C. gigantifolia, C. rubriacuta*, and *C.* sp. nov. to be inclusively monophyletic. We used the online version

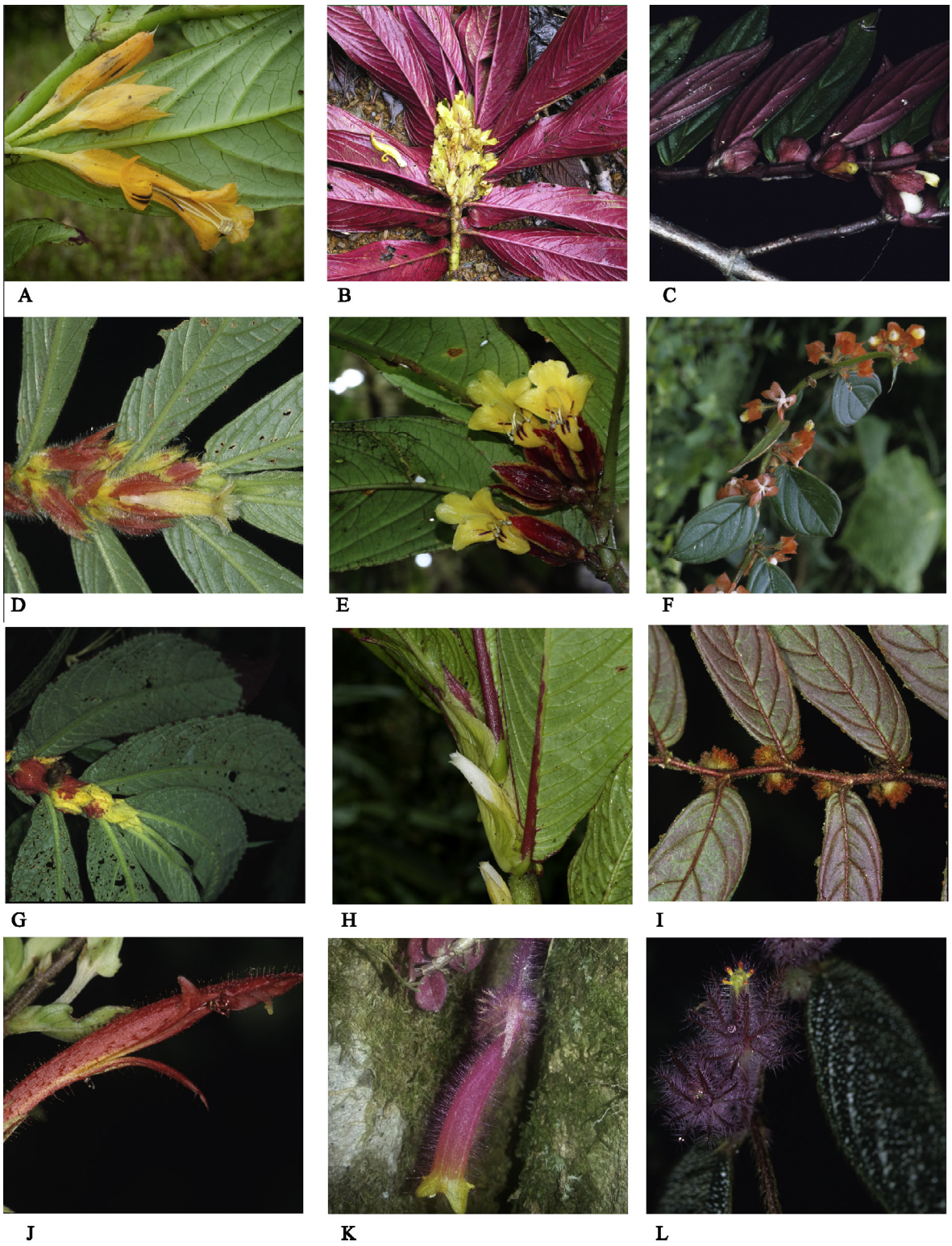

Fig. 1. Representive photos of the putative hybrids, B. *Columnea* sp. nov., E. *C. gigantifolia*, H. *C. rubriacuta*, and K. *C. moorei*. Representative photos of clade A; A. *C. picta*, D. *C. schimpfiii*, and G. *C. asteroloma*. Representative photos of clade C; C. *C. rubricalyx*, F. *C. herthae*, and I. *C. fimbricalyx*. Additionally, *C. minor* (L), and *C. scandens* as a representative of clade B. (J) both as putative parents of *C. moorei*.

(Cummings et al., 2008; http://molecularevolution.org/software/phylogenetics/gsi/citation). We manually pruned the results of the Bayesian analyses to the final 500 sampled trees to reduce computational time. The analyses used all trees for 10,000 permutations. The analyses produce a GSI value that ranges from 0 (not monophyletic) to 1 (monophyletic). Interpreting values between these two is not always clear and we set an a priori value of GSI > 0.80 to indicate monophyly.

To further discriminate between incomplete lineage sorting and hybridization we analyzed our data with JML (Joly, 2012). As input,

the analysis requires a posterior distribution of species trees generated from *BEAST (Heled and Drummond, 2010). We generated species trees for nrDNA and cpDNA with the same species definitions used for GSI for 10 million generations, selecting the "piecewise constant" option for the population size model as recommended by Joly (2012). We made comparisons across species trees (here actually gene trees because we analyzed each dataset separately) against the two data sets independently (i.e. cpDNA tree and simulated cpDNA data with observed cpDNA data as well as cpDNA tree and simulated cpDNA data with observed nrDNA data). The heredity scalar was set to 0.5 for the cpDNA simulated data and 2.0 for the nrDNA simulated data. The seqgencommand used the model that was recommended from jmodeltest for the observed cpDNA and nrDNA separately. All other factors used the default option. Because so many comparisons are made, caution must be used to interpret significance of the results. We follow the advice of Joly (2012) by evaluating only species-pair comparisons that are relevant to the discrepant taxa. Namely *Columnea gigantifolia, C. rubriacuta, C.* sp. nov. and *C. moorei* simulated pairwise distances vs. the observed pairwise distances in clades where they are placed with their cpDNA (*C. asteroloma, C. picta, C. eburnea, C. schimpfii,* and *C. densibracteata* for *C. gigantifolia, C. rubriacuta* and *C.* sp. nov.; *C. arguta, C. bilbergiana, C. gloriosa, C. microphylla* and *C. scandens* for *C. moorei*) or nrDNA (*C. minutiflora, C. herthae, C. lucifer, C. pygmaea, C. rubricalyx,* and *C. fimbricalyx* for *C. gigantifolia, C. rubriacuta* and *C.* sp. nov.; *C. minor* for *C. moorei*).

3. Results

3.1. Incongruence test

The PHT indicated significant incongruence between the cpDNA and nrDNA datasets when all taxa were included ($p < 0.001$), but not on the dataset that excluded the putative hybrid individuals ($p = 0.065$). A comparison of MP bootstrap support between the trees derived from the separate datasets indicated that the placement of *C. rubriacuta, C. gigantifolia*, *C.* sp. nov., and *C. moorei* were in conflict with at least some (BS > 70) support (Fig. 2).

3.2. Phylogeny

The results of the MP analysis of the cpDNA dataset resulted in 144 equally parsimonious trees of 415 steps each, CI = 0.65, RI = 0.86, RC = 0.71 (Fig. 2). *Columnea rubriacuta, C. gigantifolia* and *C.* sp. nov. were recovered in clade A (Fig. 2) that includes all of the sampled species from section *Collandra*. *Columnea moorei* was recovered as part of clade B (Fig. 2) that includes all sampled species of section *Columnea*. Analyses of the dataset that included all combined data, but missing nrDNA sequences for the four species mentioned above was less resolved and with lower support for resolution in the MP, ML and BI trees (results not shown).

The nrDNA dataset yielded 196 equally parsimonious trees of 614 steps each, CI = 0.52, RI = 0.81, RC = 0.49 (Fig. 2). *Columnea rubriacuta, C. gigantifolia*, and *C.* sp. nov. were recovered as part of clade C (Fig. 2). *Columnea moorei* was recovered as sister to *C. minor* (Fig. 2). Analyses that included all combined data, but missing sequences for cpDNA for the four species mentioned above was approximately the same as the dataset that included only nrDNA in terms of topology, resolution, and support for resolution (results not shown). Convergence was achieved in all BI results based on ESS values all over 2000 at a minimum as viewed in Tracer and comparison of separate runs in AWTY indicate that the analyses were approximating the same target distribution (results not shown). The ML and majority rule BI trees for all datasets were congruent with the MP tree, but with greater resolution (Fig. 2).

3.3. Splitstree

The splitstree results place the putative hybrid taxa in agreement with the cpDNA or nrDNA independent phylogenetic analyses (Figs. 2 and 3). There is a considerable amount of network connecting many of the branches, including alternative arrangements for the placement of the putative hybrid species. However, the branches leading to the clades that contain the hybrid taxa are reasonably long (Fig. 3A and C). The splitstree of the combined dataset places the putative hybrid taxa in branches that are intermediate to their placement in the cpDNA and nrDNA independent analyses (Fig. 3B). A total of 221 splits are present in the analysis that contains all data, but only 168 are present when the putative hybrid taxa individuals are removed from the data. Although splits are still present, they are primarily near the center of network (where support for relationships is low, Fig. 2) and the branches leading to the different clades are reasonably long as they are in Fig. 3A and C.

3.4. SH/AU tests

The SH and AU tests gave almost equivalent results (Table 1). When the topology resulting from one dataset was tested against the data from the other (e.g. cpDNA tree against the nrDNA data) most placements were significantly different for both tests. The exceptions were the placement of *C.* sp. nov., *C. gigantifolia*, and *C. moorei* for the SH tests. The AU tests were significant for all alternative topologies.

The GSI results indicated that with nrDNA (including nrDNA+) all individual species that were tested were monophyletic (GSI > 0.80) and significantly so with the exception of *C. moorei* (Table 2). In contrast, the cpDNA (including cpDNA+) only recovered *C.* sp. nov., *C. citriflora, C. dissimilis, C. strigosa*, and *C. minutiflora* as monophyletic (the latter four were not in question, but including multiple individuals allowed us to test the monophyly of these species as well). *Columnea gigantifolia* and *C. moorei* were not monophyletic (GSI = 0.25, 0.268, respectively) and *C. rubriacuta* was below our accepted value of GSI > 0.80, but is closer (GSI = 0.77).

The JML analyses did not indicate any significance when simulated distances for a dataset were compared against the same observed data (e.g., simulated cpDNA distances against observed cpDNA data). The simulated cpDNA distances were not significantly shorter than observed nrDNA data implying that the discrepancies caused by cpDNA could be explained by incomplete lineage sorting. In contrast simulated nrDNA data generated some distances that were significantly shorter than observed cpDNA distances (Table 3) implying nrDNA capture. Only comparisons of the simulated nrDNA data and observed cpDNA placements are presented as all other results were not significant.

4. Discussion

Significant differences between the topologies of the cpDNA and nrDNA datasets were detected using the partition homogeneity test and were found to be the result of hard incongruence (Seelanan et al., 1997; Fig. 2). Specifically, the incongruence could be traced to the inclusion of four species; *Columnea gigantifolia, C.* sp. nov., *C. rubriacuta*, and *C. moorei*. When these species were excluded from the analyses no incongruence was detected as determined by bootstrap support over 70% for alternative placement of individuals between the datasets (results not shown). Further evidence for the incongruence is supported by SH and AU tests that compared the placement of individual and groups of species from one dataset to the data of the other. In nearly all comparisons

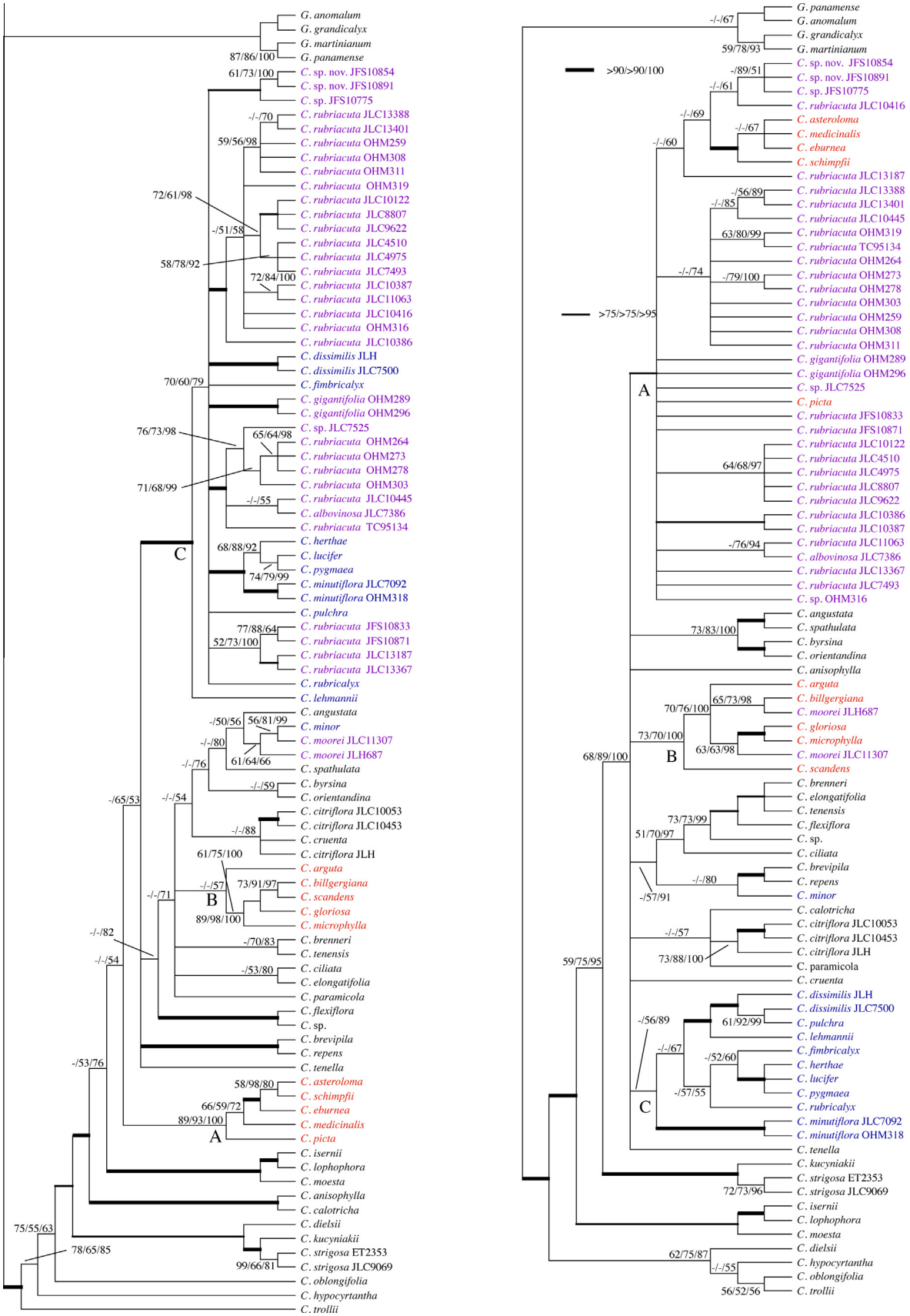

Fig. 2. Majority rule consensus tree from the Bayesian inference of A. nrDNA data. B. cpDNA. The topology of these trees are congruent with the bootstrap consensus trees from maximum parsimony and maximum likelihood and therefore both maximum parsimony bootstrap/maximum likelihood bootstrap/Bayesian inference posterior probability values are presented. Missing values (–) indicate clades not supported by bootstrap over 50. Thick lines indicate support of >75/>75/>95, double thick lines indicate support >90/> 90/100. Species names in blue are where the putative hybrids fall with nrDNA, names in red are where the same species fall in cpDNA and putative hybrids are in purple font.

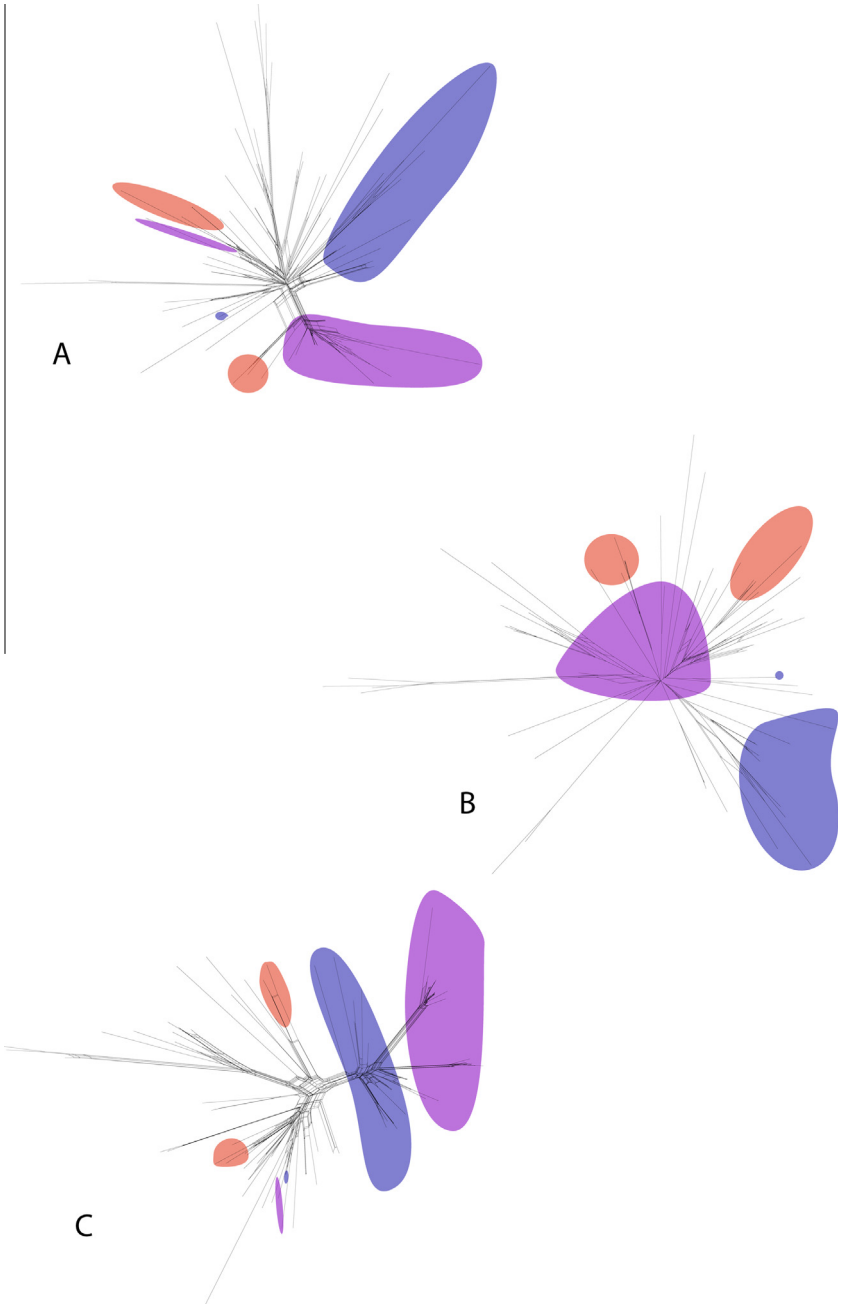

Fig. 3. Splitstree representation using the neighbornet optimization of A. cpDNA, B. combined cpDNA and nrDNA, and C. nrDNA. Taxon names have been removed for simplicity, but are available in supplementary Figs. 1–3. Violet shapes are the putative hybrid species discussed in the text. Blue-purple shapes are species of clades A and B (Fig. 2) and peach shapes are species of clade C (Fig. 2) or *C. minor*.

Table 1
Results of SH and AU tests for the placement of species from either of the datasets analyzed independently against the data from the other dataset.

Taxon	cpDNA position and nrDNA data; SH/AU	nrDNA position and cpDNA data; SH/AU
C. sp. nov.	0.373/<0.001	<0.187/<0.001
C. gigantifolia	0.052/0.05	<0.219/0.05
C. rubriacuta	<0.001/<0.001	<0.003/0.023
C. sp. nov., *C. gigantifolia,* and *C. rubriacuta*	<0.001/<0.001	<0.005/0.006
C. moorei	0.262/<0.001	0.071/<0.001
All of the above	<0.001/<0.001	<0.001/<0.001

the tests were significant (Table 1) indicating conflict between the data sets.

The conflict is further demonstrated with the splitstrees using neighbornet. The individual datasets place the putative four hybrid species with the species in the clade that they fell out with in phylogenetic analyses (Figs. 2 and 3). There are many connections in the splitstrees for these two datasets, especially toward the center (Fig. 3A and C) or what would be the "backbone" of the phylogenetic analyses where support for relationships also tends to be low (Fig. 2). However, the branches leading to the placement of the putative hybrids are reasonably long, at least longer than many of the other internal branches indicating that the discrepancies

Table 2
GSI results to test the monophyly of species or clades that were not recovered as monophyletic or with poor support in the phylogenetic analyses. Datasets were either the nrDNA or cpDNA data alone or nrDNA+ that included the combined data, but missing cpDNA for the putative hybrids and cpDNA+ that include the combined data, but missing nrDNA for the putative hybrids. Values reported are the GSI which ranges from 0 to 1 and the statistical *p* value.

Taxon	nrDNA data: GSI/*p*-value	cpDNA data: GSI/*p*-value	nrDNA+ GSI/*p* value	cpDNA+ GSI/*p* value
C. sp. nov.	1.00/0.0001	0.82/0.0003	1.00/0.001	0.82/0.0002
C. citriflora	0.79/0.0001	1.00/0.0001	0.78/0.0001	1.00/0.0001
C. dissimilis	1.0/0.0017	0.90/0.008	1.0/0.001	0.89/0.006
C. gigantifolia	1.00/0.0022	0.25/0.03	1.00/0.002	0.25/0.04
C. moorei	0.44/0.013	0.268/0.03	0.45/0.02	0.27/0.04
C. rubriacuta	0.88/0.0001	0.77/0.0001	0.912/0.001	0.77/0.001
C. strigosa	0.90/0.0036	0.98/0.002	0.99/0.009	0.98/0.006
C. minutiflora	1.00/0.0023	1.00/0.003	1.00/0.006	1.00/0.003
C. sp. nov., *C. gigantifolia,* and *C. rubriacuta*	0.77/0.0001	0.61/0.0001	0.76/0.0001	0.613/0.0001

Table 3
Results of the JML test showing the probabilities of obtaining simulated nrDNA distances compared to observed cpDNA distances without hybridization. Multiple values were obtained for some comparisons as a result of sampling more than one individual per species. Only the putative hybrid species, in comparison to the species in the clades where they fall with cpDNA were compared. Only distances where the probability is less than 0.1 are shown, all other results were insignificant including all comparisons made of simulated cpDNA distances compared to nrDNA distance. Other distances, for example any putative hybrid species and species not in any of the clades where the putative hybrids fell, were not examined.

Species pairs	
C. sp. nov.-*C. asteroloma*	0.0001
C. sp. nov.-*C. densibracteata*	0.0001
C. sp. nov.-*C. eburnea*	0.0001–0.0003
C. sp. nov.-*C. schimpfii*	0.0002–0.0026
C. sp. nov.-*C. picta*	0.01–0.039
C. gigantfolia-C. asteroloma	0.0001
C. gigantfolia-C. densibracteata	0.0001
C. gigantfolia-C. eburnea	0.0001
C. gigantfolia-C. schimpfii	0.0005
C. gigantfolia-C. picta	0.006
C. rubriacuta-C. asteroloma	0.0001–0.046
C. rubriacuta-C. densibracteata	0.0001–0.025
C. rubriacuta-C. eburnea	0.0002–0.09
C. rubriacuta-C. schimpfii	0.0018–0.06
C. rubriacuta-C. picta	0.017–0.067
C. moorei-C. gloriosa	0.0002
C. moorei-C. microphylla	0.0002–0.04
C. moorei-C. scandens	0.0002–0.058

between the two datasets is not the result of poor or insufficient data in one or both datasets (Morrison, 2009). The splitstree based on the combined data places the putative hybrid taxa in a network that is intermediate to their placements in both the cpDNA and nrDNA (Fig. 3B).

Interpreting phylogenetic incongruencies between datasets can be challenging as they may result from paralogy, incomplete lineage sorting, horizontal gene transfer, or hybridization (Buckley et al., 2006; Kubatko and Degnan, 2007; Holland et al., 2008; Maureira-Butler et al., 2008; Joly et al., 2009; Pirie et al., 2009; Polihronakis, 2009; Willyard et al., 2009; Pelser et al., 2010; de Viliers et al., 2013; Yu et al., 2011, 2013). Paralogy can be eliminated for the sequences analyzed here. Chloroplast DNA is haploid and although concerted evolution has been documented to be incomplete or lacking in some nrDNA for other members of Gesneriaceae (Denduangboripant and Cronk, 2000), all of the ITS and ETS sequences generated for this study were done using direct sequencing and results were at least as clean as the haploid cpDNA regions.

De Viliers et al. (2013) investigated potential interspecific hybridization in *Streptocarpus* species that also demonstrated discrepancies between datasets. They used the GSI as an indicator that coalescence had occurred in the lineage for either the nrDNA or cpDNA data. Haploid cpDNA will coalesce faster than diploid nrDNA and they were able to demonstrate that in species where phylogenetic discrepancy existed between cpDNA and nrDNA that the cpDNA recovered monophyletic species whereas the nrDNA did not, implying that the cpDNA had been captured in an ancestral event. Our results indicate the reverse for at least *C. gigantifolia*, *C. rubriacuta*, and *C. moorei* where the nrDNA GSI results recovered monophyletic species, but the cpDNA did not. Given that the rate of coalescence for cpDNA should be much faster than nrDNA, the data imply that nrDNA must have been transferred to these species and has coalesced to an ancestral copy within each of the species. This has not occured for the cpDNA, which implies that coalescence has not yet occured within each of the species. If there were no cross-species transfer of either material, the reverse might have occured by chance. In contrast, the species where the GSI was scored, but were recovered as monophyletic in all analyses have a greater GSI for cpDNA than nrDNA as would be expected when comparing a haploid genome to a diploid one (Table 2).

Coalescence of nrDNA and not cpDNA implies a bottleneck in the ancestor to these individuals in terms of nrDNA, but not cpDNA. An explanation could be interspecific hybridization where the F1 hybrid either self-fertilized, or continued to backcross with other members of the maternal parent, or maternal parent clade, and by chance the paternal copy of the nrDNA became fixed. This implies nrDNA capture. These data are in agreement with the morphology for *C. gigantifolia*, *C. rubriacuta*, and *C.* sp. nov., all of which would be placed in clade A (Figs. 1 and 2) that corresponds to section *Collandra* based on the sampling here using morphology alone. These species are distinctive by having dorsoventral shoots, strongly anisophyllous oblanceolate leaves that often are colored red or purple on the abaxial surface, either entirely or with spots or marginal coloration, calyces that are green or yellow in color, and large overlapping bracts subtending the flowers (Kvist and Skog, 1993). Moreover, these species have a dispersed spatial distribution, produce a few flowers per plant throughout the year, and are pollinated exclusively by hermit hummingbirds Also, the plants tend to have and autoincompatible reproductive system (Marín-Gómez, 2014; Marín-Gómez and Amaya-Márquez, 2015).

They do not fit with the morphology of species in clade C (Figs. 1 and 2) that although anisophyllous, are elliptic and with crenate margins, calyces are often red-orange in color and bracts subtending the flowers are small or lacking.

The JML test was developed as a means of detecting hybridization or incomplete lineage sorting. The program simulates a set of DNA sequences based on trees generated by real data and then compares the distances between species pairs based on the simulated vs. observed data. Species pairs that are less distant with the simulated data compared to the observed data imply that hybridization has occurred. In this study, the simulated cpDNA distances were not significantly shorter than observed nrDNA data implying that the discrepancies caused by cpDNA could be explained by incomplete lineage sorting. In contrast simulated

nrDNA data generated some distances that were significantly shorter than observed cpDNA distances (Table 3) implying nrDNA capture. These results are consistent with the GSI results in that a lack of coalescence for the cpDNA would result in simulated sequences that would be unlikely to be shorter than nrDNA distances that resulted from a more recent coalescence. However, the JML test was also able to detect shorter distances for *C.* sp. nov. that GSI recovered as monophyletic for both datasets (Tables 2 and 3).

4.1. Sympatry of parental species

It is imperative that if hybridization is occurring, that the parental species are sympatric, or at least were sympatric at the time of hybridization. Our analyses do not have the resolving power to link any of the hybrids to parental species in either group (Fig. 2). Likewise, our sampling was not conducted as a means of determining the exact parental species. In fact, section *Collandra*, where *C. gigantifolia, C. rubriacuta*, and *C.* sp. nov. are placed with cpDNA is the largest section in the genus in terms of number of species and may include over 80 species, whereas we have only sampled five here (Fig. 2). However, we do know that where *C. gigantifolia, C. rubriacuta*, and *C.* sp. nov. are found in their present day, that other species from both of the parental clades are also found. In many cases this can include multiple species from both clades (pers. obs.).

Columnea moorei presents a different challenge in terms of identifying putative parental species. This species has a narrow range, found only on Cerro Jefe in Panama. Species of section *Columnea*, where *C. moorei* is placed with cpDNA are diverse in Panama, and Central America in general (Skog, 1978). We were able to obtain recently collected wild material of this species from Cerro Jefe, along with all other species of *Columnea* that are currently known from that region including *C. arguta* and *C. billbergiana* from Cerro Jefe (Appendix A). The phylogenetic analyses of cpDNA place the recently collected wild material of *C. moorei* as sister to *C. billbergiana* (Fig. 2), implying that *C. billbergiana* may be the maternal parent. However, the cultivated specimen of *C. moorei* was not sister to *C. billbergiana* and is instead sister to a clade of *C. gloriosa/C. microphylla* (Fig. 2). These data, and the low GSI values recovered for both cpDNA and nrDNA (Table 2) for *C. moorei* suggest that hybridization may still be active with this species. However, the cultivated specimen of *C. moorei* may have also undergone more recent hybridization. This species has seldom been collected in the wild (five are cited in Skog, 1978). One of these is presumably the voucher of the wild plant for the material in cultivation that was used here. This individual was used as a parent in a series of crosses with other *Columnea* species in cultivation at Cornell University in the 1960s. It is possible that the material in cultivation represents the results of one of these crosses or a potential F2 or backcrossed hybrid. A series of vouchers at Cornell University document the F1 hybrids that were generated with *C. pilosissima* (currently considered synonymous with *C. hirta*), *C. percrassa* (currently considered synonymous with *C. billbergiana*), *C. nicaraguensis* Oerst., *C. linearis* Oerst., *C. illepida* H.E. Moore, *C. gloriosa, C. glabra* Oerst., and *C. allenii* C.V. Morton. All but *C. illepida* are considered members of section *Columnea*, represented in our analyses by the species of clade B, where *C. moorei* is placed with the cpDNA data. In many cases the vouchers clearly state that *C. moorei* was the female parent, and in all cases *C. moorei* is listed first. Assuming that the annotations are consistent then the cpDNA of these hybrids would have reflected the cpDNA of *C. moorei* and not the pollen donor species. However, we cannot rule out that other crosses were made that we no longer have documentation of (potentially also including *C. minor* which *C. moorei* is sister to with the nrDNA data, Fig. 2), that included members of the section *Columnea* clade as maternal parents, or that F2 or backcross hybrids were generated that resulted in the cultivated material of *C. moorei* that we have now. Therefore, we cannot assume multiple maternal parents for *C. moorei*.

Determining the paternal parent of *C. moorei* is also a challenge. Both accessions of *C. moorei* are with *C. minor* in the nrDNA phylogenetic analyses (Fig. 2). Although *C. minor* is a widespread and common species in Colombia and Ecuador, it is currently not known from Panama. It is also possible that the closest relative to the nrDNA of *C. moorei* remains unsampled in our analyses. *Columnea minor* is recovered as sister to a clade of Jamaican species in analyses that exclude *C. moorei* (Smith et al., 2013), but is placed within section *Angustiflorae* here (Fig. 2) when *C. moorei* is included. Analyses that used greater sampling of section *Angustiflorae* were able to exclude *C. moorei* as part of that clade (Schulte et al., 2014) implying that the placement, and possibly the close relationship of *C. moorei* to *C. minor* with nrDNA is an artefact of taxon sampling.

It does not appear that *C. moorei* is simply an F1. If it were a stable F1 hybrid, then it would be expected to have nrDNA from both parents. All sequencing of nrDNA for this species was done directly and all sequences produced clean reads without multiple peaks. This implies that either after the initial hybridization event self-fertilization resulted in the nrDNA of the *C. minor* lineage becoming fixed and the nrDNA from the maternal lineage was lost. Alternatively, it is possible that outcrossing with other members of clade B continued with the ancestor to the modern *C. moorei* with backcrossing to the hybrid or selfing such that only the nrDNA of the *C. minor* lineage was retained. This latter situation is likely given that *C. moorei* is not recovered as monophyletic in any of the analyses and may reflect a stable morphological species that is the result of several genetic lineages.

4.2. Hybrid lineage for C. gigantifolia, C. rubriacuta, and C. sp. nov.

The most parsimonious explanation for the hybrid origin of *C. gigantifolia*, *C. rubriacuta*, and *C.* sp. nov. is an ancestral hybridization event with the pollen donor being a species of clade C (Figs. 1 and 2) and the maternal lineage a member of clade A (Figs. 1 and 2). This F1 hybrid likely continued to backcross either with other individuals of its maternal species, or other members of clade A and by chance the nrDNA from its paternal parent became fixed, whereas variability in the cpDNA genome persisted. This ancestral species then gave rise to the three species sampled here, and perhaps others. Further support for this stems from the GSI results, which indicate that all three species as a clade approach our threshold of 0.8 for being monophyletic with nrDNA (GSI = 0.77, Table 2), but are less likely to be a monophyletic group with cpDNA (GSI = 0.61, Table 2). None of the three species we have recovered as being of hybrid origin were previously suspected to be hybrids and it is possible that this lineage includes other members of section *Collandra* that have yet to be sampled with molecular methods. However, we cannot preclude the possibility of three independent hybridization events for the origin of these species as the GSI results are not exceptionally strong in recognizing this clade as monophyletic.

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Table A1
Species, voucher specimens, and GenBank accession numbers for all accessions included in phylogenetic analyses. NA indicates that sequences were not generated for this region for this particular accession. ex indicates a sequence that was excluded from the analysis due to incongruence with other sequences. Accesion numbers in bold were newly generated for this study (to be added [TBA] when final acceptance has been indicated).

Taxon	Voucher	Herbarium	Collection Locality	*rpl32-trnL*$_{UAG}$ spacer	*trnQ-rps16* intron	*rps16* intron	*trnS-G* spacer	*trnH-psbA* spacer	ITS	ETS
Columnea albovinosa (M. Freiberg) J.L. Clrk & L. E. Skog	*J. L. Clark 7386*	US	Ecuador	KX912467	KX912524	KX912581	NA	KX912411	KX912354	KX912276
C. angustata (Wiehler) L. E. Skog	*J. L. Clark 8627*	UNA & US	Panama	KF005816	KF006034	KF005925	KF006137	NA	KF005727	KP260806
C. anisophylla DC.	*J. F. Smith 10773*	COL	Colombia	KX912468	KX912525	KX912582	KX912635	KX912412	KX912355	KX912277
C. arguta C. V. Morton	*J. & L. Harrison 685*	SRP	Panama	KX912469	KX912526	KX912583	KX912636	KX912413	KX912356	KX912278
C. asteroloma (Wiehler) L.E. Skog	*J. L. Clark 7950*	US	Ecuador	KX912470	KX912527	KX912584	KX912637	KX912414	KX912357	KX912279
C. billbergiana Beurl.	*J. & L. Harrison 683*	SRP	Panama	KX912471	KX912528	KX912585	KX912638	KX912415	KX912358	KX912280
C. brenneri (Wiehler) B. D. Morley	*J. F. Smith 3385*	SRP	Ecuador	KF005823	KF006040	KF005932	KF006144	KF005649	KF005734	KX912281
C. brevipila Urb.	*J. F. Smith 10058*	SRP	Cultivated, Jamaica	KF005825	KF006042	KF005934	KF006146	KF005651	KF005736	KX912282
C. byrsina (Wiehler) L. P. Kvist & L. E. Skog	*J. F. Smith 3408*	SRP	Ecuador	KF005826	KF006043	KF005935	JQ953714	KF005652	KF005737	KP260812
C. calotricha Donn. Sm.	*J. F. Smith et al. 4117*	SRP	French Guiana	KF005828	KF006045	KF005937	KF006149	KF005654	KF005739	KX912283
C. ciliata (Wiehler) L.P. Kvist & L.E. Skog	*J. F. Smith 8604*	SRP	Cultivated, Ecuador	KX912472	KX912529	KX912586	KX912639	KX912416	KX912359	KX912284
C. citriflora L. E. Skog	*J. L. Clark 10053*	UNA & US	Cultivated, Panama	KF005830	KF006047	KF005939	KF006151	KF005655	KF005741	KX912286
C. citriflora L. E. Skog	*J. L. Clark 10453*	US	Cultivated, Panama	KX912474	KX912531	KX912588	KX912641	KX912418	KX912361	KX912287
C. citriflora L. E. Skog	*J. & L. Harrison 682*	SRP	Panama	KX912473	KX912530	KX912587	KX912640	KX912417	KX912360	KX912285
C. cruenta B.D. Morley	*J. & L. Harrison 686*	SRP	Panama	KX912475	KX912532	KX912589	KX912642	KX912419	KX912362	KX912288
C. dielsii Mansf.	*J. F. Smith 1989*	WIS	Ecuador	KF005836	KF006053	KF005945	KF006157	KF005661	KF005747	KP260822
C. dissimilis C. V. Morton	*J. & L. Harrison 684*	SRP	Panama	KX912476	KX912533	KX912590	KX912643	KX912420	KX912363	KX912288
C. dissimilis C. V. Morton	*J. L. Clark 7500*	UNA	Panama	KX912477	KX912534	KX912591	KX912644	KX912421	KX912364	KX912290
C. eburnea (Wiehler) L.P. Kvist & L.E. Skog	*J. L. Clark 6353*	US	Ecuador	KF005840	KF006057	KF005949	KF006160	KF005665	KF005750	KX912291
C. elongatifolia L.P. Kvist & L.E. Skog	*J. L. Clark 10015*	US	Ecuador	KF005841	KF006058	KF005950	KF006161	KF005666	KF005751	KX912292
C. fimbricalyx L.P. Kvist & L.E. Skog	*J. L. Clark 10429*	US	Ecuador	KX912478	KX912535	KX912592	KX912645	KX912422	KX912365	KX912293
C. flexiflora L.P. Kvist & L.E. Skog	*J. L. Clark & L. Jost 6968*	US	Ecuador	KF005846	KF006063	KF005956	KF006167	KF005671	KF005755	KX912294
C. gigantifolia L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 289*	COL	Colombia	KX912479	KX912536	KX912593	KX912646	KX912423	KX912366	KX912295
C. gigantifolia L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 296*	COL	Colombia	KX912480	KX912537	KX912594	KX912647	KX912424	KX912367	KX912296
C. gloriosa Sprague	*J. L. Clark et al. 9921*	US	Ecuador	KF005848	KF006065	KF005958	KF006169	KF005673	KF005757	KX912297
C. herthae Mansf.	*J. L. Clark 7113*	US	Ecuador	KF005853	KF006069	KF005963	KF006173	KF005677	KF005761	KX912299
C. hypocyrtantha (Wiehler) J.F. Smith & L.E. Skog	*J. L. Clark & E. Rodriguez 6741*	US	Bolivia	KF005854	KF006071	KF005964	KF006174	KF005679	KF005762	KX912300
C. isernii Cuatrec.	*J. L. Clark et al. 6253*	US	Ecuador	KF005857	KF006074	KF005967	KF006177	DQ211220	AF543247	KX912301
C. kucyniakii Raymond	*T. Croat 94640*	MO	Ecuador	KX912481	KX912538	KX912595	KX912648	KX912425	KX912368	KX912302
C. lehmannii Mansf.	*J. L. Clark 13267*	US	Colombia	KX912482	KX912539	NA	KX912649	KX912426	KX912369	KX912303
C. lophophora Mansf.	*J. L. Clark et al. 7888*	US	Ecuador	KF005860	KF006076	KF005969	KF006179	KF005684	KF005767	KP260825
C. lucifer J.L. Clark	*J. L. Clark 11100*	US	Ecuador	KX912483	KX912540	KX912596	KX912650	KX912427	KX912370	KX912304
C. medicinalis (Wiehler) L.E. Skog & L.P. Kvist	*T. Croat 94600*	MO	Ecuador	KX912484	KX912541	KX912597	KX912651	KX912428	KX912371	KX912305
C. microphylla Klotsch & Hanst.	*J. L. Clark 6261*	UNA & US	Cultivated	KF005863	KF006080	KF005973	KF006182	KF005687	KF005771	KP260827 (ex)
C. minor (Hook.) Hanst.	*T. Croat 94778*	MO	Ecuador	KF005866	KF006084	KF005975	KF006185	KF005690	KF005774	KP260828 (ex)
C. minutiflora L. P. Kvist & L. E. Skog	*J. L. Clark et al. 7092*	UNA & US	Ecuador	KF005868	KF006086	KF005977	KF006187	KF005692	KF005776	KX912306
C. minutiflora L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 318*	COL	Colombia	KX912485	KX912542	KX912598	KX912652	KX912429	*KX912372*	KX912307
C. moesta Poepp.	*J. F. Smith 1829*	WIS	Bolivia	KF005870	KF006084	KF005979	KF006189	KF005694	KF005778	KP260830
C. moorei C. V. Morton	*J. & L. Harrison 687*	SRP	Panama	KX912486	KX912543	KX912599	KX912653	KX912430	KX912373	KX912308
C. moorei C. V. Morton	*J. L. Clark 11307*	UNA & US	Cultivated, originally from Panama	KX912487	KX912544	KX912600	KX912654	KX912431	KX912374	KX912309
C. oblongifolia Rusby	*J. F. Smith 1725*	WIS	Bolivia	KF005874	KF006092	KF005983	KF006193	KF005697	KF005781	KX912310
C. orientandina Mansf.	*J. L. Clark et al. 9885*	UNA	Ecuador	KF005876	KF006094	KF005985	KF006195	KF005699	KF005783	KP260833
C. paramicola (Wiehler) L.P. Kvist & L.E. Skog	Not vouchered	na	Cultivated at Smithsonian as USBG94529, Ecuador	KF005878	KF006095	KF005987	JQ954064	DQ211224	DQ211113	KX912311
C. picta H. Karst.	*T. Croat 94956*	MO	Ecuador	KF005879	KF006096	KF005988	KF006197	KF005701	KF005785	KP260837
C. pulchra C.V. Morton	*J. L. Clark 6265*	US	Cultivated	KF005880	NA	KF005990	KF006198	DQ211225	KF005786	KX912312
C. pygmaea J.L. Clark & J.F. Smith	*J. L. Clark 11180*	UNA & US	Ecuador	KX912488	KX912545	KX912601	KX912655	KX912432	KX912375	KX912313

(*continued on next page*)

Table A1 (*continued*)

Taxon	Voucher	Herbarium	Collection Locality	*rpl32-trnL*$_{UAG}$ spacer	*trnQ-rps16* intron	*rps16* intron	*trnS-G* spacer	*trnH-psbA* spacer	ITS	ETS
C. repens (Hook.) Hanst.	*J. F. Smith 8605*	SRP	Cultivated, Jamaica	KF005884	KF006100	KF005993	KF006201	KF005705	KF005790	KX912314
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 4510*	UNA & US	Ecuador	KX912501	KX912558	KX912612	KX912664	KX912444	KX912388	KX912327
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 4975*	QCNE & US	Ecuador	KX912502	KX912559	KX912613	KX912665	KX912445	KX912389	KX912328
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 7493*	UNA & US	Ecuador	KX912503	KX912560	KX912614	KX912666	KX912446	KX912390	KX912329
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 8807*	US	Ecuador	KX912504	KX912561	KX912615	KX912667	KX912447	KX912391	KX912330
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 9622*	US	Ecuador	KX912505	KX912562	KX912616	KX912668	KX912448	KX912392	KX912331
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 10122*	US	Ecuador	KX912491	KX912548	KX912604	KX912658	KX912435	KX912378	KX912317
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 10386*	US	Ecuador	KX912492	KX912549	KX912605	NA	KX912436	KX912379	KX912318
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 10387*	US	Ecuador	KX912493	KX912550	KX912606	NA	KX912437	KX912380	KX912319
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 10416*	US	Ecuador	KX912494	KX912551	NA	KX912659	KX912438	KX912381	KX912320
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 10445*	US	Ecuador	KX912495	KX912552	NA	KX912660	KX912439	KX912382	KX912321
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 11063*	US	Ecuador	KX912496	KX912553	KX912607	NA	KX912440	KX912383	KX912322
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 13187*	US	Colombia	KX912497	KX912554	KX912608	KX912661	KX912441	KX912384	KX912323
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 13367*	US	Colombia	KX912498	KX912555	KX912609	KX912662	KX912442	KX912385	KX912324
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 13401*	US	Colombia	KX912500	KX912557	KX912611	KX912663	KX912443	KX912387	KX912326
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. L. Clark 13388*	US	Colombia	KX912499	KX912556	KX912610	NA	NA	KX912386	KX912325
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*T. C. Croat 95134*	MO	Ecuador	KX912515	KX912572	KX912626	KX912678	KX912458	KX912398	KX912341
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 259*	COL	Colombia	KX912506	KX912563	KX912617	KX912669	KX912449	KX912403	KX912332
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 264*	COL	Colombia	KX912507	KX912564	KX912618	KX912670	KX912450	KX912393	KX912333
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 273*	COL	Colombia	KX912508	KX912565	KX912619	KX912671	KX912451	KX912394	KX912334
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 278*	COL	Colombia	KX912509	KX912566	KX912620	KX912672	KX912452	KX912395	KX912335
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 303*	COL	Colombia	KX912510	KX912567	KX912621	KX912673	KX912453	KX912396	KX912336
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 308*	COL	Colombia	KX912511	KX912568	KX912622	KX912674	KX912454	KX912404	KX912337
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 311*	COL	Colombia	KX912512	KX912569	KX912623	KX912675	KX912455	KX912405	KX912338
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 316*	COL	Colombia	KX912513	KX912570	KX912624	KX912676	KX912456	KX912406	KX912339
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*O. H. Marín-Gómez 319*	COL	Colombia	KX912514	KX912571	KX912625	KX912677	KX912457	KX912397	KX912340
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. F. Smith et al. 10833*	COL	Colombia	KX912489	KX912546	KX912602	KX912656	KX912433	KX912376	KX912315
C. rubriacuta (H. Wiehler) L. P. Kvist & L. E. Skog	*J. F. Smith et al. 10871*	COL	Colombia	KX912490	KX912547	KX912603	KX912657	KX912434	KX912377	KX912316
C. rubricalyx L. P. Kvist & L. E. Skog	*J. L. Clark et al. 11034*	UNA	Ecuador	KF005887	KF006103	KF005997	KF006204	KF005707	KF005792	KX912342
C. scandens L.	*J. L. Clark & S. G. Clark 6541*	US	Martinique	KF005890	KF006106	KF005999	KF006207	KF005711	KF005795	KX912343
C. schimpffii Mansf.	*J. F. Smith 8605*	SRP	Cultivated, Ecuador	KF005892	KF006109	KF006001	KF006209	KF005713	KF005797	KX912344
C. spathulata Mansf.	*J. F. Smith 1853*	WIS	Ecuador	KF005893	KF006110	KF006003	KF006211	KF005715	KF005798	KP260844
C. sp.	*J. L. Clark 7525*	US	Ecuador	KX912520	KX912577	KX912631	NA	KX912463	KX912402	KX912298
C. sp.	Not vouchered	MTJB	Cultivated	KX912516	KX912573	KX912627	KX912679	KX912459	KX912399	KX912345
C. sp. nov.	*J. F. Smith et al. 10775*	COL	Colombia	KX912517	KX912574	KX912628	KX912680	KX912460	KX912400	KX912348
C. sp. nov.	*J. F. Smith et al. 10854*	COL	Colombia	KX912518	KX912575	KX912629	KX912681	KX912461	KX912401	KX912346
C. sp. nov.	*J. F. Smith et al. 10891*	COL	Colombia	KX912519	KX912576	KX912630	KX912682	KX912462	KX912407	KX912347
C. strigosa Benth.	*J. L. Clark 9069*	US	Ecuador	KX912522	KX912579	KX912633	KX912684	KX912465	KX912409	KX912350
C. strigosa Benth.	*E. J. Tepe 2353*	SRP	Ecuador	KX912521	KX912578	KX912632	KX912683	KX912464	KX912408	KX912349
C. tenella L.P. Kvist & L.E. Skog	*T. Croat 95108*	MO	Ecuador	KX912523	KX912580	KX912634	KX912685	KX912466	KX912410	KX912351
C. tenensis (Wiehler) B.D. Morley	*J. F. Smith 3374*		Ecuador	KF005898	KF006115	KF006008	KF006216	KF005720	KF005804	KX912352
C. trollii Mansf.	*J. F. Smith 1723*	WIS	Bolivia	KF005899	KF006117	KF006010	KF006218	KF005722	KF005805	KX912353
Glossoloma anomalum J. L. Clark	*J. F. Smith 3418*	SRP	Ecuador	KF005912	KF006128	KF006021	KF006224	NA	AF543225	KP260850
Glossoloma grandicalyx (J. L. Clark & L. E. Skog) J. L. Clark	*J. F. Smith 3417*	SRP	Ecuador	KF005913	KF006129	KF006024	JQ953708	DQ211205	AF543218	KP260851
Glossoloma martinianum /ce:italic> (J. F. Smith) J. L. Clark	*J. L. Clark 6101*	US	Ecuador	KF005914	KF006130	KF006022	JQ953709	DQ211209	AF543228	KP260852
Glossoloma panamense (C. V. Morton) J. L. Clark	*L. E. Skog et al. 7641*	US	Cultivated	KF005915	KF006131	KF006023	JQ953710	DQ211202	DQ211102	KP260853

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Appendix A

See Table A1.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.10.001.

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