APPENDIX 2. Breakdown by gene of Gesneriaceae sequence data in PHYLOTA (release: 184, accessed 15 June 2011) and Genbank* data released after 15 June 2011 (accessed 11 July 2012).

| Genome | Type | *TaxIDs* | Genera | *GIs* | *GIs* plus GB | *Lmin* | *Lmax* |
|---|---|---|---|---|---|---|---|
| **/gene** | | | | | **16-July*** | | |
| ***mitochondrial*** | | | | | | | |
| genome | | | | 0 | 1 | | |
| *nad*5 | gene | 1 | 1 | 1 | 1 | 1025 | 1025 |
| *atp*1 | gene | 2 | 2 | 2 | 2 | 1049 | 1274 |
| *atp*6 | gene | 1 | 1 | 1 | 1 | 615 | 615 |
| *cox*1 | gene | 4 | 4 | 4 | 4 | 513 | 2282 |
| *mat*R | gene | 2 | 2 | 2 | 2 | 1623 | 1893 |
| *rps*3 | gene | 1 | 1 | 1 | 2 | 1407 | 1407 |
| **Sum** | **6** | | | **11** | **13** | | |
| ***chloroplast*** | | | | | | | |
| genome | | | | 0 | 1 | | |
| *rbc*L gene | gene | 40 | 33 | 47 | 89 | 1061 | 1421 |
| *ndh*F gene | gene | 150 | 84 | 159 | 161 | 1117 | 2268 |
| *mat*k/intron | intron/gene | 5 | 4 | 5 | 57 | 1136 | 2478 |
| *rpl*16 intron | intron | 114 | 31 | 122 | 122 | 553 | 919 |
| *rps*16 intron | intron | 48 | 32 | 59 | 62 | 667 | 946 |
| *trn*L-*trn*F spacer | intron/spacer | 613 | 178 | 787 | 850 | 433 | 1281 |
| *psb*A-*trn*H spacer | spacer | 230 | 25 | 261 | 264 | 208 | 363 |
| *atp*B-*rbc*L spacer | spacer | 203 | 72 | 215 | 225 | 371 | 1278 |
| *trn*E-*trn*T spacer | spacer | 89 | 48 | 99 | 99 | 386 | 834 |
| *trn*S-*trn*G spacer | spacer | 84 | 14 | 84 | 84 | 382 | 796 |
| *trn*T-*trn*L spacer | spacer | 83 | 12 | 84 | 84 | 382 | 708 |
| *rpl*20-*rps*12 spacer | spacer | 62 | 44 | 63 | 63 | 652 | 784 |
| *trn*K-*psb*A spacer | spacer | 51 | 31 | 61 | 61 | 240 | 313 |
| *trn*C-*trn*D spacer | spacer | 5 | 1 | 57 | 57 | 1707 | 1726 |
| other (<10 seq.) | 17 diverse | diverse | diverse | 29 | 36 | | |
| **sum** | **31** | | | **2132** | **2315** | | |
| ***nuclear*** | | | | | | | |
| *CYC* | gene | 126 | 63 | 432 | 432 | 389 | 1177 |
| 26S | gene | 19 | 18 | 19 | 19 | 1166 | 3289 |
| *STM* | gene | 32 | 7 | 43 | 43 | 118 | 714 |
| *ncpGS* | gene/intron | 188 | 63 | 202 | 202 | 349 | 677 |
| *CHS-B* | gene/spacer | 33 | 16 | 148 | 148 | 743 | 760 |
| 5S spacer | spacer | 32 | 2 | 55 | 55 | 329 | 368 |
| ETS | spacer | 74 | 2 | 98 | 98 | 405 | 421 |
| ITS region | gene/spacer | 846 | 138 | 1195 | 1247 | 614 | 732 |
| ITS1 | spacer | 140 | 45 | 170 | 170 | 218 | 251 |
| ITS2 | spacer | 59 | 41 | 59 | 59 | 252 | 260 |
| other (<10 seq) | 60 diverse | diverse | diverse | 98 | 98 | na | na |
| microsatellite markers | 25 | 25 | 3 | 20 | 25 | 254 | 682 |
| **Sum** | **95** | | | **2539** | **2596** | | |
| **Total** | **130** | | | **4682** | **4924** | | |

Notes: *TaxID*=number of distinct taxa; *GIs*=number of sequences; *-plus Genbank releases after 15 June 2011; *Lmin*=minimum aligned length; *Lmax*=maximum aligned length.

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# SEARCHING FOR MONOPHYLY IN THE SUBGENERIC CLASSIFICATION SYSTEMS OF *COLUMNEA* (GESNERIACEAE)

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ABSTRACT. The use of morphological characters to define species, genera, and higher taxa within the Gesneriaceae has often been problematic with convergences causing unrelated taxa to be classified together. Recent molecular phylogenetic analyses have allowed greater insights into relationships across the family and as a result better systems of classification that reflect the common ancestry of taxa rather than convergent evolutionary history have been proposed. *Columnea* is the largest Neotropical genus in Gesneriaceae subfamily Gesnerioideae and has had a complex and confusing taxonomic history. The species that are now considered *Columnea* have been placed in 14 genera and at times up to nine sections within the genus. More recently it has been recognized as five genera or a single genus with six sections. The phylogenetic analyses presented here sampled 68 species and for the first time resolved relationships among them. None of the recent subgeneric classification systems are in complete agreement with the phylogenetic relationships. The results here also indicate that there may be greater cryptic speciation in *Columnea* than had previously been assumed as some morphologically determined species are not recovered as monophyletic. Although our sampling consists of only two morphologically divergent species from Jamaica, they are supported as sister, implying that the endemic *Columnea* species in Jamaica may be derived from a single introduction event.

*Key words:* cpDNA, Jamaica, ITS, monophyly, Neotropical, phylogeny

## INTRODUCTION

Morphological variation has been the mainstay of systematics and classification systems since their inception and continues to play an important and often critical role amidst the ever-growing field of molecular systematics. However, unless morphology is carefully and critically evaluated in a context of evolutionary or genetic development, convergence can create seemingly homologous characters and character states across unrelated organisms. When these states are used as the basis for a classification system, the erroneously scored morphologies result in systems where organisms that do not share a most recent common ancestor are considered a single taxon. This is especially the case for *Columnea* L. and the artificial subgeneric classification that will be addressed in this study.

Misinterpreting homology among morphological characters has been common in the taxonomic history of Gesneriaceae. This has become apparent in numerous groups in both the Old World subfamily Cyrtandroideae (Smith 1996; Moeller & Cronk 1997; Smith et al. 1997, 1998; Mayer et al. 2003; Li & Wang 2007; Moeller et al. 2009; Wang et al. 2010, 2011) as well as the New World subfamily Gesnerioideae (Clark & Zimmer 2003; Smith et al. 2004; Roalson et al. 2005a, 2005b, 2008; Clark et al. 2006; Clark et al. 2012; Smith & Clark 2013). To be fair to early taxonomists, many of these characters are not easy to interpret as having multiple independent origins. It is only in the light of modern molecular and phylogenetic methods that we can better assess homology and determine which characters are the most informative to define monophyletic groups. Gesneriaceae are certainly not the only family where this has been important and other groups of angiosperms have seen major reclassifications as a result of phylogenetic analyses, most notably in the temperate counterpart to Gesneriaceae, the former Scrophulariaceae (Olmstead et al. 2001, Oxelman et al. 2005, Albach et al. 2005, Xia et al. 2009).

As was the case for many groups, early classification systems in Gesneriaceae relied heavily on floral form (Hanstein 1854, 1856, 1859, 1865; Oersted 1858; Bentham 1876; Fritsch 1894; Morton 1971; Morley 1976). Wiehler (1983) was among the first gesneriad researchers

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to question the utility of floral form to reflect the ancestry of organisms. Instead he viewed many of the common morphologies seen across the genus and genera of the Neotropical Gesneriaceae to be a reflection of pollinator selection. As a result, he proposed a sweeping re-classification of the Gesnerioideae that relied on characters other than those of the corolla.

*Columnea* has not been exempt from a classification system that has relied on analogous rather than homologous characters and states. The type for the species, *C. scandens* L., was described by Linnaeus in 1753. In the early 1800s several additional species were described that were later combined into *Columnea* by Hanstein (1854) who retained generic status for *Ortholoma* Benth. and *Collandra* Lem., and added four new genera. Hanstein later (1865) combined all the genera into *Columnea* at the rank of subgenera and added one additional subgenus, bringing the total to seven. Fritsch (1894) recognized *Trichantha* Hook. as a genus distinct from *Columnea* but considered the subgenera of Hanstein (1865) to be sections. Fritsch also combined into *Columnea* the genera *Stygnanthe* J. Hanst. and *Systolostoma* Benth., both as sections. This classification system was altered slightly by Morton (1971) and Morley (1974a, 1976) who mostly recognized a different number of sections than had Fritsch (1894).

Wiehler (1973, 1975, 1983), in an attempt to minimize the use of corolla characters in classification, revised *Columnea* by splitting it into four genera based on vegetative and nectary characters. He added a fifth genus to the columneoid alliance, *Bucinellina* (Wiehler) Wiehler (Wiehler 1977, 1981). This classification was controversial mostly in that *Columnea* was not considered a single genus. The presence of an opaque, white to pale colored berry rather than a capsule was considered an important unifying character for a single genus encompassing these species. As such, Kvist and Skog (1993) combined Wiehler's five genera into a single genus and recognized six sections. The sections largely corresponded to Wiehler's five genera, the exception being that section *Pentadenia* (Planch.) Benth. comprised only a single species, *C. strigosa* Benth., and the remaining members of Wiehler's genus *Pentadenia* (Planch.) J. Hanst. were placed in section *Stygnanthe* (Hanst.) Benth.

Smith & Sytsma (1994a–c) conducted phylogenetic analyses of Kvist & Skog's (1993) sections *Pentadenia* and *Stygnanthe* using morphological and chloroplast DNA restriction site data. Although they did not have evidence for the monophyly of either of these sections, Smith (1994) retained them both in his revision, pending further sampling of molecular data. Smith (1994) considered section *Pentadenia* more broadly than did Kvist & Skog (1993) and retained nine species in this group.

Since these studies, species of *Columnea* have been sampled in numerous DNA sequence based phylogenetic analyses (Smith & Carroll 1997; Smith 2000; Zimmer et al. 2002; Clark & Zimmer 2003; Clark et al. 2006, 2012) and has been recovered as monophyletic or unresolved (Smith & Carroll 1997) among other closely related genera. However, in none of these studies has there been sufficient resolution or support for resolved clades to test the subgeneric classification of the genus. The goal of the present study is to examine the relationships among species within *Columnea* to generate a well-supported topology that can test the monophyly of recent classification systems within the genus (Wiehler 1983, Kvist & Skog 1993, Smith 1994). We use phylogenetic analyses of sequences from five chloroplast DNA (cpDNA) regions (*rpl32-trnL*$_{UAG}$ and *trnQ-rps16* spacers: both from Shaw et al. 2007; *rps16* intron: Oxelman et al. 1997; *trnS-trnG* spacer: Hamilton 1999; and *trnH-psbA* spacer: Clark et al. 2006), along with the nuclear ribosomal internal transcribed spacer region (ITS1, 5.8S, and ITS2; hereafter referred to as ITS).

## MATERIALS AND METHODS

A complete list of samples, voucher specimens, and GenBank accession numbers can be found in TABLE 1. Our ingroup included 93 accessions of *Columnea* representing 68 species based on morphology. These species represented multiple individuals from each of Wiehler's (1983) segregate genera as well as the sections of Kvist and Skog (1993) with the exception that only one of the two species of *Bucinellina* was included (TABLE 1). Our outgroup samples were chosen on the basis of Clark et al.'s (2006, 2012) study of Episcieae and included species of *Alloplectus* Mart., *Corytoplectus* Oerst., *Crantzia* Scop., *Drymonia* Mart., *Glossoloma* Hanst., *Neomortonia* Wiehler, and *Pachycaulos* J.L.Clark & J.F.Smith (TABLE 1).

DNA was extracted from silica-dried leaf material using Qiagen DNeasy plant mini kits (Valencia, California, USA) according to manufacturer's instructions. Double-stranded DNA was amplified via PCR following the methods of Smith et al. (1997). Sequences were obtained either through the methods described in Smith et al. 2004, or purified PCR products were sent to Genewiz (Plainfield, New Jersey, USA) and chromatograms were viewed and sequences edited and aligned by hand in PhyDe (http://www.phyde.de/).

Nearly every region had missing data at the beginning and end of each region in the full alignment. Additionally, the alignment produced regions of ambiguity due to single base pair or microsatellite repeats. Areas of missing data and ambiguous alignments were excluded from phylogenetic analyses. The alignments also resulted in gaps to account for indel events. While the inclusion of indels can often be of phylogenetic significance (Simmons & Ochoterena 2000), the indels generated here were either autapomorphic, found only in the outgroup species, or were found in two or more individuals of the same species. A total of eight indels have potential phylogenetic significance (five in *trnQ-rps16* and one each in *rpl32-trnL*$_{UAG}$, *trnS-trnG* and *psbA-trnH*). We opted to treat indels as missing data in the phylogenetic analyses and use them as further support for recovered clades.

The partition homogeneity test (Farris et al. 1994) was performed as implemented in PAUP*4.0 b10 (Swofford 2002) with 10,000 bootstrap replicates (using a heuristic search, simple addition, and no branch swapping). The cpDNA and ITS regions were treated as separate partitions. 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 supported (Seelanen et al. 1997).

Phylogenetic trees were estimated using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). Maximum parsimony analyses were performed using PRAP2 (Müller 2004) in conjunction with PAUP*4.0b10 (Swofford 2002). Bootstrap support (BS) for nodes (Felsenstein 1985) was estimated with 1000 heuristic replicates using PRAP2. Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analysis were given by consistency index (CI; Kluge & Farris 1969), retention index (RI; Farris 1989), and the resulting rescaled consistency index (RC).

Maximum likelihood analyses were performed using optimal substitution models suggested by Modeltest 3.6 (Posada & Crandall 1998) for both ITS and cpDNA regions separately and combined. The Akaike Information Criterion (AIC), which allows non-nested models to be evaluated, was used as a selection criterion (Posada & Buckley 2004). The GTR + $\Gamma$ + I model was chosen for both partitions and the combined data. Analyses of ML were completed using GARLI v0.96 (Zwickl 2006) with 100 bootstrap replicates.

Bayesian inference analyses were completed using MrBayes 3.1.1 (Huelsenbeck & Ronquist 2003) with the GTR + $\Gamma$ + I model and run with 4 to 1 heated chains, for ten million generations. Convergence was determined by viewing in Tracer v1.3 (Rambaut & Drummond 2005), and a burnin of 50,000 generations was discarded prior to sampling the posterior distribution. The analyses were repeated twice to ensure that parameter estimates converged to similar values. The separate runs were compared using the online version of AWTY (http://king2.scs.fsu.edu/CEBProjects/awty/awty.php?fromStart=1&sessionDir=tmp18595; Nylander et al. 2008) as a means of determining if the separate chains approximated the same target distribution. We report the 50% majority-rule consensus tree sampled from the posterior probability distribution.

The trees did not produce clades that agreed with previous classification systems, therefore, Shimodaira-Hasegawa (SH) tests (Shimodaira & Hasegawa 1999, Goldman et al. 2000) were employed to determine whether the clades were significantly different from either Wiehler's (1983) genera, or the sections of Kvist and Skog (1993). The one exception was the single species of *Bucinellina*. Constraint trees were created in MacClade (Maddison & Maddison 2003) by altering the topology of the optimal tree. Topologies were altered to test each set of taxa separately while retaining the remainder of the tree to the topology recovered without constraints. These constraint trees were tested to determine whether they had significantly poorer fit to the data than the unconstrained tree using the SH test as implemented in PAUP*, with full optimization and 1000 bootstrap replicates (one-tailed test).

## RESULTS

Amplifications were successful for all regions for all individuals with some exceptions for each DNA region except for the *trnS-trnG* spacer (TABLE 1). Length for the aligned sequence, and the aligned sequences with missing and ambiguous regions removed were 1222/928, 1223/1246, 996/668, 1038/622, 542/429, and 723/477 for *trnQ-rps16* intron, *rpl32-trnL*$_{UAG}$ spacer, *rps16* intron, *trnH-psbA* spacer, and ITS, respectively for a total of 4219 included base pairs in the phylogenetic analyses. The 5.8S gene between ITS1 and ITS2 was identical across ingroup species and was excluded from the analyses and calculations. The aligned matrix contained 4219 base pairs and of these, 3013 were constant and 723 were uninformative. The matrix contained 483 (11.4%) phylogenetically informative base pairs. An inversion in *trnH-psbA* was detected in *C. lophophora* Mansf., *C. moesta* Poepp. (*J.L. Clark 6690*), *C. eburnea* (Wiehler) L.P.Kvist & L.E.Skog, *C. picta* H. Karst., *C.* sp. nov., (*J.L. Clark 8898*) and *C. schimpfii* Mansf.. The inversion was reversed and complemented for all individuals prior to analyses. All datasets have been submitted to TreeBASE (study number 14207).

TABLE 1. Species, voucher, herbarium, locality, and GenBank numbers, NA = not sequenced.

| Species | Voucher | Herbarium | Country of origin | *trnQ-rps16* spacer | *rpl32-trnL$_{UAG}$* spacer | *rps16* intron | *trnS-trnG* spacer | *trnH-psbA* spacer | ITS |
|---|---|---|---|---|---|---|---|---|---|
| *Alloplectus hispidus* (Kunth.) Mart. | *J.L. Clark 7720* | US | Ecuador | KF006030 | KF005812 | KF005921 | JQ953700 | DQ211219 | DQ211111 |
| *Columnea albiflora* L.P.Kvist & L.E.Skog | *J.L. Clark & J. Rea 8015* | UNA | Ecuador | KF006031 | KF005813 | KF005922 | KF006135 | KF005640 | KF005725 |
| *C. ambigua* (Urb.) B.D. Morley | *J. Smith 3701* | SRP | cultivated, originally Puerto Rico | KF006032 | KF005814 | KF005923 | JQ953713 | KF005641 | JQ953789 |
| *C. angustata* (Wiehler) L.E.Skog | *J. Smith 1433* | WIS | Colombia | KF006033 | KF005815 | KF005924 | KF006136 | KF005642 | KF005726 |
| *C. angustata* (Wiehler) L.E.Skog | *J.L. Clark 8627* | UNA | Panama | KF006034 | KF005816 | KF005925 | KF006137 | NA | KF005727 |
| *C. anisophylla* DC. | *E. Tepe 1081* | SRP | Panama | KF006035 | KF005817 | KF005927 | KF006138 | KF005643 | KF005729 |
| *C. anisophylla* DC. | *J.L. Clark & J. Rea 8019* | UNA | Ecuador | KF006037 | KF005819 | KF005926 | KF006141 | KF005646 | KF005728 |
| *C. atahualpae* J.F.Smith & L.E.Skog | *J.L. Clark et al. 8000* | UNA | Ecuador | KF006038 | KF005821 | KF005930 | KF006142 | KF005647 | KF005732 |
| *C. bilabiata* Seem. | *J.L. Clark et al. 11157* | UNA | Ecuador | KF006039 | KF005822 | KF005931 | KF006143 | KF005648 | KF005733 |
| *C. brenneri* (Wiehler) B.D. Morley | *J. Smith 3385* | SRP | Ecuador | KF006040 | KF005823 | KF005932 | KF006144 | KF005649 | KF005734 |
| *C. brenneri* (Wiehler) B.D. Morley | *J.L. Clark & M. Mailloux 7842* | UNA | Ecuador | KF006041 | KF005824 | KF005933 | KF006145 | KF005650 | KF005735 |
| *C. brevipila* Urb. | *J. Smith 10058* | SRP | cultivated, originally from Jamaica | KF006042 | KF005825 | KF005934 | KF006146 | KF005651 | KF005736 |
| *C. byrsina* (Wiehler) L.P.Kvist & L.E.Skog | *J. Smith 3408* | SRP | Ecuador | KF006043 | KF005826 | KF005935 | JQ953714 | KF005652 | KF005737 |
| *C. byrsina* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark & O. Meija 6291* | UNA | Ecuador | KF006044 | KF005827 | KF005936 | KF006148 | KF005653 | KF005738 |
| *C. calotricha* Donn. Sm. | *J. Smith et al. 4117* | SRP | French Guiana | KF006045 | KF005828 | KF005937 | KF006149 | KF005654 | KF005739 |
| *C. ciliata* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark et al. 7508* | UNA | Ecuador | KF006046 | KF005829 | KF005938 | KF006150 | NA | KF005740 |
| *C. citriflora* L.E.Skog | *J.L. Clark 10053* | UNA | cultivated, originally from Panama | KF006047 | KF005830 | KF005939 | KF006151 | KF005655 | KF005741 |
| *C. colombiana* (Wiehler) L.P.Kvist & L.E.Skog | *J. Smith 1126* | WIS | cultivated | KF006049 | KF005832 | KF005941 | KF006153 | KF005657 | KF005743 |
| *C. colombiana* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark et al. 10024* | UNA | cultivated | KF006048 | KF005831 | KF005940 | KF006151 | KF005656 | KF005742 |
| *C. consanguinea* Hanst. | *E. Tepe 1082* | SRP | Panama | KF006050 | KF005833 | KF005942 | KF006154 | KF005658 | KF005744 |
| *C. crassicaulis* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark 8859* | US | cultivated | KF006051 | KF005834 | KF005943 | KF006155 | KF005659 | KF005745 |
| *C. cruenta* B.D. Morley | *J. Smith 8606* | SRP | cultivated, originally from Panama | KF006052 | KF005835 | KF005944 | KF006156 | KF005660 | KF005746 |
| *C. dielsii* Mansf. | *J. Smith 1989* | WIS | Ecuador | KF006053 | KF005836 | KF005945 | KF006157 | KF005661 | KF005747 |
| *C. dissimilis* C. V. Morton | *E. Tepe 1070* | SRP | Panama | KF006054 | KF005837 | KF005946 | KF006158 | KF005662 | KF005748 |

TABLE 1. Continued.

| Species | Voucher | Herbarium | Country of origin | *trnQ-rps16* spacer | *rpl32-trnL*$_{UAG}$ spacer | *rps16* intron | *trnS-trnG* spacer | *trnH-psbA* spacer | ITS |
|---|---|---|---|---|---|---|---|---|---|
| *C. dodsonii* Wiehler | *B. Stewart s. n.* | SRP | cultivated | KF006055 | KF005838 | KF005947 | KF006159 | KF005663 | KF005749 |
| *C. domingensis* (Urb.) B.D. Morley | *L. Hahn 445* | SRP | Dominican Republic | KF006056 | KF005839 | KF005948 | JQ953715 | KF005664 | JQ953790 |
| *C. eburnea* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark et al. 6353* | UNA | Ecuador | KF006057 | KF005840 | KF005949 | KF006160 | KF005665 | KF005750 |
| *C. elongatifolia* L.P.Kvist & L.E.Skog | *J.L. Clark et al. 10015* | UNA | cultivated, originally from Ecuador | KF006058 | KF005841 | KF005950 | KF006161 | KF005666 | KF005751 |
| *C. ericae* Mansf. | *J.L. Clark et al. 6920* | UNA | Ecuador | KF006059 | KF005842 | KF005951 | KF006162 | KF005667 | KF005752 |
| *C. ericae* Mansf. | *E. Tepe 1570* | SRP | Ecuador | NA | KF005818 | KF005928 | KF006139 | KF005644 | KF005730 |
| *C. erythrophaea* Decne. ex Houllet | *J. Smith 3727* | SRP | cultivated | KF006060 | KF005843 | KF005952 | KF006163 | KF005668 | AF543246 |
| *C. filifera* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark et al. 7140* | UNA | Ecuador | KF006061 | KF005844 | KF005954 | KF006165 | KF005669 | KF005753 |
| *C. fimbricalyx* L.P.Kvist & L.E.Skog | *J.L. Clark et al. 7395* | UNA | Ecuador | KF006062 | KF005845 | KF005955 | KF006166 | KF005670 | KF005754 |
| *C. flexiflora* L.P.Kvist & L.E.Skog | *J.L. Clark & L. Jost 6968* | UNA | Ecuador | KF006063 | KF005846 | KF005956 | KF006167 | KF005671 | KF005755 |
| *C. gallicauda* Wiehler | *J.L. Clark 6283* | UNA | cultivated | KF006064 | KF005847 | KF005957 | KF006168 | KF005672 | KF005756 |
| *C. gloriosa* Sprague | *J.L. Clark et al. 9921* | UNA | Ecuador | KF006065 | KF005848 | KF005958 | KF006169 | KF005673 | KF005757 |
| *C. guianensis* C. V. Morton | *J. Smith 3711* | SRP | Guyana | KF006066 | KF005849 | KF005959 | JQ953718 | KF005674 | JQ953791 |
| *C. guttata* Poepp. | *J.L. Clark & L. Jost 6974* | UNA | Ecuador | KF006067 | KF005850 | KF005960 | KF006170 | KF005675 | KF005759 |
| *C. herthae* Mansf. | *J.L. Clark et al. 4960* | UNA | Ecuador | KF006070 | KF005852 | KF005961 | KF006172 | KF005676 | KF005760 |
| *C. herthae* Mansf. | *J.L. Clark et al. 7113* | UNA | Ecuador | KF006069 | KF005853 | KF005963 | KF006173 | KF005677 | KF005761 |
| *C. herthae* Mansf. | *J.L. Clark et al. 11055* | UNA | Ecuador | KF006068 | KF005851 | KF005962 | KF006171 | NA | NA |
| *C. hypocyrtantha* (Wiehler) J.F.Smith & L.E.Skog | *J.L. Clark & E. Rodriguez 6741* | US | Bolivia | KF006071 | KF005854 | KF005964 | KF006174 | KF005679 | KF005762 |
| *C. isernii* Cuatrec. | *J. Smith 2010* | WIS | Ecuador | KF006073 | KF005856 | KF005966 | KF006176 | KF005681 | KF005764 |
| *C. isernii* Cuatrec. | *J.L. Clark et al. 6253* | UNA | Ecuador | KF006074 | KF005857 | KF005967 | KF006177 | DQ211220 | AF543247 |
| *C. katzensteiniae* (Wiehler) L.P.Kvist & L.E.Skog | *J.L. Clark et al. 7625* | UNA | Ecuador | KF006075 | KF005858 | KF005968 | KF006178 | KF005683 | KF005766 |
| *C. lophophora* Mansf. | *J.L. Clark et al. 7888* | US | Ecuador | KF006076 | KF005860 | KF005969 | KF006179 | KF005684 | KF005767 |
| *C. magnifica* Klotzsch ex. Oerst. | *J. Smith 8602* | SRP | cultivated | KF006078 | KF005861 | KF005971 | KF006181 | KF005685 | KF005769 |
| *C. medicinalis* (Wiehler) L.E.Skog & L.P.Kvist | *J. Smith 1972* | WIS | Ecuador | KF006079 | KF005862 | KF005972 | KF006164 | KF005686 | KF005770 |
| *C. microphylla* Klotsch & Hanst. | *J.L. Clark 6261* | UNA | cultivated | KF006080 | KF005863 | KF005973 | KF006182 | KF005687 | KF005771 |
| *C. minor* (Hook.) Hanst. | *B. Stewart s. n.* | SRP | cultivated | KF006082 | KF005865 | KF005974 | KF006184 | KF005689 | KF005773 |
| *C. minor* (Hook.) Hanst. | *J.L. Clark 2934* | SRP | Ecuador | KF006081 | KF005864 | NA | KF006183 | KF005688 | KF005772 |
| *C. minor* (Hook.) Hanst. | *T. Croat 94778* | MO | Ecuador | KF006084 | KF005866 | KF005975 | KF006185 | KF005690 | KF005774 |

TABLE 1. Continued.

| Species | Voucher | Herbarium | Country of origin | *trnQ-rps16* spacer | *rpl32-trnL*$_{UAG}$ spacer | *rps16* intron | *trnS-trnG* spacer | *trnH-psbA* spacer | ITS |
|---|---|---|---|---|---|---|---|---|---|
| *C. minutiflora* L.P.Kvist & L.E.Skog | *J.L. Clark et al. 10832* | UNA | Ecuador | KF006085 | KF005867 | KF005976 | KF006186 | KF005691 | KF005775 |
| *C. minutiflora* L.P.Kvist & L.E.Skog | *J.L. Clark et al. 7092* | US | Ecuador | KF006086 | KF005868 | KF005977 | KF006187 | KF005692 | KF005776 |
| *C. mira* B.D. Morley | *J. Smith 2450* | WIS | cultivated, originally from Panama | KF006087 | KF005869 | KF005978 | KF006188 | KF005693 | KF005777 |
| *C. moesta* Poepp. | *J. Smith 1829* | WIS | Bolivia | KF006084 | KF005870 | KF005979 | KF006189 | KF005694 | KF005778 |
| *C. moesta* Poepp. | *J.L. Clark & M. Zeballos 6850* | UNA | Bolivia | KF006089 | KF005872 | KF005981 | KF006191 | KF005695 | KF005779 |
| *C. moesta* Poepp. | *J.L. Clark & D. Barrientos 6690* | US | Bolivia | KF006088 | KF005871 | KF005980 | KF006190 | DQ211242 | DQ211123 |
| *C. moesta* Poepp. | *J.L. Clark et al. 8211* | UNA | Peru | KF006091 | KF005873 | KF005982 | KF006192 | KF005696 | KF005780 |
| *C. oblongifolia* Rusby | *J. Smith 1721* | WIS | Bolivia | KF006092 | KF005874 | KF005983 | KF006193 | KF005697 | KF005781 |
| *C. orientandina* Mansf. | *J. Smith 3421* | SRP | Ecuador | KF006093 | KF005875 | KF005984 | KF006194 | KF005698 | KF005782 |
| *C. orientandina* Mansf. | *J.L. Clark et al. 9885* | UNA | Ecuador | KF006094 | KF005876 | KF005985 | KF006195 | KF005699 | KF005783 |
| *C. ovatifolia* L.P.Kvist & L.E.Skog | *J. Smith 1921* | WIS | Ecuador | KF006091 | KF005877 | KF005986 | KF006196 | KF005700 | KF005784 |
| *C. paramicola* (Wiehler) L.P.Kvist & L.E.Skog | no voucher USBRG94529 | NA | cultivated | KF006095 | KF005878 | KF005987 | JQ954064 | DQ211224 | DQ211113 |
| *C. picta* H. Karst. | *T. Croat 94956* | MO | Ecuador | KF006096 | KF005879 | KF005988 | KF006197 | KF005701 | KF005785 |
| *C. pulchra* (Wiehler) L.E.Skog | no voucher | - | cultivated | KF006097 | KF005881 | KF005989 | KF006199 | KF005702 | KF005787 |
| *C. pulchra* (Wiehler) L.E.Skog | *J.L. Clark 6265* | US | cultivated | NA | KF005880 | KF005990 | KF006198 | DQ211225 | KF005786 |
| *C. purpureovittata* (Wiehler) B.D. Morley | *J.L. Clark et al. 11448* | UNA | Peru | KF006098 | KF005882 | KF005991 | KF006200 | KF005703 | KF005788 |
| *C. purpureovittata* (Wiehler) B.D. Morley | *J. Smith s. n.* | SRP | cultivated | KF006072 | KF005855 | KF005965 | KF006175 | KF005680 | KF005763 |
| *C. purpusii* Standl. | *A. Rincon et al. 2302* | XAL | Mexico | KF006099 | KF005883 | KF005992 | JQ953719 | KF005704 | JQ953792 |
| *C. repens* (Hook.) Hanst. | *J. Smith 8605* | SRP | cultivated, originally from Jamaica | KF006100 | KF005884 | KF005993 | KF006201 | KF005705 | KF005790 |
| *C. rileyi* (Wiehler) J.F.Smith | *J. Smith 1944* | WIS | Ecuador | KF006101 | KF005885 | KF005994 | KF006202 | KF005706 | KF005791 |
| *C. rileyi* (Wiehler) J.F.Smith | *J.L. Clark 6263* | US | Ecuador | KF006102 | KF005886 | KF005995 | KF006203 | DQ211250 | AF543239 |
| *C. rubricalyx* L.P.Kvist & L.E.Skog | *J.L. Clark et al. 11034* | UNA | Ecuador | KF006103 | KF005887 | KF005997 | KF006204 | KF005707 | KF005792 |
| *C. rubricalyx* L.P.Kvist & L.E.Skog | *T. Croat 95236* | MO | Ecuador | KF006104 | KF005888 | KF005996 | KF006205 | KF005708 | KF005793 |
| *C. sanguinea* (Pers.) Hanst. | *J. Smith 636* | WIS | cultivated | KF006105 | KF005889 | KF005998 | KF006206 | KF005709 | KF005794 |
| *C. scandens* L. | *J.L. Clark & S. G. Clark 6541* | UNA | Martinique | KF006106 | KF005890 | KF005999 | KF006207 | KF005711 | KF005795 |

TABLE 1. Continued.

| Species | Voucher | Herbarium | Country of origin | *trnQ-rps16* spacer | *rpl32-trnL*$_{UAG}$ spacer | *rps16* intron | *trnS-trnG* spacer | *trnH-psbA* spacer | ITS |
|---|---|---|---|---|---|---|---|---|---|
| *C. schiedeana* Schltdl. | *J. Smith 288* | WIS | cultivated, originally from Mexico | KF006107 | KF005891 | KF006000 | KF006208 | KF005712 | KF005796 |
| *C. schimpfii* Mansf. | *J. Smith 8603* | SRP | cultivated, originally from Ecuador | KF006109 | KF005892 | KF006001 | KF006209 | KF005713 | KF005797 |
| *C. segregata* (B.D. Morley) Wiehler | *J.L. Clark et al. 10029* | UNA | cultivated | KF006108 | NA | KF006002 | KF006210 | KF005714 | NA |
| *C.* sp. DC. | *J.L. Clark & N. Harris 7295* | UNA | Ecuador | KF006036 | KF005820 | KF005929 | KF006140 | KF005645 | KF005731 |
| *C.* sp. nov. | *J.L. Clark et al. 8898* | UNA | Ecuador | KF006077 | KF005859 | KF005970 | KF006180 | KF005684 | KF005768 |
| *C. spathulata* Mansf. | *J. Smith 1853* | WIS | Ecuador | KF006110 | KF005893 | KF006003 | KF006211 | KF005715 | KF005798 |
| *C. spathulata* Mansf. | *J.L. Clark et al. 7485* | UNA | Ecuador | KF006111 | KF005894 | KF006004 | KF006212 | KF005716 | KF005799 |
| *C. strigosa* Benth. | *J. Smith 1200* | WIS | Venezuela | KF006112 | KF005895 | KF006005 | KF006214 | KF005717 | KF005800 |
| *C. strigosa* Benth. | *T. Croat 94580* | MO | Ecuador | KF006113 | KF005896 | KF006006 | KF006213 | KF005718 | KF005801 |
| *C. tenella* L.P.Kvist & L.E.Skog | *M. Amaya M. & J. Smith 603* | COL | Colombia | KF006114 | KF005897 | KF006007 | KF006215 | KF005719 | KF005802 |
| *C. tenensis* Wiehler | *J.L. Clark et al. 9500* | UNA | Ecuador | KF006116 | KF005899 | KF006009 | KF006217 | KF005721 | KF005803 |
| *C. tenensis* Wiehler | *J. Smith 3374* | SRP | Ecuador | KF006115 | KF005898 | KF006008 | KF006216 | KF005720 | KF005804 |
| *C. trollii* Mansf. | *J. Smith 1723* | WIS | Bolivia | KF006117 | KF005899 | KF006010 | KF006218 | KF005722 | KF005805 |
| *C. ultraviolacea* J.F.Smith & L.E.Skog | *J.L. Clark & V. Velaz 6603* | UNA | Bolivia | KF006118 | KF005900 | KF006011 | KF006219 | KF005723 | KF005806 |
| *C. villosissima* Mansf. | *E. Tepe 1628* | SRP | Ecuador | KF006119 | KF005901 | KF006012 | KF006220 | KF005724 | KF005807 |
| *Corytoplectus capitatus* (Hook.) Wiehler | *T. Croat 94581* | MO | cultivated | KF006120 | KF005902 | KF006013 | JQ953698 | NA | JQ953798 |
| *Corytoplectus speciosus* (Poepp.) Wiehler | no voucher, USBRG 1994-268 | | cultivated | KF006117 | KF005903 | KF006010 | JQ953699 | NA | JQ95379 |
| *Crantzia cristata* (L.) Scopoli | *J.L. Clark 6346* | US | Martinique | KF006027 | KF005809 | KF005918 | KF006134 | DQ211294 | DQ211154 |
| *Crantzia epirotes* (Leeuwenb.) J.L.Clark | *D. Clarke 10172* | US | Guyana | KF006028 | KF005810 | KF005919 | JQ953702 | DQ211293 | DQ211153 |
| *Crantzia tigrina* (Karsten.) J.L.Clark | *J.L. Clark 6892* | US | Venezuela | KF006029 | KF005811 | KF005920 | JQ953703 | DQ211295 | DQ211155 |
| *Drymonia coccinea* (Aubl.) Wiehler | *J. Smith 3373* | SRP | Ecuador | KF006121 | KF005905 | KF006014 | JQ953704 | DQ211266 | DQ211132 |
| *Drymonia pendula* (Poepp.) Wiehler | *J. Smith 3384* | SRP | Ecuador | KF006122 | KF005906 | KF006015 | KF006221 | NA | NA |
| *Drymonia pilifera* Wiehler | *E. Tepe 1065* | SRP | Panama | KF006123 | KF005907 | KF006016 | KF006222 | DQ211272 | DQ211137 |
| *Drymonia serrulata* (Jacq.) Mart. | *J. Smith 4202* | SRP | French Guiana | KF006124 | KF005908 | KF006017 | JQ953705 | DQ211267 | DQ211133 |
| *Drymonia strigosa* (Oerst.) Wiehler | *A. Rincon 2301* | XAL | Mexico | KF006125 | KF005909 | KF006018 | JQ953706 | DQ211280 | DQ211143 |
| *Drymonia turrialvae* Hanst. | *E. Tepe 1063* | SRP | Panama | KF006126 | KF005910 | KF006019 | KF006223 | DQ211278 | DQ211141 |
| *Drymonia urceolata* Wiehler | *J. Smith 3416* | SRP | Ecuador | KF006127 | KF005911 | KF006020 | JQ953707 | DQ211289 | AF543265 |

TABLE 1. Continued.

| Species | Voucher | Herbarium | Country of origin | *trnQ-rps16* spacer | *rpl32-trnL$_{UAG}$* spacer | *rps16* intron | *trnS-trnG* spacer | *trnH-psbA* spacer | ITS |
|---|---|---|---|---|---|---|---|---|---|
| *Glossoloma anomalum* J.L.Clark | *J. Smith 3418* | SRP | Ecuador | KF006128 | KF005912 | KF006021 | KF006224 | NA | AF543225 |
| *Glossoloma grandicalyx* (J.L.Clark & L.E.Skog) J.L.Clark | *J. Smith 3417* | SRP | Ecuador | KF006129 | KF005913 | KF006024 | JQ953708 | DQ211205 | AF543218 |
| *Glossoloma martinianum* (J.F.Smith) J.L.Clark | *J.L. Clark 6101* | US | Ecuador | KF006130 | KF005914 | KF006022 | JQ953709 | DQ211209 | AF543228 |
| *Glossoloma panamense* (C.V.Morton) J.L.Clark | *L.E. Skog et al. 7641* | US | cultivated | KF006131 | KF005915 | KF006023 | JQ953710 | DQ211202 | DQ211102 |
| *Neomortonia rosea* Wiehler | no voucher, USBRG 94230 | | cultivated | KF006133 | KF005917 | KF006026 | JQ953712 | DQ211197 | DQ211099 |
| *Pachycaulos nummularium* (Hanst.) J.L.Clark & J.F.Smith | *J. Smith 3944* | SRP | cultivated | KF006132 | KF005916 | KF006025 | JQ953711 | DQ211308 | AF543266 |

### Test of Incongruence

The result of the partition homogeneity test ($P = 0.01$) indicated significant differences between partitions. However, as has been reported on many occasions, this test often indicates incongruence when none exists (Reeves et al. 2001, Yoder et al. 2001). Alternatively, comparing support for partitions is a better indicator of incongruence (Seelanen et al. 1997). All regions were in complete topological congruence or received BS <50 for the individual analyses. Therefore a combined analysis of the DNA regions was performed and is the basis for all results and discussion.

### Phylogenetic Analyses

Maximum parsimony analysis resulted in 1684 trees of 2335 steps (CI = 0.65, RI = 0.70, RC = 0.45). The strict consensus is presented in FIGURE 1. The ML analyses used the GTR + $\Gamma$ + I model as suggested by Modeltest 3.6. The analysis produced one tree (-lnL = 220803.2141). The BI analyses recovered similar trees and supported clades as with the ML and MP analyses (FIGURE 1). The output from AWTY indicated that the separate chains approximated the same target distribution for both the full and reduced analyses. Support for clades is represented by maximum parsimony bootstrap (MPBS), maximum likelihood bootstrap (MLBS) and Bayesian posterior probabilities (PP) on FIGURE 1 and is reported as MPBS/MLBS/PP hereafter in the text.

In all analyses, *Columnea* is recovered as a monophyletic group (FIGURE 1; 96/100/0.95) with *Glossoloma* supported as sister (FIGURE 1; 87/86/0.95). Support among clades within the genus is not strong. Clade A (FIGURE 1) is only supported as monophyletic by ML (54/76/0.90) and supported as sister to the remainder of the genus from all methods (98/99/0.95). Clade B is supported as monophyletic (82/88/0.97) but is not supported for its placement as sister to the remainder of the genus excluding clade A. There is no support from any analyses for the relationships among the remainder of the clades, but several of the clades themselves (clades C-G of FIGURE 1) are each supported. There are two additional clades that are recovered in all analyses, but are not supported by any analysis and four individuals that represent four species do not group in any clade (FIGURE 1). The SH tests rejected the monophyly of all genera and sections following the systems of Wiehler (1983), Kvist and Skog (1993) and Smith (1994) for *Columnea* based on the phylogenetic results presented here.

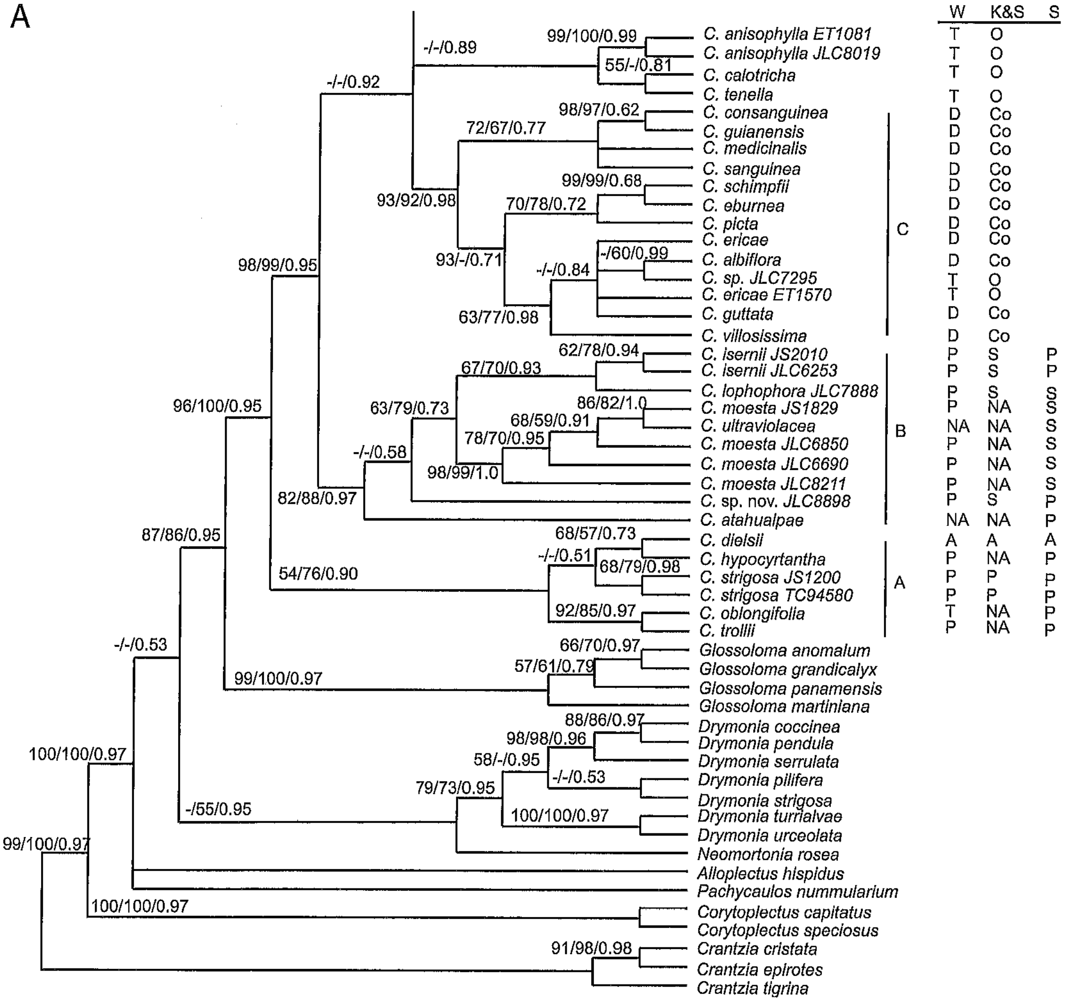

FIGURE 1. Majority rule consensus tree from the Bayesian analysis. The topology of this tree is almost completely congruent with the trees from maximum parsimony and maximum likelihood. Branches that collapse in the strict consensus of the maximum parsimony tree are marked with an asterisk (*). Values along branches are maximum parsimony bootstrap/maximum likelihood bootstrap/Bayesian posterior probabilities. A dash indicates that support was less than 50 for that analysis. Letters to the right of the tree indicate clades that are discussed in the text. Columns to the right of the tree designate how the species was classified by Wiehler (1983; C- genus *Columnea,* D – genus *Dalbergaria,* T – genus *Trichantha,* P – genus *Pentadenia,* B – genus *Bucinellina*), Kvist & Skog (1993; C- section *Columnea,* Co – section *Collandra,* B – section *Bucinellina,* O – section *Ortholoma,* P – section *Pentadenia,* S - section *Stygnanthe*) and Smith (1994; abbreviations follow Kvist & Skog). Note that Smith (1994) only considered sections *Pentadenia* and *Stygnanthe.* NV=no voucher, *purpur* =*purpureovittata.*

## DISCUSSION

Although we have not fully resolved all relationships among the species sampled here, there are still several supported conclusions that can be drawn from these results. *Columnea* is a monophyletic group that is supported as the sister to *Glossoloma* (FIGURE 1). While the monophyly of *Columnea* has been supported in previous studies that sampled more than a single species (Smith 2000; Zimmer et al. 2002; Clark & Zimmer 2003; Clark et al. 2006, 2012), this is the first study that has identified clades within the genus.

The clades that are resolved here do not agree with previous subgeneric classification systems based on morphology alone. The SH tests reject the monophyly of all of Wiehler's segregate genera and Kvist & Skog's (1993) sections except for *Bucinellina* which is represented here by one species, *C. paramicola* (Wiehler) L.P.Kvist & L.E.Skog, and Kvist & Skog's section *Pentadenia* that encompassed only *C. strigosa* Benth.

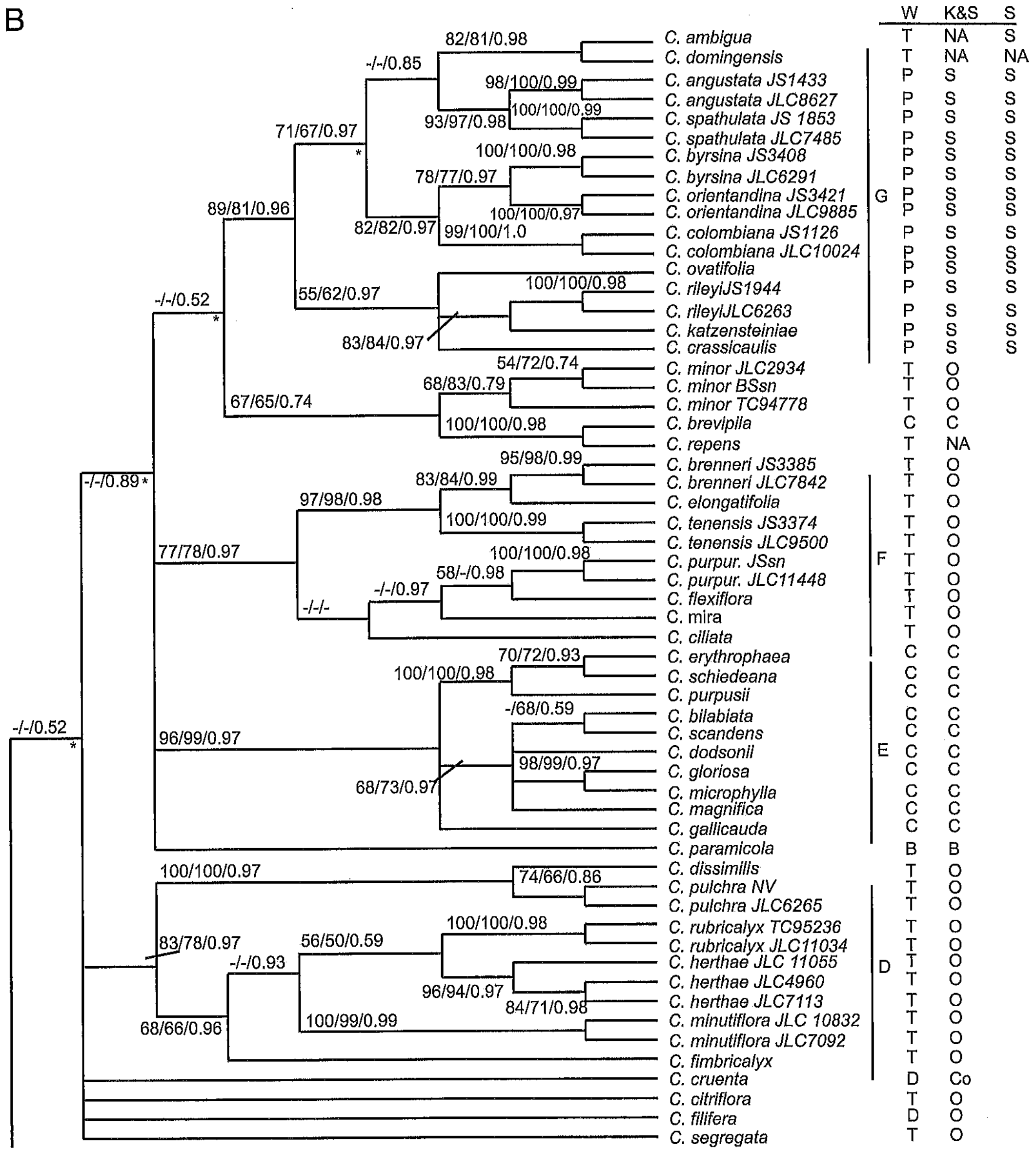

FIGURE 1. Continued.

Likewise, Smith's (1994) revised classification of Kvist & Skog's (1993) sections *Pentadenia* and *Stygnanthe* can all be rejected as monophyletic based on SH tests.

### Clade A

Clade A is supported as the sister group to the remainder of *Columnea* (98/99/0.95). This clade (based on four sampled species in common) was also recovered as sister to the remainder of *Columnea* by Clark et al. (2006). Support for the monophyly of this clade is only from ML (54/76/0.90) although an eight bp indel in the *rpl32-trnL*$_{UAG}$ spacer is also common to all species in Clade A plus *C. rubricalyx* L.P.Kvist & L.E.Skog (*J.L. Clark 11034*), albeit the latter has a single bp substitution compared to the species of Clade A. The opposite leaves are isophyllous (equal in size) or weakly anisophyllous. Most *Columnea* are strongly anisophyllous. This vegetative feature is useful for defining clade A. Species in this clade

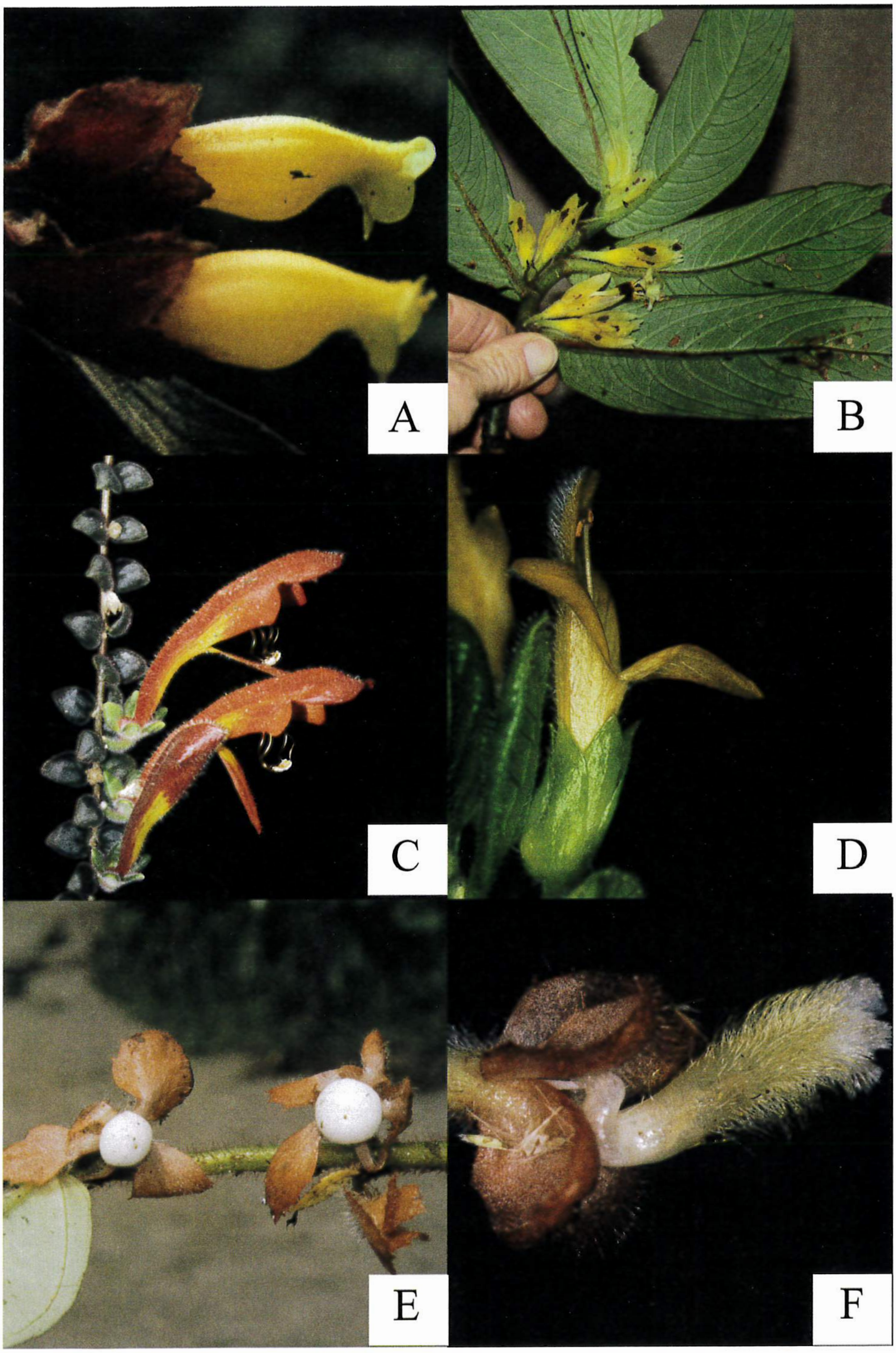

FIGURE 2. Photographs illustrating morphologies in *Columnea*. **A**. Ventricose corolla of *C. dielsii* (*J.L. Clark 5813*). **B**. Dorsiventral habit of *C. picta* (*T. Croat 94956*). **C.** Bilabiate corolla of *C. microphylla* (*J.L. Clark 6261*). **D.** Bilabiate corolla of *C. proctorii* (*J.L. Clark 8880*) with reflexed lateral lobes. **E.** Flattened fruit of *C. herthae* (*J.L. Clark 4960*). **F.** Flower of *C. herthae* (*J.L. Clark 7113*) with apical pubescence. A, and C-F photographed by J.L. Clark. B by Chris Davidson.

are generally robust herbs with erect to scandent stems and are either terrestrial or epiphytic. The corollas are generally large for *Columnea* (smallest ones approximately 3.0 cm in length, but reaching 9.5 cm in *Columnea strigosa*; Smith 1994) and are strongly to weakly ventricose on the lower apical region of the corolla (FIGURE 2A). Two species, (*Columnea trollii* Mansf. and *C. hypocyrtantha* (Wiehler) J.F.Smith & L.E. Skog) have tubular corollas with a constricted throat and developed pouch on the lower surface. This corolla form is termed hypocyrtoid and is common in other genera (e.g., *Drymonia, Nematanthus* Schrad., and *Pachycaulos*), but not found elsewhere in *Columnea.* Species in this clade are typically found at higher elevations than most other species in the genus, generally above 2000 m although some collections of *C. strigosa* are known from 1500 m and a few of *C. trollii* have been documented at 1200 m (Smith 1994).

This clade comes closest to matching Smith's (1994) section *Pentadenia*, although he included three species (*C. isernii* Cuatrec., *C. lophophora,* and *C. atahualpae* J.F.Smith & L.E.Skog) that are in clade B of this analysis. Kvist and Skog (1993) included only *C. strigosa* in their section *Pentadenia* and Wiehler's genus *Pentadenia* included many of the species here, but excluded *C. oblongifolia* Rusby (placed in his genus *Trichantha*) and many of the species that shared the five-lobed nectary that defined Wiehler's genus *Pentadenia* are here placed in clade G (FIGURE 1). Four species from Clade A, *C. strigosa, C. oblongifolia, C. trollii*, and *C. dielsii* Mansf. were sampled by Smith and Sytsma (1994b) in a cpDNA restriction site analysis and all but *C. strigosa* were recovered as a monophyletic group that also was sister to the remainder of the genus.

Clade A also includes *Columnea dielsii* (FIGURE 2A) that had been excluded from most previous classifications of *Columnea* although both Bentham (1876) and Fritsch (1894) had included it in *Columnea.* Based on habit, distribution, and corolla form, this species fits well with the other species of this clade. *Columnea dielsii* was supported as belonging to *Columnea* based on molecular data prior to this study (Smith & Sytsma 1994b; Clark et al. 2006). The generic placemement of this taxon is historically confusing and it was usually classified as belonging to *Alloplectus* instead of *Columnea* because it has a fleshy dehiscent capsule instead of a fleshy non-dehiscent berry (Wiehler 1973, 1983; Kvist & Skog 1993; Smith 1994). Two other species in this clade that at least sometimes have fleshy dehiscent capsules are *Columnea trollii* (Smith, pers. obs.) and an undescribed species from Carpish, Peru (*J.L. Clark 8188*) that is not sampled here, but was supported as being a member of *Columnea* in Clark et al. (2006). Some of the species in this clade (e.g., *C. trollii*) may contain berries that are fleshy and indehiscent, but split along the septum when pressure is applied (Smith, pers. obs.).

This clade includes the following two generic type species in *Columnea*: *Pentadenia* with the type of *C. strigosa* and *Systolostoma* with the type of *C. dielsii.* The former name has priority and this clade will likely be considered section *Pentadenia* in forthcoming formal classification systems.

### Clade B

Clade B is also supported as monophyletic (82/88/0.97) and receives some support from BI as sister to the remainder of *Columnea* excluding clade A (PP = 0.92). Vegetatively, Clade B is like Clade A in that the opposite leaves are isophyllous (equal in size) or weakly anisophyllous. Species in this clade are erect herbs. The corollas are slightly ventricose on the lower surface, but not as strongly ventricose or hypocyrtoid as some species in clade A. They also are primarily terrestrial in habit and generally have petiolate leaves although this latter character is also found in other clades. The four species in this clade are found between 200 and 2450 m elevation (Smith 1994).

As with clade A, these species would have been included by Wiehler (1973, 1983) in the genus *Pentadenia* (FIGURE 1). However, two species in the present analysis (*C. ultraviolacea* J.F.Smith & L.E.Skog and *C. atahualpae*) were never treated by Wiehler. The species in clade B were recognized in section *Stygnanthe* by Kvist and Skog (1993). Most of these species in clade B would have been placed by Smith (1994) in his section *Pentadenia.* However, both *C. moesta* and *C. ultraviolacea* are both strongly placed in clade B and would have been included in Smith's (1994) section *Stygnanthe* with most of the species in clade G (FIGURE 1). The classification system promoted by Wiehler emphasized the number of nectaries surrounding the ovary. The results presented here therefore suggest that the presence of five nectaries surrounding the ovary is convergent in clades, A, B, and G. Smith and Systma (1994b) did show a close relationship between *C. ultraviolacea* and *C. moesta*, but did not place these two species close to *C. isernii,* the only other species included here that was sampled in both studies. The results presented here differ from the cpDNA restriction site variation (Smith & Sytsma 1994b) and can be attributed to the limited taxon sample size in that study (21 species from sections *Stygnanthe* and *Pentadenia* and six other taxa) and limited resolving power of the cpDNA restriction site variation among clades.

*Columnea ultraviolacea* is supported as nested within a clade of several individuals of *C. moesta*

(FIGURE 1). *Columnea ultraviolacea* has ternate leaves that is relatively uncommon in the Gesneriaceae and in *Columnea* is only known in *Columnea fritschii* (Rusby) J.F.Smith. This species is known only from three collections in Bolivia and is geographically found close to *C. ultraviolacea*. *Columnea moesta* is also found in Bolivia and southern Peru. Further examination of specimens will be necessary to resolve whether *C. ultraviolacea* and *C. fritschii* represent unique morphological shifts within *C. moesta*, or if the several different lineages of *C. moesta* resolved here are each representative of undescribed species. With the exception of missing data for *C. moesta* (*J.L. Clark 6850*), all individuals of *C. moesta* and *C. ultraviolacea* share a three bp indel in the *trnQ-rps16* spacer.

The *C. lophophora* specimen *J.L. Clark et al. 7888* is from near the village of Huigra in Chimborazo, Ecuador, near where the type for *C. lophophora* was collected. The placement of this species as sister to *C. isernii* is additionally supported by a five bp indel in the *trnQ-rps16* spacer. The voucher of *C. lophophora* lacked flowers at the time of collection, but based on earlier collections from this region, *C. lophophora* is characterized by a violet to blue corolla (Smith 1994). The other collection initially identified here as *C.* sp. nov., *J.L. Clark et al. 8898* is from Zamora-Chinchipe, Ecuador and has a yellow corolla with red striations. This latter collection likely represents an undescribed species that superficially resembles *C. lophophora*. This clade includes the type for the name *Stygnanthe, C. moesta,* that will likely be the name for this clade pending further sampling.

### Clade C

Clade C is supported as monophyletic (93/92/0.98). Species in this clade have climbing shoots with strongly anisophyllous leaves at each node, the leaves are subsessile arranged in distichous pairs. The epiphytic stems generally grow nearly perpendicular to the trunk and the leaves are held in a single plane parallel to the ground (FIGURE 2B). Most species have conspicuous red or purple spots on the underside of the leaves, and large bracts subtending the corollas are common. The corolla tube can be cylindrical, subventricose, or less common salverform; the corolla limb is either actinomorphic or bilabiate, but in the latter case never with a galea as occur in the bilabiate corollas of section *Columnea*. The species of this clade occur at elevations ranging from sea level to 2600 m elevation.

Wiehler's genus *Dalbergaria* Tussac and section *Collandra* (Lem.) Benth. of Kvist and Skog (1993) are mostly found in this clade. The non-monophyly is created by the absence of *C. filifera* (Wiehler) L.P. Kivst & L.E.Skog and *C. cruenta* B.D. Morley which fall outside of this clade, and outside of any other clade (FIGURE 1). *Columnea cruenta* is endemic to Panama and based on its suite of morphological characters (Skog 1978) would be expected to nest with other species of clade C. Further examination of this species will be essential if it continues to fall outside of clade C in subsequent analyses that include more species and DNA sequences.

Kvist and Skog initially described *C. filifera* as *C. fililoba* L.P.Kvist & L.E.Skog in their 1993 treatment of *Columnea* in Ecuador. They tentatively placed this species in section *Collandra* based on its leaves and habit, but noted that the presence of a globose rather than ovoid berry raised uncertainty in this placement. Additionally they commented that the elongate slender corolla lobes present in this species may in fact not be homologous to corolla lobes of other species of *Columnea* but may be similar to the corolla appendages that occur between the lobes of species in their section *Ortholoma* Benth., such as *C. minor* (Hook.) Hanst. Further analyses have shown that several species of section *Collandra* form a somewhat distinct group characterized by having long, free corolla lobes, which can be filiform (*C. filifera*, *C. incredibilis* L.P.Kvist & L.E.Skog, *C. nematoloba* L.P.Kvist & L.E.Skog) or laminar (*C. coronata* Amaya, L.E.Skog & L.P. Kivst). In addition, these species (except *C. nematoloba*) present a set of appendages at the base of the corolla limb, each appendage is located in front of the corolla lobe on the inside, forming a corona. The corona is an uncommon trait in *Columnea* (Amaya-Márquez et al. 2003). Some species of section *Ortholoma* also present corolla appendages, but those are located between the corolla lobes on the outside of the corolla tube. Based on the phylogenetic results here and the unique morphological characters, it may be that *C. filifera*, *C. incredibilis,* and *C. coronata* represent a unique linage in *Columnea*, or may yet be placed in clade C pending sampling of additional species and DNA regions. It will be interesting to see where *C. nematoloba* nests since this species has the elongated corolla lobes, but lacks the corona. This clade also includes the type for the name *Collandra* (*Columnea sanguinea* (Pers.) Hanst.) and this will likely be the name for the clade.

### Clade D

Most of the corollas in this supported (83/78/0.97) clade appear cleistogamous, the corolla lobes enclosed around the throat and never fully reflexed. There are two supported subgroups. One group consists of two species, *C. dissimilis* C. V. Morton and *C. pulchra* (Wiehler) L.E.Skog (their monophyly is also supported by two indels, one (three bp) in the *trnQ-rps16* spacer and the other (five bp) in the *psbA-trnH* spacer). The two species are morphologically similar with the notable exceptions of corolla color and vestiture. The other subclade consists of a group that is characterized by persistent calyx lobes that become truncate in fruit. The fruit is usually white and flattened (FIGURE 2E) instead of elongate or globose as found in other clades of *Columnea.* The calyx lobes are usually cordate at the base and reddish-orange (FIGURE 2E–F). With the exception of *Columnea rubricalyx,* most of corollas in the group are glabrous near the base and heavily pubescent near the apex (FIGURE 2F). This differs from the more uniform pubescence or glabrous corollas found in other species of *Columnea.* One remarkable species that nests in this clade is *C. fimbricalyx* L.P.Kvist & L.E.Skog with laciniate calyx lobes. However, the laciniate margin is an autapomorphic character state for the clade (albeit shared with other species of *Columnea*) and the cleistogamous corolla is typical of other members from this group.

Clade D represents the core of the species that Wiehler (1983) placed in his genus *Trichantha* and therefore the core of species that Kvist and Skog (1993) considered as section *Ortholoma* (FIGURE 1). There are still many species unsampled from this group, but regardless, it is not monophyletic due to the absence of *C. minor, C. anisophylla* DC., *C. calotricha* Donn. Sm., and *C. tenella* L.P.Kvist & L.E.Skog. The other species that would represent *Trichantha* (sensu Wiehler) or *Ortholoma* (sensu Kvist & Skog) are nesting in clade F (FIGURE 1). Although there is not BS support to separate clades D and F there is some PP support (0.89) that places clade D apart from clade F. *Columnea minor* is the type for *Trichantha,* which is currently not supported as belonging to one of the defined clades in this study (FIGURE 1). Instead, it is placed as the sister taxon to two Jamaican species without support (FIGURE 1; 67/65/0.74). Morphologically, *C. calotricha* would also be predicted to nest with other members of Clade D because it is morphologically similar to *C. pulchra* and *C. dissimilis* (e.g., cleistogamous corollas that are heavily pubescent apically). However, *C. calotricha* has a disjunct distribution and is found in Central America from Guatemala to Panama, and in South America from Surinam and French Guiana. The specimen included here is from French Guiana and may represent an undescribed species. It will be essential to include individuals from Central America in future analyses. There are no prior sectional or generic names associated with any of the species in this clade.

### Clade E

Based on the sequences analyzed here this clade is supported as monophyletic (97/99/0.97) and gets additional support by the presence of two indels; one (four bp) in the *trnS-trnG* spacer and the other (five bp) in *trnQ-rps16* spacer. Species in this clade have large showy corollas that widen gradually from the base, and are strongly bilabiate (never ventricose) forming a galea by the fusion of the upper two corolla lobes (FIGURE 2C). This clade is congruent with Wiehler's genus *Columnea* and Kvist & Skog's section *Columnea* with the exception that *C. brevipila* Urb. is supported a sister to *C. repens* (Hook.) Hanst. (100/100/0.98). Both of these latter species are endemic to Jamaica (Morley 1974b) but *C. repens* has never been considered to be close to the other species placed in clade E whereas *C. brevipila* has the corolla typical of section *Columnea.*

There are 13 species of *Columnea* in Jamaica, all of them endemic to the island (Morley 1974). Both *C. repens* and *C. grisebachiana* Kuntze have unusual corolla morphologies that have made them difficult to place in a subgeneric classification which was largely the reason they were initially described in the genus *Pterygoloma* Hanst. Wiehler placed both *C. grisebachiana* and *C. repens* in his genus *Trichantha* along with another Jamaican endemic, *C. pubescens* (Griseb.) Kuntze, whereas the other Jamaican endemics were all included in *Columnea.* The sister relationship of two Jamaican species with widely divergent corolla morphologies raises the question whether Jamaican *Columnea* species are all a monophyletic lineage or, as had previously been thought, are the result of two or three independent introductions.

Although the corollas of *C. brevipila* match those of section *Columnea* in a superficial sense, it is likely that they are the result of convergence. Convergence in corolla morphology has been a common occurrence among Neotropical Gesneriaceae and it is possible that selection has resulted in the origin of this corolla independently in Jamaica. Examination of specimens of *C. brevipila* at IJ, SRP, US, and UCWI indicates that the lateral corolla lobes are strongly reflexed (FIGURE 2D), a trait that is not common among other members of section *Columnea,* but is shared among other Jamaican species such as *C. proctorii* Stearn and *C. subcordata* C. V. Morton (Smith pers. obs.).

### Clade F

Species in Clade F are characterized by hispid trichomes on the stems and a warty appearance to older stems that likely arises when the trichomes are lost leaving a swollen base. This clade receives support for being monophyletic from the molecular data (77/78/0.97). Although not present in all species in this clade, and not unique among the clades in *Columnea* recovered here, there is a predominance of corollas that are yellow and purple striped. Only *C. ciliata* (Wiehler) L.P.Kvist & L.E.Skog and *C. flexiflora* L.P.Kvist & L.E.Skog lack this corolla color of the species that are found in this clade. The inclusion of *C. flexiflora* here is unusual based on corolla morphology alone in that this species has a strongly bilaterally symmetric corolla. Other species in this clade have weakly bilateral to nearly radial limb.

All species here were included in Wiehler's genus *Trichantha* and Kvist & Skog's section *Ortholoma* (FIGURE 1). See discussion for clade D for further discussion of the lack of monophyly for this section. As with clade D, there are no prior sectional or generic names that are associated with this clade.

### Clade G

The final supported clade (89/81/0.96) based on our current sampling within *Columnea* has two subclades that receive PP support but no BS support. Species in this clade are characterized as epiphytic herbs with slender or creeping stems, short pedicels that are nearly absent in some individuals and corollas that are generally relatively small (<5 cm long), and a five-lobed nectary surrounding the ovary.

This is the clade that has the remainder of species that Wiehler (1983) included in his genus *Pentadenia* and would make up the remainder of the species in both Kvist & Skog's (1993) and Smith's (1994) section *Stygnanthe* (FIGURE 1). The primary exception to the latter is that Smith included both *C. ultraviolacea* and *C. moesta* (clade B in FIGURE 1) in his section *Stygnanthe*. The lack of monophyly for Wiehler's genera is further exacerbated by the presence of both *C. ambigua* and *C. domingensis* (Urb.) B.D. Morley in this clade, two endemic Caribbean species that Wiehler included in his genus *Trichantha*.

Smith and Sytsma (1994b) sampled many of the species that are included in clade G using cpDNA restriction site variation, however they were unable to recover a monophyletic clade. They were able to resolve the subclades that are recovered here: *C. angustata* (Wiehler) L.E. Skog/*C. spathulata* Mansf. (also supported by a five bp indel in the *trnQ-rps16* spacer), *C. byrsina* (Wiehler) L.P.Kvist & L.E.Skog/*C. orientandina* Mansf./*C. colombiana* (Wiehler) L.P.Kvist & L.E.Skog, and *C. rileyi* (Wiehler) J.F.Smith/*C. katzensteiniae* (Wiehler) L.P.Kvist & L.E.Skog/*C. ovatifolia* L.P.Kvist & L.E.Skog/*C. crassicaulis* (Wiehler) L.P.Kvist & L.E.Skog. None of the species included in clade G are associated with any prior sectional or generic name.

### Species Outside of any Clade

In addition to *Columnea cruenta* and *C. filifera* and the two unsupported clades discussed above, there are two additional species that fall outside of any of the fully resolved clades. These are *C. paramicola* and *C. segregata* (B.D. Morley) Wiehler. *Columnea paramicola* was classified as *Bucinellina* (genus sensu Wiehler 1983, section sensu Kvist & Skog 1993). There is only one additional species in this group, *C. nariniana* (Wiehler) Kvist and L. Skog and sampling this species will be essential to resolve whether these two are retained as their own section or not. Their separation from other species is based on the presence of a flattened berry and small corollas, the flattened berry being unique in the genus.

*Columnea segregata* is also morphologically similar to both *C. grata* C. V. Morton and *C. sanguinolenta* (Klotzsch ex Oerst.) Hanst., all from Central America. These species may also form a distinct clade in the genus as *C. grata* was initially described as *Stenanthus heterophyllus* Oerst. and will necessarily await additional taxon sampling.

This is the first study to resolve supported relationships within *Columnea* and sample widely from across the genus. Support for some clades remains low and with the exception of identifying the clade that is sister to the remainder of the genus, there is not much support for relationships among clades. However, additional sampling of species, individuals and DNA regions is currently underway. We anticipate that the inclusion of these additional data will help resolve relationships that are currently un-supported and will lead to a forthcoming formal subgeneric classification for *Columnea*.

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