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

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A Publication Devoted to Tropical Plants,
with Emphasis on Epiphytic Plant Families

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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. *Columnnea* is the largest Neotropical genus in Gesneriaceae subfamily Gesnerioideae and has had a complex and confusing taxonomic history. The species that are now considered *Columnnea* 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 *Columnnea* 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 *Columnnea* 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 *Columnnea* 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.

Columnnea 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 *Columnnea* 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 *Columnnea* 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 *Columnnea* but considered the subgenera of Hanstein (1865) to be sections. Fritsch also combined into *Columnnea* 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 *Columnnea* by splitting it into four genera based on vegetative and nectary characters. He added a fifth genus to the columnneoid alliance, *Bucinellina* (Wiehler) Wiehler (Wiehler 1977, 1981). This classification was controversial mostly in that *Columnnea* 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 *Columnnea* 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 *Columnnea* 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 *Columnnea* 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 + Γ + 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 + Γ + 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	<i>tmQ-rps16</i> spacer	<i>rpl32-trnL-UG</i> spacer	<i>rps16</i> intron	<i>trnS-trnG</i> spacer	<i>trnH-psbA</i> spacer	ITS
<i>Alloplectus hispidus</i> (Kunth.) Mart.	J.L. Clark 7720	US	Ecuador	KF006030	KF005812	KF005921	JQ953700	DQ211219	DQ211111
<i>Columnea albiflora</i> L.P.Kvist & L.E.Skog	J.L. Clark & J. Rea 8015	UNA	Ecuador	KF006031	KF005813	KF005922	KF006135	KF005640	KF005725
<i>C. ambigua</i> (Urb.) B.D. Morley	J. Smith 3701	SRP	cultivated, originally Puerto Rico	KF006032	KF005814	KF005923	JQ953713	KF005641	JQ953789
<i>C. angustata</i> (Wiehler) L.E.Skog	J. Smith 1433	WIS	Colombia	KF006033	KF005815	KF005924	KF006136	KF005642	KF005726
<i>C. angustata</i> (Wiehler) L.E.Skog	J.L. Clark 8627	UNA	Panama	KF006034	KF005816	KF005925	KF006137	NA	KF005727
<i>C. anisophylla</i> DC.	E. Tepe 1081	SRP	Panama	KF006035	KF005817	KF005927	KF006138	KF005643	KF005729
<i>C. anisophylla</i> DC.	J.L. Clark & J. Rea 8019	UNA	Ecuador	KF006037	KF005819	KF005926	KF006141	KF005646	KF005728
<i>C. atahualpae</i> J.F. Smith & L.E.Skog	J.L. Clark et al. 8000	UNA	Ecuador	KF006038	KF005821	KF005930	KF006142	KF005647	KF005732
<i>C. bilabiatata</i> Seem.	J.L. Clark et al. 11157	UNA	Ecuador	KF006039	KF005822	KF005931	KF006143	KF005648	KF005733
<i>C. bremeri</i> (Wiehler) B.D. Morley	J. Smith 3385	SRP	Ecuador	KF006040	KF005823	KF005932	KF006144	KF005649	KF005734
<i>C. brevipila</i> Urb.	J.L. Clark & M. Mailloux 7842	UNA	Ecuador	KF006041	KF005824	KF005933	KF006145	KF005650	KF005735
<i>C. byrsina</i> (Wiehler) L.P.Kvist & L.E.Skog	J. Smith 10058	SRP	cultivated, originally from Jamaica	KF006042	KF005825	KF005934	KF006146	KF005651	KF005736
<i>C. byrsina</i> (Wiehler) L.P.Kvist & L.E.Skog	J. Smith 3408	SRP	Ecuador	KF006043	KF005826	KF005935	JQ953714	KF005652	KF005737
<i>C. byrsina</i> (Wiehler) L.P.Kvist & L.E.Skog	J.L. Clark & O. Mejia 6291	UNA	Ecuador	KF006044	KF005827	KF005936	KF006148	KF005653	KF005738
<i>C. calotricha</i> Donn. Sm.	J. Smith et al. 4117	SRP	French Guiana	KF006045	KF005828	KF005937	KF006149	KF005654	KF005739
<i>C. ciliata</i> (Wiehler) L.P.Kvist & L.E.Skog	J.L. Clark et al. 7508	UNA	Ecuador	KF006046	KF005829	KF005938	KF006150	NA	KF005740
<i>C. citriflora</i> L.E.Skog	J.L. Clark 10053	UNA	cultivated, originally from Panama	KF006047	KF005830	KF005939	KF006151	KF005655	KF005741
<i>C. colombiana</i> (Wiehler) L.P.Kvist & L.E.Skog	J. Smith 1126	WIS	cultivated	KF006049	KF005832	KF005941	KF006153	KF005657	KF005743
<i>C. colombiana</i> (Wiehler) L.P.Kvist & L.E.Skog	J.L. Clark et al. 10024	UNA	cultivated	KF006048	KF005831	KF005940	KF006151	KF005656	KF005742
<i>C. consanguinea</i> Hanst.	E. Tepe 1082	SRP	Panama	KF006050	KF005833	KF005942	KF006154	KF005658	KF005744
<i>C. crassicaulis</i> (Wiehler) L.P.Kvist & L.E.Skog	J.L. Clark 8859	US	cultivated	KF006051	KF005834	KF005943	KF006155	KF005659	KF005745
<i>C. cruenta</i> B.D. Morley	J. Smith 8606	SRP	cultivated, originally from Panama	KF006052	KF005835	KF005944	KF006156	KF005660	KF005746
<i>C. dielsii</i> Mansf.	J. Smith 1989	WIS	Ecuador	KF006053	KF005836	KF005945	KF006157	KF005661	KF005747
<i>C. dissimilis</i> C. V. Morton	E. Tepe 1070	SRP	Panama	KF006054	KF005837	KF005946	KF006158	KF005662	KF005748

TABLE 1. Continued.

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

TABLE 1. Continued.

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

TABLE 1. Continued.

Species	Voucher	Herbarium	Country of origin	<i>trnQ-rps16</i> spacer	<i>rpl32-trnL_{UAG}</i> spacer	<i>rps16</i> intron	<i>trnS-trnG</i> spacer	<i>trnH-psbA</i> spacer	ITS
<i>C. schiedeana</i> Schldl.	<i>J. Smith</i> 288	WIS	cultivated, originally from Mexico	KF006107	KF005891	KF006000	KF006208	KF005712	KF005796
<i>C. schimpffii</i> Mansf.	<i>J. Smith</i> 8603	SRP	cultivated, originally from Ecuador	KF006109	KF005892	KF006001	KF006209	KF005713	KF005797
<i>C. segregata</i> (B.D. Morley) Wiehler	<i>J.L. Clark et al.</i> 10029	UNA	cultivated	KF006108	NA	KF006002	KF006210	KF005714	NA
<i>C. sp.</i> DC.	<i>J.L. Clark & N. Harris</i> 7295	UNA	Ecuador	KF006036	KF005820	KF005929	KF006140	KF005645	KF005731
<i>C. sp.</i> nov.	<i>J.L. Clark et al.</i> 8898	UNA	Ecuador	KF006077	KF005859	KF005970	KF006180	KF005684	KF005768
<i>C. spathulata</i> Mansf.	<i>J. Smith</i> 1853	WIS	Ecuador	KF006110	KF005893	KF006003	KF006211	KF005715	KF005798
<i>C. spathulata</i> Mansf.	<i>J.L. Clark et al.</i> 7485	UNA	Ecuador	KF006111	KF005894	KF006004	KF006212	KF005716	KF005799
<i>C. strigosa</i> Benth.	<i>J. Smith</i> 1200	WIS	Venezuela	KF006112	KF005895	KF006005	KF006214	KF005717	KF005800
<i>C. strigosa</i> Benth.	<i>T. Croat</i> 94580	MO	Ecuador	KF006113	KF005896	KF006006	KF006213	KF005718	KF005801
<i>C. tenella</i> L.P.Kvist & L.E.Skog	<i>M. Amaya M. & J. Smith</i> 603	COL	Colombia	KF006114	KF005897	KF006007	KF006215	KF005719	KF005802
<i>C. tenensis</i> Wiehler	<i>J.L. Clark et al.</i> 9500	UNA	Ecuador	KF006116	KF005899	KF006009	KF006217	KF005721	KF005803
<i>C. tenensis</i> Wiehler	<i>J. Smith</i> 3374	SRP	Ecuador	KF006115	KF005898	KF006008	KF006216	KF005720	KF005804
<i>C. trollii</i> Mansf.	<i>J. Smith</i> 1723	WIS	Bolivia	KF006117	KF005899	KF006010	KF006218	KF005722	KF005805
<i>C. ultravioleacea</i> J.F.Smith & L.E.Skog	<i>J.L. Clark & V. Velaz</i> 6603	UNA	Bolivia	KF006118	KF005900	KF006011	KF006219	KF005723	KF005806
<i>C. villosissima</i> Mansf.	<i>E. Tepe</i> 1628	SRP	Ecuador	KF006119	KF005901	KF006012	KF006220	KF005724	KF005807
<i>Corytoplectus capitatus</i> (Hook.) Wiehler	<i>T. Croat</i> 94581	MO	cultivated	KF006120	KF005902	KF006013	JQ953698	NA	JQ953798
<i>Corytoplectus speciosus</i> (Poepp.) Wiehler	no voucher, USBRG 1994-268		cultivated	KF006117	KF005903	KF006010	JQ953699	NA	JQ953779
<i>Crantzia cristata</i> (L.) Scopoli	<i>J.L. Clark</i> 6346	US	Martinique	KF006027	KF005809	KF005918	KF006134	DQ211294	DQ211154
<i>Crantzia epirotas</i> (Leeuwenb.) J.L.Clark	<i>D. Clarke</i> 10172	US	Guyana	KF006028	KF005810	KF005919	JQ953702	DQ211293	DQ211153
<i>Crantzia tigrina</i> (Karsten.) J.L.Clark	<i>J.L. Clark</i> 6892	US	Venezuela	KF006029	KF005811	KF005920	JQ953703	DQ211295	DQ211155
<i>Drymonia coccinea</i> (Aubl.) Wiehler	<i>J. Smith</i> 3373	SRP	Ecuador	KF006121	KF005905	KF006014	JQ953704	DQ211266	DQ211132
<i>Drymonia pendula</i> (Poepp.) Wiehler	<i>J. Smith</i> 3384	SRP	Ecuador	KF006122	KF005906	KF006015	KF006221	NA	NA
<i>Drymonia pilifera</i> Wiehler	<i>E. Tepe</i> 1065	SRP	Panama	KF006123	KF005907	KF006016	KF006222	DQ211272	DQ211137
<i>Drymonia serrulata</i> (Jacq.) Mart.	<i>J. Smith</i> 4202	SRP	French Guiana	KF006124	KF005908	KF006017	JQ953705	DQ211267	DQ211133
<i>Drymonia strigosa</i> (Oerst.) Wiehler	<i>A. Rincon</i> 2301	XAL	Mexico	KF006125	KF005909	KF006018	JQ953706	DQ211280	DQ211143
<i>Drymonia turrialvae</i> Hanst.	<i>E. Tepe</i> 1063	SRP	Panama	KF006126	KF005910	KF006019	KF006223	DQ211278	DQ211141
<i>Drymonia urceolata</i> Wiehler	<i>J. Smith</i> 3416	SRP	Ecuador	KF006127	KF005911	KF006020	JQ953707	DQ211289	AF543265

TABLE 1. Continued.

Species	Voucher	Herbarium	Country of origin	<i>trnQ-rps16</i> spacer	<i>rpl32-trnL_{UAG}</i> spacer	<i>rps16</i> intron	<i>trnS-trnG</i> spacer	<i>trnH-psbA</i> spacer	ITS
<i>Glossoloma anomalum</i> J.L.Clark	<i>J. Smith 3418</i>	SRP	Ecuador	KF006128	KF005912	KF006021	KF006224	NA	AF543225
<i>Glossoloma grandicalyx</i> (J.L.Clark & L.E.Skog)	<i>J. Smith 3417</i>	SRP	Ecuador	KF006129	KF005913	KF006024	JQ953708	DQ211205	AF543218
<i>Glossoloma martinianum</i> (J.F.Smith) J.L.Clark	<i>J.L. Clark 6101</i>	US	Ecuador	KF006130	KF005914	KF006022	JQ953709	DQ211209	AF543228
<i>Glossoloma panamense</i> (C.V.Morton) J.L.Clark	<i>L.E. Skog et al. 7641</i>	US	cultivated	KF006131	KF005915	KF006023	JQ953710	DQ211202	DQ211102
<i>Neomortonia rosea</i> Wiehler	no voucher, USBRG 94230		cultivated	KF006133	KF005917	KF006026	JQ953712	DQ211197	DQ211099
<i>Pachycaulos nummularium</i> (Haust.) J.L.Clark & J.F.Smith	<i>J. Smith 3944</i>	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 + Γ + 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, *Columnnea* 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 *Columnnea* based on the phylogenetic results presented here.

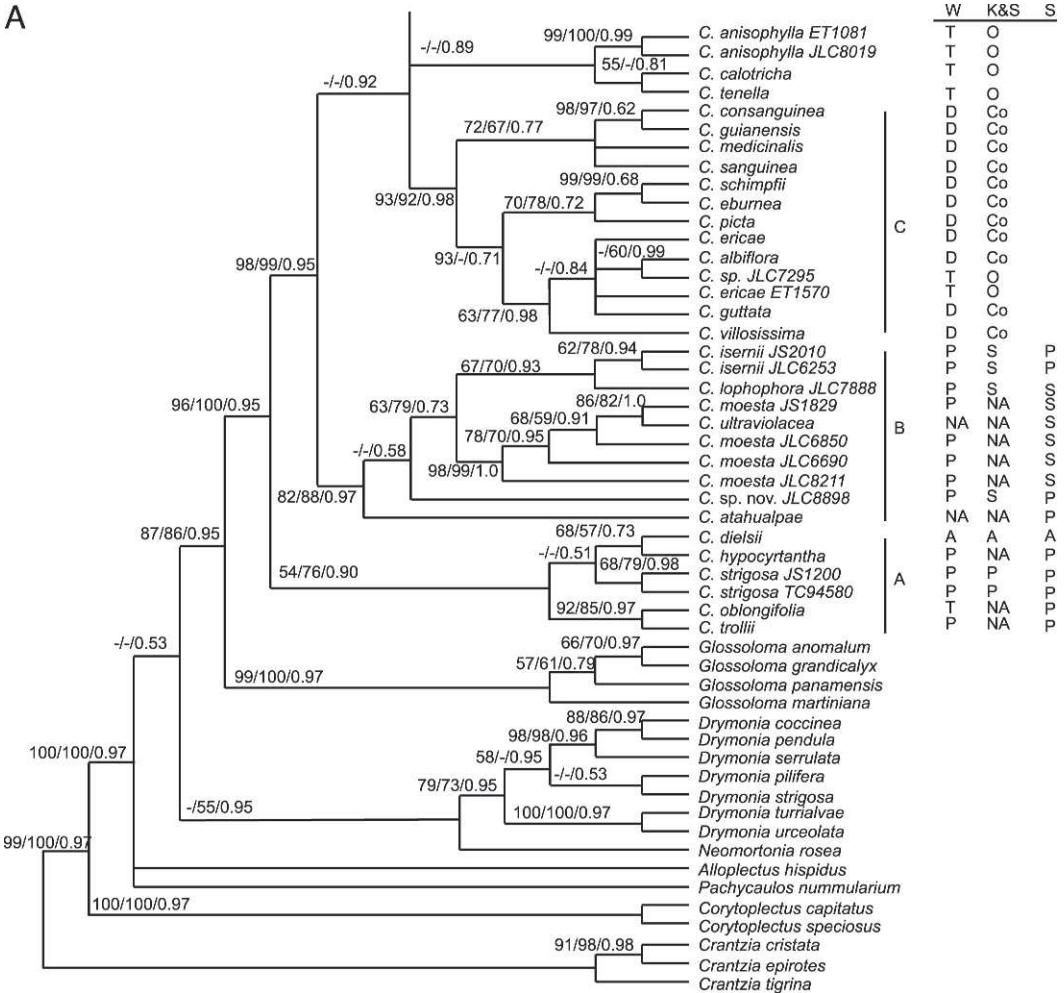


FIGURE 1. Majority rule consensus tree from the Bayesian analysis. The topology of this tree is almost completely congruent with the trees from maximum parsimony and maximum likelihood. Branches that collapse in the strict consensus of the maximum parsimony tree are marked with an asterisk (*). Values 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 *Columnnea*, D - genus *Dalbergaria*, T - genus *Trichantha*, P - genus *Pentadenia*, B - genus *Bucinellina*), Kvist & Skog (1993; C- section *Columnnea*, 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. *Columnnea* is a monophyletic group that is supported as the sister to *Glossoloma* (FIGURE 1). While the monophyly of *Columnnea* 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.

B

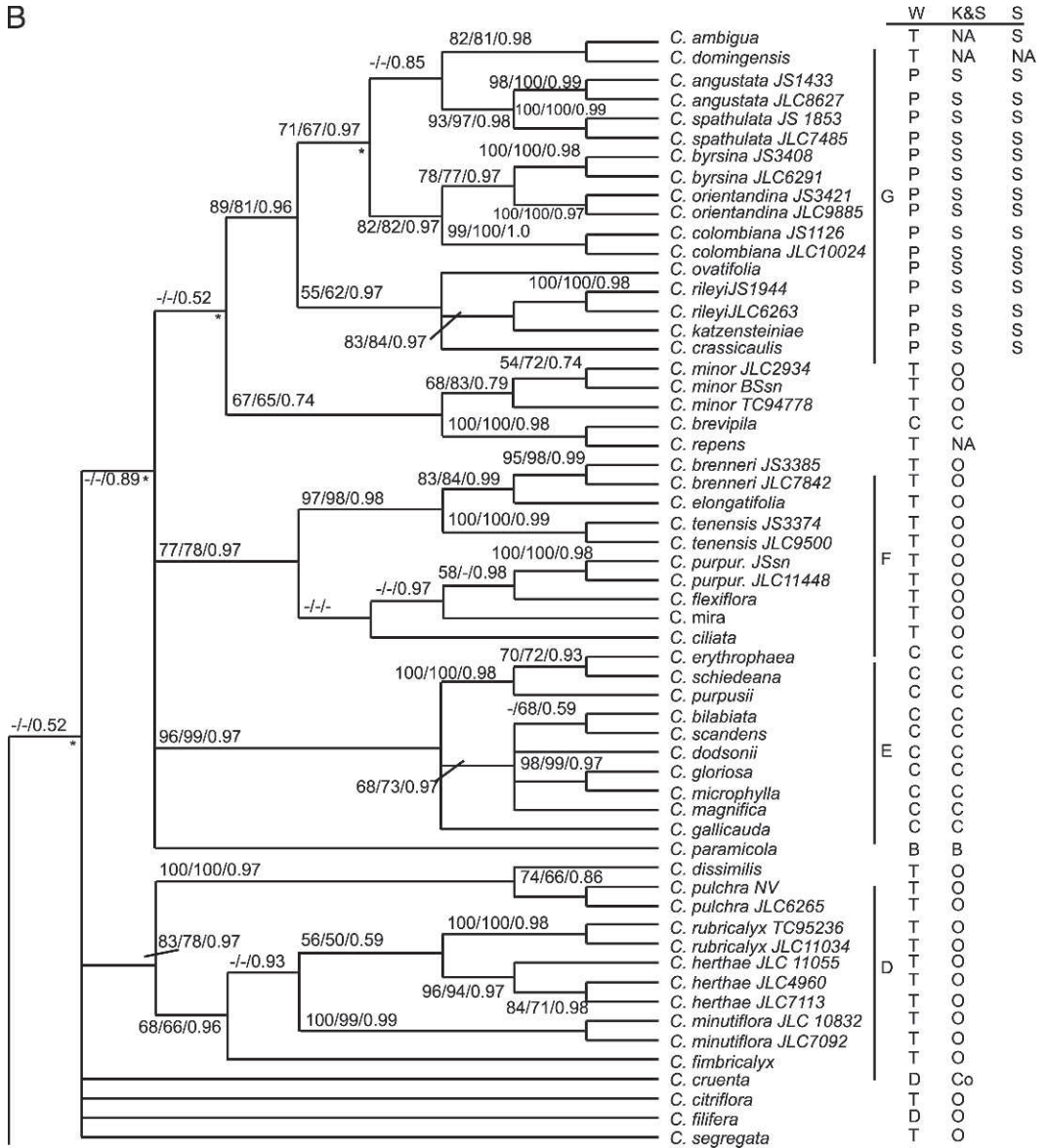


FIGURE 1. Continued.

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 *Columnnea* (98/99/0.95). This clade (based on four sampled species in common) was also recovered as sister to the remainder of

Columnnea 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-trnLUAG* 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 *Columnnea* are strongly anisophyllous. This vegetative feature is useful for defining clade A. Species in this clade

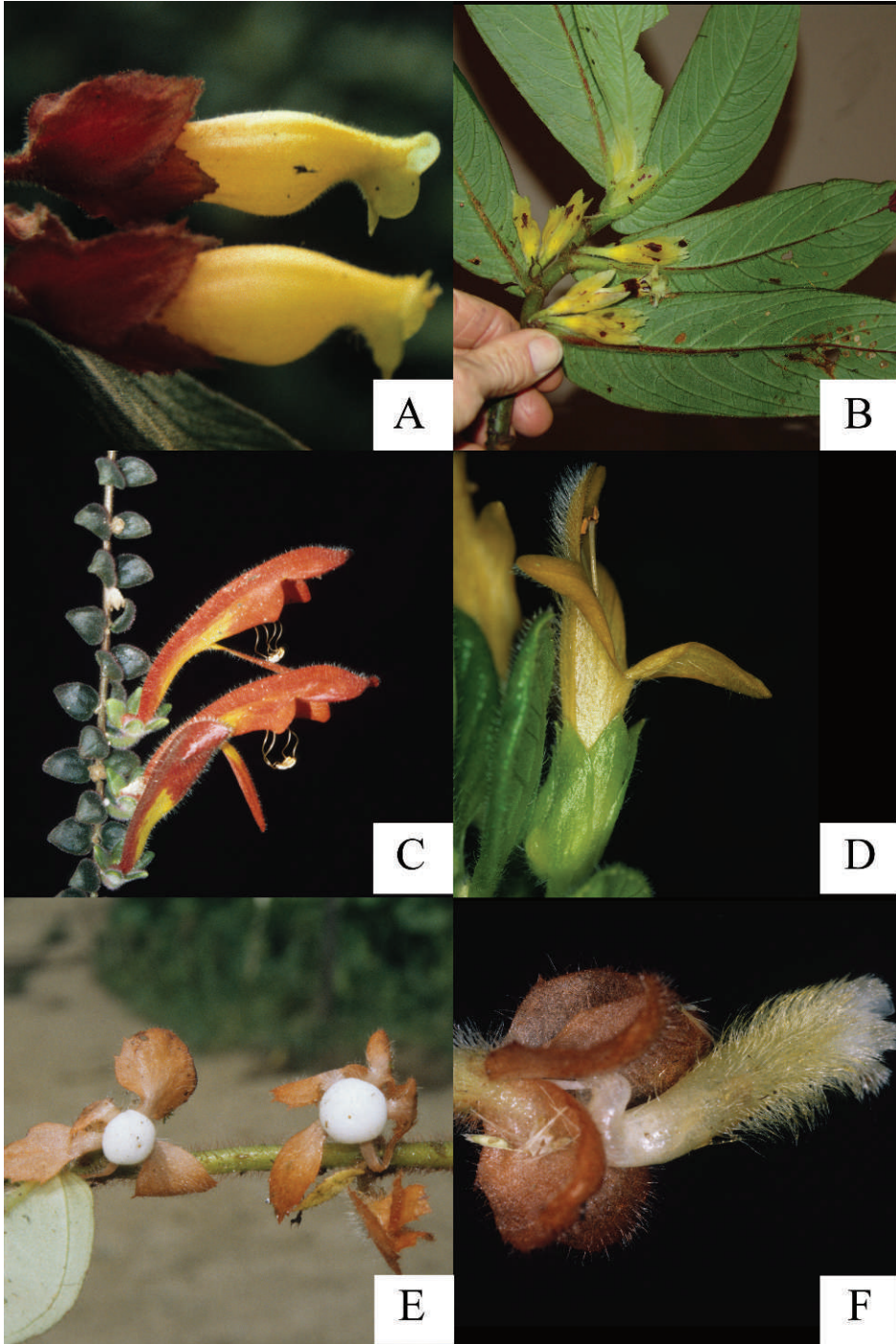


FIGURE 2. Photographs illustrating morphologies in *Columnea*. **A.** Ventricose corolla of *C. dielsii* (J.L. Clark 5813). **B.** Dorsiventral habit of *C. picta* (T. Croat 94956). **C.** Bilabiate corolla of *C. microphylla* (J.L. Clark 6261). **D.** Bilabiate corolla of *C. proctorii* (J.L. Clark 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 *Columnnea* (smallest ones approximately 3.0 cm in length, but reaching 9.5 cm in *Columnnea strigosa*; Smith 1994) and are strongly to weakly ventricose on the lower apical region of the corolla (FIGURE 2A). Two species, (*Columnnea 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 *Columnnea*. 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 *Columnnea dielsii* (FIGURE 2A) that had been excluded from most previous classifications of *Columnnea* although both Bentham (1876) and Fritsch (1894) had included it in *Columnnea*. Based on habit, distribution, and corolla form, this species fits well with the other species of this clade. *Columnnea dielsii* was supported as belonging to *Columnnea* based on molecular data prior to this study (Smith & Sytsma 1994b; Clark et al. 2006). The generic placement of this taxon is historically confusing and it was usually classified as belonging to *Alloplectus* instead of *Columnnea* 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 *Columnnea 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 *Columnnea* 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 *Columnnea*: *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 *Columnnea* 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 Sytsma (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.

Columnnea 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. isemii* 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-Chinchiipe, 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 sessile 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. Kvist & 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. Kvist). 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 lineage 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 *Columnnea*. The calyx lobes are usually cordate at the base and reddish-orange (FIGURE 2E–F). With the exception of *Columnnea 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 *Columnnea*. One remarkable species that nests in this clade is *C. fimbriicalyx* L.P.Kvist & L.E.Skog with lacinate calyx lobes. However, the lacinate margin is an autapomorphic character state for the clade (albeit shared with other species of *Columnnea*) 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. *Columnnea 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 *Columnnea* and Kvist & Skog's section *Columnnea* with the exception that *C. brevipila* Urb. is supported as 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 *Columnnea*.

There are 13 species of *Columnnea* 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 *Columnnea*. The sister relationship of two Jamaican species with widely divergent corolla morphologies raises the question whether Jamaican *Columnnea* 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 *Columnnea* 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 *Columnnea*, 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 *Columnnea* 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 *Columnnea* 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 *Columnnea 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. *Columnnea 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.

Columnnea 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 *Columnnea* 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 *Columnnea*.

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