Molecular Phylogenetics and Evolution xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

# Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate

Lacie J. Schulte [a], John L. Clark [b], Stephen J. Novak [a], Shandra K. Jeffries [a], James F. Smith [a,*]

[a] Department of Biological Sciences, Boise State University, 1910 University Drive, Boise, ID 83725-1515, USA
[b] University of Alabama, Department of Biological Sciences, Box 870345, Tuscaloosa, AL 35487, USA

ARTICLE INFO

*Article history:*
Received 23 May 2014
Revised 12 December 2014
Accepted 16 December 2014
Available online xxxx

*Keywords:*
Ancestral character states
Climate
Nectar robbing
Pollinators
SEEVA
SIMMAP

ABSTRACT

Despite many advances in evolutionary biology, understanding the proximate mechanisms that lead to speciation for many taxonomic groups remains elusive. Phylogenetic analyses provide a means to generate well-supported estimates of species relationships. Understanding how genetic isolation (restricted gene flow) occurred in the past requires not only a well-supported molecular phylogenetic analysis, but also an understanding of when character states that define species may have changed. In this study, phylogenetic trees resolve species level relationships for fourteen of the fifteen species within *Columnea* section *Angustiflorae* (Gesneriaceae). The distributions of sister species pairs are compared and ancestral character states are reconstructed using Bayesian stochastic mapping. Climate variables were also assessed and shifts in ancestral climate conditions were mapped using SEEVA. The relationships between morphological character states and climate variables were assessed with correlation analyses. These results indicate that species in section *Angustiflorae* have likely diverged as a result of allopatric, parapatric, and sympatric speciation, with both biotic and abiotic forces driving morphological and phenological divergence.



## 1. Introduction

Darwin (1859) elegantly identified natural selection as a major means of generating biological diversity. Yet, over 150 years later the specific historical processes that drive evolutionary divergence remain unknown for most organisms. Physical (geographic) isolation is an important component in initiating the speciation process and allopatric speciation is widely accepted as a means by which populations diverge (Bush, 1975b). However, populations can diverge even when gene flow is maintained (Bush, 1975a; Feder, 1998; Savolainen et al., 2006). Divergence can result from selection by the environment or through interactions with other organisms (Givnish, 1997; Schluter, 2000), or even through stochastic processes (e.g., genetic drift) that result in species which may or may not be adaptive (Gittenberger, 2008). All of these scenarios create challenges when trying to infer forces that are currently involved in the speciation process, and they become amplified when trying to uncover the processes that generated species diversity in the past.

Advances in obtaining large amounts of sequence data and the methodology to interpret those data have greatly enhanced phylogenetic analyses such that species level evolutionary relationships can be estimated and the confidence of those relationships can be assessed (Swofford, 2002; Huelsenbeck and Ronquist, 2003; Rambaut and Drummond, 2005; Stamatakis, 2006; Zwickl, 2006; Drummond and Rambaut, 2006; Stamatakis et al., 2008). However, understanding the relationships of species only reveals the pattern of speciation. To interpret the processes that led to divergence, various factors can be evaluated across the evolutionary history of the species and deep divergences can be evaluated using molecular dating and paleoclimatic history (Graham, 1997; Symmank et al., 2011). Ideally specific historical events can be examined against evolutionary history (Lemmon et al., 2007), but more often multiple factors are examined across an evolutionary tree to determine where shifts in climatic tolerance or morphological changes have occurred (Kay et al., 2005; Drummond, 2008; Symmank et al., 2011; Valente et al., 2012; De Vos et al., 2014). Identifying biotic or abiotic factors involved in the diversification of extant species is not a direct observation of events; instead the products of selection (adaptations) are examined as proxies for natural selection. While this poses some limits on our ability to assess past speciation processes, the effects of selective agents on extant species with similar adaptations can corroborate that the shifts in character states across a phylogenetic tree are likely the result of similar selective processes in the past.

* Corresponding author.
*E-mail address:* jfsmith@boisestate.edu (J.F. Smith).

http://dx.doi.org/10.1016/j.ympev.2014.12.008
1055-7903/

Tropical forests provide numerous opportunities to study the forces driving speciation (Martén-Rodríguez et al., 2010; Viljanen et al., 2010; Tolley et al., 2011). Over half the world's plant and animal species are found in tropical forests, which cover only a small portion of the Earth's surface, resulting in a high concentration of biodiversity (Wills et al., 2006; Mittelbach et al., 2007). Although tropical forests have less striking seasonal temperature variation, compared to temperate regions, their climate varies depending upon latitude, elevation, and physiogeographic features, creating a broad array of both biotic and abiotic factors that may contribute to speciation (Haffer, 1969; Bush, 1994; Graham, 1997; Haffer and Prance, 2001; Struwe et al., 2011), both in the past and presently.

The Neotropics experienced climate oscillations in the Pleistocene and along with volcanism in Central America, the closing of the Isthmus of Panama, and the Andean orogeny, climates would have been varied and changing across northern South America and the Caribbean (Gentry, 1982; Graham, 1997). Over the past three to five million years such shifts created an array of selective environments that resulted in numerous species adapted to them. The combination of abiotic and biotic interactions have been suggested to accelerate speciation in the tropics (Gentry, 1989; Schemske, 2002), particularly as a result of plant-pollinator interactions (Johnson and Steiner, 2000). Plant-pollinator interactions and shifts in morphology as a response to pollinator-driven selection have also increased diversity in other regions such as South Africa, where one third of all lineage splits in *Gladiolus* are the result of pollinator shifts (Valente et al., 2012).

Gesneriaceae is a tropical plant family whose evolutionary history provides insights into the speciation process. Wide variation in flower shape and color have been hypothesized to be the result of selection by different pollinator syndromes (Harrison et al., 1999; Perret et al., 2007; Martén-Rodríguez et al., 2010), different vegetative growth forms have been shown to have high rates of lability (Möller and Cronk, 2001), and long distance dispersal events have contributed to allopatric speciation of sister species on islands arrayed across the Pacific Ocean (Woo et al., 2011). These previous studies have implied a number of biotic and abiotic factors that drive speciation in the family. Within Gesnerioideae, *Columnea* L. is the largest genus. The 200+ species of *Columnea* are distributed in the Caribbean islands and throughout the Andes, from Mexico to Bolivia and eastward into northern Brazil (Smith, 1994). Recent molecular phylogenetic analyses show that the 200+ species of *Columnea* are divided into seven clades. These clades are still the focus of ongoing investigations, are supported by molecular synapomorphies (Smith et al., 2013), and preliminary data indicate that some clades are further supported by morphological synapomorphies, geographic distribution, or a combination of the two. One of these clades is section *Angustiflorae* which has been defined by morphological characters (Schulte et al., 2014).

Species of *Angustiflorae* are characterized by small tubular corollas that are radially to subradially symmetric, narrow calyx lobes loosely clasping the corolla, and sparse pubescence on the corolla (Fig. 1; Schulte et al., 2014). The species also have opposite leaves that range from isophyllous to strongly anisophyllous, with a dorsiventral arrangement in *Columnea byrsina*, *C. orientandina*, *C. manabiana*, *C. tandapiana*, and *C. spathulata*. Leaf coloration is green, sometimes purple, suffused with pink, or with violet spots. Corollas range from cream to lemon-yellow, orange, red or violet, with darker colored lobe spots in some species (Fig. 1). The species of section *Angustiflorae* cover nearly the full geographic and climatic range of *Columnea*, from sea level to 4000 meters in elevation. Their distributions vary from narrow endemics, such as *C. ambigua* and *C. domingensis* on Caribbean islands, to species that are widespread, such as *C. angustata* that ranges from Costa Rica to Ecuador (Schulte et al., 2014). All species are either epiphytic, or both epiphytic and epipetric (*C. orientandina*). A wide range of morphological variation, distributions (allopatric, parapatric, and sympatric), and climate requirements makes section *Angustiflorae* a good model system for studying factors influencing speciation events.

This study aimed to estimate species trees for section *Angustiflorae* with five unlinked gene regions. Species that belong in section *Angustiflorae* have been identified based on molecular analyses and morphological characters, however, previous molecular data have not resolved phylogenetic relationships among the species of *Angustiflorae* (Schulte et al., 2014). We sampled 36 accessions representing 14 of the 15 species of section *Angustiflorae* (Appendix A). *Columnea antiocana* is a rare Colombian endemic known only from three collections. We were unable to procure sufficient DNA material to include this species in our phylogenetic analyses but based on morphological data this species would likely be related to *C. crassicaulis*, *C. katzensteiniae*, *C. ovatifolia*, and *C. rileyi* (Schulte et al., 2014). *Columnea antiocana* shares corolla lobe spots with this clade, but has entire calyx margins. It is the only species known from Colombia among this clade and would thus be allopatric from its most likely sister species. Phylogenetic analyses were conducted using five chloroplast DNA (cpDNA) gene regions along with nuclear ribosomal internal transcribed spacers (ITS1 and ITS2, hereafter referred to as ITS), and the external transcribed spacer (ETS). In addition, two low-copy nuclear genes, glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*; Strand et al., 1997) and NADP-dependent isocitrate dehydrogenase (*idh*; Weese and Johnson, 2005), were used to increase phylogenetic support for major branching events within *Angustiflorae*. All gene regions were chosen because they have proven successfull in resolving species level phylogenetic relationships (Linder et al., 2000; Ingram and Doyle, 2003; Levin et al., 2005; Johnson and Johnson, 2006; Huertas et al., 2007; Smith et al., 2008a; Ruiz-Sanchez and Sosa, 2010; Steele et al., 2010).

Our objectives were to investigate the processes of speciation in section *Angustiflorae* by (1) reconstructing a species level phylogeny to estimate patterns of specation, (2) estimating the impact of geographic isolation on speciation in this group, (3) determining ancestral character states for morphological and phenological character states that have been used to differentiate these species, (4) determining the lability of these morphological and phenological character states, (5) estimating differences in climatic tolerance across the phylogeny, and (6) correlating shifts in morphology with shifts in climate tolerance to determine whether climate may have driven shifts in morphology.

## 2. Materials and methods

### 2.1. DNA extraction, amplification, and alignment

A complete list of samples, voucher specimens, and GenBank numbers for all sequences used in all analyses is in Appendix A. DNA was extracted from silica-dried leaf material using Qiagen DNeasy plant mini kits (Valencia, CA) according to manufacturer's instructions. Two separate datasets were used, the first dataset included sampling for all accessions (31 species, 54 individuals) for seven gene regions and is herein referred to as the full dataset. The second dataset sampled from monophyletic clades (24 species, 30 individuals) using analyses of the full dataset to minimize the total number of *Columnea* species that were not members of section *Angustiflorae* and reduce the number of accessions for each species of section *Angustiflorae*. This reduced dataset included more DNA sequence data through the addition of two low-copy nuclear genes.

The ingroup for phylogenetic analyses included 36 accessions for the full dataset and a subset of 20 accessions for the reduced



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

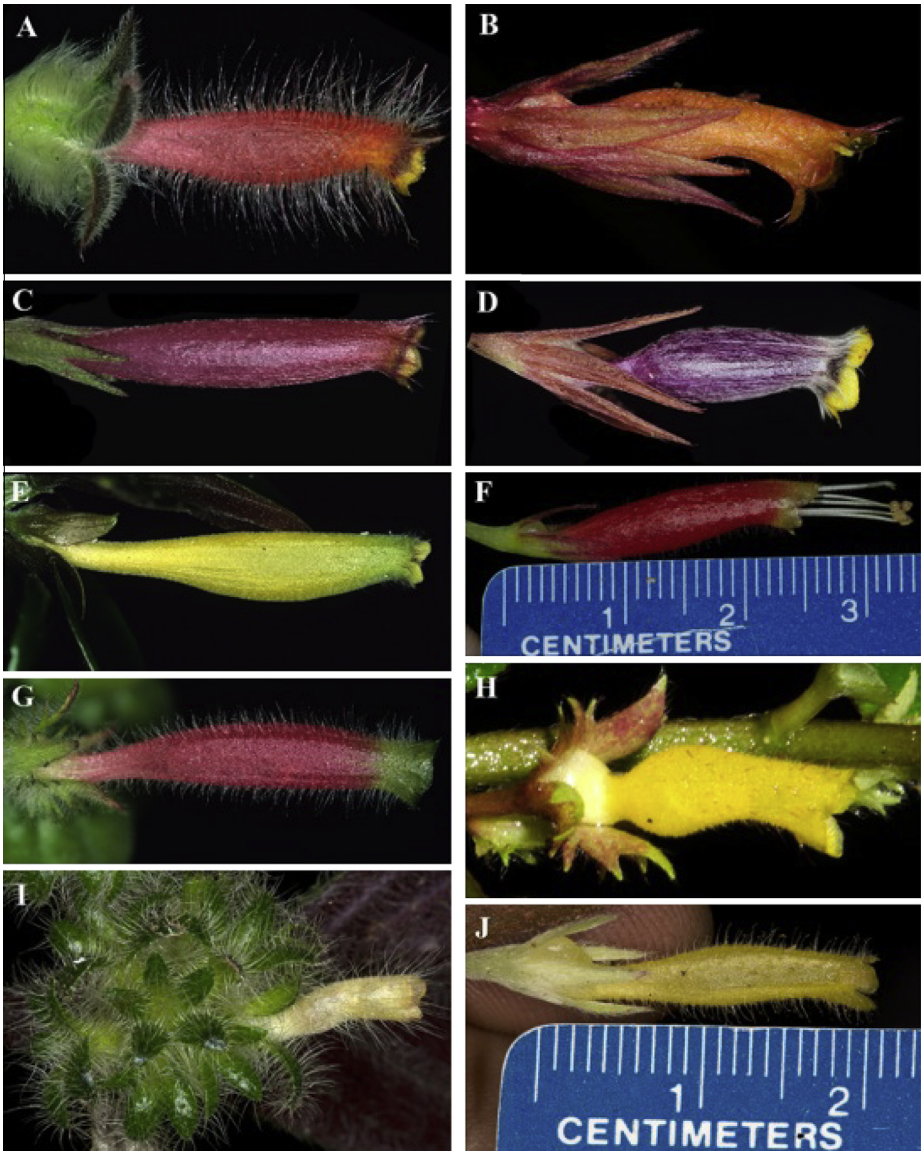


**Fig. 1.** Species of *Columnea* section *Angustiflorae* and their voucher collection. A. *Columnea rileyi J.L. Clark 7077*. B. *Columnea angustata J.L. Clark 13654*. C. *Columnea ovatifolia J.L. Clark 8461*. D. *Columnea katzensteiniae J.L. Clark 8915*. E. *Columnea crassicaulis J.L. Clark 8859*. F. *Columnea byrsina J.L. Clark 13552*. G. *Columnea colombiana J.L. Clark 8874*. H. *Columnea ambigua J.L. Clark 13758*. I. *Columnea spathulata J.L. Clark 10926*. J. *Columnea orientandina J.L. Clark 11424*. Photos J.L. Clark.

dataset (Appendix A); accessions in both datasets represented 14 of the 15 species in section *Angustiflorae*. The outgroup for phylogenetic analyses included one accession each for 18 species in the full dataset and 10 species in the reduced dataset. Outgroup accessions for both datasets included species representing the remaining six clades of *Columnea*, identified by Smith et al. (2013) and Schulte et al. (2014) in addition to species of the sister genus *Glossoloma* Hanst. (Clark et al., 2012) in the full dataset.

Five cpDNA gene regions were chosen; *trnQ-rps16* spacer (Shaw et al., 2007), *rpl32-trnL*$_{UAG}$ spacer (Shaw et al., 2007), *rps16* intron (Oxelman et al., 1997), *trnS-G* spacer (Hamilton, 1999), and *trnH-psbA* spacer (Clark et al., 2006). The cpDNA gene regions were treated as a single partition in each of the datasets because they are inherited as a single non-recombining unit. The two nuclear DNA gene regions, ITS (Baldwin et al., 1995) and ETS (Baldwin and Markos, 1998) were each treated as separate partitions for all analyses. These three gene partitions were concatenated to form the full dataset (cpDNA, ITS, and ETS). The sequences from cpDNA, ITS, and ETS for 20 accessions were added to the sequences from *G3pdh*A and *idh*B to create the reduced dataset with a smaller taxon sampling, but increased DNA sequence data.

Double-stranded DNA was amplified via PCR, following the methods of Smith et al. (1997) and details on the amplification and cloning for *G3pdh*A and *idh*B are described in Schulte (2012). Sequences were obtained either through the methods of Smith et al. (2004) or through Genewiz (South Plainfield, NJ); chromatograms were viewed and sequences edited and aligned by hand in PhyDE (Müller et al., 2005). Summaries of the alignments are presented in Table 1.

Areas of missing data (typically at the beginning and end of the alignments) and regions of ambiguous alignment were excluded from phylogenetic analyses. Single base and microsatellite repeats can be unambiguously aligned; however the homology of these repeats is uncertain. To assess the utility of including single base and microsatellite repeats, the resolution and support within the tree was compared with and without the repeats. Thus, two additional maximum parsimony (MP) analyses were performed for both the full and reduced datasets that included or excluded these

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

**Table 1**
Summary of alignment, maximum parsimony (MP) results, model used in Bayesian inference (BI) and maximum likelihood (ML).

| Alignment length | Constant sites | Uninformative sites | Phylogenetically informative sites (percentage) | Indels (length range) | MP tree number, length, CI, RI, RC | BI one model | ML model (−lnL) |
|---|---|---|---|---|---|---|---|
| *Full dataset* | | | | | | | |
| 4282 | 3604 | 410 | 268 (6.3%) | 55 (2–66) | 175, L = 1045, CI = 0.56, RI = 0.78, RC = 0.58 | TVM + I + Γ | TVM + I + Γ (13484.81) |
| *Reduced dataset* | | | | | | | |
| 6281 | 5340 | 612 | 276 (4.4%) | 27 (2–66) | 4, L = 1160,CI = 0.79, RI = 0.64, RC = 0.51 | TVM + I + Γ | TVM + I + Γ (15833.22) |

repeats. These MP analyses resulted in reduced resolution and consistency index (CI; Kluge and Farris, 1969), therefore single base and microsatellite repeats were excluded from further analyses for both the full and reduced datasets.

Each indel for both the full and reduced datasets was scored using the simple indel coding of Simmons and Ochoterena (2000). The datasets that included the indel event scores were analyzed using MP in PAUP* v4.0 b10 (Swofford, 2002) and for Bayesian inference (BI) indels were analyzed with the Mk1 model (Lewis, 2001) in MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2003). Indels were not included in the maximum likelihood (ML) analysis.

### 2.2. Test of incongruence

The partition homogeneity test (Farris et al., 1994) was performed as implemented in PAUP* v4.0 b10 (Swofford, 2002) with 10,000 bootstrap replicates (using a heuristic search, simple addition, and no branch swapping). As an additional measure of congruence among partitions, bootstrap analyses were performed (full and reduced) on each partition separately (full dataset: cpDNA, ITS, and ETS; reduced dataset: cpDNA, ITS, ETS, *G3pdh*A, and *idh*B) to assess areas of conflict and to determine if any conflict was strongly supported (>70% support; Seelanen et al., 1997). Specific sequences that could be individually identified as incongruent with other partitions were removed. Analyses were then repeated to verify that deleted regions were the source of incongruence based on increased resolution and branch support in combined analyses (Mason-Gamer and Kellogg, 1996; Smith, 2000). All loci that were removed from the analyses due to conflict were assumed to be paralogs in that they provided support for the placement of species that was in conflict with support from multiple loci. The potential exists that we have selected for use in this analysis the paralogs that do not reflect the species trees. However, congruence of data is currently our best criterion for selecting homologs, and inclusion of all data reduced both resolution and support for the relationships that was recovered (results not shown).

### 2.3. Phylogenetic analyses

Phylogenetic trees were estimated using MP, ML, and BI for both datasets. Maximum parsimony analyses were performed using PRAP2 (Müller, 2004; using the default settings but uploading the nexus file for each dataset) in conjunction with PAUP* v4.0 b10 (Swofford, 2002). Bootstrap support (BS; Felsenstein, 1985) was estimated with 1000 heuristic replicates using PRAP2 (Müller, 2004). Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analysis were given by the CI (Kluge and Farris, 1969), retention index (RI; Farris, 1989), and the resulting rescaled consistency index (RC; Farris, 1989).

Maximum likelihood and BI analyses were performed using optimal substitution models suggested by Modeltest 3.6 (Posada and Crandall, 1998). The Akaike information criterion (AIC), which allows non-nested models to be evaluated, was used as a selection criterion (Posada and Buckley, 2004) for all partition regions separately and as combined datasets (full and reduced).

Three BI analyses were completed using MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2003). All three BI analyses were run for the full and reduced datasets. The one model analyses were performed using a single model. The partition model analyses were performed using individual models for each partition (full dataset: cpDNA, ITS, and ETS; reduced dataset: cpDNA, ITS, ETS, *G3pdh*A, and *idh*B). The final BI analyses, referred to as the indel analyses, used a single model for all sequence data and a second model for indels.

All analyses were run with four chains, for ten million generations. Convergence was determined by viewing in Tracer v1.3 (Rambaut and Drummond, 2005), and a burnin of 50,000 generations was discarded prior to sampling the posterior distribution for all BI analyses. All BI analyses were repeated twice to ensure that parameter estimates converged to similar values. The separate runs were compared using the online version of Are We There Yet (AWTY; Nylander et al., 2008) as a means of determining if the separate chains approximated the same target distribution. The ML analyses were completed using GARLI v0.96 (Zwickl, 2006) with 1000 bootstrap replicates using a single model for both the full and reduced datasets. Indels were not analyzed with ML.

### 2.4. Species distributions

A total of 493 herbarium collection records of the 14 species of *Angustiflorae* were used to determine geographic distributions of the species (Supplemental Appendix A). All species were represented by more than one specimen, ranging from three specimens of *C. colombiana* to 155 specimens of *C. angustata*. Each herbarium collection locality was georeferenced using Google Earth to obtain latitude and longitude coordinates. Only specimens with recorded locations known to the nearest minute were included in analyses. Latitude and longitude coordinates were converted to decimal degrees, and then converted to a point shapefile in ArcMap version 10.0 (ESRI, Redlands, CA), to generate distribution maps (Schulte et al., 2014).

To determine if species were allopatric, parapatric, or sympatric, only geographic distributions and climate ranges of sister species pairs were compared because ancestor species distributions could not be readily inferred with extant data. If sister species did not overlap at all in their geographic distribution, they were classified as allopatric. Sister species pairs with overlapping geographic distributions were examined for differences in climatic variables. Using the output from SEEVA version 1.00 (Heiberg, 2012) sister species pairs that had at least one significant difference in a BioClim variable were determined to be parapatric. Sympatric sister species were those that had overlapping geographic distributions and no significant differences in BioClim variables.

### 2.5. Morphological characters

Fourteen morphological characters and one phenological character were scored for 14 species in section *Angustiflorae* (Appendix B). These floral and vegetative characters were chosen because they vary among the species of section *Angustiflorae* and



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

potentially represent character state shifts that may have been important in the process of species divergence. These characters differ among the species in section *Angustiflorae* and quantitative states could be placed into discrete, non-overlapping categorical states as determined by graphically examining the range of each quantitative trait for all species. Character states for the 14 species for all 14 morphological, and one phenological character were obtained from Smith (1994) with the exception of *C. domingensis* (Schulte et al., 2014). The following characters were scored: habit, leaf dimorphism, adaxial leaf pubescence, abaxial leaf pubescence, abaxial leaf color, calyx margin, corolla color, and corolla lobe color. Petiole length, number of flowers per inflorescence, floral bract length, and corolla length were scored based on the maximum value of variation within individual species. Leaf surface area was determined using the maximum value of the larger leaf (in anisophyllous species) and calculated by multiplying the longest length by the widest width for each species. The corolla to calyx ratio was determined using the maximum values for the corolla and calyx lengths and calculated by dividing the corolla length by the calyx length. Phenology of flowering was determined from collection dates for all herbarium collections for each species (Supplemental Appendix A) and those cited by Smith (1994).

Character state categories for continuously variable characters were determined by plotting raw values for each morphological character for all 14 species and creating categories based on non-overlapping ranges as determined by graphically examining the range of each quantitative trait for all species. Character state definitions and scoring for each of the 14 morphological characters are provided in Appendix B.

Ancestral state reconstructions for each of the 14 morphological characters and one phenological character were conducted with a single topology for species relationships using a ML approach in Mesquite v. 2.75 (Maddison and Maddison, 2011) and Bayesian stochastic character mapping (Huelsenbeck et al., 2003) in SIMMAP v. 1.5 (Bollback, 2006). Both methods were used to test if the different modeling systems interpreted ancestral character states differently. In Mesquite, ML ancestral state reconstructions used the Mk1 model (Lewis, 2001) with all character state changes equally probable. Ancestral state reconstructions are given as ML probabilities (MLP). For Bayesian stochastic character mapping, the bias parameter was set to the empirical prior, and the rate parameter was set to the branch length prior with the character state changes unordered for all 14 morphological characters and the single phenological character. The Bayesian stochastic character mapping analyses will hereafter be referred to as the branch length model (BL model). Ancestral character state reconstructions are given as Bayesian posterior probabilities (BPP).

### 2.6. Climate variables

Climate data for each georeferenced herbarium collection (Table 2; Supplemental Appendix A) were extracted using 19 available BioClim layers (Hijmans et al., 2005) at 30s Arc (~1 km) accuracy. Bioclimatic variables were derived from monthly temperature and rainfall values provided by Hijmans et al. (2005). Eleven variables were related to the temperature and reported in degrees Celsius, with the exception of isothermality and temperature seasonality. Isothermality was calculated by dividing the mean diurnal range (mean monthly maximum temperature minus mean monthly minimum temperature) by the temperature annual range then multiplying by 100, resulting in a possible range from 0 to 100. A higher value for isothermality indicates that the temperature does not vary throughout the year, and a lower value indicates that the temperature has more annual variation. Temperature seasonality was calculated by multiplying the standard deviation by 100. The remaining seven variables were related to the amount of precipitation and reported in mm with the exception of precipitation seasonality. Precipitation seasonality was expressed as the coefficient of variation (Hijmans et al., 2005). ArcMap v.10.0 was used to combine each of the BioClim layers with a 500 m buffer zone around all data points, and climate information was collected for all herbarium collection records (Supplemental Appendix A).

Extracted environmental data were analyzed to determine if climate variables differed across the phylogenetic tree for species of section *Angustiflorae* in SEEVA. The topology of the species level molecular phylogeny was used to map environmental data. SEEVA divided each of the 19 BioClim qualitative variables into four quartiles, spanning the variation of each variable for all species (Table 2; Heiberg and Struwe, 2012). Significance of SEEVA analyses was determined by comparing the two sister clades at each node below the species level. All variables were analyzed independently according to Heiberg and Struwe (2012). For each of the 19 BioClim variables, *p*-values were calculated at each node using a chi-squared test and Fisher's exact test ($H_0$: data distribution at the node is not different between the two sister clades). The Fisher's exact test was used for all analyses because it provides a more accurate *p*-value when analyzing smaller sample sizes. A Bonferroni correction was used to account for multiple comparisons for each climatic variable based on the number of nodes in the phylogenetic tree ($n = 12$), and significance was established at $p < 0.00417$ for all SEEVA analyses. SEEVA also calculated the divergence index ($D_i$) at all nodes for each climate variable independently (Heiberg and Struwe, 2012). The $D_i$ value ranges from 0.0 (no difference between sister clades) to 1.0 (maximum possible difference between sister clades). Significant $D_i$ values were determined as $D_i > 0.75$ according to Struwe et al. (2011).

Ancestral state reconstructions of the 19 BioClim variables were also conducted using Bayesian stochastic character mapping in SIMMAP to examine correlations between climate variables and morphology. SIMMAP cannot use multiple data points for each species (493 herbarium collections). Therefore, environmental data extracted from ArcMap for SEEVA analyses were converted to scores prior to stochastic character mapping. The entire range for each species was taken into account when they were scored prior to stochastic character mapping because potentially important speciation data would be lost by averaging climate variables or using only the maximum or minimum for each species. Species that fell within one of the four categories determined by SEEVA were scored as being only in that range (e.g., category A = character state 0). Species that fell within two sequential categories (e.g., A and B, B and C, etc.) were scored as a unique state for each of the three possible combinations. Species that fell within two of the four categories that were not sequential (e.g., category A and C) were assumed to occupy the entire climatic range (e.g., categories A, B, and C) but were lacking collections representing the entire variation within the species. Species that had collections with any three or four categories were scored as polymorphic (character state 4). Two variables, annual precipitation and precipitation of the wettest month, had no variation among species, and were not included in any further analyses. A complete list of character states and scores for each species are presented in Schulte (2012).

Although re-scoring extracted climate data is not ideal for comparing results from SEEVA to SIMMAP, we could not determine a better approach to score climate variables while maintaining the range of variability within each species. SEEVA analyses pool the climate data at each node below the species level, which may not be a reflection of the ancestral history. To avoid this error, we conducted ancestral state reconstruction via stochastic character mapping to account for the probability of each character state over the ancestral history. However, the re-scored BioClim variables may be a poor reflection of the actual variability within each



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

**Table 2**
SEEVA results for *p*-value and $D_i$ value results for all 19 climatic variables at each node (Fig. 5). Numbers in bold represent significant $D_i$ values ($D_i \geqslant 0.75$) and *p*-values ($p \geqslant 0.05$) and are presented in that order.

| | Node 1 | Node 2 | Node 3 | Node 4 | Node 5 | Node 6 |
|---|---|---|---|---|---|---|
| Annual mean temperature of coldest month | 0.532413/**0.000006** | 0.077719/0.015909 | 0.081647/**0.000556** | 0.569443/**0** | 0.415032/**0** | 0.326935/0.448353 |
| Mean diurnal range | **0.882633/0** | 0.268263/**0** | 0.122125/**0.000027** | 0.301325/**0** | 0.314791/**0** | 0.403255/0.085883 |
| Isothermality | 0.002063/0.534161 | **0.761602/0** | 0.254957/**0** | 0.048442/0.107844 | 0.069867/0.057564 | 0.283918/0.648725 |
| Temperature seasonality | 0/1 | **0.790549/0** | 0.20081/**0** | 0.277741/**0.000001** | 0.184778/**0.000031** | 0.396319/0.37112 |
| Maximum temperature of warmest month | 1/0.006073 | 0.056172/0.084022 | 0.070927/**0.002392** | 0.509041/**0** | 0.340399/**0** | 0.462738/0.248758 |
| Minimum temperature | 0.62504/**0** | 0.292491/**0** | 0.048239/0.015831 | 0.642852/**0** | 0.402092/**0** | 0.344609/0.401908 |
| Temperature annual range | 0.416136/**0** | 0.601985/**0** | 0.007221/0.661439 | 0.326248/**0** | 0.248879/**0** | 0.415606/0.068864 |
| Mean temperature of wettest quarter | 0.456978/**0.000067** | 0.014994/0.609926 | 0.110607/**0.00048** | 0.571876/**0** | 0.372421/**0** | 0.434632/0.273569 |
| Mean temperature of driest quarter | 0.402716/**0.000251** | 0.251994/**0** | 0.044728/0.022463 | 0.602524/**0** | 0.369459/**0** | 0.343507/0.40466 |
| Mean temperature of warmest quarter | 0.377304/**0.00031** | 0.025721/0.383179 | 0.088731/0.000358 | 0.599818/**0** | 0.369939/**0** | 0.398613/0.380051 |
| Mean temperature of coldest quarter | 0.586696/**0.000001** | 0.180916/**0.000011** | 0.064809/**0.00219** | 0.608225/**0** | 0.384666/**0** | 0.302805/0.53334 |
| Annual precipitation | 0.680629/**0** | 0.151859/**0.00008** | 0.281886/**0** | 0.348779/**0** | 0.175425/**0.000232** | 0.119906/0.642976 |
| Precipitation of wettest month | 0.573293/**0** | 0.28824/**0** | 0.018372/0.325243 | 0.401396/**0** | 0.473611/**0** | 0.22679/0.294586 |
| Precipitation of driest month | **0.911308/0** | 0.231674/**0** | 0.384456/**0** | 0.231645/**0.000087** | 0.14766/**0.00059** | 0.126348/1 |
| Precipitation seasonality | 0.444444/**0.000004** | 0.42571/**0** | 0.519882/**0** | 0.37766/**0** | 0.165428/**0.000003** | 0.393305/0.196702 |
| Precipitation of wettest quarter | **0.77214/0** | 0.268805/**0** | 0.021707/0.239888 | 0.387005/**0** | 0.447902/**0** | 0.204993/0.310403 |
| Precipitation of driest quarter | **0.904393/0** | 0.174873/**0** | 0.399351/**0** | 0.245978/**0.000043** | 0.19741/**0.000055** | 0.127229/0.717004 |
| Precipitation of warmest quarter | 0.617361/**0** | 0.276223/**0** | 0.078212/**0.000488** | 0.250452/**0** | 0.323318/**0** | 0.109273/0.845734 |
| Precipitation of coldest quarter | 0.671828/**0** | 0.246863/**0** | 0.36326/**0** | 0.290296/**0.000001** | 0.331688/**0** | 0.111323/0.706327 |

| | Node 7 | Node 8 | Node 9 | Node 10 | Node 11 | Node 12 |
|---|---|---|---|---|---|---|
| Annual mean temperature | 0.423858/**0.000151** | 0.12692/0.059532 | 0.197788/0.062681 | 0.040747/0.584586 | 0.29269/**0.000318** | **0.893073/0.000012** |
| Mean diurnal range | 0.035449/0.531605 | 0.081779/0.245493 | 0.125309/0.276743 | 0.104611/0.310533 | 0.154718/0.046006 | 0.459485/0.031131 |
| Isothermality | 0.707627/**0** | 0.00466/1 | 0.283256/0.013904 | 0.21009/0.115497 | 0.217005/**0.001604** | 0.461596/0.030818 |
| Temperature seasonality | **0.782528/0** | 0.201521/0.040413 | 0.464293/**0.000251** | 0.055847/0.547216 | 0.18447/0.028553 | **0.830809/0.000152** |
| Maximum temperature of warmest month | 0.7/**0** | 0.141914/0.065037 | 0.004295/1 | 0.005785/1 | 0.310775/**0.000223** | **0.869948/0.000082** |
| Minimum temperature of coldest month | 0.3183/**0.00317** | 0.094584/0.101411 | 0.08192/0.2365 | 0.004048/1 | 0.194443/0.010939 | **0.835998/0.000059** |
| Temperature annual range | 0.159647/0.045177 | 0.139145/0.373639 | 0.27173/0.016262 | 0.338058/0.057051 | 0.136103/0.105073 | 0.074982/0.80267 |
| Mean temperature of wettest quarter | 0.442247/**0.000185** | 0.087333/0.115257 | 0.147837/0.051958 | 0.019184/1 | 0.278689/**0.000405** | **0.886699/0.00001** |
| Mean temperature of driest quarter | 0.606946/**0.000002** | 0.200666/0.01307 | 0.025813/0.668139 | 0.109656/0.39455 | 0.264112/**0.000888** | **0.873476/0.000014** |
| Mean temperature of warmest quarter | 0.508958/**0.000025** | 0.070488/0.161162 | 0.07996/0.222575 | 0.019184/1 | 0.335539/**0.000049** | **0.905124/0.00001** |
| Mean temperature of coldest quarter | 0.491071/**0.000108** | 0.12692/0.059532 | 0.090312/0.25832 | 0.019099/0.776021 | 0.246391/**0.000883** | **0.884129/0.000019** |
| Annual precipitation | 0.118812/0.344315 | 0.105532/0.436045 | 0.243794/0.097658 | 0.44106/0.038234 | 0.036842/0.670018 | 0.418151/0.037227 |
| Precipitation of wettest month | 0.05871/0.463893 | 0.020929/0.862865 | 0.055081/0.731578 | 0.465193/0.013523 | 0.176478/0.060582 | 0.374348/0.051104 |
| Precipitation of driest month | 0.444595/**0.000315** | 0.28244/0.04824 | 0.293926/0.027244 | 0.239915/0.328381 | 0.019897/0.520659 | 0.4715/0.017664 |
| Precipitation seasonality | 0.385606/**0.000783** | 0.094624/0.257953 | 0.365669/**0.000881** | 0.043506/0.549148 | 0.044643/0.201898 | 0.649123/**0.000334** |
| Precipitation of wettest quarter | 0.110904/0.253162 | 0.032258/0.811693 | 0.019619/0.938229 | 0.367612/0.028144 | 0.094992/0.279567 | 0.310762/0.090561 |
| Precipitation of driest quarter | 0.167602/0.037164 | 0.180606/0.125233 | 0.245322/0.027946 | 0.039364/0.919908 | 0.088285/0.091777 | 0.553774/**0.003616** |



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

**Table 2** (*continued*)

| | Node 1 | Node 2 | Node 3 | Node 4 | Node 5 | Node 6 |
|---|---|---|---|---|---|---|
| Precipitation of warmest quarter | 0.05174/ 0.752634 | 0.01816/ 0.841773 | 0.04272/ 0.693352 | 0.387339/ 0.029628 | 0.092529/ 0.203892 | 0.247295/ 0.219814 |
| Precipitation of coldest quarter | 0.014831/ 0.874264 | 0.398675/ **0.002454** | 0.087208/ 0.657263 | 0.129366/ 0.526609 | 0.003723/ 0.984038 | **0.763163/ 0.000667** |

species, potentially losing important climate boundaries. These ancestral character state transitions were compared to the results from SEEVA to see where character state transitions and significant climate variables between lineages co-occured.

### 2.7. Correlation analyses

Correlated character state evolution was evaluated with Bayesian stochastic character mapping in SIMMAP that estimated the associations among character states over the phylogenetic tree. The probability of correlations between character states is proportional to the amount of time the character states co-occur in the given state over the history of the phylogenetic tree. Expected character state associations were calculated by multiplying the frequency of individual character states for each combination of two character states (Huelsenbeck et al., 2003; Bollback, 2006). This method allows for detection of associations if character states co-occur, even if evolutionary transitions are rare.

Correlation analyses were conducted with the bias parameter set to the empirical prior and the rate parameter set to the branch length prior with all character state changes unordered. The analyses were conducted with 32 characters (14 morphological, one phenological, and 17 climatic variables), the number of samples was set to 2000, the number of prior draws was set to 1, and the number of predictive samples was set to 1000. Values were chosen to generate an observed sample size $\geqslant$2000 and a predictive sample size $\geqslant$1000 (Meredith et al., 2011).

Correlation analyses generate two separate test statistics, $D_c$ and $M_c$ that are calculated for the state-by-state associations between two characters. The $D_c$ statistic measures the overall association between individual states of each character. The $M_c$ statistic measures the overall association along the phylogeny between states of each character. SIMMAP reported significant results at $p \leqslant 0.05$; however, due to the high number of relationships tested, $p$-values were corrected using the false discovery rate test (FDR; Benjamani and Hochberg, 1995) in SAS software version 9.2 (SAS Institute, Cary, NC). The FDR is comparable to a Bonferroni correction in that it increases the stringency for determining statistical significance when so many comparisons are being made that significance will be detected by chance alone. The FDR is more suitable for the correlation analyses we conducted. Prior to performing the FDR test, morphological characters that were unchanging in the ancestral state reconstructions, polymorphic climate variable character states, and correlations between two morphological characters (including flowering phenology), or two climatic characters were removed from correlation analyses. Polymorphic BioClim characters were excluded because ancestral character state shifts to or from a polymorphic character state are not informative. Correlations between two morphological characters or two climatic characters were not included because we were only interested in relationships between morphological characters and climatic characters. The FDR test was run for the $D_c$ and $M_c$ statistic $p$-values separately, because the two statistics represented separate tests in SIMMAP. The FDR test minimized the number of false positives given the total number of positive tests and reported an adjusted $p$-value. The adjusted $p$-values from the FDR test were reported as significant at $p \leqslant 0.05$.

## 3. Results

### 3.1. 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), and as a result, comparing MPBS support for individual partitions may be a better indicator (Seelanen et al., 1997). The comparison of the full dataset partitions resulted in incongruencies between ETS and cpDNA and ITS (Schulte, 2012). Because we had support from two independently inherited sources of data (cpDNA and ITS) that were in agreement with morphological character states, the ETS sequences that were discrepant and in conflict with morphology for three species (*C. microphylla*, *C. minor*, and *C. purpusii*) were removed from further analyses. The comparison of the MPBS tree topologies of the five individual partitions of the reduced dataset also resulted in incongruencies. As a result, the *C. katzensteiniae G3pdh*A sequence and the *C. crassicaulis idh*B sequence were removed from all further analyses as these two sequences placed these species in conflict with the other four partitions when examined separately.

All other regions of the final analyses were in complete topological congruence or received BS < 50 for the individual analyses. Therefore, a combined analysis of DNA regions for each dataset was performed and is the basis for all results and discussion.

### 3.2. Phylogenetic analyses

Summary statistics are presented in Table 1. The AWTY (Nylander et al., 2008) output indicated that the separate chains approximated the same target distribution for both datasets in all analyses (Schulte, 2012).

All analyses produced trees with congruent topologies with varying amounts of resolution for both the full and reduced datasets. The BI partition model produced the most resolved topology for both datasets. The BI partition model tree that displays the results from the MP, ML, and BI one model analyses are presented in Figs. 2 and 3 for the full dataset and reduced dataset, respectively. Support for clades is represented by MPBS, maximum likelihood BS (MLBS), BI one model PP (OBPP), and BI partition model PP (PBPP) and is reported as MPBS/MLBS/OBPP/PBPP hereafter in the text. Support for each clade was considered strong if BS was >75 and/or PP >95.

### 3.3. Phylogenetic tree topology

In the full dataset, section *Angustiflorae* was recovered as monophyletic (Fig. 2; –/–/100/100). Alternative analyses supported different clades as sister to section *Angustiflorae*. There was support for the monophyly of each of the other six clades of *Columnea* identified by Smith et al. (2013) and Schulte et al. (2014).

There was phylogenetic support for subclades and relationships among species (Fig. 2) within section *Angustiflorae*. Subclade A had strong support for *C. ambigua* and *C. domingensis* as sister (Fig. 2; 98/99/100/100; Fig. 3: 98/88/100/100) and is supported as sister

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

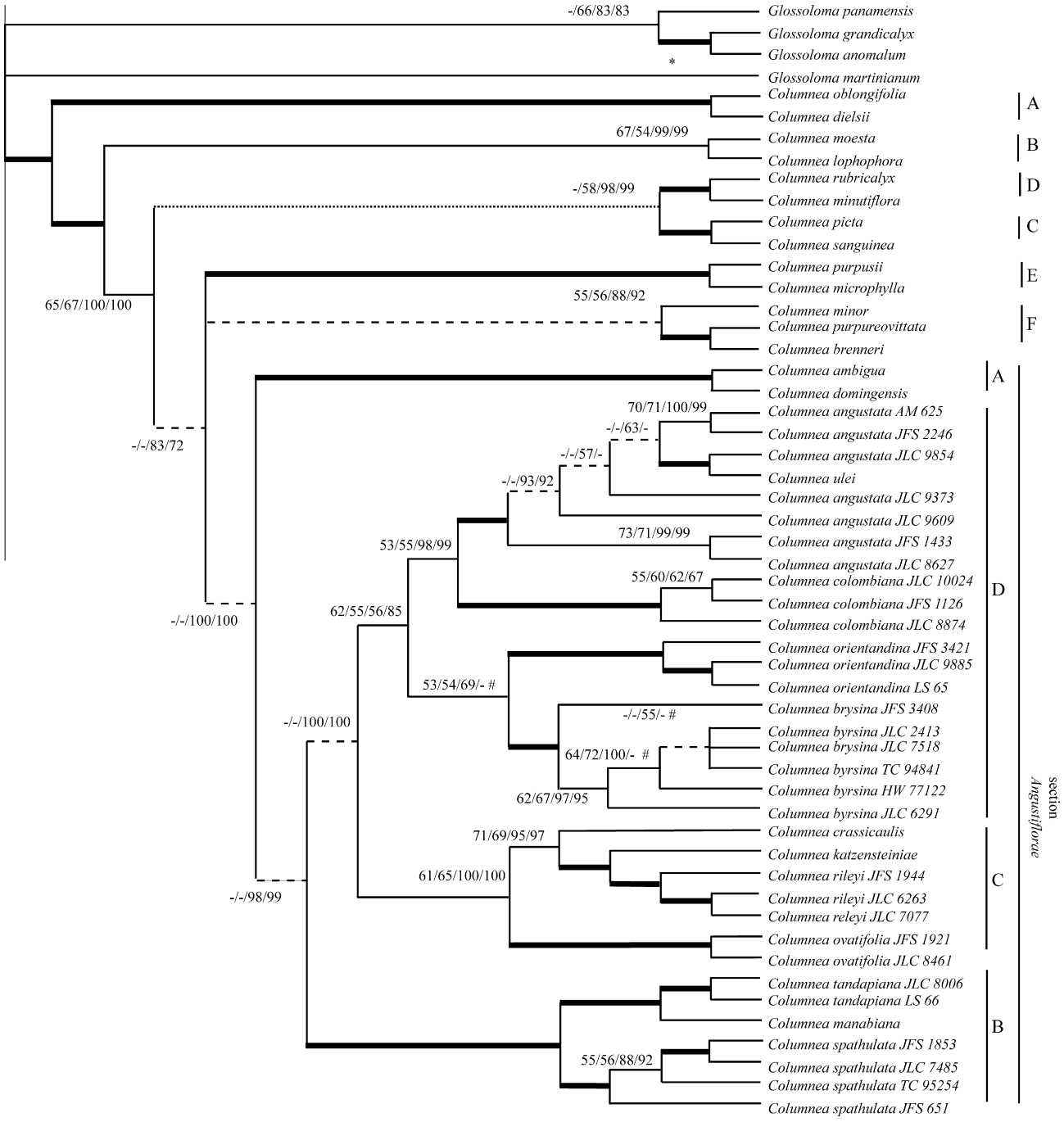


**Fig. 2.** Phylogenetic summary of maximum parsimony (MP), maximum likelihood (ML), Bayesian inference (BI) one model, and BI partition model analyses mapped onto the BI partition analysis tree topology for the full dataset. Numbers above branches represent MP bootstrap (BS)/MLBS/BI one model posterior probability (PP)/BI partition model PP. Bold branches are strongly supported in all four analyses (BS, ML $\geqslant$ 75; PP $\geqslant$ 95). Dashed and dotted lines represent branches that collapse in the MP and ML analyses, respectively and a pound symbol (#) next to support values indicates a clade that collapsed in both the MP and ML analyses. Letters to the far right of the tree indicate clades that were also recovered in Smith et al. (2013) and those immediately to the right of the tree are subclades within section *Angustiflorae*.

to all other species of *Angustiflorae* (Fig. 2: –/–/98/99; Fig. 3: –/–/93/89).

Subclade B included three species: *C. spathulata*, *C. manabiana*, and *C. tandapiana* (Fig. 2: 87/93/100/100; Fig. 3: –/–/100/100). Within subclade B, *C. manabiana* was recovered as sister to *C. tandapiana* (Fig. 2: 99/100/100/100; Fig. 3: 99/100/100/100). *Columnea spathulata* was supported as sister to these two species (Fig. 2: 87/93/100/100; Fig. 3: 100/100/100/100). Subclade B was sister to the remaining species of *Angustiflorae* (Fig. 2: –/–/100/100; Fig. 3: –/–/96/91).

Subclade C included four species: *C. crassicaulis*, *C. katzensteiniae*, *C. rileyi*, and *C. ovatifolia* (Fig. 2: 61/65/100/100; Fig. 3: 73/82/100/100). It was recovered as sister to the five remaining species of *Angustiflorae*. *Columnea katzensteiniae* and *C. rileyi* were well-supported as sister in both the full and reduced dataset analyses (Fig. 2: 93/95/100/100; Fig. 3: 50/53/90/97). *Columnea crassicaulis* was recovered as sister to the remaining two species (Fig. 2: 71/69/95/97; Fig. 3: –/–/55/60). Both datasets recovered *C. ovatifolia* as the sister to the remaining three species within subclade C (Fig. 2: 61/65/100/100; Fig. 3: 73/82/100/100). Subclade D had

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

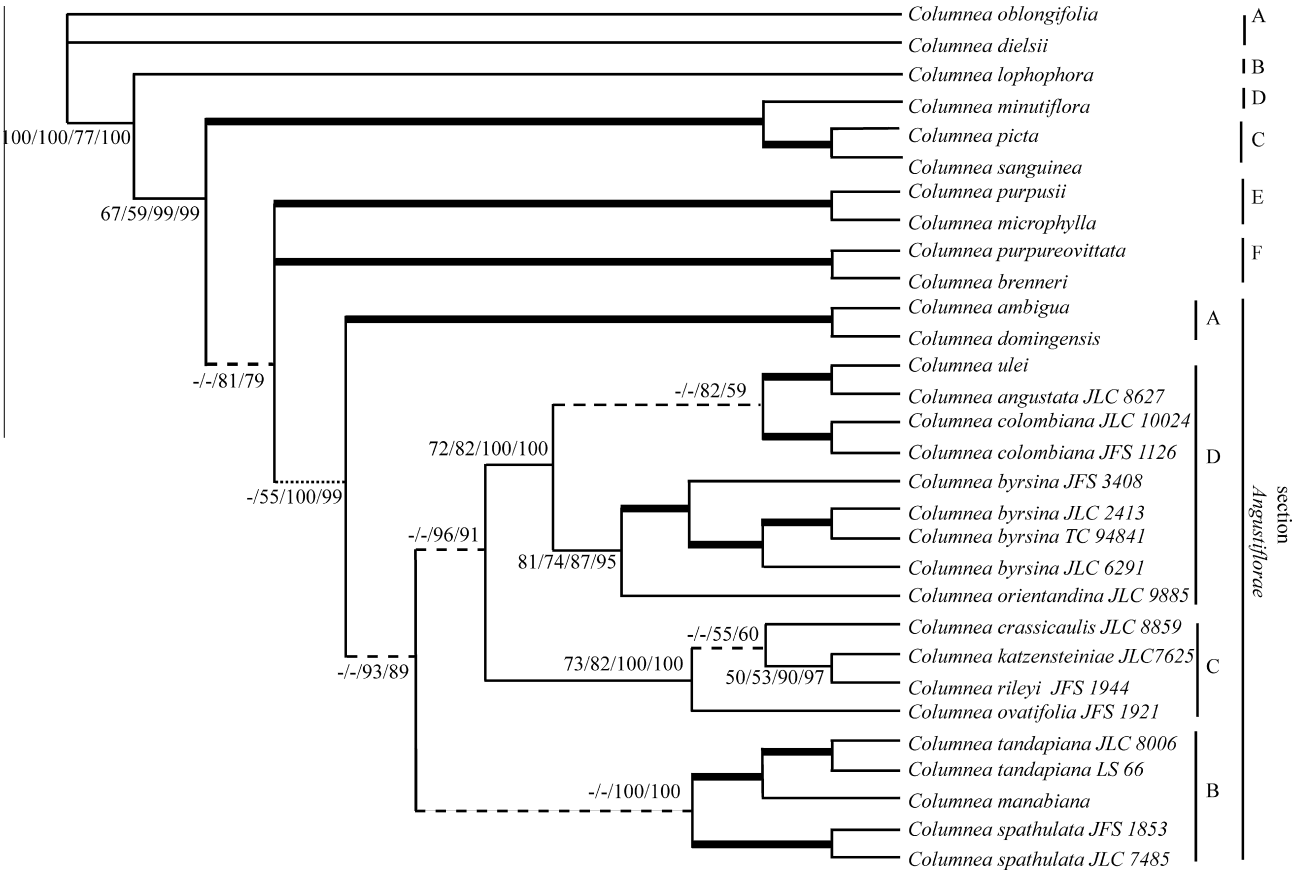


**Fig. 3.** Phylogenetic results from the reduced dataset. Summary of maximum parsimony (MP), maximum likelihood (ML), Bayesian inference (BI) one model, and BI partition model analyses mapped on the BI partition analysis tree topology for the reduced dataset. Numbers on branches represent MP bootstrap (BS)/MLBS/BI one model posterior probability (PP)/BI partition model PP. Bold branches are strongly supported in all four analyses (BS, ML $\geqslant$ 75; PP $\geqslant$ 95). Letters to the far right of the tree indicate clades that were also recovered in Smith et al. (2013) and those immediately to the right of the tree are subclades within section *Angustiflorae*. Dashed and dotted lines represent branches that collapse in MP and ML analyses, respectively.

low support in the full dataset (Fig. 2: 62/55/86/85), but was recovered by all analyses and was more strongly supported in the reduced datatset (Fig. 3: 72/82/100/100). This clade included five species: *C. angustata*, *C. ulei*, *C. colombiana*, *C. byrsina*, and *C. orientandina*. In both the full and reduced dataset analyses, *C. byrsina* and *C. orientandina* were recovered as sister to one another (Fig. 2: 53/54/69/–; Fig. 3: 81/74/87/95). In the analyses of the full dataset, *C. colombiana* was recovered as sister to *C. angustata*/*C. ulei* with moderate support (Fig. 2; 53/55/98/99). This relationship was also recovered by the reduced dataset analyses but with less support than the full dataset analyses (Fig. 3; –/–/82/59). *Columnea angustata* is recovered as paraphyletic due to the inclusion of *C. ulei* (Fig. 2) as has been seen previously (Schulte et al., 2014). It is possible that these two species are conspecific, but because *C. ulei* has seldom been collected, and represents a population widely disjunct from the remainder of *C. angustata*, it was decided to not synonymize the name at present (Schulte et al., 2014). However, there are no characters that differ between *C. ulei* and *C. angustata* that were used in the present study and to minimize calculations and data analyses, *C. ulei* is treated as synonymous with *C. angustata* throughout the manuscript.

Including indels as characters in the full dataset resulted in loss of resolution within subclade D for MP and BI analyses, otherwise relationships within section *Angustiflorae* were identical and the topology of the full tree was nearly identical (Schulte, 2012). In the reduced dataset, inclusion of indels placed *C. colombiana* as sister to *C. brysina*/*C. orientandina* albeit with minimal support (MPBS = 51, PP = 54). Likewise, inclusion of indels placed *C. katzensteiniae* as sister to *C. crassicaulis*, and again, with minimal support (MPBS = 51, PP = 53). Therefore, all discussion is based on topologies without indels.

### 3.4. Species distributions

Within section *Angustiflorae* there were five sister species pairs (Figs. 2 and 3), excluding *C. ulei*/*C. angustata* as discussed above. *Columnea ambigua* and *C. domingensis* are both endemic to Caribbean islands, *C. ambigua* to Puerto Rico and *C. domingensis* to Hispaniola. Because the two species do not overlap in their distribution, they were considered allopatric. If *C. antiocana*, the species not sampled here, were part of subclade C then it would likely represent an additional allopatric relationship (depending on whether it was sister to any one species or sister to a group of species) because it is the only species in this clade known from Colombia.

The four other sister species pairs had overlapping geographic distributions, at least in part. The results from SEEVA (Table 2) for each species in a pair were then compared to determine if species pairs were parapatric or sympatric. *Columnea byrsina*/*C. orientandina* and *C. manabiana*/*C. tandapiana* did not have the same climatic range for all 19 BioClim variables. *Columnea byrsina* and *C. orientandina* had significant differences in eleven climatic variables (annual mean temperature; isothermality; temperature seasonality; maximum temperature of the warmest month; minimum temperature of the coldest month; mean temperatures of the wettest, driest, warmest, and coldest quarters; precipitation of

the driest month; and precipitation seasonality) with a significant $D_i$ value for temperature seasonality. *Columnea manabiana* and *C. tandapiana* also had significant differences in eleven climatic variables (annual mean temperature; temperature seasonality; maximum temperature of the warmest month; minimum temperature of the coldest month; mean temperatures of the wettest, driest, warmest, and coldest quarters; precipitation seasonality; and precipitation of the driest and coldest quarters) with significant $D_i$ values for nine variables (annual mean temperature; temperature seasonality; maximum temperature of the warmest month; minimum temperature of the coldest month; mean temperatures of the wettest, driest, warmest, and coldest quarters; and precipitation of the coldest quarter). Because both sister species pairs had significant differences in climatic variables, they were classified as parapatric. The remaining two sister species pairs, *C. angustata*/*C. colombiana* and *C. katzensteiniae*/*C. rileyi*, had overlapping geographic distributions and no significant differences in the 19 BioClim variables and were thus classified as sympatric.

### 3.5. Morphological characters

No character states of the 14 sampled characters differed between *C. angustata* and *C. ulei*. Given the possible conspecifity of these two species, and to simplify calculations and the results, only *C. angustata* is discussed here to include both species. Ancestral character state reconstructions for the 14 morphological characters and phenology resulted in five morphological characters that shifted states for terminal taxa rather than internal nodes in both Mesquite and SIMMAP analyses (adaxial pubescence, abaxial pubescence, abaxial coloration, number of flowers per inflorescence, and corolla length). Thus, these five autapomorphic characters were removed from further analyses.

The Mesquite and SIMMAP analyses resulted in mostly similar ancestral state reconstructions for the nine remaining morphological characters (habit, leaf dimorphism, lamina surface area, petiole length, floral bract size, corolla to calyx ratio, calyx margin, corolla color, and corolla lobe color) and one phenological character (Supplemental Appendix B). Ancestral state reconstructions had MLP and BPP mostly within 0.01 probability of each other for habit, and within 0.03 probability for phenology, with one exception in the ancestral state reconstruction of phenology at node 12 (Supplemental Appendix B). The ancestral state reconstructions of the other eight morphological characters had differences between MLP and BPP ranging from 0.01 to 0.74 (Supplemental Appendix B). In many cases MLP was not capable of resolving probabilities other than equivocal for all possible ancestral character states, whereas BPP clearly indicated one state as more likely than the other. This is likely due to the different model of stochastic mapping that included branch lengths as a factor in the probability of character state transition that maximum likelihood did not. The discussion will therefore focus on the results of stochastic mapping (Fig. 4) although the results of maximum likelihood are presented in Supplemental Appendix B.

### 3.6. Climate variables

Results from SEEVA for *p*-values and $D_i$ values at all nodes for each climate variable are in Table 2. All nodes except 6 and 10 had at least one climate variable with a significant *p*-value (Table 2). Only nodes 1, 2, 7, and 12 had climate variables with significant $D_i$ values (Table 2).

Ancestral state reconstructions of the 17 BioClim variables resulted in ten variables that were unchanging throughout the ancestral history and thus, are not presented for these ten climatic variables (mean diurnal range; isothermality; maximum temperature of the warmest month; minimum temperature of the coldest month; temperature annual range; precipitation of the driest month; precipitation seasonality; and precipitation of the wettest, driest, and warmest quarters). Ancestral state reconstructions for the precipitation of the wettest, driest, warmest, and coldest quarters resulted in the exact same BPP. All shifts in the ancestral state reconstructions of the seven BioClim variables (results not shown) were from a polymorphic character state and therefore do not provide valuable information for correlation analyses.

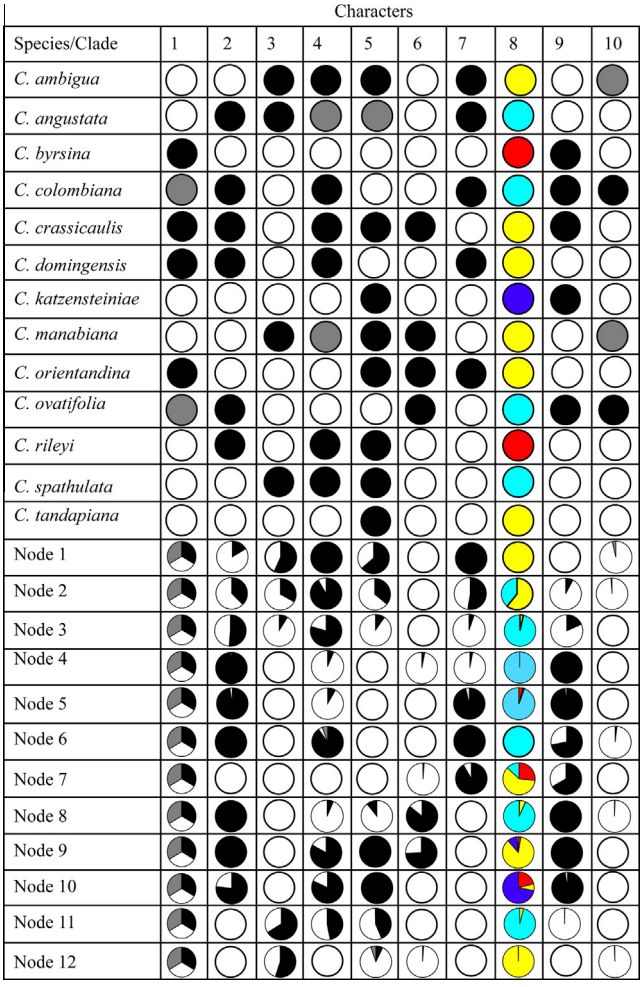


**Fig. 4.** Ancestral state reconstructions and species character states for nine morphological traits and phenology. Circles show the probability of a node having a character state for each character based on stochastic character mapping. Specific values are in Supplemental Appendix B. Posterior probabilities from Mesquite were similar to those presented here, if not identical. 1. Stem orientation, white = upright, black = horizontal, gray = pendent. 2. Leaf dimorphism, white = anisophyllous, black = isophyllous. 3. Leaf surface area, white = 0–30 $cm^2$, black > 30 $cm^2$. 4. Petiole length, white = 0–5 mm, black = 5–20 mm, gray > 20 mm. 5. Floral bract size, white = 0–6 mm, black = 6–13 mm, gray > 13 mm. 6. Corolla to calyx ratio, white = 2–2.5, black > 2.5. 7. Calyx margin, white = entire, black = serrate. 8. Corolla color, yellow = yellow, red = red, purple = purple, blue = polymorphic (more than one of the previous states). 9. Corolla lobe color, white = equal to corolla color, black = different from corolla color. 10. Phenology, white = flowering continuously, black = flowering from January to March, gray = flowering March to October. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.7. Correlations

SIMMAP analyzed 5597 correlations each for the $D_c$ and $M_c$ statistic. The FDR test using SAS compared 1204 correlations each for the $D_c$ and $M_c$ statistic after removing five morphological characters, polymorphic character states for BioClim variables, and

**Table 3**
Correlation results from the false discovery rate (FDR) test. Morphological variable and climate variable are the two sets of characters that were compared for each correlation. Stat represents the two character states (Appendix B) that were compared for either the $D_c$ (d) or $M_c$ (m) statistic test. Raw *p*-value is the *p*-value as given by SIMMAP correlation analyses. FDR Adjusted *p*-value is the adjusted *p*-value from FDR test that was used to determine significance of correlations with $p \leqslant 0.05$ as significant; only FDR *p*-values $\leqslant 0.01$ are presented. Bold values indicate significant *p*-values from the FDR test ($p \leqslant 0.05$).

| Morphological variable | Climate variable | Stat | Value | Raw *p*-value | FDR adjusted *p*-value |
|---|---|---|---|---|---|
| Habit | Mean Diurnal Range | *d* (2,1) | 0.002201 | 0.000 | 0.000 |
| Lamina surface area | Precipitation of Driest Quarter | *d* (0,1) | −0.002165 | 0.000 | 0.000 |
| Lamina surface area | Precipitation of Driest Quarter | *d* (1,1) | 0.002066 | 0.000 | 0.000 |
| Corolla color | Mean Temperature of Coldest Quarter | *d* (0,0) | −0.001453 | 0.000 | 0.000 |
| Phenology | Temperature Annual Range | *d* (0, 2) | −0.002196 | 0.000 | 0.000 |
| Phenology | Precipitation of Driest Month | *d* (0,1) | −0.002697 | 0.000 | 0.000 |
| Habit | Precipitation of Wettest Quarter | *d* (0,1) | −0.002550 | 0.001 | 0.080267 |
| Adaxial pubescence | Maximum Temperature of Warmest Month | *d* (0,2) | −0.002221 | 0.001 | 0.080267 |
| Adaxial pubescence | Maximum Temperature of Warmest Month | *d* (1,2) | 0.002160 | 0.001 | 0.080267 |
| Calyx margin | Temperature Annual Range | *d* (0,1) | −0.001358 | 0.001 | 0.080267 |
| Calyx margin | Temperature Annual Range | *d* (1,1) | 0.001265 | 0.001 | 0.080267 |
| Corolla color | Mean Diurnal Range | *d* (0,1) | −0.002308 | 0.001 | 0.080267 |
| Phenology | Precipitation of Driest Month | *d* (2,1) | 0.002289 | 0.001 | 0.080267 |
| Phenology | Precipitation of Driest Quarter | *d* (0,1) | −0.002867 | 0.001 | 0.080267 |
| Phenology | Precipitation of Driest Quarter | *d* (2,1) | 0.002919 | 0.001 | 0.080267 |
| Habit | Mean Diurnal Range | *m* (2,1) | 0.002585 | 0.000 | 0.000 |
| Lamina surface area | Precipitation of Driest Quarter | *m* (0,1) | −0.001805 | 0.000 | 0.000 |
| Lamina surface area | Precipitation of Driest Quarter | *m* (1,1) | 0.002419 | 0.000 | 0.000 |
| Corolla color | Mean Temperature of Coldest Quarter | *m* (0,0) | −0.000641 | 0.000 | 0.000 |
| Phenology | Temperature Annual Range | *m* (0,2) | −0.001634 | 0.000 | 0.000 |
| Phenology | Precipitation of Driest Month | *m* (0,1) | −0.002097 | 0.000 | 0.000 |
| Phenology | Precipitation of Driest Quarter | *m* (2,1) | 0.003513 | 0.000 | 0.000 |
| Habit | Precipitation of Wettest Quarter | *m* (0,1) | −0.002189 | 0.001 | 0.086 |
| Adaxial pubescence | Maximum Temperature of Warmest Month | *m* (0,2) | −0.001773 | 0.001 | 0.086 |
| Adaxial pubescence | Maximum Temperature of Warmest Month | *m* (1,2) | 0.002605 | 0.001 | 0.086 |
| Calyx margin | Temperature Annual Range | *m* (1,1) | 0.001639 | 0.001 | 0.086 |
| Corolla color | Mean Diurnal Range | *m* (0,1) | −0.001888 | 0.001 | 0.086 |
| Phenology | Precipitation of Driest Month | *m* (2,1) | 0.002859 | 0.001 | 0.086 |
| Phenology | Precipitation of Driest Quarter | *m* (0,1) | −0.002315 | 0.001 | 0.086 |
| Habit | Annual Mean Temperature | *m* (0,0) | −0.001220 | 0.002 | 0.09632 |
| Habit | Annual Mean Temperature | *m* (2,0) | 0.002677 | 0.002 | 0.09632 |
| Habit | Mean Diurnal Range | *m* (0,1) | −0.001913 | 0.002 | 0.09632 |
| Habit | Minimum Temperature of Coldest Month | *m* (0,3) | −0.000784 | 0.002 | 0.09632 |
| Habit | Mean Temperature of Coldest Quarter | *m* (2,3) | 0.002291 | 0.002 | 0.09632 |
| Habit | Precipitation of Coldest Quarter | *m* (0,1) | −0.001482 | 0.002 | 0.09632 |
| Leaf dimorphism | Precipitation of Coldest Quarter | *m* (0,1) | −0.000985 | 0.002 | 0.09632 |
| Leaf dimorphism | Precipitation of Coldest Quarter | *m* (1,1) | 0.001746 | 0.002 | 0.09632 |
| Calyx margin | Temperature Annual Range | *m* (0,1) | −0.000981 | 0.002 | 0.09632 |
| Phenology | Mean Diurnal Range | *m* (1,1) | 0.002344 | 0.002 | 0.09632 |
| Phenology | Mean Temperature of Coldest Quarter | *m* (1,0) | 0.002523 | 0.002 | 0.09632 |

correlations between two morphological characters or two BioClim characters. Correlations with an adjusted $p < 0.01$ from the FDR test are presented in Table 3. Of these 1204 correlations, six $D_c$ statistic correlations and seven $M_c$ statistic correlations were statistically significant (adjusted *p*-value < 0.05; Table 3). Because the six significant $D_c$ statistic correlations were a subset of the seven significant $M_c$ statistics, only significant $M_c$ statistics are further discussed. Significant correlations of morphological characters to climatic variables were as follows: a pendent habit was correlated to a mean diurnal range >9.7 °C; a smaller leaf surface area was correlated with precipitation of the driest quarter <198.43 mm; a larger leaf surface area was correlated with precipitation of the driest quarter <198.43 mm; a yellow corolla color was correlated with temperature of the coldest quarter <18.133 °C; flowering continuously was correlated with temperature annual range >11.45 °C and with precipitation of the driest month <53 mm; and flowering from March to October was correlated with precipitation of the driest quarter <198.43 mm (Table 3). Statistically significant correlations are expected to co-occur at multiple nodes throughout the phylogenetic tree. Three of the correlations were statistically significant, but only co-occurred in one extant species each. A pendent habit was correlated with a mean diurnal range of >9.7 °C. These two character states only co-occurred in *C. ovatifolia* (Appendix B). A smaller leaf surface area was correlated with a low precipitation for the driest quarter that only co-occurred in *C. tandapiana* (Appendix B). Flowering from March to October was correlated with a lower precipitation in the driest quarter and only co-occurred in *C. manabiana* (Appendix B). Because each of the correlations was found in only a single species, the significant value associated with the correlation may have been an artifact. These analyses enhanced the ability to detect correlations with transitions that were rare. This may also be a type II error due to the less conservative FDR test that was used to adjust the *p*-values for the correlation analyses. Because these correlations are only found for a single species each, they will not be considered further.

## 4. Discussion

### 4.1. Phylogenetic relationships within section Angustiflorae

The full dataset analyses showed support for the same seven clades as Smith et al. (2013) and Schulte et al. (2014) and resolved four subclades within section *Angustiflorae*, although not all clades received strong support (Fig. 2). Where multiple accessions per species were sampled, all species were shown to be monophyletic (except *C. angustata*, but see above), permitting a smaller sampling of accessions, but with additional sequence data in the reduced dataset. The reduced dataset boosted support within *Angustiflorae*



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

(Fig. 3) for most clades, but resulted in lower support values (particularly MPBS and MLBS) for the monophyly of subclade B, the placement of subclade B as sister to the remaining two subclades, relationships within subclade C, and the sister pair relationship between *C. angustata* and *C. colombiana* (Figs. 2 and 3).

The changes in support between the two datasets are almost certainly the result of the two low-copy nuclear genes that were only included in the reduced dataset. Clearly, additional sequence data supporting the relationships from Fig. 2 is the reason for increased support, and decreased support is likely the result of paralogs that were not detected when individual gene trees were examined. Two loci each for *GPD3* and *idh* were recovered in amplification and cloning of these two regions (Schulte, 2012), therefore paralogy is known to be present. Only one locus was used for each because independent analyses of the second locus produced topologies that were clearly discordant with results from ribosomal RNA and cpDNA regions. The discrepant topologies and missing data for some species justified discarding these regions from the analysis. Attempts were made to identify paralogs or alleles by excluding them individually from the analyses. While this worked to identify some regions for some species, further exclusions resulted in reduced support for clades where congruence among all regions was recovered, implying that only some substitutions were incongruent with other loci rather than indicating an entirely paralogous locus. We therefore opted to retain the sequences, knowing that the inclusion of some incongruent data was likely to create some reduced support because we wanted to demonstrate that the relationships recovered in the full dataset (Fig. 2) were supported by additional data. The clades where the inclusion of incongruent data reduced support are well supported in the full dataset, at least by BI PP. Thus, all relationships in Fig. 3 are strongly supported by a minimum of one of the analytical methods in either Fig. 2 and 3 and there are no relationships recovered from the reduced dataset based on five loci that were in conflict with the full dataset based on three loci (i.e., they were congruent). The topology of species level relationships recovered from the reduced dataset analysis (Fig. 3) was therefore used for all investigations of climate, morphology, and phenology shifts in this clade.

### 4.2. Distribution and climate as driving forces of speciation

Within *Angustiflorae*, one sister species pair is allopatric, two are parapatric, and two are sympatric (Fig. 5). One additional allopatric pair might have been recovered if *C. antiocana* had been sampled (see Results). Allopatry is one of the most widely recognized means by which speciation occurs (Bush, 1975b) but is apparently a more rare event in this clade, at least among extant species pairs. In contrast, allopatric speciation was considered the primary form of speciation in *Sinningia*, with most sympatric sister pairs recovered in a single clade (Perret et al., 2007). Allopatric speciation is likely important in other clades of Gesneriaceae and has been demonstrated in tribe Coronantheree where dispersal across the Pacific and to different islands in the South Pacific has resulted in diversification (Woo et al., 2011). *Columnea ambigua* and *C. domingensis* are on separate Caribbean islands, suggesting that geographic isolation was the primary means of disrupting gene flow in their ancestral populatons. Physical (geographic) isolation likely increased the potential for morphological divergence via other evolutionary processes, such as selection, mutation, or genetic drift.

Given that the majority of sister species pairs have either parapatric or sympatric distributions, disruption of gene flow in ancestral species within section *Angustiflorae* likely occurred in more complex ways. The frequency of parapatric and sympatric speciation has often been questioned because of the lack of empirical evidence (Barluenga et al., 2006), compared with evidence of allopatric speciation (Bush, 1975b). Savolainen et al. (2006) and Papadopulos et al. (2011) demonstrated sympatric speciation in *Howea* and other genera on Lord Howe Island using a complex array of ecological and genetic studies that were challenged (e.g., Stuessy, 2006). We are making no claims that we have demonstrated sympatric speciation here. Based on their current distribution and phylogenetic relationships, sister species pairs in *Angustiflorae* provide additonal potential examples of both parapatric and sympatric speciation. Our analyses are limited to the phylogenetic and climatic variables that were analyzed and further field investigations would be essential to verify the degree to which sister species are truly sympatric.

As defined here, parapatric species pairs have at least partially overlapping geographic distributions, but differ significantly by at least one climate variable as determined by SEEVA. Thus, while there is potential for gene flow, it may be that selection in different microclimates removes alleles from other species, or prevents pollinators from crossing climate boundaries, and serves as a barrier to gene flow. As sessile organisms, plants respond to environmental conditions (Hopkins et al., 2008) and must adapt to climatic conditions to survive and reproduce (Hancock et al., 2011). *Columnea byrsina* and *C. orientandina* differed by 11 climatic variables with a significant $D_i$ for temperature seasonality (Table 2). These two species are morphologically similar, but differ in a number of character states including anther exsertion and corolla color; implying that pollinator shifts may also have been involved in speciation (see below). *Columnea manabiana* and *C. tandapiana* also overlap in their geographic distributions and differ by 11 climatic variables with nine of them having significant $D_i$ values (Table 2). These two species are morphologically similar and have similar corollas with the major difference between the two being the size of the floral bracts. Therefore it is likely that climate differences may have been a major force in speciation for this pair, but because the two species are so similar morphologically, physiological characters that have not yet been investigated for these species may have initiated and maintained barriers to gene flow.

The final two sister species pairs, *C. angustata*/*C. colombiana* and *C. katzensteiniae*/*C. rileyi* both have overlapping distributions and no significant differences among climatic variables (Table 2). It is possible that these species may differ in climatic variables that we did not measure that may make them more parapatric than sympatric, however the similarities they share imply that forces other than geographic isolation or climatic adaptations are driving diversification in these pairs (see below).

### 4.3. Selectional forces driving speciation

#### 4.3.1. Habit and leaf characters

Changes in habit and leaf characters are likely to have driven speciation within section *Angustiflorae*. Leaf traits are under selection pressure in plants, because in most species they are responsible for the capture and utilization of light energy, transpiration, and thermoregulation (Carson, 1985; Givnish, 1979, 1984, 1987; Ezcurra et al., 1997; Hufford and McMahon, 2004; Mummenhoff et al., 2005; Hopkins et al., 2008; Jones et al., 2009; Yates et al., 2010). Adaptations in vegetative characters are likely to increase a plant's fitness allowing it to grow larger, possibly attract more pollinators, and produce more fruit and seeds (Carson, 1985; Hopkins et al., 2008). Multiple shifts between unifoliate, rosulate, and caulescent growth forms in *Streptocarpus* (Gesneriaceae, subfamily Didymocarpoideae) have been hypothesized to be adaptations to different climate conditions (Möller and Cronk, 2001). Character state shifts in one or more of the vegetative characters analyzed here occurred at nodes 1, 4, 6, 7, 11, and since divergence from their most recent ancestor for *C. angustata*, *C. crassicaulis*, *C. katzensteiniae*, and *C. ovatifolia* (Figs. 4 and 5).



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

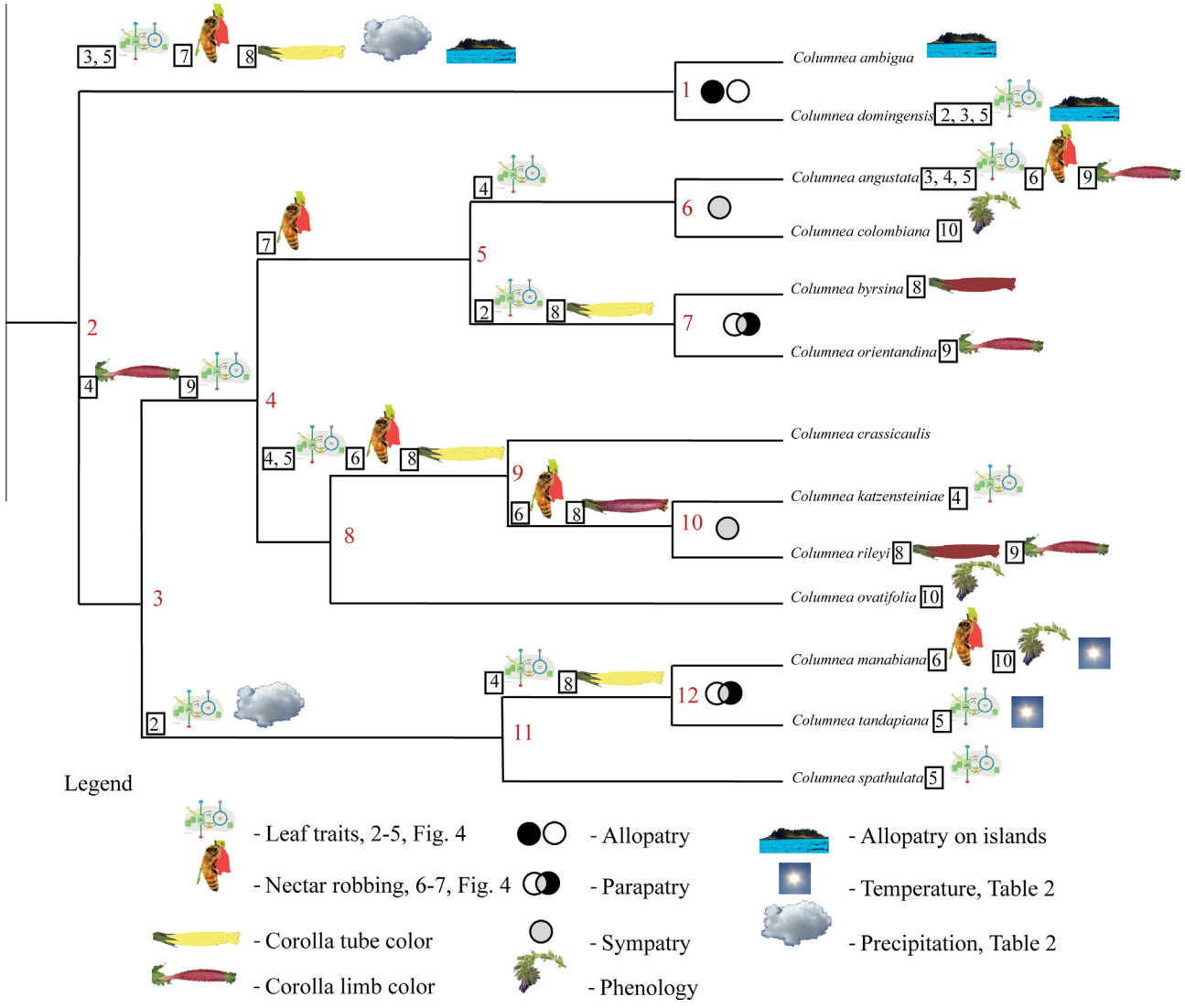


**Fig. 5.** Summary of forces driving speciation within section *Angustiflorae*. Pictures represent causes of speciation at each node and for individual species. Red numbers mark nodes for ancestral character state reconstructions that are presented in Fig. 4. Black numbers in boxes adjacent to images refer to characters in Fig. 4. Exceptions are climate factors that are in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Leaves are the primary photosynthetic organs in *Columnea*; thus leaves with a larger surface area increase the potential amount of light that can be captured for photosynthesis. However, as with most morphological traits, there are tradeoffs that limit the size of leaf surface area that were not examined as part of this study (Parkhurst and Loucks, 1972; Meinzer and Goldstein, 1985; Givnish, 1987; Roderick et al., 1999; Ristic and Jenks, 2002; Bonser, 2006). A larger leaf surface area co-occurred with lower precipitation during the driest quarter at three nodes on the phylogenetic tree (Figs. 4 and 5; nodes 1, 11, and 12; Table 3). Because these two character states did not co-occur in any of the extant species, the correlation may have been more important for past speciation. A larger leaf surface area and lower precipitation during the driest quarter separated the common ancestor of *C. ambigua* and *C. domingensis* from the ancestor of the entire section (Fig. 4: node 1). It is likely that the ancestor to *C. ambigua* and *C. domingensis* dispersed to Caribbean islands from the mainland. Results from stochastic mapping of ancestral state reconstruction for distributions indicate that the common ancestor to the entire clade (node 2) has a 99% probability of having been on the mainland rather than in the Caribbean (unpublished results). The presence of a larger leaf surface area with tolerance for less precipitation during the driest quarter may have been important to allow the colonization of *C. ambigua* and *C. domingensis* into the Caribbean islands where climatic conditions are significantly drier.

These two character states also co-occur in the common ancestor of *C. spathulata*, *C. manabiana*, and *C. tandapiana* and are retained in the common ancestor of *C. manabiana* and *C. tandapiana* (subclade B; Figs. 4 and 5). Because a larger leaf surface area does not co-occur with lower precipitation in the driest quarter for any of the three extant species within subclade B (Figs. 4 and 5), a shift away from either one or both of these character states was likely an important force in recent speciation of *C. spathulata*, *C. manabiana*, and *C. tandapiana*.

The amount of rainfall an area receives is positively correlated with the percentage of species with larger leaves (Parkhurst and Loucks, 1972; Dilcher, 1973; Dolph and Dilcher, 1980; Givnish, 1987). In section *Angustiflorae*, a larger leaf surface area was instead correlated with lower precipitation during the driest quarter (Table 3). Leaf surface area is also positively correlated with mean annual temperature and soil fertility (McDonald et al., 2003). No correlation was detected between leaf size and temperature (Table 3) and soil fertility was not assesssed. However, most species of section *Angustiflorae* are primarily epiphytic or epipetric

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

and would therefore be expected to occur in habitats with relatively low fertility. Thus, it is unlikely that either of these conditions could be counter-acting the impact of precipitation. It is possible that the influences of unquantified parameters may have resulted in a counterintuitive correlation between a larger leaf size and lower amount of precipitation.

Alternatively, the correlation between larger leaves and lower precipitation during the driest quarter may reflect a trade-off between capturing light, minimizing nutrient uptake, and the epiphytic habit. Most species with larger leaf surface area have leaves that are anisophyllous (Fig. 4; Appendix B). Exceptions to this are the anisophyllous species *C. byrsina*, *C. orientandina*, and *C. tandapiana* that were placed in the smaller leaf area category. All three of these species have leaves that are much longer than wide. Thus, the narrower width is likely to have placed them in the smaller leaf surface area category. Shoot dorsiventrality and leaf anisophylly has been proposed as an adaptation to shaded habitats to maximize light capture and minimize mutual shading of leaves on plagiotropic stems (Givinish, 1984; Dengler and Sanchez-Burgos, 1988; Dengler, 1999). In contrast, larger isophyllous leaves either overlap or require larger internodes to prevent overlap (Givnish, 1987). Epiphytes may be under stress for nutrients (reviewed in Zotz and Hietz, 2001) and rely on litter fall and precipitation to obtain nutrients. Thus, selection for efficient leaf packing may occur in habitats that experience the lowest precipitation during the driest months.

#### 4.3.2. Floral characters as adaptations: pollinator selection

The most likely forces driving speciation within section *Angustiflorae* are changes in reproductive characters as a result of pollinator selection. Selection by pollinators has been shown to drive character state changes in many plant groups (Schemske, 1981; Whitten et al., 1986; Galen, 1989; Johnson, 1996; Armbruster, 1993; Johnson et al., 1998; Beardsley et al., 2003; Perret et al., 2007; Kay et al., 2005; Ree, 2005; Irwin, 2006; Smith et al., 2008b; Martén-Rodríguez et al., 2010; Smith, 2010; Valente et al., 2012; van der Niet and Johnson, 2012). Within Gesneriaceae, shifts in pollinators have been proposed as selective forces driving speciation in *Streptocarpus* (Harrison et al., 1999). In Gesnerieae, Martén-Rodríguez et al. (2010) demonstrated that for regions where hummingbird diversity may be low, such as Caribbean islands, a transition from specialist to generalist pollination syndrome may be an evolutionarily advantage. Perret et al. (2007), however, did not find major phenotypic or phenological shifts of floral characters in sympatric pairs of *Sinningia*; implying that sympatries accumulated at random. Because pollinators directly affect gene flow, they can have a large impact on floral characters and speciation processes (Carson, 1985).

Plant-pollinator interactions are often important in driving speciation because the efficiency of pollination systems is directly related to the fitness of the plant (Proctor et al., 1996). Tropical habitats have a diverse array of potential pollinators including hummingbirds, bats, and insects (Bawa, 1990). The number and type of pollinators that visit a plant species depends upon the corolla shape, color, and size, the pollen or nectar reward, or scent among other characters (Proctor et al., 1996; Muchhala et al., 2008); however, within Gesneriaceae, the corolla color and shape are usually indicative of whether the flower is a generalist (visited by a variety of pollinators) or visited by a specific pollinator (Roalson et al., 2003; Perret et al., 2007; Martén-Rodríguez et al., 2010).

Within Gesneriaceae subfamily Gesnerioideae, red, tubular, diurnal flowers are associated with hummingbird pollinators; campanulate or tubular corollas with purple, blue, or yellow colors are associated with bee pollinators; and long tubular white or yellow flowers are associated with moth pollinators or generalists (Roalson et al., 2003; Perret et al., 2007; Martén-Rodríguez et al., 2010). These plant-pollinator interactions are likely similar for species of section *Angustiflorae* because they are found in the same tropical habitats and have similar floral morphologies, though there have not been extensive studies examining plant-pollinator interactions within the section.

Within section *Angustiflorae*, speciation due to pollinator shift was indicated by character state shifts in corolla to calyx ratio, calyx margin, corolla color, corolla lobe color, floral bract size, and flowering phenology. Pollinator selection seemed to have a large impact on speciation in *Angustiflorae* with character shifts in the above characters occurring at nodes 1, 3, 4, 5, 7, 8, 9, 10, 11, 12, and in *C. angustata*, *C. colombiana*, *C. byrsina*, *C. rileyi*, *C. ovatifolia*, *C. manabiana*, *C. tandapiana*, and *C. spathulata* (Figs. 4 and 5).

This study showed a correlation between a yellow corolla color and a lower temperature during the coldest quarter (Table 3). The correlation between corolla color and temperature may also be an indication that there was a pollinator shift. Each shift to a yellow corolla occurred from an ancestor that had a polymorphic corolla color. A species with a polymorphic corolla color may be an indication that the ancestor species was a generalist and visited by a variety of pollinators (Martén-Rodríguez et al., 2010). A lower temperature in the coldest quarter may not have been tolerated by all the pollinators of the ancestor species, eliminating them as potential pollinators. Hummingbirds would be expected to tolerate colder temperatures compared to insects, which are more temperature sensitive (Kendeigh, 1969). If a shift to hummingbird pollinators had occured one would expect red corollas rather than yellow. If yellow is truly correlated with moth pollinators, these results imply that a moth species is capable of tolerating lower temperatures during the coldest quarter, and selected for a yellow corolla.

A yellow colored corolla co-occurred with a colder temperature during the coldest quarter at four nodes on the phylogenetic tree (Table 3; Figs. 4 and 5; nodes 1, 7, 9, and 12). Of the four nodes where these character states co-occurred, all are close to the tips of the tree (Figs. 3 and 5). These character state shifts occurring in more recent divergent taxa imply that the pollinator selecting for yellow corollas is a more recent driving force in speciation within section *Angustiflorae*.

Another force driving speciation may have been nectar robbing or, more precisely, adaptations to decrease nectar robbing. Nectar robbing occurs when insects push aside the calyx lobes and chew through the corolla to eat the nectar but do not obtain and disperse pollen. Because most plants depend upon pollinators for reproduction, and nectar robbers are not performing pollination, there is likely to be a decrease in the fitness of plants where nectar robbing occurs. To counter the detrimental effect of nectar robbers, morphological and physiological characters have evolved to deter nectar robbing. One morphological adaptation is reinforcement of the calyx (Inouye, 1983; Galen, 1999) such that access to the nectar at the base of the corolla tube is limited. Examination of corollas of *Columnea* in herbaria indicate that nectar robbing does occur (J. F. Smith, pers. obs.) and may be more common than directly observed because the damaged corollas are likely to decay more rapidly and be collected less often than intact ones. Within section *Angustiflorae*, character state shifts in the calyx margin (from entire to serrate) and the corolla to calyx ratio (from a larger ratio to a smaller ratio) are likely to be adaptions reinforcing the calyx and deterring nectar robbing. A serrated calyx is likely to be more of a deterent to nectar-robbing insects compared with an entire margin; when proportionally more of the corolla is covered by the calyx it is more difficult for insects to access the base of the flower.



Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

These hypotheses however remain untested in *Columnea*. The floral adaptations may have contributed to speciation. Character state shifts toward a serrate calyx margin or smaller corolla to calyx ratio occurred at nodes 5 and 10 (Figs. 4 and 5). It is possible that the inclusion of *C. antiocana*, which has an entire calyx margin, may have altered where the shift from entire to serrate margins occurred in subclade C (Figs. 3–5). However, its inclusion here is unlikely to have altered the finding that independent shifts to a serrate margin occurred within this section.

#### 4.3.3. Phenology: adaptation to climate and pollinators

A continuously flowering phenology and a larger annual temperature range co-occurred at four nodes in the phylogenetic tree (Table 3; Fig. 5: nodes 1, 8, 9, and 10) and a phenology of flowering continuously co-occurred with lower precipitation during the driest month at three nodes (Table 3; Fig. 5: nodes 1, 11, and 12). None of these character states co-occurred in extant species of section *Angustiflorae* indicating that they may have been important in past speciation. Both correlations (Table 3) are present at node 1, which is the common ancestor to *C. ambigua* and *C. domingensis* and may have been an important factor to allow the common ancestor to move into the Caribbean, and tolerate more variable climatic conditions.

In the tropics, an aseasonal climate allows the potential for plants to flower year round (Bawa et al., 2003). However, the flowering phenology of each species is limited by both biotic and abiotic factors, such as pollinators (Bawa et al., 2003) and seasonal variation in precipitation (Gentry, 1974). Pollinator selection causes directional or divergent selection in the timing of flowering, eliminates competition and selects for the optimal time of year for a specialized pollinator (Gentry, 1974). Seasonal changes limit a plant's acquisition of resources and leads to limited time for flowering (Reich and Borchert, 1984). Tropical trees often flower during the dry season when a lack of leaves makes flowers more visible to pollinators (Bawa et al., 2003), and not during the wet season that allows plants to allocate resources to shoot elongation and growth (Reich and Borchert, 1984).

In section *Angustiflorae*, some species flower from January to March, other species flower from March to October, but the majority flower continuously (Appendix B). Species that flower continuously are expected to be found in regions with little temperature variation and consistent rainfall patterns (Gentry, 1974; Reich and Borchert, 1984; Bawa et al., 2003). Significant correlations between continuous flowering and both a greater annual temperature range and lower precipitation during the driest month were detected (Table 3). Ancestral state reconstructions indicated that a continuous flowering phenology was most likely the ancestral state of the entire section (Fig. 4). Therefore, correlations between a continuous flowering phenology and climate are not necessarily a reflection of character state shifts in both variables at the same nodes. Correlation analyses determine significance based on the frequency of character states co-occurring, and cannot detect when character state shifts co-occur. Because flowering phenology did not shift at any of these nodes, speciation is likely to have occurred due to changes in climate alone. It is also possible that continuous flowering linked with greater temperature annual range and lower precipitation during the driest month is an artefact of how the data were scored - across all collections from throughout their range. Analyses that structure a species' distribution more finely may reveal that flowering time may closely track other variables that were averaged over the entire geographic range of the species. Thus, while flowering may be continuous for a species, it does not ensure that all individuals throughout a species' range are continuously flowering.

### 4.4. Conclusions

Phylogenetic analyses using multiple DNA regions were able to resolve a strongly supported hypothesis of species relationships for members of section *Angustiflorae*. Ancestral state reconstructions and correlation analyses identified six possible forces driving speciation within section *Angustiflorae*. Correlation analyses have identified shifts between a larger leaf surface area and less precipitation during the coldest quarter, a yellow corolla and a lower temperature during the coldest quarter, and a continuously flowering phenology with both a wider annual temperature range and lower precipitation during the driest month. Only one of five sister species pairs is clearly the result of allopatric speciation. The remaining four have overlapping geographic distributions but two of these pairs have significantly different climate requirements and are thus parapatric. The final pair cannot be separated by climate or geographic distribution and potentially may be the result of sympatric speciation, but additional studies in the field will be essential to demonstrate this. Identifying patterns among distributions and morphological characters, changes in vegetative characters, adaptations against nectar robbing, pollinator shifts, geographic isolation, and climate changes in temperature or precipitation as possible forces driving evolutionary divergence. Of these forces, pollinator shifts are likely to have had the largest effect on speciation within *Angustiflorae* as shifts in floral size and color occurred most frequently throughout the phylogeny of these species (Fig. 5).

We base these conclusions on phylogenetic analyses, interpretation of character states and geographic distribution patterns mapped onto the resulting tree; based on this comparative evidence, we have thus generated hypotheses that highlight the forces of selection. Future analyses could examine the impact of diversification rates in clades that are defined by various synapomorphies and model the role of extinction on the results that were recovered here.

The analyses presented here provide a strong set of hypotheses, but verification (or refutation) of these hypotheses will require ecological field work. Future work will need to investigate the hypotheses that have been proposed by determining specific pollinators, nectar robbers, photosynthetic efficiency and the impact of potential trade-offs on all of these forces of selection.

## Acknowledgments

The authors would like to thank the following for their generosity in sharing plant material: Larry Skog, Nancy and Jerry Kast, Bob Stewart, Bill Price, Carol Ann Bonner, Julie Mavity-Hudson, and Karen Cichocki. We are also grateful to the staff of the following herbaria for permitting us to examine specimens on site, share material on loan and let us capture label data for georeferencing: AAU, COL, F, GH, IOJ, MO, NY, QCA, QCNE, S, SEL, SRP, U, UNA, US, and UWI. Funding for this project is from NSF, grant DEB0949270 to JFS and JLC and The American Gesneriad Society. This project was part of the M. S. thesis of the first author in partial fulfilment of her degree at Boise State University.

## Appendix A

See Table A1.

## Appendix B

See Table B1.

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

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

| Taxon | Voucher | Herbarium | Collection Locality | *rpl32-trnL*$_{UAG}$ spacer | *trnQ-rps16* intron | *rps16* intron | *trnS-G* spacer | *trnH-psbA* spacer | ITS | ETS | *G3pdh*A | *idh*B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. ambigua* (Urb.) B.D. Morley | *J. Smith 3701* | SRP | Cultivated originally Puerto Rico | KF005814 | KF006032 | KF005923 | JQ953713 | KF005641 | JQ953789 | **KP260803** | NA | **KP260854** |
| *C. angustata* (Wiehler) L.E. Skog | *Amaya M. & J. Smith 625* | COL | Colombia | KF554345 | KF554365 | KF554385 | KF554404 | KF554304 | KF554324 | **KP260804** | NA | NA |
| *C. angustata* (Wiehler) L.E. Skog | *J. Smith 1433* | WIS | Colombia | KF005815 | KF006033 | KF005924 | KF006136 | KF005642 | KF005726 | **KP260805** | NA | NA |
| *C. angustata* (Wiehler) L.E. Skog | *J.L. Clark 8627* | UNA | Panama | KF005816 | KF006034 | KF005925 | KF006137 | NA | KF005727 | **KP260806** | **KP260879** | **KP260855** |
| *C. angustata* (Wiehler) L.E. Skog | *J.L. Clark* et al. *9373* | US | Ecuador | KF554346 | KF554366 | KF554386 | KF554406 | KF554305 | KF554325 | **KP260807** | NA | NA |
| *C. angustata* (Wiehler) L.E. Skog | *J.L. Clark* et al. *9609* | UNA | Ecuador | KF554347 | KF554367 | KF554387 | KF554407 | KF554306 | KF554326 | **KP260808** | NA | NA |
| *C. angustata* (Wiehler) L.E. Skog | *J.L. Clark* et al. *9854* | UNA | Ecuador | KF554348 | KF554368 | KF554388 | KF554408 | KF554307 | KF554327 | **KP260809** | NA | NA |
| *C. angustata* (Wiehler) L.E. Skog | *J. Smith 2246* | WIS | Cultivated at SEL | KF554349 | KF554369 | KF554389 | KF554409 | KF554308 | KF554328 | **KP260810** | NA | NA |
| *C. brenneri* (Wiehler) B.D. Morley | *J.L. Clark & M. Mailloux 7842* | UNA | Ecuador | KF005824 | KF006041 | KF005933 | KF006145 | KF005650 | KF005735 | **KP260811** | **KP260880** | **KP260856** |
| *C. byrsina* (Wiehler) L.P. Kvist & L.E. Skog | *J.F. Smith 3408* | SRP | Ecuador | KF005826 | KF006043 | KF005935 | JQ953714 | KF005652 | KF005737 | **KP260812** | **KP260881** | **KP260857** |
| *C. byrsina* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark & O. Meija 6291* | UNA | Ecuador | KF005827 | KF006044 | KF005936 | KF006148 | KF005653 | KF005738 | **KP260813** | NA | NA |
| *C. byrsina* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark 2413* | US | Ecuador | KF554350 | KF554370 | KF554390 | KF554410 | KF554309 | KF554329 | **KP260814** | **KP260882** | **KP260858** |
| *C. byrsina* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark* et al. *7518* | US | Ecuador | KF554351 | KF554372 | KF554391 | KF554411 | KF554310 | KF554330 | **KP260815** | NA | NA |
| *C. byrsina* (Wiehler) L.P. Kvist & L.E. Skog | *H. Wiehler 77122* | SEL | Cultivated at SEL | KF554353 | KF554373 | KF554393 | KF554413 | KF554312 | KF554332 | **KP260816** | NA | NA |
| *C. byrsina* (Wiehler) L.P. Kvist & L.E. Skog | *T. Croat 94841* | MO | Ecuador | KF554352 | KF554372 | KF554392 | KF554412 | KF554311 | KF554331 | **KP260817** | NA | **KP260859** |
| *C. colombiana* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark* et al. *10024* | UNA | Cultivated | KF005831 | KF006048 | KF005940 | KF006151 | KF005656 | KF005742 | **KP260818** | NA | NA |
| *C. colombiana* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark 8874* | US | Cultivated | KF554354 | KF554374 | KF554394 | KF554415 | KF554313 | KF554333 | **KP260819** | NA | NA |
| *C. colombiana* (Wiehler) L.P. Kvist & L.E. Skog | *J.F. Smith 1126* | WIS | Cultivated at SEL | KF005832 | KF006049 | KF005941 | KF006153 | KF005657 | KF005743 | **KP260820** | **KP260883** | **KP260860** |
| *C. crassicaulis* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark 8859* | US | Cultivated | KF005834 | KF006051 | KF005943 | KF006155 | KF005659 | KF005745 | **KP260821** | **KP260884** | **KP260861 (ex)** |
| *C. dielsii* Mansf. | *J.F. Smith 1989* | WIS | Ecuador | KF005836 | KF006053 | KF005945 | KF006157 | KF005661 | KF005747 | **KP260822** | **KP260885** | **KP260862** |
| *C. domingensis* (Urb.) B.D. Morley | *L. Hahn 445* | SRP | Dominican Republic | KF005839 | KF006056 | KF005948 | JQ953715 | KF005664 | JQ953790 | **KP260823** | **KP260886** | **KP260863** |
| *C. katzensteiniae* (Wiehler) L.P. Kvist & L.E. Skog | *J.L. Clark* et al. *7625* | UNA | Ecuador | KF005858 | KF006075 | KF005968 | KF006178 | KF005683 | KF005766 | **KP260824** | **KP260887 (ex)** | **KP260864** |
| *C. lophophora* Mansf. | *J.L. Clark* et al. *7888* | US | Ecuador | KF005860 | KF006076 | KF005969 | KF006179 | KF005684 | KF005767 | **KP260825** | **KP260888** | **KP260865** |
| *C. manabiana* (Wiehler) J.F. Sm. & L.E. Skog | *Dodson & Dodson 6791* | SEL | Cultivated at SEL | KF554360 | KF554380 | KF554400 | KF554421 | KF554320 | KF554339 | **KP260826** | **KP260889** | **KP260866** |

(*continued on next page*)



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

**Table A1** (*continued*)

| Taxon | Voucher | Herbarium | Collection Locality | *rpl32-trnL*$_{UAG}$ spacer | *trnQ-rps16* intron | *rps16* intron | *trnS-G* spacer | *trnH-psbA* spacer | ITS | ETS | *G3pdh*A | *idh*B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. microphylla* Klotsch & Hanst. | *J.L. Clark 6261* | UNA | Cultivated | KF005863 | KF006080 | KF005973 | KF006182 | KF005687 | KF005771 | **KP260827 (ex)** | **TBKP260890A** | **KP260867** |
| *C. minor* (Hook.) Hanst. | *T. Croat 94778* | MO | Ecuador | KF005866 | KF006084 | KF005975 | KF006185 | KF005690 | KF005774 | **KP260828 (ex)** | NA | NA |
| *C. minutiflora* L.P. Kvist & L.E. Skog | *J.L. Clark* et al. *10832* | UNA | Ecuador | KF005867 | KF006085 | KF005976 | KF006186 | KF005691 | KF005775 | **KP260829** | **KP260891** | **KP260868** |
| *C. moesta* Poepp. | *J.F. Smith 1829* | WIS | Bolivia | KF005870 | KF006084 | KF005979 | KF006189 | KF005694 | KF005778 | **KP260830** | NA | NA |
| *C. oblongifolia* Rusby | *J.F. Smith 1721* | WIS | Bolivia | KF005874 | KF006092 | KF005983 | KF006193 | KF005697 | KF005781 | **KP260831** | **KP260892** | **KP260869** |
| *C. orientandina* Mansf. | *J.F. Smith 3421* | SRP | Ecuador | KF005875 | KF006093 | KF005984 | KF006194 | KF005698 | KF005782 | **KP260832** | NA | NA |
| *C. orientandina* Mansf. | *J.L. Clark* et al. *9885* | UNA | Ecuador | KF005876 | KF006094 | KF005985 | KF006195 | KF005699 | KF005783 | **KP260833** | **KP260893** | **KP260870** |
| *C. orientandina* Mansf. | *L. Schulte 65* | SRP | Cultivated | KF554356 | KF554375 | NA | KF554417 | KF554316 | KF554334 | **KP260834** | NA | NA |
| *C. ovatifolia* L.P. Kvist & L.E. Skog | *J.F. Smith 1921* | WIS | Ecuador | KF005877 | KF006091 | KF005986 | KF006196 | KF005700 | KF005784 | **KP260835** | **KP260894** | **KP260871** |
| *C. ovatifolia* L.P. Kvist & L.E. Skog | *J.L. Clark 8461* | US | Ecuador | KF554357 | KF554376 | KF554397 | KF554418 | KF554317 | KF554335 | **KP260836** | NA | NA |
| *C. picta* H. Karst. | *T. Croat 94956* | MO | Ecuador | KF005879 | KF006096 | KF005988 | KF006197 | KF005701 | KF005785 | **KP260837** | **KP260895** | **KP260872** |
| *C. purpureovittata* (Wiehler) B.D. Morley | *J.L. Clark* et al. *11448* | UNA | Peru | KF005882 | KF006098 | KF005991 | KF006200 | KF005703 | KF005788 | **KP260838** | **KP260896** | **KP260873** |
| *C. purpusii* Standl. | *A. Rincon* et al. *2302* | XAL | Mexico | KF005883 | KF006099 | KF005992 | JQ953719 | KF005704 | JQ953792 | **KP260839 (ex)** | NA | **KP260874** |
| *C. rileyi* (Wiehler) J.F. Smith | *J.F. Smith 1944* | WIS | Ecuador | KF005885 | KF006101 | KF005994 | KF006202 | KF005706 | KF005791 | **KP260840** | **KP260897** | **KP260875** |
| *C. rileyi* (Wiehler) J.F. Smith | *J.L. Clark 6263* | US | Ecuador | KF005886 | KF006102 | KF005995 | KF006203 | DQ211250 | AF543239 | **KP260841** | NA | NA |
| *C. rileyi* (Wiehler) J.F. Smith | *J.L. Clark 7077* | US | Ecuador | KF554358 | KF554377 | KF554398 | KF554419 | KF554318 | KF554336 | **KP260842** | NA | NA |
| *C. rubricalyx* L.P. Kvist & L.E. Skog | *T. Croat 95236* | MO | Ecuador | KF005888 | KF006104 | KF005996 | KF006205 | KF005708 | KF005793 | NA | NA | NA |
| *C. sanguinea* (Pers.) Hanst. | *J.F. Smith 636* | WIS | Cultivated | KF005889 | KF006105 | KF005998 | KF006206 | KF005709 | KF005794 | **KP260843** | NA | NA |
| *C. spathulata* Mansf. | *J.F. Smith 1853* | WIS | Ecuador | KF005893 | KF006110 | KF006003 | KF006211 | KF005715 | KF005798 | **KP260844** | **KP260898** | **KP260876** |
| *C. spathulata* Mansf. | *J.L. Clark* et al. *7485* | UNA | Ecuador | KF005894 | KF006111 | KF006004 | KF006212 | KF005716 | KF005799 | **KP260845** | **KP260899** | NA |
| *C. spathulata* Mansf. | *T. Croat 95254* | MO | Ecuador | KF554359 | KF554379 | KF554399 | KF554420 | KF554319 | KF554338 | **KP260846** | NA | NA |
| *C. spathulata* Mansf. | *J.F. Smith 651* | WIS | Cultivated at SEL | KF554361 | KF554381 | KF554401 | KF554422 | KF554321 | KF554340 | **KP260847** | NA | NA |
| *C. tandapiana* (Wiehler) L.E. Skog & L.P. Kvist | *L. Schulte 66* | SRP | Cultivated | KF554362 | KF554382 | KF554402 | KF554423 | KF554322 | KF554344 | **KP260848** | **KP260900** | **KP260877** |
| *C. tandapiana* (Wiehler) *L.E. Skog & L.P. Kvist* | *J. L. Clark* et al. *8006* | US | Ecuador | KF554355 | KF554384 | KF554395 | KF554416 | KF554315 | KF554343 | NA | NA | NA |
| *C. ulei* (Wiehler) L.E. Skog | *A. Chautems & M. Perret 10109* | G | Brazil | KF554363 | KF554383 | KF554403 | KF554424 | KF554323 | KF554341 | **KP260849** | **KP260901** | **KP260878** |
| *Glossoloma anomalum* J.L. Clark | *J.F. Smith 3418* | SRP | Ecuador | KF005912 | KF006128 | KF006021 | KF006224 | NA | AF543225 | **KP260850** | NA | NA |
| *Glossoloma grandicalyx* (J.L. Clark & L.E. Skog) J.L. Clark | *J.F. Smith 3417* | SRP | Ecuador | KF005913 | KF006129 | KF006024 | JQ953708 | DQ211205 | AF543218 | **KP260851** | NA | NA |
| *Glossoloma martinianum* (J.F. Smith) J.L. Clark | *J.L. Clark 6101* | US | Ecuador | KF005914 | KF006130 | KF006022 | JQ953709 | DQ211209 | AF543228 | **KP260852** | NA | NA |
| *Glossoloma panamense* (C.V. Morton) J.L. Clark | *L.E. Skog* et al. *7641* | US | Cultivated | KF005915 | KF006131 | KF006023 | JQ953710 | DQ211202 | DQ211102 | **KP260853** | NA | NA |



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

**Table B1**
Character state definitions and scores. Definition of character states for 14 morphological characters, phenology, and 19 climatic variables and scores for the 14 species in section *Angustiflorae* included in study. Morphological characters and phenology are numbered to match the characters that were used in the analyses and match numbers in the text and the caption to Fig. 4. Note that *C. ulei* scores were identical to those for *C. angustata* and are therefore not repeated as a separate column. Abbreviations are *amb* – *C. ambigua*, *ang* – *C. angustata*, *byr* – *C. byrsina*, *col* – *C. colombiana*, *cra* – *C. crassicaulis*, *dom* – *C. domingensis*, *kat* – *C. katzensteiniae*, *man* – *C. manabiana*, *ori* – *C. orientandina*, *ova* – *C. ovatifolia*, *ril* – *C. rileyi*, *spa* – *C. spathulata*, *tan* – *C. tandapiana*.

| Character | 0 | 1 | 2 | 3 | *amb* | *ang* | *byr* | *col* | *cra* | *dom* | *kat* | *man* | *ori* | *ova* | *ril* | *spa* | *tan* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Habit | Upright | Horizontal | Pendent | – | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 |
| 2. Leaf dimorphism | Anisophyllous | Isophyllous | – | – | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 3. Lamina surface area | 0.0–30.0 $cm^2$ | >30.0 $cm^2$ | – | – | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Adaxial pubescence | Few to no trichomes | Dense trichomes | – | – | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Abaxial pubescence | Few to no trichomes | Dense trichomes | – | – | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Abaxial coloration | Green | Purple apices | Entirely purple | Variable | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 3 | 1 |
| 4. Petiole length | 0.0–5.0 mm | 5.0–20.0 mm | >20.0 mm | – | 1 | 2 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Number of flowers per inflorescence | 1 flower per axil | >1 flower per axil | – | – | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| 5. Floral bract size | 0.0–6.0 mm | 6.0–13 mm | >13 mm | – | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 1 | 0 |
| 6. Corolla to calyx ratio | 0.0–2.5 | >2.5 | – | – | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| 7. Calyx margin | Entire | Serrate | – | – | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Corolla length | >40 mm | 10.0–40.0 mm | – | – | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| 8. Corolla color | Yellow | Red | Purple | Variable | 0 | 3 | 1 | 3 | 0 | 0 | 2 | 0 | 0 | 3 | 1 | 3 | 0 |
| 9. Corolla lobe color | Same color as corolla | Different color than corolla | – | – | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| 10. Phenology | Flowering continuously | Flowering January to March | Flowering March to October | – | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| Annual mean temperature | <18.667 °C | 21.225–23.0 °C | Polymorphic | <21 °C | 2 | 2 | 2 | 1 | 3 | 2 | 2 | 2 | 2 | 0 | 3 | 2 | 2 |
| Mean diurnal range (monthly max temp – monthly min temp) | Polymorphic | >9.7 °C | – | – | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Isothermality (mean diurnal range/ temperature annual range * 100) | <77.75 | Polymorphic | >84 | <84 | 0 | 1 | 1 | 2 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Temperature seasonality (standard deviation * 100) | Polymorphic | 265.75–439.5 °C | <439.5 °C | >703.33 °C | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Maximum temperature of warmest month | <24.925 °C | Polymorphic | <27 °C | >29.133 °C | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| Minimum temperature of coldest month | <12.533 °C | Polymorphic | <15.4 °C | >17.933 °C | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| Temperature annual range | Polymorphic | >12.84 °C | >11.45 °C | – | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean temperature of wettest quarter | <18.85 °C | Polymorphic | <21.5 °C | >23.667 °C | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| Mean temperature of driest quarter | <18.3 °C | Polymorphic | <20.98 °C | >22.633 °C | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| Mean temperature of warmest quarter | <19.375 °C | Polymorphic | <21.85 °C | >23.9 °C | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| Mean temperature of coldest quarter | <18.133 °C | Polymorphic | <20.5 °C | >22.433 °C | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| Annual precipitation | Polymorphic | – | – | – | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Precipitation of wettest month | Polymorphic | – | – | – | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Precipitation of driest month | Polymorphic | <53 mm | >53 mm | – | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Precipitation seasonality (coefficient of variation) | Polymorphic | <44 | >44 | >70.5 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Precipitation of wettest quarter | Polymorphic | <921.67 mm | – | – | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Precipitation of driest quarter | Polymorphic | <198.43 mm | >198.43 mm | – | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Precipitation of warmest quarter | Polymorphic | <698.3 mm | 486.0–1022.0 mm | – | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Precipitation of coldest quarter | Polymorphic | <382.0 mm | >382.0 mm | 158.0–829.0 mm | 3 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

## Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.12.008.

## References

Armbruster, W.S., 1993. Evolution of plant pollination systems: hypotheses and test with the Neotropical vine *Dalechampia*. Evolution 47, 1480–1505.
Baldwin, B.G., Markos, S., 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of Calycadenia. Mol. Phylogenet. Evol. 10, 449–463.
Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S., Donoghue, M.J., 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Gard. 82, 247–277.
Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439, 719–723.
Bawa, K.S., 1990. Plant-pollinator interactions in tropical rain forests. Ann. Rev. Ecol. Syst. 21, 399–422.
Bawa, K.S., Kang, H., Grayum, M.H., 2003. Relationships among time, frequency, and duration of flowering in tropical rain forests. Amer. J. Bot. 90, 877–887.
Beardsley, P.M., Yen, A., Olmstead, R.G., 2003. AFLP phylogeny of *Mimulus* section *Erytrhranthe* and the evolution of hummingbird pollination. Evolution 57, 1397–1410.
Benjamani, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 57, 289–300.
Bollback, J.P., 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinf. 7, 88.
Bonser, S.P., 2006. Form defining function: interpreting leaf functional variability in integrated plant phenotypes. Oikos 114, 187–190.
Bush, G.L., 1975a. Sympatric speciation in phytophagous parasitic insects. In: Price, P.W. (Ed.), Evolutionary Strategies of Parasitic Insects and Mites. Plenum Press, New York, pp. 187–206.
Bush, G.L., 1975b. Modes of animal speciation. Ann. Rev. Ecol. Syst. 6, 339–364.
Bush, M.B., 1994. Amazonian speciation – a necessarily complex model. J. Biogeog. 21, 5–17.
Carson, H.L., 1985. Unification of speciation theory in plants and animals. Syst. Bot. 10, 380–390.
Clark, J.L., Herendeen, P.S., Skog, L.E., Zimmer, E.A., 2006. Phylogenetic relationships and generic boundaries in the tribe Episcieae (Gesneriaceae) inferred from nuclear, chloroplast, and morphological data. Taxon 55, 313–336.
Clark, J.L., Funke, M.M., Duffy, A.M., Smith, J.F., 2012. Phylogeny of a neotropical clade in the Gesneriaceae: more tales of convergent evolution. Int. J. Plant Sci. 173, 894–916.
Darwin, C., 1859. On the Origin of Species by Means of Natural Selection. Murray, London.
de Vos, J.M., Hughes, C.E., Schneeweiss, G.M., Moore, B.R., Conti, E., 2014. Heterostyly accelerates diversification via reduced extinction in primroses. Proc. Roy. Soc. B 281, 20140075.

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

Dengler, N.G., 1999. Anisophylly and dorsiventral shoot symmetry. Int. J. Plant. Sci. 160, S67–S80.
Dengler, N.G., Sanchez-Burgos, A.A., 1988. Effect of light level on the expression of anisophylly in *Paradrymonia ciliosa* (Gesneriaceae). Bot. Gaz. 149, 158–165.
Dilcher, D.L., 1973. The Eocene floras of southeastern North America. In: Graham, A. (Ed.), Vegetation and Vegetational History of Northern Latin America. Elsevier, New York, pp. 39–59.
Dolph, G.E., Dilcher, D.L., 1980. Variation in leaf size with respect to climate in the tropics of the western hemisphere. Bull. Torrey Bot. Club 107, 154–162.
Drummond, C.S., 2008. Diversification of *Lupinus* (Leguminosae) in the western New World: Derived evolution of perennial life history and colonization of montane habitats. Mol. Phylogenet. Evol. 48, 408–421.
Drummond, A.J., Rambaut, A., 2006. BEAST, version 1.4. <http://beast.bio.ed.ac.uk/>.
Ezcurra, C., Ruggiero, A., Crisci, J.V., 1997. Phylogeny of *Chuguiraga* sect. *Acanthophyllae* (Asteraceae-Barnadesioideae) and the evolution of its leaf morphology in relation to climate. Syst. Bot. 22, 151–163.
Farris, J.S., 1989. The retention index and the rescaled consistency index. Cladistics 5, 417–419.
Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.
Feder, J.L., 1998. The apple maggot fly, *Rhagoletis pomonella*. Flies in the face of conventional wisdom about speciation? In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms. Species and Speciation. Oxford University Press, New York, pp. 130–144.
Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1–15.
Galen, C., 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. Evolution 43, 882–890.
Galen, C., 1999. Why do flowers vary? The functional ecology of variation in flower size and form within natural plant populations. Bioscience 49, 631–640.
Gentry, A.H., 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica 6, 64–68.
Gentry, A.H., 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? Ann. Missouri Bot. Gard. 69, 557–593.
Gentry, A.H., 1989. Speciation in tropical forests. In: Holm-Nielsen, L.B., Nielsen, I.C., Balslev, H. (Eds.), Tropical Forests: Botanical Dynamics, Speciation and Diversity. Academic Press, London, pp. 113–134.
Gittenberger, E., 2008. What about non-adaptive radiation? Biol. J. Linn. Soc. 43, 263–272.
Givnish, T.J., 1979. On the adaptive significance of leaf form. In: Solbrig, O.T., Jain, S., Johnson, G.B., Raven, P.H. (Eds.), Topics in Plant Population Biology. Columbia University Press, New York, pp. 375–407.
Givnish, T.J., 1984. Leaf and canopy adaptations in tropical forests. In: Medina, E., Mooney, H.A., Vásquez-Yánes, C. (Eds.), Physiological Ecology of Plants of the Wet Tropics. Dr. W. Junk, The Hague, pp. 51–84.
Givnish, T.J., 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol. 106, 131–160.
Givnish, T.J., 1997. Adaptive radiation and molecular systematics: issues and approaches. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, U.K., pp. 1–54.
Graham, A., 1997. Neotropical plant dynamics during the Cenozoic – diversification and the ordering of evolutionary and speciation processes. Syst. Bot. 22, 139–150.
Haffer, J., 1969. Speciation in Amazonian forest birds. Science 165, 131–137.
Haffer, J., Prance, G.T., 2001. Climatic forcing of evolution in Amazonia during the Cenozoic: on the refuge theory of biotic differentiation. Amazoniana 16, 579–605.
Hamilton, M.B., 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol. Ecol. 8, 521–523.
Hancock, A.M., Brachi, B., Faure, N., Horton, M.W., Jarymowycz, L.B., Sperone, F.G., Toomajian, C., Roux, F., Bergelson, J., 2011. Adaptation to climate across the *Arabidopsis thaliana* genome. Science 334, 83–86.
Harrison, C.J., Möller, M., Cronk, Q.C.B., 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. Ann. Bot. 84, 49–61.
Heiberg, E., 2012. SEEVA ver. 1.00. Software for Spatial Evolutionary and Ecological Vicariance Analysis. <http://seeva.heiberg.se>.
Heiberg, E., Struwe, L., 2012. SEEVA manual, ver. 1.00. On-line publication, Rutgers University. <http://www.rci.rutgers.edu/~struwe/seeva>.
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climat. 25, 1965–1978.
Hopkins, R., Schmitt, J., Stinchcombe, J.R., 2008. A latitudinal cline and response to vernalization in leaf angle and morphology in *Arabidopsis thaliana* (Brassicaceae). New Phytol. 179, 155–164.
Huelsenbeck, J.P., Ronquist, F., 2003. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Huelsenbeck, J.P., Nielsen, R., Bollback, J.P., 2003. Stochastic mapping of morphological characters. Syst. Biol. 52, 131–158.
Huertas, M.L., Schneider, J.V., Zizka, G., 2007. Phylogenetic analysis of *Palaua* (Malveae, Malvaceae) based on plastid and nuclear sequences. Syst. Bot. 32, 157–165.
Hufford, L., McMahon, M., 2004. Morphological evolution and systematics of *Synthris* and *Besseya* (Veronicaceae): a phylogenetic analysis. Syst. Bot. 29, 716–736.
Ingram, A.L., Doyle, J.J., 2003. The origin and evolution of *Eragrostis tef* (Poaceae) and related polyploids: evidence from nuclear *waxy* and plastic *rps16*. Amer. J. Bot. 90, 116–122.
Inouye, D.W., 1983. The ecology of nectar robbing. In: Bentley, B., Elias, T.S. (Eds.), Biology of Nectarines. Colombia University Press, New York, pp. 153–173.
Irwin, R.E., 2006. The consequences of direct verses indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. Am. Nat. 137, 315–328.
Johnson, S.D., 1996. Adaptation and speciation models in the Cape floral of South Africa. Taxon 45, 59–66.
Johnson, L.A., Johnson, R.L., 2006. Morphological delimitation and molecular evidence for alloploidy in *Collomia vilkenii* (Polemoniaceae), a new species from northern Nevada. Syst. Bot. 31, 349–360.
Johnson, S.D., Steiner, K.E., 2000. Generalization versus specialization in plant pollination systems. Trends Ecol. Evol. 15, 140–143.
Johnson, S.D., Linder, H.P., Steiner, K.E., 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). Am. J. Bot. 85, 402–411.
Jones, C.S., Bakker, F.L., Schlichting, C.D., Nicotra, A.B., 2009. Leaf shape evolution in the South African genus *Pelargonium* L'Her. (Geraniaceae). Evolution 63, 479–497.
Kay, K.M., Reeves, P.A., Olmstead, R.G., Schemske, D.W., 2005. Rapid speciation and the evolution of hummingbird pollination in Neotropical *Costus* subgenus *Costus* (Costaceae): evidence from nrDNA ITS and ETS sequences. Am. J. Bot. 92, 1899–1910.
Kendeigh, S.C., 1969. Tolerance of cold and Bergmann's rule. The Auk 86, 13–25.
Kluge, A.G., Farris, S.J., 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18, 1–32.
Lemmon, E.M., Lemmon, A.R., Cannatella, D.C., 2007. Geological and climatic forces driving speciation in the continentally distributed trilling chorus frog (*Pseudacris*). Evolution 61, 2086–2103.
Levin, R.A., Watson, K., Bohs, L., 2005. A four-gene study of evolutionary relationships in *Solanum* section *Acanthophora*. Am. J. Bot. 92, 603–612.
Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.
Linder, C.R., Goertzen, L.R., Heuvel, B.V., Francisco-Ortega, J., Jansen, R.K., 2000. The complete external transcribed spacer of 18S–26S rDNA: amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. Mol. Phylogenet. Evol. 14, 285–303.
Maddison, W.P., Maddison, D.R., 2011. Mesquite: A Modular System for Evolutionary Analysis. Version 2.75 <http://www.mesquiteproject.org>.
Martén-Rodríguez, S., Fenster, C.B., Agnarsson, I., Skog, L.E., Zimmer, E.A., 2010. Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. New Phytol. 188, 403–417.
Mason-Gamer, R.J., Kellogg, E.A., 1996. Chloroplast DNA analysis of the monogenomic Triticeae: phylogenetic implications and genome-specific markers of special interest. In: Jauhar, P. (Ed.), Methods of Genome Analysis in Plants. CRC Press, Boca Raton, pp. 301–325.
McDonald, P.G., Fonseca, C.R., Overton, J., Westoby, M., 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? Funct. Ecol. 17, 50–57.
Meinzer, F., Goldstein, G., 1985. Some consequences of leaf pubescence in the Andean giant rosette plant *Espeletia timotensis*. Ecology 66, 512–520.
Meredith, R.W., Pires, M.N., Reznick, D.N., Springer, M.S., 2011. Molecular phylogenetic relationships and the coevolution of placentotrophy and superfetation in *Poecilia* (Poeciliidae: Cyprinodontiformes). Mol. Phylogenet. Evol. 59, 148–157.
Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McCune, A.R., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M., Turelli, M., 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecol. Lett. 10, 315–331.
Möller, M., Cronk, Q.C.B., 2001. Evolution of morphological novelty: a phylogenetic analysis of growth patters in *Streptocarpus* (Gesneriaceae). Evolution 55, 918–929.
Muchhala, N., Caiza, A., Vizuete, J.C., Thomson, J.D., 2008. A generalized pollination system in the tropics: bats, birds, and *Aphelandra acanthus*. Ann. Bot. 103, 1481–1487.
Müller, K., 2004. PRAP – computation of Bremer support for large datasets. Mol. Phylogenet. Evol. 31, 780–782.
Müller, K., Quandt, D., Müller, J., Neinhuis, C., 2005. PhyDE 0.9971: Phylogenetic Data Editor. <http://www.phyde.de>.
Mummenhoff, K., Al-Shehbaz, I.A., Bakker, F.T., Linder, H.P., Muhlhausen, A., 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape Flora of southern Africa. Ann. Missouri Bot. Gard. 92, 400–424.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.
Oxelman, B., Lidén, M., Berglund, D., 1997. Chloroplast *rps16* intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). Plant Syst. Evol. 206, 393–410.
Papadopulos, A.S.T., Baker, W.J., Crayn, D., Butlin, R.K., Kynast, R.G., Hutton, I., Savolainen, V., 2011. Speciation with gene flow on Lord Howe Island. Proc. Nat. Acad. Sci. USA 108, 13188–13193.
Parkhurst, D.F., Loucks, O.L., 1972. Optimal leaf size in relation to environment. J. Ecol. 60, 505–537.

Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008

Perret, M., Chautems, A., Spichiger, R., Barraclough, T.G., Savolainen, V., 2007. The geographical pattern of speciation and floral diversification in the Neotropics: the tribe Sinningieae (Gesneriaceae) as a case study. Evolution 61, 1641–1660.
Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.
Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Proctor, M., Yeo, P., Lack, A., 1996. The Natural History of Pollination. Timber Press, Portland, Oregon.
Rambaut, A., Drummond, A.J., 2005. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.
Ree, R.H., 2005. Phylogeny and the evolution of floral diversity in *Pedicularis* (Orobanchaceae). Int. J. Plant Sci. 166, 595–613.
Reeves, G., Chase, M.W., Goldblatt, P., Fay, M.F., Cox, A.V., Lejeune, B., Suozachies, T., 2001. Molecular systematics of Iridaceae: evidence from four plastid DNA regions. Am. J. Bot. 88, 2074–2087.
Reich, P.B., Borchert, R., 1984. Water stress and tree phenology in a tropical dry forest in the lowlands of Cost Rica. J. Ecol. 72, 61–74.
Ristic, Z., Jenks, M.A., 2002. Leaf cuticle and water loss in maize lines differing in dehydration avoidance. J. Plant Phys. 156, 645–651.
Roalson, E.H., Skog, L.E., Zimmer, E.A., 2003. Phylogenetic relationships and the diversification of floral form in *Achimenes* (Gesneriaceae). Syst. Bot. 28, 593–608.
Roderick, M.L., Berry, S.L., Noble, I.R., Farquhar, G.D., 1999. A theoretical approach to linking the composition and morphology with the function of leaves. Functional Ecol. 13, 683–695.
Ruiz-Sanchez, E., Sosa, V., 2010. Delimiting species boundaries within the Neotropical bamboo *Otetea* (Poaceae: Bambusoideae) using molecular, morphological, and ecological data. Mol. Phylogenet. Evol. 54, 344–356.
Savolainen, V., Anstett, M.-C., Lexer, C., Hutton, I., Clarkson, J.J., Norup, M.V., Powell, M.P., Springate, D., Salamin, N., Baker, W.J., 2006. Sympatric speciation in palms on an oceanic island. Nature 441, 210–213.
Schemske, D.W., 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. Ecology 62, 946–954.
Schemske, D.W., 2002. Ecological and evolutionary perspectives on the origins of tropical diversity. In: Chazdon, R.L., Whitmore, T.C. (Eds.), Foundations of Tropical Forest Biology: Classic Papers with Commentaries. University of Chicago Press, Chicago, Illinois, pp. 163–173.
Schluter, D., 2000. The Ecology of Adaptive Radiation. Oxford University Press, New York.
Schulte, L.J., 2012. Phylogenetic relationships within Columnea section Angustiflorae: Insights into forces driving speciation, M.S. Thesis, Boise State University.
Schulte, L.J., Clark, J.L., Novak, S.J., Ooi, M.T.-Y., Smith, J.F., 2014. Paraphyly of section *Stygnanthe* (*Columnea*, Gesneriaceae) and a revision of the species of section *Angustiflorae*, a new section inferred from ITS and chloroplast DNA data. Syst. Bot. 39, 613–616.
Seelanen, T., Schnabel, A., Wendel, J.F., 1997. Congruence and consensus in the cotton tribe (Malvaceae). Syst. Bot. 22, 275–288.
Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am. J. Bot. 94, 275–288.
Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.
Smith, J.F., 1994. Systematics of *Columnea* section *Pentadenia* and section *Stygnanthe* (Gesneriaceae). Syst. Bot. Monog. 44, 1–89.
Smith, J.F., 2000. Phylogenetic signal common to three datasets: combining data which initially appear heterogeneous. Plant Syst. Evol. 221, 179–198.
Smith, S.D.W., 2010. Using phylogenetics to detect pollinator-mediated floral evolution. New Phytol. 188, 354–363.
Smith, J.F., Wolfram, J.C., Brown, K.D., Carroll, C.L., Denton, D.S., 1997. Tribal relationships in the Gesneriaceae: evidence from DNA sequences of the chloroplast gene *ndhF*. Ann. Missouri Bot. Gard. 8, 50–66.
Smith, J.F., Hileman, L.C., Powell, M.P., Baum, D.A., 2004. Evolution of *GCYC*, a Gesneriaceae homolog of *CYCLOIDEA*, within Gesnerioideae (Gesneriaceae). Mol. Phylogenet. Evol. 31, 765–779.
Smith, J.F., Stevens, A.C., Tepe, E.J., Davidson, C., 2008a. Placing the origin of two species-rich genera in the late Cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). Plant Syst. Evol. 275, 9–30.
Smith, S.D., Ane, C., Baum, D.A., 2008b. The role of pollinator shifts in the floral diversification of *Iochroma* (Solanaceae). Evolution 62, 793–806.
Smith, J.F., Ooi, M., Schulte, L.J., Amaya, M.M., Clark, J.L., 2013. The disintegration of the subgeneric classification of *Columnea* (Gesneriaceae). Selbyana 31, 126–142.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758–771.
Steele, P.R., Friar, L.M., Gilbert, L.E., Jansen, R.K., 2010. Molecular systematics of the Neotropical genus *Psiguria* (Cucurbitaceae): Implications for phylogeny and species identification. Am. J. Bot. 97, 156–173.
Strand, A.E., Leebens-Mack, J., Milligan, B.G., 1997. Nuclear DNA-based markers for plant evolutionary biology. Mol. Ecol. 6, 113–118.
Struwe, L., Smouse, P.E., Heiberg, E., Haag, S., Lathrop, R.G., 2011. Spatial evolutionary and ecological vicariance analysis (SEEVA), a novel approach to biogeography and speciation research, with an example from Brazilian Gentianaceae. J. Biogeog. 38, 1841–1854.
Stuessy, T.F., 2006. Evolutionary biology: sympatric plant speciation on islands? Nature 443, e12.
Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other materials), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
Symmank, L., Samain, M.-S., Smith, J.F., Pino, G., Stoll, A., Goetghebur, P., Neinhuis, C., Wanke, S., 2011. The extraordinary journey of *Peperomia* subgenus *Tildenia* (Piperaceae): insights into diversification and colonization patterns from its cradle in Peru to the Tans-Mexican volcanic belt. J. Biogeog. 38, 2337–2349.
Tolley, K.A., Tilbury, C.R., Measey, G.J., Menegon, M., Branch, W.R., Matthee, C.A., 2011. Ancient forest fragmentation or recent radiation? Testing refugial speciation models in chameleons within an African biodiversity hotspot. J. Biogeog. 38, 1748–1760.
Valente, L.M., Manning, J.C., Goldblatt, P., Vargas, P., 2012. Did pollinator shifts drive diversification in Southern African *Gladiolus*? Evaluating the model of pollinator-driven speciation. Am. Nat. 180, 83–98.
van der Niet, T., Johnson, S.D., 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. Trends Ecol. Evol. 27, 353–361.
Viljanen, H., Escobar, F., Hanski, I., 2010. Low local but high beta diversity of tropical forest dung beetles in Madagascar. Global Ecol. Biogeog. 19, 886–894.
Weese, T.L., Johnson, L.A., 2005. Utility of NADP-dependent isocitrate dehydrogenase for species-level evolutionary inference in angiosperm phylogeny: a case study in *Saltugilia*. Mol. Phylogenet. Evol. 36, 24–41.
Whitten, W.M., Williams, N.H., Armbruster, W.S., Battiste, M.A., Strekowski, L., Lindquist, N., 1986. Carvone oxide: an example of convergent evolution in euglossine pollinated plants. Syst. Bot. 11, 222–228.
Wills, C., Harms, K.E., Condit, R., King, D., Thompson, J., He, F., Muller-Landau, H.C., Ashton, P., Losos, E., Comita, L., Hubbell, S., LaFrankie, J., Bunyavejchewin, S., Dattaraja, H.S., Davies, S., Esufali, S., Foster, R., Gunatilleke, N., Gunatilleke, S., Hall, P., Itoh, A., John, R., Kiratiprayoon, S., Loo de Lao, S., Massa, M., Nath, C., Noor, M.N.S., Kassim, A.R., Sukumar, R., Suresh, H.S., Sun, I., Tan, S., Yamakura, T., Zimmerman, J., 2006. Nonrandom processes maintain diversity in tropical forests. Science 311, 527–531.
Woo, V.L., Funke, M.M., Smith, J.F., Lockhart, P.J., Garnock-Jones, P.J., 2011. New world origins of southwest Pacific Gesneriaceae: multiple movements across and within the south Pacific. Int. J. Plant Sci. 172, 434–457.
Yates, M.J., Verboom, G.A., Rebelo, A.G., Cramer, M.D., 2010. Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. Functional Ecol. 24, 485–492.
Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50, 408–424.
Zotz, G., Hietz, P., 2001. The physiological ecology of vascular epiphytes: current knowledge, open questions. J. Exp. Biol. 52, 2067–2078.
Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation. University of Texas, Austin.



Q1 Please cite this article in press as: Schulte, L.J., et al. Speciation within *Columnea* section *Angustiflora* (Gesneriaceae): Islands, pollinators and climate. Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2014.12.008