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THE GEOGRAPHICAL PATTERN OF SPECIATION AND FLORAL DIVERSIFICATION IN THE NEOTROPICS: THE TRIBE SINNINGIEAE (GESNERIACEAE) AS A CASE STUDY

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The geographical pattern of speciation and the relationship between floral variation and species ranges were investigated in the tribe Sinningieae (Gesneriaceae), which is found mainly in the Atlantic forests of Brazil. Geographical distribution data recorded on a grid system of 0.5 × 0.5 degree intervals and a near-complete species-level phylogenetic tree of Sinningieae inferred from a simultaneous analysis of seven DNA regions were used to address the role of geographical isolation in speciation. Geographical range overlaps between sister lineages were measured across all nodes in the phylogenetic tree and analyzed in relation to relative ages estimated from branch lengths. Although there are several cases of species sympatry in Sinningieae, patterns of sympatry between sister taxa support the predominance of allopatric speciation. The pattern of sympatry between sister taxa is consistent with range shifts following allopatric speciation, except in one clade, in which the overlapping distribution of recent sister species indicates speciation within a restricted geographical area and involving changes in pollinators and habitats. The relationship between floral divergence and regional sympatry was also examined by analyzing floral contrasts, phenological overlap, and the degree of sympatry between sister clades. Morphological contrast between flowers is not increased in sympatry and phenological divergence is more apparent between allopatric clades than between sympatric clades. Therefore, our results failed to indicate a tendency for sympatric taxa to minimize morphological and phenological overlap (geographic exclusion and/or character displacement hypotheses). Instead, they point toward adaptation in phenology to local conditions and buildup of sympatries at random with respect to flower morphology. Additional studies at a lower geographical scale are needed to identify truely coexisting species and the components of their reproductive isolation.

KEY WORDS: **Allopatric speciation, Brazilian Atlantic forest, floral diversification, phylogenies, *Sinningia*, sympatric speciation.**

Geographical isolation has often been considered as the most important cause of speciation (Mayr 1963; Grant 1971). However, whether speciation exists in sympatry is still much debated (Via 2001; Barluenga et al. 2006; Savolainen et al. 2006). In plants, polyploidy, hybridization, and large-effect mutations affecting pollinator preferences or flowering phenology can provide mechanisms that lead to a sudden reproductive isolation, and inbreeding or vegetative reproduction can allow these mutants or hybrids to spread from initially low numbers (Levin 1983; Arnold 1997; Rieseberg 1997; Schemske and Bradshaw 1999; Johanson et al. 2000; Bradshaw and Schemske 2003). Few studies have tested the relationship between geography and speciation in

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flowering plants, making it difficult to assess the relative frequency of allopatric versus sympatric speciation in this group. Empirical evidence is also needed to determine the interaction between geography and phenotypic evolution (Brooks and McLennan 1991; Barraclough et al. 1998, 1999). Although there is a large body of literature on the role of species differences in promoting or maintaining local species richness (e.g., Bowers and Brown 1982; Waser 1983; Feinsinger 1987; Richman and Price 1992; Martin 1996; Schluter 2001), the extent to which sympatry within plant lineages is associated with the evolution of phenotypic or ecological differences is poorly documented (Linder 2005).

These questions can now be evaluated using methodological approaches based on species-level phylogenetic trees derived from molecular data (Barraclough et al. 1998; Barraclough and Vogler 2000; Losos and Glor 2003; Fitzpatrick and Turelli 2006). Phylogenetic trees including all the living species in a higher taxonomic group provide an indirect record of the speciation events that have led to present-day species (Barraclough and Nee 2001). Phylogenetic and biogeographical data have been used together to test the geographical circumstances of speciation in several groups of animals (Lynch 1989; Chesser and Zink 1994; Schliewen et al. 1994; Joseph et al. 1995; Friesen and Anderson 1997; Berlocher 1998; Barraclough and Vogler 2000; Johnson and Cicero 2002; Fitzpatrick and Turelli 2006; Jiggins et al. 2006) and in a few groups of plants (Hart 1985; Givnish et al. 1995; Knox and Palmer 1995; Goldblatt and Manning 1996; Baldwin 1997; Givnish et al. 2000; Hughes et al. 2005). To determine which mode of speciation operates predominantly within monophyletic groups, Barraclough and Vogler (2000) have proposed to calculate the range overlap between all sister lineages on a phylogenetic tree and to consider the pattern of those measures in relation to the relative age of nodes, as estimated from DNA sequences. Then, the observed patterns between sympatry and node age can be compared with the expected patterns of range overlap under the alternative modes of speciation (Barraclough and Vogler 2000). If speciation was predominantly allopatric then recent sister species or clades would exhibit nonoverlapping ranges, whereas overlaps would increase among older splits as a result of post-speciation range shifts or dispersal (Chesser and Zink 1994; Barraclough and Vogler 2000). Alternatively, if speciation was predominantly sympatric, then ranges of newly formed species might be entirely overlapping, whereas deeper sister groups might overlap less because their ranges were more likely to have changed over time (Berlocher 1998). The success of this approach in assessing the role of geographical isolation in speciation depends on the intensity and frequency of post-speciation range changes (Barraclough and Vogler 2000; Losos and Glor 2003). When geographical ranges are highly labile, the present-day ranges may provide no information about the geographical mode of speciation, even for the most recent events; studies of modes of speciation should consider this possibility. Here, we use a null model approach to test whether closely related species display a higher or lower degree of sympatry than expected between random pairs of species.

Apart from looking at the geographical mode of speciation, comparisons between sympatric and allopatric sister clades also permit the evaluation of the role of phenotypic differentiation in species coexistence (Barraclough et al. 1998, 1999). In flowering plants, premating reproductive isolation between sympatric taxa is often associated with differences in flowering time (Soliva and Widmer 1999; Borchsenius 2002; Savolainen et al. 2006), but changes in flower morphology can also contribute to reproductive isolation through specialization to different pollinators (Grant 1971; Gentry 1976; Fulton and Hodges 1999; Sakai et al. 1999; Schemske and Bradshaw 1999; Kay and Schemske 2003; Ramsey et al. 2003; Fenster et al. 2004; Wilson et al. 2004) or by limiting pollen transfer between species sharing similar pollinators (Brown and Kodric-Brown 1979; Feinsinger 1983; Armbruster et al. 1994; Wolf et al. 2001). A positive relationship between floral divergence and sympatry could indicate that flower divergence is associated with species coexistence. Possible mechanisms would include geographical exclusion and/or character displacement resulting from either competition for pollinators or reinforcement to reduce detrimental interspecific pollen flow (Gentry 1974; Heithaus 1974; Stiles 1977; Waser 1983; Levin 1985; Armbruster 1986; Feinsinger 1987; Murray et al. 1987; Caruso 2000; Hansen et al. 2000; Silvertown et al. 2005). By contrast, a negative relationship is expected if flower differences evolve as adaptations to local environmental conditions. For example, some plant communities may display high floral similarity because the advantage of sharing the same pollinators outweighs the advantage of diverging to reduce interspecific competition (Brown and Kodric-Brown 1979; Schemske 1981). Finally, there may be no relationship between floral divergence and sympatry if floral diversity evolves at random with respect to sympatry.

Here we analyze the geographical mode of speciation and the relationship between range overlaps and the extent of floral divergence in the tribe Sinningieae (Gesneriaceae). This tribe includes three genera *Paliavana*, *Sinningia*, and *Vanhouttea*, with a total of 81 species (Clayberg 1968a; Wiehler 1983, 1984; Chautems 1990, 1991, 1995, 2002; Wiehler and Chautems 1995; Chautems et al. 2000). A recent molecular phylogenetic analysis of the Sinningieae demonstrated the monophyly of the tribe and its division into three major lineages (i.e., Corytholoma, Dircaea, and Sinningia) and two smaller early diverging clades (Perret et al. 2003). The genera *Paliavana* and *Vanhouttea* are clearly embedded within *Sinningia* (Perret et al. 2003), and a detailed taxonomic revision is in preparation to accommodate nomenclatural changes. The genus *Sinningia s. s.* is characterized by a herbaceous habit with a perennial tuber, whereas *Paliavana* and *Vanhouttea* are shrubs lacking tubers (Chautems and Weber 1999). Sinningieae

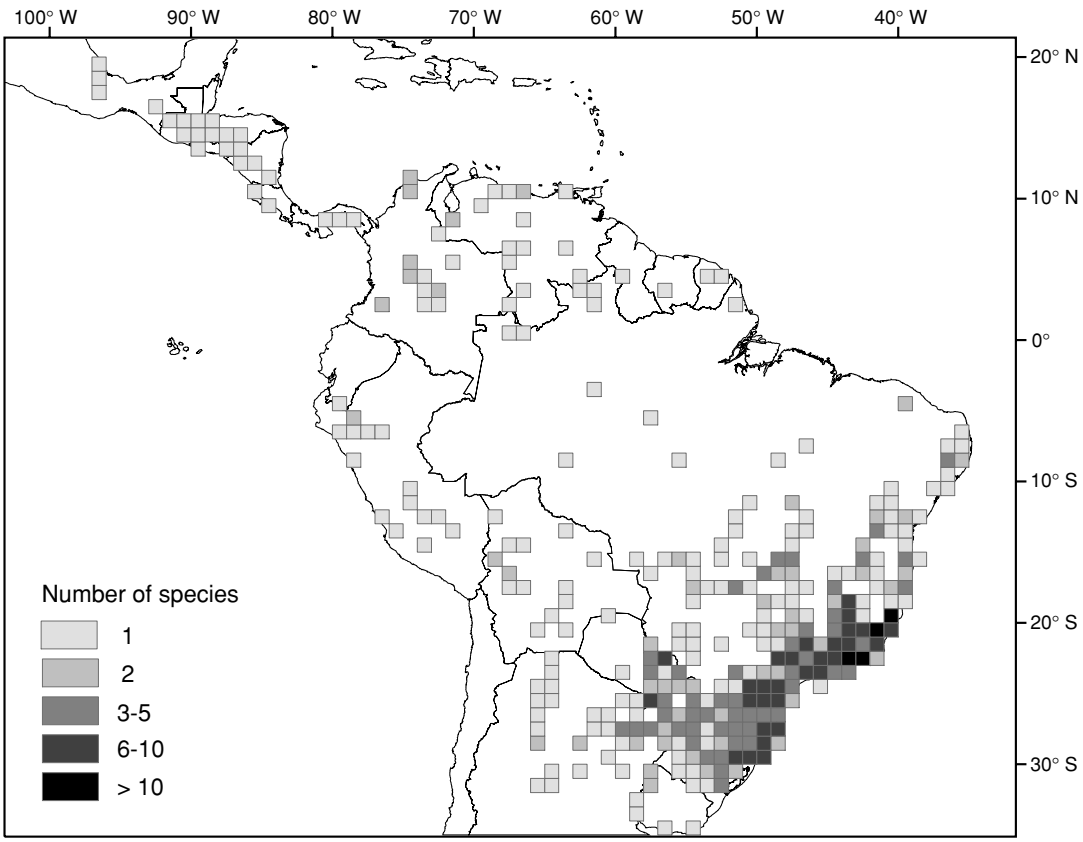


Figure 1. Geographical variation in species richness of Sinningieae in South and Central America. Species richness is calculated as the number of species within a one-degree grid cell (1° × 1°). Distribution data are derived from herbarium accessions for 69 species in the clades Corytholoma, Dircaea, and Sinningia (see text for details, clade circumscription follows Perret et al. 2003).

comprises a majority of saxicolous species, growing on "inselbergs" or on other rocky substrates, in addition to having some epiphytic and terrestrial species. The geographical distribution of Sinningieae is from Central America to northern Argentina, but by far the highest species diversity exists in the Brazilian Atlantic forests, in which several species distributions overlap (Fig. 1). Reconstruction of the biogeographical history of the Sinningieae indicates that the Brazilian Atlantic rainforests are likely to be the ancestral area of the tribe and that a large proportion (57%) of the speciation events occurred at a restricted geographical scale within single phytogeographic units in southeastern Brazil (Perret et al. 2006). Sympatric speciation may have occurred in this group, but investigations at a finer geographical scale were needed to test this hypothesis.

There is no evidence of polyploidy in Sinningieae as the only chromosome number found in this group is $n = 13$ (Clayberg 1967). Hybrids are rare in the wild, although artificial hybrids can be produced relatively easily (Clayberg 1968b, 1970, 1996). Floral phenotype and phenology could be important features limiting gene flow between sympatric species. Flowers are highly diverse in shape, color, and nectar sugar composition, associated with different pollination syndromes, that is, hummingbird, bee, bat, and moth (Perret et al. 2001). The pollinators predicted on the basis of these syndromes were largely confirmed by extensive field studies (SanMartin-Gajardo and Sazima 2004, 2005a,b).

To assess the role of geographical isolation in speciation, here we combine phylogenetic data with distribution data collected for nearly all species (69) included in three major lineages of the Sinningieae. We also investigate whether sympatry is associated with floral divergence, both in terms of flower morphology and phenology.

Materials and Methods

SAMPLING AND PHYLOGENETIC ANALYSES

Seventy-six species of Sinningieae plus two outgroups were included in the phylogenetic analysis. This sampling represents all currently recognized species in the tribe Sinningieae (Gesneriaceae) with the exception of *S. helleri*, *S. schomburkiana*, and *S. sulcata* for which no material was available. The two selected outgroups, *Smithiantha laui* and *Nematanthus villosus* belong to the tribes Gloxinieae and Episcieae, respectively, which are closely related to Sinningieae, based on molecular phylogenetic analy-

ses of the entire family (Smith et al. 1997). A full list of taxa and voucher specimen information is provided in Perret et al. (2003).

The plastid DNA spacers *trnT-trnL*, *trnL-trnF*, *trnS-trnG*, *atpB-rbcL*, introns *trnL*, and *rpl16* together with a portion of the nuclear encoded *ncpGS* gene were sequenced following the procedure described in Perret et al. (2003). All sequences have been deposited in EMBL/GenBank (accessions AJ438352-AJ438434, AJ439249-AJ439331, AJ439745-AJ439829, AJ439900-AJ439-984, AJ487702-AJ487786, AJ459606-AJ459691).

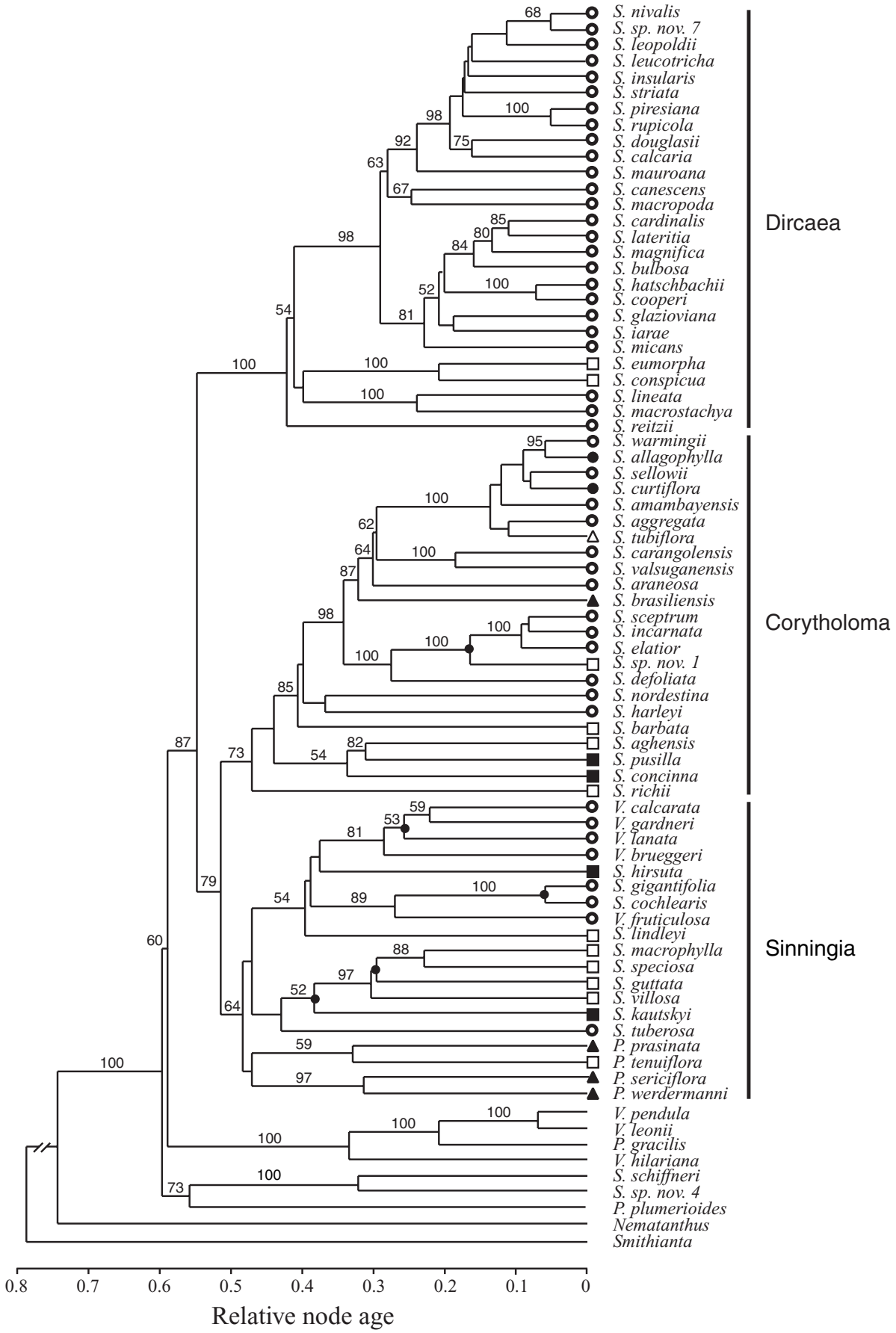
The topologies obtained from separate analyses of each of the seven DNA regions were found to be largely congruent (Perret et al. 2003), thus we performed a combined analysis with 5812 molecular characters using PAUP∗4.0b8 (Swofford 1999). A 50% majority-rule bootstrap consensus tree was obtained from 1000 bootstrap replicates (Felsenstein 1985a) using maximum parsimony (MP), tree bisection-reconnection swapping (TBR), SIMPLE addition of taxa, and a limit of 500 trees retained at each replicate (MAXTREES = 500). This tree was then used as a constraint tree in a maximum likelihood (ML) analysis. The model used in the ML analysis was chosen using a likelihood ratio test (Sanderson 1998). Starting with the HKY85 model (Hasegawa et al. 1985) and using likelihood ratio tests we tested whether incorporating the gamma-distribution among site rate variation, and the proportion of invariable sites led to a significantly improved fit to the data (Yang 1996). Parameters were all estimated from the data using the 50% majority-rule bootstrap consensus tree.

The hypothesis of a constant molecular clock was also tested using a likelihood ratio test by comparing the ML scores obtained with or without constraining a molecular clock (Sanderson 1998). Because the difference in ML scores was significant, we corrected for rate heterogeneity among lineages using the nonparametric rate smoothing (NPRS) method of Sanderson (1997) as implemented in TreeEdit version 1.0a8 (Rambaut and Charleston 2001). This approach does not assume a constant molecular clock, but instead that rates of changes tend to be similar between adjacent branches on the tree. It produces an ultrametric tree by minimizing the sum of squared changes in rates between ancestor and descendant branches across the tree (Sanderson 1997). These transformed branch lengths were used to estimate the relative age of the nodes. Standard errors of the node ages were obtained following the three-step procedure proposed by Baldwin and Sanderson (1998): (1) 100 data matrices were generated from the original one by the bootstrap procedure, (2) for each data matrix branch lengths were fitted to the ML tree of Figure 2 by using the model of sequence evolution defined for the ML search (see above), (3) the ages of each node were recalculated, and for each of these nodes a standard deviation was calculated based on the 100 ages obtained from the bootstrapped data.

SPECIES RANGE AND GEOGRAPHIC PATTERN OF SPECIATION

Distribution and range sizes of each species were measured on a grid divided into quadrats of 0.5 degree intervals of latitude and longitude. Presence or absence of species in each quadrat was based on over 3000 herbarium accessions from 67 herbaria (i.e., ALCB, ASE, B, BH, BHCB, BOTU, CAY, CGE, CEN, CEPEC, CESJ, CONN, E, EAN, ESA, F, FCQ, FUEL, G, GB, GFJP, GUA, HAS, HBR, HEPH, HUEFS, IAC, IAN, IBGE, ICN, INPA, IPA, K, MBM, MBML, MG, MO, NA, NY, P, PACA, PEUFR, PKDC, PY, R, RB, RBR, RUSU, S, SEL, SP, SPF, SPSF, UB, UC, UEC, UFG, UFMT, UFP, UPCB, UPS, US, VEN, W, WIS, WU, Z; acronyms after Holmgren and Holmgren 1998). Distribution data were obtained for all 69 species included in the phylogenetic analyses for the clades Corytholoma, Dircaea, and Sinningia. The seven species that diverged early in the phylogenetic tree of Sinningieae were not used in our analyses (see Fig. 2). A list of representative specimens for these species is provided in Perret et al. (2006); part of this chorological information is also available in national and regional floras for Argentina, Paraguay, and Brazil (Toursarkissian 1969; Chautems 1993, 2003a, 2003b; Woodgyer 1995; Araujo et al. 2005). The presence/absence matrix was used to calculate the degree of sympatry at each node of the tree: the degree of sympatry between sister species or clades was calculated as the number of quadrats in which those two species/clades co-occur divided by the number of quadrats occupied by the species /clade with a smaller range (Barraclough and Vogler 2000). The total range for a clade was given by the sum of quadrats occupied by at least one clade member. Degrees of sympatry varied from 0 (allopatric) to 1, the latter representing one clade embedded in the range of another (i.e., sympatric). The degree of sympatry was plotted against the node ages estimated from NPRS. A regression

Figure 2. Phylogenetic hypothesis for the tribe Sinningieae based on a simultaneous analysis of plastid and nuclear DNA sequences (regions *trnT-trnL*, *trnL-trnF*, *trnS-trnG*, *atpB-rbcL*, *rpl16*, and *ncpGS*). Relative node ages were estimated using ML and NPRS (node age values were scaled to one). Numbers above the branches are bootstrap proportions (only if ≥ 50%). Nodes marked with a filled circle indicate fully sympatric sister clades. Symbols at the tips indicate the main morphological types of flowers derived from the PCoA and the unweighted pair group method, arithmetic mean analyses (see Fig. 4). (⊙) "hummingbird flowers," with a red tubular corolla; (●) "hummingbird flowers" with short red tubular corolla (≤ 1 cm); (□) "bee flowers" displaying campanulate or tubular corolla with purple, blue, or yellow colors; (■) narrow small blue "bee flowers"; (▲) greenish "bat flowers"; (△) "moth flowers" with long white tubular corolla.


S. nivalis
S. sp. nov. 7
S. leopoldii
S. leucotricha
S. insularis
S. striata
S. piresiana
S. rupicola
S. douglasii
S. calcaria
S. mauroana
S. canescens
S. macropoda
S. cardinalis
S. lateritia
S. magnifica
S. bulbosa
S. hatschbachii
S. cooperi
S. glazioviana
S. iarae
S. micans
S. eumorpha
S. conspicua
S. lineata
S. macrostachya
S. reitzii
S. warmingii
S. allagophylla
S. sellowii
S. curtiflora
S. amambayensis
S. aggregata
S. tubiflora
S. carangolensis
S. valsuganensis
S. araneosa
S. brasiliensis
S. sceptrum
S. incarnata
S. elatior
S. sp. nov. 1
S. defoliata
S. nordestina
S. harleyi
S. barbata
S. aghensis
S. pusilla
S. concinna
S. richii
V. calcarata
V. gardneri
V. lanata
V. brueggeri
S. hirsuta
S. gigantifolia
S. cochlearis
V. fruticulosa
S. lindleyi
S. macrophylla
S. speciosa
S. guttata
S. villosa
S. kautskyi
S. tuberosa
P. prasinata
P. tenuiflora
P. sericiflora
P. werdermanni
V. pendula
V. leonii
P. gracilis
V. hilariana
S. schiffneri
S. sp. nov. 4
P. plumerioides
Nematanthus
Smithianta
Dircaea
Corytholoma
Sinningia
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative node age

line was fitted after arcsine transformation of the degree of sympatry because this value is bounded between 0 and 1 (Zar 1984). The intercept of the regression line with the y-axis provides information about the predominant geographic mode of speciation within a clade, and the slope of the regression informs us about the magnitude of the range changes subsequent to speciation (Barraclough and Vogler 2000). However, if species ranges have moved extensively over time, present-day ranges may retain no information on the geographical mode of speciation. To explore this possibility, we devised the following randomization test. The observed ranges were randomly shuffled among the tips of the tree and the intercept was recalculated for each of the 1000 null trials. The *P*-value of this test was determined as the proportion of random trials that gave an intercept more extreme than the one observed, multiplied by two (two-tailed test). Failure to reject the null hypothesis would indicate that closely related species do not have a significantly different pattern of range overlap than random pairs of species. Rejection of the null hypothesis indicates that closely related species do tend to have either more or less sympatry than expected between random pairs of species, that is, that there is some phylogenetic signal in the pattern of sympatry.

PHENOLOGY

Phenological data were derived from the collection dates of the herbarium samples. Only specimens with developed flowers were taken into account. These data were collected for all species of the clades Corytholoma, Dircaea, and Sinningia included in the phylogenetic analyses, with the exception of *S. richii*, which is only known in cultivation. For each species, the number of specimens in flower by month was recorded. The months totaling at least 75% of the specimens were considered as months of peak flowering for that species. Months of peak flowering versus other months were coded in a matrix with states 1 and 0, respectively (Appendix 1).

MULTIVARIATE ANALYSES OF FLORAL TRAITS

Multivariate analyses based on 16 floral characters and 64 species were performed to quantify phenotypic divergence and to define major flower types in Sinningieae. All species of the clades Corytholoma, Dircaea, and Sinningia included in the phylogenetic analyses were sampled, except *Paliavana werdermanii, S. lateritia, S. macrophylla, S. piresiana*, and *S. rupicola* for which fertile material was not available. Fresh flowers from cultivated species at the Botanical garden of Geneva (Switzerland) and pickled field-collected flowers were measured for 13 quantitative traits related to shape and size. Three other characters were also included in the analysis: nectar volume (grouped into three classes as < 5 μl, 5–40 μl, and > 40 μl), color (coded as red, i.e., hummingbird syndrome or nonred), and fragrance (coded as absent, linalool-based [i.e., moth syndrome; Perret et al. 2003], or musty [i.e., bat syndrome; SanMartin-Gajardo and Sazima 2005a]). The full list of characters used is given in Appendix 2. These data were subjected to principal coordinate analysis (PCoA, Gower 1966; Legendre and Legendre 1984, pp. 105–159) and unweighted pair group method, arithmetic mean (UPGMA) cluster analysis using the software package Progiciel R v4.0d4 (Casgrain and Legendre 2001). The distance matrix was computed using mean values for each character and the symmetric form of the Gower's coefficient. The main clusters resulting from the UPGMA analysis were mapped on a scatter plot of the two first PCoA axes to identify the major flower types. To determine which characters correlate best with the PCoA axes, the Pearson correlation coefficients (r) were calculated between all morphological characters and the PCoA scores of the first three axes (Zar 1984).

FLORAL DIVERGENCE AND SPECIES DISTRIBUTION

Phenological divergence between two sister species or clades was given by the number of months of phenological overlap divided by the total flowering period (in months) of the species/clade with the shortest flowering period. Flowering period for a clade was determined by the overlap of flowering periods across all clade members. Phenological divergence of 0 indicates that one taxon flowers within the flowering period of its sister, whereas a value of 1 indicates that there is no phenological overlap. This measure is independent of the duration of the flowering period of species, which is a desirable property if we are interested in estimating the strength of species interaction.

To compute a phenotypic distance between taxa we calculated a standardized floral divergence between each sister species/clades using independent contrasts (Felsenstein 1985b) and the Euclidian distance given by $Dij = [(Xi - Xj)^2 + (Yi - Yj)^2 + (Zi - Zj)^2]^{0.5}$, where i and j are sister taxa, and X, Y, Z are the first three principal components from the PCoA. To investigate the relationship between the evolution of floral divergence and the geographical overlap between taxa, degrees of phenological divergence and the Euclidian standardized contrasts were plotted against the degree of sympatry (Barraclough et al. 1998). Significance of the correlation was assessed using the Spearman's rank test (Zar 1984).

Results

PHYLOGENETIC ANALYSES

The HKY85 model of molecular evolution incorporating rate variation across sites and the proportion of invariable sites gave a significantly better ML score than models with fewer parameters ($P < 0.01$; likelihood ratio test, result not shown). This model was used in the ML analyses of the six noncoding plastid DNA and nuclear gene *ncpGS*. Using the same test, the constant molecular

clock model was rejected (−logL = 18564.3 vs. 18669.8 without and with clock enforced, respectively, $\chi^2 = 211$ with 76 df, $P < 0.001$). Estimates of branch lengths were therefore scaled by NPRS. The resulting ultrametric tree is shown in Figure 2. Tribe Sinningieae is monophyletic but both *Vanhouttea* and *Paliavana* are embedded in *Sinningia*, in agreement with previous phylogenetic analyses by Perret et al. (2003). Taxa are distributed in three main clades that we termed Dircaea (BS 100%), Corytholoma (BS 73%), and Sinningia (BS 64%), plus two smaller early diverging clades that were not used in later analyses (see Fig. 2).

PATTERN OF SYMPATRY WITHIN CLADES

In the Dircaea clade, 12 (54%) of 22 nodes display no sympatry, including the most recent nodes (Fig. 3). The regression line between sympatry and node ages intercepts the y-axis at 0.03 (Table 1), but this observed intercept is not significantly different from the null distribution of intercepts assuming that present-day distributions may not retain any phylogenetic information ($P = 0.334$, Table 1). Over 1000 range randomizations the mean intercept with the sympatry axis is 0.02 and the maximum intercept is 0.13 (Table 1). These low intercept values in Dircaea indicate that allopatry is most common here, irrespective of the degree of relatedness between species.

In the Corytholoma clade, the degree of sympatry among recent splits is more scattered than in Dircaea (Fig. 3). The observed degree of sympatry of 1 is due to the range overlap between *S. sp. nov.* 1 (occurring in one quadrat) and one member of its sister clade, the widespread *S. elatior* (over 250 quadrats). Ten splits, representing 45% of nodes in Corytholoma, display no sympatry between sister taxa (Fig. 3). The observed intercept with the y-axis is 0.14 (Table 1). This low intercept indicates a predominantly allopatric mode of speciation. However, it is significantly higher

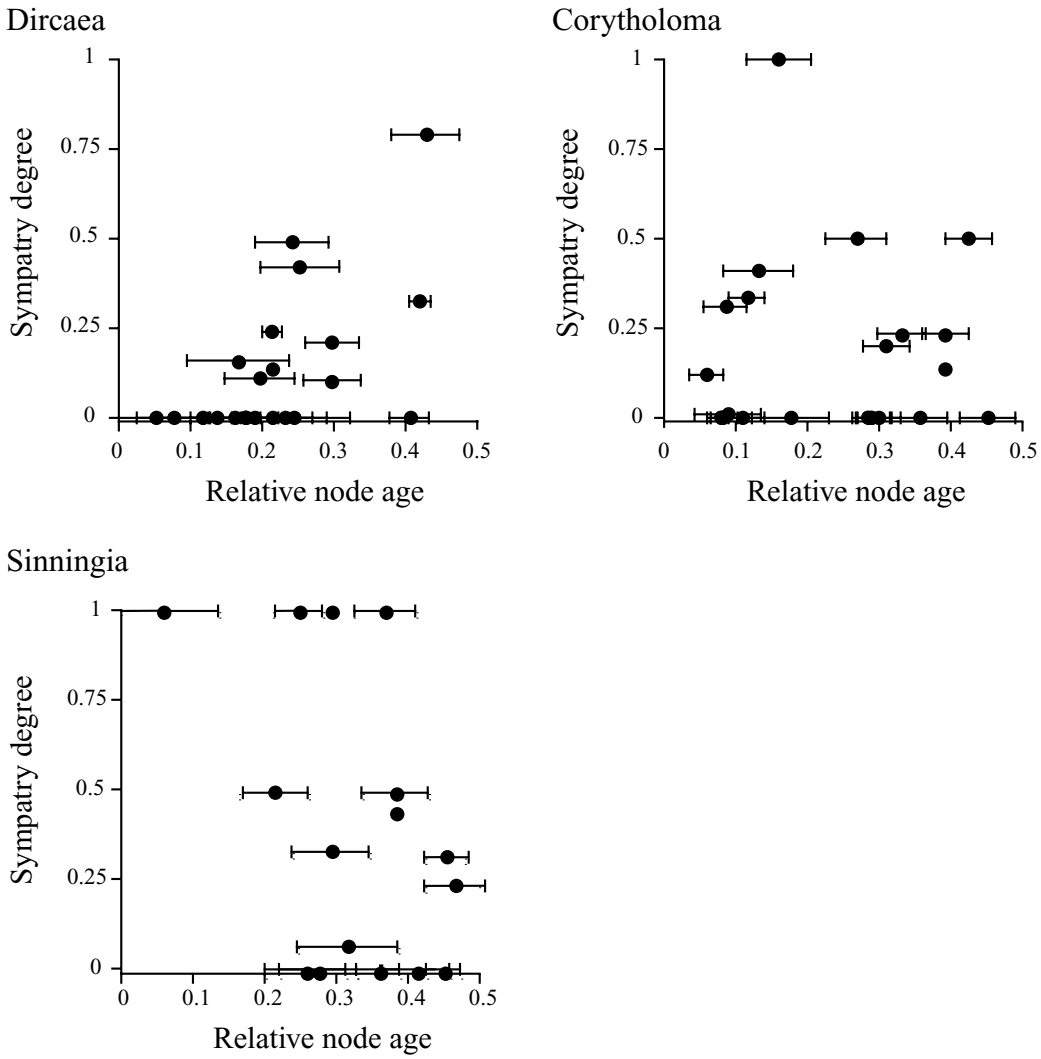


Figure 3. Scatterplots of the degree of sympatry between sister taxa (y-axis) against relative node age (x-axis) in clades Dircaea, Corytholoma, and Sinningia. Error bars associated with each node are the standard deviations of the relative node ages estimated by bootstrapping the original data matrix (see text for details). Results of the linear regression and tests of significance are provided in Table 1.

Table 1. Y-intercepts of the regression between sympatry degrees (y-axis) and relative node ages (x-axis) in the three main clades within Sinningieae (see scatterplots in Fig. 3). Intercepts were fitted by linear regression of the arcsine-transformed degrees of sympatry. The range randomization test was used to determine if observed intercepts were significantly different from intercepts obtained when the ranges of species were randomly shuffled among the tips of the tree (1000 replicates; see Materials and Methods).

Clades	Number of nodes	Observed intercept	Range randomization test ($n = 1000$)		
			Mean intercept	Minimum intercept	Maximum intercept
Dircaea	26	0.03 ns	0.02	0	0.13
Corytholoma	22	0.14*	0.02	0	0.37
Sinningia	18	0.87***	0.09	0	0.87

*$P < 0.05$; ***$P < 0.001$; ns, not significant.

Table 2. The top 11 highest correlation coefficients (r) between each morphological character and the first three axes of the PCoA. All correlations were significant ($P < 0.01$).

Character abbreviations	Characters	Axis 1	Axis 2	Axis 3
LST	Pistil length	−0.93		
LAN	Stamen length	−0.90		
LCD	Dorsal length of corolla	−0.84		
DNV	Vertical diameter of nectar chamber	−0.78		
LTU	Length of corolla tube	−0.77		
DNH	Horizontal diameter of nectar chamber	−0.75		
DLH	Horizontal diameter of corolla including lobes		0.84	
DOH	Horizontal diameter of corolla orifice		0.83	
ANG	Angle between tube and calyx axis		0.70	
DRH	Horizontal diameter of corolla constriction after the nectar chamber		0.65	
VNE	Volume of nectar			−0.63

than the intercepts generated by the randomizations ($P = 0.024$, Table 1).

In the Sinningia clade, the degrees of sympatry are widely scattered between 0 and 1 (Fig. 3). Four sister taxa are fully sympatric whereas five others (28%) have no range overlap. The intercept is 0.87, a value significantly higher than those generated by the randomizations ($P < 0.001$) indicating, as in Corytholoma, that sympatry between closely related species is not random with respect to cladogenetic history. However, the slope of the regression line and the significance value of this result are strongly influenced by one recent split between *S. cochlearis* (occurring in one quadrat) and *S. gigantifolia* (seven quadrats), which are sympatric. Excluding *S. cochlearis* from the calculation of range overlaps leads to an intercept of 0.47, a value that is not significantly higher than those generated by the randomizations ($P = 0.07$). The three other nodes with a degree of sympatry of 1 define more inclusive relationships (Fig. 2): the range of *V. lanata* (one quadrat) is included in *V. calcarata* (13 quadrats), whereas the ranges of both *S. guttata* and *S. kautskyi* (each occurring in one quadrat) are included within the range of *S. speciosa* (12 quadrats).

VARIATION IN FLOWER MORPHOLOGY

The main variation in flower phenotype is summarized in the first three axes of the PCoA. The first axis reflects variations in the width and shape of the corolla, the second axis corresponds to variations in the overall size of the corolla, whereas the third axis is associated with variations in the volume of nectar (Table 2). The scatterplot between the first two axes of the PCoA is shown in Figure 4. The main clusters resulting from the UPGMA analysis are indicated on the same figure using different symbols. Both analyses revealed a similar structure in the data and their combination allows for the circumscription of six major floral types (Fig. 4).

Of the 64 species measured, 45 are characterized by tubular, red to orange, corollas. They are distributed diagonally according to the length of their corolla tube in the lower part of the graph of Figure 4 (open and filled circles). In the left part of the graph, corolla tubes reach 4 cm and have two enlarged and fused dorsal lobes that cover the anthers exserted from the tube. In the lower part of the graph, the corollas of *S. allagophylla* and *S. curtiflora,* characterized by a tube length equal or less than 1 cm, fall into a distinct cluster with the UPGMA analysis (filled circles). All these

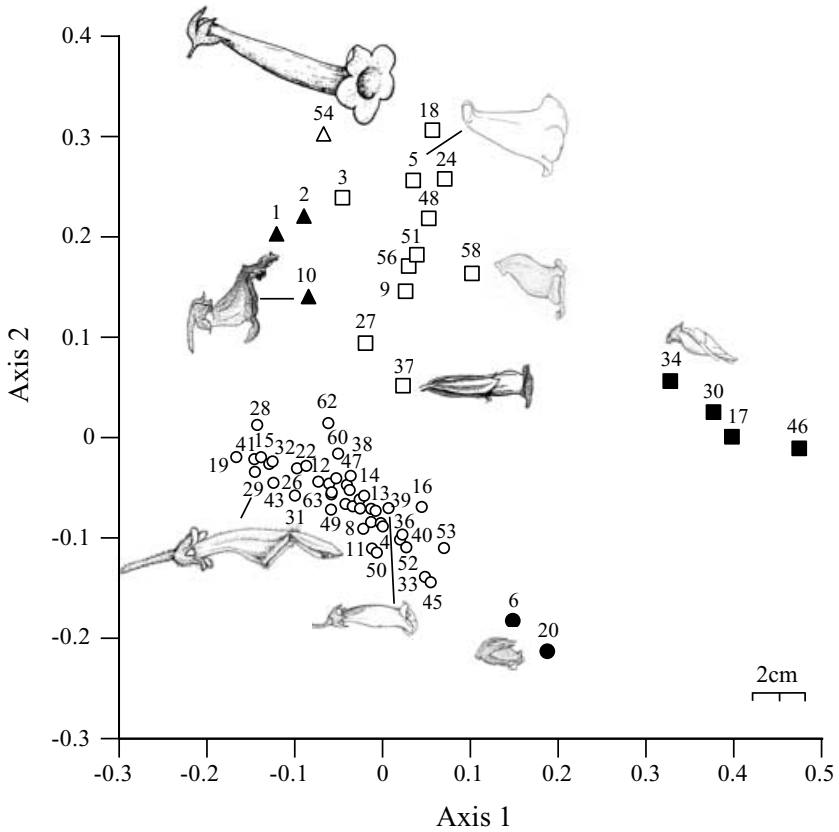


Figure 4. Scatterplot of the first two axes of the PCoA based on 16 floral characters given in Appendix 2. The floral clusters derived from the UPGMA analysis (distance $\geq$ 0.2) were represented by symbols as in Figure 2. Species are denoted by numbers given in Appendix 2. The respective size of illustrated taxa is given by the scale bar.

tubular morphologies correspond to the hummingbird syndrome (Perret et al. 2001) and hummingbird pollination for these species was confirmed by several field studies (Snow and Teixeira 1982; Sazima et al. 1996; Buzato et al. 2000; Vasconcelos and Lombardi 2000, 2001; SanMartin-Gajardo and Sazima 2005b). The remaining 19 species are clustered in four other groups and exhibit purple, blue, yellow, greenish, or white corollas. Funnel-shaped corollas of less than or up to 2 cm long are distributed in the right-hand part of the graph (filled squares), whereas campanulate corollas over 2 cm long are distributed in the upper-center part (open squares, Fig. 4). Species with these corolla morphologies were found to be pollinated by distinct groups of bees (SanMartin-Gajardo and Sazima 2004). The widely open, greenish corollas of *S. brasiliensis, P. prasinata*, and *P. sericiflora* constitute a distinct floral type (filled squares, Fig. 4). Morphological and nectar characteristics of these flowers fit the bat pollination syndrome (Vogel 1969, under *Gesneria* and *Lietzia*; Helversen 1993; Perret et al. 2001). Field studies confirmed bat pollination for *S. brasiliensis* and *P. prasinata*, but not for *P. sericiflora*, which seems to be exclusively pollinated by hummingbirds (SanMartin-Gajardo and Sazima 2005a). Finally, the flower of *S. tubiflora* has a unique floral type (open triangle, Fig. 4) characterized by a long white tubular corolla producing a sweet fragrance mainly composed of linalool (Perret et al. 2003); these features are typical of a moth pollination syndrome (Silberbauer-Gottsberger and Gottsberger 1975).

SYMPATRY AND FLORAL DIVERGENCE

In all three clades, a negative correlation was found between phenological divergence and sympatry (Fig. 5, Table 3). However, the correlation was not significant in the clade Sinningia (Spearman's rank test in Table 3). Phenological divergences above 0.5 were only recorded between allopatric taxa (Fig. 5). Maximum divergence of flowering time was observed between disjunct sister species that differed in their latitudinal distribution (e.g., *S. harleyi* vs. *S. nordestina, S. speciosa* vs. *S. macrophylla,* and *S. sceptrum* vs. *S. incarnata*). Conversely, flowering periods of sympatric taxa largely overlapped (Fig. 5).

The correlations between sympatry and floral contrasts were not significant in any clade (Fig. 6, Spearman's rank test in Table 3). In each clade, the largest floral contrasts are found between allopatric sister taxa of different floral types (Fig. 2), for example, between *S. allagophyla* and *S. warmingii* (Corytholoma clade), between *S. hirsuta* and its sisters (Sinningia clade), and between the clades including *S. eumorpha* and *S. lineata*, respec-

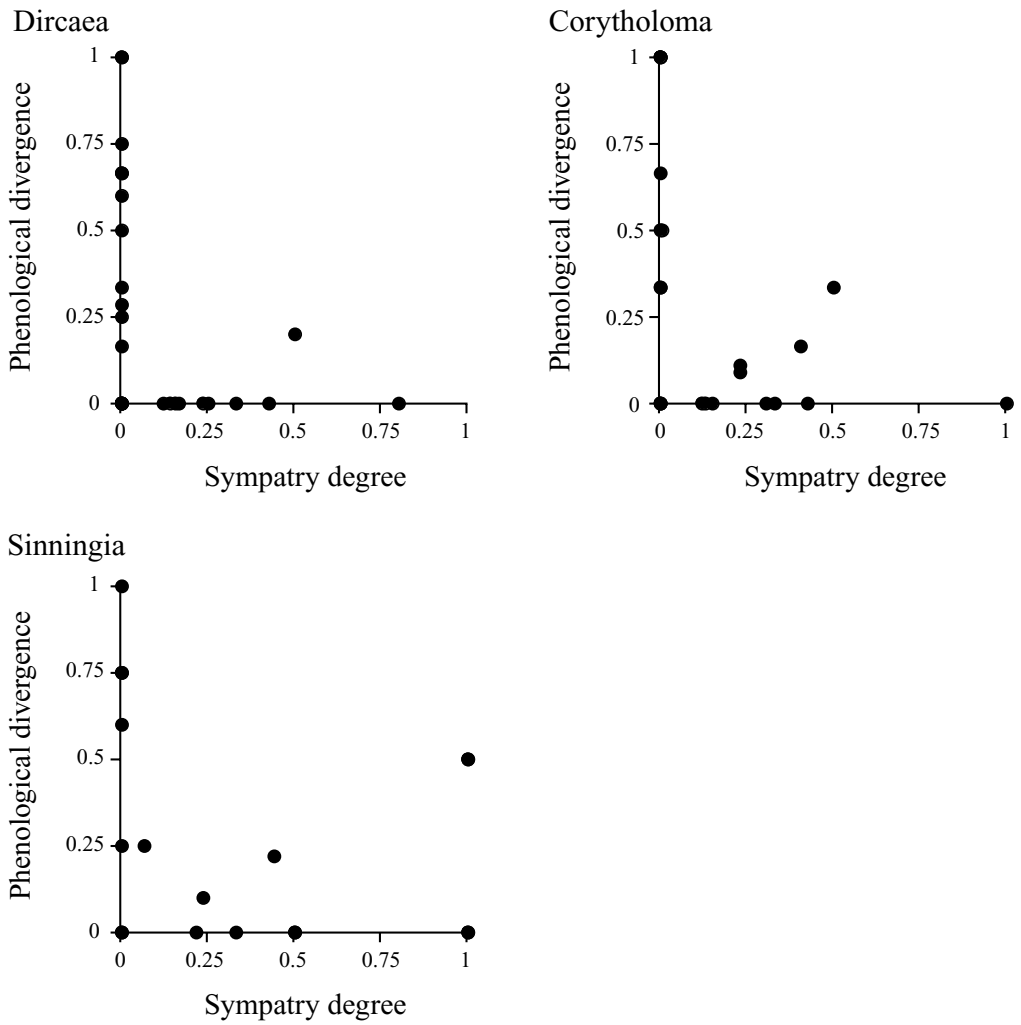


Figure 5. Scatterplots of the phenological divergence against degrees of sympatry in Dircaea, Corytholoma, and Sinningia. The y-axis represents the degree of phenological divergence between sister taxa. The x-axis represents the degree of sympatry between sister taxa. Statistical test of the correlation between phenological divergence and sympatry is provided in Table 3.

tively (Dircaea clade). Among the six splits between fully sympatric taxa only two are linked with a shift in flower types (*S. sp. nov.* 1 and *S. kautskyi* with their respective sister clades), whereas the four other splits exist within a same floral type (Fig. 2).

Table 3. Spearman's rank correlation between flower divergence (i.e., phenology and standardized Euclidian contrasts) and the degree of sympatry in the three main clades within Sinningieae (see scatterplots on Figs. 5 and 6).

Clades	Number of nodes	Spearman's rank test (r_s)	
		Phenological divergence	Standardized Euclidian contrast
Dircaea	26	−0.54*	−0.16 ns
Corytholoma	22	−0.45*	0.15 ns
Sinningia	18	−0.38 ns	0.01 ns

*$P < 0.05$; ns, not significant.

Discussion

GEOGRAPHY OF SPECIATION

The pattern of sympatry across nodes was used to investigate the geographical modes of speciation within three clades of Sinningieae. In Dircaea, the most recent nodes have no range overlap, suggesting a predominantly allopatric mode of speciation in this clade (Barraclough and Vogler 2000). Recent geographic isolation between sister taxa has been associated with discontinuous rock outcrops and/or different subdivisions of mountain ranges in southern and southeastern Brazil. Members of Dircaea are mostly saxicolous, associated with "inselbergs" or other rocky substrates scattered within different vegetation types including rainforest, semi-deciduous forest and "campo rupestre" (Meirelles et al. 1999; Safford and Martinelli 2000). Only two species (*S. douglasii* and *S. cooperi*) have independently shifted to epiphytism. These epiphytic species are also among the most widespread from the clade and their distributions largely overlap with those of other

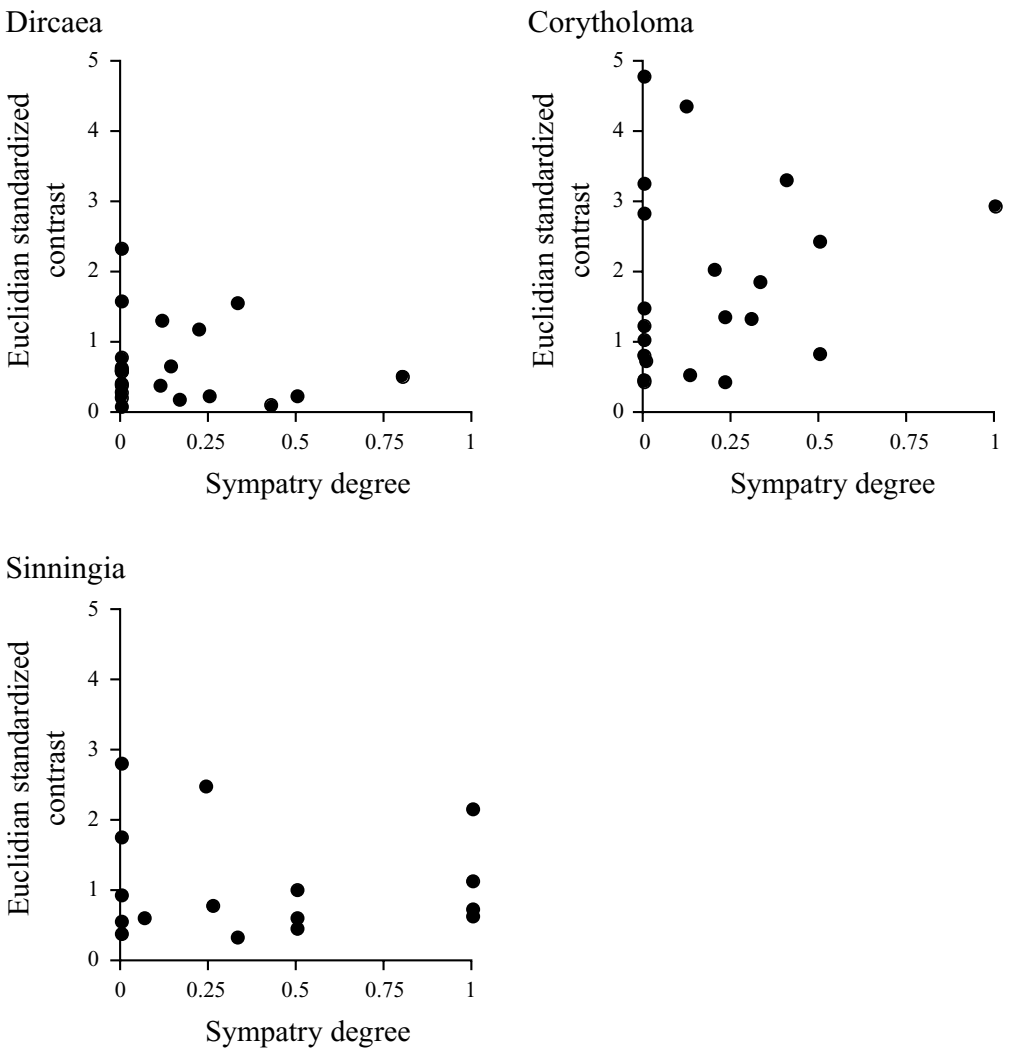


Figure 6. Scatterplots of floral contrasts against degrees of sympatry in Dircaea, Corytholoma, and Sinningia. The y-axis represents the Euclidian standardized contrast calculated from the scores of the first three axes of the PCoA. The x-axis represents the degree of sympatry between sister taxa. The statistical test of the correlation between floral contrast and sympatry is provided in Table 3.

members of Dircaea within the coastal rainforest in Brazil. This contributes to the increased degree of sympatry that we calculated among the older nodes. The fragmented distribution of suitable habitats and rare long-distance dispersal events in Dircaeae could have been the main factors that have promoted genetic isolation among populations, leading to speciation. Similar arguments have been put forward to explain the high species diversity and narrow endemism within the Brazilian Atlantic forests for other saxicolous members of the Bromeliaceae, Orchidaceae, and Velloziaceae (Porembski et al. 1998; Meirelles et al. 1999; Safford and Martinelli 2000).

Although patterns of sympatry in Dircaea are consistent with allopatric speciation, the range randomization test indicates that present-day ranges may not retain any phylogenetic signal and therefore hold little information on the mode of speciation. In this clade the observed intercept (close to zero) was not significantly different from those obtained through randomly reshuffling of the ranges (Table 1). This result can be explained by the low frequency of range overlaps between Dircaea species. When species are mostly allopatric to each other (e.g., like the pieces of a jigsaw puzzle), then each random trial would give the same low degree of sympatry (i.e., close to zero). This pattern could be interpreted as strong evidence for allopatric speciation with little subsequent range movements (Barraclough and Vogler 2000). On the other hand, this highlights the limitation of our range randomization test in determining the geographical mode of speciation when range overlap is rare. To overcome this limitation, Fitzpatrick and Turelli (2006) proposed an alternative method of generating null distributions by dropping species ranges at random within the area occupied by the clade. These randomizations of range placements could provide statistical support for allopatric speciation by testing whether the observed frequency of allopatry is

indeed lower than that expected by chance (Fitzpatrick and Turelli 2006).

In Corytholoma, the low intercept value also indicated a predominantly allopatric mode of speciation. Degrees of sympatry among recent splits were more scattered than in Dircaea, suggesting a higher level of range movements in this clade. This result is consistent with the several large-scale dispersal events reconstructed in the Corytholoma clade using dipersal-vicariance analyses (Perret et al. 2006). However, the randomizations indicated that geographical ranges were not distributed at random with respect to the phylogeny and that present-day distributions still contain information on the geography of speciation. Most of the recent cladogenetic events were found in two lineages (see Fig. 2) that alone accounted for the distribution of the Sinningieae at a continental scale in the "Cerrado," Andes, Amazonia, Guianas, and Central America (Perret et al. 2006). Within these two lineages, geographic isolation between closely related species was associated with severe disjunctions between the Brazilian Atlantic forest and other biogeographical areas like the Paraguay–Parana basin (e.g., *S. curtiflora* vs. *S. sellowii*, *S. aggregata* vs. *S. tubiflora*) and the northern South-Central America region (*S. sceptrum* vs. *S. incarnata*). The continental scale of these speciation events contrasts with the mostly local diversification observed in Dircaea showing how the geographical scale of speciation may strongly vary among lineages (Levin 2000). Increases in the degrees of sympatry among closely related species were mainly associated with the geographical range overlap of narrow endemics by widespread taxa (e.g., *S. elatior* and *S. allagophylla* vs. the narrowly distributed species *S. sp.nov.* 1 and *S. amambayensis*). In these latter cases, sympatry is likely related to relatively small range shifts or expansions of the widespread species.

In Sinningia, the high intercept value (0.87) indicates high degrees of sympatry between sister clades. Although this may indicate that sympatric speciation occurred, inferring the predominant mode of speciation in this clade is difficult because of the scarcity of recent splits and the scattered distribution of degrees of sympatry among older nodes. The recent split between the sympatric sister species *S. gigantifolia* and *S. cochlearis* is a possible case of sympatric speciation that deserves close examination. *S. cochlearis* (1 quadrat) is only known in few localities on the Serra dos Orgãos range (Rio de Janeiro State) above 1800 m, whereas *S. gigantifolia* (7 quadrats) is distributed in both the Serra dos Orgãos and Serra da Mantiqueira ranges at a lower altitude (1500–1800 m). Both species are strictly saxicolous, but they exist in different habitats. *S. gigantifolia* grows in the understory of the montane rainforest, whereas *S. cochlearis* is restricted to the open vegetation of the "campos de altitude." These two adjacent vegetation types differ in several environmental factors including solar irradiation, evaporation levels, and day/night temperature regimes (Safford 1999; Scarano et al. 2001). In addition, field studies indicate that the hummingbird species pollinating *S. gigantifolia* and *S. cochlearis* exhibit habitat specificity that may reduce interspecific pollen flow. Hermit hummingbirds (i.e., *Phaethornis eurynome*) visiting *S. gigantifolia* tend to forage low in the forest understory, whereas nonhermit hummingbirds visiting *S. cochlearis* are either generalists or tend to favor more open areas (SanMartin-Gajardo and Sazima 2005b). This clear-cut transition in environmental factors provides the means for disruptive selection and reproductive isolation, which could have driven speciation at a geographical scale below the one considered here (quadrats of $0.5^\circ \times 0.5^\circ$).

In all three lineages within Sinningieae, cladogenesis events were frequently associated with allopatry (28%, 45%, and 54% of the nodes display zero sympatry in Sinningia, Corytholoma, and Dircaea, respectively). Sympatry within clades was interpreted as a consequence of range movements post-speciation, although the possibility of sympatric speciation cannot be excluded in the clade Sinningia and deserves further investigations at a lower geographical scale. Therefore, geographical isolation and the heterogeneous environments that characterize the Brazilian Atlantic forests are the most likely factors that promoted speciation and lineage persistence in Sinningeae.

IS FLORAL DIVERGENCE INCREASED AMONG SYMPATRIC SPECIES?

The different patterns of sympatry within clades may have occurred as a result of random shifts in the distribution ranges of species over time. There also may be constraints such that only species with ecological differences can coexist because of the effect of competitive exclusion or reinforcement. These hypotheses can be distinguished by evaluating the correlation, if any, between the degrees of sympatry and levels of ecological differentiation (e.g., floral differentiation) between sister taxa (Barraclough et al. 1998, 1999). In all three major clades, flowering periods of sympatric sister taxa largely overlap and the maximum values of phenological divergence were observed between allopatric sister taxa (Fig. 5). This pattern does not support the role of competition for pollinators or reinforcement in promoting different flowering peaks among sympatric species (Waser 1983). For most of the species with a tuber and annual shoots (genus *Sinningia*), production of flowers was concentrated between December and February. This period corresponds with the wettest season in southern and southeastern Brazil and a known general flowering peak for plants of the Brazilian Atlantic forest (Morellato et al. 2000). In contrast, shrubby species (*Paliavana* and *Vanhouttea*) embedded within the clade Sinningia mostly flowered between March and May during the wet-to-dry season transition in southeastern Brazil. High phenological divergences between closely related taxa were associated with variation in local seasonal conditions. For example, nonoverlapping flowering times were observed between the sister species *S. harleyi*/*S. nordestina* and *S. speciosa*/*S. macrophylla*,

which are distributed at different latitudes along the eastern coast of Brazil in regions with contrasting periods of rainfall (November to April around the tropic of Capricorn vs. June to August at lower latitudes). Geographical isolation and adaptation to local climatic variations are likely the primary causes of the differences in flowering seasons in the tribe Sinningieae.

Within the context of competitive exclusion and reproductive character displacement, we could thus expect that differences in flower phenotypes compensated for the low phenological differentiation among sympatric taxa. However, no significant correlation was found between the degree of sympatry and the amplitude of floral contrasts in all three clades (Table 3). In our graphs, the values for floral contrasts were scattered along the axis reflecting the degree of sympatry (Fig. 6). Maximum phenotypic differences were found between allopatric clades and therefore might have evolved as a "by-product" of geographical isolation (Grant 1971, 1994).

We must acknowledge two caveats that could explain our failure to detect a clear pattern between distribution range overlap and floral phenotypic divergence. First, the outcome of our analyses may have depended on the definition of sympatry (Armbruster 1986). In this study, two species were considered sympatric if they existed in a same quadrat of a $0.5^\circ \times 0.5^\circ$ grid. This scale might be too large to detect distinct patterns but unfortunately data at a finer scale are not readily available. Second, our estimation of flower isolation using phenotypic contrasts might also need refining. Our multivariate analysis of flower characters indicates that species are separated in clusters that correspond to pollination syndromes for hummingbirds, bees, bats, and moths, and field studies have confirmed the majority of these pollinators (SanMartin-Gajardo and Sazima 2004, 2005a,b). However, specific plant–pollinator associations may also involve phenotypic variation of smaller amplitude, occurring within morphological clusters. For example, some studies have pointed to minute differences in the length of corolla tube that reduce pollen flow between coexisting species sharing the same type of pollinators (Brown and Koderic-Brown 1979; Armbruster et al. 1994; Wolf et al. 2001). Hence, our phenotypic distance derived along the main axes of flower variation may underestimate the degree of premating reproductive isolation.

CONCLUSION

Our phylogenetic analysis of species range data showed that despite the fact that many cases of sympatry exist between species, they are the product of secondary range overlap rather than sympatric speciation. However, in one clade at least (i.e., Sinningia), the scenario of sympatric or parapatric speciation on a mountain of the Serra dos Orgãos range (Rio de Janeiro State) cannot be excluded. In this latter speciation event, altitude may have played a significant role, with subsequent/concordant pollinator shifts. We did not find an increase in phenotypic or phenological disjunction for sympatric species and therefore were not able to support the role of competitive exclusion or reinforcement. Instead, our results point to location-driven phenological divergences and buildup of random sympatries with respect to flower morphology. The results provide the first insights into mechanisms of speciation in one of the major diversity hotspots for flowering plants.

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Appendix 1. **Phenology of 68 Sinningieae species from the clades Corytholoma, Dircaea, and Sinningia based on a survey of herbarium specimens. The value of (1) indicates the months in which at least 75% of herbarium specimens were collected in flower.**

	Month (from January to December)											
Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
P. prasinata	0	0	0	0	1	1	1	1	0	0	0	0
P. sericiflora	0	1	1	1	1	0	0	0	0	0	0	0
P. tenuiflora	0	0	1	1	1	1	1	0	0	0	0	0
P. werdermanii	0	1	1	1	1	0	0	0	0	0	0	0
S. aggregata	1	1	0	0	0	0	0	0	1	1	1	1
S. aghensis	0	0	0	0	0	0	0	0	0	1	1	1
S. allagophylla	1	1	0	0	0	0	0	0	0	1	1	1
S. amambayensis	1	1	1	0	0	0	0	0	0	0	1	1
S. araneosa	0	0	1	1	1	0	0	0	0	0	0	0
S. barbata	1	1	1	1	1	1	1	1	1	0	1	1
S. brasiliensis	1	1	1	1	0	0	0	0	0	0	0	1
S. bulbosa	0	0	1	1	1	0	0	0	1	1	1	1
S. calcaria	1	0	0	0	0	0	0	1	1	1	1	1
S. canescens	0	0	0	0	0	0	0	0	0	1	1	1
S. carangolensis	1	1	0	0	0	0	0	0	0	0	0	1
S. cardinalis	0	1	0	0	0	0	0	1	0	1	1	1
S. cochlearis	1	0	0	0	0	0	0	0	0	0	0	1
S. concinna	1	0	0	0	0	0	0	0	0	0	0	1
S. conspicua	1	1	1	0	0	0	0	0	0	0	0	1
S. cooperi	1	1	1	1	0	0	0	0	0	0	1	1
S. curtiflora	1	1	1	0	0	0	0	0	0	0	0	0
S. defoliata	0	0	0	0	0	0	0	1	1	1	0	0
S. douglasii	0	0	0	0	0	0	0	0	0	1	1	0
S. elatior	1	1	0	0	0	0	0	0	0	0	1	1
S. eumorpha	1	1	1	1	0	0	0	0	0	0	0	0
S. gigantifolia	1	1	1	0	0	0	0	0	0	0	0	0
S. glazioviana	0	0	0	1	1	0	0	0	1	0	0	0
S. guttata	0	0	0	0	0	0	0	0	0	0	1	1
S. harleyi	0	1	1	1	0	0	0	0	0	0	0	1
S. hatschbachii	1	1	0	0	0	0	0	0	0	0	0	0
S. hirsuta	0	1	0	0	0	0	0	1	0	0	1	1
S. iarae	0	0	1	1	1	0	0	0	0	0	0	0
S. incarnata	0	0	0	0	0	0	1	1	1	0	0	0
S. insularis	0	0	0	0	0	0	0	0	1	1	0	0
S. kautskyi	0	0	0	0	0	1	0	0	0	0	0	1
S. lateritia	1	1	1	1	0	0	0	0	0	0	0	0
S. leopoldii	0	0	1	1	1	0	0	0	0	0	0	0
S. leucotricha	0	0	0	0	0	0	1	1	1	0	0	0
S. lindleyi	1	1	0	0	0	0	0	0	0	0	1	1
S. lineata	0	0	0	0	0	0	0	0	0	1	1	1
S. macrophylla	0	1	0	0	0	0	0	0	0	0	0	0
S. macropoda	0	0	0	0	0	0	0	1	0	1	1	1
S. macrostachya	1	0	0	0	0	0	0	0	1	1	1	1
S. magnifica	1	1	1	1	0	0	0	0	0	0	0	0
S. mauroana	0	0	0	1	1	1	1	0	0	1	0	0
S. micans	0	0	0	0	0	1	0	0	0	0	0	0
S. nivalis	0	0	0	0	0	0	0	0	1	1	1	1
S. nordestina	0	0	0	0	0	0	1	1	1	0	0	0

Continued.

Appendix 1. Continued.

	Month (from January to December)											
Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
S. piresiana	0	0	0	0	0	0	0	0	1	1	1	0
S. pusilla	0	1	0	0	0	0	0	1	0	1	1	0
S. reitzii	0	0	0	1	1	0	0	0	0	0	0	0
S. rupicola	1	1	0	0	0	0	0	0	0	0	1	1
S. sceptrum	1	1	1	1	0	0	0	0	0	0	0	0
S. sellowii	1	1	0	0	0	0	0	0	0	1	1	1
S. speciosa	1	0	0	0	0	0	0	0	1	1	1	1
S. striata	1	0	0	0	0	0	0	0	0	0	0	1
S. tuberosa	1	1	1	1	1	0	0	0	0	0	0	0
S. tubiflora	1	1	0	0	0	0	0	0	0	0	0	1
S. valsuganensis	1	1	0	0	0	0	0	0	0	0	0	1
S. villosa	1	1	0	0	0	0	0	0	0	0	0	1
S. warmingii	1	1	0	0	0	0	0	0	0	0	1	1
S. sp. nov. 1	1	0	0	0	0	0	0	0	0	0	0	0
S. sp. nov. 7	0	0	0	0	0	0	0	0	0	1	1	0
V. brueggeri	0	1	1	0	0	0	0	0	0	0	0	0
V. calcarata	0	1	1	1	1	1	1	0	0	0	0	0
V. fruticulosa	0	0	1	1	1	0	1	1	1	0	0	0
V. gardneri	0	1	1	1	1	0	1	0	0	0	0	0
V. lanata	0	1	0	1	1	0	0	0	0	0	0	0

Appendix 2. Floral traits for 64 species of Sinningieae included in the clades Corytholoma, Dircaea, and Sinningia. Abbreviations are as follows: n, number of flowers measured; DLH, horizontal diameter of the corolla with lobes; LCD and LCV, lengths of the dorsal and ventral faces of the corolla; LTU, length of the tube; DOH and DOV, horizontal and vertical diameters of the corolla orifice; DRH and DRV, horizontal and vertical diameters of corolla constriction after the nectar chamber; DNH and DNV, horizontal and vertical diameters of nectar chamber; LAN, maximum length between anthers and the base of the ovary; LST, maximum length between stigma and the base of the ovary; ANG, approximate angle in degrees formed between the axis of the tube and the base of the corolla; VNE, volume of nectar; COL, color; FRA, fragrance. Mean and standard deviations were calculated for all morphometric measurements (in millimeters) over the number of flowers measured. Nectar volumes were grouped into three size classes; color and fragrances were coded as discrete values.

No	Species	n	DLH	LCD	LCV	LTU	DOH	DOV	DRH	DRV	DNH	DNV	LAN	LST	ANG	VNE	COL	FRA
1	*P. prasinata*	8	35.9±7.6	40.8±7.6	39.7±5.7	29.7±5.1	15.1±4.1	15.9±2.6	5.7±1.2	5.9±0.9	8.5±0.7	8.9±0.7	27.1±0.8	30.7±7.0	0	3	0	1
2	*P. sericiflora*	1	40.6	55.0	52.0	40.9	24.2	22.4	4.0	4.9	6.5	5.2	39.3	46.4	30	3	0	0
3	*P. tenuiflora*	2	35.4±2.0	52.0±1.4	60.8±1.1	49.2±0.1	20.6±0.6	19.1±2.3	5.5±1.3	6.2±1.1	6.5±0.2	8.6±0.8	23.8±0.4	25.4±0.5	30	2	0	0
4	*S. aggregata*	7	11.2±0.8	29.3±2.0	28.6±1.4	25.3±1.4	5.0±0.3	5.5±0.4	2.9±0.3	3.6±0.5	5.6±0.6	6.4±0.5	27.2±0.5	28.7±1.9	0	2	1	0
5	*S. aghensis*	6	35.6±3.6	41.7±2.9	50.2±2.2	33.5±1.7	22.3±2.1	13.5±0.9	4.0±0.3	7.5±0.7	7.4±0.6	8.4±1.0	18.5±0.5	27.9±2.3	40	1	0	0
6	*S. allagophylla*	8	9.3±4.2	14.9±4.7	13.7±4.9	11.1±3.3	4.3±0.5	3.6±0.4	3.4±0.6	3.7±0.7	4.1±0.9	5.0±1.1	9.7±2.9	14.0±4.1	10	2	1	0
7	*S. amambayensis*	3	15.9±1.3	39.4±1.0	36.8±0.6	31.9±0.5	6.3±0.2	6.5±0.4	2.5±0.5	4.0±0.1	5.8±0.1	6.1±0.1	45.7±1.2	42.9±1.3	0	2	1	0
8	*S. araneosa*	6	19.3±1.3	42.0±2.9	38.8±1.3	31.6±1.5	4.7±0.5	6.0±0.7	2.0±0.4	2.5±0.2	4.3±0.7	4.4±0.7	43.3±2.9	50.4±8.2	0	2	1	0
9	*S. barbata*	4	20.0±0.6	37.2±0.7	42.2±0.8	31.6±2.0	10.0±0.9	8.2±0.9	6.4±0.3	5.7±0.4	7.1±0.5	5.6±1.4	21.5±1.1	23.8±2.5	30	2	0	0
10	*S. brasiliensis*	8	24.3±3.1	39.2±2.2	27.4±2.4	19.6±1.9	11.2±0.7	19.8±1.7	3.0±0.5	7.1±0.6	9.5±0.9	10.3±0.7	31.9±4.3	37.6±3.2	0	3	0	1
11	*S. bulbosa*	2	8.2±0.6	36.2±2.6	24.1±0.1	24.1±0.1	6.6±1.1	5.5±0.7	2.4±0.1	3.2±0.1	5.6±0.6	5.5±0.7	34.0±0.1	36.0±0.1	0	2	1	0
12	*S. calcaria*	3	15.4±0.4	45.7±1.2	43.0±0.9	39.2±1.3	4.2±0.1	9.0±0.7	3.2±0.4	4.1±0.8	5.4±0.7	5.2±0.5	43.4±0.4	42.2±1.3	0	2	1	0
13	*S. canescens*	3	13.9±0.2	32.4±0.5	31.6±0.4	26.7±0.3	3.8±0.4	7.6±0.4	3.1±0.3	3.5±0.1	5.5±0.1	5.3±0.4	28.3±1.0	30.0±3.6	0	2	1	0
14	*S. carangolensis*	3	12.2±0.4	38.6±1.3	36.4±0.9	33.5±1.8	8.2±2.6	5.9±0.3	2.3±0.1	3.5±0.1	5.6±0.3	6.0±0.0	34.7±0.1	48.3±0.0	0	1	1	0
15	*S. cardinalis*	6	11.1±0.8	61.7±10.8	35.7±7.2	34.3±8.0	7.9±1.4	9.6±1.0	4.2±0.8	4.8±0.4	7.3±1.1	6.6±0.8	52.1±6.5	54.9±8.3	0	2	1	0
16	*S. cochlearis*	6	12.9±1.1	30.1±2.2	30.4±2.8	25.7±2.0	5.9±0.9	6.0±0.7	2.9±0.2	3.7±0.7	4.8±0.5	5.3±0.4	23.7±2.9	24.5±0.7	0	1	1	0
17	*S. concinna*	1	13.5	17.1	18.4	13.2	4.7	4.1	2.1	1.9	2.2	1.9	6.1	7.6	40	1	0	0
18	*S. conspicua*	2	28.1±1.8	42.3±4.5	49.6±2.7	35.0±3.5	16.2±1.7	12.4±1.5	7.2±0.5	6.0±0.1	6.8±0.7	3.8±0.4	17.6±3.4	26.2±0.5	40	1	0	2
19	*S. cooperi*	3	9.4±0.4	61.4±0.6	26.9±0.4	26.6±0.4	7.5±0.6	19.7±1.0	4.8±0.2	6.2±0.2	8.3±0.2	8.2±0.3	49.7±1.9	60.0±0.5	0	2	1	0
20	*S. curtiflora*	4	6.7±0.8	11.8±0.5	11.4±0.8	9.6±0.2	3.4±0.3	3.3±0.1	2.3±0.3	3.7±0.3	4.9±0.5	5.7±0.4	8.3±0.3	10.4±0.1	20	2	1	0
21	*S. defoliata*	1	13.9	38.2	34.3	29.8	5.2	5.6	3.1	4.3	4.3	5.2	34.5	40.1	0	2	1	0
22	*S. douglasii*	4	18.5±2.8	45.6±2.5	43.2±1.5	36.0±1.2	5.8±0.9	7.6±1.4	3.2±0.5	4.0±0.3	6.2±0.2	6.0±0.3	44.3±2.0	47.6±0.8	0	2	1	0
23	*S. elatior*	3	12.9±0.2	40.4±0.9	30.4±0.6	26.8±0.7	6.2±0.2	8.6±0.4	3.6±0.1	4.2±0.4	5.7±0.2	6.4±0.7	39.1±2.0	40.0±0.5	0	2	1	0
24	*S. eumorpha*	3	42.6±2.1	40.0±3.1	60.0±1.7	30.6±2.9	18.0±1.8	11.0±1.7	7.0±0.6	6.0±0.2	7.2±0.9	3.8±0.5	20.0±2.7	24.0±0.6	40	1	0	0
25	*S. gigantifolia*	3	13.0±1.3	44.3±1.7	42.9±0.7	38.7±0.6	5.2±0.1	6.1±0.5	2.7±0.4	3.4±0.2	4.9±0.2	5.2±0.4	33.5±0.8	33.6±0.5	0	2	1	0
26	*S. glazioviana*	2	11.3±0.6	55.7±2.0	33.0±0.1	31.6±0.3	7.6±0.2	9.6±0.1	4.4±0.4	4.6±0.1	6.4±0.3	7.9±2.3	46.3±1.8	53.9±1.0	0	2	1	0
27	*S. guttata*	3	25.9±4.4	43.7±3.7	42.5±3.3	32.3±2.8	8.1±0.6	9.9±0.5	3.4±0.3	4.4±0.5	6.5±0.4	6.0±0.6	23.0±0.4	29.8±0.9	0	2	0	0
28	*S. harleyi*	2	25.3±1.3	55.4±1.7	51.0±0.1	42.8±0.4	8.7±0.5	10.9±1.2	2.6±0.1	5.2±0.2	6.2±0.2	6.6±0.1	44.0±1.4	56.6±0.1	0	2	1	0
29	*S. hatschbachii*	3	9.1±0.4	62.6±2.8	31.0±0.9	29.9±1.1	5.7±0.4	15.4±0.6	3.9±0.2	5.2±0.2	7.2±0.3	6.9±0.2	52.5±0.1	65.2±2.6	0	2	1	0
30	*S. hirsuta*	1	16.0	15.7	17.1	10.0	5.4	4.5	2.3	1.6	3.5	3.0	8.2	7.6	45	1	0	0

Continued.

Appendix 2. Continued.

No	Species	n	DLH	LCD	LCV	LTU	DOH	DOV	DRH	DRV	DNH	DNV	LAN	LST	ANG	VNE	COL	FRA
31	*S. iarae*	4	9.4±0.2	56.3±0.3	36.2±1.3	34.6±1.2	5.2±0.6	9.2±0.2	3.7±0.3	4.0±0.3	5.5±0.3	5.9±0.5	44.3±4.6	55.5±0.4	0	2	1	0
32	*S. incarnata*	5	13.7±0.8	54.9±1.4	43.1±3.6	37.1±0.3	6.4±0.2	7.9±0.5	3.0±0.1	5.1±0.2	7.8±0.6	8.3±0.2	46.5±1.1	57.0±2.0	0	2	1	0
33	*S. insularis*	3	11.5±0.5	29.2±0.7	27.5±1.2	24.5±1.0	3.0±0.1	6.6±0.1	1.9±0.2	2.9±0.2	3.8±0.1	3.8±0.3	26.0±0.6	28.2±0.2	0	2	1	0
34	*S. kautskyi*	6	18.8±0.9	19.0±1.4	24.4±1.6	11.8±0.4	5.2±0.3	3.3±0.4	3.2±0.4	2.8±0.5	3.5±0.3	3.4±0.4	6.9±0.3	9.2±1.1	45	1	0	0
35	*S. leopoldii*	4	14.5±1.1	42.7±2.7	37.3±2.0	32.5±1.3	5.2±0.2	6.6±0.4	3.1±0.3	3.4±0.5	5.0±0.8	4.8±0.9	40.3±1.3	45.4±1.2	0	2	1	0
36	*S. leucotricha*	6	11.4±1.2	29.2±1.0	29.3±1.2	25.5±0.9	5.0±0.4	5.4±0.4	3.0±0.3	3.3±0.1	4.9±0.4	4.6±0.4	25.4±1.4	26.5±0.9	0	2	1	0
37	*S. lindleyi*	3	21.6±1.4	40.7±1.4	40.9±2.7	33.7±1.1	8.4±0.6	9.1±0.4	2.2±0.3	3.5±0.4	4.6±0.4	4.6±0.3	27.3±1.6	30.4±0.5	0	2	0	0
38	*S. lineata*	16	19.2±1.0	35.6±2.4	34.3±2.0	28.4±1.7	5.3±0.3	5.3±0.3	4.1±0.6	3.8±08	6.1±0.7	6.4±0.7	29.1±2.7	34.0±3.3	0	2	1	0
39	*S. macropoda*	5	10.8±0.9	31.4±0.5	30.7±1.1	27.0±0.8	4.9±0.1	4.7±0.3	3.7±0.2	2.9±0.1	5.8±0.3	5.8±0.3	26.8±0.4	30.6±0.8	0	2	1	0
40	*S. macrostachya*	4	10.7±0.7	30.0±0.2	25.8±0.3	24.1±0.5	4.3±0.1	4.2±0.5	4.4±0.6	2.7±0.3	5.2±0.2	5.5±0.5	27.1±0.3	28.0±1.6	0	2	1	0
41	*S. magnifica*	3	10.1±1.6	59.8±1.5	38.1±0.5	38.0±0.6	6.7±0.8	10.9±0.5	4.2±0.2	4.6±0.2	7.4±0.2	7.1±0.3	51.3±0.8	53.9±1.1	0	2	1	0
42	*S. mauroana*	3	13.8±1.1	38.3±1.1	38.3±1.3	34.7±1.2	5.9±0.7	6.7±0.7	3.1±0.1	3.6±0.1	5.5±0.2	5.5±0.6	31.8±1.8	34.0±1.9	0	2	1	0
43	*S. micans*	4	10.3±0.4	61.9±4.5	35.9±3.3	35.9±3.3	6.4±0.5	11.3±2.4	3.3±0.4	3.8±0.4	6.7±0.9	6.7±0.8	51.0±5.0	59.8±7.2	0	2	1	0
44	*S. nivalis*	5	16.6±1.1	33.2±1.8	30.4±1.3	25.3±1.6	3.8±0.6	8.3±0.6	2.8±0.2	3.6±0.3	5.5±0.3	5.1±0.6	30.2±2.7	34.7±4.0	0	2	1	0
45	*S. nordestina*	3	15.0±0.3	29.5±1.4	26.2±1.3	19.6±1.2	2.6±0.3	8.3±0.1	1.6±0.2	2.9±0.2	3.2±0.2	3.6±0.2	28.6±0.6	31.5±1.0	0	2	1	0
46	*S. pusilla*	14	14.3±1.2	17.3±0.7	20.2±2.6	11.9±0.9	1.6±0.3	2.1±0.2	1.4±0.2	1.3±0.2	2.0±0.2	1.4±0.2	8.5±0.5	10.0±0.8	45	1	0	0
47	*S. reitzii*	4	16.1±0.3	40.8±0.8	39.1±1.1	33.7±0.6	5.3±0.3	7.3±0.2	3.1±0.2	3.4±0.2	5.5±0.3	5.5±0.5	33.4±0.2	35.2±0.9	0	2	1	0
48	*S. richii*	3	27.6±4.9	42.2±3.4	43.7±4.2	35.3±2.6	12.7±0.7	11.4±1.5	5.6±0.7	5.4±0.4	6.3±0.3	6.4±0.5	22.2±0.8	26.6±2.3	45	1	0	0
49	*S. sceptrum*	4	10.9±0.5	44.0±3.3	39.8±2.1	34.4±2.7	5.0±0.9	7.5±0.6	3.0±0.2	3.1±0.2	5.3±0.6	5.6±0.5	39.5±3.5	46.5±9.2	0	2	1	0
50	*S. sellovii*	12	10.4±0.8	30.4±1.5	29.2±0.9	25.2±0.8	4.8±0.8	5.2±0.7	2.4±0.5	3.1±0.5	5.4±0.9	6.1±0.8	35.4±2.0	38.0±2.6	0	2	1	0
51	*S. speciosa*	9	25.8±3.5	37.5±4.3	38.2±3.3	31.5±1.3	15.3±2.1	16.1±1.4	4.7±0.3	5.6±0.3	5.2±0.5	6.4±0.5	19.1±1.9	27.6±0.8	45	2	0	0
52	*S. striata*	4	13.6±1.4	30.7±2.4	30.6±1.9	26.1±2.4	3.9±0.3	7.2±0.8	2.4±0.6	3.0±0.5	4.0±0.5	3.8±0.4	28.3±3.5	32.5±3.5	0	2	1	0
53	*S. tuberosa*	4	15.4±0.5	36.2±2.6	34.9±3.0	29.6±3.2	4.7±1.2	5.1±0.4	1.8±0.7	2.4±0.4	3.2±0.2	3.3±0.3	32.4±1.6	29.4±3.4	0	1	1	0
54	*S. tubiflora*	4	31.6±2.2	79.6±1.5	80.6±4.0	67.4±2.5	6.1±0.8	8.9±1.3	3.8±0.3	5.9±0.2	7.1±0.7	8.7±0.5	60.3±2.5	62.7±2.1	50	1	0	2
55	*S. valsuganensis*	3	16.6±0.5	49.2±1.1	39.8±1.8	36.3±1.3	6.2±0.2	6.7±0.6	1.9±0.4	4.4±0.2	5.7±0.3	6.7±0.2	44.9±1.7	51.8±2.5	0	1	1	0
56	*S. villosa*	6	27.5±2.9	41.1±2.2	40.8±2.6	31.2±1.8	10.0±1.1	10.3±0.6	4.3±0.6	4.6±0.5	6.8±0.5	6.6±0.7	22.8±2.3	25.9±3.1	50	2	0	0
57	*S. warmingii*	4	13.9±0.5	40.6±1.3	38.2±1.8	33.6±1.3	6.4±0.4	7.0±0.6	3.3±0.4	4.6±0.5	8.1±0.9	8.6±0.9	37.5±0.7	48.3±2.9	0	2	1	0
58	*S. sp. nov. 1*	6	27.1±3.1	30.6±2.8	28.7±0.7	20.8±2.2	13.2±0.4	10.9±1.3	5.1±0.6	5.8±0.5	5.1±0.1	5.5±0.4	15.6±1.0	22.3±1.4	45	2	0	0
59	*S. sp. nov. 7*	4	13.4±0.4	33.7±1.0	31.0±1.3	26.5±0.6	3.6±0.3	6.3±0.2	2.7±0.1	3.7±0.2	5.8±0.3	5.6±0.4	30.3±0.2	34.2±1.1	0	2	1	0
60	*V. brueggeri*	4	24.2±1.2	49.2±1.6	45.5±2.7	36.3±1.5	7.8±1.3	8.4±1.1	2.4±0.4	3.7±0.2	4.6±0.3	4.2±0.5	43.8±1.6	48.9±2.0	15	2	1	0
61	*V. calcarata*	4	17.8±3.9	37.4±9.5	33.8±7.0	28.5±7.0	5.6±2.9	7.6±1.7	2.9±0.5	3.6±1.0	4.6±1.1	4.5±0.9	35.3±11.8	40.8±13.1	0	2	1	0
62	*V. fruticulosa*	1	23.3	42.6	36.1	30.7	13.2	11.2	3.6	5.8	3.9	5.7	22.0	33.2	0	2	1	0
63	*V. gardneri*	1	17.9	45.8	43.7	37.0	6.1	7.0	2.0	4.1	5.0	4.4	40.0	51.0	0	2	1	0
64	*V. lanata*	4	17.9±1.2	30.1±3.4	29.8±1.9	23.1±0.9	4.1±0.6	8.1±0.8	2.7±0.7	4.0±0.5	5.0±0.7	5.0±0.6	29.2±1.7	25.9±1.0	0	2	1	0