Evol Ecol (2015) 29:355–377
DOI 10.1007/s10682-014-9729-4

ORIGINAL PAPER

Convergence of anti-bee pollination mechanisms in the Neotropical plant genus *Drymonia* (Gesneriaceae)

John L. Clark · Laura Clavijo · Nathan Muchhala

Received: 17 March 2014 / Accepted: 16 July 2014

Abstract The neotropical plant genus *Drymonia* displays a remarkable variety of floral shapes and colors. One feature that is particularly important to coevolution with pollinators involves the variable shapes and widths of corolla tubes. To evaluate the evolutionary context for changes in corolla shape, we constructed a phylogeny of 50 of the 75 species of *Drymonia* using molecular markers from plastid (*trn*K-*mat*K) and nuclear regions (ITS and ETS). Mapping tube shapes on the phylogeny supports open, bell-shaped (campanulate) corolla shape as the ancestral character state for *Drymonia*, with multiple independent origins of constriction in the corolla tube. Corollas with constrictions take one of three tube shapes: a constricted flower opening and throat with a large, expanded pouch on the lower surface (hypocyrtoid); a constricted flower opening and throat lacking an expanded pouch on the lower surface (urceolate); or a constricted opening and throat where the sides of the corolla appear laterally compressed. Fieldwork demonstrates euglossine bees (mostly *Euglossa* spp. and *Epicharis* spp.) visit campanulate corollas while hummingbirds visit corollas that are constricted. Results support eight independent origins of constricted corolla tubes from ancestors with campanulate corolla tubes: 3 hypocyrtoid clades, 3 laterally compressed clades, and 3 urceolate clades (one of which represents a shift from a hypocyrtoid ancestor). Constricted corollas are associated with shifts from the ancestral condition of poricidal anther dehiscence, which presents pollen to pollinators in multiple small doses, to the derived condition of longitudinal anther dehiscence, which presents all pollen to pollinators simultaneously. The association of hummingbird pollination with constricted corolla tubes suggests that narrowing evolved as a barrier mechanism that prohibits the visitation of flowers by bees.

J. L. Clark (✉) · L. Clavijo
Department of Biological Sciences, The University of Alabama, Box 870345, Tuscaloosa, AL 35487-0345, USA
e-mail: jlc@ua.edu

N. Muchhala
Department of Biology, University of Missouri-St. Louis, One University Blvd., St. Louis, MO 631121, USA

Keywords Convergence · *Drymonia* · Gesneriaceae · Hypocyrtoid corollas · Laterally compressed corollas · Pollination biology · Poricidal anther dehiscence · Urceolate corollas

Introduction

Flowers of neotropical plants of the family Gesneriaceae have diversified into a remarkable array of colors and shapes (Fig. 1), suggesting a diverse coevolutionary history with pollinators. Few pollination studies or species-level phylogenies exist for the group. An understanding of the ecology and evolution of their flowers has been further hampered by a confusing classification system and lack of monophyly for many of the traditionally recognized genera because species were frequently shifted between poorly defined genera and genera were shifted between poorly defined tribes (Hanstein 1854, 1856, 1859, 1865; Fritsch 1893–1894; Martius 1829; Ivanina 1965, 1967; Wiehler 1973, 1983; Burtt and Wiehler 1995; Möller and Clark 2013; Weber et al. 2013). Recent molecular-based studies have begun to clarify phylogenetic relationships and circumscribe monophyletic genera (e.g., Smith and Atkinson 1998; Smith et al. 1997, 1998, 2004a, b; Smith and Clark 2013; Zimmer et al. 2002, Clark et al. 2006, 2012; Möller and Clark 2013; Weber et al. 2013). The objective of this study is to provide an evolutionary and ecological context for understanding the evolution of the narrowing of corolla tubes in *Drymonia* Mart. and how this feature may function as a barrier mechanism to pollinators.

Drymonia is one of the largest genera of Neotropical Gesneriaceae, with 75 species (Weber et al. 2013; Möller and Clark 2013). Martius (1829) circumscribed *Drymonia* on the basis of a leafy calyx and large corolla, but these features are also found in many other closely related genera. More recently Moore (1955) characterized *Drymonia* from other Gesneriaceae by the presence of poricidal anther dehiscence (Fig. 2a). Instead of undergoing longitudinal dehiscence (Fig. 2b), with thecae splitting fully open along the length and presenting all pollen simultaneously, the thecae in *Drymonia* open by a short basal pore which slowly releases pollen throughout anthesis (Fig. 2c, d). Wiehler (1983) aptly described these poricidal anthers as "salt-shaker-like." In bud, the four anthers are grouped coherently around the style, with their pore-like thecae facing inward, and become connate along the length of their margins as they mature. Prior to anthesis, the curvature and the differential length of the filament pairs invert the anther structure by turning it upside down (i.e., rotating 180°), causing the basal pores to face upwards before they open. During anthesis, the strategically placed anthers are thus able to pour or "shake" their powdery pollen grains through the pores onto visitors when they tip the structure over as they enter the flower (Fig. 2f). Steiner (1985) noted that gland-tipped trichomes located inside the corollas of *Drymonia serrulata* (Jacq.) Mart. exuded oil that played a role in promoting the adhesion of pollen grains to the body of *Epicharis* bees (family Apidae, subfamily Apinae). The 'salt-shaker' structure releases the pollen in doses, such that it takes five to eight visits to fully empty the anthers (Wiehler 1983).

The majority of flowering plants have anthers that open by splitting longitudinally along the entire locule. In contrast, anthers that dehisce poricidally represent less than 10 % of flowering plants (Buchmann 1983). Poricidal anthers are almost entirely associated with vibratory pollen collection ("buzz pollination") by bees (Buchmann 1983). The transfer of pollen grains in *Drymonia* flowers is not facilitated by vibrations and is therefore unique among taxa with poricidal anther dehiscence.

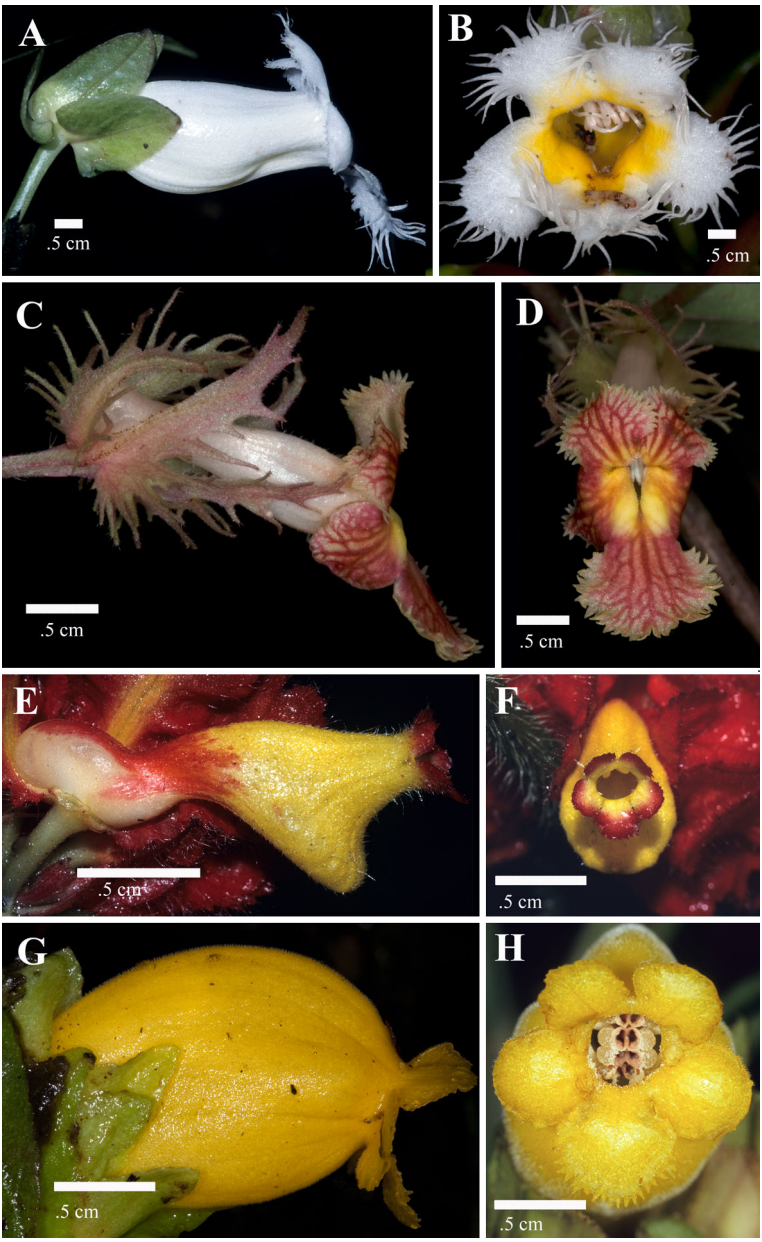

Fig. 1 Corolla shape variation evaluated in *Drymonia*. **a**, **b** Bell-shaped (campanulate) in *Drymonia brochidodroma*. **c**, **d** Laterally compressed in *Drymonia multiflora*. **e**, **f** Pouched (hypocyrtoid) in *Drymonia teuscheri*. **g**, **h** Urn-shaped (urceolate) in *Drymonia urceolata*. Photos from field collections by John L. Clark (**a**, **b** *J.L. Clark* et al. *6354*; **c**, **d** *J.L. Clark* et al. *12499*; **e**, **f** *J.L. Clark* et al. *6369*; **g** *J.L. Clark 10006*; **h** *J.L. Clark 9005*)

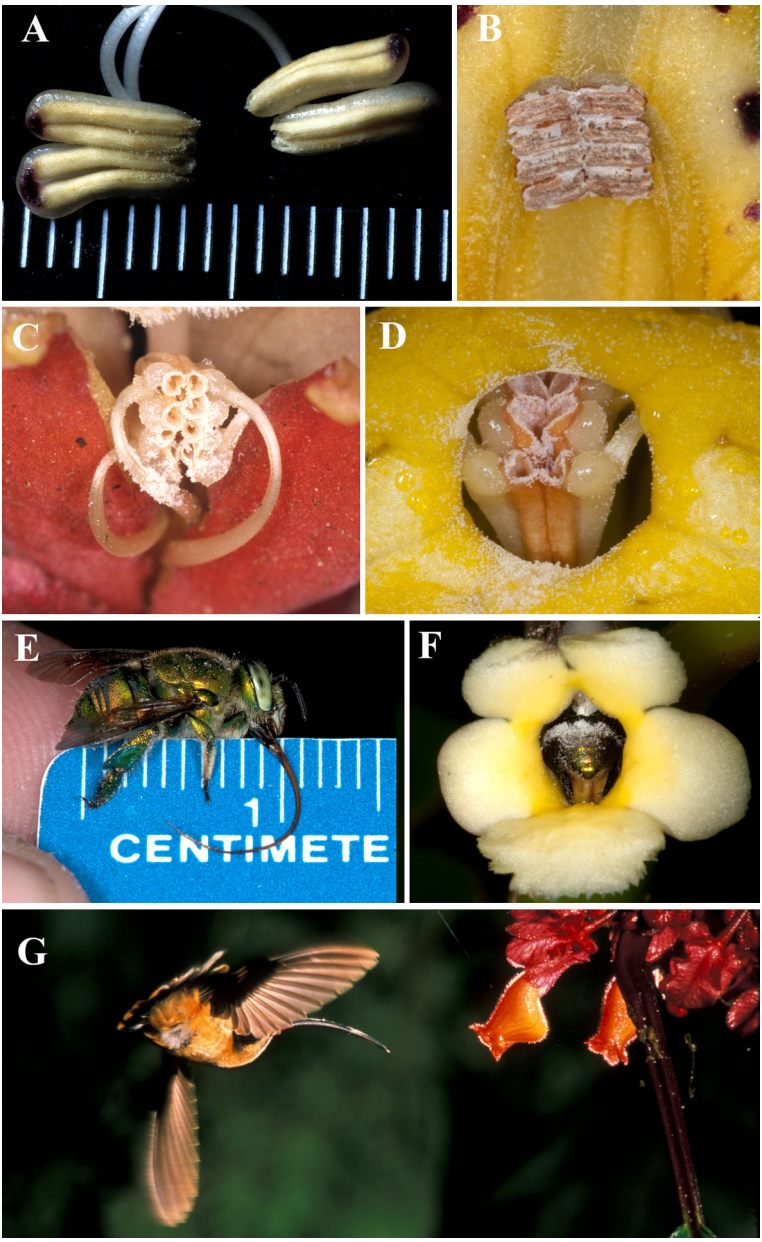

Fig. 2 Plant-pollinator interactions and anther dehiscence. **a** Poricidal anther dehiscence in *Drymonia killipii* (scale in mm). **b** Longitudinal anther dehiscence in *Columnea medicinallis*. **c**, **d** Poricidal anther dehiscence in *Drymonia urceolata.* **e** *Euglossa* bee captured and photographed from recently visited flower of *Drymonia ecuadorensis*, Rio Palenque Science Center. **f** *Drymonia ecuadorensis* visited by *Euglossa* bee. **g** *Drymonia collegarum* visited by Tawny-bellied Hermit (*Phaethornis syrmatophorus*). Photo **a** by Richard W. Dunn; **b**–**f** by John L. Clark and G by Murray Cooper (**a** R.W. Dunn s.n. from cultivated material; **b** *J.L. Clark* et al. *10006*; **c** *J.L. Clark* et al. *6906*; **d** *J.L. Clark 10006*.**e**, **f** = From Rio Palenque Science Center, Ecuador, No voucher specimen; **g** = El Pahuma Orchid Reserve, Ecuador)

The remarkable array of corolla shapes and colors (Fig. 1) across *Drymonia* has resulted in a confusing taxonomic history. The traditional or pre-phylogenetic circumscription of *Drymonia* was limited to species with campanulate corollas (Wiehler 1983; Moore 1955), as featured in Fig. 1a, b. However, more recent molecular phylogenetic work demonstrated that the genus was paraphyletic and necessitated the transfer of species into *Drymonia* from other genera, including discordant taxa that were previously in *Alloplectus* Mart., *Nautilocalyx* Linden ex Hanst., and *Paradrymonia* Hanst. (Clark 2005; Clark et al. 2006, 2012). These changes resulted in the addition of many species with different corolla shapes, making *Drymonia* one of the most morphologically variable clades in the family. The convoluted taxonomic history of *Drymonia* demonstrates why relying on floral traits can be problematic as the basis for generic circumscriptions. A classification system based on pollination syndromes, or convergent floral adaptations to different pollinator types (Faegri and van der Pijl 1979; Fenster et al. 2004), will not necessarily reflect phylogenetic relationships. In the case of *Drymonia*, the campanulate corolla and 'salt-shaker-anthers' likely represent adaptations to bee pollination (Wiehler 1983; Steiner 1985). Molecular phylogenies were important precursors to studies such as the present on *Drymonia* because they helped define monophyletic units. In contrast, traditional classifications exemplified by *Drymonia* would recognize most of the non-bee pollinated flowers in other genera (e.g., *Alloplectus, Paradrymonia,* or *Nautilocalyx*).

Another good example of an artificial circumscription is the gesneriad genus *Hypocyrta* Mart. The genus is no longer recognized, but mentioning it here helps understand the evolutionary plasticity of corolla shapes in the Gesneriaceae, as the defining character of *Hypocyrta* was the corolla shape: specifically, a constricted flower opening and throat, with a large, expanded pouch on the lower surface (Fig. 1e, f). Some of the 44 species previously classified as *Hypocyrta* are now classified in *Drymonia*, and the rest nest in seven other genera including *Besleria* L., *Codonanthe* (Mart.) Hanst., *Corytoplectus* Oerst., *Nematanthus* Schrad., *Pachycaulos* J.L. Clark and J.F. Sm., *Paradrymonia*, and *Pearcea* Regel. Phylogenetic methods based on molecular sequence data have greatly facilitated the classification of the family by discarding artificially recognized genera such as *Hypocyrta* and defining monophyletic genera that are morphologically diverse such as *Drymonia*.

In the present paper, we combine ecological and phylogenetic approaches to evaluate the evolution of corolla shapes across *Drymonia*. Flowers in the genus can be classified into four general shapes based on the width of the corolla tube and the flower opening. Bell-shaped (campanulate) corollas have a relatively constant width from the base through the throat and flower opening (5.6–17.0 mm), with lobes flaring out around the opening (Fig. 1a, b). Most *Drymonia* flowers with bell-shaped corollas are yellowish-green to white, and they tend to have fimbriate margins on the lobes. Pouched (hypocyrtoid) corollas are defined by a constricted throat and flower opening (3.0–4.2 mm) and an often greatly expanded pouch on the lower surface (9.1–15.4 mm, Fig. 1e, f). Pouched corollas tend to have yellow tubes that contrast with bright reds on the lobes. Urn-shaped (urceolate) corollas are apically constricted like pouched corollas (to a narrowest corolla width of 4.0–4.8 mm), but lack the ventral pouch (Fig. 1g, h). Finally, laterally compressed corollas have throat widths of approx. 4.0–10.0 mm, similar to those of bell-shaped corollas, but the flower openings appear pinched into narrow "key-holes" (3.0–4.5 mm wide; Fig. 1c, d).

We hypothesize that open bell-shaped flowers are pollinated primarily by bees (Fig. 2e, f), while the three constricted flower shapes (pouched, urn-shaped, and laterally compressed) are pollinated primarily by hummingbirds (Fig. 2g) and that constricted flower openings represent adaptations to prevent access by bees to nectar and pollen. Grant and

Grant (1968) originally proposed that such an association between narrow openings and hummingbird flowers serves to reduce bee visitation. Here we evaluate directionality of shifts in corolla shape and provide an initial assessment of pollination syndromes by developing a species-level phylogeny of the genus, recording pollinators of focal flowers for each of the shape classes, and surveying the literature for additional pollination records. We also map shifts in anther dehiscence (poricidal vs. longitudinal) and discuss implications for pollination.

Materials and methods

Taxon sampling and outgroup selection

Fifty-nine species were sequenced for the *trn*K-*mat*K of plastid DNA (cpDNA), the internal transcribed spacer (ITS) region and the external transcribed spacer (ETS) region of 18S-26S of nuclear ribosomal DNA (nrDNA). The ingroup included 50 of 75 *Drymonia* species. This research represents the most comprehensive phylogenetic taxon sampling to date for *Drymonia*. Most species were photographed in the field and determinations were verified with herbarium voucher specimens, photographs, and literature. The study of type specimens was necessary for the identification of many *Drymonia* species and was carried out in conjunction with an ongoing monographic revision. Some *Drymonia* species, such as *D. serrulata*, are common roadside weeds in South and Central America, but most are local endemics that are only found in intact forests. Extensive fieldwork for the present study was necessary because many *Drymonia* in the analyses are only known from one or two localities (e.g., *D. decora* J.R. Clark and J.L. Clark, *D. ignea* J.L. Clark, *D. peltata* (Oliver) H.E. Moore, *D. submarginalis* Gómez-Laurito and Chavarría, and others). All taxa have fertile voucher specimens archived at the Smithsonian Institution's U.S. National Herbarium (US) and The University of Alabama Herbarium (UNA). A complete list of samples, voucher specimens with locality, and GenBank accession numbers is provided in Appendix 1.

Outgroup samples were chosen on the basis of previous phylogenetic studies of Gesneriaceae and Episcieae (=subtribe Columneinae) (Clark et al. 2006, 2012). Given our focus on *Drymonia*, we limited outgroups to species in closely related genera from the core Episcieae clade outlined in Clark et al. (2012). Specifically, we used *Alloplectus aquatilis* C.V. Morton, *Columnea* (2 spp.), *Corytoplectus congestus* (Linden ex Hanst.) Wiehler, *Crantzia cristata* (L.) Scop. ex Fritsch, *Glossoloma* (2 spp.), *Neomortonia rosea* Wiehler, and *Pachycaulos nummularia* (Hanst.) J.L. Clark and J.F. Sm. (Appendix 1).

DNA extraction, amplification, and sequencing

Most genomic DNAs were isolated from silica-dried leaf material collected in the field. Leaf samples were ground using a ThermSavant FastPrep FP120 cell disrupter (Qbiogene, Carlsbad, CA). DNA was isolated using the Qiagen DNeasy™ DNA isolation kit (Qiagen, Valencia, CA).

Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5HP (Suh et al. 1993) and ITS4 (White et al. 1990). Additionally, the reverse and forward of the internal primers ITS2 and ITS3 (White et al. 1990) were used to obtain double stranded DNA sequence of the entire ITS region. Templates of the nrDNA external transcribed spacer region (ETS) were prepared using the primers18S-ETS (Baldwin and Markos

1998) and ETS-B developed for *Mimulus* (Phyramaceae) by Beardsley and Olmstead (2002). Templates of the cpDNA *trn*K-*mat*K were prepared using primers *trn*K1 (CTAACTCAACGGTAGAGTACTCG) and *mat*K (CTCCTGAAAGATAAGTGG).

Polymerase chain reaction (PCR) amplifications followed the procedures described by Baldwin et al. (1995) utilizing Taq DNA polymerase (Promega, Madison, WI). To reduce within-strand base pairing that can result in interference with Taq polymerase activity, we found it essential to use 5 % DMSO and 5 % BSA in PCR reactions for ETS and ITS. The PCR products were electrophoresed using a 1.0 % agarose gel in 1× TBE (pH 8.3) buffer, stained with ethidium bromide to confirm a single product, and purified using PEG 8000 (polyethylene glycol) in 2.5 M NaCl under the conditions described in Johnson and Soltis (1995). Direct cycle sequencing of purified template DNAs was performed by the Nevada Genomics Center (University of Nevada, Reno, NV).

DNA chromatograms were proofed, edited, and contigs were assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI). Sequences were deposited in GenBank (Appendix 1).

Alignment

All sequences were aligned in the multiple sequence alignment program MUSCLE (Edgar 2004). Because the sequences were not highly divergent in the ingroup (i.e., *Drymonia*), it was possible to make minor adjustments to minimize overlapping gaps. This approach allowed for single-site and multiple-site gaps to be treated with equal weight (Simmons and Ochoterena 2000). All regions were easily aligned and none were excluded from the analyses.

Assignment of corolla shape and assessment of anther dehiscence

The corollas of *Drymonia* were assigned to one of the following four corolla shapes: (1) bell-shaped (campanulate; Fig. 1a, b), (2) laterally compressed (Fig. 1c, d), (3) pouched (hypocyrtoid; Fig. 1e, f), and (4) urn-shaped (urceolate; Fig. 1g, h). Anthers were assigned as poricidally dehiscent (Fig. 2a) or longitudinally dehiscent (Fig. 2b). Some species with urn-shaped and laterally compressed corollas present an initial poricidal stage, which then rapidly develops into longitudinal dehiscence; we coded these as longitudinal in the character state reconstructions. Each taxon was carefully evaluated in the field or from herbarium specimens. All corolla shapes were photographed with images readily available on the first author's website (www.gesneriads.ua.edu).

Drymonia is strongly supported as nesting in a clade with *Glossoloma, Columnea, Alloplectus*, and *Neomortonia rosea* (Clark et al. 2006, 2013). Compared to *Drymonia,* the flowers of *Columnea, Glossoloma,* and *Alloplectus* have a more elongate tubular or bilabiate corolla and are not readily assigned to one of the four shapes outlined above. The corolla shapes of *Neomortonia rosea, Pachycaulos nummularia*, and *Corytoplectus congestus* could be assigned to one of the above corolla shapes, but it would be conjectural to evaluate them in a phylogenetic context here because they are not the focus of the present study and including them would require extensive taxon sampling of additional outgroups. Characters were unordered (Fitch 1971) and character evolution analyses were performed in Mesquite, version 4.08 (Maddison and Maddison 2011) where parsimony optimization using the unordered states assumption was implemented. The character states were mapped onto the Bayesian consensus tree obtained in the molecular analyses (Fig. 3).

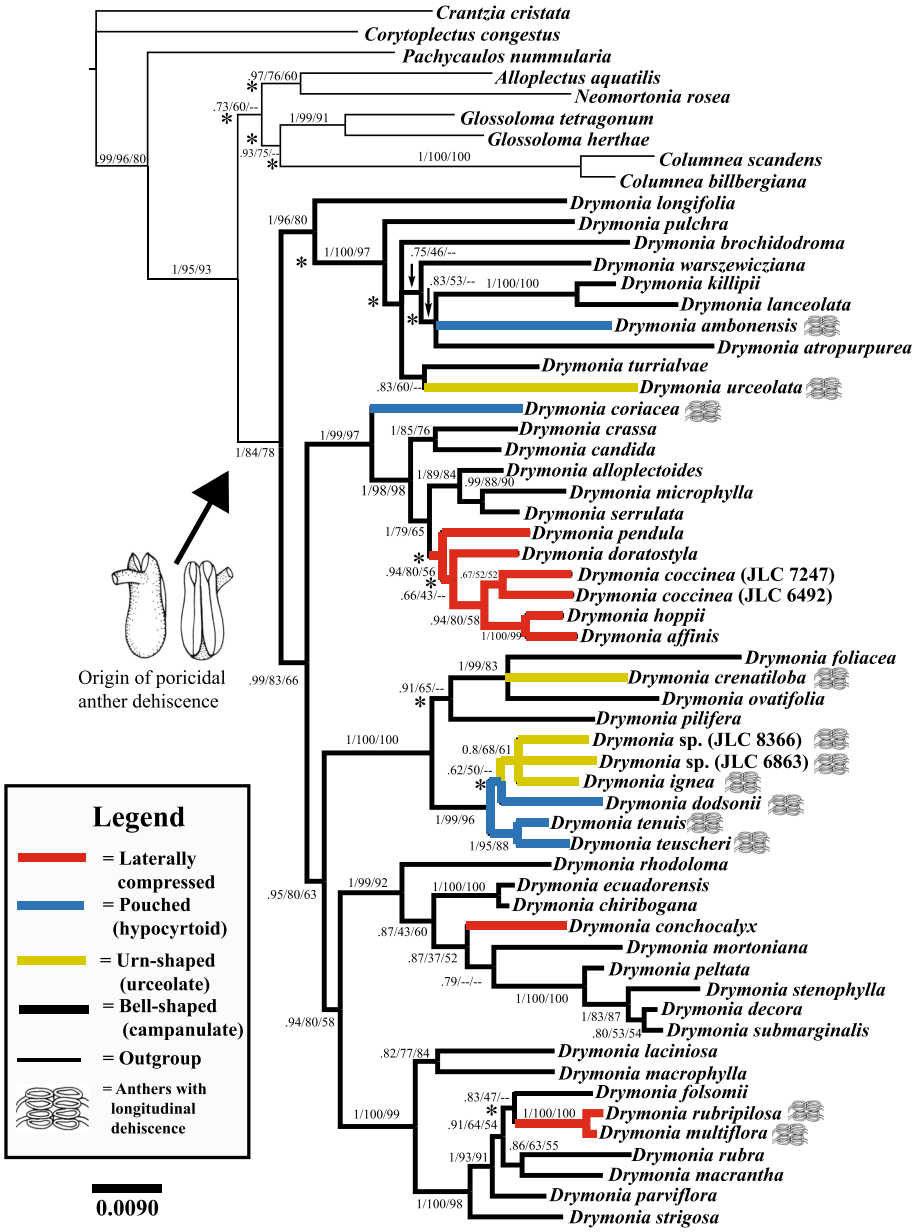

Fig. 3 Majority rule Bayesian inference tree shown with support values indicated for Maximum likelihood bootstrap and parsimony bootstrap. Based on three molecular markers (ITS, ETS and *trn*K-*mat*K spacer). Numbers correspond to Bayesian posterior probabilities/maximum likelihood bootstrap/parsimony bootstrap. Nodes that collapse in the strict consensus tree are indicated by ("*") at the base of the branch. Independent origins of longitudinal anther dehiscence (ingroup only) is shown by diagrams to right of taxon names. All other in-group taxa have poricidal anther dehiscence

Test of incongruence

The incongruence length difference test (ILD: Farris et al. 1994) was performed as the partition homogeneity test implemented in PAUP*4.0 b10 (Swofford 2003) with 1,000 bootstrap replicates (using a heuristic search, simple addition, and no branch swapping). The cpDNA, ITS, and ETS were each treated as separate partitions. As the ILD has been shown to indicate incongruence where none exists (Dolphin et al. 2000; Yoder et al. 2001; Barker and Lutzoni 2002; Dowton and Austin 2002), bootstrap analyses were performed on each partition separately to assess areas of conflict and to determine if any conflict was strongly supported (Mason-Gamer and Kellogg 1996; Seelanen et al. 1997).

Phylogenetic analyses

The parsimony analysis was performed to completion using a two stage heuristic search in PAUP* 4.0 (Swofford 2003). The first stage of the analysis was done using the following settings: 1,000 random addition cycles, holding 10 trees of equal length at each step; tree bisection-reconstruction (TBR) branch swapping with no more than 10 trees saved for each rep; MULTREES option not in effect. The second stage of the analysis was performed on all trees in memory with the same settings, but with the MULTREES option in effect. Two searches with altered settings did not find shorter trees; these included a search with 10 random addition cycles holding 1,000 trees at each step, and one with 1,000 random addition cycles holding 100 trees at each step.

Additional tree searches were done using the parsimony ratchet analysis with NONA (Goloboff 1999) and Winclada (Nixon 2002). Ten separate tree searches were conducted using the following settings: 200 iterations per search, one tree held for each iteration, 132 characters sampled (10 % of the total), and amb = poly-(only considers unambiguous support). The total evidence analysis was swapped to completion, but analyses of individual datasets were limited to 100,000 trees. Multiple ratchet searches were performed in WinClada as suggested by Nixon (1999) since the ratchet option can sometimes get stuck on suboptimal "islands" and it is therefore better to perform more separate searches with fewer iterations than one larger search with more iterations.

Clade robustness was evaluated in PAUP* with a bootstrap analysis (Felsenstein 1985). We used 1,000 heuristic bootstrap replicates with the following settings: 10 random addition cycles; tree bisection-reconstruction (TBR) branch swapping with no more than 10 trees saved for each replicate.

The parsimony analyses and clade support were evaluated for each individual dataset (ETS, ITS, *trn*K-*mat*K), a combined molecular dataset, and a total evidence analysis. Conflict between datasets was evaluated by comparing incongruence of strongly supported clades from individual datasets (e.g., ITS vs. ETS; ITS vs. *trn*K-*mat*K; and nrDNA vs. *trn*K-*mat*K).

Bayesian inference analyses were conducted using MrBayes 3.2.2. (Ronquist et al. 2012) implemented in the CIPRES web portal (http://www.phylo.org/; Miller et al. 2009). Models of nucleotide substitution for each partition were assessed with JModeltest 2.1.3. (Darriba et al. 2012). The best-fit models, selected using the Akaike information criterion (AIC), were TPM2uf + G for ETS, GTR + I + G for ITS, and GTR + G for *trnK-matK*. Two independent Markov Chain Monte Carlo (MCMC) analyses, each with 20 million generations, were run. The analyses were started from random trees, sampling each 1000th generation. Convergence of the two independent MCMC runs was analyzed in Tracer v1.5 (Rambaut and Drummond 2007) and the first 25 % of trees were discarded as burn in

before the posterior distribution was sampled. The remaining trees from both runs were used to construct a majority-rule consensus tree, and the posterior probability (PP) values were used as indicators of robustness.

Maximum Likelihood analyses were conducted in RaxML v7.6.6 (Stamatakis 2006; Stamatakis et al. 2008) implemented in CIPRES web portal (http://www.phylo.org/; Miller et al. 2009), and clade support was estimated by performing bootstrap with 1,000 replicates.

Ancestral reconstruction

Standard parsimony character optimization was implemented in Mesquite 2.75 (Maddison and Maddison 2011) to reconstruct the ancestral state for corolla tube shape and anther dehiscence. For the reconstruction we used the BI consensus topology derived from the total evidence data set, and considered the characters unordered and equally weighted. This method finds the ancestral states that minimize the number of changes required to explain the distribution of character states observed on the phylogeny (Maddison and Maddison 2011).

Flower visitation and pollination modes

To identify floral visitors, flowers from the six species of *Drymonia* were videotaped with Sony Digital Camcorders. With three cameras, we were able to videotape three different flowers (from three different plants) at any given time. Each camera was placed on a tripod approximately 2 m away from the focal flower and covered with a modified 2-L plastic bottle to protect it from rain. Flower visitor surveys were selected to focus on at least one species for each of the four different corolla shapes of *Drymonia* (Table 2). Examples of each corolla shape are shown in Fig. 1 and the coding of corolla shape for the entire matrix is provided in Table 1. The following six *Drymonia* species were videotaped: *Drymonia affinis* (Mansf.) Wiehler and *D. hoppii* (Mansf.) Wiehler (laterally compressed); *D. dodsonii* (Wiehler) J.L. Clark *and D. tenuis* (Benth.) J.L. Clark (pouched); *D. ecuadorensis* Wiehler (bell-shaped); and *D. urceolata* Wiehler (urn-shaped). A total of 164 h of filming (16–48 h per taxon) were performed in 2009, 2010, and 2011 in three localities in Ecuador. Visits were considered legitimate when the visitor entered the corolla and made contact with the anthers (Table 1).

Results

DNA sequencing and alignment

Amplifications were successful for all regions for all individuals, with some exceptions (Appendix 1). Of the three regions sampled, ITS provided the most parsimony-informative substitutions (142 or 51 % of the combined three regions; Appendix 2). The *trn*K-*mat*K spacer provided the least number of parsimony-substitutions (20 or 7.2 % of the combined three regions; Appendix 2). The aligned matrix for the full analysis contained 1,689 basepairs; of these, 1,176 were constant and 235 were uninformative. The outgroups of the analysis contributed 53 of the 278 parsimony informative substitutions. There were no ambiguously aligned sites excluded from the analysis. Sequence divergence in the ingroup was relatively conserved and no informative indels required scoring. The complete list of gene regions and statistics is provided in Appendix 2.

Table 1 Pollinator survey information outlining taxon, distribution, source of observation (literature citation if not generated for this study), and corolla shape

Taxon	Distribution	Source of observation	Pollinator	Corolla shape
Ingroup (50)				
Drymonia affinis (Mansf.) Wiehler	Central and South America	This study (Ecuador)	Bird	Laterally compressed
Drymonia alloplectoides Hanst.	Central and South America			Campanulate
Drymonia ambonensis (L.E. Skog) J.L. Clark	Central America			Hypocyrtoid
Drymonia atropurpurea Clavijo and J.L. Clark	Ecuador and Colombia			Campanulate
Drymonia brochidodroma Wiehler	Ecuador and Colombia			Campanulate
Drymonia candida Hanst.	Bolivia, Brazil, Colombia, Ecuador, Peru			Campanulate
Drymonia chiribogana Wiehler	Ecuador			Campanulate
Drymonia coccinea (Aubl.) Wiehler	South America			Laterally compressed
Drymonia coccinea (Aubl.) Wiehler	South America			Laterally compressed
Drymonia conchocalyx Wiehler	Central America	Feinsinger et al. (1987)	Bird	Laterally compressed
Drymonia coriacea (Oerst. ex Hanst.) Wiehler	Central and South America			Hypocyrtoid
Drymonia crassa C.V. Morton	Colombia and Venezuela			Campanulate
Drymonia crenatiloba (Mansf.) Wiehler	Ecuador			Urceolate
Drymonia decora J.R. Clark and J.L. Clark	Costa Rica			Campanulate
Drymonia dodsonii (Wiehler) J.L. Clark	Colombia and Ecuador	This study (Ecuador)	Bird	Hypocyrtoid
Drymonia doratostyla (Leeuwenb.) Wiehler	Bolivia and Peru			Laterally compressed
Drymonia ecuadorensis Wiehler	Ecuador	This study (Ecuador)	Bee	Campanulate
Drymonia foliacea (Rusby) Wiehler	South America			Campanulate
Drymonia folsomii L.E. Skog	Costa Rica and Panama			Campanulate
Drymonia hoppii (Mansf.) Wiehler	South America	This study (Ecuador)	Bird	Laterally compressed
Drymonia ignea J.L. Clark	Ecuador			Urceolate
Drymonia killipii Wiehler	Colombia and Ecuador			Campanulate
Drymonia laciniosa Wiehler	Ecuador and Peru			Campanulate
Drymonia lanceolata (Hanst.) C.V. Morton	Central and South America			Campanulate
Drymonia longifolia Poepp.	South America			Campanulate

Table 1 continued

Taxon	Distribution	Source of observation	Pollinator	Corolla shape
Drymonia macrantha (Donn. Sm.) D.N. Gibson	Central America			Campanulate
Drymonia macrophylla (Oerst.) H.E. Moore	Central and South America			Campanulate
Drymonia microphylla Wiehler	Panama			Campanulate
Drymonia mortoniana (Oerst.) H.E. Moore	Costa Rica			Campanulate
Drymonia multiflora (Oerst. ex Hanst.) Wiehler	Central America	Stiles and Freeman (1993)	Bird	Laterally compressed
Drymonia ovatifolia J.L. Clark	Central and South America	Enrique (1998)	Bee	Campanulate
Drymonia parviflora Hanst.	Costa Rica and Panama			Campanulate
Drymonia peltata (Oliver) H.E. Moore	Costa Rica			Campanulate
Drymonia pendula (Poepp.) Wiehler	South America			Laterally compressed
Drymonia pilifera Wiehler	Costa Rica and Panama			Campanulate
Drymonia pulchra Wiehler	Ecuador			Campanulate
Drymonia rhodoloma Wiehler	Colombia and Ecuador			Campanulate
Drymonia rubra C.V. Morton	Costa Rica and Panama			Campanulate
Drymonia rubripilosa Wiehler	Costa Rica			Laterally compressed
Drymonia serrulata (Jacq.) Mart.	Central and South America	Steiner (1985)	Bee	Campanulate
Drymonia stenophylla (Donn. Sm.) H.E. Moore	Central America			Campanulate
Drymonia strigosa (Oerst.) Wiehler	Central America	Enrique (1998)	Bee	Campanulate
Drymonia submarginalis Gómez-Laur. and M.M. Chavarría	Costa Rica and Nicaragua			Campanulate
Drymonia tenuis (Benth.) J.L. Clark	Colombia and Ecuador	This study Dziedzioch et al. (2003)	Bird	Hypocyrtoid
Drymonia teuscheri (Raymond) J.L. Clark	South America	Dziedzioch et al. (2003)	Bird	Hypocyrtoid
Drymonia turrialvae Hanst.	Central and South America	Dressler (1968)	Bee	Campanulate
Drymonia urceolata Wiehler	South America	This study Dziedzioch et al. (2003)	Bird	Urceolate
Drymonia warszewicziana Hanst.	Central and South America			Campanulate
Drymonia sp. 1—JLC 6863	Venezuela			Urceolate
Drymonia sp. 2—JLC 8366	Ecuador			Urceolate

Tests of incongruence

The incongruence length difference (ILD) tests found no significant discordance between the ITS and ETS datasets ($P = 0.100$) or between the combined nrDNA (ITS concatenated with ETS) and *trn*K-*mat*K (cpDNA) datasets ($P = 1.00$ and 0.500 respectively). Therefore, we combined these three datasets in a total evidence analysis.

Phylogenetic analyses

The parsimony analysis for the combined dataset resulted in 208 trees of length 1,230 (CI = 0.55, RI = 0.66, and RC = 0.36). The strict consensus of these trees is congruent with the tree shown in Fig. 3. Minor exceptions include polytomies for some of the crown clades. Clades that collapse in the strict consensus tree that are resolved in ML or BI are indicated by asterisks in Fig. 3.

Pollination modes

Results of videotaping were consistent with an association between constricted corollas and hummingbird pollination (Table 1). The species with bell-shaped corollas, *D. ecuadorensis*, was visited solely by euglossine bees (Fig. 2e, f) at a rate of 3.29 visits per hour. No bee visits were recorded to the five species with constricted openings (urn-shaped, pouched, and laterally compressed), while hummingbirds visited these flowers at rates of 0.04 to 0.36 visits per hour. Literature surveys further support this pattern; bees have been observed pollinating the bell-shaped *D. aciculata* Wiehler (observations by Dressler, cited in Steiner 1985), *D. serrulata* (Steiner 1985), *D. strigosa* (Enrique 1998), *D. turrialvae* Hanst. (Dressler 1968), and *D. ovatifolia* J.L. Clark (as *Nautilocalyx panamensis* (Seem.) Seem.; Enrique 1998), while hummingbirds have been observed pollinating the laterally compressed *D. conchocalyx* Hanst. (Feinsinger et al. 1987) and *D. multiflora* (Oerst. ex Hanst.) Wiehler (Stiles and Freeman 1993) as well as the pouched *D. teuscheri* (Raymond) J.L. Clark (Dziedzioch et al. 2003).

Discussion

Bayesian, maximum likelihood, and parsimony analyses all resulted in congruent topologies with high levels of node support, and corolla shapes were unambiguously optimized on the inferred phylogeny for *Drymonia* (Fig. 3). This optimization strongly supports the convergent evolution of corolla shapes in *Drymonia*, with multiple independent origins of the three types of constricted corolla tubes from campanulate ancestors (cf., legend in Fig. 3).

Traditional circumscription of *Drymonia*

The results presented here are congruent with previous phylogenetic studies that support the non-monophyly of traditional *Drymonia* (Clark et al. 2006; 2012). The traditional circumscription of *Drymonia* is artificial because it relies on corolla shape and anther dehiscence, traits that reflect pollination syndrome rather than evolutionary relatedness. Below we discuss shifts from campanulate corollas to each of the three types of constricted corollas in greater detail.

Pollination modes

Results of the pollinator observations and literature survey support the hypothesis that corolla constriction evolved multiple times in association with bird pollination. Species with the ancestral bell-shaped corollas are pollinated by bees, while species with pouched, urn-shaped, or laterally compressed corollas are pollinated by hummingbirds (Table 1). Euglossine and *Epicharis* bees found to pollinate bell-shaped *Drymonia* have thorax widths of 5–10 mm (Steiner 1985). The narrow openings of pouched (3.0–4.2 mm), urn-shaped (4.0–4.8 mm), and laterally compressed flowers (3.0–4.5 mm at the top of the throat and 1.0–2.5 mm near the base of the throat) effectively prevent access by bees to the pollen and nectar rewards of these flowers, while visitation results demonstrate they are not narrow enough to serve as barriers to hummingbird bills. Thus narrow corollas in *Drymonia* serve as a 'barrier trait', preventing or reducing visitation by bees and other insects.

We hypothesize that selection to reduce loss of pollen and nectar to insects was the main driver of evolutionary narrowing of corollas. Alternatively, constricted corollas may have evolved primarily to better guide hummingbird bills and increase precision and consistency of pollen placement. Temeles et al. (2002) demonstrated that flower width is an important factor when considering the coevolution and specialization of hummingbirds and flowers (also see Grant and Temeles 1992, Muchhala 2007). Future experimental work would be useful in evaluating the relative importance of bill-corolla fit vs. insect exclusion in the repeated evolution of constricted corollas across *Drymonia*.

Along with corolla shape, the shifts in primary pollinators across *Drymonia* are also associated with changes in anther dehiscence. The ancestral condition of poricidal dehiscence ("salt-shaker anthers") is independently lost in six clades, each of which is also associated with constricted corollas and hummingbird pollination (Fig. 3). For some species in these clades, including *D. urceolata* (Fig. 2c, d), *D. rubripilosa* Kriebel and *D. multiflora,* an initial poricidal stage can be detected before anthers fully rupture via longitudinal dehiscence (prior to anthesis). The presence of a vestigial poricidal dehiscence stage further supports the conclusion that these represent recent shifts from ancestors with poricidal anthers. We suggest that the shifts in anther dehiscence represent adaptations to more effectively present pollen to each pollinator type. To maximize male fitness, selection should favor placing specific amounts of pollen on each visitor, with the optimal dose size depending on visitation frequency and visitor behavior (Harder and Thomson 1989; Thomson and Thomson 1992; Castellanos et al. 2006). Thus, because bees tend to have high visit rates and frequently groom excess pollen off their bodies (Harder 1990), bee-pollinated plants should present their pollen in numerous small doses to as many bees as possible, while for hummingbirds, pollen would be more effectively dispersed in fewer, larger doses (Thomson et al. 2000, Castellanos et al. 2006). In line with this scenario, the hummingbird-adapted *Drymonia* species with narrow corollas have much lower visitation rates than the bee-adapted *D. ecuadorensis* (Table 2). The 'salt-shaker' poricidal anthers in *Drymonia* likely evolved to slowly dose pollen to multiple bees, while also avoiding 'over-dosing' individual bees and triggering grooming behavior. Longitudinally-dehiscent anthers present few, larger doses to the infrequent hummingbird visitors.

Pouched corollas

Pouched corollas (Fig. 1e) have three independent origins within *Drymonia* (Fig. 3); one in Central America and two in the northern Andes of South America (Fig. 3). Additional

Table 2 Results from videotaping flower visitors to six *Drymonia* species representing four corolla shapes

Drymonia species	Corolla shape	Number of plants filmed	Total hours filmed	Total visits		Visits per hour	
				Bees	Birds	Bees	Birds
D. affinis	Laterally compressed	3	16	0	1	0	0.06
D. dodsonii	Hypocyrtoid	4	47.7	0	17	0.00	0.36
D. ecuadorensis	Campanulate	3	32.5	107	0	3.29	0.00
D. hoppii	Laterally compressed	3	17	0	1	0	0.06
D. tenuis	Hypocyrtoid	5	26.7	0	1	0.00	0.04
D. urceolata	Urceolate	4	24	0	3	0	0.13

independent origins of pouched corollas occur in other New World genera such as *Besleria, Columnea, Gasteranthus*, *Nematanthus, Pachycaulos,* and *Paradrymonia.*

The possible adaptive function of the enlarged pouches at the base of the corolla is unclear. One possibility is that they may serve as an 'overflow chamber' for the accumulation of nectar (sensu Wolf and Stiles 1989), however we consider this unlikely as we have never found nectar in the pouches when flowers were dissected in the field. Wiehler (1983) suggested that pouches serve as a "target enlargement;" an increased visual display that aids in long-distance attraction of hummingbirds. Many *Drymonia* inflorescences include brightly-colored bracts, thus the pouches could also function to create a 'bi-colored display' (sensu Willson and Thompson 1982).

Urn-shaped corollas

There are three independent origins of urn-shaped corollas (Fig. 1g, h) in *Drymonia* and they are all located in South America (Fig. 3). It is noteworthy that urn-shaped corollas share recent common ancestors with bell-shaped taxa (e.g., *Drymonia turrialvae* and *D. foliaceae* + *D. ovatifolia*) and pouched taxa (e.g., *D. dodsonii, D. tenuis,* and *D. teuscheri*). This study sampled nearly all of the currently known species of *Drymonia* with urn-shaped corollas, including two species that are potentially new to science (JLC 8366 and JLC 6863).

Laterally compressed corollas

Laterally compressed corollas (Fig. 1c, b) within *Drymonia* have three independent origins; one in South America and two in Central America (Fig. 3). The clade that includes *Drymonia rubripilosa* and *D. multiflora* comprises species from Central America, and *D. conchocalyx* is also from Central America. The clade that includes *Drymonia pendula, D. doratostyla, D. coccinea, D. hoppii* and *D. affinis* comprises species from the western slopes of the northern Andes and western Amazonia. The South American clade of laterally compressed flowers is the only example in the genus where constricted corollas have retained the ancestral condition of poricidal anther dehiscence. All of the species with laterally compressed corollas are epiphytic and most have brightly colored bracts relative to sister-clades. Laterally compressed corollas are also found in most species of *Glossoloma* (Clark 2009) and in *Nematanthus*, for which hummingbird pollination is well documented (Franco and Buzato 1992; Sazima et al. 1995; Buzato et al. 2000; San Martin-Gajardo and Santana Vianna 2010).

Acknowledgments The authors would like to thank Alex Monro from The Natural History Museum in London (BM) for sharing leaf material of Gesneriaceae. This study was supported by grants from the National Science Foundation (DEB-0949270 and DEB-0841958 to JLC). Fieldwork was greatly facilitated by the following undergraduate students from The University of Alabama: Cassandra L. Coleman, Seema Kumar, and Laura A. Frost. Lucas McDonald from Hillcrest High School (Tuscaloosa, AL) also helped in collecting data during a 2011 expedition to Ecuador. Murray Cooper and Richard W. Dunn are gratefully acknowledged for contributing images. We express our appreciation to two anonymous reviewers for useful comments that improved an earlier version of the manuscript.

Appendix 1

See Table 3.

Table 3 Specimens sequenced in molecular phylogenetic study of *Drymonia* and closely related congeners with voucher specimen, institution and GenBank accession numbers for ITS, ETS, and *trn*K-*mat*K

Taxon	Voucher	Locality	ITS	ETS	*trnK-matK*
Ingroup					
Drymonia affinis (Mansf.) Wiehler	L. Clavijo 1560 (COAH)	Colombia	KM079423	KM079416	KM079491
Drymonia alloplectoides Hanst.	J.L. Clark 10049 (US)	Cultivated (Costa Rica)	KM079424	KM079422	KM079498
Drymonia ambonensis (L.E. Skog) J.L. Clark	J.L. Clark 8600 (US)	Panama	DQ211134	KM079399	KM079474
Drymonia atropurpurea Clavijo and J.L. Clark	L. Clavijo 1689 (COL)	Colombia	KM079425	KM079392	KM079467
Drymonia brochidodroma Wiehler	J.L. Clark 7360 (US)	Ecuador	DQ211166	KM079396	KM079471
Drymonia candida Hanst.	J.L. Clark 8341 (US)	Ecuador	DQ211131	KM079413	KM079488
Drymonia chiribogana Wiehler	J.L. Clark 7358 (US)	Ecuador	DQ211149	KM079385	KM079460
Drymonia coccinea (Aubl.) Wiehler	J.L. Clark 6492 (US)	Ecuador	DQ211132	KM079419	KM079495
Drymonia coccinea (Aubl.) Wiehler	J.L. Clark 7247 (US)	Ecuador	KM079426	KM079421	KM079497
Drymonia conchocalyx Wiehler	J.L. Clark 6276 (US)	Costa Rica	AF543261	KM079384	KM079459
Drymonia coriacea (Oerst. ex Hanst.) Wiehler	J.L. Clark 6590 (US)	Cultivated (Ecuador)	DQ211129	KM079414	KM079489
Drymonia crassa C.V. Morton	J.L. Clark 6865 (US)	Venezuela	KM079427	KM079412	KM079487
Drymonia crenatiloba (Mansf.) Wiehler	J.L. Clark 5462 (US)	Ecuador	AF543259	KM079375	KM079450
Drymonia decora J.R. Clark and J.L. Clark	J.L. Clark 10043 (US)	Cultivated (Costa Rica)	KM079428	KM079389	KM079464
Drymonia dodsonii (Wiehler) J.L. Clark	J.L. Clark 6205 (US)	Ecuador	AF543256	KM079379	KM079454
Drymonia doratostyla (Leeuwenb.) Wiehler	J.L. Clark 6783 (US)	Bolivia	DQ211144	KM079418	KM079493
Drymonia ecuadorensis Wiehler	J.L. Clark 6185 (US)	Ecuador	DQ211147	KM079386	KM079461
Drymonia foliacea (Rusby) Wiehler	J.L. Clark 6808 (US)	Bolivia	DQ211138	KM079373	KM079448
Drymonia folsomii L.E. Skog	J.L. Clark 8569 (US)	Panama	KM079429	KM079408	KM079483
Drymonia hoppii (Mansf.) Wiehler	J.L. Clark 5036 (US)	Ecuador	AF543263	KM079417	KM079492
Drymonia ignea J.L. Clark	J.L. Clark 5713 (US)	Ecuador	AF543257	KM079380	KM079455
Drymonia killipii Wiehler	J.L. Clark 7521 (US)	Ecuador	KF040189	KM079402	KM079477
Drymonia laciniosa Wiehler	J.L. Clark 8794 (US)	Ecuador	DQ211126	KM079411	KM079486
Drymonia lanceolata (Hanst.) C.V. Morton	J.L. Clark 8553 (US)	Panama	KF040190	KM079401	KM079476

Table 3 continued

Taxon	Voucher	Locality	ITS	ETS	*trnK-matK*
Drymonia longifolia Poepp.	J.L. Clark 6262 (US)	Ecuador	KF040191	KM079371	KM079446
Drymonia macrantha (Donn. Sm.) D.N. Gibson	J.L. Clark 10055 (SEL)	Cultivated (Costa Rica)	KM079430	KM079405	KM079480
Drymonia macrophylla (Oerst.) H.E. Moore	J.L. Clark 4776 (US)	Ecuador	KM079431	KM079410	KM079485
Drymonia microphylla Wiehler	J.L. Clark 10036 (US)	Cultivated	KM079432	KM079420	KM079496
Drymonia mortoniana (Oerst.) H.E. Moore	J.L. Clark 6585 (US)	Cultivated (Panama, Costa Rica)	KM079433	KM079400	KM079475
Drymonia multiflora (Oerst. ex Hanst.) Wiehler	J.L. Clark 8586 (US)	Panama	DQ211128	KM079406	KM079481
Drymonia ovatifolia J.L. Clark	J.L. Clark 8625 (US)	Panama	DQ211175	KM079376	KM079451
Drymonia parviflora Hanst.	J.L. Clark 8676 (US)	Panama	DQ211148	KM079404	KM079479
Drymonia peltata (Oliver) H.E. Moore	J.L. Clark 6286 (US)	Cultivated (Costa Rica)	DQ211140	KM079387	KM079462
Drymonia pendula (Poepp.) Wiehler	J.L. Clark 8870 (US)	Cultivated (Peru)	DQ211140	KM079415	KM079490
Drymonia pilifera Wiehler	J.L. Clark 8568 (US)	Panama	DQ211137	KM079374	KM079449
Drymonia pulchra Wiehler	J.L. Clark 7245 (US)	Ecuador	KM079434	KM079370	KM079445
Drymonia rhodoloma Wiehler	J.L. Clark 4843 (US)	Ecuador	AF543260	KM079383	KM079458
Drymonia rubra C.V. Morton	A. Monro 4242 (BM)	Costa Rica	KM079435	KM079409	KM079484
Drymonia rubripilosa Wiehler	A. Monro 4911 (BM)	Costa Rica	KM079436	KM079407	KM079482
Drymonia serrulata (Jacq.) Mart.	J.L. Clark 8843 (US)	Cultivated (Central and South America)	DQ211133	KM095651	KM079494
Drymonia stenophylla (Donn. Sm.) H.E. Moore	J.L. Clark 8872 (US)	Cultivated (Panama)	KM079437	KM079388	KM079463
Drymonia strigosa (Oerst.) Wiehler	J.L. Clark 8854 (US)	Cultivated (Mexico)	DQ211143	KM079403	KM079478
Drymonia submarginalis Gómez-Laur. and M.M. Chavarría	J.L. Clark 8871 (US)	Cultivated (Costa Rica)	DQ211143	KM079390	KM079465
Drymonia tenuis (Benth.) J.L. Clark	J.L. Clark 4586 (US)	Ecuador	AF543254	KM079381	KM079456
Drymonia teuscheri (Raymond) J.L. Clark	J.L. Clark 8174 (US)	Peru	KM079438	KM079382	KM079457
Drymonia turrialvae Hanst.	J.L. Clark 8552 (US)	Panama	DQ211141	KM079398	KM079473
Drymonia urceolata Wiehler	J.L. Clark 5225 (US)	Ecuador	KF040192	KM079372	KM079447
Drymonia warszewicziana Hanst.	J.L. Clark 8614 (US)	Panama	DQ211127	KM079397	KM079472
Drymonia sp. 1	J.L. Clark 6863 (US)	Venezuela	DQ211142	KM079378	KM079453

Table 3 continued

Taxon	Voucher	Locality	ITS	ETS	*trnK-matK*
Drymonia sp. 2	J.L. Clark 8366 (US)	Ecuador	DQ211130	KM079377	KM079452
Outgroup					
Alloplectus aquatilis C.V. Morton	J. L. Clark 6875 (US)	Venezuela	DQ211110	KM079394	KM079469
Columnea billbergiana Beurl.	J. L. Clark 8630 (US)	Panama	DQ211115	KM079366	KM079441
Columnea scandens L.	J. L. Clark 6541 (US)	Martinique	KM079439	KM079367	KM079442
Crantzia cristata (L.) Scop.	J.L. Clark 6546 (US)	Martinique	DQ211154	KM079365	KM079440
Corytoplectus congestus (Linden ex Hanst.) Wiehler	J.L. Clark 6868 (US)	Venezuela	DQ211162	KM079395	KM079470
Glossoloma herthae (Mansf.) J.L. Clark	J.L. Clark 4958 (US)	Ecuador	AF543230	KM079391	KM079466
Glossoloma tetragonum Hanst.	J.L. Clark 8547 (US)	Panama	DQ211104	KM079393	KM079468
Neomortonia rosea Wiehler	J. L. Clark 7582 (US)	Ecuador	DQ211099	KM079369	KM079444
Pachycaulos nummularia (Hanst.) J.L. Clark and J.F. Sm.	J.L. Clark 6248 (US)	Ecuador	AF543266	KM079368	KM079443

Herbarium acronyms follow Thiers (2013)

Appendix 2

See Table 4.

Table 4 Statistics of ITS, ETS and *trn*K-*mat*K genic regions

Statistic	ETS	ITS	*trn*K-*mat*K	All three regions
Aligned length	462	632	595	1,689
Mean GC content (%)	46.7 (46.8)	58.2 (58.2)	32.7 (32.7)	45.7 (45.7)
Mean pair-wise divergence (%)	8.0 (5.7)	5.7 (5.2)	8.9 (8.8)	4.1 (3.8)
Parsimony uninformative substitutions	87 (82)	86 (71)	62 (52)	235 (205)
Parsimony informative substitutions	116 (87)	142 (120)	20 (18)	278 (225)
Constant characters	259 (293)	404 (441)	513 (525)	1176 (1,259)
Consistency index	.59 (.65)	.50 (.57)	.90 (.90)	.55 (.60)
Retention index	.70 (.76)	.67 (.73)	.86 (.87)	.66 (.72)
Rescaled consistency index	.41 (.50)	.34 (.41)	.77 (.78)	.36 (.43)
Tree length	464 (337)	625 (449)	97 (81)	1,230 (906)

Values in parentheses are for the ingroup only (i.e., *Drymonia*)

References

Baldwin BG, Markos S (1998) Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). Mol Phylogenet Evol 10:449–463

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Missouri Bot Gard 82:247–277

Barker FK, Lutzoni FM (2002) The utility of the incongruence length difference test. Syst Biol 51:625–637

Beardsley PM, Olmstead RG (2002) Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. Am J Bot 89:1093–1102

Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 73–113

Burtt BL, Wiehler H (1995) Classification of the family Gesneriaceae. Gesneriana 1:1–4

Buzato S, Sazima M, Sazima I (2000) Hummingbird-pollinated floras at three Atlantic forest sites. Biotropica 32:824–841

Castellanos MC, Wilson P, Keller SJ, Wolfe AD, Thomson JD (2006) Anther evolution: pollen presentation strategies when pollinators differ. Am Nat 167:288–296

Clark JL (2005) A Monograph of *Alloplectus* (Gesneriaceae). Selbyana 25:182–209

Clark JL (2009) The systematics of *Glossoloma* (Gesneriaceae). Syst Bot Monogr 88:1–128

Clark JL, Herendeen PS, Skog LE, Zimmer EA (2006) Phylogenetic relationships and generic boundaries in the tribe Episcieae (Gesneriaceae) inferred from nuclear, chloroplast, and morphological data. Taxon 55:313–336

Clark JL, Funke MM, Duffy AM, Smith JF (2012) Phylogeny of a Neotropical clade in the Gesneriaceae: more tales of convergent evolution. Int J Plant Sci 173:894–916

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Dolphin K, Belshaw R, Orme CDL, Donald L, Quicke J (2000) Noise and incongruence: interpreting results of the incongruence length difference test. Mol Phylogenet Evol 17:401–406

Dowton M, Austin AD (2002) Increased congruence does not necessarily indicate increased phylogenetic accuracy: the behavior of the incongruence length difference test in mixed-model analyses. Syst Biol 51:19–31

Dressler RL (1968) Pollination by euglossine bees. Evolution 22:202–210

Dziedzioch C, Stevens AD, Gottsberger G (2003) The hummingbird plant community of a tropical montane rain forest in southern Ecuador. Plant Biol 5:331–337
Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797
Enrique ERA (1998) Resources foraged by *Euglossa atroveneta* (Apidae: Euglossinae) at Union Juárez, Chiapas, Mexico. A palynological study of larval feeding. Apidologie 29:347–359
Faegri K, van der Pijl L (1979) The principles of pollination ecology. Pergamon, Oxford
Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10:315–319
Feinsinger P, Beach JH, Linhart YB, Rusby WH, Murray KG (1987) Disturbance, pollinator predictability, and pollination success among Costa Rican cloud forest plants. Ecology 68:1294–1305
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. Annu Rev Ecol Evol S 35:375–403
Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–415
Franco ALM, Buzato S (1992) An assemblage of hummingbird-pollinated flowers in a montane forest in southeastern Brazil. Bot Acta 109:149–160
Fritsch K (1893–1994) Gesneriaceae. In: Engler A, Prantl K (eds) Die Nat. Pflanzenfam., vol 4 (3b). Engelmann, Leipzig, Germany, pp 133–185
Goloboff P (1999) NONA, version 2. Published by the author, Tucumán
Grant KA, Grant V (1968) Hummingbirds and their flowers. Columbia University Press, New York
Grant V, Temeles EJ (1992) Foraging ability of rufous hummingbirds on hummingbird flowers and hawkmoth flowers. Proc Natl Acad Sci 89(20):9400–9404
Hanstein J (1854) Die Gesneraceen des Königlichen Herbariums und der Gärten zu Berlin, nebst Beobachtungen über die Familie im Ganzen I. Abschnitt. Linnaea 26:145–216
Hanstein J (1856) Die Gesneraceen des Königlichen Herbariums und der Gärten zu Berlin, nebst monographischer Uebersicht der Familie im Ganzen, II. Abschnitt. Gattungen und Arten. Erstes Stück. Die Niphaeen und Achimeneen. Linnaea 27:693–785
Hanstein J (1859) Die Gesneraceen des Königlichen Herbariums und der Garten zu Berlin, nebst monographischer Uebersicht der Familie im Ganzen, II. Abschnitt. Gattungen und Arten. Zweites Stück. Die Brachylomateen. Linnaea 29:497–592
Hanstein J (1865) Die Gesneraceen des Königlichen Herbariums und der Gärten zu Berlin, nebst monographischer Uebersicht der Familie im Ganzen, II. Abschnitt. Gattungen und Arten. Drittes Stück. Die Eugesnereen, Rhytidophylleen, und Beslerieen. Linnaea 34:225–462
Harder LD (1990) Pollen removal by bumble bees and its implications for pollen dispersal. Ecology 71:1110–1125
Harder L, Thomson JD (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. Am Nat 133:325–334
Ivanina LI (1965) Application of the carpological method to the taxonomy of Gesneriaceae. Notes Roy Bot Gard Edinburgh 26:383–402
Ivanina LI (1967) The family Gesneriaceae (The Carpological Review). Komarov Bot. Inst, Leningrad, USSR, 126 pp
Johnson LA, Soltis DE (1995) Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. Ann Missouri Bot Gard 82:149–175
Maddison WP, Maddison DR (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org. Accessed March 2014
Martius CFP (1829) Gesneriaceae. Nova Genera et Species Plantarum, vol 3. Impensis auctoris, Munich, pp 27–73
Mason-Gamer RJ, Kellogg EA (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst Biol 45:524–545
Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T (2009) The CIPRES Portals. CIPRES. 2009–08–04. http://www.phylo.org/sub_sections/portal. Accessed March 2014
Möller M, Clark JL (2013) The state of molecular studies in the family Gesneriaceae. Selbyana 31:95–125
Moore HE (1955) *Drymonia macrophylla*. Baileya 3:109–112
Muchhala N (2007) Adaptive tradeoff in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. Am Nat 169:494–504

Nixon KC (1999) The Parsimony Ratchet, a new method for rapid parsimony analysis. Cladistics 15:407–414
Nixon KC (2002) WinClada, version 1.00.08. Published by the author, Ithaca, New York
Rambaut A, Drummond AJ (2007) Tracer v1.4: MCMC trace analyses tool. http://beast.bio.ed.ac.uk/Tracer. Accessed October 25, 2013
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542
San Martin-Gajardo I, Santana Vianna JR (2010) Pollination of *Namatanthus brasiliensis*: an epiphytic Gesneriaceae endemic to the southeastern Atlantic forests of Brazil. Selbyana 30:216–220
Sazima I, Buzato S, Sazima M (1995) An assemblage of hummingbird-pollinated flowers in a montane forest in southeastern Brazil. Bot Acta 109:149–160
Seelanen T, Schnabel A, Wendel JF (1997) Congruence and consensus in the cotton tribe (Malvaceae). Syst Bot 22:259–290
Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381
Smith JF, Atkinson S (1998) Phylogenetic analysis of the tribes Gloxinieae and Gesnerieae (Gesneriaceae): data from *ndhF* sequences. Selbyana 19:122–131
Smith JF, Clark JL (2013) Molecular phylogenetic analyses reveal undiscovered monospecific Genera in the tribe Episcieae (Gesneriaceae). Syst Bot 38:451–463
Smith JF, Wolfram JC, Brown KD, Carroll CL, Denton DS (1997) Tribal relationships in the Gesneriaceae: evidence from DNA sequences of the chloroplast gene *ndhF*. Ann Missouri Bot Gard 84:50–66
Smith JF, Kresge M, Møller M, Cronk QCB (1998) The African violets (*Saintpaulia*) are members of *Streptocarpus* subgenus *Streptocarpella* (Gesneriaceae): combined evidence from chloroplast and nuclear ribosomal genes. Edinb J Bot 55:1–11
Smith JF, Draper SB, Hileman LC, Baum DA (2004a) A phylogenetic analysis within tribes Gloxinieae and Gesnerieae (Gesnerioideae: Gesneriaceae). Syst Bot 29:947–958
Smith JF, Draper SB, Hileman LC, Baum DA (2004b) Evolution of *GCYC*, a Gesneriaceae homolog of *CYCLOIDEA*, within Gesnerioideae (Gesneriaceae). Mol Phylog Evol 31:765–779
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 fast bootstrapping algorithm for the RAxML web-servers. Syst Biol 5:758–771
Steiner KE (1985) The role of nectar and oil in the pollination of *Drymonia serrulata* (Gesneriaceae) by *Epicharis* bees (Anthophorideae) in Panama. Biotropica 17:217–229
Stiles FG, Freeman CE (1993) Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. Biotropica 25:191–205
Suh Y, Thien LB, Reeve HE, Zimmer EA (1993) Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. Am J Bot 80:1042–1055
Swofford DL (2003) PAUP*: phylogenetic analysis using parsimony (* and other methods), Version 4.0. Sinauer Associates, Sunderland, Massachusetts
Temeles EJ, Linhart RB, Masonjones M, Masonjones HD (2002) The role of flower width in hummingbird bill length-flower length relationships. Biotropica 34:68–80
Thiers B (2013) [continuously updated]: Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden. http://sweetgum.nybg.org/ih/. Accessed March 2013
Thomson JD, Thomson BA (1992) Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. In: Wyatt R (ed) Ecology and evolution of plant reproduction: new approaches. Chapman and Hall, New York, pp 1–24
Thomson JD, Wilson P, Valenzuela M, Malzone M (2000) Pollen presentation and pollination syndromes, with special reference to *Penstemon*. Plant Species Biol 15:11–29
Weber A, Clark JL, Möller M (2013) A new formal classification of Gesneriaceae. Selbyana 31:68–94
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322
Wiehler H (1973) 100 Transfers from *Alloplectus* and *Columnea* (Gesneriaceae). Phytologia 27:309–329
Wiehler H (1983) A synopsis of the Neotropical Gesneriaceae. Selbyana 6:1–219
Willson MF, Thompson JN (1982) Phenology and ecology of color in bird-dispersed fruits, or why some fruits are red when they are "green"? Can J Bot 60:701–713
Wolf LL, Stiles FJ (1989) Adaptations for the 'Fail-safe' pollination of specialized ornithophilous flowers. Am Midl Nat 121:1–10

Yoder AD, Irwin JA, Payseur BA (2001) Failure of the ILD to determine data combinability for slow loris phylogeny. Syst Biol 50:408–424

Zimmer EA, Roalson EH, Skog LE, Boggan JK, Idnurm A (2002) Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* spacer region sequences. Am J Bot 89:296–311