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PHYLOGENY OF A NEOTROPICAL CLADE IN THE GESNERIACEAE: MORE TALES OF CONVERGENT EVOLUTION

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The Gesneriaceae is a family known for convergent evolution of complex floral forms. As a result, defining genera and resolving evolutionary relationships among such genera using morphological data alone has been challenging and often does not accurately reflect monophyletic lineages. The tribe Episcieae is the most diverse within Neotropical Gesneriaceae in terms of its number of species and morphological diversity. As a result, defining genera using floral characters has been historically troublesome. Here we investigate relationships among genera of the tribe using an array of chloroplast DNA, nuclear ribosomal genes, and low-copy nuclear genes to provide resolution for the monophyly of the genera and relationships among the monophyletic groups. All known genera in the tribe (with the exception of the monospecific Lampadaria) have been sampled, and most have been sampled to provide an assessment to determine their monophyly. Of the 17 genera in the tribe that comprise more than a single species, we have sampled 15 with at least two species. The following six genera are identified as para- or polyphyletic: Neomortonia, Episcia, Paradrymonia, Nautilocalyx, Codonanthe, and Nematanthus. Our results strongly support at least three independent origins of fleshy fruits, which are defined here as fleshy display capsules or indehiscent berries.

Keywords: Columnea, Drymonia, display capsules, Episcia, Episcieae, Gesneriaceae, Neomortonia, splash-cup seed dispersal, stolons.

Online enhancements: appendix table and figure.

Introduction

The exploration and discovery of morphological variation in a phylogenetic context is a mainstay of plant systematics and is one of the most revealing aspects of generating a species-level phylogeny that allows us to differentiate homology from convergence. An increased understanding of evolution and homology, coupled with an explosion in the number of studies that have relied on molecular data to resolve phylogenetic history (Palmer and Zamir 1982; Chase et al. 1993; Soltis et al. 1998; Leebens-Mack et al. 2005; Burleigh et al. 2011), has resulted in a greater appreciation of the convergence of character states across relatively large evolutionary distances (Chase et al. 1993; APG III 2009; Endress 2011) as well as among closely related species where morphological similarities were presumed to be homologous (Mummenhoff et al. 1997; Clark and Zimmer 2003; Jousselin et al. 2003; Vences et al. 2003; Smith et al. 2004b; Roalson et al. 2005a, 2005b).

Phylogenetic analyses of Gesneriaceae over the past 20-plus years have helped resolve the homology of many morphological characters and revealed the convergence of many more. Broader-level sampling has provided support for the division of the family into two clades, the primarily Paleotropical Cyrtandroideae and almost exclusively Neotropical Gesnerioideae (Smith et al. 1997; Mayer et al. 2003; Möller et al. 2009), and for the monophyly of many of the tribes within the Gesnerioideae (Smith 1996, 2000a, 2000b, 2000c; Smith and Carroll 1997; Smith et al. 1997; Smith and Atkinson 1998; Zimmer et al. 2002; Woo et al. 2011). In contrast, there has been little support for many of the tribes of the Cyrtandroideae except Epithematae (Smith et al. 1997; Mayer et al. 2003; Möller et al. 2009), and in the Gesnerioideae the tribe Sphaerorrhizeae was discovered with molecular data, which was not foreseen from morphological data alone (Roalson et al. 2005a).

At finer scales in Gesneriaceae, the problems of defining monophyletic groups are exacerbated by the convergence of corolla morphologies. The monophyly of numerous genera in the Gesnerioideae has been questioned, including Neomortonia and Paradrymonia (Smith 2000b); Alloplectus, Nautilocalyx, and Paradrymonia (Clark and Zimmer 2003; Clark et al. 2006); and Phinaea, Capanea, Kobleria, and Gloxinia (Smith et al. 2004a; Roalson et al. 2005a, 2005b, 2008; Clark et al. 2011). Similar problems at the generic level exist in the Cyrtandroideae (Möller and Cronk 1997; Smith et al. 1998; Möller et al. 2009; Wang et al. 2010, 2011).

The utility of molecular phylogenetic analyses to recover monophyletic clades whose constituent species can then be surveyed for character states to support the clade has become...
the new mainstay in plant systematics (Tekle et al. 2009). Within Gesneriaceae, this method has been useful within the tribe Gloxinieae, where several monospecific genera have been found to belong to a single clade and combined (Gloxinia erinoides [DC] E. H. Roalson & J. K. Boggan, G. xanthophylla [Poepig] E. H. Roalson & J. K. Boggan), and other genera (Monopyle and Phinacea) have been found to be polyphyletic (Smith et al. 2004b; Roalson et al. 2005b; Clark et al. 2011). Once monophyletic groupings have been identified, it is possible to reexamine the morphology of the species that fall into the separate clades and determine traits that define the taxa and redefine generic boundaries. A striking example is Phinacea s.l. These plants form small rosettes and produce scapose inflorescences with several flowers with nearly actinomorphic white corollas. Actinomorphy is relatively rare among Neotropical Gesneriaceae, and therefore the tendency to include these species in a single genus was not surprising. However, phylogenetic analyses placed these species in two unrelated clades (Smith et al. 2004a; Roalson et al. 2005b, 2008). When examined more closely, it turned out that one clade had nodding flowers (Amalophyllum) and the other had erect flowers (Phinacea; Boggan et al. 2008). Recently, another species, Phinacea pulchella, has been found to be phylogenetically unrelated to either of the two previously recognized clades and may merit an additional generic name (Clark et al. 2011).

Another major clade of Neotropical Gesneriaceae where similar problems are being discovered with increased taxon sampling is the tribe Episcieae. Like Gloxinieae, the tribe Episcieae is morphologically diverse (fig. 1) but has long been considered monophyletic on the basis of its three-trace trilacunar nodal anatomy, axillary flowers derived from reduced pair-flowered cymes, and a base chromosome count of $x = 8$ or 9 (Wieher 1983; Weber 2004). The combination of these traits is not known among other Neotropical Gesneriaceae. Molecular-based phylogenetic analyses have supported the monophyly of this tribe (Smith and Carroll 1997; Smith et al. 1997; Smith 2000b; Zimmer et al. 2002; Smith et al. 2004a, 2004b; Roalson et al. 2005a, 2005b; Clark et al. 2006), although support and number of species sampled has varied. Despite this, the monophyly of several genera in the tribe have been suspect on the basis of the cpDNA region, including Neomortonia (Smith and Carroll 1997; Smith 2000b), Paradrymonia (Clark et al. 2006; Smith 2000b), and most notably Alloplectus, which was recovered in no less than six clades across the tribe (Clark and Zimmer 2003; Clark et al. 2006).

Clark et al. (2006) sampled Episcieae most recently and more thoroughly in terms of species than previous studies. Despite this, several clades were not strongly supported or fully resolved on the basis of molecular data alone, and the inclusion of morphology was necessary to boost support and resolution for many clades, including the monophyly of Neomortonia, which contradicted previous studies based on molecular data alone (albeit poorly supported; Smith and Carroll 1997; Smith 2000b). The goal of this study is to revisit the phylogenetic analyses of Episcieae and to sample more broadly among chloroplast DNA (cpDNA; Shaw et al. 2007) and low-copy nuclear DNA (Emshwiller and Doyle 1997; Perret et al. 2003; Smith et al. 2004a, 2004b; Woo et al. 2011) regions, which have been shown to increase support among taxa that have been particularly recalcitrant at revealing evolutionary relationships. In particular, we were interested in resolving the monophyly and sister relationships among the genera associated with Glossoloma and Columnea, as these genera have been the research focus for two of the authors in previous studies (Smith and Sytsma 1994a, 1994b, 1994c; Smith 1994; Clark and Zimmer 2003; Clark 2009).

### Material and Methods

A complete list of samples, voucher specimens, and GenBank accession numbers can be found in appendix A. DNA was extracted using either CTAB (Doyle and Doyle 1987) or DNeasy Plant Mini kits (Qiagen, Valencia, CA). In some instances, different individuals of the same species were used for different gene regions. These are not considered problematic since they were from species that we are confident are monophyletic.

Our analyses were at two levels. For the full analysis, we sampled comprehensively among species from all genera of tribe Episcieae except Lamypadaria. For these species, we sampled the following cpDNA regions: $trnL-trnF$ and the $trnL$ intron (using primers c and d and primers e and f of Taberlet et al. 1991), the $rps16$ intron (using primers from Oxelman et al. 1997), the $rpl20-rps12$ spacer (Hamilton 1999), the nuclear ribosomal internal transcribed spacer region (ITS1, 5.8S, and ITS2; hereafter referred to as ITS), the low-copy nuclear loci GGYC (using the primers of Citerne et al. [2000] and Smith et al. [2004a]), and nuclear-encoded chloroplast-expressed glutamine synthetase loci (ncpGS herein, using primers 687f and 956e of Emshwiller and Doyle 1997). Two loci of different size are typically recovered from Gesneriaceae with the ncpGS primers used here (Smith et al. 2004a) and are referred to herein as ncpGS1 and ncpGS2.

For the reduced analysis, we focused on the clade recovered from the full analysis, which included Alloplectus, Columnea, Corytoplectus, Cranzia, Drymonia, and Neomortonia. We removed some species in genera that had been recovered as monophyletic in the full analysis to focus our effort on increasing the number of DNA regions sampled per individual, thus improving support for relationships among genera. Therefore, in addition to the eight regions sampled for the full analysis, an additional six DNA regions were sampled, including five cpDNA regions ($rpl32-trnL\_LAG$ and $trnQ-rps16$ spacers, both from Shaw et al. [2007], and $trnS-trnG$ and $trnD-T$ spacers, from Demesure et al. [1995]), along with the nuclear DNA phosphoenolpyruvate carboxylase ($PepC$, from Malcomber [2002]).

Most cpDNA regions, GGYC, and ncpGS were amplified and sequenced according to Smith et al. (2004b). The ITS regions and $trnH-psbA$ regions were amplified following Clark et al. (2006). Sequences were obtained either through the methods described in Smith et al. (2004a) or by sending samples to Genewiz (South Plainfield, NJ). Chromatograms were viewed and sequences edited and aligned by hand in PhyDe (http://www.phyde.de/). Most regions had missing data at the beginning and end in the full alignment. Additionally, the
alignment produced regions of ambiguity due to single base pair or microsatellite repeats. Areas of missing data and ambiguous alignments were excluded from phylogenetic analyses. The alignments also resulted in gaps to account for indel events. While the inclusion of indels can often be of phylogenetic significance (Simmons and Ochoterena 2000), the indels generated here were primarily either autapomorphic or found only in the outgroup species. A total of 19 indels have potential phylogenetic significance in the full analysis (three in ITS, one in GGCY, four in ncpGS1, two in ncpGS2, three in the trnL intron, two in the trnL-F spacer, three in the rps16 intron, and one in the rpl12-rps20 spacer). In the reduced analysis, 13 indels had potential phylogenetic utility (two in the rpl32-trnL spacer, two in the trnQ-rps16 spacer, one in the trnD-T spacer, four in the trnS-G spacer, and one in the trnH-psbA spacer). Indels were treated as missing data and then recored as presence or absence characters. This approach allowed for single-site and multiple-site gaps to be treated with equal weight (Simmons and Ochoterena 2000).

The outgroup samples were chosen on the basis of previous phylogenetic studies of Gesneriaceae and Episcieae, and we follow Woo et al. (2011) in recognizing eight tribes within subfamily Gesnerioideae: Beslerieae, Episcieae, Gesnerieae, Gloxinieae, Napeantheae, Sinningieae, Coronantherae, and Sphaerorrizeae. Because our analysis was focused on tribe Episcieae, we included outgroups from it and Gesnerieae, Gloxinieae, Napeantheae, Sinningieae, Coronantherae, and Sphaerorrizeae, since these five tribes have been demonstrated to form a well-supported monophyletic clade separate from the remaining tribes of Gesnerioideae (Woo et al. 2011). The reduced analysis was rooted using Crantzia, as indicated from results of the full analysis.

Test of Incongruence

The incongruence length difference (ILD) test (Farris et al. 1994) was performed as the partition homogeneity test implemented in PAUP* 4.0b10 (Swofford 2002) with 1000 bootstrap replicates (using a heuristic search, simple addition, and no branch swapping). The cpDNA, ITS, ncpGS1, ncpGS2, PepC, and GGCY were each treated as separate partitions for both full and reduced analyses. As the ILD test has been shown to indicate incongruence where none exists (Dolphins et al. 2000; Yoder et al. 2001; Barkworth and Lutzoni 2002; Dowton and Austin 2002), bootstrap analyses were performed on each partition separately to assess areas of conflict and to determine whether any conflict was strongly supported (Mason-Gamer and Kellogg 1996; Seelanan et al. 1997; Smith 2000c).

Phylogenetic Analyses

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

ML analyses were performed using optimal substitution models suggested by Modeltest 3.6 (Posada and Crandall 1998). The Akaike Information Criterion (AIC), which allows nonnested models to be evaluated, was used as a selection criterion (Posada and Buckley 2004). The GTR + Γ model was chosen for the full analysis and GTR + Γ + I for the reduced. Analyses of ML were completed using GARLI 0.96 (Zwickl 2006) with 100 bootstrap replicates.

BI analyses were completed using MrBayes 3.1.1 (Huelsenbeck and Ronquist 2003), using either a single model across the entire data set with the models used for ML, or partitioned models with a different model for each of the partitions as determined by AIC in Modeltest 3.6 (full analysis: ITS = GTR + Γ, GGCY = HKY + Γ, ncpGS1 = GTR + Γ, ncpGS2 = K81uf + Γ, cpDNA = TVM + Γ; reduced analysis: ITS = GTR + Γ, GGCY = TVM + Γ, ncpGS1 = TVM + Γ, ncpGS2 = HKY + Γ, PepC = HKY + Γ, cpDNA = GTR + Γ). All analyses were run with four to one heated chains for 10 million generations. Convergence was determined by viewing in Tracer 1.3 (Rambaut and Drummond 2005), and a burn in of 50,000 generations was discarded prior to sampling the posterior distribution. The analyses were repeated twice to ensure that parameter estimates converged to similar values. The separate runs were compared using the online version of AWTY (http://king2.scs.fsu.edu/CEBProjects/awty/awty.php?fromStart=1&sessionDir=TMP18595; Nylander et al. 2008) as a means of determining whether the separate chains approximated the same target distribution. We report the 50% majority-rule consensus tree sampled from the posterior probability distribution.

The phylogenetic trees and data sets used in the full and reduced analyses have been submitted to TreeBASE (study 12668). SIMMAP 1.5 used Bayesian stochastic character mapping to perform the ancestral state reconstructions. The bias parameter was set to the empirical prior, and the rate parameter was set to the branch length prior with the character state changes unordered. Ancestral character state reconstructions using the branch length (BL) model in SIMMAP 1.5 are given as Bayesian posterior probabilities.

Results

Amplifications were successful for all regions for all individuals, with some exceptions for each DNA region (app. A). Table 1 presents a complete list of gene regions and combined data matrix statistics for the full analysis. Of the eight gene regions sampled in the full analysis, ITS provided the most parsimony-informative substitutions (317, or 47%), whereas the trnL intron provided the least number of parsimony-informative substitutions (86, or 15%; table 1). The aligned matrix for the full analysis contained 4815 characters (4796 bp and 19 indels); of these, 2109 were constant and 1432 were uninformative. The complete matrix for the full analysis contained 1274 (26.5%) phylogenetically informative characters.

The aligned matrix for the reduced analysis contained 8966 characters (8953 bp and 13 indels); of these, 7236 were constant and 1329 were parsimony uninformative. The ma-
The primary difference between the models was that a single
both the full and the reduced analysis (tree not shown) showed
was sister to all remaining species in the genus. The
were supported as monophyletic, and five genera (Alloplectus, Cobanathus, Cremeria, Lembocarpus) were represented by only a single
(PP = 96). Output from AWTY analyses showed that the independent runs of each data set were close in parameter (tree) space; thus,
we could conclude that the two separate runs approximated the same target (tree) distribution.
The analyses recovered 10 clades consisting of more than a single genus with MPBS, MLBS, and PP > 95 within Epis- cieae. Six of these are labeled in figures 2 and 3. The remaining four strongly supported clades are either found within one of the labeled clades or consist of more than one of them. Relationships among the clades are mostly well supported with the exception of the clade sister to the remainder of the tribe and the relationship among the Episcia s.s., Central American, and Southeastern Brazilian Atlantic Forest clades. Seven genera (Chrysothemis, Columnea, Corytoplectus, Crantzia, Glossoloma, Rhogeton, and Rufadoras) for which more than a single species was sampled were strongly supported as monophyletic (MPBS, MLBS, PP > 95 for all except Glossoloma, with MPBS = 68, MLBS = 100; fig. 3). Another eight genera (Alsobia, Codanathus, Drymonia, Episcia, Nautilocalyx, Nematanthus, Neomortonia, and Paradrymonia) were not supported as monophyletic, and five genera (Alloplectus, Cobanathus, Codonanthopsis, Cremeria, and Lembocarpus) were represented by only a single species and thus were not tested here, although all are monophyletic.
The MP analysis for the reduced sampling with indels scored as missing resulted in two trees of length 2262 (CI = 0.57, RI = 0.67, RC = 0.56). The two trees differed only in whether Glossoloma martintinum was sister to Glossoloma panamense or was sister to all remaining species in the genus. The strict consensus is shown in figure 4. Rescoring indels resulted in the same two trees (length = 2265, CI = 0.57, RI = 0.67, RC = 0.56), and support was not altered. All genera except Neomortonia were recovered as monophyletic with strong support (fig. 4). Relationships among all genera were strongly supported in the reduced analysis, providing sister-group relationships among these genera for the first time using only molecular data. The ML analysis
| Statistic                        | ITS | GYC  | ncpGS1 | ncpGS2 | trnL intron | trnL-F spacer | rps16 intron | rpl12-rps20 spacer | rpl32-trnL spacer | trnQ-rps16 spacer | trnD-T spacer | PepC intron | trnS-G spacer | trnH-psbA |
|---------------------------------|-----|------|--------|--------|-------------|--------------|--------------|-------------------|-----------------|-----------------|--------------|-------------|------------|-------------|------------|
| Aligned length                  | 458 | 576  | 535    | 458    | 523         | 396          | 642          | 770               | 1113            | 893             | 970          | 463         | 594         | 323        |
| Mean GC content (%)             | 60.4| 40.7 | 38.1   | 43.1   | 36.0        | 54.3         | 33.4         | 35.2              | 27.6            | 26.4            | 35.5         | 38.5        | 31.6        | 34.7       |
| Mean pairwise divergence (%)    | 8.99| 3.72 | 3.40   | 4.02   | 1.57        | 2.40         | 1.21         | 1.61              | 2.17            | 2.63            | 2.38         | 2.72        | 2.09        | 2.17       |
| Parsimony-uninformative subs.   | 107 | 139  | 112    | 74     | 72          | 78           | 39           | 84                | 135             | 139             | 156         | 72          | 66          | 31         |
| Parsimony-informative subs.     | 88  | 34   | 20     | 35     | 8           | 8            | 8            | 18                | 22              | 39              | 32          | 19          | 18          | 14         |
| Constant characters             | 263 | 403  | 403    | 349    | 443         | 310          | 642          | 664               | 939             | 722             | 786         | 372         | 510         | 278        |
| Unambiguous indels              | ... | ...  | ...    | ...    | ...         | ...          | ...          | ...               | ...             | ...             | ...         | ...         | ...         | ...        |
| Consistency index               | .646|.893 | .925   | .829   | .977        | .970         | .909         | .944              | .853            | .916            | .956        | .905        | .903        | .940       |
| Retention index                 | .619|.730 | .600   | .771   | .905        | .769         | .860         | .833              | .700            | .762            | .873        | .789        | .833        | .930       |
| Rescaled consistency index      | .400|.651 | .555   | .639   | .884        | .746         | .782         | .787              | .597            | .704            | .835        | .714        | .752        | .8744      |
| Tree length                     | 387 | 215  | 160    | 146     | 86          | 99           | 66           | 126               | 225             | 213             | 203         | 116         | 103         | 50         |
resulted in a single tree (−ln likelihood = 28, 400.094456) that was completely congruent with the MP tree that placed G. martinianum as sister to all other species included in Glossoloma (fig. 4). The two BI runs (both with a unique model for each data partition and with a single model across all data) produced a majority-rule consensus tree in full agreement with the ML analysis, and PP values were identical between runs. Output from AW TY analyses showed that the independent runs of each data set were in close parameter (tree) space; thus, we can conclude that the two separate runs approximated the same target (tree) distribution.

The reduced analysis (fig. 4) is the first molecular-based phylogeny that provides strong support for intergeneric relationships in this clade. Our results strongly support that Glossoloma is sister to Columnea and places Neomortonia rosea as sister to Drymonia, with the latter clade sister to the Glossoloma/Columnea clade. Alloplectus is sister to these four taxa, with Neomortonia nummularia as sister to all other genera except Coryplectus and Crantzia.

Discussion

The results of this analysis provide a well-supported phylogenetic estimate for most clades within tribe Episcieae based entirely on molecular data (Smith and Carroll 1997; Smith 2000b; Clark et al. 2006). Although Clark et al. (2006) sampled more broadly among the species of Episcieae, particularly among species-rich genera, their analyses were not able to resolve all relationships with molecular data alone, and support for some clades was not strong. In this analysis, we were able to take advantage of those data and sample fewer species in the clades that were recovered as monophyletic by sampling additional DNA regions, including several low-copy nuclear loci that provide an independent source of data for the evolutionary history of these taxa. Each of the well-supported clades is discussed in detail below.

Paradrymonia, Nautilocalyx, and Chrysothemis

The Paradrymonia + Nautilocalyx + Chrysothemis clade received strong support from all analyses (figs. 2, 5) and has been recovered (at least in part) in other analyses (Smith and Carroll 1997; Smith 2000b; Zimmer et al. 2002; Clark and Zimmer 2003; Clark et al. 2006). Paradrymonia is not monophyletic in this analysis, as species nest in other clades (e.g., Paradrymonia maculata shown in fig. 2) as well as the paraphyly of the majority of Paradrylonia, with the inclusion of a polyphyletic Nautilocalyx and a monophyletic Chrysothemis (figs. 2, 5). Smith and Carroll (1997) and Smith (2000b) also failed to recover a monophyletic Paradrymonia based on ndBF and a combined ITS and ndBF data set, respectively; however, this was mainly due to Paradrymonia densa falling outside the clade, as only three species of Paradrymonia and only one each of Nautilocalyx and Chrysothemis were sampled. The results recovered here are more similar to those of Clark et al. 2006, who also noted that Drymonia longifolia (=Paradrymonia longifolia in Clark et al. 2006) nested in Drymonia and that Paradrymonia anisophylla (not sampled here) was sister to Codonanthe + Codonanthopsis.

While it is clear that species of Paradrymonia falling outside this clade will need to be reassigned to other genera, it is not immediately obvious how to solve the problem of paraphyly among the bulk of the species in this clade. One option is to recognize a single large genus encompassing all species of Nautilocalyx and Chrysothemis. A second option is to divide Paradrymonia and Nautilocalyx into multiple genera. Greater species sampling will be essential to resolve this problematic clade, and it is the current focus of a PhD dissertation (M. Mora, in preparation).

This study strongly supports the sister group relationship of the clade comprising Paradrymonia + Nautilocalyx + Chrysothemis as the sister group to the remainder of Episcieae (all three analyses with >95% support values). Smith and Carroll (1997) and Smith (2000b) also recovered this relationship, but with no support. The results presented here differ from those of Clark et al. (2006), where the sister clade to Episcieae was a strongly supported clade comprising Cremersia + Lembocarpus + Rhoogoton. Taxon sampling representing species from these two clades differ by inclusion of additional species from the Guyana Shield (e.g., Episcia xantha and P. maculata) in the current study. In contrast, taxon sampling in Clark et al. (2006) was limited to Rhoogoton viviparum, Rhoogoton cyclophyllus, Cremersia platula, and Lembocarpus amoenuis. Additional ongoing studies of Paradrymonia (M. Mora, personal communication) that have more extensive taxon sampling also suggest that the Paradrymonia, Nautilocalyx, and Chrysothemis complex is the sister clade to the rest of Episcieae.

Guyana Shield Clade

The Guyana Shield clade is strongly supported as monophyletic on the basis of support values from the three analyses and includes the monospecific genera Cremersia and Lembocarpus as well as the two species of Rhoogoton. Smith and Carroll (1997) did not sample any of these species, and Smith (2000b) included an ITS sequence for one species of Rhoogoton derived from herbarium material that was likely partly fungal in origin, as its placement on the tree varied depending on the analysis. Clark et al. (2006) recovered Cremersia, Lembocarpus, and a monophyletic Rhoogoton as sister to the remainder of Episcieae. The current study is the first to include P. maculata and E. xantha, and as a result neither species is found to be with the remainder of their genus, making Episcia polyphyletic for the first time.

Species in this clade have unique morphologies compared with other members of Episcieae, but there are no known morphological characters that unite them. For example, both Lembocarpus and Rhoogoton are tuberous with one to a few basal leaves and flowers borne on a scapose stem. In contrast, P. maculata is an herbaceous vine with a large bracteate pendent inflorescence (fig. 1A).

One aspect that all these species have in common is that they are endemic to the Guyana Shield, indicating that additional species from this region should be sampled to verify their placement. While the placement of most species in this clade has not been overly surprising given their unusual mor-
Fig. 2  Strict consensus tree from total evidence analysis of eight molecular markers (ITS, GCYC, ncpGS1, ncpGS2, trnL intron, trnL-F spacer, rps16 intron, and rpl12-rps20 spacer). The strict consensus tree is from 48 most-parsimonious trees of 6411 steps. Numbers above branches are maximum parsimony (MP) bootstrap/maximum likelihood (ML) bootstrap/Bayesian inference (BI) posterior probabilities. Thick bars indicate nodes where support for all three analyses was >95. Note that the topology based on ML and BI is nearly identical. Incongruence between ML/BI and MP is explained in “Discussion” and is shown in figs. 5 and 6. Different fruit types are explained in detail in “Discussion” and are depicted using the images shown in the key in fig. 3 (i.e., dry capsules, semifleshy capsules, fleshy capsules, and indehiscent berries).
Lampadaria recognize an important character by Feuillet and Skog (2003) to characterize may be overemphasized, as some genera that were described in the fully reflexed to nearly truncate bivalved capsules in Lampadaria. The elongate pedunculate and subcapitate inflorescence of Lampadaria was included in this analysis to verify that the original material collected in French Guiana (J. F. Smith et al. 4116; app. A) was neither misidentified nor contaminated with other material. DNA from the two individuals was extracted, amplified, and sequenced years apart in time; therefore, the chances of contamination are minimal. The two individuals are maximally supported as monophyletic (figs. 2, 5). The fruits of E. xantha have been observed as dry dehiscent capsules. Leeuwenberg (1980) differentiated between globose and laterally compressed capsules when he described E. xantha and referred to most Episcia as globose (fig. 7F) and only E. xantha as laterally compressed (fig. 7E). The fruit of E. xantha is a dry capsule and therefore more similar to Lembocarpus (fig. 7G) than to the semifleshy capsules in Episcia (fig. 7F). The phylogenetic placement of E. xantha is not surprising given the convergence of stolons in multiple lineages, as discussed below.

A remarkable feature in P. maculata is an inflexed ventral corolla lobe that completely closes the opening of the tube (fig. 1B). The completely occluded throat was noted and illustrated by Hooker (1890). More recently, Feuillet (2009) described the inflexed ventral corolla lobe as a barrier mechanism that can be opened by exerting pressure on both sides of the apical third of the tube. Field observations for a doctoral dissertation by Hentrich (2008) noted that the corolla remained closed during the entire flowering period (2 d) and was forcibly opened by large euglossine bees (Eulaema sp.) by pulling down the petals.

The monospecific genus Lampadaria is not included in this analysis. There are few collections of Lampadaria rupestris Feuillet & L. E. Skog, and it is known only from the Potaros-Siparuni region of Guyana. The most recent collection is from 2001 by H. David Clarke (H. D. Clarke 8897, US). The elongate pedunculate and subcapitate inflorescence of Lampadaria are similar to Rboogoton. In contrast, the capsular fruits of Lampadaria do not reflex at maturity, compared with the fully reflexed to nearly truncate bivalved capsules in Rboogoton. The presence of inflorescence bracts was considered an important character by Feuillet and Skog (2003) to recognize Lampadaria as a member of the Episcieae, but this character may be overemphasized, as some genera that were thought to lack bracts (such as Resia) have been found to have bracts (e.g., the recently described species Resia bracteoides; Fernando-Alonso 2006; Skog and de Jesus 1997).

Episcia s.s.

In the Episcia s.s. clade, three species of Episcia are recovered as a monophyletic group that is strongly supported in all three analyses (>95%). One difference between the MP and ML/BI is that this clade is sister to the primarily Central American clade (e.g., Alsobia, Rufodorsia, Oerstedina, and Cobananthus) in the MP/BI (fig. 2). The MP analysis suggests that Episcia is sister to the Central American clade, the Southeastern Brazilian Atlantic Forest clade, and the remaining genera shown in figure 3. The genus Episcia is not recovered as monophyletic in this analysis because of the position of E. xantha (discussed above), but the placement of the remainder of the genus here in the tribe is in agreement with previous phylogenetic analyses (Smith 2000b; Clark et al. 2006). The monophyly of Episcia has only been questioned previously depending on whether species of Alsobia, which also share the characteristic of stolons, are considered part of Episcia (Smith and Carroll 1997).

Central American Clade

The species recovered in the Central American clade are all native to Central America. This clade has been recovered as monophyletic in all other analyses that have sampled some or all of these genera (Smith and Carroll 1997; Smith 2000b; Zimmer et al. 2002; Clark and Zimmer 2003; Clark et al. 2006). The data here support the separation of Alsobia from Episcia. These two genera differ by the presence of one stolon per node and epiphytic habit in Alsobia as opposed to two stolons per node and terrestrial habit in Episcia (Wiehler 1983; Weber 2004). The same two leaf samples of Alsobia were included in Clark et al. (2006) and resulted in a monophyletic Alsobia with a 74% BS value. We recovered lower support for the monophyly of Alsobia, but this is likely because of the absence of an adequate sequence for ncpGS1 for Alsobia punctata. Although this region amplified and was sequenced, it gave a result incongruent with other regions (A. punctata sister to Cobananthus) and was therefore removed from the analyses, as it is likely a paralog.

The genus Rufodorsia was recently expanded to include one of the three traditionally recognized species of Oerstedina (Kriebel 2010) with the new combination Rufodorsia cerricola (Wiehler) Kriebel. The new combination published by Kriebel (2010) did not consider or discuss the other two species of Oerstedina (O. mexicana Wiehler and O. suffrutescens L. E. Skog), and therefore we have retained the name Oerstedina cerricola. Wiehler (1975b) differentiated Oerstedina from Rufodorsia by relatively larger corollas that lack red coloration on the dorsal surface and the presence of pointed berries in contrast to globose berries. These differences are consistent for differentiating these two genera. These two clades share a recent common ancestor, and the recognition of two separate genera or the reduction of the genus Oerstedina in Rufodorsia should be considered in the context of future phylogenetic and monographic work of Central American Gesneriaceae. The sister-group relationship of Oerstedina and Rufodorsia is strongly supported in this study and in previous phylogenetic analyses (Smith 2000b; Clark et al. 2006).

Convergence of Stolons

The presence of stolons in the Gesneriaceae is known only in Episcia and Alsobia. The data presented here and in Clark et al. (2006) strongly support that the majority of Episcia species form a monophyletic group that is not sister to any other single genus of Episcieae. Clearly, the presence of sto-
ions is convergent on the basis of the placement of these two genera in previous and the current phylogenetic analyses (figs. 3, 6). The stolons in Alloplectus are produced one per node in alternating leaf axils. The successive stolons give the appearance of a single pendent stem (Wiehler 1983). In contrast, the stolons in Episcia are produced in pairs at each node (Wiehler 1983; Weber 2004). In this analysis, the placement of E. xantha is supported as an additional clade that has evolved stolons separate from traditional Episcia (fig. 2). Thus, the distinguishing traits of terrestrial habit with two stolons per node is convergent in “Episcia” xantha and other species of Episcia.

Southeastern Brazilian Atlantic Forest Clade

Members of the Southeastern Brazilian Atlantic Forest clade are widespread in the New World but are most diverse in the Atlantic Forest of southeastern Brazil. Members of this clade are mostly unique in Episcieae in that they have haploid chromosome counts of n = 8 rather than n = 9, which is otherwise found throughout the tribe. The exception to this is Codonanthes dissimulata, which has been reported as n = 9 (Wiehler 1978). Nematanthus savannarum was recently transferred from Alloplectus (Clark 2005), and its chromosome count is not known. Other than chromosome counts, there are no other nonmolecular traits that define this clade. This clade has been recovered as monophyletic in previous analyses of Episcieae, although sampling has varied widely (Smith 2000b; Clark et al. 2006).

Both this study and Clark et al. (2006) failed to recover a monophyletic Codonanthe, although there is no overlap in species sampling. Codonanthes gracilis, which falls amid the Brazilian Nematanthus species, is also from Brazil, as is Codonanthes carnosa, which Clark et al. (2006) also found to be part of the Nematanthus clade. The other species of Codonanthes sampled here and by Clark et al. (2006) are species not found in Brazil. Further sampling of both Brazilian and non-Brazilian species will be essential for evaluating generic boundaries for Codonanthes, Codonanthopsis, and Nematanthus and is the current focus of research by Alain Chautems and collaborators.

Clark (2005) transferred the taxon and published the combination N. savannarum (C. V. Morton) J. L. Clark from Alloplectus savannarum as a best fit for a species whose inclusion in Alloplectus was clearly in error. Its placement in Nematanthus was uncertain, as it occurs in the Guyana Shield and is disjunct from the remainder of Nematanthus, which occur in southeastern Brazil. This species was not supported as sister to Nematanthus by Clark et al. (2006), but it is strongly supported as the sister to all other members of this clade in the current analysis, making its inclusion in Nematanthus less supported. It is likely that this species represents yet another monospecific genus from the Guyana Shield, but one that is not part of the clade recognized here as the Guyana Shield (fig. 2). Here again there is a convergence of several morphological characters—such as tubular corolla shape, fleshy capsular fruits, and epiphytic habit—that caused this species to be initially described in Alloplectus (Morton 1948).

The Alloplectus/Columnnea/Drymonia Alliance (Figs. 3, 4, 6)

The Alloplectus/Columnnea/Drymonia clade is well supported (100/97/100), and, with the exception of Drymonia, all of the genera are well supported. Beyond the grade of Crantzia, Corycophyllum, and Neomortonia nummularia, there is little support for resolution among the genera (fig. 3). Most of these genera have been recovered as monophyletic in previous studies (Smith 2000b; Zimmer et al. 2002; Clark et al. 2006), but with little support for intergeneric relationships.

Outside of Paradrymonia, this clade has faced some of the most difficult challenges with respect to generic delimitations. As a result of extensive taxon sampling by Clark and colleagues (Clark and Zimmer 2003; Clark 2005, 2009; Clark et al. 2006), most of these genera have been resolved fairly recently by identifying monophyletic lineages in an otherwise polyphyletic Alloplectus that resulted in the resurrection of Crantzia and Glossoloma and the transfer of A. savannarum to Nematanthus. As with previous studies (Smith 2000b; Clark et al. 2006), the monophyly of Columnnea as a single genus rather than five is supported here, and with a revised circumscription of Drymonia (sensu Clark et al. 2006) this genus is also monophyletic.

Further relationships among genera will be discussed below on the basis of the reduced analysis.

The Reduced Analysis: Alloplectus/Columnnea/Drymonia Alliance

Glossoloma is strongly supported as sister to Columnnea (93/92/100; fig. 4). Resolving these two genera as sister will be critical to further resolve phylogenetic relationships within Columnnea, which is the largest Neotropical genus of Gesneriaceae in terms of its total number of species (J. F. Smith, M. T. Ooi, L. J. Schulte, M. Amaya Marquez, R. Pritchard, and J. L. Clark, unpublished manuscript). Likewise, the placement of Neomortonia rosea (figs. 2, 4, 5) as sister to the sampled species of Drymonia will be important as future studies investigate the evolutionary relationships of this latter genus (L. Clavijo, personal communication). Last, these data resolve Alloplectus as sister to all of the aforementioned genera with moderate (MPBS = 78, MLBS = 70) to strong (PP = 100) support (fig. 4). Only a single species of Alloplectus was sampled here, and it may be that the inclusion of additional species would have stabilized this relationship with greater support.

This study provides additional support and evidence for the nonmonophyly of Neomortonia. Although early studies that included both species of this genus failed to recover a monophyletic group (Clark and Zimmer 2003), low support and relative proximity of the two species in the tree led to uncertainty about the nonmonophyly of the genus. This uncertainty was increased when Neomortonia was recovered as monophyletic by Clark et al. (2006); however, the two species were in a single clade with the inclusion of morphological data—molecular data alone failed to bring the two species together (J. L. Clark, unpublished results). Although the corollas of the two species are dramatically different (fig. 3)
Fig. 3  *Alloplectus/Columnea/Drymonia* clade. See the fig. 2 legend for details.
1E, 1F), they both have an indehiscent fleshy orange berries (fig. 1C, 1D). See the section below for further discussion of
the convergence of berries in traditional Neomortonia. Here, for the first time, there is strong support for the placement of
N. rosea as sister to Drymonia (86/86/100) and for N. num-
mularia as sister to the majority of the genera in this clade
except Corytoplectus and Crantzia (fig. 4). The type for
the genus is N. rosea, meaning that a new generic name is neces-
sary for N. nummularia.

The strong support for the monophyly of this clade and
the strong support for the placement of Crantzia as sister to
the remainder of this clade allowed for further sampling of
DNA regions within each of these genera and the use of
a fewer number of outgroups. This can be especially critical
when attempting to resolve relationships at the species level,
as more rapidly evolving DNA regions will be essential to
provide sufficient data. The inclusion of more distant out-
groups could result in excessive homoplasy in the analysis or

Fig. 4 Summary of maximum likelihood (–ln likelihood = 28.400.094556), strict consensus of maximum parsimony analysis, and majority-
rule Bayesian inference topology for reduced clade (25 taxa) based on 13 molecular markers (ITS, gycy, ncpGS1, ncpGS2, trnL intron, trnL-F
spacer, rps16 intron, rpl12-rps20 spacer, rpl32-trnL spacer, trnQ-rps16 spacer, trnD-T spacer, PepC intron, and trnS-G spacer). Asterisks (*)
indicate nodes that collapse in the strict consensus of the maximum parsimony analysis of two trees of length 2262 (consistency index = 0.57,
retention index = 0.67, rescaled consistency index = 0.56). The topology from the Bayesian analysis using either a single model or different
models for each of the data partitions resulted in the same topology. Numbers above branches are maximum parsimony bootstrap/maximum
likelihood bootstrap/Bayesian inference posterior probabilities. Thick bars indicate nodes where support for all three analyses was >95.
Fig. 5  Stochastic character mapping of fruit types using the 50% majority-rule tree generated from Bayesian inference (BI) of eight molecular markers (ITS, GCYC, ncpGS1, ncpGS2, trnL intron, trnL–F spacer, rps16 intron, and rpl12–rps20 spacer). Pie charts represent ancestral states at each node that were calculated as the marginal posterior probability of each possible character state. Note that the BI and maximum likelihood topologies are congruent.
Fig. 6  See the fig. 5 legend for details.
limit the ability to unambiguously align sequences. For example, the use of Glossoloma alone as the outgroup for Columnea has permitted the use of some rapidly evolving low-copy nuclear genes to resolve species-level relationships within the genus without compromising the ability to assess homology accurately across all taxa (J. F. Smith, unpublished results).

Evolution of Fleshy Display Capsules, Semifleshy Capsules, and Berries

Our results strongly support two independent origins of fleshy fruits (figs. 2, 3, 5). It should be noted that fleshy fruits are defined here as indehiscent berries (figs. 1G, 1D, 1G, 1H, 7C) or fleshy display capsules (figs. 1I, 7A, 7B). The bivalved capsules of semifleshy fruits typically open to 45°, and the valves are not reflexed or showy (fig. 7D, 7H). Fleshy capsules (figs. 1I, 7A, 7B) are fully reflexed at maturity and contrast in color with an erect cone-shaped mass of seeds embedded in brightly colored funiculi. The seeds are sometimes clumped together (fig. 1I) or remain attached to the septum of reflexed valves (fig. 7A, 7B). Fleshy capsules are often referred to as display capsules because of their contrasting color and putative role in attracting animals that eat them, resulting in seed dispersal. Reports of the role in seed dispersal from display capsules include fruit-eating bats, birds, and possibly monkeys (Wiehler 1983). Use of parsimony to map fruit character states demonstrates a clear transition to fleshy fruits prior to the divergence of the Central American clade (fig. 2) with a single reversal to dry capsule (fig. 2). The ML/BI topology differs from the MP tree in the positions of the Episcia s.s., Central American, and Southeastern Brazilian Atlantic Forest clades, but using stochastic mapping we also show two clear origins of fleshy fruits (fig. 5). There is a 99% posterior probability of fleshy fruits at the node prior to the divergence of the Southeastern Brazilian Atlantic Forest clade (97.5% fleshy capsule, 1.5% berry; app. B, available in the online edition of the International Journal of Plant Sciences). The ML/BI topology places the Episcia s.s. clade as sister to the Central American clade, and at this node there is still a greater posterior probability of reconstructing the ancestral state as fleshy (fig. 5). As with MP, there is a second origin of fleshy fruits with the presence of a berry in P. metamorphophylla (fig. 5). If we interpret fleshiness even more broadly to include the semifleshy capsules, then both MP and stochastic mapping would indicate a third origin of fleshiness in Chrysothemis (figs. 2, 5).

Fleshy fruits, whether berries or capsules, is a synapomorphy that defines clades with high diversity in the Andes, Central America, and southeastern Brazil. In contrast, Paradrymonia and Nautilocalyx are defined by the presence of semifleshy capsules and are mostly found in the Guiana Shield of northwestern South America and the foothills of the eastern slopes of the Andes. There are exceptions to this trend. For example, two species of Chrysothemis (fig. 5) are common in Central America, and they share a recent ancestor with Nautilocalyx melittifolius from the Caribbean. The sister taxon to Chrysothemis (2 spp.) and N. melittifolius is Nautilocalyx adenosiphon from French Guiana. Taxon sampling for Nautilocalyx and Paradrymonia is biased from collections that were made in South and Central America because of the geographic focus from extensive fieldwork by the first author. Taxon sampling for species native to northwestern South America (especially Venezuela) are limited to material readily available in cultivation.

Our results strongly support independent origins of berries in at least seven clades, regardless of the methods used to map ancestral character states (figs. 2, 3, 5). Three are independent origins in clades that otherwise are characterized by dehiscent fruits (P. metamorphophylla, C. gracilis, and Drymonia urceolata). Two other origins of berries can be mapped at lower levels in the topologies and include the ancestor of the Central American clade (figs. 2, 5) and species of Columnea other than C. dielsii (figs. 3, 6). The MP and stochastic mapping differ in the final origins of berries. Parsimony is equivocal on the ancestral state of the N. rosea Drymonia clade (fig. 3), implying a potentially independent origin of a berry in N. rosea. Stochastic mapping clearly indicates that the ancestor to the N. rosea/Drymonia clade is a berry (fig. 6), but the ancestor to Drymonia as a whole is clearly a fleshy capsule (fig. 6). Although the placement of the ancestral state differs between the two analyses, there are still two independent origins of berries. The MP mapping is equivocal on whether the ancestor to the Allopectus/Columnea/Drymonia clade is a berry or fleshy capsule, potentially giving rise to two origins of berries independently in N. nummularia and Corytopectus (fig. 3), bringing the total independent origins of berries to eight with parsimony. Stochastic mapping, on the other hand, indicates that the ancestor to the Allopectus/Columnea/Drymonia clade and the Central American/Episica clade has a greater probability of being a berry (fig. 5) and a slightly greater probability as the ancestral state to the Allopectus/Columnea/Drymonia clade itself (fig. 6). This implies that N. nummularia and Corytopectus berries have a single ancestral origin and would imply seven independent origins of berries. The convergence of berries in the Gesneriaceae has been documented in previous phylogenies (Smith 2000a; Clark et al. 2006), but not with strong support and not with as many independent origins.

This is the first phylogeny that strongly supports the non-monophyly of traditionally recognized Neomortonia. Wiehler (1975a) defined Neomortonia by the presence of bright-orange berries that appear laterally compressed (fig. 1D). Further investigation of N. nummularia from recent fieldwork in Ecuador has documented that the berries are more globose and not laterally compressed, as reported by Wiehler (1975a; fig. 1C). The only character that traditional Neomortonia share in common is that both berries are bright orange (fig. 1C, 1D). The corollas of these two species are very different, with N. rosea having a hypocyrtoid or pouched corolla (fig. 1E) and N. rosea having a campanulate corolla with fimbriate margins (fig. 1F). The morphologically divergent corollas, different berry shape, and phylogenetic results presented here warrant the separation of this polyphyletic genus.

It should be noted that Columnea is traditionally defined by the presence of an indehiscent fleshy berry (fig. 1G, 1H). There are only two known exceptions where Columnea has a fleshy capsule, and both are basal members of the clade. Columnea dielsii and an undescribed species from Peru have
fleshy capsules that are similar to Glossoloma, and the for-
mer is shown to be the sister taxon to all other Columnea
(figs. 3, 6). The undescribed species from Peru was included
in Clark et al. (2006) as Columnea sp. nov. (J. L. Clark
8188, US) and was shown to be a basal member of Colum-
nea. Focused fieldwork to assess fruit morphology is nec-
essary to verify whether other basal members of Columnea
have fleshy capsules. For example, Columnea hypocyrtan-
thia is reported as having a berry by Smith (1994), but the illus-
tion of the fruits (fig. 6 in Smith 1994) appears laterally
compressed, and the seeds were noted as not observed in the
description. It should be noted that other basal members of
Columnea—C. strigosa and C. kucyriactii—have fleshy inde-
sisent berries and not fleshy capsules.

Two independent origins of berries are strongly supported
in P. metamorphophylla and D. urceolata. These two species
are strongly supported in clades that are mostly defined by
the presence of capsules. Thus, indehiscent berries is autapo-
morphic in P. metamorphophylla and synapomorphic within
Drymonia. Both of these taxa have been collected on numer-
ous occasions, and sequences have been obtained from inde-
pendent extractions. The berry of P. metamorphophylla is
bright white and globose (fig. 7C), and it is the only known
species in Paradrymonia and closely related genera (e.g.,
Nautilocalyx and Chrysothemis) that lack semisucculent cap-
sules. The berry of D. urceolata is pointy and light green.
Clark et al. (2006) showed that D. urceolata shared a recent
common ancestor with Drymonia turriata and D. ambo-
nensis (not sampled here), which also have berries.

Accurate assessment of fruit morphology based on herbari-
um specimens in the Episcieae is challenging because their
fruits are fleshy and are often destroyed when pressed and
dried. Many descriptions and flora treatments have inaccu-
rate or inadequate descriptions of fruit morphology. Addi-
tionally, fruits are often difficult to locate in the field because
they are presumably rapidly consumed by dispersers, in con-
trast to frequently collected specimens with showy persistent
flowers. As a result, fruit variation in the Episcieae is poorly
documented relative to variation in flowers, and future stud-
ies will likely discover additional taxa with fleshy berries
in the Episcieae that are presumed to have fleshy or dry
capsules.

**Dry Fruits, Splash-Cup Seed Dispersal,
and Terrestrial Herbs**

The presence of splash-cup seed dispersal is an important
feature that needs to be explored in the basal lineages of the
Episcieae. Habit is also important, as many species are de-
scribed on the basis of herbarium specimens. Further evalua-
tion of these two traits may suggest that they are correlated
and underreported in the literature.

One trait that is often overlooked when evaluating epi-
phytic or terrestrial habit is the designation of a species as
facultative epiphyte, obligate epiphyte, or obligate terrestrial.
It should be noted that a combination of these characters is
necessary to describe some species. Fieldwork is essential for
assessing habit, because herbarium specimens represent only
one stage in the life cycle of the plant. For example, Paradry-
monia ciliosa is an elongate climber when immature and
then develops into an epiphyte with a basal rosette of leaves
when mature. Thus, the mature stage appears as an obligate
epiphyte. The presence of an obligate or primarily terrestrial
habit in the Episcieae is especially challenging to determine
when the plants are herbs (i.e., nonshrubs, as in many of the
unbranched shrubs found in Glossoloma).

A majority of the species in the tribe Episcieae are epi-
phytic. The Guiana Shield clade is exceptional for the Epis-
cieae in being comprised of nonfacultative terrestrial (i.e.,
obligate) herbs. The known exception is P. maculata, which
is described as being epiphytic or terrestrial (Skog and Feuil-
let 2008; Feuillet 2009). A feature that many of the species in
the Guiana Shield clade have in common is dry dehiscent
capsules with fully reflexed valves at maturity (e.g., L. amo-
nus; fig. 7G). It is possible that these dry dehiscent fruits em-
ploy a mechanism of seed dispersal from splashing caused by
rainfall. This combination is also associated with smaller
seeds and may be associated with carriage on the feet of ani-
mals (Burtt 1970, 1976). Capsules that are reported to have
splash-cup seed dispersal represented in the Guiana Shield
clade are Lembocarpus, Cremersia, and Rhoogen (J. Ertelt,
unpublished manuscript).

Many of the species in Chrysothemis, Paradrymonia, and
Nautilocalyx that are epiphytic (e.g., P. metamorphophylla)
have berries or capsules. In contrast, obligate terrestrial
herbs, such as Nautilocalyx panamensis, have dry capsules
that appear to be splash-cup dispersed (fig. 7H). One of the
challenges for understanding and evaluating character states
is that we do not have ready access to fruits or fruit images
for many members in these clades, where fruits are described
as subglobose, conical, succulent, or semisucculent. It was
surprising to discover that P. metamorphophylla (fig. 7C) is
a berry because it is autapomorphic in a clade that is entirely
capsular. Future research may discover that other species in
this clade are also indehiscent berries. Future evaluation of
habit and fruit morphology will allow for the correlation be-
tween obligate terrestrial habit and the presence of splash-
cup seed dispersal.

**Assessing Fruit Types for Future Studies**

Information on fruit types can be challenging to obtain.
Many fruits in closely related tribes are dry capsules that are
derived from semi-inferior ovaries (Diastema, Monopyle,
Gesneria, Rhytidophyllum, etc.). The relative succulence of
fruits in the Episcieae is variable and usually oversimplified
in the literature. For example, when the fruits of Episcia (fig.
7F) are compared with those of Gloxinieae (e.g., Diastema
or Monopyle), they would easily be described as succulent
(Skog 1978; Weber 2004). When the fruits of Episcia are
compared with those of Drymonia, they are relatively dry.
Likewise, the fruits of many species of Chrysothemis, Nauti-
localyx, and Paradrymonia are described as succulent in the
literature. One of the challenges of assessing fruit types in
this study was critically evaluating the character states
assigned to fruits. For example, Chrysothemis is described as
a succulent globose capsule by Weber (2004). In contrast,
an illustration of Chrysothemis friedrichsthaliana Wiehler (2002)
suggests that the capsule is conical and dry.
Utility of DNA Regions: Does It Take 14 Tortoises to Make One Hare?

It has been a challenge to find DNA regions that provide sufficient variability without excessive homoplasy or paralogy to resolve relationships among the Gesneriaceae and provide strong support for that resolution. Despite initial attempts to rely on single regions, the multi-gene approach has become the standard for molecular systematics in the family (Møller and Cronk 1997; Smith et al. 1997, 2004a, 2004b; Smith 2000b; Zimmer et al. 2002; Mayer et al. 2003; Perret et al. 2003; Roalson et al. 2005a, 2005b, 2008; Clark et al. 2006; Møller et al. 2009; Wang et al. 2011; Woo et al. 2011). For example, all the major genera were supported in the reduced and full analysis, but it was not until additional genic regions were added in the reduced analysis that a phylogeny was produced (fig. 4). In contrast, the expanded analysis of fewer base pairs yielded only 400 bp of potentially phylogenetically informative characters were able to fully resolve and provide strong support for the intergeneric relationships in the Alloplectus/Columnea/Drymonia clade in the reduced analysis (fig. 4). These results are similar to those of Miller et al. (2009), where nine noncoding cpDNA regions were screened for utility to resolve relationships within Solanaceae; three were discovered to have particularly strong utility, whereas another three provided little in the analysis. We likewise would advocate screening of sequence data prior to a larger-scale analysis at the species level and would advocate the inclusion of rpl32-trnL, trnQ-rps16, trnH-psbA, and ITS for further investigation among species in Gesneriaceae.

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Appendix A

Voucher Information

Provided here are species included in the analyses, voucher information, and GenBank accession numbers for ITS, GCYC, ncpGS1, ncpGS2, trnL intron, trnL-F, rps16 intron, rpl12-rps20, rpl32-trnL, trnQ-rps16, trnD-T, PepC, trnS-G, and trnH-psbA. Unvouchered samples taken from live material growing at the Smithsonian’s U.S. Botany Research Greenhouses (USBRG) are designated by their live accession number. Sequences not obtained are designated by a dash (—), sequences not applicable to the analyses are designated by “NA,” and generic type species are designated with an asterisk (*). Herbarium acronyms follow Thiers (2011). Taxon: voucher and herbarium; locality; ITS, GCYC, ncpGS1, ncpGS2, trnL intron, trnL-F spacer, rps16 intron, rpl12-rps20 spacer, rpl32-trnL spacer, trnQ-rps16 spacer, trnD-T spacer, PepC intron, trnS-G spacer.

Ingroup

Alloplectus hispidus (Kunth) Mart.; J. L. Clark 7720 (US); Ecuador; DQ211111, JQ953835, JQ954073, JQ953724, JQ954233, JQ953896, JP953137, JQ953984, JQ953960, JQ954208, JQ953811, JQ954048, JQ953700.

Alsobia dianthiflora (H. E. Moore & R. G. Wilson) Wiehler; J. Hall s.n. (SEL); cultivated (Costa Rica); DQ211160, JQ953837, JQ954074, JQ953726, JQ954235, JQ953898, JP954139, JQ953896, NA, NA, NA, NA. Alsobia punctata (Lind.) Hanst.; J. L. Clark 8851 (US); cultivated (Mexico); DQ211159, JQ953836, —, JQ953725, JQ954234, JQ953897, JQ954138, JQ953985, NA, NA, NA, NA.
Guyana; JQ953802, JQ953872, JQ954106, —, JQ954271, JQ954180, JQ954024, NA, NA, NA, NA, NA.

**Glossoloma anomalum** J. L. Clark; J. L. Clark 6020 (US); Ecuador; AF543225, JQ953855, JQ954090, JQ953744, JQ954253, JQ953917, JQ954160, JQ954005, JQ953968, JQ954216, JQ953818, JQ954055. *Glossoloma baguense* (L. E. Skog) J. L. Clark; J. L. Clark 5448 (US); Ecuador; AF543226, JQ953853, JQ954089, JQ953743, —, JQ953915, JQ954157, JQ954003, NA, NA, NA. *Glossoloma grandicalyx* (J. L. Clark & L. E. Skog) J. L. Clark; J. L. Clark 5449 (US); Ecuador; AF543218, JQ953854, —, JQ954252, JQ953916, JQ954159, JQ954004, JQ953969, JQ954217, JQ953819, JQ954056, JQ953708. *Glossoloma herbeae* (Ens.) J. L. Clark; J. L. Clark 4598 (US); Ecuador; AF543230, JQ953856, JQ954091, JQ953745, JQ954254, JQ953918, JQ954006, NA, NA, NA, NA, NA.

**Paradrymonia pedunculata** (J. F. Smith) Wiehler; J. F. Smith 4134 (SRP); French Guiana; JQ953806, JQ953887, JQ954121, JQ953777, JQ954288, JQ953952, JQ954041, NA, NA, NA. *Glossoloma oblongicalyx* (C. L. Leewenb.) Wiehler; J. L. Clark 8700 (US); cultivated (Brazil); AF543270, —, —, —, —, —, —, —, NA, NA, NA, NA, NA.

**Nautilocalyx adnexitifolius** (L.) Wiehler; J. L. Clark 6540 (US); Martinique; AY047086, JQ953874, JQ954108, JQ953763, JQ954274, JQ953937, JQ954008, NA, NA, NA, NA, NA.

**Nematanthus fissus** (Vell.) L. E. Skog; J. L. Clark 6266 (US); cultivated (Brazil); AF543270, —, —, —, —, —, —, —, NA, NA, NA, NA, NA.

**Nematanthus savannarum** (C. V. Morton) J. L. Clark; K. Redden 1339 (US); Guyana; DQ211188, JQ953886, JQ54190, JQ54031, JQ53972, JQ54220, JQ53882, JQ53940, NA, NA, NA, NA, NA.

**Neomortonia nummularia** (Enst.) Wiehler; J. L. Clark 6248 (US); Ecuador; AF543266, JQ953879, JQ954113, JQ953768, JQ54279, JQ53942, JQ54190, JQ54031, JQ53972, JQ54220, JQ53882, JQ53940, JQ53971. *Neomortonia rosea* Wiehler; J. L. Clark 7582 (US); Ecuador; DQ211099, JQ53880, —, JQ53769, JQ54280, JQ53943, JQ54191, JQ54032, JQ53973, JQ54221, JQ53823, JQ54060, JQ53712.

**Oerstedinia coccinella** Wiehler; J. L. Clark 8700 (US); Panama; DQ211150, JQ53881, JQ54114, JQ53770, JQ54281, JQ53944, JQ54192, JQ54038, NA, NA, NA, NA.

**Paradrymonia aurea** Wiehler; L. E. Skog 7979 (US); cultivated (Ecuador); AF206232, JQ953932, JQ954115, JQ53771, JQ54282, JQ53945, JQ54034, NA, NA, NA, NA. *Paradrymonia aurea* Wiehler; J. L. Clark 5409 (US); cultivated (Ecuador); AF543274, JQ53896, JQ54120, JQ53776, JQ54287, JQ53950, JQ54198, JQ54039, NA, NA, NA. *Paradrymonia campostyla* (Leewenb.) Wiehler; J. L. Clark 8855 (US); cultivated (French Guiana); DQ211180, —, —, —, —, —, —, —, NA, NA, NA, NA. *Paradrymonia campostyla* (Leewenb.) Wiehler; J. F. Smith 4137 (SRP); French Guiana; —, JQ53882, JQ54116, JQ53772, JQ54283, JQ53946, JQ54194, JQ54035, NA, NA, NA, NA, NA. *Paradrymonia ciliata* (Mart.) Wiehler; D. Clarke 10239 (US); Guyana; DQ211182, —, —, —, —, —, —, —, NA, NA, NA, NA. *Paradrymonia ciliata* (Mart.) Wiehler; J. F. Smith 4114 (SRP); French Guiana; —, JQ53883, JQ54117, JQ53773, JQ54284, JQ53947, JQ54195, JQ54036, NA, NA, NA. *Paradrymonia ciliata* (Mart.) Wiehler; R. Stewart s.n. (SRP); cultivated (Central America); JQ53805, JQ53884, JQ54118, JQ53774, JQ54285, JQ53948, JQ54037, NA, NA, NA, NA. *Paradrymonia densa* (C. H. Wright) Wiehler; K. Redden 1060 (US); Guyana; DQ211184, —, —, —, —, —, —, —, NA, NA, NA, NA. *Paradrymonia densa* (C. H. Wright) Wiehler; J. F. Smith 4115 (SRP); French Guiana; —, JQ53885, JQ54119, JQ53775, JQ54286, JQ53949, JQ54197, JQ54038, NA, NA, NA, NA. *Paradrymonia maculata* (Hook. f.) Wiehler; J. F. Smith 4134 (SRP); French Guiana; JQ53806, JQ53887, JQ54121, JQ53777, JQ54288, JQ53951, JQ54199, JQ54040, NA, NA, NA. *Paradrymonia metamorphophylla* (Donn. Sm.) Wiehler; J. L. Clark 6028 (US); Ecuador; DQ211178, JQ53888, JQ54122, JQ53778, JQ54289, JQ53952, JQ54200, JQ54041, NA, NA, NA, NA, NA, NA.
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- **Rhoogeton cyclophyllus** Leewenb.; D. Clarke 10350 (US); Guyana; DQ211163, JQ953893, JQ954127, JQ953783, JQ954294, JQ953937, JQ954205, JQ954045, NA, NA, NA, NA.
- **Rhoogeton viviparus** Leewenb.; D. Clarke 9255 (US); Guyana; DQ211164, JQ953892, JQ954126, JQ953782, JQ954293, JQ953936, JQ954204, JQ953892, NA, NA, NA, NA.
- **Rufodorsia major** Wiehler; J. F. Smith 3948 (SRP); cultivated (Costa Rica and Panama); JQ953807, JQ953890, JQ954124, JQ953780, JQ954291, JQ953954, JQ954202, JQ954043, NA, NA, NA, NA.
- **Rufodorsia minor** Wiehler; J. F. Smith 3934 (SRP); cultivated (Costa Rica and Panama); JQ953808, JQ953891, JQ954125, JQ953781, JQ954292, JQ953955, JQ954203, JQ954044, NA, NA, NA, NA.

### Outgroups

- *Achimenes cetoana* H. E. Moore; USBRG 1994-235; cultivated (Mexico); AY623371, AY623134, AY623176, AY623220, AY623265, JQ954136, AY623325, NA, NA, NA, NA, NA.
- **Amalophyllon albiflorum** (Rusby) Boggan, L. E. Skog & E. H. Roalson; USBRG 1994-503; cultivated (Colombia); AY373232, AY363914, AY623197, AY623242, AY364266, AY364288, JQ954134, AY623348, NA, NA, NA, NA, NA.
- **Gesneria pedicellaris** (Lodd.) Hiern; J. F. Smith 4512 (SRP); cultivated (Brazil); AY372337/AY372354, AY363942, AY623218, AY623263, JQ953983, NA, NA, NA, NA, NA.
- **Gesneria richii** (Aubl.) D. L. Denham; USBRG 1994-554; cultivated (Brazil); AY372337/AY372354, AY363942, AY623218, AY623263, JQ954135, AY623325, NA, NA, NA, NA, NA.
- **Gesneria sativa** (Rusby) Boggan, L. E. Skog & E. H. Roalson; USBRG 1994-503; cultivated (Costa Rica and Panama); JQ953807, JQ953890, JQ954124, JQ953780, JQ954291, JQ953954, JQ954202, JQ954043, NA, NA, NA, NA.
- **Gesneria xanthocarpa** (Paxt.) Wiehler; USBRG 1994-524; cultivated (Hispaniola and Puerto Rico); AF272232/AF272233, AY363927, AY623208, AY623252, AY623279, AY364301, JQ954135, AY623358, NA, NA, NA, NA, NA.
- **Sinningia cooperi** (Paxt.) Wiehler; USBRG 1994-340; cultivated (Brazil); JQ953784, JQ953833, JQ954294, JQ954044, NA, NA, NA, NA, NA.
- **Sinningia incarnata** (Aubl.) D. L. Denham; USBRG 1994-554; cultivated (Brazil); AY372337/AY372354, AY363942, AY623218, AY623263, JQ954135, AY623325, NA, NA, NA, NA, NA.
- **Sinningia richii** (Aubl.) D. L. Denham; USBRG 1994-554; cultivated (Brazil); AY372337/AY372354, AY363942, AY623218, AY623263, JQ954135, AY623325, NA, NA, NA, NA, NA.

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