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THE STATE OF MOLECULAR STUDIES IN THE FAMILY GESNERIACEAE: A REVIEW

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ABSTRACT. Sparked by the publication of large phylogenetic studies and major generic redefinitions in the Gesneriaceae, we review this growing body of molecular studies on the family. Different aspects of molecular data and their use in Gesneriaceae systematics are considered including conceptual challenges on the phylogenetic work undertaken to date as well as an overview of taxon sampling in the family. Molecular data are currently available for 70 of 72 recognized New World genera and 64 of 68 Old World genera. Many of the smaller genera and some of the larger genera are completely sampled. Current knowledge of tribal and generic delineations and relationships among the New World genera is relatively advanced. In contrast, intergeneric relationships and tribal arrangements are mostly unresolved for the Old World genera. In this paper we illustrate and summarize the published phylogenetic work in composite phylogenies with an emphasis on the most pertinent and accurate molecular systematic studies. This paper provides the molecular-based background for a new formal classification of the family Gesneriaceae.

Key words: Composite phylogenies, Gesneriaceae, molecular data, phylogenetic relationships, sampling density

INTRODUCTION

Darwin (1837–1838, p. 36) was the first to suggest in his sketched tree that classification should be genealogical, and systematists have conceived classifications since then to reflect evolutionary relationships through phylogenies. Over the last 25 years advances in molecular techniques have allowed an unparalleled progress in this area. Particularly the invention of automated sequencing techniques and machines resulted in a surge of data available for analysis. In addition, improved cladistic phylogenetic methods have greatly facilitated progress in the understanding of plant relationships, not only at the species level, but down to the population level and up to genus and higher taxonomic levels, to bring classifications in line with genealogical relationships. At the family level this has been exercised to its fullest conclusion in APG III (<http://www.mobot.org/MOBOT/research/APweb/>).

The Gesneriaceae is a mid-sized family in the order Lamiales with over 3300 species and 160 genera (Weber 2004). In the most recent formal classification, Burttt and Wiehler (1995) divided the Gesneriaceae into the following three subfamilies: 1) the neotropical Gesnerioideae; 2) the southern hemisphere Coronantheroideae; and 3) the chiefly Old World Cyrtandroideae (now

Didymocarpoideae for priority reasons, see Weber et al. 2013). The distribution of genera and species between the Neotropics/southern hemisphere and Paleotropics is roughly equivalent. The late B.L. Burttt, a traditional taxonomist by training, recognized the value of molecular approaches, particularly for placing difficult taxa in Gesneriaceae whose characters were not always conclusively resolved (B.L. Burttt pers. comm., 2003). Burttt (1956) stated “There is probably no group of flowering plants whose generic classification is more in need of revision than the [American] Gesneriaceae.” Although progress in understanding relationships within the family is significant, the Gesneriaceae has lagged behind relative to other plant groups in phylogenetic systematics, even though molecular studies of the Gesneriaceae began with the invention of PCR and automated sequencing techniques more than 25 years ago. Other plant groups may have benefited from their agronomic importance, such as the Brassicaceae, Fabaceae, Poaceae or Solanaceae. Current literature on the phylogenetic relationships in the Gesneriaceae is starting to corroborate across different studies, but the work is far from complete. In the following, an overview is provided particularly of the molecular phylogenetic research carried out on the Gesneriaceae to present the impact these studies have had on our evolutionary understanding of the family.

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There are more than 100 molecular-based papers published on the Gesneriaceae since 1991 (TABLE 1). In some, sequence data were used for population genetics studies (e.g., Pico et al. 2002, Xiao & Wang 2007, Xiao et al. 2012). In others, Gesneriaceae samples were used solely as outgroups. Publications with Gesneriaceae samples as the target group (e.g., ingroup) cover a wide range of topics, such as taxonomy and systematics, the placement of problematic taxa, biogeography, and divergence time estimations. On a more fundamental level, Gesneriaceae taxa were used for topics such as patterns of sequence evolution and evolutionary development (EvoDevo). A brief historical overview on molecular work is presented followed by current state-of-the-art studies and closing remarks.

HISTORIC OVERVIEW OF MOLECULAR STUDIES IN GESNERIACEAE

Publications on molecular research began in earnest in the early 1990s. By the end of this decade, research increased and continued to appear at a steady rate, with an average of 4–5 papers per year up to the present. Studies range from the population level (e.g., Hughes et al. 2005), species and genus level, to tribal and family-wide studies (e.g., Smith 2000c; Möller et al. 2009, 2011a).

The first paper to appear that included molecular sequence data of Gesneriaceae was by Martin and Dowd (1991), a study on angiosperm phylogeny using protein sequences. Only a few years later Smith (1993, 1994) and Smith and Sytsma (1994a, 1994b) published papers on *Columnea* L. relationships using chloroplast DNA restriction fragment length polymorphisms. The first study to include Gesneriaceae samples using nucleotide sequence data was from Olmstead and Reeves (1995). A few years later, studies on Old World and New World Gesneriaceae were published. The Old World studies focused at the species level in *Cyrtandra* J.R.Forst. & G.Forst. (Samuel et al. 1997), *Streptocarpus* Lindl. and *Saintpaulia* H.Wendl. (Möller & Cronk 1997a, 1997b). The New World studies focused on the tribe Episcieae (Smith & Carroll 1997). In addition, a family-wide study (Smith et al. 1997a) focused on the phylogenetic placement of uncertain species (Smith et al. 1997b).

Initial acquisition of sequence data was relatively slow and the matrices were limited to one or two markers and less than 20 samples. Analyses increased in sample size and marker diversity, sometimes including 100+ species (e.g., Perret et al. 2003, Clark et al. 2006, Hughes et al. 2006, Clark et al. 2009, Wei et al. 2010). The largest analyses to date include more than two hundred

Gesneriaceae samples (Weber et al. 2011a for Old World; Perret et al. 2013 for New World).

CONCEPTUAL ISSUES

Phylogenetic Methods Applied

The analytical approaches to generate and evaluate phylogenetic hypotheses were historically challenging for larger datasets. Computer performance in the early 1990s was a limiting factor because processors were slow compared to today's standards. In addition, the number of computer software programs for phylogenetic analyses were primarily limited to PAUP, then version 3.1.1 (Swofford 1993), and NONA (Goloboff 1998) by Lindqvist and Albert (2001). PAUP* version 4 replaced earlier versions from 2001 onwards (first used in Gesneriaceae studies including Atkins et al. 2001, Smith 2001, Denduangboripant et al. 2001), by offering an improved maximum parsimony (MP) algorithm, and the integration of distance and maximum likelihood (ML) methods (Swofford 2002).

Distance methods such as UPGMA and neighbor joining were practiced and accepted. Software programs continue to use them, but it is important to note that they are not phylogenetic-based. For example, neighbor joining is a distance based method that is effectively used to evaluate gene trees in EvoDevo studies to confirm sequence homology by comparing sequence similarities (e.g., Zhou et al. 2008, Mantegazza et al. 2009, Nishii et al. 2010, Tononi et al. 2010, Yang et al. 2010).

Phylogenetic thoughts on studying the evolutionary relationships among organisms go back to Aristotle with the parsimony principle hinted at in his Great Chain of Being, a principle that William of Ockam formulated in the 1300s. Camin and Sokal (1965) were the first to use a computer program for parsimony analyses. Fitch parsimony (i.e., on unordered characters) was introduced in 1971 (Fitch 1971). Initial publications applying parsimony criteria (vs. distance-based methods) to infer plant phylogenies were focused on higher-level relationships among angiosperms (e.g., Bremer 1988). Many plant phylogenetic studies include a parsimony algorithm.

Parsimony is a non-parametric statistical method where the preferred phylogenetic trees are chosen on the basis of minimizing assumptions to explain evolutionary changes of the observed data. In contrast, ML is a parametric statistical method applied to data given a statistical model of evolution. The maximum-likelihood method provides estimates for the parameters of the model. Edwards and Cavalli-Sforza (1963) were the first to use ML in phylogenetics and Neyman (1974)

was the first to use ML for nucleotide sequences. Felsenstein (1981) developed a computationally efficient algorithm. Initial ML algorithms (Jukes & Cantor 1969) were limited to assuming equal base frequency and equally likely substitution types. Substitution models became more sophisticated over time with the Generalised time-reversible (GTR) being the most complex, allowing a probability for each of the six substitution types (Tavaré 1986). Initially ML was not widely applied to plant phylogenetic work because of model limitations, but with the inclusion of this method in PAUP* version 4 (ca. 2000 onwards), it became common to include ML to the then standard MP approach. The addition of ML methods in phylogenetic systematics allowed for independent support for topological hierarchies, particularly where the MP topology received little support (e.g., Smith 2000a, Möller & Cronk 2001a). However, ML has the disadvantage of a high computational complexity, and hence analyses are slow for larger matrices (>100 samples) (e.g., Yang 1994, but see Schadt et al. 1998).

Bayesian inference (BI) is an additional parametric statistical method that incorporates a substitution model often implemented with the software MrBayes (Huelsenbeck & Ronquist 2001, 2007). The Bayesian inference of phylogeny is based on the posterior probability distribution of trees. BI analyses have been used to address phylogenetic relationships in the Gesneriaceae from around 2004 to present (e.g., Smith et al. 2004a, 2004b; Wang et al. 2004a). The ability to incorporate BI methods became more user-friendly with the introduction of MrBayes version 3.0 (Huelsenbeck & Ronquist 2001). MrBayes version 3.1.2 released in 2007 (Huelsenbeck & Ronquist 2007) and version 3.2.1 released in 2011 (Ronquist et al. 2012) show improvements such as multiple Markov Chain Monte Carlo (MCMC) runs in parallel and average standard deviation of split frequencies, a measure of convergence of two runs.

Comparison of results generated from multiple methods (e.g., ML, MP, or BI) is common in current phylogenetic studies of Gesneriaceae and throughout systematics. Applying multiple methods allows a comparison of independently derived evolutionary hypotheses and provides a measure of confidence where the tree topologies are in congruence.

Molecular Markers Used for Phylogenetic Reconstructions

Many thousand Gesneriaceae nucleotide sequences are deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>). However, these are not easily categorized. PHYLOTA (www.phylota.net) allows

for the examination of GenBank data and arranges the accessible submissions in aligned clusters by gene regions. Release 184 (15 June 2011) includes 4668 Gesneriaceae sequences (APPENDIX 1). For our purpose, they have been complemented here by manual addition of Gesneriaceae sequences from GenBank released after 15 June 2011 (APPENDIX 2). Combined, these amount to 4924 sequences available in the public domain at that point in time.

Contrary to nuclear data, which are inherited biparentally and recombine, potentially confounding the retrieval of phylogenetic signal (e.g., McDade 1992, Xiao et al. 2010), data from the mitochondrial and plastid genomes are uniparentally inherited (e.g., Mogensen 1996). Chloroplast DNA (cpDNA) in Gesneriaceae and most angiosperms is maternally inherited (Möller et al. 2004, Puglisi et al. 2011b). Mitochondrial gene regions were found to be of no practical use for phylogenetic work in Gesneriaceae due to their low evolutionary rate. Consequently, only 13 submissions of 6 mitochondrial genes are presently available (APPENDIX 2). Around 2300 (ca. 47%) of Gesneriaceae accessions in GenBank are from the chloroplast genome (APPENDIX 2). There are 223 sequence clusters of cpDNA listed in PHYLOTA. They include large clusters of *matK* (57 accessions) and *rbcL* (89 accessions), these being the core DNA barcodes for land plants (CBOL Plant Working Group 2009), and have recently been used for this purpose in New World Gesneriaceae (iBOL on GenBank). The cpDNA marker *rbcL* has been used for inferring phylogenetic relationships by Smith (2000c). The chloroplast marker *ndhF* has also been used for phylogenetic work at the level of subfamily and tribe (e.g., Smith & Carroll 1997; Smith et al. 1997a, 1997b, 2004a, 2004b; Smith & Atkinson 1998; Smith 2000a, 2000b, 2000c, 2001). Preliminary work on the phylogeny of the Episcieae (Clark et al. 2006) showed that variation for *rbcL* and *ndhF* is not adequate for inferring phylogenetic relationships at the generic or tribal level. In addition, Smith (2000b) concluded that, because of the low resolution in the *ndhF* parsimony tree, any comparison to the ITS parsimony tree to be “mostly irrelevant.” Lack of support for most branches in Smith’s results and the grouping of distantly related species (e.g., *Kohleria grandiflora* L.P.Kvist & L.E.Skog from the tribe Gloxinieae as sister species to *Paradrymonia* Hanst. spp. in the Episcieae) suggest that *ndhF* is inappropriate for inferring phylogenetic relationships in the Gesneriaceae. In conclusion, results from early studies of New World Gesneriaceae based on *rbcL* and *ndhF* (Smith & Carroll 1997; Smith 2000a, 2000b) should be treated with caution.

TABLE 1. Guide to publications involving molecular work on Gesneriaceae arranged by main topic and taxonomic level of analysis. Taxonomic level refers to the maximum taxonomic range of the samples included.

Subject	Taxonomic level within studies					
	Species	Genus	Tribe	Subfamily	Family	Family placement
Population genetics	Picó et al. 2002 Xiao & Wang 2007 Xiao et al. 2012					
Phylogeny and systematics		Bramley et al. 2004 Caro et al. 2006 Chautems et al. 2000 Denduangboripant & Cronk 2001 Denduangboripant et al. 2001 DeVilliers 2008 Li & Wang 2007 Lindqvist & Albert 1999 Lindqvist & Albert 2001 MacMaster et al. 2005 Möller 2003 Möller & Cronk 1997a Möller & Cronk 1997b Möller & Cronk 1999 O'Sullivan 1999 Qiu 2011 Samuel et al. 1997 Smith 1993 Smith 1994 Smith & Sytsma 1994a Smith & Sytsma 1994b Atkins et al. 2001 Clark et al. 2008 Clark et al. 2009 Cronk et al. 2005 Möller & Cronk 2001b	Clark et al. 2006 Möller et al. 2011b Perret et al. 2003 Perret et al. 2006 Puglisi et al. 2011a Roalson et al. 2006 Roalson et al. 2008 Smith 2000b Smith & Carroll 1997 Wang & Li 1998 Wang et al. 2011 Weber et al. 2011b Weber et al. 2011c	Möller et al. 2011 Roalson et al. 2005a Roalson et al. 2005b Smith 2000a Smith 2001 Smith & Atkinson 1998 Smith et al. 2004a Smith et al. 2004b Smith et al. 2006 Weber et al. 2011a Woo et al. 2011 Zimmer et al. 2002	Cierner et al. 2000 Mayer et al. 2003 Möller et al. 2009 Perret et al. 2013 Smith 2000c Smith et al. 1997a Smith et al. 1998 Wang et al. 2004a Wei et al. 2010	Albach et al. 2001 Bremer et al. 2002 Bremer et al. 2001 Donoghue et al. 1998 Hilu et al. 2003 Martin & Dowd 1991 Olmstead & Reeves 1995 Olmstead et al. 2000 Olmstead et al. 2001 Oxelman et al. 1999 Oxelman et al. 2005 Qiu et al. 2010 Savolainen et al. 2000 Schäferhoff et al. 2010 Soltis et al. 2000 Soltis et al. 2011 Wortley et al. 2005 Xia et al. 2009
Biogeography	Hughes et al. 2005 Kokubugata et al. 2011		Perret et al. 2006		Perret et al. 2013	
Divergence time dating					Perret et al. 2013	Bremer et al. 2004 Palmer 2010

TABLE 1. Continued.

Subject	Taxonomic level within studies					
	Species	Genus	Tribe	Subfamily	Family	Family placement
Placement of uncertain taxa			Araújo et al. 2010	Möller et al. 2011	Smith et al. 1997b	Bremer et al. 2002
			Clark et al. 2010	Smith 2001	Wang et al. 2004a	Bremer et al. 2001
			Middleton & Möller 2012		Wei et al. 2010	Bremer et al. 2004
Character evolution			Smith & Clark 2013			Oxelman et al. 1999
		Clark & Zimmer 2003	Clark et al. 2011	Smith 2000a		Oxelman et al. 2005
		Denduangboripant & Cronk 2000	Clark et al. 2012	Woo et al. 2011		Xia et al. 2009
		Denduangboripant & Cronk 2001	Martén-Rodríguez et al. 2010			Donoghue et al. 1998
		Harrison et al. 1999	Perret et al. 2007			
		Hughes et al. 2006	Wang et al. 2010			
		Möller & Cronk 2001a				
		Palee et al. 2006				
		Roalson et al. 2003				
		Harrison et al. 2005				
Evo-Devo		Nishii et al. 2010				
	Du & Wang 2008				Citerne et al. 2000	
	Gao et al. 2008				Möller et al. 1999	
	Mantegazza et al. 2007					
	Mantegazza et al. 2009					
	Song et al. 2009					
	Tonomi et al. 2010					
	Wang et al. 2004b					
Yang et al. 2010						
Zhou et al. 2008						

In the chloroplast genome of the Gesneriaceae, spacer regions evolve twice as fast as introns (e.g., Möller et al. 1999b, Perret et al. 2003). The *trnL-F* intron spacer region is the most commonly used cpDNA marker in Gesneriaceae of all chloroplast sequences (850 sequences), and has been applied from species to family level in >25 publications (APPENDIX 2). Other spacer regions that are often used include *atpB-rbcL* (Mayer et al. 2003; Wang et al. 2004a; Möller et al. 2009; Perret et al. 2003, 2006, 2007; Samuel et al. 1997; Chautems et al. 2000) and *psbA-trnH* (Clark JL et al. 2006, Clark JR 2008, 2009).

For Gesneriaceae, as well as most other plant groups, the most widely applied marker is the internal transcribed spacer (ITS) region of 18S–26S nuclear ribosomal DNA (nrDNA) (APPENDIX 2). The region is readily amplified because there are multiple copies of ITS paralogues that have evolved through concerted evolution (Baldwin et al. 1995) and are greatly homogenized by concerted evolution and molecular drive (Dover 1994). For example, hundreds of copies of ITS were detected in a single locus of plants (e.g., ~250 in *Arabidopsis* L., Copenhaver & Pikaard 1996). They are, as all nuclear genes, biparentally inherited. At least in *Streptocarpus* ITS is inherited as one cluster without significant levels of recombination (Denduangboripant et al. 2007), and is therefore useful for phylogenetic reconstructions. In some groups where hybridization is involved, ITS can result in sequencing problems (e.g., Bailey et al. 2003, Saito et al. 2007, Puglisi et al. 2011b).

The 18S and 26S nrDNA genes have not been used widely in Gesneriaceae because of their relative slow rates of evolution. Only Wang et al. (2004a) used 26S sequences at the family level in combination with other gene regions to infer the phylogenetic placement of *Titanotrichum oldhamii* Soler.

The nrDNA ITS sequences evolve about five times faster than the 5.8S nrDNA genes (Palee et al. 2006) and 5 to 10 times faster than 26S (data compiled from Wang et al. 2004a and Möller et al. 2011a). The ITS spacer sequences have been widely applied in molecular studies that range from the population level (Hughes et al. 2005) to the species level (>40 publications). They have been used at the subfamily level, though here alignment ambiguities begin to indicate the limits of ITS (Möller et al. 2009, 2011a).

The external transcribed spacer (ETS) evolves about twice as fast as ITS and can generate congruent tree topologies (e.g., Baldwin & Markos 1998). ETS sequences have been included in a study of *Cyrtandra* species to obtain sufficient sequence diversity (Clark et al. 2008, 2009).

Where ITS shows no inter-specific variation, the 5S nrDNA gene spacers can provide more

resolution (Lindqvist & Albert 1999). For example, this was the case in *Saintpaulia* where six species had identical ITS sequences (Möller et al. 1997b). However, in this instance 5S spacer sequences approach population level variation and show intraspecific variation (Lindqvist & Albert 1999, 2001). A similar example was shown for ETS in *Cyrtandra* where nucleotide sequence data perhaps no longer reflect the species boundary (Clark et al. 2009).

Contrary to multicopy chloroplast and nuclear ribosomal DNA genes, developmental genes are often single or low copy in number and establish mutations faster at a close taxonomic distance even though they are under functional constraints (Möller et al. 1999). In Gesneriaceae, the low copy nuclear gene *CYCYLOIDEA* (*CYC*) was first explored for phylogenetic studies (e.g., Möller et al. 1999, Citerne et al. 2000). In the Gesnerioideae no duplication event has so far been observed, and only *GCYC1* is present (e.g., Smith et al. 2004a, Clark et al. 2012), making it an ideal marker for inferring phylogenetic relationships in this subfamily where it has been used in thirteen studies. Several duplications (e.g., *GCYC1*, *GCYC2*) of the gene have been detected outside the Gesnerioideae at various levels ranging from genus to tribe and one that predates the first diversification of the Gesneriaceae. A duplication was detected at the genus level in the African *Streptocarpus/Saintpaulia* clade (*GCYC1A*, *GCYC1B*, Citerne et al. 2000, Wang et al. 2004a). Other duplications have occurred in the advanced Asiatic and Malesian didymocarpooids (*GCYC1C*, *GCYC1D*, Wang et al. 2004a, Song et al. 2009) and a *GCYC* duplication predating the divergence of Coronanthereae (*GCYC1E*, *GCYC1F*, Wang et al. 2004a, Smith et al. 2006). The few samples included within the duplicated clades show a topology principally congruent with those recovered from neutrally evolving genes, with few exceptions, such as *Diastema* Benth. (Smith et al. 2004a), which was explained by homoplasies due to saturation of nucleotide substitutions.

Housekeeping genes, constitutive genes required for the maintenance of basic cellular function, such as the nuclear plastid-expressed *GLUTAMINE SYNTHETASE* gene (*ncpGS*) that includes introns, have been isolated and amplified for phylogenetic analyses from New World Gesneriaceae (Sinningieae: Perret et al. 2003, 2006, 2007, Gloxinieae and Gesnerieae: Smith et al. 2004b, Episcieae: Clark et al. 2012). Among the Sinningieae, the *ncpGS* intron divergence levels were found to be comparable to *GCYC* (Citerne et al. 2000), and substitution rates that were higher than in plastid non-coding regions.

Caro et al. (2006) used exon 2 of a *CHALCONE SYNTHASE* (*CHS*) gene to infer phylogenetic

relationships in *Saintpaulia*. They found that the genus comprised two *CHS* genes (*SaCHSA* and *SaCHSD*), and used *SaCHSD* to construct a gene tree of six *Saintpaulia* species. The sequences distinguished four of five closely related species of the *S. ionantha*-complex that were previously not resolved by ITS data alone (Möller & Cronk 1997b).

Taxonomic Level of Analysis and Sampling Coverage

The Gesneriaceae contain more than 3340 species (Weber 2004). After the latest major taxonomic changes (cf. section “Systematic Consequences of Molecular Phylogenetic Work” below), the Gesneriaceae *s.str.* include ca. 148 genera (Weber et al. 2013), a reduction from the 160 genera given in Weber (2004) and Weber and Skog (2007onw.). The New World gesnerioids and the South American and southern hemisphere coronantheroids include 66 and nine genera respectively, while the Old World epithematoids and didymocarpoids include seven and 66 genera respectively (TABLE 2, Weber et al. 2013). A complete taxon sampling would be ideal for phylogenetic studies, but some genera are very large, making the computational analyses awkward, or DNA samples for some do not exist in the first place. At the generic level, samples of all but two Old World Asian and Malesian genera (*Championia* C.B.Clarke and *Deinostigma* W.T.Wang & Z.Y.Li) have been included in recent phylogenetic analyses (Möller et al. 2009, 2011a), discounting the presumed extinct epithematoid genus *Gyrogyne* (Wang 2003). Of the nine didymocarpoid African genera (TABLE 2), all but two (i.e., *Nodonema* B.L.Burt and *Trachystigma* C.B.Clarke) are sequenced and include molecular data deposited in GenBank. Molecular data are available for 73 of 75 New World and southern hemisphere genera. Only *Lampadaria* Feuillet & L.E.Skog and *Cremospermopsis* L.E.Skog & L.P.Kvist have not been investigated using molecular sequence data.

A complete species sampling of monotypic and small genera are relatively easy to accomplish, unless they are rare (e.g., *Deinostigma*), or presumed extinct (e.g., *Gyrogyne* W.T.Wang). In contrast, there are difficulties when assessing large genera, for example in the collection of sufficient samples to represent their morphological diversity or cover their geographic distribution. Some large genera have received particular attention and efforts were undertaken to include a significant number of species. For example, among the Old World genera, 50 species (~30%) out of ~170 species of *Aeschynanthus* Jack were included by Denduangboripant et al. (2001). Of *Paraboea*

(C.B.Clarke) Ridl. in its new circumscription 42 species (~42%) out of >100 species were included by Puglisi et al. (2011a). About half of the species of *Oreocharis* Benth. in its newly defined circumscription (39 out of ~80 species) were included by Möller et al. (2011b). For *Streptocarpus* 77 species (~48%) out of ~160 species were included by Möller and Cronk (2001a, 2001b). Ongoing studies of *Streptocarpus* have increased molecular data to >90 species (DeVilliers (2008). A complete species sampling is envisaged here, and so far ITS data for 125+ species are available (Bellstedt & Möller unpublished). Clark et al. (2008, 2009) included samples of 70 species of *Cyrtandra*, though the genus is huge (652–818 species, Atkins et al. 2013) and sampling density is under 10%. A similar level of sampling was achieved by Roalson and Clark (2006) for the large New World genus *Besleria* L. (ca. 22 out of 165+). In the same study (Roalson & Clark 2006) there were 19 out of 35 species (over 50%) analyzed in *Gasteranthus* Benth. Roalson et al. (2003) included all 24 species of *Achimenes* Pers. The most comprehensive taxon sampling for a phylogenetic study to date for New World Gesneriaceae is for the tribe Sinningieae, where Perret et al. (2003, 2006, 2007) sampled 59 out of 60+ species of *Sinningia* Nees, all eight species of *Vanhouttea* Lem. and all six species of *Paliavana* Vell. ex Vand.

SYSTEMATIC CONSEQUENCES OF MOLECULAR PHYLOGENETIC WORK

Family Placement

The phylogenetic position of Gesneriaceae has been addressed in more than 25 research papers since 1991 (TABLE 1). Early analyses aimed at addressing higher-level phylogenetic relationships such as family, class or order, were limited by computational constraints and limited taxon sampling. Samples representing families were often limited to a single species to represent Gesneriaceae. This resulted in varying phylogenetic positions of the Gesneriaceae because of different sampling emphases, though always in the Lamiales.

A first attempt to place the family based on molecular sequence data was by Martin and Dowd (1991) who used *rbcL* protein sequences and found the Gesneriaceae clustering with Pedaliaceae (*Sesamum* L., *Ceratotheca* Endl.), Bignoniaceae (*Jacaranda* Juss., *Pandorea* Spach) and Scrophulariaceae s.l. (*Digitalis* L., *Paulownia* Siebold & Zucc.). This was a reasonable position given the inclusion of two generic samples per family (e.g., *Sinningia* and *Saintpaulia* to represent the Gesneriaceae). Later work that utilized

TABLE 2. Classification of the family Gesneriaceae based principally on molecular phylogenetic results (unless otherwise stated), referring to formal and informal categories presently in use. Species numbers and distribution ranges from Skog and Boggan (2006) with additional data incorporated from recent literature (i.e., 2006 to present) and Weber and Skog (2007onw).

Genus	No of species	General distribution
Subfamily Gesnerioideae (including Coronantheroideae)		
Tribe Beslerieae		
<i>Besleria</i> L.	165+	From Mexico to Central & South America, Caribbean
<i>Anetanthus</i> Hiern ex Benth. & Hook.f.,	3+	Colombia to Bolivia & Brazil
<i>Cremosperma</i> Benth.	27	Costa Rica to Peru
<i>Cremospermopsis</i> L.E.Skog & L.P.Kvist ¹	2	Colombia
<i>Gasteranthus</i> Benth.	41	Mexico to Bolivia
<i>Reldia</i> Wiehler	6	Costa Rica to Peru
<i>Resia</i> H.E.Moore ¹	5	Colombia, Ecuador & Venezuela
<i>Shuaria</i> D.A.Neill & J.L.Clark	1	Ecuador
<i>Tylopsacas</i> Leeuwenb.	1	Brazil, Guyana & Venezuela
Tribe Napeantheae		
<i>Napeanthus</i> Gardner	>20	Mexico to Bolivia & Brazil, Guianas, Trinidad
Tribe Coronanthereae		
<i>Asteranthera</i> Hansl.	1	Argentina & Chile
<i>Fieldia</i> A.Cunn.	1	Australia
<i>Coronanthera</i> Vieill. ex C.B.Clarke	13–20	New Caledonia
<i>Depanthus</i> S.Moore	1	New Caledonia
<i>Lenbrassia</i> G.W.Gillett	1	Australia
<i>Mitraria</i> Cav.	1	Argentina & Chile
<i>Negria</i> F.Muell.	1	Pacific
<i>Rhabdothamnus</i> A.Cunn.	1	New Zealand
<i>Sarmienta</i> Ruiz & Pav.	1	Argentina & Chile
Tribe Gesneriaceae		
<i>Bellonia</i> L.	2	Cuba & Hispaniola
<i>Gesneria</i> L.	71	Caribbean
<i>Pheidonocarpa</i> L.E.Skog	2	Cuba & Jamaica
<i>Rhytidophyllum</i> Mart.	25	Caribbean
Tribe Gloxinieae		
<i>Achimenes</i> Pers.	27	Mexico, Central America & N South America, Caribbean
<i>Amalophyllum</i> Brandege	13	Mexico to NW South America
<i>Chautemsia</i> A.O.Araujo & V.C.Souza	1	Brazil
<i>Diastema</i> Benth.	20+	Mexico to NW South America, Brazil
<i>Eucodonia</i> Hanst.	2	Mexico
<i>Gloxinella</i> (H.E.Moore) Roalson & Boggan	1	Peru
<i>Gloxinia</i> L'Hér.	4	Mexico, Central American, N South America
<i>Gloxiniopsis</i> Roalson & Boggan	1	Colombia
<i>Goyazia</i> Taub.	3	Brazil
<i>Heppiella</i> Regel	4	NW South America & Brazil
<i>Kohleria</i> Regel	27+	Mexico to NW South America, Caribbean
<i>Mandirola</i> Decne.	3	Brazil
<i>Monopyle</i> Moritz ex Benth. & Hook.f.	21+	Guatemala to NW South America
<i>Moussonia</i> Regel	15	Mexico to Panama
<i>Niphaea</i> Lindl.	4	Mexico to Nicaragua, Peru, Venezuela
<i>Nomopyle</i> Roalson & Boggan	2	Ecuador & Peru
<i>Pearcea</i> Regel	20+	Colombia to Bolivia
<i>Phinaea</i> Benth.	4	Mexico to NW South America, Brazil, Caribbean
<i>Seemannia</i> Regel	4	Ecuador to Bolivia
<i>Smithiantha</i> Kuntze	6	Mexico
<i>Solenophora</i> Benth.	18	Mexico to Panama
Tribe Episcieae		
<i>Alloplectus</i> Mart.	6	Costa Rica, NW South America
<i>Alsobia</i> Hanst.	4	Costa Rica NW South America
<i>Christopheria</i> J.F.Smith & J.L.Clark	1	Guyana & French Guyana

TABLE 2. Continued.

Genus	No of species	General distribution
<i>Chrysothemis</i> Decne.	6	Guatemala to Ecuador, Brazil, Guianas & Caribbean
<i>Cobananthus</i> Wiehler	1	Guatemala & Honduras
<i>Codonanthe</i> (Mart.) Hanst.	19	Mexico to Bolivia, Brazil, Guianas SE Caribbean
<i>Codonanthopsis</i> Mansf.	4	NW South America, Brazil & Guianas
<i>Columnea</i> L.	205+	Mexico to Bolivia, Brazil, Guianas & Caribbean
<i>Corytoplectus</i> Oerst.	13	NW South America to Bolivia, Brazil & Guyana
<i>Crantzia</i> Scop	4	NW South America, Brazil & Guianas
<i>Cremerisa</i> Feuillet & L.E.Skog	1	French Guiana
<i>Drymonia</i> Mart.	75+	Mexico to Bolivia, Brazil, Guianas & Caribbean
<i>Episcia</i> Mart.	11	Mexico to Peru, Brazil, Guianas & Martinique
<i>Glossoloma</i> Hanst.	27+	Mexico to Bolivia
<i>Lampadaria</i> Feuillet & L.E.Skog	1	Guyana
<i>Lembocarpus</i> Leeuwenb.	1	French Guiana & Surinam
<i>Lesia</i> J.L.Clark & J.F.Smith	1	N South America
<i>Nautilocalyx</i> Linden ex Hanst.	57	Mexico to Bolivia, Brazil, Guianas & E Caribbean
<i>Nematanthus</i> Schrad.	31	Brazil & N South America
<i>Neomortonia</i> Wiehler	1	Mexico, Costa Rica, Panama to Ecuador
<i>Oerstedia</i> Wiehler	3	Mexico, Costa Rica, Panama
<i>Pachycaulos</i> J.L.Clark & J.F.Smith	1	N South America
<i>Pagothyra</i> (Leeuwenb.) J.F.Smith & J.L.Clark	1	Guianas and W Venezuela
<i>Paradrymonia</i> Hanst.	45	Mexico to Bolivia, Brazil & Guianas
<i>Rhoogeton</i> Leeuwenb.	2	Brazil, Guyana & Venezuela
<i>Rufodorsia</i> Wiehler	4	Nicaragua to Panama
Tribe Sphaerorrhizeae		
<i>Sphaerorrhiza</i> Roalson & Boggan	2	Brazil
Tribe Sinningieae		
<i>Paliavana</i> Vell. ex Vand.	6	Brazil
<i>Sinningia</i> Nees	73	Mexico to Panama, Colombia to Bolivia, Argentina, Paraguay, Guianas & Brazil
<i>Vanhouttea</i> Lem.	12	Brazil
Tribe Titanotricheae		
<i>Titanotrichum</i> Soler.	1	China, Taiwan, Japan
Subfamily Didymocarpoideae		
Tribe Epithemateae (missing: <i>Gyogyne</i>)		
<i>Epithema</i> Blume	20+	S & SE Asia, one species in W Africa
<i>Gyogyne</i> W.T.Wang ²	1	China
<i>Loxonia</i> Jack	3	W Malesia
<i>Monophyllaea</i> R.Br.	30+	Malesia
<i>Rhynchoglossum</i> Blume	10	from India and S China to New Guinea, one spp in C America
<i>Stauranthera</i> Benth.	5+	from NE India and S China throughout Malesia to New Guinea
<i>Whytockia</i> W.W.Sm.	8	S China, Taiwan
Tribe Didymocarpoideae		
Basal Asian and Malesian genera		
<i>Beccarinda</i> Kuntze ³	8	NE India, Burma, S China, Vietnam, Sumatra
<i>Boeica</i> C.B.Clarke	12	Bhutan, S China, N & NE India, Myanmar, N Vietnam, NW Malaya
<i>Championia</i> C.B.Clarke ⁴	1	Sri Lanka
<i>Corallodiscus</i> Batalin	3–5	Bhutan, China, W, E, N and NE India, Nepal, Thailand
<i>Jerdonia</i> Wight.	1	India
<i>Leptoboea</i> Benth.	3	Bhutan, N and NE India, China, Myanmar, Thailand
<i>Platystemma</i> Wall.	1	E & W Himalayas (Nepal, Bhutan, N India), SW China
<i>Rhynchotechum</i> Blume	18	NE India, Nepal, Bhutan, SW & S China, SE Asia and Malesia to New Guinea
<i>Tetraphyllum</i> Griff. ex C.B.Clarke	3	NE India, Bangladesh, Burma, Thailand

TABLE 2. Continued.

Genus	No of species	General distribution
European genera		
<i>Haberlea</i> Friv.	1 ⁵	Balkan peninsula (Bulgaria)
<i>Jancaea</i> Boiss.	1	Greece
<i>Ramonda</i> Rich.	3	Spain and Balkan peninsula
African and Madagascan genera⁶		
<i>Acanthonema</i> Hook.f.	2	S Nigeria, Fernando Po, Cameroun, Gabon
<i>Colpogyne</i> B.L.Burt	1	Central Madagascar
<i>Hovanella</i> A.Weber & B.L.Burt	2(3)	E & C Madagascar
<i>Linnaeopsis</i> Engl.	3	E Africa (N Tanzania, Tanganyika Terr.)
<i>Nodonema</i> B.L.Burt ⁴	1	Nigeria, Cameroun
<i>Saintpaulia</i> H.Wendl.	11	E Africa (SE Kenya, N Tanzania)
<i>Schizoboea</i> (Fritsch) B.L.Burt	1	Cameroun, Fernando Po; Ruanda, Zaire; S Tanzania
<i>Streptocarpus</i> Lindl.	135+	tropical and southeastern Africa, Madagascar, Comoro Islands
<i>Trachystigma</i> C.B.Clarke ⁴	1	Gabon
Advanced Asian and Malesian twisted-fruited genera		
<i>Boea</i> Comm. ex Lam.	14	S China, NE India, N Thailand, Vietnam, Philippines, Sulawesi, Sumbawa, Flores, New Guinea, Solomon Islands, Bismarck and Louisiade archipelago, Australia.
<i>Damrongia</i> Kerr ex Craib	6	Thailand and Peninsular Malaysia
<i>Emarhendia</i> Kiew, A.Weber & B.L.Burt	1	Malay Peninsula (Pahang)
<i>Kaisupeea</i> B.L.Burt	3	Myanmar, Thailand, S Laos
<i>Loxocarpus</i> R.Br.	20+	S Thailand and W Malesia
<i>Orchadocarpa</i> Ridl.	1	Malay Peninsula
<i>Ornithoboea</i> Parish ex C.B.Clarke	11	China, Malaysia, E Myanmar, Thailand, Vietnam
<i>Paraboea</i> (C.B.Clarke) Ridl.	110+	throughout Malesia, from Sumatra to New Guinea and from S China to Borneo, including the Philippines
<i>Rhabdothamnopsis</i> Hemsl.	1	S China
<i>Senyumia</i> Kiew, A.Weber & B.L.Burt	1	Malay Peninsula
<i>Somrania</i> D.J.Middleton ⁷	2	Thailand
<i>Spelaeanthus</i> Kiew, A.Weber & B.L.Burt	1	Malay Peninsula, Batu Luas
Advanced Asian and Malesian straight-fruited genera		
<i>Aeschynanthus</i> Jack	185	from S China, N & S India throughout Malesia to New Guinea and the Solomon Islands
<i>Agalmyla</i> Blume	96	Sumatra, Malay Peninsula, Borneo, Java, Sulawesi, New Guinea
<i>Allocheilos</i> W.T.Wang	2	S China
<i>Allostigma</i> W.T.Wang	1	S China
<i>Anna</i> Pellegr.	4	China, N Vietnam
‘‘ <i>Briggsia</i> ’’ ⁸	4	China
<i>Briggsiopsis</i> K.Y.Pan	1	S China
<i>Cathayanthe</i> Chun	1	S China
<i>Codonoboea</i> Ridl.	120	from southern Thailand and throughout Malesia
<i>Conandron</i> Siebold & Zucc.	1	S Japan, E China, Taiwan.
<i>Cyrtandra</i> J.R.Forst. & G.Forst.	652–818 ⁹	from the Nicobar Islands and S Thailand through Malesia and the S Pacific to the Hawaiian Islands
<i>Deinostigma</i> W.T.Wang & Z.Y.Li ⁴	1	Vietnam
<i>Didymocarpus</i> Wall.	60+	from N and NE India, Nepal and S China southwards to the Malay Peninsula and N Sumatra
<i>Didymostigma</i> W.T.Wang	3	SE China
<i>Gyrocheilos</i> W.T.Wang	4	S China
<i>Hemiboea</i> C.B.Clarke	25	C & S China, Taiwan, N Vietnam, Japan
<i>Henckelia</i> Spreng.	56	Sri Lanka, S and NE India, Nepal, Bhutan, S China, N Vietnam, N Laos, N Thailand
<i>Hexatheca</i> C.B.Clarke	4	Borneo (W Kalimantan, Sarawak to Sabah)

TABLE 2. Continued.

Genus	No of species	General distribution
<i>Liebigia</i> Endl.	12	Sumatra, Java and Bali
<i>Loxostigma</i> C.B.Clarke	7+	S China, N Vietnam
<i>Lystionotus</i> D.Don	29	from N India and Nepal eastwards through N Thailand, N Vietnam and S China to S Japan
<i>Metapetrocosmea</i> W.T.Wang	1	S China
<i>Microchirita</i> (C.B.Clarke) Yin Z.Wang	18	W India, the foothills of the Himalayas, through continental Southeast Asia to Borneo, Sumatra and Java
<i>Oreocharis</i> Benth.	80+	China, Thailand, Myanmar, Bhutan, NE India, Japan
<i>Petrocodon</i> Hance	18+	China, N Vietnam, NE Thailand
<i>Petrocosmea</i> Oliv.	30	NE India, S China, Myanmar, Thailand, S Vietnam
<i>Primulina</i> Hance	131	W & S China, Vietnam
<i>Pseudochirita</i> W.T.Wang	1	S China, Vietnam
<i>Raphiocarpus</i> Chun	11+	S China and N & C Vietnam
<i>Ridleyandra</i> A.Weber & B.L.Burt	20+	Malay Peninsula and Borneo
Unplaced:		
<i>Didissandra</i> C.B.Clarke & C.DC.	8	W Malesia (Sumatra, Malay Peninsula, Borneo, Java)
<i>Litostigma</i> Y.G.Wei, F.Wen & Mich.Möller	2	China
<i>Tribounia</i> D.J.Middleton	2	Thailand

¹ *Resia* and *Cremospermopsis* are placed in Beslerieae based on morphological characteristics (see Weber et al., 2013). Molecular data for *Resia* places the genus in tribe Beslerieae (Smith et al. 2000).

² Likely extinct (Wang 2003); placement based on morphological grounds (Weber 2004).

³ Placement based on preliminary molecular data (M.Möller et al., unpubl.).

⁴ Placement based on Weber (2004).

⁵ Based on Petrova et al. (2013) only one species exists.

⁶ Until further data becomes available the status quo of the African genera is maintained.

⁷ Placement based on morphological grounds (Middleton & Triboun 2012).

⁸ The type species *B. longifolia* has been sunk into *Oreocharis*, see Möller et al. (2011b).

⁹ Based on Atkins, 2013.

nucleotide sequences continued to represent families with rather few placeholders (e.g., Olmstead & Reeves 1995, Olmstead et al. 2000, Donoghue et al. 1998, Savolainen et al. 2000, Soltis et al. 2000, Albach et al. 2001). In earlier studies the Gesneriaceae included uncertain taxa (Oxelman et al. 1999; Bremer et al. 2001, 2002, 2004). The Gesneriaceae always fell in a grade of lineages near the base of the Lamiales, but often in polytomies with other families. It was not until samples of the Calceolariaceae were included in analyses (Olmstead et al. 2001, Oxelman et al. 2005, Wortley et al. 2005) that this family was found to be sister to Gesneriaceae. Support for this Gesneriaceae/Calceolariaceae relationship is the presence of pair-flowered cymes (Weber et al. 2013), and the presence of cornoside and the absence of iridoids (Jensen et al. 2005). By combining three fast evolving cpDNA regions (*trnK-matK* intron, *trnL-F* intron/spacer, *rps16* intron) and four unambiguous Gesneriaceae rep-

resentatives (*Mitraria* Cav., *Kohleria* Regel, *Rhynchoglossum* Blume, *Streptocarpus*) it was recently possible to reconstruct a resolved and strongly supported phylogeny of the Lamiales that showed the Gesneriaceae + Calceolariaceae as first branch in the Lamiales with zygomorphic flowers (Schäfer-hoff et al. 2010, but see Perret et al. 2013). The same result was obtained in a recent analysis of 17 gene regions and 640 taxa (Soltis et al. 2011).

Placement of Uncertain and Excluded Taxa

Several genera have an uncertain position in Lamiales (Weber 2004), and have been classified in and out of Gesneriaceae, mainly based on morphological characters. Genera that have an uncertain family affiliation for which molecular data are available are: *Sanango* G.S.Bunting & Duke, *Cubitanthus* Barringer, *Jerdonia* Wight, *Charadrophila* Marloth, *Cyrtandromoea* Zoll.,

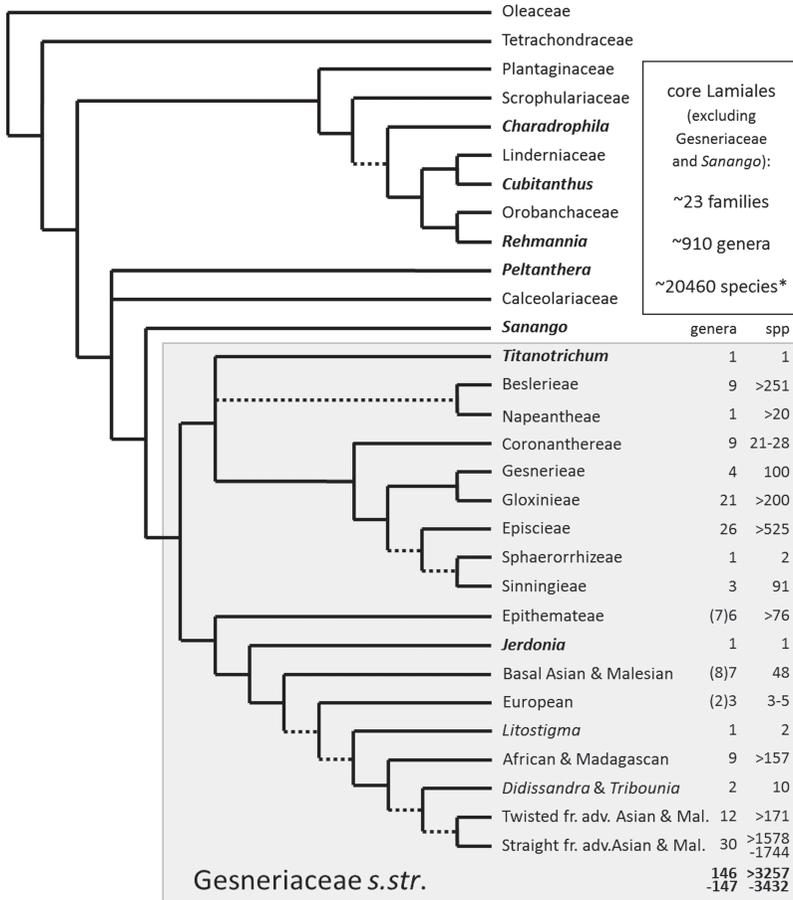


FIGURE 1. Simplified composite phylogenetic tree of the Gesneriaceae as currently defined, and uncertain taxa, based on published analyses. Only those relationships are shown as resolved that received high statistical support. Dotted branches indicate differences in topology between studies or low branch support. The number of outgroup lineages is reduced to the relevant lineages including uncertain taxa. Their relationships are based on Oxelman et al. (2005), and Perret et al. (2013). To the right the approximate number of species are given for each lineage (based primarily on numbers given in TABLE 2). *Figures from APG III (<http://www.mobot.org/MOBOT/research/APweb/>).

Rehmannia Fisch. & C.Mey, and *Titanotrichum* Soler. The placement of these genera can be more precisely ascertained with the advent of molecular phylogenetic methods and recent studies to do so have appeared since Weber (2004) (FIGURE 1). The genera are addressed here only in a molecular context. More comprehensive treatments, including historical and morphological aspects, are presented by Weber et al. (2013).

Sanango G.S.Bunting & Duke. After placement in various families, *Sanango* was for the first time considered to belong to Gesneriaceae by Wiehler (1994). This was initially confirmed in a molecular phylogenetic analysis by Smith et al. (1997b) and Smith and Carroll (1997) using cpDNA *ndhF* data, and their molecular data supported inclusion of the genus in tribe Gesnerieae. But this placement proved erroneous and the results were refuted by later

studies. The analysis by Oxelman et al. (1999) supported a position as sister to Gesneriaceae, though only two representatives of Gesneriaceae, *Streptocarpus* and *Nematanthus* Schrad., were included in their analysis. In Bremer et al. (2001, 2002) and Oxelman et al. (2005) *Sanango* was included, but their studies were limited to one taxon of Gesneriaceae. A recent paper by Perret et al. (2013) based on three plastid loci and a broad sampling of Gesneriaceae and Lamiales strongly supports the placement of *Sanango* as sister to the Gesneriaceae.

Cubitanthus Barringer. This monotypic genus was first included in molecular work by Perret et al. (2013) who investigated the Gesneriaceae and its relationship with other lineages of Lamiales based on broad taxon sampling and three plastid loci. Their results strongly supported *Cubitanthus* outside of the Gesneriaceae and

as sister to *Stemodiopsis* Engl. at the base of the Linderniaceae.

Jerdonia Wight. This monotypic genus was originally described in the Gesneriaceae and later considered a member of Scrophulariaceae s.l. Based on three datasets, cpDNA *trnL-F*, cpDNA *atpB-rbcL* and nuclear ITS, Möller et al. (2009) found that the Indian *Jerdonia* is highly supported as sister to all Old World didymocarpoide Gesneriaceae. This work is based on the analysis of a single collection and confirmation from additional samples would be desirable.

Charadrophila Marloth. The genus was described in Scrophulariaceae s.l. by Marloth (1899), but moved between that family and Gesneriaceae thereafter. Based on three chloroplast sequence data sets, *ndhF*, *trnL-F* and *rps16*, *Charadrophila* was found to be a member of Stilbaceae (Oxelman et al. 2005). This position outside Gesneriaceae is confirmed by preliminary analyses using *trnL-F* and *atpB-rbcL* data (Möller et al. unpubl. data).

Cyrtandromoea Zoll. Initially, this genus was placed in the Gesneriaceae but moved to Scrophulariaceae s.l. by Burt (1965). The only molecular study that attempts to place *Cyrtandromoea* is that of Smith et al. (1997b) using *ndhF* data. In Smith (1997b), *Cyrtandromoea acuminata* was supported as sister to *Rhynchosglossum* and *Monophyllaea* R.Br., two members of the epithematoids. The presence of isocotylous seedlings was suggested as a synapomorphy for the close relationship of *Cyrtandromoea* with *Charadrophila*, *Jerdonia*, and *Rehmannia* (Burt 1977). The phylogenetic placement of the isocotylous *Cyrtandromoea* (Smith 1997b) near the extremely anisocotylous Epithemateae is highly unlikely. It is the Old World Gesneriaceae that show anisocotily, the uneven post-germination development of seedlings, while New World Gesneriaceae have isocotylous seedlings. Preliminary molecular data for several *Cyrtandromoea* spp. places them firmly outside and distant from the Gesneriaceae (J. Luna-Castro, pers. comm.).

Rehmannia Libosch. ex Fisch. & C.Mey. The genus was variously placed in both the Scrophulariaceae s.l. and Gesneriaceae. In Oxelmann et al. (2005), *Rehmannia* appears related to *Mazus* Lour. and *Lancea* Hook.f. & Thomson in the Scrophulariaceae s.l. Xia et al. (2009) expanding on the sampling by including six species of *Rehmannia* and two of *Triaenophora* Soler., found these two genera as sister to the Orbanchaceae.

Titanotrichum Soler. This monotypic genus was originally described in Scrophulariaceae s.l., but later sometimes placed in Gesneriaceae (see also Weber et al. 2013). Recent studies by Soltis et al. (2000) and Albach et al. (2001) suggested that *Titanotrichum* was sister to Gesneriaceae, but their sampling of the family included no New World

taxa and only three Old World genera. A more comprehensive taxon sampling of the Gesneriaceae was carried out by Smith et al. (1997b) and Wang et al. (2004a) using *ndhF* data (Smith et al. 1997b) as well as a combination of *atpB-rbcL*, *trnL* intron, *trnL-F* spacer, nr 26S and *GCYC* data (Wang et al. 2004a). These studies strongly support the placement of *Titanotrichum* inside the Gesneriaceae. However, differences were seen in the position within the family between the two phylogenetic studies. In Smith et al. (1997b) *Titanotrichum* was sister to the rest of the Old World Gesneriaceae, though with no Bremer branch support (=decay index). In Wang et al. (2004a), *Titanotrichum* fell in the New World Gesnerioideae/Coronantherioideae clade, thus contradicting its distribution in the Old World (China, Taiwan and Japan). The position in the New World Gesneriaceae was confirmed in recent work by Perret et al. (2013). It was found to be sister to *Napeanthus* Gardner, but lacked branch support, likely as a result of taxon sampling limitation (e.g., this study only included three Old World samples and few Besleriaceae samples).

Peltanthera Benth. This monotypic genus has been referred to various families, including Scrophulariaceae s.l., Loganiaceae and Buddlejaceae. Bremer et al. (2001, 2002, 2004) considered it part of the Gesneriaceae. In Bremer et al. (2001, 2002) and Oxelman et al. (1999), where both *Peltanthera* and *Sanango* were included, *Sanango* was consistently more closely related to the Gesneriaceae than *Peltanthera*. In an analysis that included Calceolariaceae taxa, both genera were close to Gesneriaceae (Oxelman et al. 2005). With a comprehensive taxon sampling, Wang et al. (2004a) and Perret et al. (2013) also placed *Peltanthera* between Calceolariaceae and Gesneriaceae, but more distant to the Gesneriaceae. Two phylogenies that strongly support that *Peltanthera* does not share a recent common ancestor with the Gesneriaceae are based on four mitochondrial genes (Qiu et al. 2010) and 17 multigenome regions (Soltis et al. 2011). Here, *Peltanthera* was outside a clade Calceolariaceae + Gesneriaceae (not supported in Qiu et al. 2010), though each family was represented by only one sample. Overall the strongest evidence suggests that *Peltanthera* should not be considered a member of Gesneriaceae. The placement of *Peltanthera* in a family needs further investigation.

In conclusion, of the eight uncertain genera discussed here and based on the current knowledge, only *Jerdonia* and *Titanotrichum* belong inside Gesneriaceae. A third genus, *Sanango*, is the sister taxon to all other members of the Gesneriaceae and its inclusion in that family is an option (see Weber et al. 2013).

Molecular Para- and Polyphyly

Many molecular phylogenies of Gesneriaceae indicated problems in the taxonomic system at genus and higher levels. One of the first examples was *Saintpaulia* that was included as outgroup samples in the study by Möller and Cronk (1997a) on species relationships of *Streptocarpus*. They used ITS sequences and found that the species of *Saintpaulia* were in fact nested inside *Streptocarpus*. In a later study, other African genera were found nesting inside *Streptocarpus* as well. These genera include *Schizoboea* (Fritsch) B.L.Burt, *Linnaeopsis* Engl. (Möller & Cronk 2001a, 2001b) and *Acanthonema* Hook.f. (Möller et al. 2009) and the Madagascan genera *Hovanella* A.Weber & B.L.Burt and *Colpogyne* B.L.Burt (O'Sullivan 1999, Möller 2003). At that time, it was too early to make firm taxonomic changes because many African Gesneriaceae genera were not included in the analysis (Möller & Cronk 1999). Attempts to lump most of the African genera into one genus were made without new data or analyses (Darbyshire 2006, Christenhusz 2012). The taxonomic changes by Christenhusz (2012) are considered here premature because there are significant gaps in taxon sampling and key species have not been included in the analyses. More phylogenetic research is needed on the African genera before a new classification is proposed.

The non-monophyly of *Chirita* was first indicated by Mayer et al. (2003), and this was further elaborated on by Li and Wang (2007) who included seven samples of *Chiritopsis* W.T.Wang and 15 samples *Chirita* Buch.-Ham. ex D.Don. They found a polyphyletic *Chiritopsis* nesting in a paraphyletic *Chirita* sect. *Gibbosaccus*. Recently, Möller et al. (2009, 2011a), found high levels of polyphyly among didymocarpoid genera such as *Briggsia* Craib, *Chirita*, *Henckelia* Spreng., *Lagarosolen* W.T.Wang, *Oreocharis* and *Raphiocarpus* Chun based on several molecular datasets. As a consequence of these molecular phylogenetic studies, *Chirita* was split into five genera (*Damrongia* Kerr ex Craib, *Liebigia* Endl., *Microchirita* (C.B.Clarke) Yin Z.Wang, *Primulina* Hance, and a redefined *Henckelia*) (Weber et al. 2011a). On the other hand, based on molecular data, the following ten Chinese genera were all synonymized into *Oreocharis* (Möller et al. 2011b): *Ancylostemon* Craib, *Bournea* Oliv., *Briggsia* s.str., *Dayaoshania* W.T.Wang, *Deinocheilos* W.T.Wang, *Isometrum* Craib, *Opithandra* B.L.Burt, *Oreocharis*, *Paraisometrum* W.T.Wang, *Thamnocharis* W.T.Wang, *Tremacron* Craib. The following six genera were sunk into *Petrocodon* Hance (Wang et al. 2011, Weber et al. 2011b): *Calcareaoboea* C.Y.Wu ex H.W.Li, *Didymocarpus* Wall. pro parte, *Dolicholoma* D.Fang & W.T.Wang, *Lagarosolen*, *Para-*

lagarosolen Y.G.Wei, *Tengia* Chun. In addition to the above six genera, *Wentsaiboea tiandengensis* Yan Liu & B.Pan was also lumped into *Petrocodon*. The small genus *Metabriggsia* W.T.Wang with two species was included in *Hemiboea* C.B.Clarke (Weber et al. 2011c). For the *Briggsia* species not included in *Oreocharis* (basically caulescent species) and *Raphiocarpus*, more samples and sequence data are required to elucidate and evaluate the phylogenetic relationships and affiliations of these species.

Among the twisted-fruit advanced didymocarpoids, Puglisi et al. (2011a), on the basis of *trnL-F* and ITS data found *Paraboea* to be paraphyletic, and synonymized *Phylloboea* and *Trisepalum* with the larger genus *Paraboea*. The polyphyly of *Boea* Comm. ex Lam. was shown by Möller et al. (2009). Available molecular data on *Loxocarpus* R.Br. (previously *Henckelia* sect. *Loxocarpus*) suggest that it is not monophyletic (Weber et al. 2011a), but more molecular and morphological work is needed.

Among the New World Gesneriaceae, early work (Smith & Carroll 1997, Smith & Atkinson 1998) indicated problems with monophyly of certain genera, e.g., *Columnnea*, *Episcia* Mart., *Paradrymonia* and *Sinningia*. Clark et al. (2006) working on generic boundaries in the New World tribe Episcieae analyzed 155 species of 21 of the 22 genera of the tribe with molecular and morphological data, and found that most of the traditionally recognized genera were not monophyletic. Some genera such as *Columnnea* have only minor exceptions such as *Columnnea dielsii* Mansf., which had been recognized as a member of *Alloplectus* Mart. (Smith & Sytsma 1994a, Skog 1999). A reclassification of Episcieae based on Clark et al. (2006) resulted in the segregation of *Glossoloma* Hanst. and *Crantzia* Scop. from *Alloplectus*, a polyphyletic genus found to be nested in seven different clades (Clark & Zimmer 2003, Clark et al. 2006). Other members of *Alloplectus* were shown to nest in *Drymonia* Mart. and *Nematanthus*. The most problematic genera remaining in the Episcieae are *Paradrymonia* (highly polyphyletic) and *Nautilocalyx* Linden ex Hanst. (paraphyletic) (FIGURE 2D). Further phylogenetic studies in the Episcieae established the non-monophyly of *Neomortonia* Wiehler and *Episcia* (Clark et al. 2012), and *Codonanthe* (Mart.) Hanst. (Perret et al. 2013). Four monotypic genera were circumscribed (Smith & Clark 2013) to accommodate the non-monophyly of *Episcia*, *Neomortonia*, *Paradrymonia*, and *Nematanthus* as shown in Clark et al. (2012). These recently described genera are outlined below in section "Discovery and Confirmation of Placement of New Species and Genera."

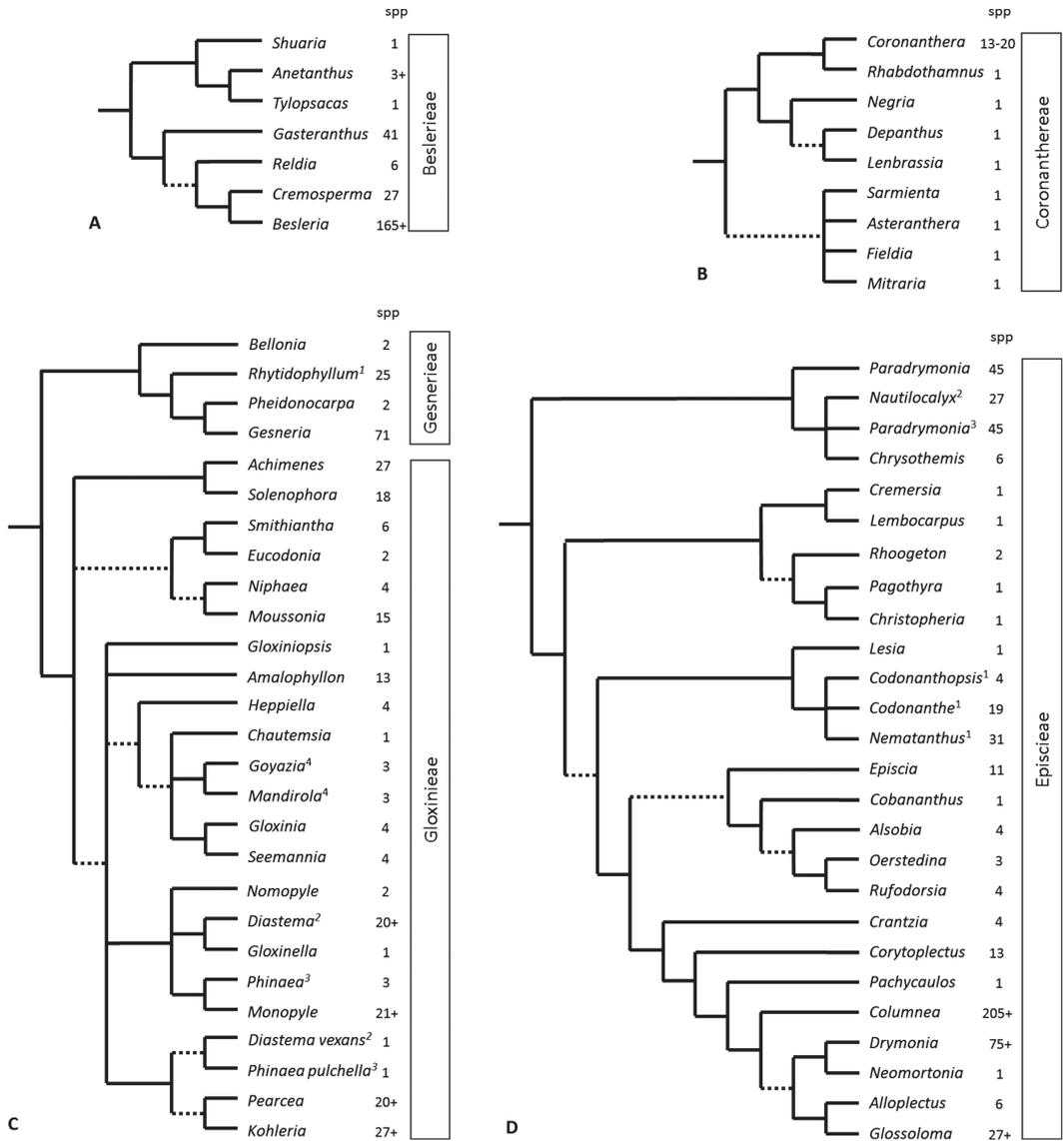


FIGURE 2. Composite phylogenetic trees of A) tribe Beslerieae based on Roalson and Clark (2006) and Clark et al. (2010). Dotted branch indicates difference in topology (collapsed branch in Roalson & Clark 2006) between the two studies. B) tribe Coronanthereae based on Smith et al. (2006 topology of GCYC 1F), Möller et al. (2009), and Woo et al. (2011). Dotted branches indicate differences in topology between and within studies [e.g., (*Lenbrassia* (*Depanthus* + *Negria*)) or (*Negria* (*Lenbrassia* + *Depanthus*)) in Woo et al. 2011; for the subtribe Mitrariinae each publication proposes different generic relationships]. C) tribe Gesnerieae based on Martén-Rodríguez et al. (2010), and tribe Gloxinieae based on Zimmer et al. (2002), Smith et al. (2004b), Roalson et al. (2008), Araújo et al. (2010), Clark et al. (2011) and Perret et al. (2013). Differences in topologies between studies are given as polytomies. Dotted branches indicate no or low branch support. ¹ Plus a few species of *Gesneria*; ^{2,3} Non-monophyletic genera; ⁴ In Araújo et al. (2010) forming a mixed clade. D) tribe Episcieae based on Clark et al. (2012), Smith and Clark (2013) and Perret et al. (2013). Dotted branches indicate differences in topology between studies. ¹ Polyphyletic *Codonanthe* (with *Codonanthis*) and *Nematanthus*; ^{2,3} Mixed clade with *Chrysothemis*, *Nautilocalyx* and *Paradrymonia* species. To the right, approximate species numbers as given in Table 2.

Perret et al. (2003, 2006) included >70 species around *Sinningia* and confirmed previous studies (Smith & Carroll 1997, Smith & Atkinson 1998) that *Paliavana* and *Vanhouttea* nest in a paraphyletic *Sinningia*. However, taxonomic changes have yet to be made.

Zimmer et al. (2002) found a widely scattered polyphyletic *Gloxinia* L'Hér. (with *G. sarmentiana* Gardner ex Hook. sister to the Episcieae & *Sinningia* clade). Relationships among the tribe Gloxinieae were further studied by Roalson et al. (2005a, 2005b) and Clark et al. (2011). The paraphyly of *Kohleria* with respect to *Capanea* Decne. resulted in an expanded circumscription of *Kohleria* (mostly terrestrial) to include *Capanea* (mostly epiphytic). Strongly supported phylogenetic relationships from Roalson et al. (2005a) resulted in major shifts of generic concepts for species traditionally recognized as *Gloxinia* (cf. section "Splitting of Existing Species or Genera" below) and a revision of three genera for species with actinomorphic corollas that were traditionally recognized as *Phinaea* Benth. (Boggan et al. 2008). The non-monophyly of *Diastema* was strongly supported because of the phylogenetic placement of *Diastema vexans* H.E. Moore sharing a more recent common ancestor with *Pearcea* than with other species of *Diastema* (Roalson et al. 2005a).

The non-monophyly of *Gesneria* L. in the tribe Gesnerieae was strongly supported in phylogenetic results from two markers (ITS and *GCYC*) and morphology presented by Martín-Rodríguez et al. (2010). The two largest genera in the Gesnerieae are *Rhytidophyllum* Mart. and *Gesneria* with the later displaying bird pollination traits and the former displaying bat pollination traits. Research presented by Martín-Rodríguez et al. (2010) suggests some traditionally recognized members of *Gesneria* with red flowers should be transferred to *Rhytidophyllum*.

Splitting of existing species or genera. The Madagascan *Streptocarpus ibityensis* Humbert was long believed to occur on Mt Itremo and Mt Ibity, until flowering plants were studied in detail in the field and in cultivation. Many morphological differences were detected in material originating from the two mountain localities such as differences in flower size, flower coloration, corolla shape, number of staminodes, and leaf indumentum. The molecular phylogenetic work by MacMaster et al. (2005) unequivocally showed that plants from the two localities represented different lineages. The phylogenetic results and morphological differences of the populations thus confirmed that *S. lanatus* MacMaster occurring on Mt Itremo is a different species from *S. ibityensis* on Mt Ibity.

A recent morphological and molecular investigation of *Didymocarpus venosus* Barnett, which has long been recognized as misplaced in *Didymocarpus* (Weber & Burt 1998, Weber et al. 2000, Burt 2001) on the grounds of flower shape and the long stipe of the fruit, resulted in the establishment of a new genus and species *Tribounia venosa* (Barnett) D.J.Middleton. The molecular data placed the species distantly from *Didymocarpus s.str.* (Middleton & Möller 2012).

Many new boundaries in the tribe Gloxinieae were based on results from molecular phylogenetic analyses of ITS and *trnL-F* and morphological data by Roalson et al. (2005b). They resurrected the genera *Mandirola* Decne. and *Seemannia* Regel, redefined *Gloxinia* and *Kohleria*, described four new genera [*Gloxinella* (H.E.Moore) Roalson & Boggan, *Gloxiniopsis* Roalson & Boggan, *Nomopyle* Roalson & Boggan, *Sphaerorrhiza* Roalson & Boggan] and defined the new monogeneric tribe Sphaerorrhizeae (Roalson et al. 2005a).

Recent molecular phylogenetic analyses in the Episcieae (Clark et al. 2012) confirmed that many genera are polyphyletic. As a result, the circumscriptions of four monospecific genera were published for taxa that were strongly supported and morphologically unique. These new genera were described as *Christopheria xantha* (Leeuwenberg) J.F.Smith & J.L.Clark (= *Episcia xantha* Leeuwenb.), *Lesia savannarum* (C.V.Morton) J.L.Clark & J.F.Smith [= *Nematanthus savannarum* (C.V.Morton) J.L.Clark = *Alloplectus savannarum* C.V.Morton], *Pachycaulos nummularium* (Hanst.) J.L.Clark & J.F.Smith (= *Hypocyrtia nummularia* Hanst. = *Columnnea nummularia* (Hanst.) Wiehler = *Neomortonia nummularia* (Hanst.) Wiehler), and *Pagothyra maculata* (Hook.f.) J.F.Smith & J.L.Clark [= *Episcia maculata* Hook.f. = *Paradrymonia maculata* (Hook.f.) Wiehler] (Smith & Clark 2013).

Discovery and Confirmation of Placement of New Species and Genera

It is becoming more common to include molecular data in descriptions of new species or genera, and some journals do not publish substantial taxonomic changes without support from molecular analyses. For example, Chautems et al. (2000) demonstrated the independent species status of *Sinningia nordestina* Chautems, Baracho & J.A.Siqueira and showed it to nest in the *Sinningia* clade, though it is morphologically distinct from other congeners by the presence of vestigial tubers that are more common in annuals.

A new Gesneriaceae taxon was collected from Brazil with a unique combination of morphological features that placed it in tribe Gloxinieae, and

its placement in this tribe was confirmed by phylogenetic analysis of five sequence regions (Araújo et al. 2010). This taxon was described as a new genus, *Chautemsia* A.O.Araújo & V.C.Souza, on the basis of its relatively isolated position in the tribe. The epithet is in honor of Alain Chautems for his more than 20 years of work on Brazilian Gesneriaceae.

Clark et al. (2010) described a new, monotypic genus, *Shuaria* D.A.Neill & J.L.Clark, from the Cordillera del Cóndor region of eastern Ecuador. The following unusual characteristics made the placement of *Shuaria* in the Gesneriaceae difficult: arborescent habit (vs. herbaceous habit for all other members of the clade); phyllotaxy of opposite and alternate leaves; and lepidote trichomes on vegetative and floral structures. The phylogenetic placement of *Shuaria* in the tribe Beslerieae was strongly supported by ITS and *trnL*-F data. The molecular sequence data allowed for a critical evaluation of morphological features with closely related genera and it was discovered that septicidally dehiscent capsules are a unique morphological synapomorphy that *Shuaria* shares with *Anetanthus* Hiern ex Benth. & Hook.f. and *Tylopsacas* Leeuwenb.

A new genus in the Old World Gesneriaceae was described by Wei et al. (2010) based on phylogenetic analyses of ITS and *trnL*-F data. The two species in the new genus *Litostigma* Y.G.Wei, F.Wen & Mich.Möller have morphological features linking basal and derived didymocarpoid lineages (e.g. unsculptured v. sculptured seed testa cells and tetrandry v. diandry, respectively). Molecular data was important for the circumscription of the genus because one species, *L. crystallinum* Y.M.Shui & W.H. Chen, was first illustrated in a book (Shui & Chen 2006) in another genus under the illegitimate name, *Petrocosmea crystallina*. The second species, *L. coriaceifolium* Y.G. Wei, F.Wen & Mich.Möller, was to be described as a new monospecific genus. Only when analyzing the molecular data for both taxa together it became clear that a new genus with two entities existed (Wei et al. 2010).

CURRENT KNOWLEDGE OF PHYLOGENETIC RELATIONSHIPS AT GENERIC AND TRIBAL LEVELS

From the extensive multigene analyses reviewed here, an overview of the family can now be given from a phylogenetic point of view. A summary of the present tribal (New World) or equivalent (Old World) groupings are outlined in TABLE 2. Here we focus mainly on selected studies using the latest generic delineations available with results of comprehensive taxon sampling and strongly supported phylogenies (where available).

It should be noted that results in earlier studies may be inconsistent because of limited taxon sampling, or single gene analyses. Relationships that are uncertain or not supported are indicated by dashed lines in the FIGURES 2–4.

New World Gesneriaceae

The Gesneriaceae fall roughly into an Old World clade and New World clade (Möller et al. 2009). Two isolated exceptions are the Old World *Titanotrichum* that nests in the New World clade (Wang et al. 2004a) (FIGURE 1) and *Rhynchoglossum azureum* (Schltdl.) B.L.Burt that is geographically found in the New World and falls in the Old World clade (Mayer et al. 2003). A larger discontinuity is represented by the Coronantheroideae. They reside in the New World clade (plus *Titanotrichum*), though they occur across the southern hemisphere, including Australia and New Zealand. The New World clade includes the coronantheroids and gesnerioids of Weber (2004). The gesnerioids are not monophyletic. *Titanotrichum*, and tribe Beslerieae and Napeantheae fall in a polytomy, and the coronantheroids reside between the remaining gesnerioids (Wang et al. 2004a). In Zimmer et al. (2002) and Roalson et al. (2005b, 2008), the Beslerieae and Napeantheae are sister clades to the remaining gesnerioids, but *Titanotrichum* and the coronantheroids were not sampled. In Perret et al. (2013), in which the latter were included the relationship of Beslerieae and Napeantheae to the remaining gesnerioids was confirmed. Additionally, *Titanotrichum* was sister to *Napeantheae*, though with a low posterior probability value of 0.8, a value that is not necessarily suggesting a strong relationship (see Möller et al. 2009 for a comparison of parsimony bootstrap values and Bayesian posterior probability values). The remaining gesnerioid tribes form a well-supported clade, with the Gesnerieae sister to the Gloxinieae (Zimmer et al. 2002; Roalson et al. 2005b, 2008; Möller et al. 2009; Perret et al. 2013). The Episcieae, the Sinningieae and Sphaerorrhizeae form a clade, the latter as sister to the Sinningieae in e.g., Roalson et al. (2008), but with no branch support. In Perret et al. (2013) the Sinningieae fall as sister to a polytomy of Episcieae and Sphaerorrhizeae and the Gesnerieae/Gloxinieae clade, but also with no statistical support (FIGURE 1). These tribal relationships are poorly supported or unresolved and need additional data.

The tribal relationship between Napeantheae and Beslerieae is still unresolved, though the monophyly of the tribes themselves is well supported. In Smith (2000a) they are sister (based on *ndhF*). This is not supported with an increased sampling by Clark et al. (2010) (based on ITS,

trnL-F), while in Woo et al. (2011) (based on ITS, *trnL-F*, *trnE-T*, *psbA-trnK*) one analysis shows a weakly supported sister relationship of the two tribes, while in the same paper a reduced dataset (22 ingroup taxa) shows them in grades to the Gesnerioideae and Coronantheroideae with high branch support.

Generic relationships within Beslerieae show partly different topologies between the studies by Smith (2000a) and Roalson and Clark (2006) and Clark et al. (2010), though for the first many intergeneric branches receive no or low support, and the topology of the latter studies is presented (FIGURE 2A). The relationships between *Gasteranthus* and *Reldia* Wiehler are in conflict between Clark et al. (2010) and Roalson and Clark (2006), or less resolved in the latter, though this study included only four Beslerieae genera.

For the Coronanthereae, three studies are relevant, Smith et al. (2006), Möller et al. (2009), and Woo et al. (2011). The first employed *GCYC* sequences, the next was based on ITS and *trnL-F* data, while the last used ITS, and three chloroplast spacers, *trnL-F*, *trnE-T* and *psbA-trnK*. For the phylogenetic relationships of *Asteranthera* Hansl., *Fieldia* A.Cunn., *Mitraria* and *Sarmienta* Ruiz & Pav., all putative relationships are available, even significantly different ones in one publication (Woo et al. 2011). Relationships between the remaining genera are more consistent (FIGURE 2B), with *Coronanthera* Vieill. ex C.B. Clarke sister to *Rhabdothermus* A.Cunn., but alternative, significant relationships between *Depanthus* S. Moore, *Lenbrassia* G.W. Gillett and *Negria* F. Muell. are possible (Woo et al. 2011).

For Gesnerieae, *Rhytidophyllum*, *Bellonia* L. and *Pheidonocarpa* L.E. Skog are monophyletic (Martén-Rodríguez et al. 2010). The genus *Gesneria* is paraphyletic, but taxon sampling is still relatively limited for the wide range of habits and morphologies that are displayed for this genus. Further taxon sampling is necessary before changes to the current generic circumscriptions are made. Molecular-based phylogenies by Martén-Rodríguez et al. (2010) and others (Smith 2004b, Roalson et al. 2005b, Clark et al. 2011) have shown lack of monophyly for *Gesneria*, but support is weak (FIGURE 2C).

Relationships in tribe Gloxinieae begin to settle, with all 21 genera now included in phylogenetic studies. The first work by Zimmer et al. (2002) and Smith et al. (2004b) indicated some relationships that were confirmed in later papers by Roalson et al. (2008) and Clark et al. (2011), though overall the branch support of many internal branches and the backbone are still low and therefore resolving inter-generic relationships need additional data (FIGURE 2C).

All but 1 of the 26 genera of tribe Episcieae have been included for analysis by Clark et al. (2006). Only *Lampadaria*, described only recently (Feuillet & Skog 2003), has not been included so far. Based on morphological similarities of a well-developed peduncle and dry capsule, a placement near *Cremeria* Feuillet & L.E. Skog and *Rhoogeton* Leeuwenb. is proposed. The molecular generic relationships are relatively ascertained, with similar topologies to Clark et al. (2006) and Clark et al. (2012) revealed first in Zimmer et al. (2002) and Smith (2000b) considering the differences in generic sampling (FIGURE 2D).

The very comprehensive work by Perret et al. (2003, 2006, 2007) using 6 gene sequences, resulted in a stable topology in tribe Sinningieae, though none of the three genera included here were monophyletic. A new classification with five entities is possible, but no formal changes have yet been proposed (FIGURE 3A).

Old World Gesneriaceae

The Old World clade includes the epithematoid and didymocarpoid Gesneriaceae of Weber (2004) as sister clades (Mayer et al. 2003, Möller et al. 2009; FIGURE 1). The tribe Epithemateae was studied with two chloroplast gene regions by Mayer et al. (2003). They found strong genetic intergeneric differentiation and phylogenetic relationships that were in accordance with relationships inferred from plant architectural and floral characters (FIGURE 3B).

While generic relationships within the epithematoids are well understood (Mayer et al. 2003), this is very limited for the didymocarpooids. The pattern of evolution for the latter is strongly affected by geography. The basal Asian clades form several grades, followed by a European clade and the genus *Litostigma* (FIGURE 3C). The following predominantly twisted-fruited African & Madagascan clade is strongly supported as a lineage (FIGURE 3C). Here, all five of the non-*Streptocarpus* genera included fall inside the largest genus, *Streptocarpus*. Though, nomenclatural realignments have been attempted (see above), some molecular data are ambiguous and some relationships are not strongly supported.

Next is a clade with two straightfruited genera, *Didissandra* C.B. Clarke & C.D.C., and *Tribounia*, followed by the *Boea*-clade, consisting predominantly of twisted fruited advanced Asian and Malesian genera. Internal branches are currently poorly supported (FIGURE 3C), and to fully understand the intergeneric relationships in this clade, more molecular data are needed.

The largest clade by far in the family is represented by the straight-fruited advanced Asian and Malesian didymocarpooids, representing 1600

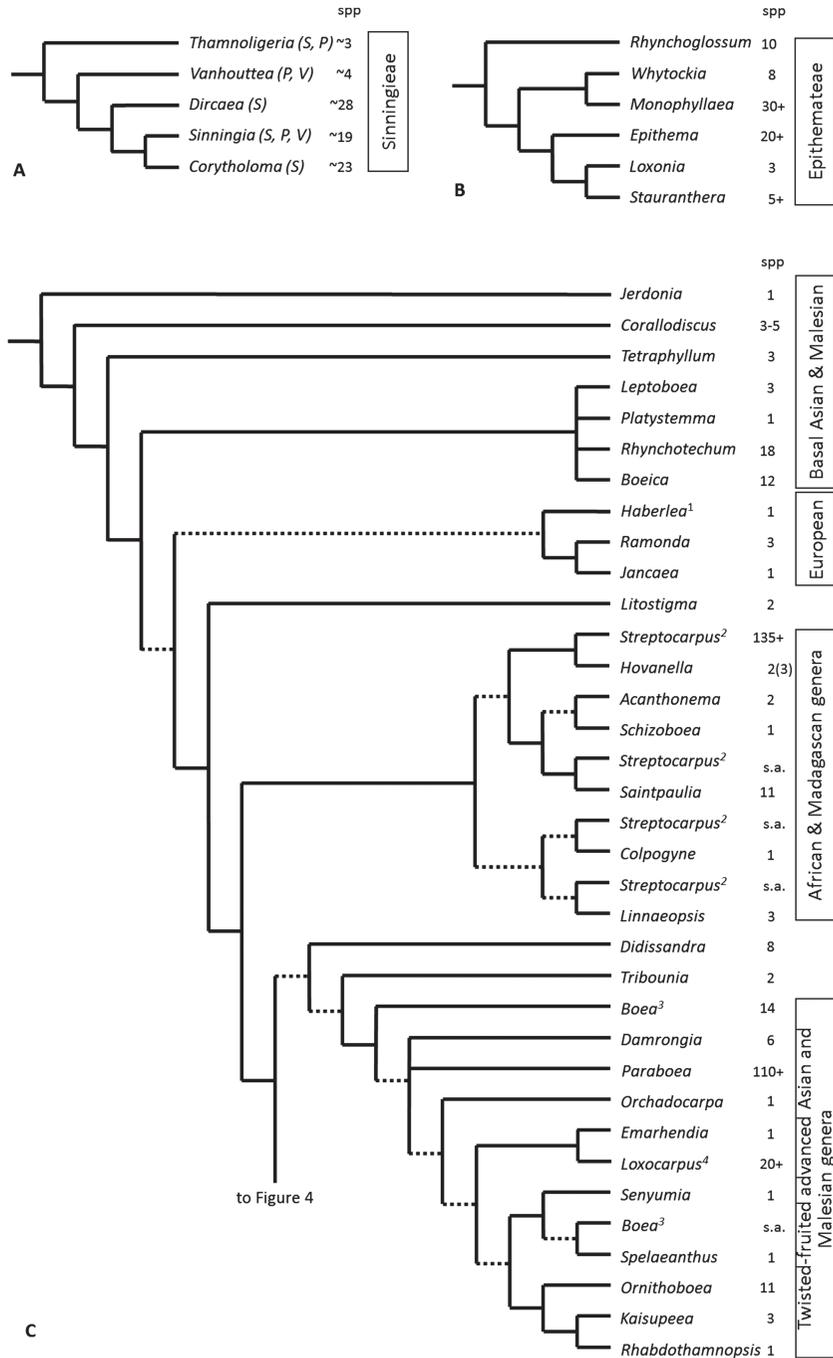


FIGURE 3. Composite phylogenetic trees of A) tribe Sinningieae based on Perret et al. (2003, 2006, 2007, 2013), illustrating the five main lineages in this tribe. Letters in brackets indicate presence of species of different genera. S—*Sinningia*, P—*Paliavana*, V—*Vanhouttea*. B) tribe Epithemateae based on Mayer et al. (2003). C) Basal Asian and European genera based on Möller et al. (2009), of African & Madagascar genera based on Möller and Cronk (1997a, 2001a, 2001b), and Möller et al. (2009), and of the twisted-fruited Asian and Malesian genera, the *Boea*-clade, based on Möller et al. (2009, 2011), Weber et al. (2011), and Puglisi et al. (2012). Dotted branches indicate differences in topologies between the studies and/or low branch support. Dotted branches indicate differences in topologies between the studies and/or no or low branch support. ¹ Only one species *H. rhodopensis* (Petrova et al. 2013); ^{2,3} Polyphyletic *Streptocarpus* and *Boea*, respectively; ⁴ Perhaps not monophyletic; s.a.—See above. To the right, approximate species numbers as given in Table 2.

species (FIGURE 1). Traditionally this group was divided into three tribes (Burt & Wiehler 1995). However, none of these were found to represent monophyletic groups, and the tribal structure consequently was abandoned (Weber 2004, Möller et al. 2009). While the phylogenetic resolution in the clade is high, the backbone support is low or non-existing (Möller et al. 2009, 2011a; Weber et al. 2011a; FIGURE 4). This might be due to the relatively low sample coverage of this large clade, since even the 183 samples included in the most comprehensive molecular phylogenetic analysis (Weber et al. 2011a) only represent ~12% of the species. Though, if the large genus *Cyrtandra* (652–818 spp.) is excluded, the sample coverage would represent ~20% at the species level. While in these analyses (e.g., Möller et al. 2009, 2011a; Weber et al. 2011a) inter-generic relationships were often tenuous, the generic boundaries were highly supported, except where the genera were not monophyletic (i.e., *Chirita*, *Henckelia*, *Briggsia*, *Oreocharis*, *Petrocodon*; Möller et al. 2011a). Most of these genera were consecutively redefined to represent monophyletic entities (see above section ‘Molecular Para- and Polyphyly and Möller et al. 2011b; Weber et al. 2011a, 2011b, 2011c).

EVOLUTIONARY SCENARIOS

Character Evolution

Cladistic analysis in the Gesneriaceae has been employed initially using morphological characters to construct phylogenies (e.g., Smith 1996, Roalson et al. 2002). However, with the development of automated DNA sequencing technology, the analysis of molecular data became increasingly feasible and has since predominantly been employed in phylogeny reconstructions. With these phylogenies being independent of morphology, morphological character evolution can be explored independently from molecular data on these trees. By mapping morphological traits on supported DNA-based phylogenies, homoplasies in character evolution (i.e., the parallel origin of superficially identical morphological characteristics) can be readily detected without the bias of the morphology on the topology of the phylogenetic tree.

Earliest studies on Gesneriaceae morphological character evolution using molecular phylogenies included that of Smith and Carroll (1997) on the tribe Episcieae (fruit type, epithytism, presence of tuber). Many characteristics in Gesneriaceae have been found to have multiple origins, some have been studied in great detail. Not all of these studies can be described here, and the reader is referred to TABLE 1 for a list of publications. However, a few key characters may be elaborated on here.

The ancestral state of fruit type in Gesneriaceae is a dry dehiscent capsule, from which independently fleshy fruits have evolved in several tribes, and often repeatedly within the tribes, e.g., Beslerieae (Smith 2000a), Coronanthereae (Woo et al. 2011), Episcieae (Clark et al. 2012), Basal Asian genera (*Rhynchoetechum* Blume) and Advanced Asian & Malesian straight fruited genera (*Cyrtandra*) (Möller et al. 2009). In the latter two, the independent evolution of berries (and non-monophyly of the tribe Trichosporeae possessing seed appendages), resulted in the abandonment of the tribal structure in the Old World Gesneriaceae (Möller et al. 2009). A new fleshy fruit type, fleshy carpels with loculicidal split of the upper suture, has independently evolved in *Cathayanthus* Chun, a monotypic genus from Hainan (Wei et al. 2010, Möller et al. 2011a).

The Gesneriaceae flowers are fundamentally zygomorphic but six parallel losses of symmetry were found in the Old World didymocarpooids (Möller et al. 1999, 2009, 2011a, 2011b; Wang et al. 2010). Similarly, Clark et al. (2011) found the repeated evolution of actinomorphic flowers in the New World tribe Gloxinieae. The evolution of flower traits with view to pollination syndromes have been studied at the species level, first by Harrison et al. (1999) on *Streptocarpus* and *Saintpaulia*, and later by Hughes et al. (2006). The diversification of flowers in New World genera was studied by Roalson et al. (2003) in *Achimenes*, by Perret et al. (2003, 2007) in the tribe Sinningieae, and by Martén-Rodríguez et al. (2010) in *Gesneria* and *Rhytidophyllum*. Common to most studies was the realization that pollination syndromes often have multiple origins, in the case of the African genera from a small pouch type, favoring autogamy, diversifying into showy flowers in pollinator driven co-evolution scenarios. The reverse is the case of *Gesneria* and *Rhytidophyllum* where from bird pollinated flowers, due to paucity of ancestral pollinators (such as on islands), shifts in pollination syndromes including generalists and autogamy were observed (Martén-Rodríguez et al. 2010). The convergence of resupinate flowers in three different lineages was shown to be an important feature in the Episcieae that resulted in the non-monophyly of *Alloplectus* (Clark & Zimmer 2003).

From a simple character mapping exercise (Möller et al. 2009), tetrandry can be deduced to be the ancestral state for the Gesneriaceae, since virtually all New World clade members, most epithematoid genera and the basal didymocarpooid lineages have four fertile stamens. Diandry has repeatedly evolved, very rarely in the New World clade (e.g., *Sarmienta scandens*), separately in two epithematoid genera, *Rhynchoglossum* (Old World species) and *Epithema*, and once for the African/

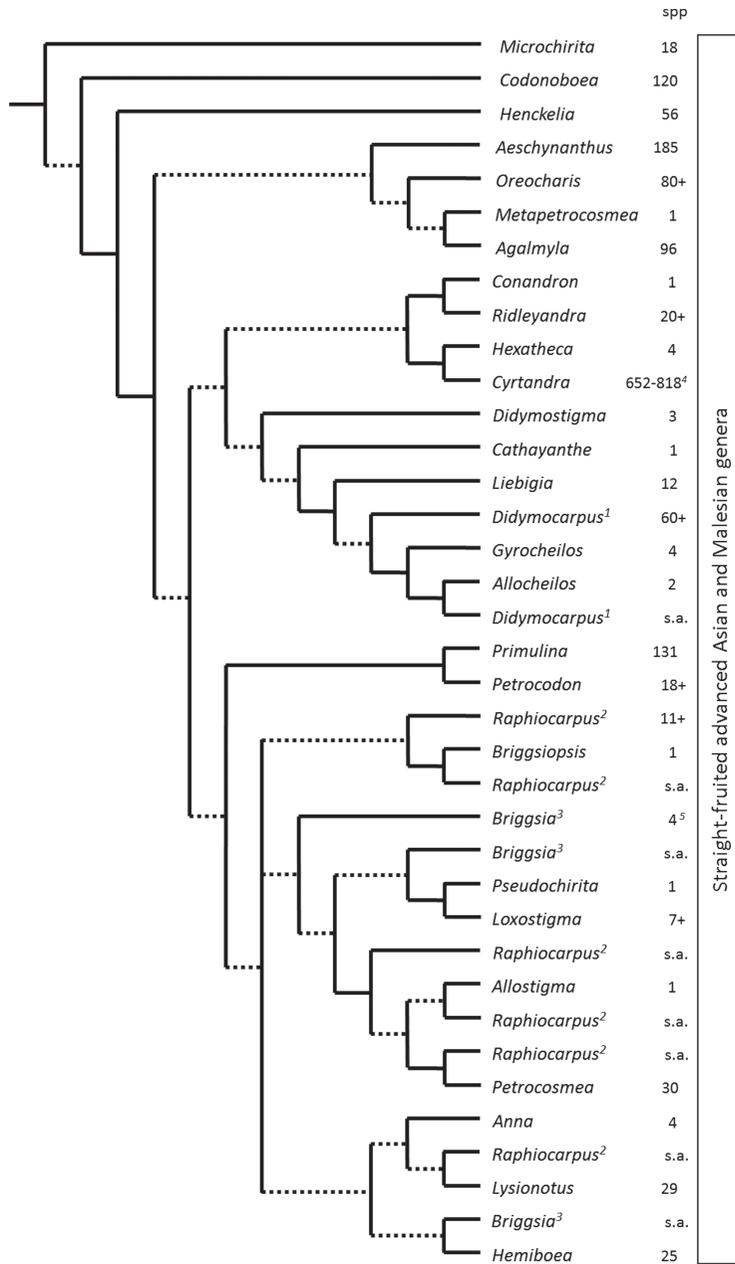


FIGURE 4. Composite phylogenetic tree of the straight-fruited Asian and Malesian genera based on Möller et al. (2009, 2011a,b) and Weber et al. (2011a,b,c). Dotted branches indicate differences in topologies between the studies and/or no or low branch support. ¹⁻³ Denotes the polyphyletic genera *Didymocarpus*, *Raphiocarpus*, and *Briggsia* respectively. ⁴ According to Atkins et al. (2013); ⁵ Remaining species in the genus, the type species *B. longifolia* and five other species have been sunk into *Oreocharis*, see Möller et al. (2011b). s.a.—See above. To the right, approximate species numbers as given in Table 2.

Madagascan and advanced Asiatic and Malesian genera, with several reversals to tetrandry.

Parallel origins of stolons in the tribe Episcieae were shown in Clark et al. (2012). Glands on the leaf surface and their correlation to sections in

Didymocarpus was investigated by Palee et al. (2006). Möller and Cronk (2001a) demonstrated the evolution of the labile vegetative growth forms in *Streptocarpus*, with repeated shifts between caulescent, rosulate and unifoliate forms.

The evolution of basic chromosome numbers was studied at the species level in *Streptocarpus* (Möller & Cronk 2001a), *Aeschynanthus* and *Agalmyla* Blume (Möller et al. 2008), showing contrasting patterns of genome evolution with uniformity of chromosome number in *Agalmyla* and *Streptocarpus*, but repeated dysploid changes in basic number for *Aeschynanthus*, but only one in *Streptocarpus*. At the subfamily level in New World Gesnerioideae a high level of across genus conservation of basic chromosome numbers was found, following roughly the tribal arrangement (Zimmer et al. 2002).

Evolutionary Development Studies

It was not long after the rise of molecular sequencing methods that molecular phylogenetic approaches were integrated with developmental genetics (e.g., Möller et al. 1999), where development genes important for major morphological traits of taxonomic value such as flower shape were studied, and their expression and function compared in an evolutionary framework. The flower symmetry gene *CYCLOIDEA* (*CYC*), important for the expression of flower symmetry (Luo et al. 1996, 1999; Almeida et al. 1997), has been first explored in Gesneriaceae (Möller et al. 1999) and its expression studied (e.g., Du & Wang 2008, Gao et al. 2008, Song et al. 2009).

The evolution, diversification and expression of other developmental genes have been studied since in Gesneriaceae, such as the floral development genes *RADIALIS* (*RAD*) (Zhou et al. 2008, Yang et al. 2010), and *DIVARICATA* (*DIV*) (Zhou et al. 2008), the floral meristem identity gene *FLORICULA* (*FLO*) (Wang et al. 2004b), and vegetative meristem genes such as *SHOOTMERISTEMLESS* (*STM*) (Harrison et al. 2005, Mantegazza et al. 2007), *WUSCHEL* (*WUS*) (Mantegazza et al. 2009), *BREVIPEDICELLUS* (*BP*), *ARP* (Nishii et al. 2010), *GRAMINIFOLIA* (*GRAM*) (Tonomi et al. 2010), though the latter ones mostly for homology testing with characterized genes from model plants. These genes have not been widely used for reconstructing phylogenies, perhaps due to their sometimes complex duplication patterns.

Reconstructing Biogeographic Histories

Studying the distribution of species in the light of molecular phylogenies is an elegant approach to investigate the history of a plant species or group. Recent publications including dated phylogenies allow a glimpse into the past of the Gesneriaceae. While early work used phylogenetic hypotheses to infer biogeographic patterns, more recent work added more sophisticated analyses involving molecular dating approaches and diverse ancestral-area-reconstructions. The strength and weak-

nesses of such approaches were tested by Clark et al. (2008).

Perret et al. (2013), reconstructed the biogeographic history using dated phylogenies and biogeographical reconstruction analyses, primarily of the New World subfamily, and suggested an origin of the family in South America during the late Cretaceous, and the split between Old World and New World lineages (including the Coronantheroideae) at around 44.7 Mya. The Gesnerioideae were hypothesized to have colonized Neotropical biomes since 34 Mya, followed by local adaptations and specification in these areas. Roalson et al. (2008), studying the biogeographic history of Gesnerioideae and Gloxinieae in South America and the Caribbean on the basis of Fitch parsimony optimization and dispersal vicariance (DIVA) analyses, found the Greater Antilles/Aves landbridge to be critical in the dispersal events for the tribes that arrived in the Central America/Caribbean zone as early as 26 Mya.

Two independent long-distance dispersals, or overland migrations from South America to Australasia through Antarctica, during the late Oligocene (24.7 Mya) and Miocene (17.2 Mya) are postulated on the basis of phylogenetic analyses, ancestral-area reconstruction, and molecular dating, to account for the Gesneriaceae in Australasia (Woo et al. 2011, Perret et al. 2013). This would refute a notion that the coronantheroids represent a paleopolyploid Gondwanan vicariant lineage (e.g., Weber 2004). Perret et al. (2013) proposed the arrival of the Gesneriaceae in the Paleotropics to result from a long-distance dispersal in the late Paleocene/Early Eocene (44.7 Mya) via the Antarctic route to Australasia, but did not hypothesize how they might have entered Asia or Africa.

Early biogeographic work in Gesneriaceae at the genus level often involved an interpretation of biogeographic patterns on phylogenetic trees, rather than dedicated phylogeographic analyses. Nevertheless, strong biogeographic patterns could be inferred this way. By linking divergence points with species distributions the African genus *Streptocarpus*, a strong North to South migration and speciation gradient was inferred in Africa, from Kenya/Tanzania to the Cape Province in South Africa (Möller & Cronk 2001b). While the origin of the genus (Africa or Madagascar) could not be ascertained, an exchange of lineages via a land bridge between the two areas was postulated to have occurred 45–26 million years ago (Mya) and an age of the genus of 50–25 Mya. On a finer geographic scale, *Streptocarpus* migrated southwards from Natal along the coast to the Cape Province and then inland into highland areas to the north and further south along the coast in the most recent history. Since they showed no genetic

intrapopulation variation, the plants in the most southern distribution points on the African continent appeared to have, in evolutionary terms, just arrived in this area (Hughes et al. 2005).

Perhaps the most studied genus biogeographically is *Cyrtandra*, with 750+ spp. also the largest and most widespread in Gesneriaceae (Weber 2004). Atkins et al. (2001) comparing the distribution of *Cyrtandra* species in SE Asia in the light of phylogenetic hypotheses, showed a significant split between species in the Sundaland region and the Philippines and found support for the biogeographic significance of Huxley's line. They showed ancient and recent dispersal events transgressing the line, suggesting ongoing dispersals in the region.

Bramley et al. (2004) used molecular phylogenies and a regional taxonomic treatment approach to tackle *Cyrtandra* species on Mt Kerinci, Sumatra. Their phylogeny gave an insight into the mode of species assemblage, resulting from a gradual accumulation of species over time (the museum hypothesis, cf. Richardson et al. 2001).

Cronk et al. (2005), working on the Pacific Ocean species of *Cyrtandra*, inferred a single origin from SE Asia by stepping-stone migrations from phylogenies and that they represented a supertramp clade *sensu* Diamond (1974). The evolution of the fleshy fruit was thought to represent a key innovation to the expansion success of the lineage that adapted the seeds to highly effective bird dispersals. The evolution of fleshy fruits was also proposed to represent a key innovation to the expansion in the primarily Andean genera in the tribe Episcieae (Clark et al. 2012).

Clark et al. (2008, 2009), sampling around 70 species across the genus *Cyrtandra* with emphasis on the Pacific Ocean area, applied molecular dating and ancestral range reconstruction methods to estimate divergence times of major lineages and their geographic origins. While the exact origin of the Pacific lineage remained unresolved, the authors suggested Fiji to be the first stepping stone of *Cyrtandra* into the Pacific about 20 Mya, with subsequent radiation into other archipelagos. Population level analyses were proposed to perhaps better clarify species relationships in the phylogeographic scenario of *Cyrtandra*.

In Kokubugata et al. (2011) the biogeographic links between the triangle China, Taiwan and Japan were investigated, using ITS and *trnC-D* data on the widely distributed species *Lysionotus pauciflorus* Maxim. as a model. The authors found that species apparently originated in China and dispersed by a single dispersal event via a land-bridge during low sea levels in a Pleistocene glacial maximum. Three oversea dispersal events were also hypothesized, probably through anemochory (seed dispersal by wind), to Japan proper,

across the Tokara Gap, to Okinawa islands crossing the Kerama Gap, and from Taiwan to Lanyu Island.

The studies above on the New World taxa, and the *Streptocarpus* example, are perhaps in line with the relatively poor dispersal ability in the tribes, and perhaps in most species of Gesneriaceae with dehiscent fruits and small seeds without specialized means of dispersal that are not suitable for long distance dispersal, unlike the fleshy fruits of the Pacific Ocean lineage of *Cyrtandra*. The wind dispersals proposed for *Lysionotus* are also in line with their seeds possessing long appendages.

CONCLUDING REMARKS

Over the last two decades, and especially the last few years, major steps have been made towards a better understanding of the molecular systematics, morphological evolution and biogeographic history of the Gesneriaceae. The generic delineations are more or less complete, and follow greatly an alignment along boundaries drawn by phylogenetic relationships. This has required a conceptual shift in the approach to delineation of genera, often away from those based on floral characters towards vegetative ones. Genera with a wide range of corolla shapes (e.g., *Drymonia*, Clark et al. 2006; *Oreocharis*, Möller et al. 2011b; *Petrocodon*, Weber et al. 2011b), have resulted. This would not seem out of place. B.L. Burt (1977) envisaged that "taxonomists must think in terms of diversity, and not try to reduce every group to maximum uniformity." Perhaps it is time to rethink our approach to defining generic diagnostic characters and let phylogenies be used to point to useful synapomorphies. In most cases above where genera were redefined, synapomorphies are present (e.g., leaf arrangement or fruit characters). In others it is more difficult. A departure from our traditional suite of characters may be required and additional, e.g., anatomical or cytological, characters need to be investigated in more detail. They are not new, but require systematic work to be carried out to determine their diagnostic value.

The tribal delineations in the New World and coronantheroids are nearing completion, but the Old World taxa require more work, particularly with view to species sampling. At the highest taxonomic level, there are three major clades recognizable: gesnerioids + coronantheroids, the epithematoids, and the didymocarpoids. From a purely molecular systematics point of view, these three clades could be recognized at the subfamily level. However, the epithematoids and the didymocarpoids are not only sister groups in the molecular phylogenies, but there are also strong morphological and phylogeographical arguments

to keep them in a subfamily (see Weber et al. 2013). The gesnerioids are a lineage that have basically diversified at the diploid level in South America. The coronantheroids are not a relict lineage as once thought, but apparently the result of recent long distance dispersals into Australasia from within the gesnerioids (Woo et al. 2011, Perret et al. 2013) for which polyploidy has been argued to be particularly advantageous (Stebbins 1950, Barrier et al. 1999) by allowing an increased fitness through fixed heterozygosity (e.g., Soltis et al. 2004). The epithematoids are an odd assemblage of morphologically discontinuous lineages that might have experienced a high level of extinction events. They also appear to have evolved at a much higher rate than the rest of Gesneriaceae (e.g., FIGURE 3 in Mayer et al. 2003), which may reflect their often short life cycles (often annuals) (cf. Li et al. 1996, Kay et al. 2006).

A subtribal structure for the Old World didymocarpoideis is still elusive, as is certain knowledge of the history of this lineage since their splitting from the gesnerioids. Some insight has been gained recently, but not enough to fully understand their present day distribution.

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APPENDIX 1. Simplified summary output from PHYLOTA for a ‘Gesneriaceae’ query, showing number of species, genera and sequences (release 184).

NCBI taxon name	Descendant species	Descendant genera	Sequences (GIs)
all Gesneriaceae	1038	148	4668
Coronantheroideae	20	9	222
Cyrtandroideae	526	79	2154
Gesneriaceae <i>incertae sedis</i>	3	2	30
Gesnerioideae	488	58	2257
unclassified Gesneriaceae	1	0	5

¹ Taxa at species rank as determined by NCBI (including this node). Note that some of these have not been formally named yet and are not retrieved in certain NCBI Taxonomy searches (e.g., *Marina* sp. *Lavin* 5341), but they are associated with sequence(s) and are counted as species here.

APPENDIX 2. Breakdown by gene of Gesneriaceae sequence data in PHYLOTA (release: 184, accessed 15 June 2011) and Genbank* data released after 15 June 2011 (accessed 11 July 2012).

Genome	Type	TaxIDs	Genera	GIs	GIs plus GB	Lmin	Lmax
/gene				16-July*			
mitochondrial							
genome				0	1		
<i>nad5</i>	gene	1	1	1	1	1025	1025
<i>atp1</i>	gene	2	2	2	2	1049	1274
<i>atp6</i>	gene	1	1	1	1	615	615
<i>cox1</i>	gene	4	4	4	4	513	2282
<i>matR</i>	gene	2	2	2	2	1623	1893
<i>rps3</i>	gene	1	1	1	2	1407	1407
Sum	6			11	13		
chloroplast							
genome				0	1		
<i>rbcl</i> gene	gene	40	33	47	89	1061	1421
<i>ndhF</i> gene	gene	150	84	159	161	1117	2268
<i>matk</i> /intron	intron/gene	5	4	5	57	1136	2478
<i>rpl16</i> intron	intron	114	31	122	122	553	919
<i>rps16</i> intron	intron	48	32	59	62	667	946
<i>trnL-trnF</i> spacer	intron/spacer	613	178	787	850	433	1281
<i>psbA-trnH</i> spacer	spacer	230	25	261	264	208	363
<i>atpB-rbcL</i> spacer	spacer	203	72	215	225	371	1278
<i>trnE-trnT</i> spacer	spacer	89	48	99	99	386	834
<i>trnS-trnG</i> spacer	spacer	84	14	84	84	382	796
<i>trnT-trnL</i> spacer	spacer	83	12	84	84	382	708
<i>rpl20-rps12</i> spacer	spacer	62	44	63	63	652	784
<i>trnK-psbA</i> spacer	spacer	51	31	61	61	240	313
<i>trnC-trnD</i> spacer	spacer	5	1	57	57	1707	1726
other (<10 seq.)	17 diverse	diverse	diverse	29	36		
sum	31			2132	2315		
nuclear							
<i>CYC</i>	gene	126	63	432	432	389	1177
26S	gene	19	18	19	19	1166	3289
<i>STM</i>	gene	32	7	43	43	118	714
<i>ncpGS</i>	gene/intron	188	63	202	202	349	677
<i>CHS-B</i>	gene/spacer	33	16	148	148	743	760
5S spacer	spacer	32	2	55	55	329	368
ETS	spacer	74	2	98	98	405	421
ITS region	gene/spacer	846	138	1195	1247	614	732
ITS1	spacer	140	45	170	170	218	251
ITS2	spacer	59	41	59	59	252	260
other (<10 seq)	60 diverse	diverse	diverse	98	98	na	na
microsatellite markers	25	25	3	20	25	254	682
Sum	95			2539	2596		
Total	130			4682	4924		

Notes: *TaxID*=number of distinct taxa; *GIs*=number of sequences; *-plus Genbank releases after 15 June 2011; *Lmin*=minimum aligned length; *Lmax*=maximum aligned length.