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A duplication of g*cyc* predates divergence within tribe Coronanthereae (Gesneriaceae): Phylogenetic analysis and evolution

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Abstract. Recent investigations in Gesneriaceae have indicated that the *cycloidea* homolog, g*cyc*, remains functional at the DNA level and rates of sequence divergence in this gene are not statistically different across all taxa regardless of floral symmetry. A duplication of g*cyc* has been detected within Coronanthereae, a tribe that has phylogenetic affinities to subfamily Gesnerioideae and includes two genera with radially symmetrical corollas. Duplication of g*cyc* has been detected in all Coronanthereae except *Sarmienta*. All paralogs appear functional at the DNA level. Likewise, there is no increased sequence divergence between the two copies, nor between species with radially symmetrical flowers to those with bilateral symmetry. The duplication of g*cyc* in Coronanthereae is most likely a result of polyploidy since Coronanthereae have the highest chromosome counts of all Gesneriaceae.

Key words: Gesneriaceae, Coronanthereae, paralogs, g*cyc*, floral symmetry, phylogeny, polyploidy.

Floral symmetry is a trait of systematic and ecological importance in Asteridae (Cronk and Möller 1997, Donoghue et al. 1998) and aspects of symmetry in Asteridae are thought to be dependent on a small set of floral symmetry genes (Luo et al. 1996, Almeida et al. 1997, Reeves and Olmstead 1998). By studying mutant plants of *Antirrhinum majus* L. (snapdragon) that lack the wild type floral bilateral symmetry, Luo et al. (1996, 1999) identified two loci that are essential for the development of bilaterally symmetrical flowers. These are *cycloidea* (*cyc*) and *dichotoma* (*dich*). Both genes are essential for full bilateral symmetry in *Antirrhinum* but the role of *cyc* is more important and acts early in floral development. The developmental aspects of these flower symmetry genes in *Antirrhinum* have stimulated interest in the evolution of plant groups with both bilateral and radial floral symmetry (Coen and Nugent 1994; Coen et al. 1995; Coen 1996; Cronk and Möller 1997; Endress 1997; Running 1997; Donoghue et al. 1998; Baum 1998; Möller et al. 1999; Citerne et al. 2000; Theißen 2000; Cronk 2002; Cubas 2002; Gillies et al. 2002; Hileman and Baum 2003; Hileman et al. 2003; Smith et al. 2004a, b).

One family of Asteridae that manifests shifts in floral symmetry is Gesneriaceae (Cronk and Möller 1997). Gesneriaceae and nearly all close relatives of the family (placed in Lamiales sensu APGII 2003) have bilaterally symmetrical flowers. Additionally, peloric

mutants of *Saintpaulia* Wendl., *Sinningia speciosa*, and *Sinningia cardinalis* (Lehm.) H. E. Moore indicate that shifts from bilateral to radial symmetry can occur within a species as it has been seen for *Antirrhinum* (Luo et al. 1996, 1999) and *Linaria* Mill. (Cubas et al. 1999). Phylogenetic analyses (Smith 1996, 2000; Smith et al. 1997) indicate that the ancestral condition for Gesneriaceae is bilateral symmetry and that radially symmetrical flowers have been derived several times from within otherwise bilaterally symmetrical flowered clades (Cronk and Möller 1997, Möller et al. 1999, Smith 2000). Möller et al. (1999) identified a homolog to *cyc* in Gesneriaceae, g*cyc*. Investigations into the function of g*cyc* in taxa with radially symmetrical taxa have not revealed any mutations that may be directly responsible for the shift in floral symmetry (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004a, b; Wang et al. 2004). Likewise, these same studies did not discover a statistically significant increased rate of sequence divergence in taxa with radially symmetrical flowers in comparison to their bilaterally symmetrical flowered sister taxa, indicating that g*cyc* is still under positive selection and may have functions beyond determining floral symmetry in Gesneriaceae.

For most Gesneriaceae, g*cyc* exists as a single copy, at least based on the means that have been used to detect multiple copies such as PCR and cloning (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004a, b; Wang et al. 2004). However, duplications have been detected within Gesneriaceae, and based on current phylogenetic understanding, imply at least four separate duplication events, one during the early divergence of the family giving rise to g*cyc*1 and g*cyc*2 that may have occurred prior to the divergence between Gesnerioideae and Cyrtandroideae or may only be ancestral to Cyrtandroideae. Another duplication has been detected within *Saintpaulia* and *Streptocarpus* (Citerne et al. 2000), and the remaining two consist of a duplication that preceded the divergence of *Loxostigma* and *Didymocarpus* and another within tribe Coronanthereae (Wang et al. 2004).

Tribe Coronanthereae has been poorly studied within Gesneriaceae (Weber 2004). All species placed in this tribe have long been considered a monophyletic group and are unique among all Gesneriaceae in having nectaries embedded in the ovaries (Wiehler 1983, Weber 2004). Additionally, chromosome counts for these species are among the highest for all Gesneriaceae with counts ranging from estimates of n = 37 to n = +/− 45 (Wiehler 1983, Skog 1984, Weber 2004). These counts are relative to n = 8–14 and 16 for other Gesnerioideae (Wiehler 1983, Skog 1984). Seedlings are isocotylous (Weber 2004), indicating that there may be a closer link to Gesnerioideae since members of Cyrtandroideae are distinguished by anisocotylous seedlings (Burtt 1963).

Early phylogenetic analysis of Gesneriaceae indicated that Coronanthereae were sister to the remainder of subfamily Gesnerioideae based either on morphological (Smith 1996) or molecular characters (Smith et al. 1997). Subsequent analyses at the family level have, 1) supported this position (Smith 2000), 2) shown an unresolved polytomy among the Coronanthereae, Napeantheae, Beslerieae, and the remainder of Gesnerioideae (Mayer et al. 2003), 3) shown that Coronanthereae are a part of Gesnerioideae, but the relationships between Coronanthereae, Beslerieae and Napeantheae are poorly supported (Smith et al. 2004a).

The tribe comprises nine genera distributed either in the South Pacific (*Negria*, *Coronanthera*, *Depanthus*, *Rhabdothamnus*), Australia (*Lenbrassia*, *Fieldia*) or Southern South America (*Sarmienta*, *Asteranthera*, *Mitraria*). This tribe often has been treated as a separate subfamily of Gesneriaceae, Coronantheroideae (Wiehler 1983, Burtt and Wiehler 1995) since its distribution bridges that of the two other subfamilies, Gesnerioideae with a primarily New World distribution, and Cyrtandroideae that is nearly exclusively Old World.

With the exception of *Coronanthera* which comprises 11 species and *Depanthus* with two species, all other genera are monotypic. Burtt (1998) has hinted that *Depanthus* may be congeneric with *Coronanthera*. Both genera are known from New Caledonia and *Depanthus* is separated from *Coronanthera* on the basis of having a radially symmetrical corolla with five fertile stamens as opposed to the bilabiate corolla with four fertile coherent stamens found in *Coronanthera*. Thus the species of *Depanthus* represent a shift from bilateral to full radial symmetry. Likewise, *Lenbrassia* has been suggested as being synonymous with *Fieldia* (Burtt 1999). The species are similar morphologically, but differ in habit, *Lenbrassia* is a small tree and *Fieldia* is an epiphytic subshrub with a nearly regular corolla (Weber 2004), potentially again representing a shift from bilateral to radial symmetry, at least in the corolla. Both species are from Australia.

The duplication of g*cyc* within Coronanthereae allows for a further investigation on the potential functionality of this gene within Gesneriaceae. This study proposes to examine sequences of g*cyc* in Coronanthereae to 1) determine if both copies retain functionality at the DNA level regarding the presence of premature stop codons and frameshift mutations, 2) determine if rates of sequence divergence between the two copies differ, implying a release from selection in at least one copy, and 3) determine if *Depanthus* and/or *Fieldia* show any increased rate of sequence divergence relative to its sister taxa since they are the only member of Coronanthereae with radially symmetrical corollas.

Materials and methods

Species used in the analysis, voucher information and Genbank accession numbers for all sequences are listed in Table 1. DNA was extracted either with a modified CTAB procedure (Smith et al. 1992) or using Qiagen DNeasy extraction kits following the manufacturer's instructions. The amplification, cloning and sequencing of the low copy number nuclear gene, g*cyc* followed the protocols of Smith et al. (2004a). Up to 20 clones per species were sequenced in order to detect both paralogs. Sequences were obtained from both strands of all gene regions to be examined via PCR amplification and sequencing with a Li-Cor LongreadIR 4200 automated sequencer. The pGEM-T vector system kit (Promega) was used to clone g*cyc*. Wang et al. (2004) detected paralogs of g*cyc* in *Fieldia* in their analysis. To determine which of each of the paralogs in Coronanthereae matched these sequences, both *Fieldia* g*cyc*1E (AY423151) and g*cyc*1F (AY423152) were included in one phylogenetic analysis to resolve which of the two paralogs from within *Fieldia* matched g*cyc*1E and g*cyc*1F. The g*cyc*1E of *Mitraria* (AY423153) from Wang et al. (2004) was also included.

The data were analyzed using PAUP* 4.0b10 (Swofford 2000) utilizing both maximum parsimony (MP) and maximum likelihood (ML). Indels were either treated as missing data, or rescored following the methods of Simmons and Ochoterena (2000). Additionally, since g*cyc* lacks introns, indels were rescored as multi-state characters using the amino acid translation as a template and scoring characters as groups of three base pairs corresponding to codons (Baum et al. 1994). For the MP analyses, the data sets were analyzed independently using the search option of Olmstead and Palmer (1994) where five searches are performed using 1000 replicates with nearest neighbor interchange and MulTrees Off saving all shortest trees. The results of each of these searches are then used as the starting trees for a search using tree-bisection reconnection (TBR) and MulTrees on. The shortest trees from all searches were combined and a strict consensus was created. Bootstrap (BS; Felsenstein 1985) replicates were performed with 100 searches with 10 random replicates using full heuristic search with TBR and MulTrees on.

Modeltest version 3.06 (Posada and Crandall 1998) was used to determine the best model that fit the data for maximum likelihood and Bayesian analyses. The test indicated that the Hasegawa et al. (1985) model allowing sites to vary according to a gamma distribution (4.1782) and some sites invariable (0.0919) (HKY + G + I) best fit the data according to the AIC criteria. The hLR criteria selected a similar model except without invariable sites. Both models were applied in both

Table 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. SI - Smithsonian Institution living collection. Letters in parentheses indicate herbarium acronyms where vouchers are deposited. For *Streptocarpus* and *Ramonda*, the paralogs used are not the equivalent of g*cyc*1E and are indicated as such with the Genbank submission numbers. The voucher for the *Mitraria* g*cyc*1F is from Wang et al. (2004).

Species	Voucher	g*cyc*1F	g*cyc*1E
Alloplectus panamensis C. V. Morton	*Skog et al. 7641* (US)	AY363933	NA
Asteranthera ovata Hanst.	*Stewart 12234* (SRP)	AY363950	AY363949
Besleria sp.	*Amaya & Smith 525* (COL)	AY363943	NA
Columnea byrsina (Wiehler) Kvist & L. Skog	*Smith 3408* (SRP)	AY363931	NA
Coronanthera clarkeana Schltr.	*Motley 2191* (NY)	DQ406720	AY363952
Depanthus glaber (C. B. Clarke) S. Moore	*Woo 05–010 & Dawson* (WELTU)	DQ406727	DQ406723
Fieldia australis A. Cunn.	*Stewart s.n.* (SRP)	DQ406721	AY363954
Gasteranthus sp.	*Amaya M. & Smith 515* (COL)	AY363946	NA
Gesneria christii Urban	SI 94-507	AY363923	NA
Kohleria spicata (Kunth) Oerst.	*Skog 7701* (US)	AY363919	NA
Lenbrassia australiana (C. T. White) G. W. Gillett	*Telford & Rudd 11314* (E)	DQ406726	DQ406722
Mitraria coccinea Cav.	*Smith 3936* (SRP)	AY363953	AY423153
Napeanthus apodemus J. D. Smith	*Amaya & Smith 605* (COL)	AY363947	NA
Napeanthus macrostoma Leeuwenberg	*Feuillet s. n.* (US)	AY363948	NA
Negria rhabdothamnoides F. Mueller	*Nordenstam 8608* (S)	DQ406725	DQ406724
Primulina tabacum Hance	Citerne et al. 2000	AF208320	NA
Ramonda myconi (L.) Rchb.	Citerne et al. 2000	AF208323	= g*cyc*2 AF208318
Rhabdothamnus solandri A. Cunn.	*Smith 4393* (SRP)	AY363956	AY363955
Rhytidophyllum auriculatum Hook.	SI 94–524	AY363927	NA
Sarmienta repens Ruiz & Pav.	*Smith 3933* (SRP)	AY363951	NA
Sinningia richii Clayb.	SI 94–554	AY363935	NA
Sinningia speciosa Hiern. peloric mutant	*Smith 4512* (SRP)	AY363942	NA
Solenophora obliqua D. L. Denham & D. N. Gibson	*Breedlove 71542* (CAS)	AY363921	NA
Streptocarpus primulifolius Gandoger	Citerne et al. 2000 and Möller et al. 1999	= g*cyc*1a AF208340	= g*cyc*1b AF208336

the maximum likelihood applications of PAUP* and the Bayesian methods using MRBAYES 3.0B4 (Huelsenbeck and Ronquist 2001).

One means of determining if selection is acting differently on the different copies of g*cyc*, or to determine if g*cyc* is evolving differently in lineages with radially symmetrical flowers is to determine the ratio of the rate of nonsynonymous to synonymous substitutions ($d_N/d_S = \omega$). Ratios significantly less than 1 are suggestive of purifying selection and ratios greater than 1 suggest directional selection. To test for shifts in selective constraint, three likelihood models were applied to the MP tree with indels scored as missing using the codeml program in the PAML package (Yang 2000). The first model (A) assumes a single ω for all branches (Goldman and Yang 1994). The other models (B and C) allow for variation among lineages (Yang 1998, Yang and Nielsen 1998, Bielawski and Yang 2001). Model B assumes two ω ratios, one for the duplication of g*cyc* in Coronanthereae, the other for all other sequences. Model C assumes an independent ω ratio for each branch of the phylogeny.

The likelihood values of the pairs of models A to C were compared using a likelihood ratio test (Felsenstein 1981, Goldman 1993, Yang et al. 1995, Huelsenbeck and Rannala 1997). If the ratio (twice the difference between the natural logs of the likelihoods) is significant as determined from a χ^2 distribution with the appropriate degrees of freedom (difference in number of ω ratios estimated), the parameter rich model was considered the better explanation of the data.

Results

Agarose gels resolved only a single band for all amplifications of g*cyc*. However, sequencing of the cloned products revealed two copies for each member of Coronanthereae with the exception of *Sarmienta* and *Mitraria*. Twenty different clones were sequenced from two different amplifications in an attempt to detect paralogs for these two genera. for other species up to 10 clones were used from a single amplification. Duplicates of g*cyc* were not detected in *Mitraria* in a previous study (Wang et al. 2004).

Translation of the DNA sequences to amino acids in MacClade (Maddison and Maddison 2000) indicated no premature stop codons in any of the sequences. Indels were necessary for alignment of sequences, but always in multiples of three, indicating that no frameshift mutations are present.

Maximum parsimony analysis treating indels as missing data resulted in 52 trees of 911 steps, consistency index (CI) = 0.66, retention index (RI) = 0.77. The inclusion of the *Fieldia* g*cyc*1E and g*cyc*1F of Wang et al. (2004) determined which of the paralogs used in this analysis matched the 1E and 1F paralogs of *Fieldia* and other Coronanthereae obtained for this study. This nomenclature has been used in this paper. The *Mitraria* g*cyc*1E sequence of Wang et al. (2004) included in the phylogenetic analyses here was a different paralog from the g*cyc* sequence obtained for *Mitraria* in this study. Therefore it was retained in all analyses. A single tree selected at random is presented in Fig. 1.

Rescoring of indels resulted in the placement of *Besleria* and *Gasteranthus* (tribe Beslerieae) as members of Coronanthereae (trees not shown). These two genera are clearly a monophyletic group based on the g*cyc* sequences alone, but their sequences are widely divergent from other Gesneriaceae sequences in some regions. As a result, there are often indels in *Besleria* and *Gasteranthus* that are the same as those of other Gesneriaceae that Beslerieae are not assumed to have any close relationship with. Other analyses have indicated that the placement of Beslerieae is not congruent with the analysis of g*cyc* indels seen here (Smith et al. 1997, Zimmer et al. 2002, Wang et al. 2004). When combined with other sequence data, g*cyc* of Beslerieae is more in accordance with these other analyses (Smith and Funke 2005). Therefore, both *Besleria* and *Gasteranthus* were removed from all additional analyses.

The analysis of g*cyc* with indels rescored as multi-state characters and Beslerieae excluded resulted in 16 trees of 830 steps each, CI = 0.71, RI = 0.81. One of these trees selected at random is presented in Fig. 2. With indels rescored following the methods of Simmons

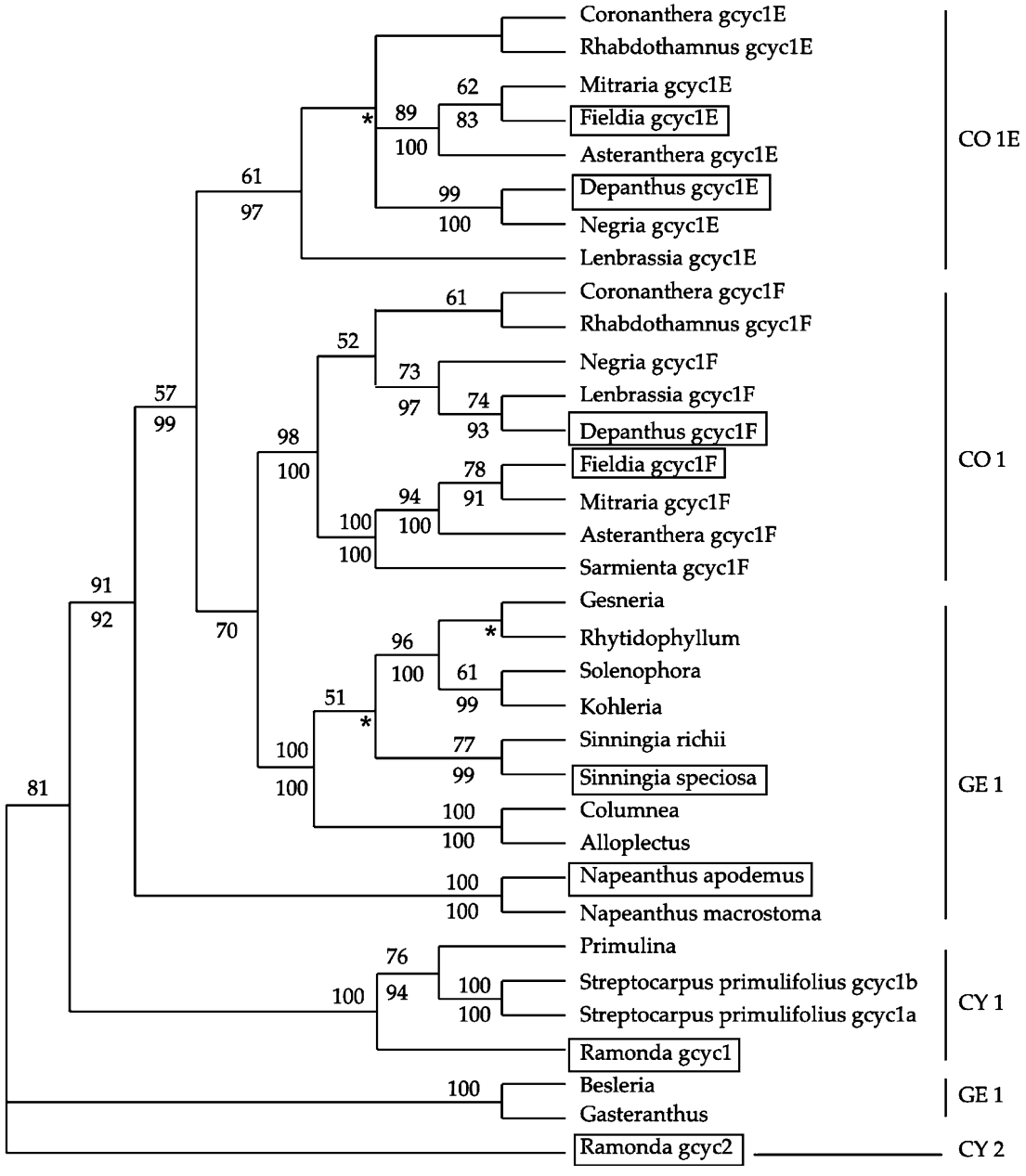

Fig. 1. One of 52 most-parsimonious trees of 911 steps each (CI = 0.66, RI = 0.77) of g*cyc* sequences with indels treated as missing data. Numbers above branches are bootstrap values, numbers below branches are posterior probabilities from a Bayesian analysis. Posterior probability values absent within each of the g*cyc* clades reflect relationships that differed between Bayesian, ML and MP analyses. Asterisks mark nodes that collapse in the strict consensus of all 52 trees. Taxa marked with boxes indicate a radially symmetrical corolla or fully radially symmetrical flower. CY – Cyrtandroideae, GE – Gesnerioideae, CO – Coronanthereae. Numbers refer to the paralog of g*cyc* that has been detected, the letters following the numbers refer to further duplications within these paralogs. Gene nomenclature follows that of Wang et al. (2004) that first designated the duplication within Coronanthereae as 1F and 1E

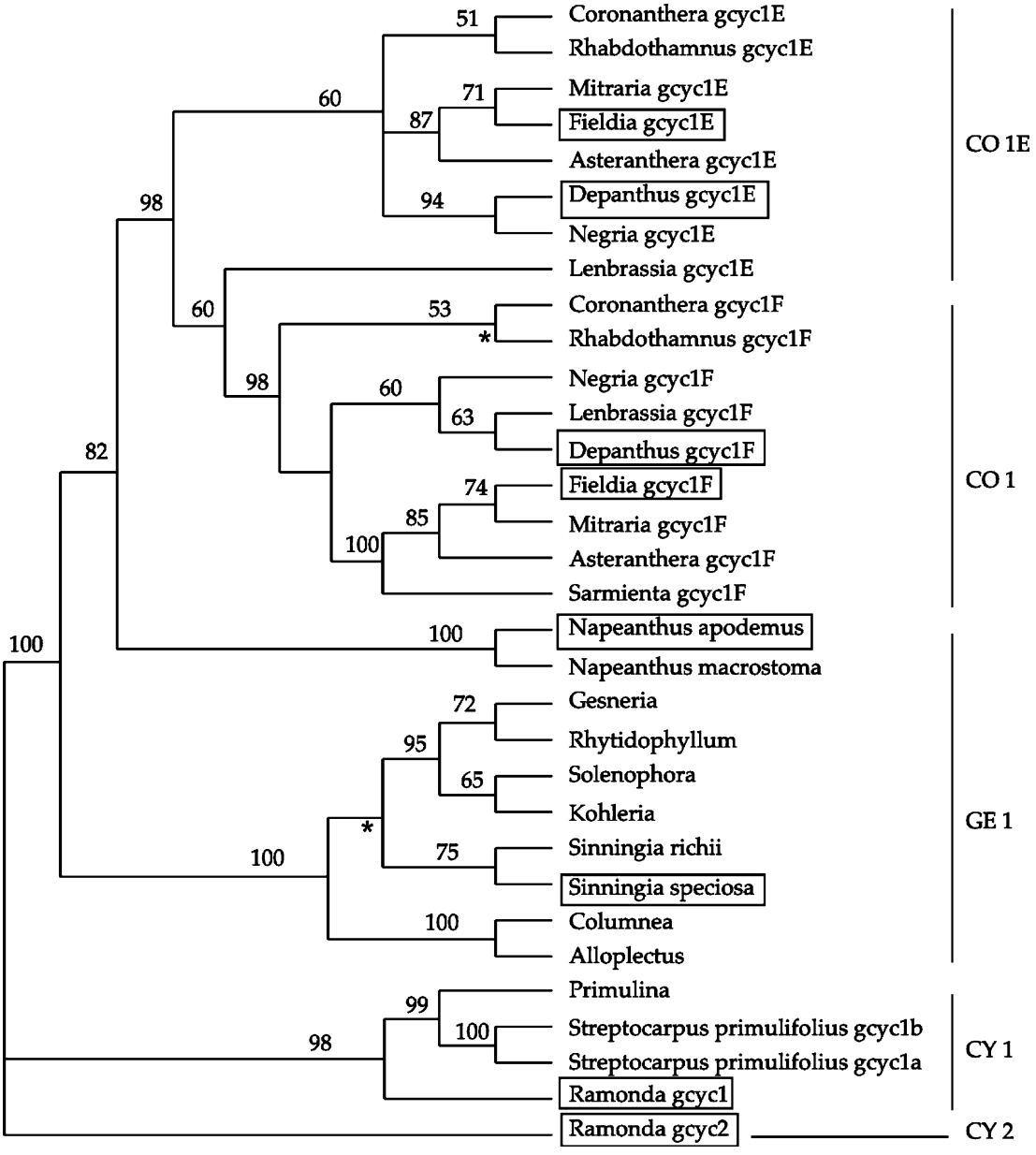

Fig. 2. One of 16 most-parsimonious trees of 830 steps each (CI = 0.71, RI = 0.81) of g*cyc* sequences with indels treated as multi-state data and Beslerieae excluded from the analysis. Numbers above branches are bootstrap values. Asterisks mark nodes that collapse in the strict consensus of all 16 trees. Taxa marked with boxes indicate a radially symmetrical corolla or fully radially symmetrical flower. Numbers refer to the paralog of g*cyc* that has been detected, the letters following the numbers refer to further duplications within these paralogs. Gene nomenclature follows that of Wang et al. (2004) that first designated the duplication within Coronanthereae as 1F and 1E

and Ochoterena (2000) the analysis resulted in 198 trees of 577 steps each, CI = 0.65, RI = 0.77 (trees not shown). The strict consensus of these 198 trees was consistent with the topology of Fig. 2, but less resolved regarding the relationships in the Coronanthereae g*cyc*1E

clade. Rescoring indels differed from the analysis that treated indels as missing data in that both paralogs of g*cyc* in Coronanthereae were monophyletic and both species of *Napeanthus* were sister to Coronanthereae (Fig. 2). Relationships within each of the Coronanthereae paralogs differed slightly, but only at nodes that are poorly supported (Figs. 1 and 2).

Maximum likelihood analyses and Bayesian analyses resulted in topologies (trees not shown) that were similar to the MP tree with indels treated as missing data (Fig. 1). The trees differed from each other and the MP tree in the relationships within the g*cyc*1E and g*cyc*1F clades at nodes that are poorly supported in the MP tree (Fig. 1). The HKY + G and HKY + I + G models did not affect the results for either ML or Bayesian analyses.

The likelihood ratio tests that were used to search for evidence of different rates of nonsynonymous to synonymous substitutions on different lineages were not able to reject a single ω for the entire tree.

Discussion

A duplication of g*cyc* in Coronanthereae is widespread among the members of this tribe, being found in all but one species. Phylogenetic analyses of these sequences indicate that the duplication event preceded the divergence within the tribe (Figs. 1 and 2) and that the absence of the duplication in *Sarmienta* may be a secondary loss, or inability to detect the duplication using the methods explored herein. It also is possible that g*cyc* paralogs have become isolated in different accessions of *Sarmienta* and only g*cyc*1F was present in the individual used in this analysis. Similarly, Wang et al. (2004) detected only g*cyc*1E from *Mitraria*, but from a different plant than that used here. It is possible that within *Sarmienta* and *Mitraria*, some individuals may possess only one paralog. The absence of the second paralog may be the result of a loss of this locus altogether or perhaps both loci are homozygous for the same paralog. Coronanthereae are hypothesized to be polyploids (see below). If these species are autopolyploids, it is possible that homeologous chromosomes may align in meiosis and crossing over could create gametes with only one of the two paralogs present. Further information on the chromosomal location of the g*cyc* paralogs, and behavior of the chromosomes during meiosis will be essential to resolve this.

The duplications did resolve phylogenetic relationships within Coronanthereae similarly in both copies (Figs. 1 and 2) and differ only at nodes that receive low support. The resolution that is present is consistent with results from other genes (Woo et al. unpubl. data). The phylogenetic analyses of these data also indicate that regardless of the phylogenetic method or how indels are scored, Coronanthereae are best considered a part of Gesnerioideae and not sister to it (Figs. 1 and 2).

*Evolution of g**cyc***

Neither duplication of g*cyc* within Coronanthereae showed any indication of loss of function at the DNA level in terms of premature stop codons or frameshift mutations. This was true for both paralogs in Coronanthereae and for the radially symmetrical flowered species within Coronanthereae, *Depanthus* and *Fieldia*. These results are consistent with other studies that have compared sequences of g*cyc* in bilaterally and radially symmetrical flowered taxa (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004a, b) as well as studies that have looked at paralogs in this gene (Citerne et al. 2000, Wang et al. 2004).

Additionally, comparing nonsynonymous to synonymous substitutions ($d_N/d_S = \omega$) did not indicate that the divergence in Coronanthereae paralogs or the sequences in the species with radially symmetrical flowers showed a different rate of sequence divergence. Again these results parallel what has been seen in other members of Gesneriaceae (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004a, b). The implications of the lack of sequence divergence are that g*cyc* likely has a function beyond controlling floral symmetry such as

floral initiation as has been seen in the related gene *TCP* in *Arabidopsis* (Cubas et al. 2001). It is likely that such a function exists in Coronanthereae and although *Depanthus* has clearly shifted from bilateral to full radial symmetry, and *Fieldia* has a radially symmetrical corolla, neither species lost function at the DNA level nor resulted in relaxed selection at either of the g*cyc* loci. G*cyc* may play a significant role in determining floral symmetry in Gesneriaceae but may be regulated at the transcriptional level. Such a model does not preclude the function of g*cyc* beyond floral symmetry and indeed, its lack of increased sequence divergence implies that it likely does have other functions.

However, the puzzle remains within Coronanthereae with the duplication of g*cyc*. Although this locus may have important functions in both floral symmetry and presumably other important developmental processes, a single locus is sufficient for all other members of subfamily Gesnerioideae. The lack of any loss of function mutations or increased rate of sequence divergence in both copies implies that both are still under purifying selection. Classical models suggest that either a duplicate gene will diverge and acquire a new function or more frequently will be lost (Ohno 1970, Nei and Roychoudhury 1973, Bailey et al. 1978, Ohta 1988, Walsh 1995, Hileman and Baum 2003). Hileman and Baum (2003) hypothesize that the duplication of *cyc* that resulted in the gene *dich* in the Antirhineae (Plantaginaceae) occurred by a three step process of, 1) gene duplication, 2) relaxed selection in the paralogs which allows one paralog to acquire a novel function, and 3) purifying selection on both gene copies although function may now differ between the two. This was used to explain why *cyc* contributes more significantly to floral symmetry than does *dich* since *dich* may have accumulated deleterious mutations during the period of relaxed selection. It is possible that a similar process is occurring within Coronanthereae, but is instead in an early stage of this process. Both paralogs may be in the stage of relaxed selection such that some deleterious mutations are occurring, but prior to the increase in purifying selection on both loci. As such there are not a sufficient number of nonsynonymous substitutions to be statistically significant with the methods employed herein. Alternatively, since developmental analyses have not been performed on these species, it may be that the duplication of g*cyc* has resulted in important, but divergent functions, for both loci. It will require further developmental studies to resolve the relative importance of each of these loci in determining floral symmetry in Coronanthereae.

Polyploidy

The chromosome counts in Coronanthereae are higher than most counts in other Gesneriaceae. These higher chromosome counts in the entire tribe imply either a chromosome duplication event in the origin of the tribe such as autopolyploidy or the division of the chromosomes into smaller units. If duplication has occurred, multiple copies of nuclear genes, such as g*cyc,* should be detected (Helentjaris et al. 1988, Ahn and Tanksley 1993). The presence of g*cyc* paralogs in all members of the tribe (except *Sarmienta*) provides evidence that the high chromosome counts in this tribe may be attributed to polyploidy. Additional support for polyploidy in Coronanthereae comes from another nuclear gene, chloroplast expressed glutamine synthetase (ncpGS; Emshwiller and Doyle 1999, Perret et al. 2003, Smith et al. 2004b). This gene appears to have two copies of distinctly different size in Gesnerioideae (Smith et al. 2004b). Analysis of ncpGS for Coronanthereae also produced two amplified products of distinctly different size, however, cloning and sequencing indicated there were at least four different sequences among these two copies (unpublished results). The large number of paralogs made determining orthology of ncpGS for phylogenetic analyses impractical. These data also imply a duplication of the entire genome relative to other Gesnerioideae. Studies of additional nuclear

genes may provide answers as to whether these are auto- or allopolyploids. The ITS regions are unlikely to produce duplicate copies in all taxa if the high chromosome counts are the result of autopolyploidy, but duplicate or disparate copies may be detected if the duplication is the result of allopolyploidy, provided that concerted evolution is not complete. Examining ITS may provide further insights into the type of polyploidy that appears to characterize Coronanthereae.

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