

A Phylogenetic Analysis of Tribes Beslerieae and Napeantheae (Gesneriaceae) and Evolution of Fruit Types: Parsimony and Maximum Likelihood Analyses of *ndhF* Sequences

JAMES F. SMITH

Department of Biology, Boise State University, 1910 University Drive, Boise, Idaho 83725

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ABSTRACT. A cladistic analysis of tribes Beslerieae and Napeantheae revealed that both are monophyletic groups and that Napeantheae is sister to Beslerieae. These relationships are supported by parsimony and most models of maximum likelihood analysis, although with the F81 model, maximum likelihood places Napeantheae within Beslerieae making the latter tribe paraphyletic. All analyses indicate that within Beslerieae, *Besleria* and *Gasteranthus* are sister genera. In parsimony analysis these two genera are sister to the remainder of the tribe whereas in the maximum likelihood analyses, *Cremosperma* is sister to *Besleria*/*Gasteranthus*. Regardless of the analysis, there is a close relationship between clades comprised of dehiscent fleshy-fruited genera and those with indehiscent fleshy fruits. This is similar to Episcieae, implying that the transition from dry capsular fruits to berries may require a two-step process where a fleshy dehiscent capsule is the first stage followed by an indehiscent fleshy berry. The origin of stomata in islands, a characteristic common among Beslerieae and Napeantheae is discussed.

Gesneriaceae comprise approximately 2,500–3,700 species in 120–147 genera, distributed primarily in the tropics with a few temperate species in Europe, China, and Japan (Heywood 1978; Burt and Wiehler 1995). The majority of species in Gesneriaceae are herbaceous perennials, but others are annuals, shrubs, lianas, or trees. Leaves are opposite in the majority of the family but anisophylly, leading to an apparent alternate arrangement following abscission of the smaller leaf, is common. The family is divided into subfamilies with Gesnerioideae found almost exclusively in the neotropics (Burt and Wiehler 1995; Smith et al. 1997b). Gesnerioideae are divided further into six tribes and 60 genera (Burt and Wiehler 1995; Smith et al. 1997b).

The Beslerieae have a complicated taxonomic history. Originally placed in the Old World subfamily Cyrtandroideae on the basis of their superior ovaries (Hanstein 1865), this tribe became a “wastebasket” for many neotropical Gesneriaceae with superior ovaries. Subsequent revisions removed many taxa to Episcieae, leaving a tribe containing primarily the single, large genus *Besleria* (Plum.) L. (Bentham 1876). Later revisions and work on many other South American genera have resulted in new generic descriptions and transfers of genera into this tribe (Wiehler 1983) such that it now encompasses eight genera (Burt and Wiehler 1995).

Beslerieae, as circumscribed by Wiehler (1983) are defined on the basis of 1) a lack of prophylls

and subtending bracts on the inflorescence, 2) one trace, unilacunar nodal anatomy, 3) a petiole with a deep vascular crescent in cross section, 4) seeds without fleshy funiculi, and 5) an annular or semi-annular nectary. Despite these characters, members of Beslerieae tend to include representatives that lack one or more of these traits, opening the possibility that the tribe still represents a “waste basket” for unusual members of Gesneriaceae. For example annular nectaries are also known from Gloxinieae and Gesnerieae (Wiehler 1983) and therefore do not serve as a unique synapomorphy for the tribe. *Tylopsacas* Leeuw. does not have the deep vascular crescent in the cross sections of the petiole and has unique pustulate and semi-globose seeds (Leeuwenberg 1958; Beaufort-Murphy 1983). The nectary of *Tylopsacas* also is unusual in having a ring with enlarged dorsal lobes (Wiehler 1983) although this same character state is observed in some species of *Gasteranthus* Benth.

Anetanthus Hiern. ex Benth. similarly poses a classification problem in having several unusual traits for Gesnerioideae. These include 1) a nectary of a five-pronged sheath that almost surrounds the 2) superior ovary, 3) septicidally dehiscent bivalved capsules (most capsules are loculicidally dehiscent), and 4) seeds that are discoid and winged (Wiehler 1983). This combination of unique traits prompted Ivanina (1966) to conclude that *Anetanthus* is best considered a member of Scrophulariaceae.

More recently, Skog and de Jesus (1997) have described a new subspecies of *Resia* H. E. Moore (Beslerieae) that clearly has bracts subtending the inflorescence, implying that this character is not consistent within the tribe or that *Resia* belongs elsewhere in the family.

Lastly, *Cubitanthus* Barringer is a rarely collected, small herb from the coastal range of Brazil (Barringer 1984). The plant has intermediate characteristics between Gesneriaceae and Scrophulariaceae such as parietal placentation, coherent anthers and striate seeds. All characters are known from Scrophulariaceae, but not in the combination seen in *Cubitanthus*. Therefore, Barringer (1984) concluded that this species belonged in Gesneriaceae, but with no clear affinity to any previously described genus. *Cubitanthus* also has flattened winged seeds and septically dehiscent capsules, traits shared with *Anetanthus*. In fact this species had been classified as *Anetanthus* previously (Bentham 1876). This species is unique among Gesneriaceae in that it has a winged stem and a densely bearded lower corolla lip (Barringer 1984). Its placement in Beslerieae appears to be due to lack of prophylls and bracts in the inflorescence, annular nectary and the similarity of seeds shared with *Anetanthus*.

The core genera of Beslerieae that are not in question are *Besleria*, *Gasteranthus*, *Reldia* H. Wiehler, and *Cremosperma* Benth. (Burt and Wiehler 1995). These genera consistently have the tribal characteristics described above (Wiehler 1983; Kvist and Skog 1988, 1989). The majority of species within Beslerieae are within *Besleria* which encompasses ~200 species (Wiehler 1983). This genus is readily recognized in the field by its combination of tubular red, orange or yellow corollas, terrestrial herbaceous or shrubby habit (a few species are small trees), and a berry.

The berry is of particular interest within *Besleria* as it is the only genus within Gesnerioideae outside of Episcieae and Coronanthereae to have a berry fruit. However, the berries of the three tribes are not homologous. The pulp in the berries of Episcieae is derived from the funiculi of the seeds whereas in *Besleria* the pulp is derived from the placenta (Wiehler 1983). The berries of Coronanthereae are different from either of the above since the nectary is fused to the carpel wall (Wiehler 1983).

Gasteranthus also has fleshy fruits, but they are laterally compressed bivalved capsules, reminiscent of the "display fruits" (Wiehler 1983) of Episcieae. *Gasteranthus* also is characterized by stomata occurring in islands (clusters) on the undersides of the

leaves. Both the stomata in islands and laterally compressed bivalved capsules are shared with *Reldia*, although the fruits of the latter are not fleshy at maturity. *Cremosperma* also has a fleshy capsule, but its stomata are scattered over the undersides of the leaves as they are in *Besleria* (Wiehler 1983).

The Napeantheae are the smallest tribe in Gesnerioideae comprising a single genus and ~30 species. The tribe was circumscribed by Wiehler (1983) principally due to the lack of a set of synapomorphies with other tribes. Napeantheae lack the three trace, trilacunar nodal anatomy of Episcieae and although they have a vascular crescent in the cross section of the petioles as do Beslerieae, they have prophylls and subtending bracts in the inflorescence. Napeantheae lack nectaries, a trait also found in Gloxinieae but the superior ovary and septically dehiscent fruits remove *Napeanthus* from Gloxinieae (sensu Burt and Wiehler 1995 which also includes Sinnigieae) and Gesnerieae.

Species of *Napeanthus* are low-growing rosulate herbs found in humid areas on mossy rocks, on clay soils of river banks, or cloud forests. The stomata occur in islands as they do in *Gasteranthus* and *Reldia*. The presence of these stomatal characters, the vascular crescent in the cross section of the petiole, and the septically dehiscent capsules shared with *Anetanthus* and *Cubitanthus* all imply a relationship of Napeantheae to Beslerieae, including the possibility that Napeantheae has been derived from within Beslerieae as a bracteate lineage.

Recent phylogenetic analyses have focused on the tribal relationships within Gesneriaceae (Smith et al. 1997a, 1997b) as well as relationships within Episcieae (Smith and Carroll 1997), Gloxinieae and Gesnerieae (Smith and Atkinson 1998), and Sinnigieae (Chautems pers. comm.). Of the neotropical subfamily Gesnerioideae (sensu Smith et al. 1997b), only three tribes remain that have not been thoroughly investigated cladistically; Beslerieae, Napeantheae, and Coronanthereae. This paper investigates the relationships within two exclusively South and Central American tribes, Beslerieae and Napeantheae, using *ndhF* sequences. Analyses are performed using parsimony and maximum-likelihood estimates (MLE). Several models of molecular evolution are tested with the MLE methods and the resulting trees are examined in terms of the relationships within and between Beslerieae and Napeantheae.

MATERIALS AND METHODS

The gene sequences used in this analysis were generated by thermal cycle sequencing (Innis et al.

TABLE 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. Letters in parentheses indicate herbarium where vouchers are deposited.

Species	Voucher	Genbank Number
Gesnerioideae: Coronanthereae		
<i>Asteranthera ovata</i> (Cav.) Hanst.	Smith et al. 1997a	U62204
<i>Fieldia australis</i> Cunn.	Smith et al. 1997a	U62196
<i>Mitraria coccinea</i> Cav.	Smith et al. 1997a	U62193
<i>Negria rhabdothermoides</i> F. Muell.	Smith et al. 1997a	U62195
<i>Sarmienta repens</i> R. & P.	Smith et al. 1997a	U62194
Gesnerioideae: Episcieae		
<i>Alloplectus</i> sp.	Smith & Carroll 1997	AFO13686
<i>Alsobia punctata</i>	Smith & Carroll 1997	AFO13688
<i>Cobananthus calochlamys</i> (J. D. Sm.) Wiehler	Smith & Carroll 1997	AFO13692
<i>Codonanthe elegans</i> Wiehler	Smith et al. 1997a	U62178
<i>Codonanthopsis peruviana</i> Wiehler	Smith & Carroll 1997	AFO13693
<i>C. sanguinea</i> (Pers.) Hanst.	Smith & Carroll 1997	AFO13697
<i>Corytoplectus speciosus</i> (Poepp.) Wiehler	Smith & Carroll 1997	AFO13698
<i>Drymonia urceolata</i> Wiehler	Smith & Carroll 1997	AFO13699
<i>Episcia fimbriata</i> Fritsch	Smith & Carroll 1997	AFO13700
<i>Nautilocalyx adenosiphon</i> (Leeuw.) Wiehler	Smith & Carroll 1997	AFO13702
<i>Neomortonia rosea</i> Wiehler	Smith & Carroll 1997	AFO13704
<i>Paradrymonia fuquaiana</i> Wiehler	Smith & Carroll 1997	AFO13707
Gesnerioideae: Gesnerieae		
<i>Gesneria pedicellaris</i> Alain	Smith et al. 1997a	U62192
<i>Rytidophyllum tomentosum</i> (L.) Mart.	Smith et al. 1997a	U62200
<i>Sanango racemosum</i> (R. & P.) Barringer	Smith et al. 1997b	U62144
Gesnerioideae: Gloxinieae		
<i>Diastema racemiferum</i> Benth.	Smith et al. 1997b	U62156
<i>Koellikeria erinoides</i> (DC.) Mansf.	Smith & Carroll 1997	AFO13709
<i>Niphaea oblonga</i> Lindl.	Smith et al. 1997ab	U62160
<i>Solenophora obliqua</i> D.L. Denham & D.N. Gibson	Smith et al. 1997b	U62202
<i>Heppiella ulmifolia</i> (Kunth) Hanst.	Smith and Atkinson 1998	AFO40147
<i>Anodiscus xanthophyllus</i> (Poepp.) Mansf.	Smith & Atkinson 1998	AFO40143
<i>Achimenes skinneri</i> Lindl.	Smith et al. 1997b	U62177
<i>Smithiantha cinnabarina</i> (Linden) Kuntze	Smith & Atkinson 1998	AFO40152
Gesnerioideae: Napeantheae		
<i>Napeanthus costaricensis</i> Wiehler	Smith et al. 1997b	U62198
<i>N. macrostoma</i> Leeuwenberg	Smith et al. 1997b	U62161
<i>N. sp.</i>	Amaya M. & Smith 520 (COL)	AF176622
<i>N. sp.</i>	Amaya M. & Smith 605 (COL)	AF176623
Gesnerioideae: Sinningieae		
<i>Paliavana prasinata</i> (Ker-Gawl.) Fritsch	Smith et al. 1997b	U62174
<i>S. cooperi</i> (Paxt.) Wiehler	Smith et al. 1997b	U62201
<i>Vanhouttea lanata</i> Fritsch	Smith et al. 1997b	U62203

1988) of previously amplified regions. The *ndhF* gene was amplified in two overlapping sections (positions 1-1,350, and 972-2,044) from genomic DNA isolated from fresh, frozen, or silica gel dried material for most species (Smith et al. 1992). Amplification and sequencing procedures followed that

of Smith et al. (1997b) used for other members of Gesneriaceae. Sequences were obtained for *Resia*, one *Reldia* specimen, and *Anetanthus* from DNA extracted from herbarium specimens (Savolainen et al. 1995) using the DNEasy Plant miniprep kits (Qiagen) following the manufacturers instructions.

TABLE 1. Continued.

Species	Voucher	Genbank Number
Gesnerioideae: Beslerieae		
<i>Anetanthus gracilis</i> Hiern.	Irwin et al. 14055 (US)	AF176624
<i>Besleria affinis</i> Morton	Smith et al. 1997b	U62162
<i>Besleria mirifica</i> Morton	Smith 3377 (US)	AF176625
<i>Besleria</i> sp.	Amaya M. & Smith 525 (COL)	AF176626
<i>Besleria</i> sp.	Amaya M. & Smith 575 (COL)	AF176627
<i>Cremosperma ecuadoranum</i> Kvist & L. Skog	Smith 3400 (SRP)	AF176628
<i>Gasteranthus corallinus</i> (Fritsch) Wiehler	Smith et al. 1997b	U62163
<i>Gasteranthus</i> sp.	Amaya M. & Smith 515 (COL)	AF176629
<i>Gasteranthus delphinoides</i> (Seem.) Wiehler	Amaya M. & Smith 545 (COL)	AF176630
<i>Resia nimbicola</i> H. E. Moore	Forero et al. 802 (US)	AF176631
<i>Reldia minutiflora</i> (L. Skog) Kvist & L. Skog	Folsom & Renteria 10344 (US)	AF176632
<i>Reldia minutiflora</i> (L. Skog) Kvist & L. Skog	Amaya M. & Smith 531 (COL)	AF176633
<i>Reldia</i> sp.	Amaya M. & Smith 578 (COL)	AF176634
<i>Tylopsacas cuneatum</i> (Gleason) Leeuw.	Smith 3712 (SRP)	AF176635

The first fragment is smaller than those reported previously since amplifying the DNA from herbarium specimens required successive amplifications using internal primers. Initial amplifications followed procedures for DNA as described elsewhere (Smith et al. 1997b) using primers 1 and 1,350R for the first half and 803 and 2,044R for the second half. Subsequent amplifications required the use of 172 and 972 as forward primers although the same reverse primers resulted in successful amplifications. Alignments of all sequences were done by hand.

This analysis focused on the relationships within Napeantheae and Beslerieae, therefore every attempt was made to gather all genera within both tribes and several species within some of the larger genera. Two to four species each were used to represent the larger genera (*Besleria*, *Gasteranthus*, *Napeanthus*, and *Reldia*). Material of *Cubitanthus* was not obtained for this analysis and is the only member of Beslerieae not included. The species used in the analysis, voucher information, and Genbank accession numbers are included in Table 1. The data matrix contains 2.67% missing cells based on total sequence alignments.

Outgroups were selected to root the tree based on recent morphological and molecular analyses of tribal relationships within Gesneriaceae (Smith 1996; Smith et al. 1997b). The most appropriate outgroups for Beslerieae and Napeantheae were Coronantheae. To determine if Beslerieae are monophyletic and if Napeantheae are sister to Beslerieae as indicated by previous cladistic analyses of *ndhF* sequences, an initial analysis was used with rep-

resentatives of all tribes within Gesnerioideae. Bootstrap and decay analyses were not performed for this full sampling.

Phylogenetic Analysis. Phylogenetic divergence was reconstructed using PAUP version 4.0d64 to implement parsimony (Farris 1970; Farris et al. 1970; Swofford and Maddison 1987) and maximum likelihood. In the parsimony analysis, trees were generated using the general heuristic option. To search for islands of equally parsimonious trees (Maddison 1991), the search strategy of Olmstead and Palmer (1994) was implemented searching for 1,000 trees each in five subsequent analyses with the nearest neighbor interchange (NNI) search option in effect and mulpars "off." Each of the results from the five NNI searches was used as the starting tree(s) for a search with tree bisection reconnection (TBR) and mulpars "on."

MLE trees were conducted using the heuristic search option with TBR and mulpars "on." Using the MLE option, three different models of nucleotide substitution were examined. The first corresponds to Felsenstein's (1981) model (F81), where transitions and transversions are equally likely. The other models are more complex in that the Hasegawa et al. (1985) model (HKY) allows for differential rates of transitions and transversions and the final model (GTR; Yang 1994) calculates the rate matrix for the substitution of one nucleotide to the other. All models allow for unequal nucleotide frequencies. The assumed nucleotide frequencies for all data were estimated from the data: A = 0.28360, C = 0.15042, G = 0.17557, and T = 0.39041.

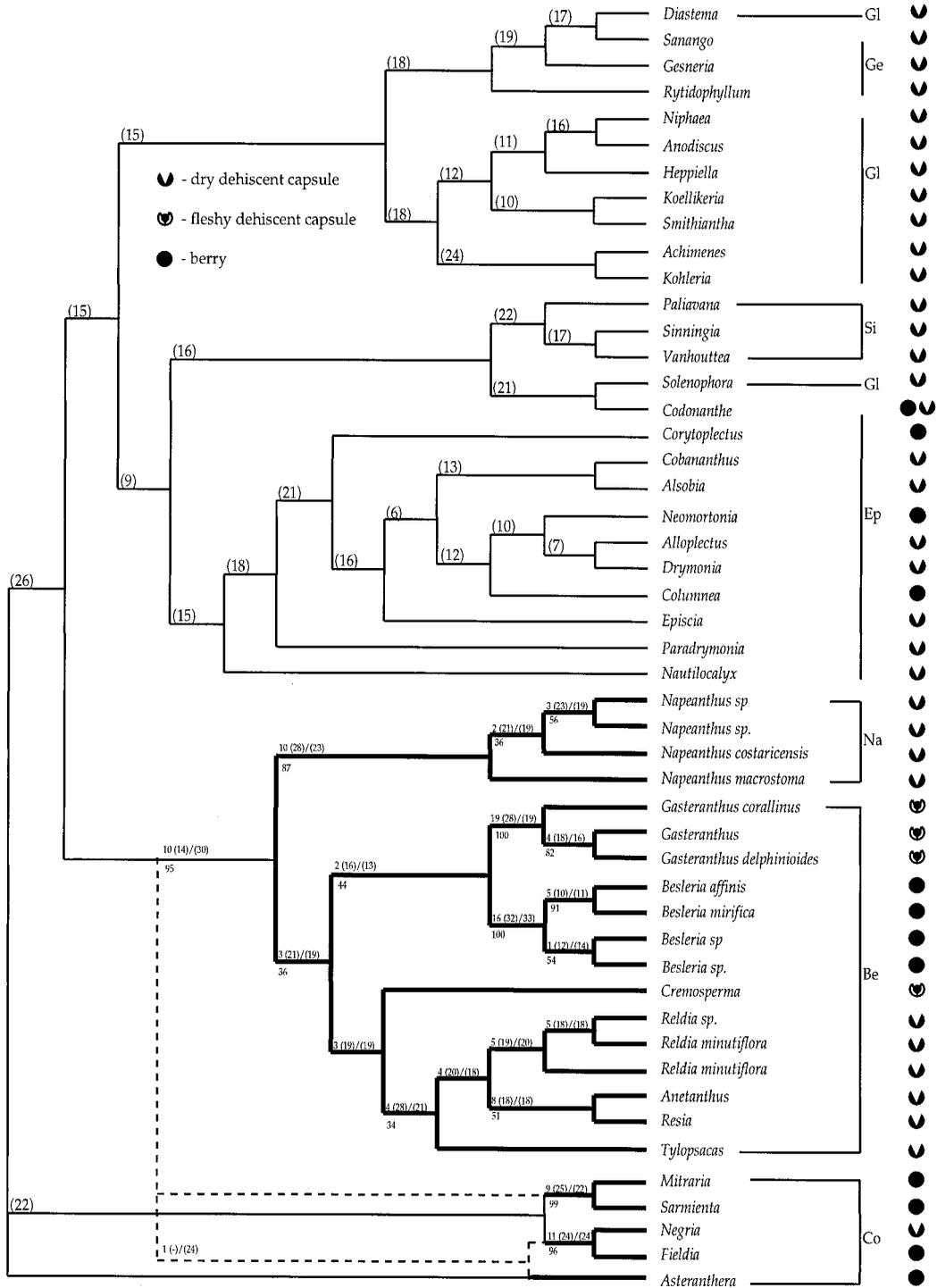


FIG. 1. Single most-parsimonious tree of 3,421 steps each, CI = 0.29, RI = 0.41 including representatives from all tribes within Gesnerioideae and rooted using Coronanthereae. This tree is not intended to represent relationships within and among tribes other than Beslerieae and Napeantheae. Numbers in parentheses are branch lengths using the acctran

Branch support analysis was performed to examine trees that were up to 11 steps longer than the most-parsimonious trees (Bremer 1988, 1994; Donoghue et al. 1992). This type of analysis provides an indication of the robustness of the data by determining which clades persist in a consensus tree as parsimony is relaxed. Clades that persisted in strict consensus trees 11 steps beyond the most-parsimonious trees were examined using the constraints option to search for the shortest tree that did not contain that clade. Bootstrap analysis (Felsenstein 1985) was performed using 100 replicates with TBR and mulpars "off."

RESULTS

The parsimony analysis of all Gesnerioideae resulted in a single most-parsimonious tree of 3,421 steps, consistency index (CI) = 0.29, retention index (RI) = 0.41. This tree is presented in Fig. 1. This tree indicated that Napeantheae and Beslerieae are sister taxa, confirming earlier results with a smaller sampling within these two tribes (Smith et al. 1997b) and that both tribes are monophyletic.

The reduced analysis, using only members of Beslerieae and Napeantheae as ingroups and Coronanthereae as the outgroup produced a single most-parsimonious tree of 1,467 steps, CI = 0.53, RI = 0.45 (Fig. 1). This tree places Napeantheae as sister to Beslerieae and Beslerieae is divided into two clades; one of *Besleria* and *Gasteranthus*, the other with all remaining genera.

The MLE analyses resulted in three additional trees (Fig. 2). The HKY and GTR models produced nearly identical trees (Fig. 2A) with $-\ln$ likelihoods of 10,833.91880 and 10,843.91025, respectively. These trees differ from each other only in the relationships within Napeantheae and Coronanthereae. The HKY MLE tree differs from the parsimony tree only in the position of *Creemosperma*, which is sister to *Besleria/Gasteranthus* in the MLE trees (Fig. 2A) and sister to the remaining genera of Beslerieae in the parsimony tree (Fig. 1). The F81 model of

MLE produced a third topology with $-\ln$ likelihood of 10,846.41765. This tree differs substantially from all other trees in that one species of *Reldia* is sister to *Creemosperma*, both of which are sister to *Besleria/Gasteranthus* and *Napeanthus* is sister to this clade, creating a paraphyletic Beslerieae (Fig. 2B).

DISCUSSION

From all analyses of *ndhF* sequence data, it is apparent that Beslerieae and Napeantheae are monophyletic and together form a monophyletic group (Figs. 1 and 2; Smith et al. 1997b). Both tribes are closely related in the cladistic analysis of morphological data, although *Besleria/Gasteranthus* are sister to Episcieae, which together are sister to a monophyletic *Anetanthus/Napeanthus* (Smith 1996). The monophyly of Napeantheae and Beslerieae also is supported by several morphological traits that are found only in these tribes within Gesnerioideae. These include septically dehiscent fruits, a petiole cross sectional anatomy of a deep vascular crescent and stomata in islands.

Comparison of Parsimony and MLE Analyses. Parsimony and MLE analyses are largely in congruence with Napeantheae and Beslerieae as sister tribes although the F81 analysis indicates that Napeantheae are derived from within Beslerieae (Fig. 2B). There is some support for this from morphological data. The Beslerieae are largely circumscribed by lacking prophylls and bracts in the inflorescence whereas these are present in Napeantheae (Wiehler 1983). A recently described subspecies of *Resia* (Beslerieae) clearly has bracteate inflorescences (Skog and de Jesus 1997). Therefore the lack of inflorescence bracts is no longer a consistent diagnostic trait for Beslerieae and implies that Napeantheae could be derived from within Beslerieae as a reversal to the bracteate state as it was in *Resia*. Additionally Napeantheae and some members of Beslerieae have stomata in islands. This trait is relatively rare in Gesnerioideae and a single origin of this trait would require inclusion of Na-

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option of PAUP. Terminal branch lengths are not shown. Abbreviations for tribes are as follows: Be – Beslerieae, Co – Coronanthereae, Ep – Episcieae, Ge – Gesnerieae, Gl – Gloxinieae, Na – Napeantheae, Si – Sinningieae. The portion of the tree in bold represents the parsimony analysis that includes only Beslerieae, Napeantheae and Coronanthereae, rooted with the latter tribe. The single most-parsimonious tree is 1,467 steps long, CI = 0.53, RI = 0.45. The dashed line indicates the position of the outgroup taxa in this reduced analysis. Numbers above clades are decay values, numbers in parentheses following the slash are branch lengths using the acctran option of PAUP for the reduced analysis. Numbers in parentheses before the dash represent branch lengths in the full analysis. Numbers below clades indicate bootstrap values. Fruit types are indicated for each genus in a column to the right of the tree.

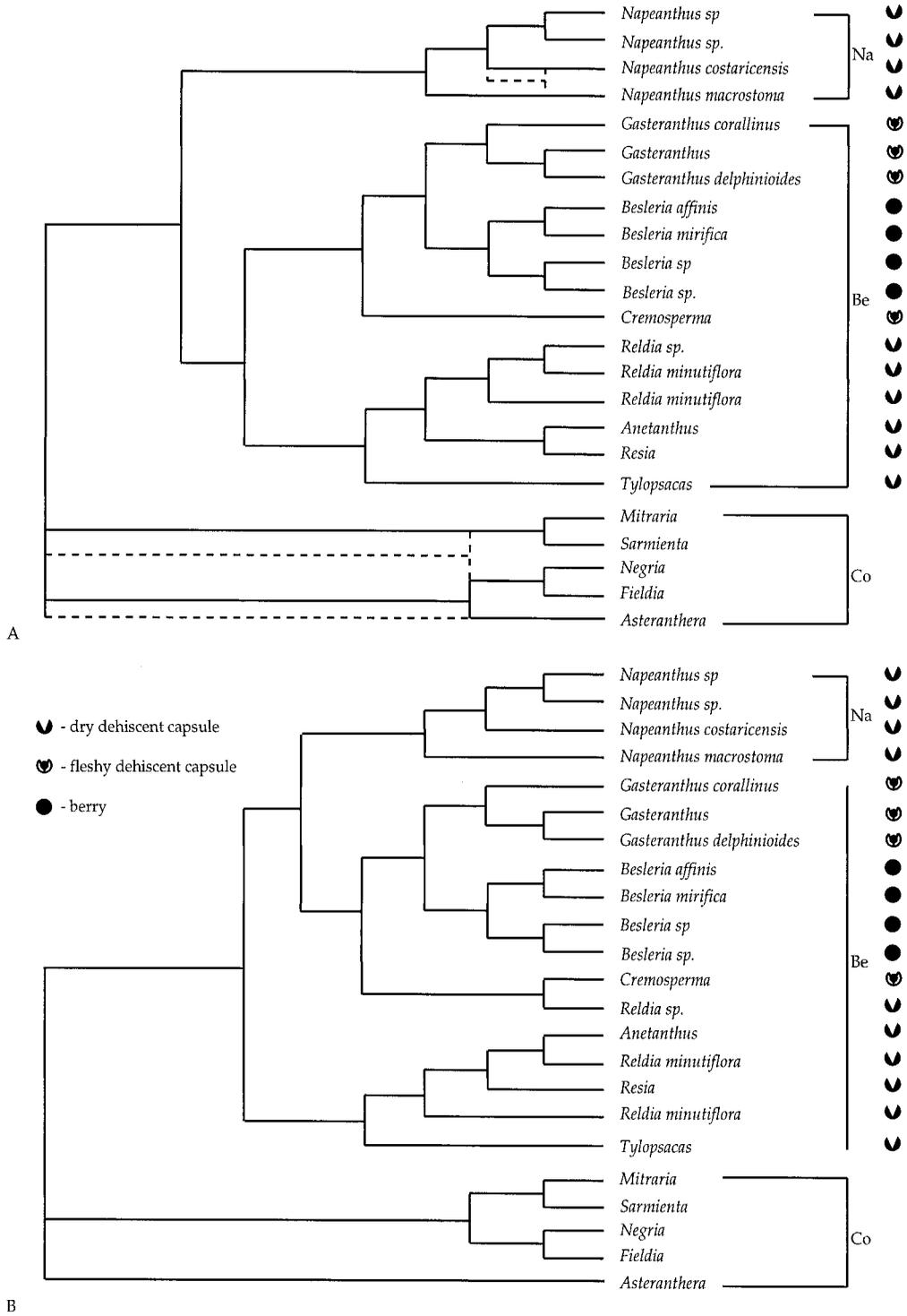


FIG. 2. A. Maximum likelihood estimate trees based on the HKY and GTR models, $-ln$ likelihood = 10,833.91880 and 10,843.91025, respectively. The dashed lines within Napeantheae and Coronanthereae represent the relationships within these tribes following the GTR model. All other relationships were identical between the two models. B. Maxi-

peantheae within Beslerieae. However, the distribution of this trait throughout these two tribes is not in agreement with a single origin based on the phylogenetic results presented here (see below).

The best explanation for the presence of Napeantheae within Beslerieae in the F81 model of MLE is that the model does not accurately reflect the evolutionary history of the DNA in this group. This model assumes equal rates of substitution across all sites and equal probabilities of transitions and transversions (Felsenstein 1981). This is likely to represent an oversimplification of the possible substitutions in the data and is therefore less accurate than other methods as compared in Yang et al. (1994).

Relationships within Napeantheae and Beslerieae. Within Beslerieae there is support for two distinct clades, although the groupings vary in the different analyses. In all analyses, *Besleria* and *Gasteranthus* are sister genera and belong in a single clade (Figs. 1 & 2). Likewise, *Anetanthus*, *Tylopsacas*, most species of *Reldia*, and *Resia* are always in a single clade (Figs. 1 and 2) and the relationships among these genera are the same with the exception of the F81 MLE model (Fig. 2B). It is only the positions of *Cremosperma* and a species of *Reldia* that vary (Figs. 1 and 2). The discrepancy in the position of the one *Reldia* specimen is only found in the F81 MLE model (Fig. 2B) and most likely reflects the erroneous nature of this model as discussed above. However, it should be noted that Beaufort-Murphy (1983) in her monograph on the seeds of Gesneriaceae divided Beslerieae into two groups. One group contained *Besleria*, *Gasteranthus* and *Reldia*, the other had *Cremosperma* alone. Therefore a relationship between *Reldia* and *Besleria*/*Gasteranthus* has been hypothesized previously.

A remaining question is whether *Cremosperma* is sister to the *Besleria*/*Gasteranthus* clade as indicated by all MLE analyses (Fig. 2), or whether it is sister to the remaining genera of the tribe as indicated by parsimony analysis (Fig. 1). The morphological data support the MLE analysis. Among Beslerieae, there are only three genera with fleshy fruits, these are *Besleria*, *Gasteranthus*, and *Cremosperma*. The placement of these three genera in a single clade would allow for a single origin of fleshy fruits within the

tribe. Therefore the MLE analyses may reflect more accurately the phylogenetic relationships within Beslerieae.

Fruit Evolution. Cladistic analyses of *ndhF* and ITS sequences within Episcieae have revealed a close relationship between the presence of berry fruits and fleshy dehiscent capsules (display fruits) (Smith and Carroll 1997). Berries appear to have three separate origins within the tribe and display fruits have two origins. In only one instance does a berry occur (*Rufodorsia* H. Wiehler) without having a close relative with a display fruit (Smith, in mss.). In one instance, the display fruit appears to have been derived after the berry evolved and in another the reverse seems true, although limited sampling in the latter example prevents strong conclusions from being made.

Besleria is unique among Gesnerioideae in its fruit type. Members of *Besleria* have a berry and although berries are also known from Episcieae and Coronanthereae, the berry of *Besleria* is structurally different. The fleshy pulp of berries in Episcieae is from funiculi whereas the funiculi of *Besleria* fruits are not fleshy and instead the pulp of the fruit is derived from the placenta (Wiehler 1983). Therefore the fruit types are not homologous and the berries of *Besleria* are the only ones within Gesnerioideae to have such a structure. The fruits of Coronanthereae have the nectary fused to the carpel wall and are themselves unique within Gesnerioideae (Wiehler 1983). The fruits of *Gasteranthus* are fleshy, and dehiscent. This is reminiscent of the display fruit of Episcieae where the fleshy fruit dehisces to reveal brightly colored interior pericarp walls that contrast with the fleshy funiculi of the seeds (Wiehler 1983). Within Beslerieae there is a close affinity between fleshy dehiscent and fleshy indehiscent fruits (Figs. 1 and 2). Regardless of the analysis, *Besleria* and *Gasteranthus* are sister genera and the relationship is strongly supported (Figs. 1 and 2).

Although it is ambiguous comparing simply *Besleria* and *Gasteranthus* as to whether berries preceded dehiscent fleshy capsules or vice versa, by comparing the sister to these two genera a better estimate can be established (Maddison et al. 1984). In the parsimony analysis, the *Besleria*/*Gasteranthus*

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mum likelihood estimate trees based on the F81 model, $-\ln$ likelihood = 10,846.41765. Abbreviations for tribes are as follows: Be – Beslerieae, Co – Coronanthereae, Na – Napeantheae. Fruit types are indicated for each genus in a column to the right of the tree.

clade is sister to the remainder of the tribe, all of which have dehiscent fruits (Fig. 1). Therefore the most-parsimonious explanation is that the ancestor to the *Besleria*/*Gasteranthus* clade was also dehiscent and fleshy. This trait passed unaltered into *Gasteranthus* and indehiscence evolved along the line to *Besleria*. The HKY and GTR models of MLE also support this hypothesis in that *Cremosperma* is sister to the *Besleria*/*Gasteranthus* clade and all three are sister to the remainder of the tribe (Fig. 2A). *Cremosperma* is the only other genus within Beslerieae to have fleshy dehiscent fruits implying that the ancestor to this clade also had fleshy dehiscent fruits and that the ancestor to the entire tribe was most likely dehiscent but dry since fruits of Napeantheae, the sister to Beslerieae, also are dry capsular fruits. The relationships of these taxa suggest the first step in the evolution of the berry in Beslerieae was a fleshy but dehiscent capsule and the dehiscence was lost as a second step. This pattern of berry evolution is also seen within one clade in Episcieae and potentially may have occurred with others but the intermediates have been lost (Smith in mss.).

Stomatal Evolution. Within Gesnerioideae there are three basic models of stomatal distribution, 1) irregularly scattered, 2) singly on mounds, and 3) clustered in islands (Wiehler 1983). The first model of distribution is most likely symplesiomorphic and the second is found only within tribes Gloxinieae and Gesnerieae. The third model, stomata clustered in islands, is found within *Gasteranthus*, *Reldia*, *Napeanthus*, and *Gesneria* L. (Gesnerieae). That Napeantheae and members of Beslerieae share this trait implies a close affinity between the two tribes and implies that the ancestor to these two tribes also had stomata in islands. In only one analysis is it possible that the state of clustered stomata could represent a single origin among Napeantheae and Beslerieae. This is the F81 model of MLE (Fig. 2B) and even within this tree, two losses of the state would need to be invoked for *Besleria* and *Cremosperma* and two additional gains in the remaining *Reldia* specimens for a total of five state changes. There is reason to doubt the F81 model without considering stomatal evolution, but these data provide additional support. In all other trees generated by parsimony and HKY and GTR models of MLE, three independent gains of stomata in islands is necessary for all genera and is a more parsimonious explanation of stomatal evolution.

Although it seems less likely that three closely related genera would have derived a trait indepen-

dently three different times, it is clear that there is selection for this trait since it has arisen elsewhere in *Gesneria*, which is phylogenetically distant from Napeantheae and Beslerieae (Smith et al. 1997b). The majority of plants with stomata in islands occur in humid shaded environments along river banks or among moss-covered cliffs and rocks. Stomata in islands may serve as a means of maximizing transpiration as they are generally slightly raised above the epidermis of the leaf and most leaves with stomata in islands are glabrous and at least somewhat coriaceous (Wiehler 1983). Therefore there may be substantial selection pressure for this trait to have evolved multiple times and it may be other characteristics pertaining to the physiology of Beslerieae that permit them to occupy more humid shaded environments.

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