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Phylogenetic Patterns of Diversification in the Beslerieae (Gesneriaceae)

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ABSTRACT

The tribe Beslerieae constitutes one of the eight tribes currently recognized in Gesneriaceae subfamily Gesnerioideae and is presently considered to include seven genera: *Anethanthus*, *Besleria*, *Cremosperma*, *Gasteranthus*, *Reldia*, *Resia*, and *Tylopsacas*. Phylogenetic relationships among the major genera of Beslerieae, namely *Besleria*, *Cremosperma*, *Gasteranthus*, and *Reldia*, are explored here using nrDNA ITS sequences to explore generic monophyly, previous classification and phylogenetic hypotheses, and patterns of fruit-type evolution, stomatal cluster evolution, and biogeography. ITS data supports the monophyly of *Besleria*, *Cremosperma*, *Gasteranthus*, and *Reldia*, the intermediate position of fleshy capsules in the transformation of dry capsules to irregularly splitting or indehiscent berries, a single loss of stomatal clusters in the Beslerieae, and complex biogeographic patterns in the tribe, but perhaps is a single origin of Caribbean *Besleria* originating from northeastern South America.

Key Words: *Besleria*, Beslerieae, *Cremosperma*, *Gasteranthus*, Gesneriaceae

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INTRODUCTION

The tribe Beslerieae constitutes one of eight tribes currently recognized in Gesneriaceae subfamily Gesnerioideae [20, 28, 35] and is presently considered to include seven genera: (1) *Anetanthus* Hiern. ex Benth., (2) *Besleria* L., (3) *Cremosperma* Benth., (4) *Gasteranthus* Benth., (5) *Reldia* Wiehler, (6) *Resia* H.E.Moore, and (7) *Tylopsacas* Leeuw. [25, 28, 33]. *Cubitanthus* Barringer was recognized as a member of the tribe Beslerieae by Burt and Wiehler [2], but is not included here because it is most likely a member of the Scrophulariaceae sensu lato following Weber [28]. While the core genera *Besleria*, *Cremosperma*, and *Gasteranthus* have been consistently considered members of the Beslerieae [1, 8, 33], the placement of *Reldia*, *Resia*, *Tylopsacas*, and *Anetanthus* has either been questioned or was without previous tribal affiliation. The tribe is dominated by the large genus *Besleria* with ~200 recognized species [2, 33], although circumscriptions for many species are unclear and the genus is in need of revision. *Gasteranthus*, previously recognized within *Besleria* [15], was recently revised [24] and morphological cladistic analyses have explored relationships in the group [17].

Members of the Beslerieae have diverse morphological characteristics and the variation in floral form, fruit type, and the distribution of stomatal clusters have been of particular interest to previous authors (Fig. 1) [17, 24, 25, 33]. Particularly, fruit characteristics and the distribution of stomata on the abaxial leaf surface have been suggested to be important for distinguishing among the genera [31, 33]. Floral form variation is extreme within the genera *Besleria* and *Gasteranthus* (Figs. 1A & B) [17, 24] relative to fruit and vegetative characters (Figs. 1C & D) and assessing homology has been a major challenge to previous classification systems that lacked DNA sequence data.

Phylogenetic analyses consistently recognize a monophyletic Beslerieae [21, 25, 35], although most of these studies have had minimal sampling within the tribe. The only detailed phylogenetic study [21] sampled *ndhF* gene sequences for 14 species of all genera of the tribe, although in this study the largest genus *Besleria* was represented by only four species, and *Gasteranthus* and *Cremosperma* by three and one species, respectively. Additionally, statistical support of branches grouping the genera of the tribe together was low and different analyses suggested different topologies using this gene [21]. The two contrasting topologies presented by Smith [21] are

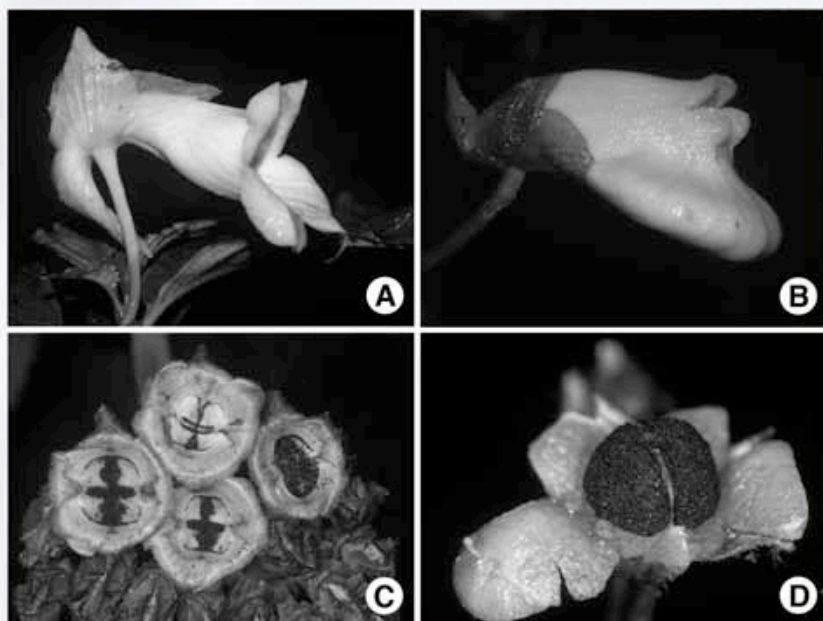


Fig. 1 Flower and fruit types in Beslerieae. A. Flower of *Gasteranthus tenellus* L.E. Skog & L.P. Kvist from Ecuador (J.L. Clark 5573). B. Flower of *Gasteranthus pansamalanus* (J.D. Smith) Wiehler from Ecuador (J.L. Clark 6863). C. Capsule of *Gasteranthus corallinus* (Fritsch) Wiehler from Ecuador (J.L. Clark 7540). D. Rupturing berry of *Besleria* sp. from Peru (J.L. Clark 8197). All photographs taken in the field by John L. Clark. Numbered collections correspond to voucher specimens deposited at US.

reconstructed in Fig. 2 and represent the starting hypotheses of relationships in the tribe.

The phylogenetic relationships among the major genera of the tribe Beslerieae: *Besleria*, *Cremosperma*, *Gasteranthus*, and *Reldia* are detailed below. Samples were not available for the small genera *Anetanthus*, *Resia*, and *Tylopsacas* so their relationships to the genera sampled will require further study. We have greatly expanded taxon sampling of the larger genera over previous studies using nrDNA (nuclear ribosomal DNA) internal transcribed spacer (ITS) sequences to address the following questions: (1) Are the genera *Besleria*, *Cremosperma*, *Gasteranthus*, and *Reldia* monophyletic? (2) Do previously applied sections, subsections, and informal groupings within these genera reflect phylogenetic clades? (3) Does the ITS data support the previously suggested phylogenetic relationships among genera proposed in Smith [25] using *ndhF*?, and (4) What are the patterns of (a) fruit-type evolution, (b) stomatal cluster evolution, and (c) biogeography?

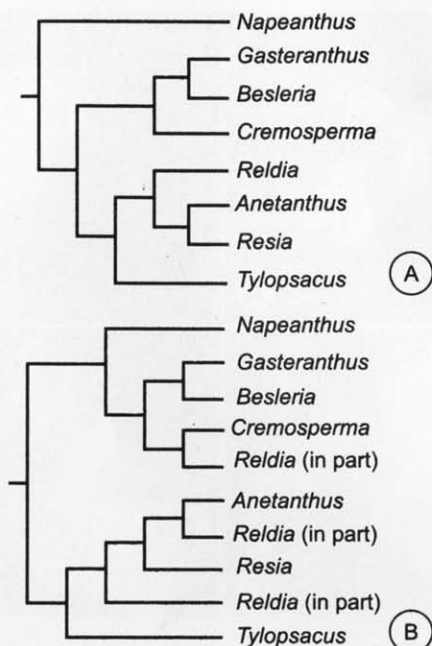


Fig. 2 Diagrammatic representations of contrasting phylogenetic hypotheses for generic relationships in the Beslerieae presented by Smith [25] based on *ndhF* sequences. Fig. 1A is based on a single most-parsimonious tree while Fig. 1B is based on a maximum likelihood estimate.

Materials and Methods

Sampling

Most genomic DNAs were isolated from silica-dried leaf material collected in the field by the second author throughout five years of intensive fieldwork throughout the Neotropics. Other samples were obtained from live plants grown at the Smithsonian's National Museum of Natural History Botany Research Greenhouses (Table 1). Samples of *Napeanthus* were used as the outgroup to the Beslerieae based on previous results supporting Napeantheae as the sister group of the Beslerieae [21, 25, 35].

DNA Sequencing

DNA was isolated using the Quiagen DNeasy™ DNA isolation kit or traditional CTAB extraction protocols [5]. Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5HP [26] and ITS4 [30].

Table 1. Samples sequenced in phylogenetic study of Beslerieae (Gesneriaceae) with voucher specimen and GenBank accession number. All voucher specimens are deposited at US, unless otherwise noted. Herbarium acronyms are: RSA = Rancho Santa Ana Botanic Garden; and US = United States National Herbarium, Smithsonian Institution, Washington, DC, USA.

Species	Voucher	GenBank Accession
<i>Besleria aggregata</i> (Mart.) Hanst.	J.L. Clark 6790	DQ070479
<i>B. angustiflora</i> Fritsch	J.L. Clark 4575	DQ070480
<i>B. barbata</i> (Poepp.) Hanst.	J.L. Clark 5705	DQ070481
<i>B. barbata</i> (Poepp.) Hanst.	J.L. Clark 5880	DQ070482
<i>B. comosa</i> C.V. Morton	J.L. Clark 6008	DQ070483
<i>B. comosa</i> C.V. Morton	J.L. Clark 6055	DQ070484
<i>B. emendata</i> C.V. Morton	J.L. Clark 8177	DQ070485
<i>B. filipes</i> Urb.	J.L. Clark 6559	DQ070486
<i>B. formicaria</i> Nowicke	J.L. Clark 8611	DQ070487
<i>B. formosa</i> C.V. Morton	Burger and Baker 9474 (RSA)	DQ070488
<i>B. gracilentia</i> C.V. Morton	J.L. Clark 8202	DQ070489
<i>B. hirsutissima</i> C.V. Morton	J.L. Clark 6901	DQ070490
<i>B. labiosa</i> Hanst.	Skog 7631	AY047041
<i>B. lutea</i> L.	J.L. Clark 6544	DQ070491
<i>B. modica</i> C.V. Morton	J.L. Clark 6056	DQ070492
<i>B. mortoniana</i> Steyerem.	J.L. Clark 6900	DQ070493
<i>B. notabilis</i> C.V. Morton	J.L. Clark 8548	DQ070494
<i>B. ovalifolia</i> Rusby	J.L. Clark 6707	DQ070495
<i>B. pendula</i> Hanst.	J.L. Clark 6866	DQ070496
<i>B. reticulata</i> Fritsch	J.L. Clark 5848	DQ070497
<i>B. rotundifolia</i> Rusby	J.L. Clark 6786	DQ070498
<i>B. solanoides</i> Kunth	J.L. Clark 6113	DQ070499
<i>B. tambensis</i> C.V. Morton	J.L. Clark 7116	DQ070504
<i>B. variabilis</i> C.V. Morton	J.L. Clark 5629	DQ070505
<i>B. sp.</i>	J.L. Clark 6057	DQ070502
<i>B. sp.</i>	J.L. Clark 6869	DQ070503
<i>B. sp.</i>	J.L. Clark 5654	DQ070500
<i>B. sp.</i>	J.L. Clark 5916	DQ070501
<i>Cremosperma castroanum</i> C.V. Morton	J.L. Clark 7104	DQ070506
<i>C. hirsutissimum</i> Benth.	J.L. Clark 6292	DQ070507
<i>C. humidum</i> L.P. Kvist & L.E. Skog	J.L. Clark 7108	DQ070508
<i>C. nobile</i> C.V. Morton	J.L. Clark 7143	DQ070509
<i>C. reldioides</i> L.P. Kvist & L.E. Skog	J.L. Clark 7573	DQ070510
<i>C. veraguanum</i> Wiehler	J.L. Clark 8618	DQ070511
<i>Gasteranthus calcaratus</i> (Kunth)	J.L. Clark 5702	DQ070514
Wiehler subsp. <i>calceolus</i> (Fritsch)		
L.E. Skog & L.P. Kvist		

Table 1. Contd.

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<i>G. calcaratus</i> (Kunth) Wiehler subsp. <i>calceolus</i> (Fritsch) L.E. Skog & L.P. Kvist	J.L. Clark 5695	DQ070513
<i>G. calcaratus</i> (Kunth) Wiehler subsp. <i>oncogastrus</i> (Hanst.) L.E. Skog & L.P. Kvist	J.L. Clark 6207	DQ070516
<i>G. calcaratus</i> (Kunth) Wiehler subsp. <i>oncogastrus</i> (Hanst.) L.E. Skog & L.P. Kvist	J.L. Clark 6186	DQ070515
<i>G. carinatus</i> Wiehler	J.L. Clark 7502	DQ070517
<i>G. giganteus</i> M.Freiberg	J.L. Clark 7426	DQ070518
<i>G. glaber</i> L.E. Skog & L.P. Kvist	J.L. Clark 6130	DQ070519
<i>G. glaber</i> L.E. Skog & L.P. Kvist	J.L. Clark 8408	DQ070512
<i>G. lateralis</i> (C.V. Morton) Wiehler	J.L. Clark 4494	DQ070520
<i>G. leopardus</i> M. Freiberg	J.L. Clark 7403	DQ070521
<i>G. magentatus</i> M. Freiberg	J.L. Clark 6127	DQ070523
<i>G. magentatus</i> M. Freiberg	J.L. Clark 6120	DQ070522
<i>G. mutabilis</i> L.E. Skog & L.P. Kvist	J.L. Clark 6102	DQ070524
<i>G. pansamalanus</i> (J.D. Smith) Wiehler	J.L. Clark 6003	DQ070525
<i>G. pansamalanus</i> (J.D. Smith) Wiehler	J.L. Clark 6139	DQ070526
<i>G. quitensis</i> Benth.	Mendoza-T. et al. 525	AY047042
<i>G. tenellus</i> L.E. Skog & L.P. Kvist	J.L. Clark 5573	DQ070527
<i>G. villosus</i> L.E. Skog & L.P. Kvist	J.L. Clark 5590	DQ070528
<i>G. wendlandianus</i> (Hanst.) Wiehler	J.L. Clark 5882	DQ070529
<i>Napeanthus jelskii</i> Fritsch	Skog 7697	AY047044
<i>N. bracteatus</i> C.V. Morton	J.L. Clark 8744	DQ070531
<i>N. robustus</i> Fritsch	J.L. Clark 5651	DQ070532
<i>N. rupicola</i> Feuillet & L.E. Skog	H.D. Clarke 10304	DQ070533
<i>N. subacaulis</i> (Griseb.) Benth. & Hook.f. ex Kuntze	J.L. Clark 6898	DQ070534
<i>Reldia minutiflora</i> (L.E. Skog) L.P. Kvist & L.E. Skog	J.L. Clark 5685	DQ070535
<i>R. minutiflora</i> (L.E. Skog) L.P. Kvist & L.E. Skog	J.L. Clark 5868	DQ070536
<i>R. minutiflora</i> (L.E. Skog) L.P. Kvist & L.E. Skog	Hammel 16030	AY047043
<i>R. sp. nov.</i>	J.L. Clark 8835	DQ070537
<i>R. aff. veraguensis</i> Wiehler	J.L. Clark 8655	DQ070530

For some material it was necessary to amplify and sequence the ITS1 and ITS2 spacers as individual units. The ITS1 spacer was amplified with primers ITS5HP and ITS2 [30] and the ITS2 spacer with primers ITS4 and ITS3 [30]. Polymerase chain reaction (PCR) amplifications followed standard procedures described by Zimmer et al. [35] utilizing Taq DNA polymerase (Promega).

The PCR products were electrophoresed using a 1.0% agarose gel in 1X TBE (pH 8.3) buffer, stained with ethidium bromide to confirm a single product, and purified using the PEG precipitation procedure [10].

Direct cycle-sequencing of purified template DNAs followed the manufacturer's specifications, using the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems). Sequencing of the ITS region utilized the primers ITS5HP and ITS4 for the entire ITS region, or ITS5 and ITS2 for the ITS1 spacer and ITS4, and ITS3 for the ITS2 spacer, when these two regions were amplified separately. Sequencing was performed using an Applied Biosystems Model 377 Automated DNA Sequencing System or the ABI Prism 3730 Genetic Analyzer.

Automated DNA sequencing chromatograms were edited, and contigs were assembled using Sequencher 3.0 or 4.1 (Gene Codes Corporation, Inc.). The sequences were truncated to include only ITS1, 5.8S, and ITS2 regions. Identification of the ends of ITS1 and ITS2 was determined by comparisons with other Gesneriaceae sequences [35]. All sequences were manually aligned. Gaps had not been coded as separate characters in any of the following analyses due to the fact that most of them were single base indels and clearly uninformative. The exception to this is one 4 bp gap, which is found exclusively in *Cremosperma*. Newly acquired sequences were deposited in GenBank (accessions DQ070479 to DQ070537).

Maximum Parsimony Analysis

Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 [27]. The analysis used heuristic searches (ACCTRAN; 1,000 random addition cycles; TBR branch swapping; MULTREES option in effect). Clade robustness was estimated using 1,000 heuristic bootstrap replicates (100 random addition cycles with 100 trees saved per cycle, TBR branch swapping; MULTREES option in effect) [6, 9].

Maximum Likelihood Analysis

Maximum likelihood (ML) analysis was performed using PAUP* 4.0b10 [27]. Heuristic searches were employed (TBR branch swapping). Clade support was estimated using 100 heuristic bootstrap replicates (100 random addition cycles and 1,000 total rearrangements per

replicate, TBR branch swapping) [6, 9]. ML analyses employed the SYM model [34] with a gamma shape (G) parameter and equal base frequencies (six substitution types: A/C = 1.55351307, A/G = 2.81705422, A/T = 1.40104248, C/G = 0.52167553, C/T = 5.23640530, G/T = 1.00000000; G = 0.5563). This model was chosen based on the results of analysis using DT_ModSel [13]. The DT_ModSel analysis uses a Bayesian information criterion to select a model using branch-length error as a performance measure in a decision theory framework that also includes a penalty for model overfitting.

Results

Sequencing and Alignment Characteristics

The two or four ITS sequencing primers produced overlapping fragments that collectively covered the entire spacer and 5.8S nrDNA regions along both strands. The aligned ITS data matrix was 655 base pairs (bp) long with 297 variable sites (45.3%), of which 207 (31.6%) were parsimony informative. The length of the unaligned complete sequences varied from 615 to 633 bp. Seven sequences are missing a portion (9-105 aligned bp) of the 3' end of the ITS 2 spacer due to poor sequencing reads of these regions. Additionally, 11 sequences are missing a portion (14-57 bp) of the 5.8S gene due to incomplete overlap of separate sequencing of the ITS 1 and ITS 2 spacers. The alignment resulted in 44 gaps ranging from 1 to 4 bp in length. This data alignment resulted in uncorrected pairwise sequence divergence within the ingroup of 0-10.9%.

Phylogenetic Analyses

Maximum parsimony analysis of the ITS *Besleriaeae* data set resulted in 57,918 most-parsimonious trees (length = 581 steps, CI = 0.683, RI = 0.875, RC = 0.598). Figure 3 is the strict consensus of these trees. The maximum likelihood analysis resulted in a single tree (-lnL = 4120.89397; Fig. 4).

The MP strict consensus and ML tree both support the monophyly of all sampled genera of the *Besleriaeae*. The MP and ML topologies are

R. = *Reldia*; N. = *Napeanthus*). Numbers above branches are bootstrap percentages where branch support is equal to or greater than 50%. Numbers to the right of species names are collection numbers from Table 1 where more than one collection of the same name are included.

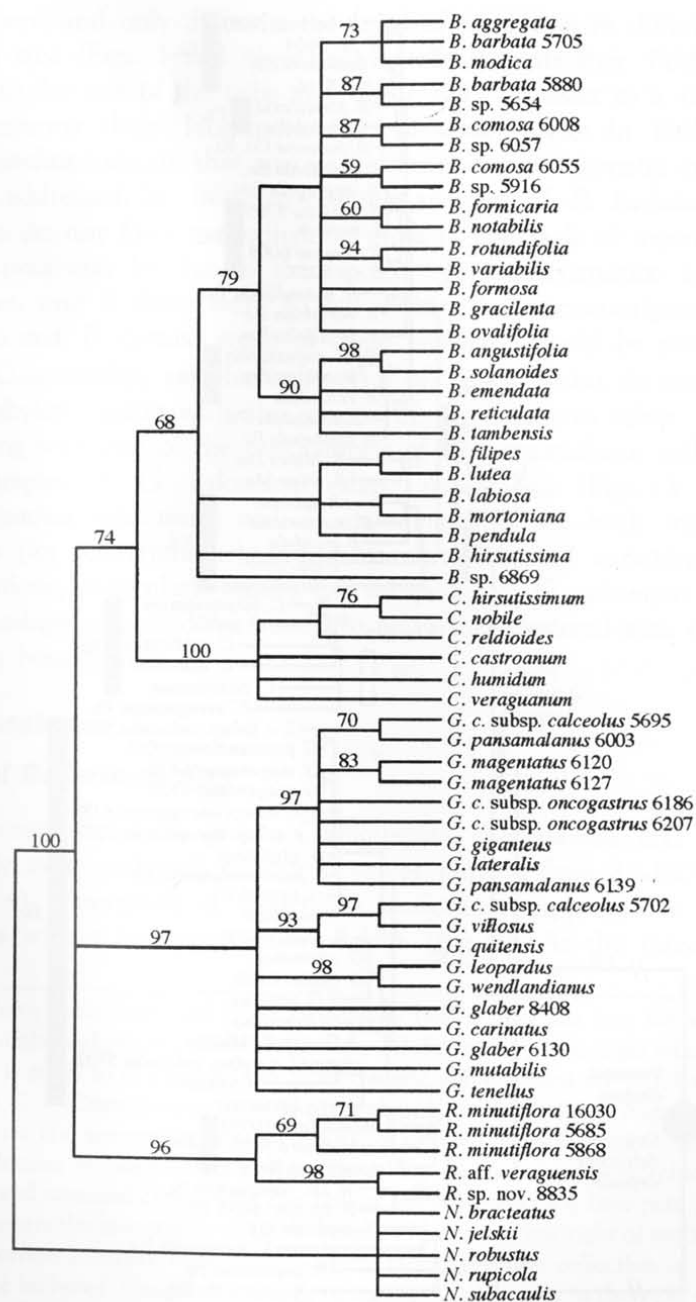


Fig. 3 Strict consensus of 57918 most-parsimonious trees (length = 581 steps; CI = 0.683, RI = 0.875, RC = 0.598) from parsimony analysis of nrDNA ITS sequence data of the tribe Beslerieae (B. = *Besleria*; C. = *Cremosperma*; G. = *Gasteranthus*;

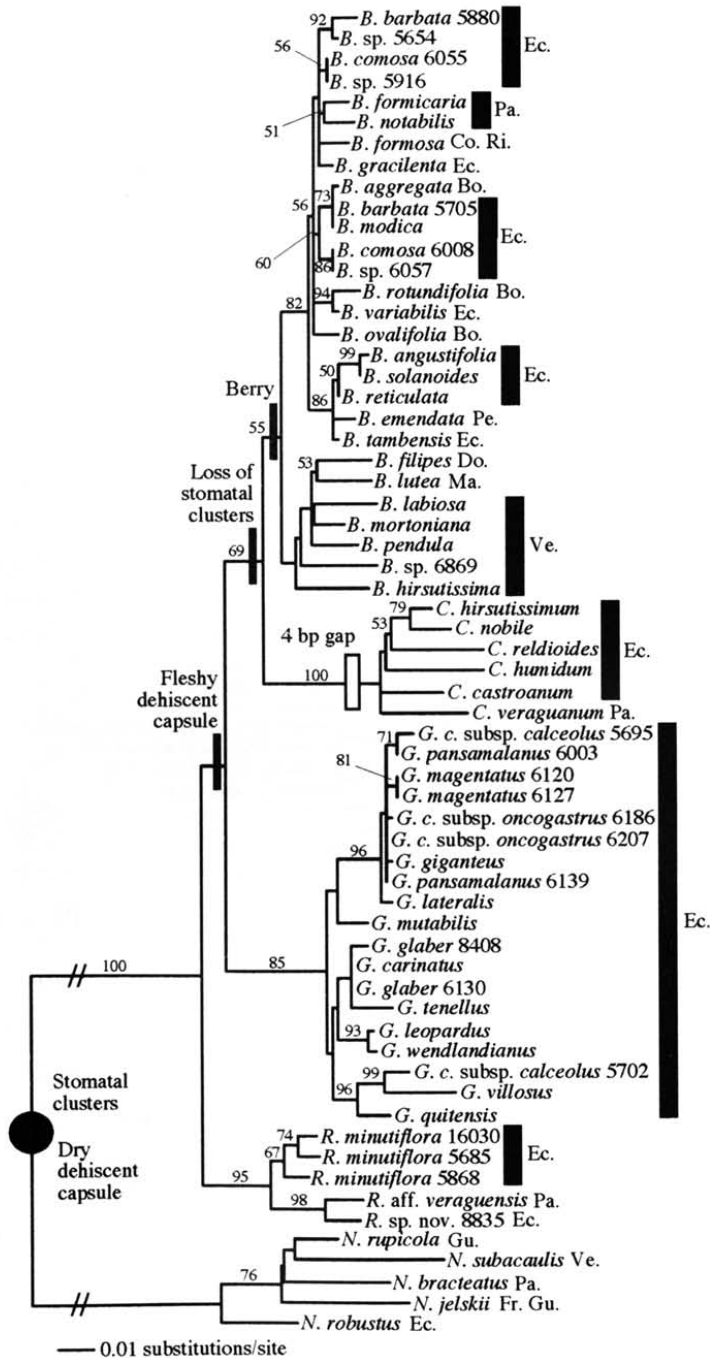


Fig. 4 The single maximum likelihood phylogram (-lnL = 4120.89397) based on nrDNA ITS region. Branch lengths are proportional except those denoted by “//”

congruent and only differ in the level of resolution in different parts of the tree (Figs. 3 and 4). The ML tree suggests that *Reldia* is the sister to the rest of the tribe and *Gasteranthus* is sister to a *Besleria* + *Cremosperma* clade. Non-monophyly of some species in *Besleria* and *Gasteranthus* indicate that species delineation is problematic and needs to be addressed. In *Besleria*, multiple samples of *B. barbata* and *B. comosa* do not form monophyletic groups. The lack of monophyly in these taxa may be due to the unclear species designation of several samples, and if these were to fall within the circumscription of *B. barbata* and *B. comosa* some of these problems would be resolved.

In *Gasteranthus*, sampled subspecies of *G. calcaratus* do not form a monophyletic group with the sample of *G. calcaratus* subsp. *calceolus* grouping with one of the two samples of *G. pansamalanus* rather than the samples of *G. calcaratus* subsp. *oncogastrus* (Figs. 3 and 4). *Gasteranthus calcaratus* and *G. pansamalanus* are both widespread species (for *Gasteranthus*) and show morphological variation among populations, particularly among the subspecies of *G. calcaratus* and the relationships suggested here need to be further explored with regard to species boundaries.

Discussion

Tribal Relationships

The monophyly of the genera *Cremosperma*, *Gasteranthus*, and *Reldia* is strongly supported with bootstrap values ranging from 85-100% (Figs. 3 and 4). Monophyly of *Besleria* is moderately supported in the MP analysis with a bootstrap value of 68% (Fig. 3). As the three genera

which have been shortened by approximately half so that the tree fits within the page. Numbers above or below branches are ML bootstrap percentages where branch support is equal to or greater than 50%. Generic abbreviations are as follows: *B.* = *Besleria*; *C.* = *Cremosperma*; *G.* = *Gasteranthus*; *R.* = *Reldia*; *N.* = *Napeanthus*. The filled circle and surrounding notation outlines inferred pleisiomorphic conditions for the Beslerieae + Napeantheae, while filled bars represent synapomorphic changes of fruit and stomatal characters. The hollow bar represents a 4 base pair (bp) indel that supports the monophyly of *Cremosperma*. Numbers to the right of species names are collection numbers from Table 1 where more than one collection of the same name are included. Geographic origins of specimens are noted to the right of species names and are abbreviated as follows: Bo. = Bolivia; Co. Ri. = Costa Rica; Do. = Dominica; Ec. = Ecuador; Fr. Gu. = French Guiana; Gu. = Guyana; Ma. = Martinique; Pa. = Panama; Pe. = Peru; and Ve. = Venezuela.

Anethanthus, *Resia*, and *Tylopsacas* have not been sampled here, the relationships of these small genera to the others cannot be addressed and will require further study. The monophyly of these genera (given current levels of sampling) supports Wiehler's [31] separation of *Gasteranthus* from *Besleria* and the MP and ML analyses suggest that *Cremosperma* is the sister lineage of *Besleria* rather than *Gasteranthus*, as has been previously suggested [25]. The suggestion pertaining to some of Smith's [25] analyses that *Reldia* is polyphyletic, is not supported here although sampling in this genus is still low.

The monophyly of *Besleria* and *Gasteranthus* contradicts previous classifications of these taxa [15] because of the emphasis on corolla morphology and the assessment of homology in pouched corollas. The intrageneric and intergeneric variation in corolla morphology in *Besleria* and *Gasteranthus* is remarkable and has been addressed in a morphological cladistic analysis [17] and the recent monographic revision of *Gasteranthus* [24]. Other groups of Gesneriaceae where generic circumscription was largely based on floral characteristics have proved to often be para- or polyphyletic [3, 14, 16, 19, 35]. In contrast to the non-monophyly of other genera in Episcieae [4] and Gloxinieae [19], *Besleria* and *Gasteranthus* have non-floral morphological characters that help define generic circumscriptions.

Infrageneric Classifications

Current infrageneric classifications for Beslerieae genera are poorly defined. *Gasteranthus* was recently revised by Skog and Kvist [24]. They did not formalize an intergeneric classification, but rather suggested three informal groupings. When these groupings are compared to the phylogeny, two are polyphyletic ("A" and "C") while the third ("B") is unresolved in the phylogeny with members of the other groups. Given the low level of resolution and sampling within *Gasteranthus*, it is unclear how large a problem this might be, but these informal groupings have also been suggested to be non-monophyletic, based on cladistic analyses of morphology [17].

The non-monophyly of some *Gasteranthus* species is most likely the result of broad circumscriptions by Skog and Kvist [24]. For example, *Gasteranthus calcaratus* and the three subspecies circumscribed by Skog and Kvist [24] need further evaluation. Two sympatric samples that keyed out to *Gasteranthus calcaratus* subsp. *calceolus* and used in this

analysis (J.L. Clark 5702 and J.L. Clark 5695) resulted in one taxon nesting in a basal clade of *Gasteranthus* and the other nesting in a crown clade of *Gasteranthus*. From field work conducted by the second author, it was noted on the herbarium labels that these two samples differed significantly in vegetative characters. One collection had a succulent and prostrate stem, and the other collection had an erect and woody stem. The field-based observation of succulence and presence of prostrate stems would be difficult to note on pressed specimens. Other non-monophyletic species in *Gasteranthus* can be attributed to the synonymy and confusing last-minute species publications by Martin Freiberg while Skog and Kvist were finishing a monographic revision of the genus. For example, Freiberg's limited type collections of *Gasteranthus magentatus* posed a major challenge in incorporating this name in the monograph of the genus. Thus, it was included as a synonym of *Gasteranthus pansamalanus*, but is clearly a separate species. The lack of citation and synonymy in Skog & Kvist [24] of other validly published Freiberg species (e.g. the three new species from Ecuador [7]) is because the monograph of *Gasteranthus* and Freiberg's new species descriptions were in press at the same time. Nevertheless, Freiberg's most recent descriptive taxonomy paper on *Gasteranthus* was published on August 18, 2000 and the monograph of *Gasteranthus* [24] appeared on July 17, 2000. Despite the priority of the names in Skog and Kvist's monograph of *Gasteranthus* [24] over Freiberg's names [7], reconciling the delineation of some *Gasteranthus* species is confusing and has complicated the taxonomy of this genus.

Morton [15] published a classification of *Besleria* recognizing four sections and 18 subsections, although he included *Gasteranthus* within his circumscription of *Besleria*. Where we have sampled multiple species within one of Morton's classification units, they do not form monophyletic groups. Morton [15] primarily used characteristics of the corolla and calyx for defining sections and subsections, and this has proved to be a difficult set of characters to use in a number of groups in the Gesneriaceae [4, 18, 19]. To underline the problems with these characters, Morton included within section *Neobesleria* Morton, both *B. pendula* and a number of species currently considered to be members of *Gasteranthus*, including *G. wendlandianus*. The presence of *B. pendula* and *G. wendlandianus* in Morton's section *Neobesleria* is an example of the homoplasy in corolla morphology that Morton used to support his artificial sections. The presence of a posterior spur in these unrelated

species is a poorly defined trait which Morton emphasized in his sectional classification. Wiehler's extraction of *Gasteranthus* from *Besleria* [31] emphasized fruit characters and vegetative features instead of corolla morphology. Based on the results presented here, Wiehler's classification changes are well-substantiated and the berries in *Besleria* are a well-supported synapomorphy that helps define this genus as monophyletic (Fig. 4).

Morphological Characteristics and Biogeography

Morphology is quite variable in the Beslerieae, particularly floral form, and especially within the genera *Besleria* and *Gasteranthus*. The plasticity in corolla formed within *Gasteranthus* is shown by the pouched corolla with an apical constriction in *G. pansamalanus* (Fig. 1B) and campanulate to funnellform corolla in *G. tenellus* (Fig. 1A).

Additional molecular markers and sampling will be necessary to fully explore the evolution of floral forms within the large genera *Besleria* and *Gasteranthus*. Two other characters have been of particular interest in the past [25]: fruit type, and presence of stomatal clusters. In the Beslerieae plus Napeantheae clade [35] three fruit types can be found, including dry dehiscent capsules (e.g. *Anetanthus*, fruit not shown), fleshy dehiscent capsules (e.g. *Gasteranthus*, Fig. 1C), and berries (e.g. *Besleria*, Fig. 1D) [28]. Previous phylogenetic studies [25] have suggested that dry dehiscent capsules are paraphyletic with regard to fleshy dehiscent capsules and berries, and that fleshy dehiscent capsules are paraphyletic with regard to berries. These patterns are supported here and lend additional support to the hypothesis that the gain of fruit fleshiness originated prior to the loss of regular dehiscence in this lineage (Fig. 4). Most *Besleria* species have indehiscent fleshy berries. Although not mentioned in the literature, the second author has observed some instances where the berries of *Besleria* rupture, thereby the fruit wall dehisces irregularly or unevenly (Fig. 1D). Rupturing berries have also been observed in *Solenophora* (J.L. Clark, pers. obs.), which was recently monographed by Weigend and Förther [29], where the fruits were described as capsules.

The presence of stomatal clusters has been used as an important character for separating *Gasteranthus* from *Besleria* [31]. Within Gesneriaceae, stomatal clusters are also found in all species of *Napeanthus* (Fig. 4), many species of *Gesneria* and a few species of

Rhytidophyllum [22, 31]. The distribution of stomatal clusters in the Beslerieae and Napeantheae was interpreted as having independent origins by Smith [25] with the exception of one analysis using a likelihood model. Smith [25] proposed that the likelihood of independently derived stomatal clusters was plausible because of the tendency of these groups to inhabit humid shaded environments along river banks or moss-covered cliffs. Smith further elaborated Wiehler's interpretation [33] that the islands of stomata served as a means of maximizing transpiration because they are raised above the leaf epidermal level. In contrast to Smith [25], our results support a single origin of stomatal clusters in Napeantheae and Beslerieae and a loss of stomatal clusters for *Besleria* + *Cremosperma* (Fig. 4). Thus, only a single loss of stomatal clusters is necessary to map this character when it is considered the plesiomorphic condition of the Beslerieae + Napeantheae (Fig. 4) when the distribution of stomatal clusters described by Wiehler [31, 32], Skog [23], and Smith [25] is followed. It should be noted, however, that the presence of stomatal clusters in *Reldia* has been questioned [12] and the distribution of this character in the tribe needs to be further explored.

Given the phylogenetic hypotheses presented here, morphological synapomorphies are present for each of the branches separating the genera of the Beslerieae (Fig. 4). How the other Beslerieae genera will fit into this phylogeny is unclear. Previous phylogenies did not provide consistent placement of the genera [25], and if the genera *Anetanthus*, *Resia*, and *Tylopsacas* are members of the tribe, morphological characters do not clearly support their placement. Particularly, none of these genera have been described to have stomatal clusters, potentially supporting a placement with *Besleria* and *Cremosperma*, but are described as having dry dehiscent fruits, suggesting a placement with *Reldia*. As these genera were found to be in the general vicinity of *Reldia* in previous studies [25], we might expect that there have been multiple losses of stomatal clusters in the tribe.

Given current sampling, only a few comments on patterns of biogeographic patterns can be presented. Within genera, there is no clear grouping of species from particular geographic areas, at least where multiple geographic areas have been sampled (Fig. 4). A majority of the samples of the Beslerieae tribe have so far come from Ecuador and this limits our ability to make some of these comparisons. One interesting pattern, though, is the monophyly of *Besleria* species

sampled from Caribbean islands (Dominica and Martinique) and their phylogenetic association with species from Venezuela (Fig. 4). This would suggest that there could possibly have been a single introduction to the Caribbean from northern South America. Additionally, several clades of *Besleria* contain species from both Ecuador and Bolivia. This suggests that some species groups might be widely spread rather than restricted to smaller geographic areas and emphasizes the need to sample more *Besleria* species from Peru.

CONCLUSION

Here we have provided a set of phylogenetic hypotheses of relationships and diversification within the Beslerieae based on nrNDA ITS sequences. It appears that with increased sampling, the genera of the tribe are monophyletic and that some of the morphological characteristics of fruits and stomatal distribution may help in defining the characteristics of clades. Additionally, issues of species circumscription are apparent in both *Besleria* and *Gastaranthus* and need to be further explored. Previous phylogenetic relationships among genera of the tribe are not supported, and many of the previously recognized intrageneric classification units or informal groupings appear to be para- or polyphyletic. While the tribe is widespread in Central and South America, few species are present in the Caribbean and preliminary sampling suggests that this might be a monophyletic lineage derived from northeastern South America. While revisions of *Cremosperma* for Ecuador [11], *Reldia* [12], and *Gastaranthus* [24] were recently completed, species circumscriptions for some groups need to be further evaluated. The largest and most challenging taxonomic task for Neotropical gesneriads will be a revision of *Besleria* (not revised since Morton [15]).

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