PHYLOGENETIC PLACEMENT OF \textit{RESIA} AND \textit{CREMOSPERMOPSIS} (GESNERIACEAE)

by

JASON MICHAEL MARTIN

JOHN L. CLARK, COMMITTEE CHAIR

PHILLIP M. HARRIS
MARTHA POWELL
MICHAEL MÖLLER

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ABSTRACT

*Resia* and *Cremospermopsis* are two small genera that are known from less than 50 collections. Recent fieldwork in Ecuador has resulted in the discovery of *Resia* outside of Colombia, which greatly expands its geographic distribution. The geographic range of *Cremospermopsis* was also recently expanded by the discovery of a population in northern Peru. A phylogenetic analysis was generated from nuclear (ITS & ETS) and chloroplast (*matKR* & *trnL-F*) markers. The present study includes the most comprehensive taxon sampling for the tribe Beslerieae. Results from this research strongly support that *Cremospermopsis* is the sister group to *Cremosperma* and nests in the subtribe Besleriinae. *Resia* is weakly supported in a clade with *Anetanthus, Shuaria*, and *Tylopsacas*, in the subtribe Anetanthinae.
DEDICATION

This thesis is dedicated to everyone who helped me and guided me through the trials and tribulations of creating this manuscript. In particular, my parents, Ed and Susan Martin, who continually encouraged me to better myself in all aspects of life.
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1: INTRODUCTION

The tribe Beslerieae includes nine genera and is divided into the subtribes Anetanthinae and Besleriinae (Weber et al. 2013). Previous classifications included the Beslerieae in the primarily Old World subfamily Cyrtandroideae on the basis of a superior ovary (Hanstein 1865, Bentham & Hooker 1883). The New World members were later transferred by Burtt (1962) when he separated the Old World genera (Cyrtandroideae, now Didymocarpoideae; Weber et al. 2103) from the New World genera (Gesnerioideae) based on the unequal size of the seedling cotyledons in the Old World genera compared to the equal sized cotyledons of the New World genera.

Ivanina (1965) published a classification based on carpels and transferred all Beslerieae into the tribe Episcieae on the basis of similarity of carpological characters such as fruit types, corolla shapes, and seed size. In this classification, the Episcieae tribe was distinguished from the rest of the family by having relatively small (0.2-0.7mm long) round or elliptic seeds and a spherical berry-like capsule that is 1.5-2 mm in diameter.

Wiehler (1983) re-circumscribed the Beslerieae tribe and recognized the following genera: Besleria L., Cremosperma Benth., Gasteranthus Benth., Reldia Wiehler, Resia H. E. Moore, Tylopsacas Leeuwenb., and Anetanthus (Hiern ex) Benth. All of these genera were previously classified in the Episcieae tribe because they lacked bracts and had non-fleshy berries (Wiehler 1983). More recent classifications based on molecular phylogenetic data have confirmed Wiehler’s classification (1983) and most of the genera are strongly supported as
monophyletic (Roalson & Clark 2005, Clark et. al. 2010, Perret et. al. 2013) (Figure 1). Of the nine genera _Cremospermopsis_ L.E.Skog & L.P.Kvist is the only member in the Beslerieae that has not been phylogenetically evaluated using molecular sequence data. The only other genus in the New World Gesneriaceae that has not been evaluated using molecular data is _Lampadaria_ Feuillet & L. E. Skog.

Smith (2000) extracted and included _Resia nimbicola_ H.E. Moore in a molecular phylogenetic analysis based on the chloroplast marker _ndhF_. The tissue samples were obtained from a herbarium collection from 1973 (_E. Forrero 803_). The phylogenetic position of _Resia_ was inconsistent in Smith (2000) and it nested in two different clades (Figure 1) that were presented for two different methodologies (parsimony and Maximum Likelihood). Support values were low for both results presented in Smith (2000). In addition, the sample was from a DNA sample extracted from a herbarium collection that was more than 25 years old and it is likely that the integrity of the DNA was marginal.

The most recent classification of the Gesneriaceae divides the tribe Beslerieae into the two subtribes Besleriinae and Anetanthinae (Weber et al. 2013). The tribe contains a broad range of morphological characters (vegetative structures, floral characters, fruit structure) without a single morphological synapomorphy. Besleriinae include the genera _Besleria, Cremosperma, Gasteranthus_, and _Reldia_ and are recognized by seed surfaces that are reticulated or striated. The subtribe Anetanthinae is shares a unique seed morphology that readily differentiates it from other Gesneriaceae. The subtribe Anetanthinae contains _Anetanthus, Tylopsacas, Shuaria_ D.A. Neill & J.L. Clark, _Resia, and Cremospermopsis_ and are recognized by seed surfaces that are papillated or pustulated due to the bulging cell walls. Skog and Kvist (2002) suggested that _Cremospermopsis_ was a member of the Anetanthinae because of the presence of papillate
seed surface, despite some morphological similarity to *Cremosperma* (adnate filaments to the corolla tube base and connate calyx lobes).

Additionally, Clark et al. (2010) showed moderate support separating the two subtribes, including the new genus of *Shuaria* within Anetanthinae, using nuclear (ITS) and plastid (*trnL-F*) markers. The relationship of genera within the subtribes had Bootstrap Support values of 82% in the Anetanthinae and 68% within the Besleriinae (Clark et al., 2010) (Figure 2). *Resia* and *Cremospermopsis* were not included in Clark et al. (2010) because tissue samples were not yet available. Thus, the classification of Weber et al. (2013) included *Resia* and *Cremospermopsis* in the Beslerieae tribe based on their interpretation of morphological data.

![Figure 1. Representations of contrasting phylogenetic hypotheses for generic relationships in the Beslerieae tribe presented by Smith (2000) based on molecular sequences. A is based on a single most-parsimonious tree. B is based on a maximum likelihood estimate. Reproduced from Roalson and Clark (2005).](image)
*Resia* was only known from 23 collections dating from 1930 to 1993. During the last decade there has been a surge in *Resia* collections by Colombian biologists. As a result, there are now more images and information available from recent collections than from the previous five decades. *Resia* is named after the botanist, Richard Evans Schultes, who made significant scientific contributions to the field of ethnobotany while he was a professor at Harvard. *Resia* are shrubs with fibrous roots that grow on damp sandstone cliffs or shaded stream sides (Moore, 1962). Flower color is conserved intraspecifically, but differs between species. For example, corollas are consistently orange in *Resia nimbicola* Moore. Corollas are uniformly white in *R. bracteata* J.L. Clark & L.E. Skog and *R. ichthyodoides* Leeuwenb. The flowers of *R. umbratica* Fern. Alonso are uniformly yellow (Figure 3). It is important to note that *Resia bracteata* is the only species that contains floral bracts whereas all other species are ebracteate. Wiegler’s circumscription of the Beslerieae (Wiegler 1983) was based on the absence of bracts. Thus, the presence of bracts in *R. bracteata* is unusual and represent the only species in the tribe with bracts.
Figure 2. A comprehensive phylogeny of the Beslerieae tribe sister to the Napeantheae tribe based on both chloroplast and nuclear genetic markers. Bootstrap percentages placed above the branches; Bremer Decay Values shown below branches. Reproduced from Clark et. al. 2010.
Figure 3. Resia nimbicola (A-C) (J.L. Clark et al. 12918) and Resia umbratica (D-F). (J.L. Clark et al. 13000)
Resia nimbicola is endemic to Colombia where it grows in damp shaded areas on cliff faces. Many of the herbarium labels noted that there are abundant populations near waterfalls. Collections are from 1300 to 2300m and one collection was from 400m (J. Cuatrecasas 8849). R. ichthyoides is endemic to Venezuela, where it grows on mica-schist shaded ledges between 1200 and 1350 meters. Resia bracteata is mostly in Colombia (Figure 4) and grows from 630 to 1950 m. A disjunct population of Resia bracteata was collected by the author along a stream in sandstone in south-central Ecuador (J.L. Clark & J. Martin 13689). R. bracteata was originally described as a subspecies of R. ichthyoides (R. ichthyoides subsp. bracteata L.E.Skog & de Jesus). However, R. bracteata was recently classified as a distinct species (Clark et al. 2012) because of the presence of bracts in R. bracteata (Figure 5).

Resia umbratica is endemic to the departments of Antioquia and Tolima in Colombia. It is similar in appearance to R. nimbicola because of its serrated leaf margins and flower and fruit morphology (Figure 3). It differs by elongate capsules, wider leaf margins with a less serrated edge, a wider inflorescence with fewer flowers, and relatively large sepals and corollas.
Figure 4. Distribution of *Resia* in Colombia, Venezuela and Ecuador; *R. bracteata* (triangles); *R. ichthyoides* (squares); *R. nimbicola* (circles) and *R. umbratica* (diamonds).
Figure 5. *Resia bracteata*. A. Lateral view of flower of *R. bracteata* (ca., 1cm). B. Top view of inflorescence of *R. bracteata*. C. Front view of flower. D. Bottom view of inflorescence.
of *R. bracteata* with subtending bracteoles. E. Lithophytic habit of *R. bracteata* near streamside. (J.L. Clark et al. 13689)

Skog and Kvist (2002) discovered *Cremospermopsis* because most of the material was in the herbarium as Rubiaceae and Acanthaceae. They compared the material to the morphologically similar *Cremosperma* and recognized a few unidentified species of *Cremosperma* as *Cremospermopsis*. Diagnostic characters that differentiate *Cremospermopsis* from other members of the tribe Beslerieae tribe are the following: spherical papillate seeds (vs. striate seeds in other members of Beslerieae and Napeantheae tribes); zygomorphic calyces (vs. actinomorphic calyces in the Napeantheae); and corolla throat with gland-tipped trichomes (vs. non-glandular trichomes or glabrous in all other members of the Beslerieae tribe). *Cremospermopsis* also contains inflorescence bracts, which further distinguishes it from *Cremosperma* and potentially complicates its placement in the Beslerieae tribe based on Wiehler’s 1983 definition (Skog and Kvist 2002).

*Cremospermopsis parviflora* and *C. cestroides* are readily differentiated by flower color and corolla shape. The corollas of *C. parviflora* have an elongate tube with the dorsal pink lobes pink or purple and white or yellow ventral corolla lobes similar to the flower coloration of some *Cremosperma* (e.g., *C. humidum*) (Figure 6). The corollas of *C. cestroides* are smaller and uniformly white or yellow (Figure 7). A few other morphological features similar to *Cremosperma* are adnate filaments to the corolla tube base and connate calyx lobes. However, the primary distinguishing features of *Cremospermopsis* from *Cremosperma* are the presence of bracts, unequal calyx lobes, and papillate seeds, whereas *Cremospemra* lacks bracts, has equal calyx lobes, and striated seeds (Skog and Kvist 2002).
Figure 6. *Cremospermopsis parviflora*. A. Lateral view of corolla. B. Front view of flower. C. Lateral view of inflorescence showing paired bracts. D. Upper view of mature fruits. E. Habit (A, D & E from J.L. Clark et al. 12898; B & C from J.L. Clark et al. 12912).
Figure 7. *Cremospermopsis cestroides*. A. Lateral view of flower. B. Front view of flower. C. Lateral view of inflorescence showing paired bracts. D. Annular nectary. E. Habit (from J.L. Clark et al. 12900).
*Cremospermopsis* had only been collected in the Department of Antioquia (Colombia) until a recent population of *C. parviflora* was collected in the lowlands of northern Peru (I. Salinas, Pers. Comm.). No populations of *Cremospermopsis* are known between Peru and Colombia and the recent range extension into Peru was unexpected.

The current classification of *Cremospermopsis* as belonging to the Beslerieae (Weber et al. 2013) is based entirely on morphological data. Skog and Kvist did not assign *Cremospermopsis* to a tribe at the time of describing the genus because it has bracts and the absence of bracts in the Beslerieae was considered by Wiehler (1983) an important diagnostic feature of the tribe. Weber (2004) provided an updated classification of all tribes recognized in the Gesneriaceae. *Cremospermopsis* was listed in Weber’s classification (2004) as one of six genera with uncertain tribal affiliation. Skog and Boggan (2006) listed *Cremospermopsis* as a member of the Napeantheae tribe where it remained until Weber et al. (2013) suggested that *Cremospermopsis* belonged in the newley described subtribe Anetanthinae based on its papillate seed surface (vs. reticulated or striated seed surface in the subtribe Besleriinae). However, Weber et al. (2013) noted the lack of molecular data was necessary for evaluating the phylogenetic placement of *Cremospermopsis* in the Anetanthinae subtribe. This study is the first to evaluate the placement of *Cremospermopsis* based on molecular sequence data.

Moore (1962) published *Resia* as a new genus based on the absence of bracts, nearly free calyx lobes, and a cylindrically elongated corolla tube. Moore also commented on the similarities between *Resia, Anetanthus*, and *Cremosperma*, but noted the differences within the rhizomes and capsules of each of the species. However, Moore (1962) proposed that *Resia* might be more closely related to *Napeanthus* Gardner in the Napeantheae than the Beslerieae due to several shared characters, such as habitat, leaf shape, anthers with confluent cells, thickened and

Smith (2000) performed a molecular phylogenetic analysis of the tribes Beslerieae and Nepeantheae. His results suggested that *Resia* belongs in the Beslerieae tribe with *Anetanthus* and *Reldia* (Figure 1). The samples of *Resia*, *Reldia*, and *Anetanthus* used for Smith’s study were from herbarium samples. The results of Smith 2000 were inconclusive in regards to the phylogenetic placement of *Resia*, *Reldia*, *Anetanthus*, and *Cremosperma*.

The goal of this study is to generate a phylogenetic hypothesis to evaluate the placement of *Resia* and *Cremospermopsis* and produce a comprehensive phylogeny of the Beslerieae tribe using both chloroplast and nuclear DNA markers. A revised phylogeny for *Resia* was generated using molecular data. Understanding the phylogenetic placements of *Resia* and *Cremospermopsis* are essential for resolving higher classification of the tribes and will help address the evolution of floral bracts.
2: MATERIALS AND METHODS

Taxon Sampling — Sixty-five species are included in the analyses representing 3 of 4 species in *Resia*. The only unsampled *Resia* species is *R. ichthyoides*. All samples in the current study were collected in the field with leaf samples dried in silica gel. Samples of *R. bracteata* were collected from southeastern Ecuador a few kilometers from the small town of El Pescado.

Previous studies support a monophyletic Beslerieae and Napeantheae (Clark et al. 2010), thus taxon sampling included members of these two tribes. Specimens from the tribes Gesnerieae, Episcieae, Sinningieae, and Napeantheae were chosen as outgroups for the Beslerieae tribe. Additionally, *Peltanthera floribunda* and *Sanango racemosum* were chosen as outgroups based on previous phylogenies (Perrett et al. 2013). These two taxa have been supported as closely related to the Gesneriaceae family (Smith et al. 1997, Clark et al. 2010) with some analysis showing *Peltanthera* as sister to Gesneriaceae and Calceolariaceae based on four mitochondrial genes (Qiu et al. 2010) and 17 multigenome regions (Soltis et al. 2011). The phylogenetic analyses are rooted with *Peltanthera* based on Wang et al. (2004), which supports the sister-group relationship of *Peltanthera* to the rest of Gesneriaceae.

DNA Extraction, Amplification and Sequencing - Leaf samples were ground using a ThermSavant FastPrep FP120 cell disrupter (Qbiogene, Carlsbad, California). All samples were extracted using the OMEGA bio-tek E.Z.N.A. HP Plant DNA extraction kit. Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5HP (Suh et al. 1993) and ITS4 (White et al. 1990). The other nrDNA analyzed was ETS forward primer
ETS-B (Beardsley and Olmstead 2002) and reverse primer 18S-ETS (Baldwin and Markos 1998). The cpDNA trnL intron and trnL-trnF intergenic spacer were amplified using the primers trnLc and trnLf (Taberlet et al. 1991).

Polymerase Chain Reaction (PCR) amplification was followed based from the descriptions in Clark et al. (2010) using TAQ DNA polymerase (Promega). To reduce within-strand base pairing that can result in interference with Taq polymerase activity, we found it essential to use 5% DMSO and 5% BSA in PCR reactions for ITS. The PCR products were electrophoresed using a 1.0% agarose gel in 1 × TBE (pH 8.3) buffer, stained with ethidium bromide to confirm a single product, and purified using PEG 8000 (polyethylene glycol) in 2.5 M NaCl under the conditions described in Johnson and Soltis (1995). Purified samples for each marker were sent to the Nevada Genomics Center for DNA direct cycle sequencing. Apart from the PCR primers, the reverse and forward of the internal primers ITS2 and ITS3 (White et al. 1990) were used in cycle sequencing to obtain independent and overlapping sequence reads for the entire ITS region. DNA electropherograms were edited and contigs were assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan).

Alignment and Phylogenetic Analyses — All sequences were initially aligned using ClustalW (Larkin et al. 2007) program within Geneious v5. All samples in the analyses are represented with four markers except for Cremospermopsis cestroides, which is based on ETS only due to low DNA yield of extracted materials. Gaps in the sequences were treated as missing data and characters were weighted equally. Because the sequences were not highly divergent, it was possible to make minor adjustments so that overlapping gaps were minimized.

The parsimony analysis was performed to completion using a two stage heuristic search in PAUP* 4.0b10 (Swofford 2002). The first stage of the analysis was done using the following
settings: 10,000 random additions, holding 100 trees at each step; tree bisection-reconstruction (TBR) branch swapping with no more than 100 trees saved for each rep. The second stage of the analysis was performed on all most parsimonious trees in memory with the same settings, but with the MULTREES option in effect. Other searches were conducted, but did not find shorter trees using the settings above with the following changes: 100 random addition cycles limited to 10,000 trees of equal length for each of the replicates; 1,000 random addition cycles limited to 100 trees of equal length for each of the replicates. A strict consensus tree was generated from a filtered list of the best trees to produce the equally most-parsimonious trees.

A bootstrap analyses (Felsenstein 1985) was performed in PAUP * to evaluate clade robustness. The bootstrap analysis was based on 10,000 replicates with the full heuristic search option in effect with the following settings for each search: 10 random addition cycles, holding 10 trees at each step; tree bisection-reconstruction (TBR) branch swapping, saving a maximum of 100 trees for 1,000 replicates. The parsimony analyses and clade support were evaluated for the individual ITS, ETS, matK-R, and trnL-F regions (data not shown). Conflict between datasets was evaluated by comparing incongruence of strongly supported clades from individual datasets. The data also were analyzed with a combined dataset of all four markers, which included 65 taxa and 2505 nuclear characters.

Bayesian Inference (BI) analysis and jModelTest 0.1.1 (Posada 2008) were used to search for the models of best fit for the individual markers and the combined dataset of all four markers. All markers used the same 6-substitution-rate model with variable sites based from the results of jModelTest 0.1.1. The Bayesian analysis was run using MrBayes 3.2.5, consisting of four Markov Chain Monte Carlo (MCMC) chains running for 2,000,000 generations with .25 of trees as the burn-in period, sampling every 1,000th generation. Posterior probability values were
generated for the top 50% majority-rule tree of for all trees, excluding those trees from the “burn-in period.”

Maximum Likelihood was performed through the CIPRES Science Gateway supercomputer version 3.1, using RAxML (v. 7.2.7). A general time reversal (GTR) model was set for each run, including runs with separate markers and combined markers. Bootstrap replication was automatically performed within the RAxML analysis, using 1,000 iterations to add the values to the maximum likelihood tree. Bootstrap values are shown using a 50% majority-rule. The morphological character state of the presence of bracts was used through ML character tracing of the ML consensus tree in MESQUITE v. 2.75 (Madison and Madison 2011) to examine the ancestral character states of bracts.
3: RESULTS

*DNA Sequencing and Alignment* – The two nuclear DNA markers (ETS and ITS) had a total length of 550 bps and 560 bps, respectively. The two chloroplast DNA markers (*matKR* and *trnLF*) had total length of 538 bps and 854 bps. Missing characters were due to difficulties in PCR amplification. *Gasteranthus carinitus Ex. 1 & 2* and *Reldia sp. nov. Ex. 1 & 2* represent two extractions of a different plant within the same population. Other samples of the same species are from different populations and are marked with collection numbers. The results of this analysis provide a well-supported phylogenetic estimate of all genera in the Beslerieae tribe.

*Maximum Parsimony Analysis* – The parsimony analysis of the combined dataset resulted in one most-parsimonious tree (length = 2,549 steps, consistency index [CI] = 0.61, retention index [RI] = 0.71, rescaled consistency index [RC] = 0.43). Figure 8 shows the most-parsimonious tree. The phylogeny including all four markers supports *Resia* as monophyletic (BS=97%). *R. nimbicola* and *R. umbratica* are sister taxa and are strongly supported (BS=100%). *R. bracteata* is also strongly supported as the sister taxon to both *R. nimbicola* and *R. umbratica* (BS=97%). Support was low (BS-65%) for the genus *Resia* as sister to the Beslerieae tribe. In the phylogenies of individual markers, *Resia* was placed sister to *Shuaria* for the nuclear dataset. However, in the phylogenies of all four combined markers, *Resia* was placed sister to the Beslerieae tribe.

*Cremospermopsis* is weakly supported as monophyletic (BS=52%). *Cremospermopsis* is the sister clade to *Cremosperma* (BS=71%). The individual marker analyses were congruent with the total evidence analysis. With *Resia* placed as sister to the Beslerieae tribe, the monophyly of
the rest of Beslerieae is strongly supported (BS=96%). The subtribe of Anetanthinae is strongly supported as monophyletic with a (BS=92%).

**Bayesian Analysis** – In the Bayesian analysis presented here the four datasets were partitioned, which allowed each marker to be modeled under different parameter values. The first 25% of trees were excluded as burn-in and the analysis achieved stationarity after 3,002 generations. The Beslerieae tribe is strongly supported as monophyletic (PP=1) (Figure 9). The phylogenies of the individual markers differed in the placement of Resia. ITS resulted in the placement of Resia in the Anetanthinae. Markers ETS, trnL-F, and matK-R resulted in the placement of Resia as sister to the rest of Beslerieae, which is the same result as the MP tree. The total evidence analysis resulted in the placement of Resia in the Anetanthinae (PP=.55). The branch supporting Cremosperma and Cremospermopsis had a posterior probability of 1.

**Maximum Likelihood Analysis** - The Maximum Likelihood analysis resulted in a single tree that was congruent with the BI tree that placed Resia within the Anetanthinae subtribe and a bootstrap value of 57%. Cremospermopsis came out monophyletic within the Cremosperma clade of the Besleriinae subtribe with a BS value of 70%. The Cremosperma clade had a 91% BS value, including the genus Cremospermopsis (Figure 10).
Figure 8. Maximum Parsimony. Strict Consensus Tree Based on the Total Evidence Analysis from all Four Markers for Maximum Parsimony. Bootstrap values are above branches.
Figure 9. Bayesian Consensus Inference Analysis Based on the Combined Four Markers from the Dataset. Posterior Probability Values are represented above the branches.
Figure 10. Maximum Likelihood Analysis from all Four Markers for Maximum Likelihood. Bootstrap percentages placed above branches.
Comparisons to Previous Phylogenies – This study is mostly congruent with previously published phylogenies (Roalson & Clark 2005, Clark et al. 2010). Weber et al. (2013) placed *Cremospermopsis* in the subtribe Anetanthinae because of the presence of papillate seed surface. However, Weber et al. (2013) recognized the limitations of classifying *Cremospermopsis* using only morphology and suggested that molecular data be used for a true phylogenetic placement. Thus, despite *Cremospermopsis* sharing a defining morphological character with the Anetanthinae subtribe, our molecular results strongly support its placement in the Besleriineae as sister to *Cremosperma*.

The molecular results of *Resia* are congruent with previous projections based on morphological features by Weber et al. (2013), who recognized the subtribe Anetanthinae based on seed coat morphology. Prior molecular phylogenies, such as Roalson and Clark (2006), utilized one marker (nrDNA ITS) while Clark et al. (2010) utilized two markers (nrDNA ITS and cpDNA trnL-F) in their analysis of the Beslerieae tribe. Clark et al. (2010) resulted in moderate support (BS = 82%) for the Anetanthinae subtribe. It should be noted that the current study had lower support values (BI=55 and ML = 57), despite additional markers. The lack of support for the Anetanthinae subtribe in the present study is likely the result of the inconsistent placement of *Resia* either within the Anetanthinae tribe or sister to the Beslerieae tribe.

Placement of *Resia* – *Resia* was consistently recovered as a monophyletic group in all analyses. Its phylogenetic placement based on the MP analysis is weakly supported as the sister clade to the Beslerieae tribe (65 BS) (Figure 8). However, the BI and ML phylogenies weakly
Phylogenies based on nuclear markers (ITS & ETS), *Resia* was recovered residing within the Anetanthinae subtribe with higher support (PP=0.77) than when combined with all four markers. The nrDNA trees were not included, since they were congruent with the BI and ML trees except for their support values.

Weber et al. (2013) classified *Resia* in the Anetanthinae subtribe based on morphological features outlined in Clark et al. (2010), such as equal calyx lobes, striated seed surface, and having a capsule with parietal placentation. *Resia* has apomorphies that differentiate it from other members of the Beslerieae such as the presence of alternate leaves (in contrast to opposite), the presence of bracts, and capsules that are likely splash-cup seed dispersed. Another genus that was initially difficult to place is *Shuaria*. Molecular sequence data strongly support its placement with Anetanthinae, which is not intuitive because *Shuaria* has an arborescent habit, presence of both opposite and alternate leaves, and lepidote trichomes and none of these characters are shared with current members of the Anetanthinae. However, Clark et al. (2010) had strong molecular support (99 BS), which placed *Shuaria* as the sister taxon to *Tylopsacas + Anetanthus* using both nuclear and chloroplast markers (Clark et al., 2010).

Even though there is low support for *Resia* in the Anetanthinae subtribe, there are morphological similarities to the other members of the subtribe that corroborate the placement of *Resia* within Anetanthinae. *Resia* has an alternate phyllotaxy, while *Shuaria* has a distinctive phyllotaxy of both opposite and alternate leaves (Clark et al. 2010). *Anetanthus* is a genus with unique characters similar to *Resia* in that one species (*Anetanthus gracilis*) contains bracteoles, while *Anetanthus sp.* lacks bracteoles. This same apomorphy is present in *Resia bracteata*, but not in the other members of *Resia*. Therefore, when combining both molecular and
morphological data, *Resia* should be placed within the Anetanthinae subtribe because of its shared characters within the subtribe as well as its unique characters that are also found in at least one other member of Anetanthinae.

*Placement of Cremospermopsis* – The placement of *Cremospermopsis* is strongly supported as a member of the Beslerieae tribe and Besleriinae subtribe. Due to low DNA yield of extracted materials, *Cremospermopsis cestroides* was only available from ETS, which combined with missing data might affect the low support values for its monophyly (Figure 8). However, each species of *Cremospermopsis* did nest either within *Cremosperma* (BI, ML analysis) or sister to *Cremosperma* (MP analysis). Additionally, *Cremospermopsis* shares morphological similarities with *Cremosperma* (such as a unique flower morphology of connate calyx lobes and filaments adnate to the corolla tube base), which originally led to its misidentification as a member of that genus. Thus, *Cremospermopsis* should be located within the Besleriinae subtribe and sister to *Cremosperma*.

*Origin of Bracts in Beslerieae* – Wiehler’s circumscription of the Beslerieae (Wiehler 1983) was based on the absence of bracts in the Beslerieae tribe. However, more recent studies by Skog and de Jesus (1997), Skog and Kvist (2002), and Clark et al. (2010) have questioned the use of bracts as a unifying character for the Beslerieae because of anomalous species that contain bracts such as *Anetanthus gracilis*, *Resia bracteata*, and *Cremospermopsis*. In accordance with each analysis from this study, all three origin events of bracts are independently derived from ancestors that are eubracteate. New World Gesneriaceae are remarkable for convergent evolution of morphological features. For example, fruit morphology in the Episcieae clade has at least three independent origins of fleshy fruits, where fleshy has evolved as berries in seven clades (Clark et al. 2012) and fleshy capsules in XX clades. Thus, the absence of bracts is an important
morphological feature for circumscription within the Beslerieae tribe, but they should be taken into consideration along with other important characters as well (seed coat, fruit type, calyx lobe connation, etc…).

**Future Taxon Sampling** – Additional sampling of *Resia* and *Anetanthus* (e.g. *A. rubra*) are needed, as these are rare and infrequently sampled species (Clark et al. 2010). Even though members of these genera are present in this study, additional specimens are needed for future DNA extractions, which would include more markers. Additionally, the inclusion of *R. ichthyoides* could improve support values of the *Resia* clade as well as help resolve the placement of *Resia* within the Anetanthinae subtribe (Graybeal 1997).

Recently, a population of *Cremospermopsis parviflora* was collected in the lowlands of northern Peru (I. Salinas, Pers. Comm.), which is a large gap from its previously known range of Colombia. No populations of *Cremospermopsis* are known between Peru and Colombia, study within the expanded range would help resolve these hypotheses.

Shaw et. al. (2005) presented several noncoding cpDNA regions that are better suited for generic-level investigation that are much more variable than other commonly used markers that lack variation. Although the present study used more markers than any previous phylogenetic analysis within the Beslerieae tribe, specific markers used for generic-level analysis might be more informative than more commonly used markers in phylogenetic analyses. Markers such as *trnH-psbA, trnD-trnT, rpoB-trnC, trnS-trnG, trnS-trnfM*, and *trnT- trnL*, had higher potentially informative characters within the family levels when compared to other cpDNA, which were better suited for broader phylogenetic studies (Shaw et al. 2005). Thus, including additional cpDNA markers should help resolve the phylogenetic placement of *Resia* within the Beslerieae tribe as well as *Cremospermopsis* as sister to *Cremosperma*.
REFERENCES


