A PHYLOGENY AND STUDY OF FLORAL TRAITS IN THE NEOTROPICAL GENUS *GASTERANTHUS* (GESNERIACEAE)

by

CASSANDRA LYNN COLEMAN

JOHN L. CLARK, COMMITTEE CHAIR
PHILLIP M. HARRIS
RYAN L. EARLEY
NATHAN MUCHHALA

A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biological Sciences
in the Graduate School of
The University of Alabama

TUSCALOOSA, ALABAMA

2012

ABSTRACT

The genus *Gasteranthus* (Gesneriaceae), with 41 currently described species, including 3 subspecies, occurs in Central and South America. This genus has a center of diversity in Ecuador and is comprised of herbs or subshrubs that grow predominately in humid to wet tropical forests and is especially abundant near streams and waterfalls. Species of *Gasteranthus* have two morphologically different corolla shapes. The hypocyrtoid (pouched) shape is defined by an inflated ventral pouch and constricted throat while the campanulate (non-pouched) shape is defined by a funnelform corolla. Molecular sequence data generated from nrDNA (ITS and ETS) and cpDNA (*matKR* and *trnL-F*) were generated for 57 taxa representing 32 species of *Gasteranthus* and 25 species from closely related genera. This study strongly supports that shifts between campanulate and hypocyrtoid flowers have occurred several times within *Gasteranthus*, though the result is equivocal as to which floral form is plesiomorphic. Phylogenetic analyses support that *Gasteranthus dressleri* should be transferred to *Cremosperma* and given a new combination. Phylogenetic results and fieldwork have shown that new circumscriptions are necessary for names previously synonymized in *Gasteranthus pansamalanus* and *Gasteranthus lateralis*. Finally, *Gasteranthus aurantiacus* is recognized as a synonym of *Gasteranthus mutabilis*.

DEDICATION

This thesis is dedicated to my family. The lifetime of unconditional support from them in all of my endeavors, academic and personal, has been a foundation that I continue to build upon. In particular, to my parents, Greg and Teresa Coleman, who never quelled my inquisitiveness as a child and encouraged me to explore and enjoy all aspects of the learning process.

ACKNOWLEDGEMENTS

I would like to acknowledge the faculty of the University of Alabama, colleagues and friends whose academic and professional guidance has made this thesis possible. I would like to acknowledge my committee members Dr. Phillip Harris, Dr. Ryan L. Earley and Dr. Nathan Muchhala whose guidance during this thesis was invaluable. I am especially grateful for the guidance of my committee chair, Dr. John L. Clark. This project, from its inception to the final draft, could not have been possible without his continued and diligent involvement and expertise.

I would also like to acknowledge all of those who were directly involved in my research, from undergraduates in the lab to my assistants in the field. I would be remiss to not give particular thanks to my lab and field assistants, Laura A. Frost and Seema Kumar as well as my field guide and assistant Winter J. Navarrete. My lab and field work would be incomplete without the long hours and dedication they put into assisting me. Special thanks go to Steven L. Ginzbarg, Marcela Mora, and Laura Clavijo for all of their help during the analysis and editing portion of this study. Additionally, I would like to thank Martin Freiberg and Laurence E. Skog for their permission to utilize their illustrations as a part of my work.

This research would not have been possible without the support of the University of Alabama, the Graduate School, the Department of Biological Sciences, the Alabama Louis Stokes Alliance for Minority Participation Program, my fellow graduate students, friends and family. I am grateful for everyone who was a part of this process and whose encouragement made the completion of this project and thesis possible.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Gasteranthus Benth. is a genus of 41 species that occurs throughout Central and South America (Freiberg 1996, 1998, 2000; Skog and Kvist 2000; Clark 2012). The etymology of the genus stems from the floral shape of the type species *Gasteranthus quitensis* Benth. that is derived from the Greek γαστήρ (gaster) meaning stomach, belly or pot-belly and ἄνθος (anthos) meaning flower (Bentham 1846; Wiehler 1975). This genus contains herbs and subshrubs with a large number of endemic species with highly restricted ranges (Skog and Kvist 2000).There are 19 endemic species of *Gasteranthus* from Ecuador, making it the country with the most number of endemics followed by Colombia with 5 endemic species. *Gasteranthus* is closely related to *Besleria* L. and *Cremosperma* Benth. in the Beslerieae tribe which share the same range of habitats, habits and some characteristics such as funnelform corolla shapes, opposite leaves and herb to subshrub habits. *Gasteranthus* had been synonymized with *Besleria* by Morton (1939) was later reestablished by Wiehler (1975) based on the recognition of clustered leaf stomata, a dorsal to semiannular nectary and fleshy or semi-fleshy capsule fruit. *Gasteranthus* is easily distinguished from closely related genera by the presence of usually conspicuous stomatal clusters and semi-fleshy capsule fruit. In contrast, *Besleria* lacks stomatal clusters and has a fleshy berry. Within the Gesneriaceae the clustered stomata in *Gasteranthus* is the primary distinguishing feature. They are usually obvious without a hand lens, but are sometimes inconspicuous as in *Gasteranthus corallinus* (Fritsch) Wiehler and *Gasteranthus glaber* L.E. Skog and L.P. Kvist. Clustered stomata are unusual in the Gesneriaceae and are limited to a few

species of *Napeanthus* Gardner*, Gesneria* L.*,* and *Reldia* Wiehler (Skog and Kvist 2000). Other morphological differences that distinguish *Gasteranthus* from other species in these genera include an alternate leaf arrangement in *Reldia* and *Gesneria* and presence of bracts in *Napeanthus* (Skog and Kvist 2000).

Species of *Gasteranthus* have two morphologically divergent corolla shapes that can be defined as hypocyrtoid (pouched) or campanulate (non-pouched) (Fig. 1). Fourteen species of *Gasteranthus* have yellow to white campanulate corolla tubes (Figs. 1B, 1D, 1F). The remainder of the *Gasteranthus* species (27 species) have uniformly orange, red, or pink hypocyrtoid corollas that have a constricted throat and dorsal pouch (Figs. 1A, 1C,1E).

The term hypocyrtoid is not defined in most widely used botanical glossaries (e.g., Endress 1994; Harris and Harris 1994; Proctor et al. 1996; Simpson 2006; Judd et al. 2010). Despite the lack of use for the term hypocyrtoid it has been used in recent literature when pertaining to the Gesneriaceae (Wiehler 1978, 1983; Skog and Kvist 2000; Freiberg 2007). The term was first used to define the genus *Hypocyrta* Mart. (Martius 1829) and has since been applied to other genera. The etymology of the genus *Hypocyrta* originated from the Greek ὑπό (hypo) meaning under and κυρτό (cyrta) meaning curved (Martius 1829). A type species was not originally named within the five species known to the genus, which contained both campanulate and hypocyrtoid corolla shapes, and in 1853, Hanstein referred to *Hypocyrta hirsuta* Mart. as the species that typified the genus *Hypocyrta* (Hanstein 1853). Later, Morton and Denham (1972) lectotypified the genus with *H. hirsuta*. The hypocyrtoid corolla shape would come into common use for describing corollas with ventral pouches and apically constricted tubes in the Gesneriaceae by Wiehler (1978, 1983). The term hypocyrtoid is used to describe the principle characteristic of *Hypocyrta* with its typically red corollas (also pink, orange or bright yellow)

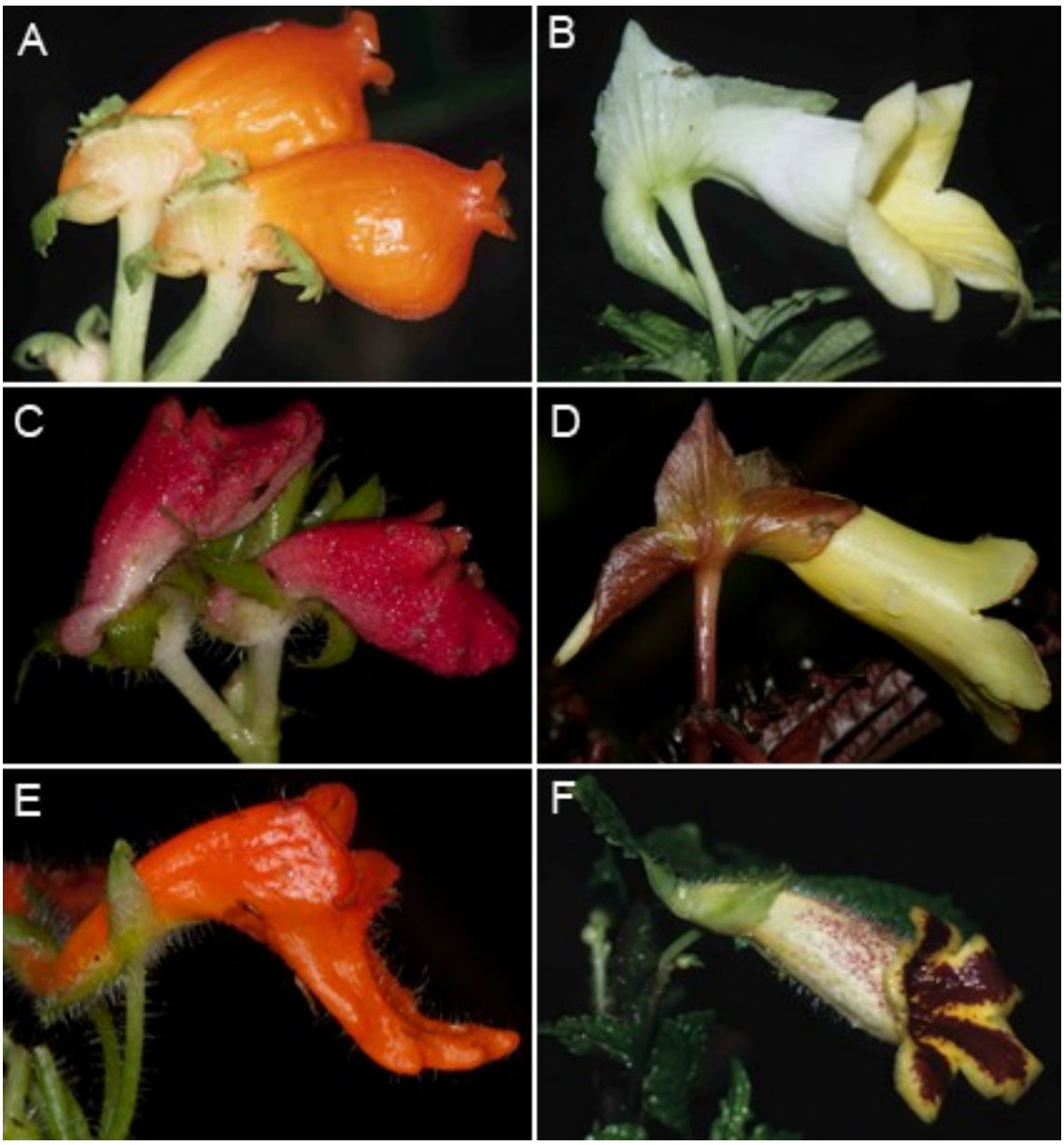

Figure 1. Lateral Views of Flowers in *Gasteranthus* Showing Different Corolla Shapes. Hypocyrtoid: A. *Gasteranthus corallinus* (Fritsch) Wiehler, C. *Gasteranthus quitensis* Benth., E. *Gasteranthus diverticularis* J. L. Clark. Campanulate: B. *Gasteranthus tenellus* L.E. Skog and L.P. Kvist, D. *Gasteranthus atratus* Wiehler, F. *Gasteranthus leopardus* M. Freiberg. (Voucher specimens: A. *J. L. Clark 6923*, B. *J. L. Clark 5573*, C. *J. L. Clark 10910*, D. *J. L. Clark 10066*, E. *J. L. Clark 10000*; Photos by J. L. Clark)

with prominent pouches below a constricted neck (Wiehler 1978, 1983). The hypocyrtoid corolla shape has independently evolved in unrelated lineages in the subfamily Gesnerioideae, one of two subfamilies of the Gesneriaceae that is limited to the Neotropics. Within the Gesnerioideae, the following three of five tribes contain species with hypocyrtoid pouches: Episcieae (*Alloplectus* Mart., *Corytoplectus* Oerst., *Drymonia* Mart., *Nematanthus* Schrad., *Neomortonia* Wiehler, and *Paradrymonia* Hanst.), Beslerieae (*Besleria and Gasteranthus*) and Gloxinieae (*Pearcea* Regel).

The diversity of corolla shapes and corolla color in *Gasteranthus*, along with the high number of endemic species, makes *Gasteranthus* an interesting group from a phylogenetic and conservation context. This diversity of corolla shapes and colors were suggested to be a result of co-evolution between flower and pollinator (Skog and Kvist 2000). A phylogenetic framework for understanding the origins of flower shapes in *Gasteranthus* is an important first step in understanding the evolution of hypocyrtoid flowers in the New World members of Gesneriaceae. Recent phylogenies containing samples of *Gasteranthus* strongly support the monophyly of the genus (Roalson et al. 2002; Roalson and Clark 2006; Clark et al. 2010). Roalson et al. (2002) was based on a morphological cladistic analysis and did not include molecular sequence data (Fig. 2A). Only two previous studies (Roalson et al. 2002; Roalson and Clark 2006) sampled broadly within *Gasteranthus* (i.e., five or more species). Roalson and Clark (2006) (Fig. 2B) and Clark et al. (2010) (Fig. 2C) were molecular phylogenetic analyses and sampled heavily in the Beslerieae tribe. This is the first molecular phylogenetic study to focus on *Gasteranthus* with significant sampling from a broad geographic range and morphological diversity. This analysis is also the first time that the majority of species of *Gasteranthus* were analyzed in a phylogenetic context. Prior to this study the following species had not been included in a molecular analysis:

G. acropodus (Donn. Sm.) Wiehler , *G. acuticarinatus* M. Freiberg, *G. atratus* Wiehler, *G. bilsaensis* L.E. Skog and L.P. Kvist , *G. columbianus* (C.V. Morton) Wiehler, *G. corallinus* (Fritsch) Wiehler, *G. crispus* (Mansf.) Wiehler, *G. delphinioides* (Seem.) Wiehler, *G. diverticularis* J. L. Clark, *G. imbaburensis* M. Freiberg, *G. otongensis* M. Freiberg, and *G. trifoliatus* M. Freiberg.

One reason for the importance of conducting research on *Gasteranthus,* as well as other tropical plant species, is the astonishing rate at which rainforests in this part of the world are disappearing. The US National Science Board (1989) predicted that as much as 25% or more of the Earth's living species are at risk of extinction within the next 25 years (Wheeler 1995). Currently 12 species of *Gasteranthus* are listed on the IUCN Red List of Endangered Species as Endangered to Critically Endangered (Clark and Skog 2011). Myers et al. (2000) found that as many as 44% of all vascular plant species are confined within 25 identified biodiversity hotspots, one of those hotspots being the Chocó in Western Ecuador where many species of *Gasteranthus* are endemic. Recent analysis has placed the number of yet undiscovered flowering plants at about 15% while finding that in the area covering Ecuador to Peru as much as 29% of species have not been discovered due to high endemism and extensive habitat loss (Joppa et al. 2011). Understanding the biology of highly endemic plants and their interactions with pollinators that often drives corolla shape diversification will foster a better understanding and awareness for efforts in the conservation of rapidly disappearing biodiversity hotspots.

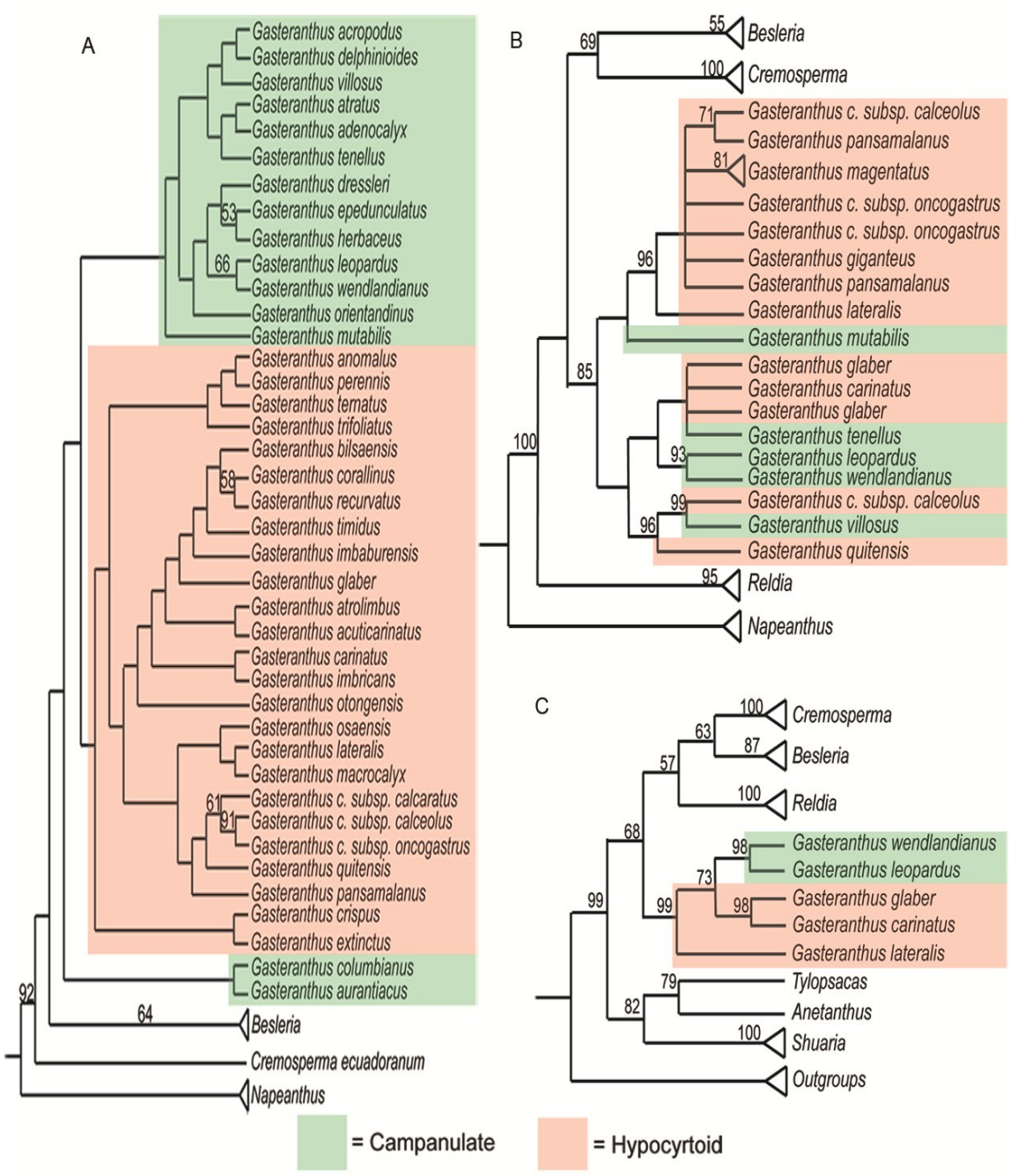

Figure 2. Diagrammatic Representation of Previous Phylogenetic Analyses with *Gasteranthus.* A. Based on a morphological data set in Roalson et al. (2002). B. Based on a molecular data set using nrDNA ITS in Roalson and Clark (2006). C. Based on a molecular data set using ITS and *trnL-F* in Clark et al. (2010).

MATERIALS AND METHODS

Taxon Sampling – Fifty-seven species were included in this study (Table 1). Within this, 26 are known species of *Gasteranthus*, six are currently undescribed species of *Gasteranthus*, two are outgroup species, and the remaining 23 species are from closely related genera within the tribe Beslerieae which includes *Anetanthus* (Hiern ex) Benth.*, Besleria, Cremosperma, Gasteranthus, Resia* H. E. Moore*, Reldia* and *Tylopsacas* Leeuwenb. Of these genera, *Resia* and *Tylopsacas* are absent from this analysis. *Resia* is known from limited localities from Venezuela, Colombia, and Ecuador (Skog and de Jesus 1997). *Cremospermopsis* L. E. Skog and L. P. Kvist was originally classified as a member of the Beslerieae (Skog and Kvist 2002), then stated by Weber (2004) to have similarities with the Beslerieae though the tribal affiliation was uncertain, and has since been classified as a member of the tribe Napeantheae (Skog and Boggan 2006). *Shuaria* D. A. Neill and J. L. Clark, recently described as a new genus in the tribe Beslerieae (Clark et al. 2010), is also included in this analysis. Taxon sampling focused on species found within Ecuador, where a high percentage of endemics are located. Additional samples were obtained from Panama (3 species).

Outgroups used in this analysis were *Peltanthera floribunda* and *Sanango racemosum*. *Peltanthera* and *Sanango* have been used as outgroups in previous molecular phylogenetic studies of the Beslerieae tribe where they are strongly supported as being closely related to the Beslerieae (Smith et al. 1997; Wang et al. 2004; Clark et al. 2010).

Table 1.

Specimens in Analysis. Species included in the analyses, voucher information, pouch formation, and GenBank accession numbers for ITS, ETS, *trnL-F*, and *matKR*. Sequences not obtained are designated by "—"

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Outgroup:							
Peltanthera floribunda Benth.	*Hammel 24426*	Bolivia, Colombia, Costa Rica, Ecuador, Panama, Peru					Campanulate
Sanango racemosum (Ruiz and Pav.) Barringer	*J. L. Clark 8863*	Ecuador, Peru					Campanulate
Ingroup:							
Anetanthus gracilis Hiern	*J. L. Clark 10003*	South America	GQ119595		———		Campanulate
Besleria angustiflora Fritsch	*J. L. Clark 4575*	Colombia, Ecuador	DQ070480/ GQ119597		GQ166797		Hypocyrtoid
Besleria beltranii Salinas	*J. L. Clark 8198*	Peru					Hypocyrtoid

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Besleria divaricata Poepp. in Poepp. and Endl.	*J. L. Clark 9188*	Peru, Ecuador, Bolivia					Hypocyrtoid
Besleria hirsutissima C.V. Morton, nom. illeg.	*J. L. Clark 6901*	Venezuela	DQ070490		———		Campanulate
Besleria ovalifolia Rusby	*J. L. Clark 6852*	Bolivia	GQ119599		GQ166799		Hypocyrtoid
Besleria parviflora L.E. Skog and Steyerm.	*H.D. Clarke 11475*	Brazil, Guyana, Venezuela					Campanulate
Besleria sp.	*J. Betancur 11582*	Colombia					Campanulate
Cremosperma castroanum C.V. Morton	*J. L. Clark 7104*	Colombia, Ecuador	DQ070506/ GQ119601		GQ166801		Campanulate
Cremosperma congruens C.V. Morton	*J. L. Clark 9888*	Colombia, Ecuador, Peru			———		Campanulate
Cremosperma maculatum L.E. Skog	*J. L. Clark 8576*	Colombia, Panama, Costa Rica				———	Campanulate

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Cremosperma muscicola L.P. Kvist and L.E. Skog	*J. L. Clark 7600*	Colombia, Ecuador					Campanulate
Cremosperma veraguanum Wiehler	*J. L. Clark 8618*	Colombia, Panama	DQ070511/ GQ119603		GQ166803		Campanulate
Cremosperma sp.	*J. L. Clark 5587*	Ecuador			_____		Campanulate
Diastema vexans H. E. Moore	*J. L. Clark 11259*	Colombia					Campanulate
Gasteranthus acropodus (Donn. Sm.) Wiehler	*J. L. Clark 12722*	Panama, Costa Rica					Campanulate
Gasteranthus acuticarinatus M. Freiberg	*J. L. Clark 8408*	Ecuador	DQ070512				Hypocyrtoid
Gasteranthus atratus Wiehler	*J. L. Clark 10066*	Ecuador			_____		Campanulate
Gasteranthus bilsaensis L.E. Skog and L.P. Kvist	*J. L. Clark 8810*	Ecuador					Hypocyrtoid

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Gasteranthus calcaratus (Kunth) Wiehler subsp. *calceolus* (Fritsch) L.E. Skog and L.P. Kvist	*J. L. Clark 5702*	Ecuador, Colombia, Peru					Hypocyrtoid
Gasteranthus calcaratus (Kunth) Wiehler subsp. *oncogastrus* (Hanst.) L.E. Skog and L.P. Kvist	*J. L. Clark 6207*	Ecuador, Colombia					Hypocyrtoid
Gasteranthus AFF *calcaratus* subsp. *oncogastrus*	*J. L. Clark 6208*	Ecuador					Hypocyrtoid
Gasteranthus columbianus (C.V. Morton) Wiehler	*J. L. Clark 10273*	Ecuador, Colombia					Campanulate

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Gasteranthus corallinus (Fritsch) Wiehler	*J. L. Clark 7169*	Ecuador, Colombia, Peru					Hypocyrtoid
Gasteranthus crispus (Mansf.) Wiehler	*J. L. Clark 7370*	Ecuador					Hypocyrtoid
Gasteranthus delphinioides (Seem.) Wiehler	*J. L. Clark 12730*	Panama, Colombia, Costa Rica					Campanulate
Gasteranthus dressleri Wiehler	*J. L. Clark 12720*	Panama	———		———		Campanulate
Gasteranthus diverticularis J. L. Clark	*J. L. Clark 10000*	Ecuador	———		———		Hypocyrtoid
Gasteranthus giganteus M. Freiberg	*J. L. Clark 7426*	Ecuador	DQ070518				Hypocyrtoid
Gasteranthus glaber L.E. Skog and L.P. Kvist	*J. L. Clark 6130*	Ecuador, Colombia	DQ070519/ GQ119605		GQ166805		Hypocyrtoid
Gasteranthus AFF *glaber* Wiehler	*J. L. Clark 7502*	Ecuador	DQ070517/ GQ119604		GQ166804		Hypocyrtoid

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Gasteranthus AFF *glaber* Wiehler	*J. L. Clark 8475*	Ecuador					Hypocyrtoid
Gasteranthus imbaburensis M. Freiberg	*C. L. Coleman 107*	Ecuador					Hypocyrtoid
Gasteranthus lateralis (C.V. Morton) Wiehler	*J. L. Clark 7619*	Ecuador	GQ119606		GQ166806		Hypocyrtoid
Gasteranthus leopardus M. Freiberg	*J. L. Clark 6331*	Ecuador, Colombia	GQ119607		GQ166807		Campanulate
Gasteranthus magentatus M. Freiberg	*J. L. Clark 6127*	Ecuador	DQ070523				Hypocyrtoid
Gasteranthus mutabilis L.E. Skog and L.P. Kvist	*J. L. Clark 6102*	Ecuador	DQ070524				Campanulate
Gasteranthus otongensis M. Freiberg	*J. L. Clark 6131*	Ecuador					Hypocyrtoid
Gasteranthus pansamalanus (Donn. Sm.) Wiehler	*J. L. Clark 5695*	Ecuador, Colombia, Guatemala, Mexico	DQ070513				Hypocyrtoid

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Gasteranthus quitensis Benth.	*J. L. Clark 6107*	Ecuador, Colombia					Hypocyrtoid
Gasteranthus tenellus L.E. Skog and L.P. Kvist	*J. L. Clark 10125*	Ecuador					Campanulate
Gasteranthus trifoliatus M. Freiberg	*J. L. Clark 7365*	Ecuador					Hypocyrtoid
Gasteranthus villosus L.E. Skog and L.P. Kvist	*J. L. Clark 8785*	Ecuador, Colombia					Campanulate
Gasteranthus wendlandianus (Hanst.) Wiehler	*J. L. Clark 6421*	Ecuador, Colombia, Bolivia, Costa Rica, Panama, Peru	GQ119608		GQ166808		Campanulate
Gasteranthus sp.	*J. L. Clark 7130*	Ecuador					Hypocyrtoid
Gasteranthus sp.	*J. L. Clark 11011*	Ecuador					Hypocyrtoid
Gasteranthus sp.	*J. L. Clark 12192*	Ecuador					Hypocyrtoid

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Mitraria coccinea Cav.	*J. L. Clark 10651*	Argentina, Chile					Campanulate
Napeanthus rupicola Feuillet and L.E. Skog	*H.D. Clarke 10351*	Guyana	GQ119590		GQ166794		Campanulate
Napeanthus sp.	*J. L. Clark 6898*	Ecuador	DQ070534/ GQ119591		GQ166795		Campanulate
Reldia minutiflora (L.E. Skog) L.P. Kvist and L.E. Skog var. *minutiflora*	*J. L. Clark 8017*	Colombia, Ecuador, Panama, Peru	GQ119610		GQ166810		Campanulate
Reldia minutiflora var. *veraguensis* (Wiehler) L.P. Kvist and L.E. Skog	*J. L. Clark 8619*	Colombia, Costa Rica, Ecuador, Panama	GQ119611		GQ166811		Campanulate
Reldia sp.	*J. L. Clark 8835*	Ecuador	DQ070537/ GQ119609		GQ166809		Campanulate

Table 1 cont.

Taxon	Voucher	Locality	ITS	ETS	*trnL-F* spacer	*matKR*	Campanulate or Hypocyrtoid
Sarmienta scandens (J.D. Brandis) Pers.	*J. L. Clark 10652*	Chile					Campanulate
Shuaria ecuadorica D.A. Neill and J.L. Clark	*Neill 15912*	Ecuador					Campanulate

DNA Extraction, Amplification and Sequencing- All samples were collected in the field with tissue samples dried in silica gel. The voucher specimens are located at the Smithsonian Institution's National Museum of Natural History (US), The University of Alabama Herbarium (UNA), and host country herbaria such as the National Herbarium of Ecuador (QNCE). Genomic DNA extractions were obtained using the Qiagen DNeasyTM DNA isolation kit (Qiagen, Valencia, California). In a few instances the Promega ReliaPrep gDNA Tissue Miniprep System (Promega, Madison, Wisconsin) was used for samples that yielded low DNA or failed to amplify when extracted with the Qiagen DNeasyTM DNA isolation kit (Qiagen, Valencia, California). All samples were sequenced for the internal transcribed spacer ITS (nrDNA), external transcribed spacer ETS (nrDNA), *matKR* (cpDNA) and *trnL-F* (cpDNA). In this analysis the spacer between the *trnK* and *matK* gene will be referred to as *matKR* (Shaw et al. 2005). Sequence data obtained from Genbank from Roalson and Clark (2006) was included in this analysis for 21 taxa for ITS and 14 taxa for *trnL-F*. All other sequence data were generated for this study.

The template of the nrDNA ITS was prepared using the forward primer ITSL (Suh et al. 1993) and reverse primer ITS4 (White et al. 1990). The internal primers ITS3 and ITS2 (White et al. 1990) were also included in some cases when the amplicon reads were short. The other nrDNA analyzed was ETS forward primer ETS-B (Beardsley and Olmstead 2002) and reverse primer 18S-ETS (Baldwin and Markos 1998). The template of the cpDNA region for *trnL-F* was amplified using the forward primer *trnLc* and reverse primer *trnLf* (Taberlet et al. 1991). The cpDNA *matKR* forward primer *trnK1-F* and reverse primer *matK-R* were used to analyze the spacer between the *trnK* and *matK* gene (Shaw et al. 2005). Polymerase Chain Reaction (PCR) amplification was performed according to the protocol explained in Clark et al. (2006). PCR

reactions were begun using a hot start where the temperature of the reaction was raised to 94°C before addition of the Taq Polymerase. In amplification of all nrDNA it was necessary to use 5% DMSO (Dimethyl sulfoxide) and 5% BSA(bovine serum albumin**)** or 5% Betaine in all PCR reactions. PCR amplification protocols were as follows: ITS and ETS - 94°C for 1 min, 35 cycles of (94°C for 30 sec, 47°C for 30 sec, and 68°C for 1 min), 68°C for 6 min and 4°C hold; *matKR* - 94°C for 2 min, 40 cycles of (94°C for 30 sec, 47°C for 1 min, and 68°C for 2 min), 68°C for 7 min and 4°C hold; *trnL-F* - 94°C for 2 min, 35 cycles of (94°C for 30 sec, 50°C for 30 sec, and 72°C for 2 min), 72°C for 6 min and 4°C hold. Gel electrophoreses was performed on the PCR reactions with loading dye to illuminate results. Gel electrophoreses was performed in 1% agarose gel solution in 1 X TBE buffer stained with ethidium bromide and visually compared to a 100-bp DNA ladder. Clean-up protocol was then performed by PEG Precipitation using PEG 8000 in 2.5 M NaCl (Johnson and Soltis 1995). In some cases multiple bands were visualized on the minigel and in these instances gel purification was performed and each band was sequenced. This was performed using the QIAquick Gel Extraction Kit and QIAprep Spin Columns (Qiagen, Valencia, California). Cycle sequencing of the purified DNA was performed following the provided specifications and the same primers as were used as in amplification. ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California) was utilized with 1 µl BigDye in all the reactions. The cycle sequencing protocol was followed by a Sodium Acetate clean-up and plate shock before being sequenced using an Applied Biosystems Model 377 Automated DNA Sequencing System (PE Biosystems).

Sequence Alignment and Phylogenetic Analyses **-** Sequencher 3.0 was used to proof and edit forward and reverse sequences, chromatograms, and to assemble contigs (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were automatically aligned in ClustalW (Larkin

et al. 2007) before being imported into SeAl version 2.0a11 Carbon (Rambaut 1996) for final alignment, modification and editing. Previously generated consensus sequences were downloaded from Genbank and added to the appropriate matrices. Genbank accession numbers are provided in Table 1. For some taxa in this analysis amplification of all four markers was not possible due to low DNA yield of extracted materials. Gaps in the sequences were treated as missing data and characters were weighted equally.

Maximum parsimony (MP) analysis was performed using a heuristic search in PAUP* 4.0b10 (Swofford 2002) using tree bisection-reconnection (TBR) branch swapping with character states unordered and unweighted. A heuristic search was performed on the resulting trees and a strict consensus tree was generated from the equally most parsimonious trees. Bootstrap values (Felsenstein 1985) were analyzed in PAUP*, determined by creating a majority-rule consensus tree, and were based on 100 replications with maxtrees set to 1000. The bootstrap analysis was based on 1000 replicates with the following heuristic search settings: 10 random addition cycles, holding one tree at each step with tree bisection-reconstruction (TBR) branch swapping. Sequences were analyzed as individual markers and as a combined total evidence dataset.

For the Bayesian inference (BI) analysis jModelTest 0.1.1 (Posada 2008) was used to determine the models that best fit the four molecular markers used in this study. ETS and *trnL-F* both resulted in model TPM2uf+G, ITS had model SYM+G and *matKR* had model GTR+G (Table 2). All of the models chosen were congruent with a 6-substitution-rate model with variable sites. The Bayesian analysis was run using MrBayes version 3.1.2 (Ronquist and Huelsenback 2003). Markov Chain Monte Carlo was performed for two simultaneous runs with four chains that ran for 5,000,000 generations with the first 25% of trees as the burn-in period

with every 1,000th generation being sampled. Posterior probability values were created when computing the 50% majority-rule consensus tree of all the compatible trees, excluding those trees from the "burn-in period."

The morphological character state in question, corolla shape, was optimized through MP character tracing of the MP and BI consensus trees in MESQUITE v. 2.75 (Madison and Madison 2011) to examine floral morphology.

RESULTS

DNA Sequencing and Alignment - The two nrDNA markers, ETS and ITS, had aligned lengths of 550 bp and 696 bp, with 226 (41%) and 223 (32%) parsimony-informative sites, respectively (Table 2). The two cpDNA markers, *matKR* and *trnL-F,* had aligned lengths of 615 bp and 860 bp, with 44 (7%) and 79 (9%) parsimony-informative sites, respectively (Table 2). Of the 57 taxa in this analysis, all were represented in ETS, 55 represented in *matKR* (2 missing), 52 represented in ITS (5 missing) and 43 represented in *trnL-F* (14 missing). Missing taxa were due to difficulties in PCR amplification. Taxa that produced double banded PCR products were then separated using gel extraction and purification. Mean pairwise divergence percentages resulted in: 11.41% (ETS), 8.69% (ITS), 2.91% (*trnL-F)*, 1.79% (*matKR)* and 6.13% (total evidence analysis) (Table 2).

Table 2.

Statistics for Individual Markers. Statistics of cpDNA and nrDNA genic regions for the analysis. Values in parentheses are for the ingroup only (i.e., Beslerieae).

Statistic	ETS	ITS	*matKR*	*trnL-F*	Combined Data Set
Aligned Length	550	696	615	860	2721
Mean pair-wise divergence (%)	11.41% (10.37%)	8.69% (7.78%)	1.79% (1.62%)	2.91% (2.70%)	6.13% (5.61%)
Parsimony uninformative characters	113 (108)	128 (119)	77 (68)	182 (159)	500 (454)
Parsimony informative characters	226 (205)	223 (203)	44 (39)	79 (70)	572 (517)

Table 2 cont.

Statistic	ETS	ITS	*matKR*	*trnL-F*	Combined Data Set
Percentage of Parsimony Informative Characters	41.09% (37.27%)	32.04% (29.17%)	7.15% (6.37%)	9.19% (8.14%)	21.02% (19.00%)
Number of Most Parsimonious Trees	185 (180)	30 (46)	38 (57)	2 (2)	53 (84)
Constant characters	211 (237)	345 (374)	494 (508)	599 (631)	1649 (1750)
Consistency index (CI)	0.5847 (0.6234)	0.5959 (0.6269)	0.8471 (0.8358)	0.9238 (0.9403)	0.6431 (0.6704)
Retention index (RI)	0.7256 (0.7638)	0.6882 (0.7215)	0.8730 (0.8706)	0.8979 (0.9249)	0.7194 (0.7508)
Rescaled consistency index (RC)	0.4242 (0.4762)	0.4101 (0.4523)	0.7396 (0.7277)	0.8295 (0.8697)	0.4626 (0.5033)
Tree length	874 (717)	871 (721)	157 (134)	315 (268)	2261 (1884)
Model Selected	TPM2uf+ G	SYM+G	GTR+G	TPM2uf+ G	GTR+G

Maximum Parsimony Analysis – Comparing the phylogenies of the separate analyses of ETS, ITS, *matKR* and *trnL-F* revealed many large polytomies within the *Gasteranthus* clade that did not bring about any new understanding of species relationships (individual marker trees not shown here). Combining the four data sets led to a significant increase in resolution over the individual analyses and so a total evidence analysis was used to analyze the relationships within the combined data set (Kluge 1989; Nixon and Carpenter 1996; Graham et al. 1998).

The Beslerieae tribe was strongly supported in this analysis with a bootstrap value (bs) of 100% (Fig. 3). Our results show that traditionally circumscribed *Gasteranthus* is not monophyletic because *Gasteranthus dressleri* is strongly supported (bs = 100%) as the sister

taxon to *Cremosperma* (Fig. 3). The remaining species of *Gasteranthus* are strongly supported in their clade with a 98% bootstrap value (Fig. 3).

The analyses of the individual marker data showed resolution among the different genera in the phylogenies, but failed to resolve the *Gasteranthus* clade well (individual phylogenies not shown). In all of the individual markers the *Gasteranthus* clade was represented for the most part as a large polytomy, or several large polytomies (results not shown). This was particularly true for the cpDNA markers *matKR* and *trnL-F* which lacked sufficient parsimony informative characters and resulted in topologies that not only failed to resolve the *Gasteranthus* clade, but had inconclusive results for the whole Beslerieae tribe where no genera were strongly supported as monophyletic (results not shown). The limited variation in the cpDNA markers was not able to resolve intrageneric relationships. While these two markers did not result in resolved phylogenies, both markers supported the placement of *G. dressleri* as the sister taxon to *Cremosperma*. This result is also congruent with the nrDNA ETS individual results. Sequence data could not be obtained for *G. dressleri* for the ITS or *trnL-F* markers due to poor amplification and was not included in this analysis.

Bayesian Analysis – Model selection implemented in jModelTest 0.1.1 (Posada 2008) resulted in TPM2uf+G for ETS, SYM+G for ITS, GTR+G for *matKR*, TPM2uf+G for *trnL-F* and GTR+G for the combined data set (Table 2). The Bayesian analysis presented here was analyzed under a single parameter established by the jModelTest (Posada 2008), though the model results were all different they were all translated to be equivalent within MrBayes (Ronquist and Huelsenback 2003). The first 25% of the trees were excluded as burn-in. This analysis was rooted with *Sanango racemosum* (Fig. 4).

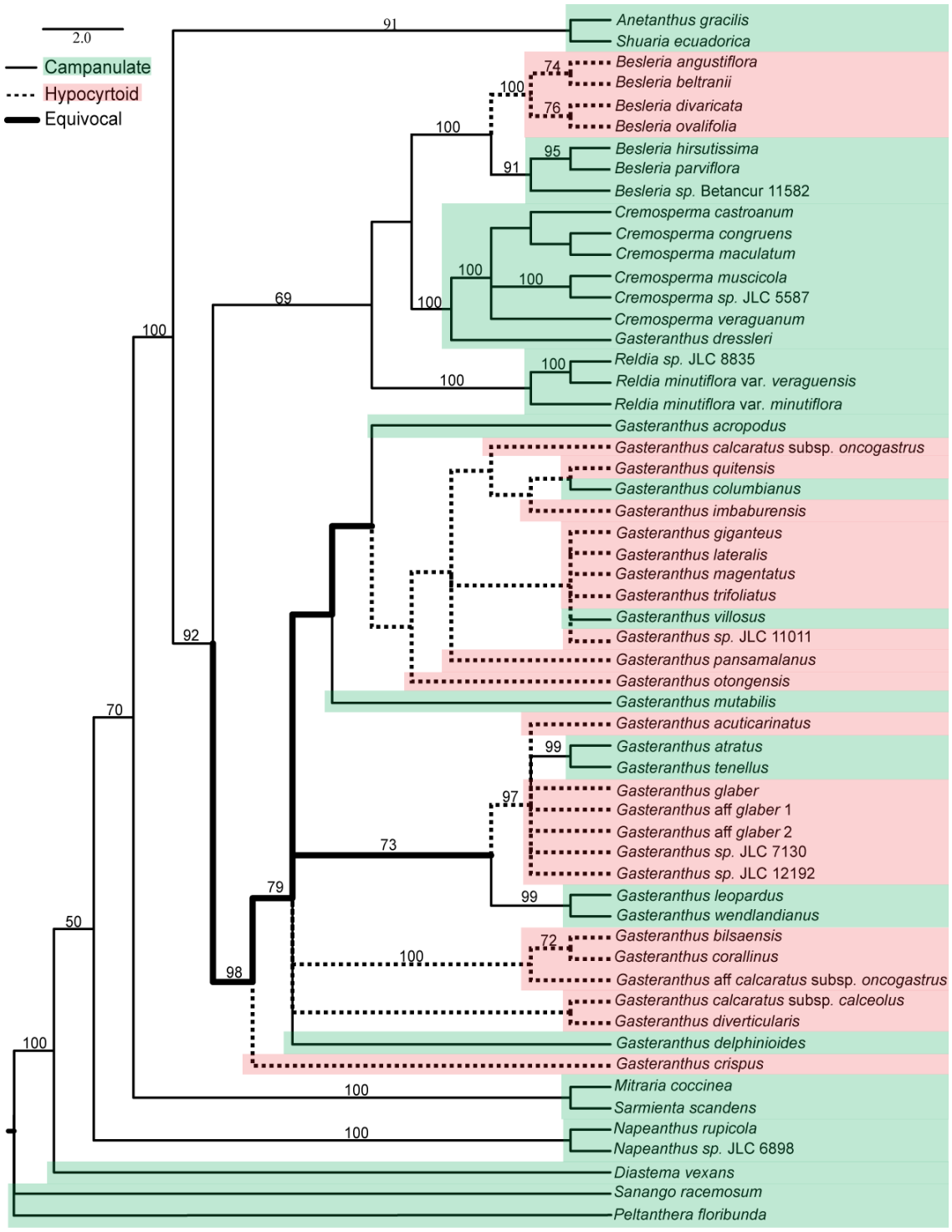

Figure 3.Strict Consensus Tree Based on the Total Evidence Analysis from Maximum Parsimony. Bootstrap percentage values are above the branches.

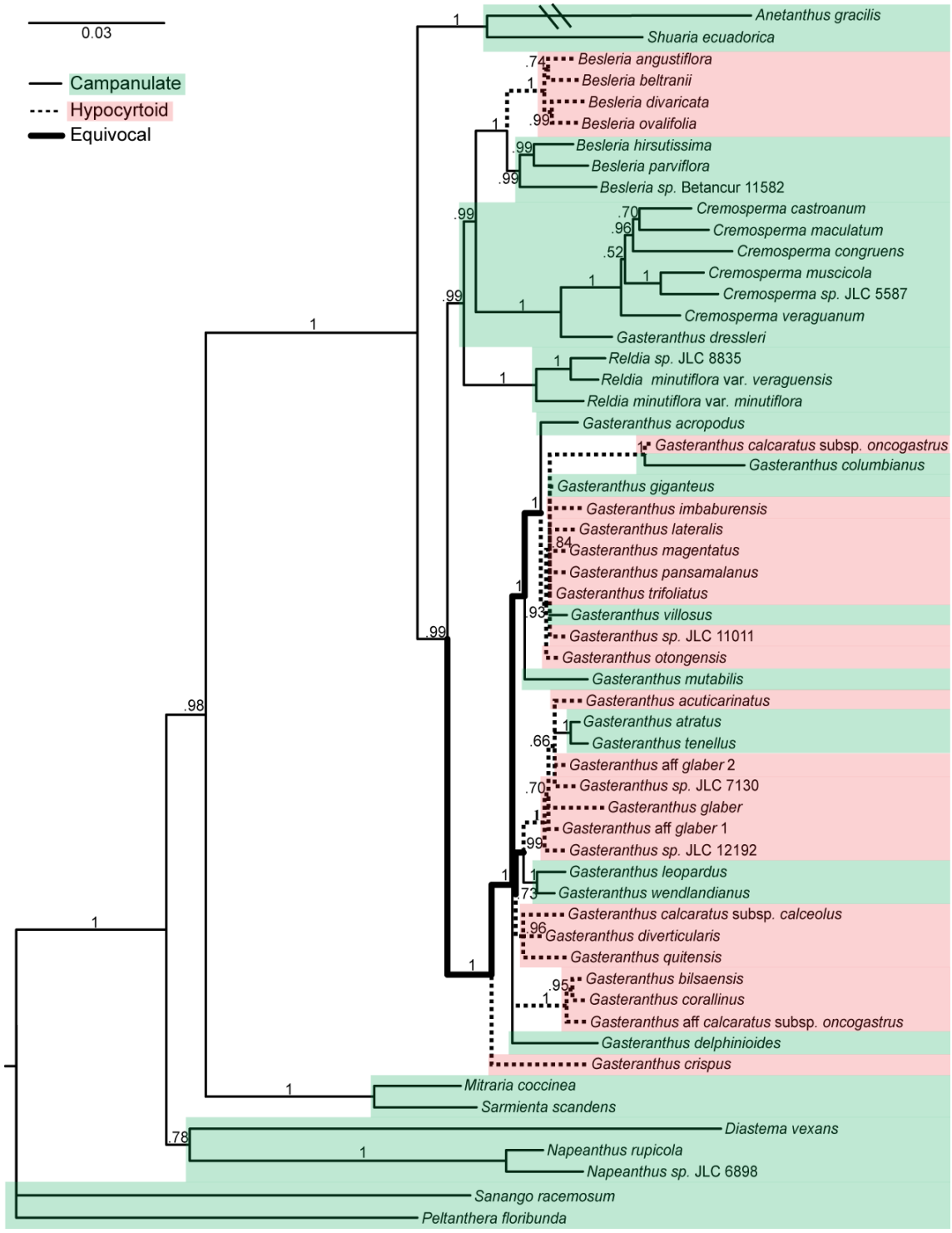

Figure 4. Bayesian Consensus Inference Analysis Based on the Total Evidence Combined Dataset. Numbers above the branches represent posterior probability values.

The topology of the BI tree (Fig. 4) was congruent with the MP tree (Fig. 3), but with greater resolution in the *Gasteranthus* clade. The Beslerieae tribe was strongly supported in this analysis with a Posterior Probability (PP) value of 1.00. However, in this analysis, as in the maximum parsimony analysis, *Gasteranthus* was not monophyletic as a result of *Gasteranthus dressleri* being more closely related to *Cremosperma* (PP = 1.00). The remaining species of *Gasteranthus* form a clade that is supported with a 1.00 pp value (Fig. 4).

Character Evolution – Corolla shapes were coded as hypocyrtoid = 1 and campanulate = 0 and then mapped on both the MP and BI trees using the character history tracing feature in Mesquite v. 2.75 (Madison and Madison 2011) (Figs. 3, 4). Assessment of corolla forms was based on field observations, the study of museum collections, original species descriptions, and the monograph of *Gasteranthus* (Skog and Kvist 2000). The MP and BI hypotheses were congruent (Figs. 3, 4) and presented equivocal shifts in corolla shapes as the same for both phylogenetic analyses.

DISCUSSION

Comparisons to Previous Phylogenies - The previous morphological cladistic analysis of *Gasteranthus* supported one evolutionary transition from campanulate to hypocyrtoid corollas (Roalson et al. 2002) (Fig. 2A) while the molecular study by Clark et al. (2010) supported one transition from hypocyrtoid to campanulate corollas (Fig. 2C). In contrast, the molecular based phylogeny by Roalson and Clark (2006) suggested multiple evolutionary shifts between hypocyrtoid and campanulate flowers (Figs. 2B). Roalson and Clark (2006) utilized one marker, nrDNA ITS, while Clark et al. (2010) utilized nrDNA ITS and cpDNA *trnL-F.* The morphological analysis (Roalson et al. 2002) was inclusive of all known species of *Gasteranthus* at the time while the molecular phylogenies were limited to 5 species (Clark et al. 2010) and 18 species (Roalson and Clark 2006) of *Gasteranthus*.

Placement of Gasteranthus dressleri - This analysis is the first phylogeny to show that *Gasteranthus dressleri* is more closely related to *Cremosperma* than to other *Gasteranthus* species. To maintain a monophyletic genus it will be necessary to provide a new combination of this taxon in *Cremosperma*. The morphological analysis of Roalson et al. (2002) supported the placement of this taxon in *Gasteranthus,* but this study was based on morphology and did not sample broadly from other closely related genera. *Gasteranthus dressleri,* described by Wiehler (1977), was placed within *Gasteranthus* based on its morphology of having supposed clustered stomata, bivalved capsule fruit, and similar habit to *G. crispus*. *Gasteranthus dressleri* is similar to *Cremosperma* in having opposite leaves and a basal rosette habit. However, there had

previously been enough morphological similarities with *Gasteranthus* that it had been placed in the genus as *G. dressleri*. *Gasteranthus dressleri* contains the diagnostics based on the description of Wiehler (1977), the most prominent being clustered stomata, as well as having a flower very similar in form to *G. herbaceus.* But in the recent monograph Skog and Kvist (2000) describe *G. dressleri* as having stomatal clusters that are not conspicuous. Recent collectors of *G. dressleri* reported not seeing the defining clustered stomata (J.L. Clark, pers. comm..) and the recently collected specimens, even when examined under the microscope, did not appear to have any sort of stomatal clustering. In the original description by Wiehler (1977) it was noted that the plant was similar morphologically to *G. crispus,* having a basal rosette. This analysis shows, however, that while morphologically similar to *G. crispus* and remaining *Gasteranthus* taxa, *G. dressleri* should not be included as a species of *Gasteranthus* and should be transferred to *Cremosperma.*

Gasteranthus calcaratus species complex **-** The MP and BI analyses strongly support that the three subspecies of *Gasteranthus calcaratus* do not share a recent common ancestor (Figs. 3, 4). The non-monophyly of this species complex was also shown in Roalson and Clark (2006). All specimens of the three subspecies of *Gasteranthus calcaratus* have orange, hypocyrtoid corollas, but it should be noted that there is a wide range of vegetative and reproductive variation. Of the three subspecies, all lack conspicuous indumentum on the corollas except for *Gasteranthus calcaratus* subsp. *calceolus* which is noted as having conspicuous indumentum on both the corollas and calyx. This subspecies should be easily identified as it has strongly anisophyllous leaves, though Skog and Kvist (2000) describe some specimens of it from the eastern Andes that lack this character. The specimens that lack anisophyllous leaves should be reexamined as potential new species as they do not conform to the recognized characteristics of

this subspecies, but are located merely in the same geographic area. *Gasteranthus calcaratus* subsp. *oncogastrus* can be easily identified among the three as having a small calyx that appears very light green and an inflorescence with upwards of 15-20 flowers at a time. The results presented here show that the three subspecies of *Gasteranthus calcaratus* as defined by Skog and Kvist (2000) are based on geographic localities and morphology.

Our results strongly support that there are multiple species from Colombia, Western Ecuador and Eastern Ecuador and that the subspecies outlined in Skog and Kvist (2000) are artificial. While they had previously been known by individual species names, Skog and Kvist (2000) synonymized the individual species into *G. calcaratus* and then recognized these taxa as subspecies. Based on this analysis and the previous analysis from Roalson and Clark (2006), it is clear that additional field and molecular work must be done to separate and properly define what is actually *G. calcaratus* and to distinguish between the possible species that were recognized as *G. calcaratus* subsp. *calcaratus*, *G. calcaratus* subsp. *calceolus*, and *G. calcaratus* subsp. *oncogastrus* (Skog and Kvist 2000). As this particular floral shape and coloration is common in *Gasteranthus* it is not surprising that it is difficult to distinguish between species considering the lack of specimens available from South America. *G. calcaratus*, as it is currently known and divided into subspecies, appears to be composed of separate species that are in need of revised circumscriptions.

Gasteranthus glaber complex **-** A species complex that is in need of revision includes *G. atrolimbus, G. glaber, G. carinatus* and *G. acuticarinatus* (Figs. 5, 6). The type localities for *G. atrolimbus* and *G. acuticarinatus* were visited to establish their morphological differences, especially in regards to *G. glaber.* Visiting the type localities and observing the morphological similarities in the descriptions made it possible to show that *G. atrolimbus* should be recognized

as a synonym of *G. glaber*. Once this was correctly established, then the differences between *G. glaber* and *G. acuticarinatus* were explored (Fig. 5). This was initially difficult as both were morphologically similar to one another and were thought to be synonyms. An easily confused *Gasteranthus* species that is similar is *G. carinatus*. An isotype of *G. glaber* (*P. Mendoza-T. et al. 624*) was studied to better understand the original description. As explained below, the differences in locality, corolla and calyx morphology, size and color as well as leaf shape distinguish these two species. *Gasteranthus glaber* is an much more widespread species with smaller, purple to wine colored hypocyrtoid corollas with a less conspicuous calyx on a plant that was found to normally be between one and two meters tall. *Gasteranthus acuticarinatus* is usually one meter tall, with large red to wine colored hypocyrtoid corollas with a large and exaggerated calyx of the same color. The recent monograph of *Gasteranthus* (Skog and Kvist, 2000) did not include species from Freiberg (2000), such as *G. acuticarinatus*. A study of herbarium specimens and field work in type localities supports that *G. acuticarinatus* should be recognized as a distinct species from *G. glaber*.

Taxonomy of Gasteranthus species with dorsal keels **-**. Wiehler (1977) originally named *G. carinatus* for its distinct dorsal keel (Fig. 6). However, when studying specimens for the monograph, Skog and Kvist (2000) described many of the specimens as having reduced or absent dorsal keels from certain areas in Ecuador. While this species is usually quite easy to identify with its dorsal keel, orange coloration, and small hypocyrtoid corollas, those specimens that lacked dorsal keels were extremely similar to *G. glaber* or to *G. acuticarinatus*, a species that also has a slight dorsal keel but with much larger corollas and different coloration. Specimens that were cited and annotated by Skog and Kvist (2000) as *G. carinatus* that lacked a dorsal keel appear to be a slight color morph of *G. acuticarinatus* or *G. glaber*. Further

evaluation of these specimens suggest that they have considerably larger corollas than *G. carinatus.* Once herbarium specimens for *G. carinatus* with a dorsal keel were examined, it became clear that those specimens lacking the dorsal keel had probably been misidentified as *G. carinatus* based on their similar orange and yellow coloration. Specimens cited as *G. carinatus* that lacked or had a reduced a dorsal keel on an obviously larger corolla were changed to *G. acuticarinatus* or *G. glaber.* Tissue samples and recent collections of *G. carinatus* were not included in this analysis and it is therefore difficult to evaluate the specific phylogenetic placement of *G. carinatus*. I would predict that it would nest with *G. glaber, G. acuticarinatus, G.* aff *glaber* 1 or *G.* aff *glaber* 2 because of the morphological similarities outlined above.

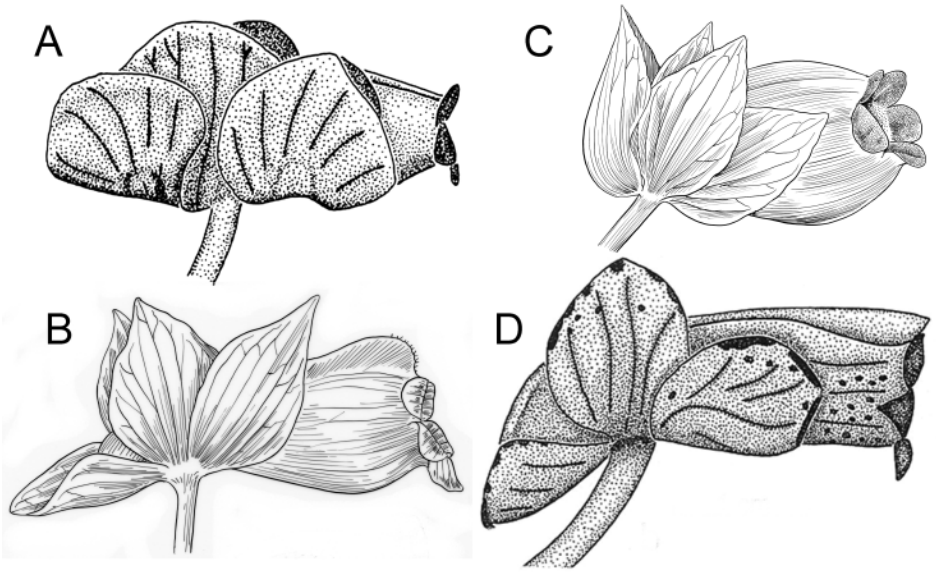

Figure 5. *Gasteranthus glaber* complex. A. *Gasteranthus atrolimbus* M. Freiberg (Freiberg 2000) B. *Gasteranthus carinatus* Wiehler (Skog and Kvist 2000). C. *Gasteranthus glaber* L. E. Skog and L. P. Kvist (Skog and Kvist 2000). D. *Gasteranthus acuticarinatus* M. Freiberg (Freiberg 2000).

New Circumscriptions – Species concepts for this study were based on the recent monographic revision by Skog and Kvist (2000). Additional recent *Gasteranthus* descriptions of species that do not appear in the monograph include Freiberg (2000) and Clark (2012). The three *Gasteranthus* species published by Freiberg (2000) were not included in the monograph by Skog and Kvist (2000) because both manuscripts were in review at the same time and published within one month of each other. Some of Freiberg's species are considered here to be conspecific with species recognized by Skog and Kvist (2000).

Freiberg (2000) came out in August, just a month after the monograph in July of the same year (Skog and Kvist 2000). Two of three species of *Gasteranthus* described in Freiberg (2000) should be synonymized as species previously included in Skog and Kvist (2000). *G. aurantiacus* and *G. atrolimbus* should be synonymized as *G. mutabilis* and *G. glaber*, respectively. The only species described in Freiberg (2000) that does not need to be synonymized is *G. acuticarinatus*.

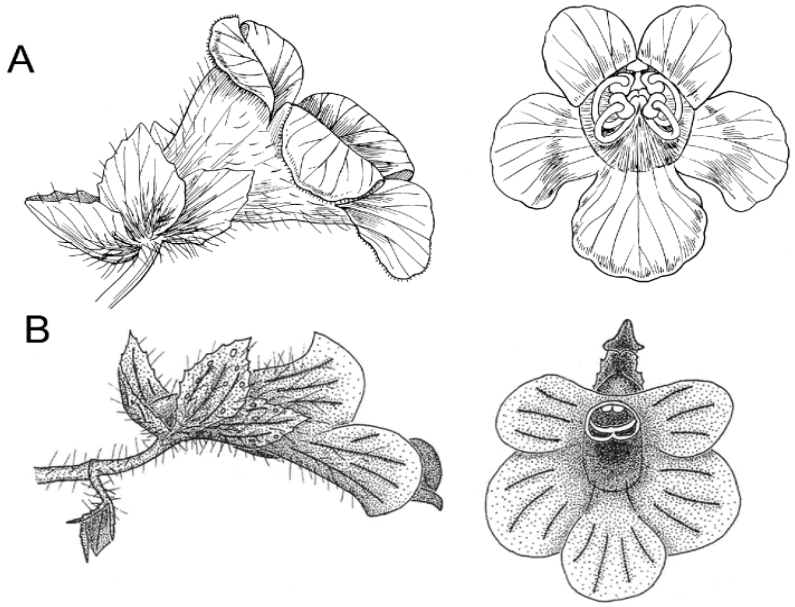

Figure 6. *Gasteranthus mutabilis* and *Gasteranthus aurantiacus*. A. *Gasteranthus mutabilis* L. E. Skog and L. P. Kvist (Skog and Kvist 2000). B. *Gasteranthus aurantiacus* Freiberg (Freiberg 2000)

Skog and Kvist (2000) synonymized *G. magentatus* (Freiberg 1998) as *G. pansamalanus* and *G. giganteus* (Freiberg 1996) as *G. lateralis* based on their descriptions and dried herbarium specimens. It is clear that these species have been incorrectly synonymized and should be recognized as distinct species. This conclusion is based on visits to type localities for both species described by Freiberg while they were in flower, studying the descriptions, a study of specimens from multiple herbaria (MO, SEL, UNA, US), previously documented photos, and the results of these analyses.

G. magentatus is a strikingly different species from *G. pansamalanus* when studied in the field. The herbarium specimens of these two species look similar and both species can be found in similar habitats and vary in height from one to three meters. The corolla color is strikingly different and consistent within populations that were visited from multiple localities in western Ecuador. *G. pansamalanus* is usually a very distinct orange color with a protruding and ridged hypocyrtoid pouch and a uniformly green calyx (Skog and Kvist 2000). *G. magentatus*, named for its conspicuous magenta colored corolla, has a less protruding and more rounded hypocyrtoid corolla shape (Freiberg 2000). The calyx of this flowering plant species is much larger than the calyx of *G. pansamalanus* and is a very light green at the base of the flower. The openings of the corolla tubes also differ greatly as *G. pansamalanus* has an opening that is obvious while *G. magentatus* does not create such a distinct opening with the corolla lobe tips falling slightly over the corolla neck opening.

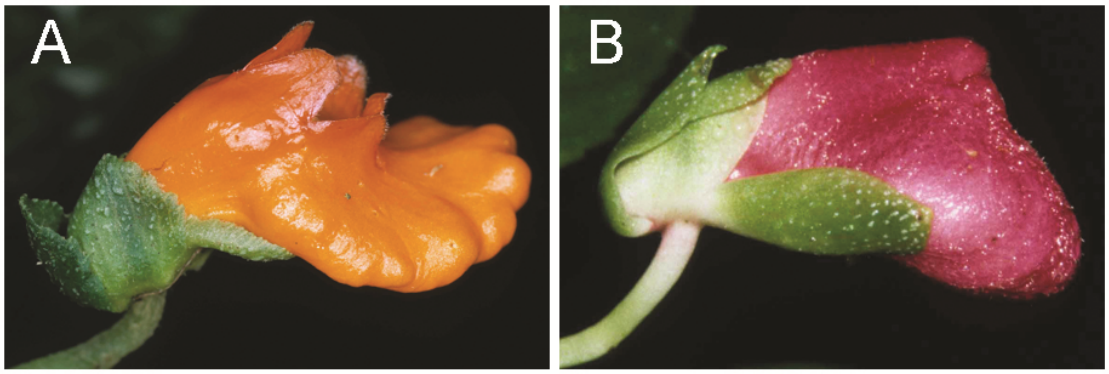

Figure. 7. Previously Synonymized as *Gasteranthus pansamalanus*. A. *G. pansamalanus* (Donn. Sm.) Wiehler. B. *G. magentatus* M. Freiberg. (Vouchers: A. *J. L. Clark 7749*, B. *J. L. Clark 6127*; Photos by J. L. Clark)

G. lateralis and *G. giganteus* should be recognized as two species instead of one (Kvist and Skog 2000). *Gasteranthus lateralis* is pilose to villose on its inflorescences, calyx and corolla (Skog and Kvist 2000) In contrast, *G. giganteus* is glabrous to subglabrous on its inflorescences, calyx and corolla (Freiberg 2000). Both species share similarly colored corollas, though *G. giganteus* usually is a much brighter orange. One distinguishing feature of *G. giganteus* is the texture of the hypocyrtoid corolla as it reaches maturity. The immature corolla surface is non-rugose/smooth in buds and as the flower matures and opens for pollination, the protruding part of the pouch becomes rugose, wrinkled and acquires small orange verrucose nodules that resemble acne.

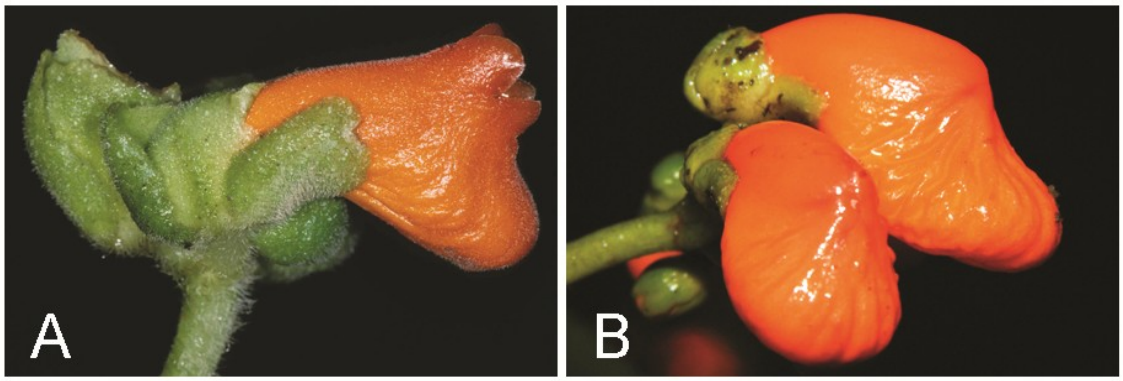

Figure 8. Previously Synonymized as *Gasteranthus lateralis.* A. *Gasteranthus lateralis* (C. V. Morton) Wiehler. B. *Gasteranthus giganteus* M. Freiberg. (Vouchers: A. *J. L. Clark 7619*, B. *C. L. Coleman 108*;Photo A. by J. L. Clark, Photo B. by C. L. Coleman)

The Phylogenetic placement of Gasteranthus in the Beslerieae – The most specious genera in the tribe Beslerieae are *Gasteranthus, Besleria* and *Cremosperma.* The relationship between these three genera based on the current and previous studies is incongruent (Smith 2000; Roalson and Clark 2006; Clark et al. 2010) (Fig. 9A). Smith (2000), in all three condensed trees, always found *Gasteranthus* to be most closely related to *Besleria* (Fig. 9A). More recent studies with expanded taxon sampling (Roalson and Clark 2006; Clark et al. 2010) are congruent with the results presented here (Fig. 9B). In the total evidence analysis (MP and BI) and in all but the ETS individual marker, *Besleria* and *Cremosperma* are shown to be sister taxa with *Gasteranthus* ancestral to them (Fig. 9C).

While *Besleria* and *Gasteranthus* do share some morphological similarities such as having nearly free calyx lobes and usually containing a calyx spur, *Besleria* and *Cremosperma* share some as well such as having scattered stomata and annular nectaries (Skog and Kvist 2000). *Gasteranthus* is set apart from them both by having clustered stomata. The lack of clustered stomata is a defining character that makes it unsurprising that *Besleria* and *Cremosperma* would be sister genera.

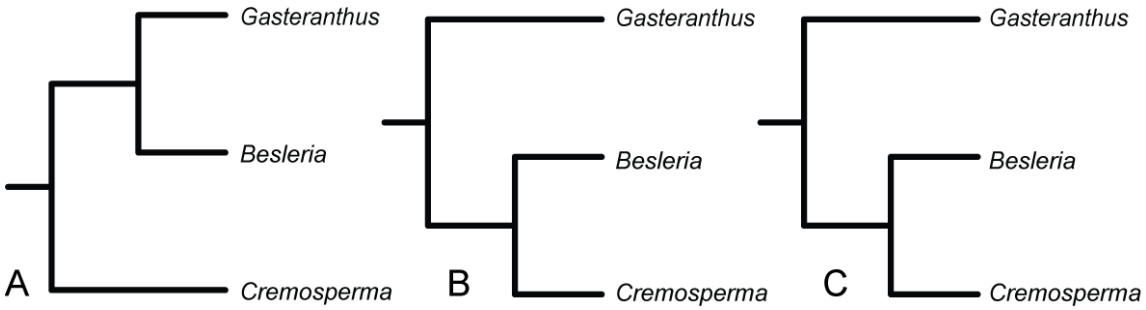

Figure 9. Phylogenetic placement of *Gasteranthus* in the Beslerieae. A. Diagrammatic representation of Beslerieae genera relationships in Smith (2000). B. Diagrammatic representation of Beslerieae genera relationships in Roalson and Clark (2006) and Clark et al. (2010). C. Diagrammatic representation of Beslerieae genera relationships using ITS, *matKR, trnL-F,* and total evidence from this analysis.

Corolla Shape and Pollinator Correlations **-** Understanding the origins of different flower shapes in *Gasteranthus* is an important step in understanding the evolution of hypocyrtoid flowers in Gesneriaceae where this corolla shape is also found in *Alloplectus, Besleria, Corytoplectus, Drymonia, Nematanthus, Neomortonia, Paradrymonia,* and *Pearcea.* The presence of hypocyrtoid flowers and their putative convergence in different genera has been mentioned in previous studies (Wiehler 1983; Freiberg 2007). Wiehler (1983) speculated that the hypocyrtoid structure of an enlarged pouch-shaped corolla may serve as a target enlargement making the corolla more conspicuous resulting in more effective pollination by attracting pollinators. Skog and Kvist (2000) proposed another explanation for the presence of a pouch, a potential advantage to inhibit self-pollination. The flowers of *Gasteranthus* are protandrous and the stamens, during the flowering of the female stage, are located at the base of the pouch, far below the stigma in the corolla throat. The separation acts as a barrier from self-pollinating the stigma (Skog and Kvist 2000). They also suggested that the restricted neck of the corolla may act

as a form of sympatric isolation, prohibiting hybridization with other species that are pollinated by insects (Wiehler 1983; Skog and Kvist 2000).

The hypocyrtoid and campanulate corolla shapes in the Gesneriaceae, may have evolved as a form of adaption to specific pollinators. Variation in corolla shape may be enhanced by the presence of many closely related plant species in a single geographical locality. Under these conditions it could be advantageous to attract effective and efficient pollinators and promote outcrossing. It has been documented in other plant systems that corolla shapes often evolve from generalist pollinators to specialist pollinators, though studies of the last decade have not always been congruent with this idea as pollination biology becomes a more popularly studied topic (McDade 1992; Armbruster and Baldwin 1998; Johnson and Steiner 2000; Tripp and Manos 2008).

The shift between hypocyrtoid and campanulate corollas shown in *Gasteranthus* may be an important factor in the diversification between closely related taxa. Within this analysis there is not only the shift from campanulate to hypocyrtoid pouch shapes in the Beslerieae, but the reversals within the *Gasteranthus* clade from hypocyrtoid back to campanulate floral shapes (Figs. 3, 4). A reversal to earlier generalist pollinator modes is often unlikely and rare in most plant families, especially given that specialist pollinators like hummingbirds are much more effective at transferring pollen than bees. However, the ability or plasticity of such a reversal or shift would be an evolutionary advantage in the life history of *Gasteranthus* if it had repeatedly dispersed to new areas that either lacked or had very small specialist pollinator populations (Tripp and Manos 2008). Plants with specialized pollinators run the risk of extinction should they lose their pollinator. Plants with generalist pollination systems are much more resilient to pollinator loss (Johnson and Steiner 2000).

While no previous studies have confirmed specific natural pollinators within *Gasteranthus*, there has been limited preliminary work within the Gesneriaceae (Skog and Kvist 2000). Because no specific studies have been done to identify the pollinators of *Gasteranthus,* the understanding of the pollinators of this genus has been based on conjecture related to pollination syndromes rather than fieldwork (Skog and Kvist 2000). Pollination syndromes were previously intended as a way to formally describe patterns of convergent evolution. The intent of having pollination syndrome guidelines was not meant to be a substitute for field work (Johnson and Steiner 2000). While the use of floral characteristics and morphology can be an indicator of pollinator type and syndrome, the need for comprehensive pollination field based studies should not be ignored. This is especially important in floral forms that do not fit classic pollination syndrome descriptions, like the hypocyrtoid shape found in *Gasteranthus.*

REFERENCES

Armbruster, W. S. and B. G Baldwin. 1998. Switch From Specialized to Generalized Pollination. *Nature* 394: 632.

Baldwin, B. G. and S. Markos. 1998. Phylogenetic Utility of the External Transcribed Spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS Trees of *Calycadenia* (Compositae*). Molecular Phylogenetics and Evolution* 10(3): 449-463.

Beardsley, P. M. and R. G. Olmstead. 2002. Redefining Phrymaceae: the Placement of *Mimulus*, tribe Mimuleae, and *Phryma*. *American Journal of Botany* 89: 1093-1102.

Bentham, G. 1839-1857. *Plantae Hartwegianae*. London: Gulielmus Pamplin.

Clark, J. L. 2012. *Gasteranthus diverticularis,* a New Species of Gesneriaceae from Southern Ecuador. *Brittonia* 64(1): 1-5.

Clark, J. L. and L. E. Skog. 2011. Gesneriaceae. Pp. 344-359 In: S. León-Yánez, R. Valencia, N. Pitman, L. Endara, C. Ulloa Ulloa, and H. Navarrete (eds). *Libro rojo de las plantas endémicas del Ecuador, segunda edición.* Publicaciones del Herbario QCA, Pontificia Universidad Católica del Ecuador, Quito.

Clark, J. L., D. A. Neill, A. Weber, J. A. Gruhn, and T. Katan. 2010. *Shuaria* (Gesneriaceae), an Arborescent New Genus from the Cordillera del Condor and Amazonian Ecuador. *Systematic Botany* 35(3): 662-674.

Endress, P. K. 1994. *Diversity and Evolutionary Biology of Tropical Flowers*. United Kingdom: Cambridge University Press.

Felsenstein, J. 1985. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* 39(4): 783-791.

Freiberg, M. 1996. Gesneriad Flora of the Los Cedros Biological Reserve, Northwest Ecuador, part 1: Four New Species in *Gasteranthus* (Gesneriaceae). *Phyton (Horn)* 36: 303-309.

Freiberg, M. 1998. Two remarkable new species of *Gasteranthus* (Gesneriaceae) from Central Ecuador. *Phyton (Horn)* 38: 167-173.

Freiberg, M. 2000. Three New Species of *Gasteranthus* (Gesneriaceae) from Ecuador. *Brittonia* 52(2): 203-209.

Freiberg, M. 2007. Evolutionary Trends in Pollination Syndromes of Neotropical Gesneriaceae. *Phyton (Horn)* 46: 207-209.

Graham, S. W., J. R. Kohn, B. R. Morton, J. E. Eckenwalder, and S. C. H. Barrett. 1998. Phylogenetic Congruence and Discordance Among one Morphological and three Molecular Data Sets from Pontederiaceae. *Systematic Biology* 47: 545-567.

Hanstein, J. 1854. "1853". *Linnaea* 26: 145-216.

Harris, J. G. and M. W. Harris. 1994. *Plant Identification Terminology: An Illustrated Glossary* (2nd ed.). Spring Lake, UT: Spring Lake Publishing.

Johnson, L. A. and D. E. Soltis. 1995. Phylogenetic Inference in Saxifragaceae sensu strict and *Gilia* (Polemoniaceae) using *matKR* Sequences. *Annals of the Missouri Garden* 82: 149-175.

Johnson, S. D. and K. E. Steiner. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* 15(4): 140-143.

Joppa, L. N., D. L. Roberts, N. Myers, and S. L. Pimm. 2011. Biodiversity Hotspots House most Undiscovered Plant Species. *Proceedings of National Academy of Science* 108: 13171-13176.

Judd, W. S., C. S. Campbell, E. A. Kellogg, P. F. Stevens, and M. J. Donoghue. 2010. *Plant Systematics: A Phylogenetic Approach* (3rd ed.). Sunderland, MA: Sinauer Associates.

Kluge, L. P. 1989. A Concern for Evidence and a Phylogenetic Hypothesis of Relationships among *Epicrates* (Boidae, Serpentes). *Systematic Zoology* 38: 7-25.

Kvist, L. P., L. E. Skog, J. L. Clark, and R. W. Dunn. 2004. The family Gesneriaceae as example for the Biological extinction in Western Ecuador. *Lyonia* 6(2): 127-151.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. Higgins. 2007. ClustalW and ClustalX version 2. *Bioinformatics* 23: 2947-2948.

Maddison, W. P. and D. R. Maddison. 2011. Mesquite: a Modular System for Evolutionary Analysis. Version 2.75 <http://mesquiteproject.org>.

Marten-Rodriguez, S., C. B. Fenster, I. Agnarsson, L. E. Skog, and E. A. Zimmer. 2010. Evolutionary Breakdown of Pollination Specialization in a Caribbean Plant Radiation. *New Phytologist* 188: 403-417.

Martius, C. F. P. von. 1829. Nova Genera et Species Plantarum. 3: 48-53.

McDade, L. A. 1992. Pollinator Relationships, Biogeography and Phylogenetics. *BioScience* 42: 21-26.

Morton, C. V. 1939. A revision of *Besleria*. *Contributions from the United States National Herbarium*. 26: 395-474.

Morton, C. V. and D. Denham. 1972. Lectotypifications of Some Generic Names of Gesneriaceae. *Taxon* 21(5/6):669-678.

Myers N., R. A. Mittermeier, C. G. Mittermeier, G. A. B da Fonseca, and J. Kents. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.

National Science Board, US. 1989. *Loss of biological diversity: a global crisis requiring international solution.* 19 pp. Washington: US National Science Foundation.

Nixon, K. C. and J. M. Carpenter. 1996. On Simultaneous Analysis. *Cladistics* 12: 221-241.

Posada, D. 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and *Evolution* 25(7): 1253-1256.

Proctor, M., P. Yeo, and A. Lack. 1996. *The Natural History of Pollination*. Portland, OR: Timber Press.

Rambaut, A. 1996. *Se-Al Sequence Alignment Editor*, ver. 1.0 alpha 1. Oxford: Department of Zoology, University of Oxford.

Roalson, E. H. and J. L. Clark. 2006. Phylogenetic Patterns of Diversification in the Beslerieae (Gesneriaceae). Pp. 251-268 in *Plant Genome: Biodiversity and Evolution, Phanerogams 1C*, eds. A. K. Sharma and A. Sharma. Science Publishers: Enfield, New Hampshire.

Roalson, E. H., A. E. Senters, L. E. Skog, and E. A. Zimmer. 2002. A Morphological Cladistic Analysis of the Neotropical Flowering Plant Genus *Gasteranthus* (Gesneriaceae). *Systematic Botany* 27(3): 573-591.

Ronquist, F. and J. P. Huelsenback. 2003. MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. *Bioinformatics* 19: 1572-1574.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, and R. L. Small. 2005. The Tortoise and the Hare II: Relative Utility of 21 Noncoding Chloroplast DNA Sequences for Phylogenetic Analysis. *American Journal of Botany* 92(1): 142-166.

Simpson, M. G. 2006. *Plant Systematics*. London: Elsevier Academic Press.

Skog, L. E. and F. F. de Jesus. 1997. A Review of *Resia* (Gesneriaceae). *Biollania* 6: 515-525.

Skog, L. E. and J. K. Boggan. 2006. A New Classification of the Western Hemisphere Gesneriaceae. *Gesneriads* 56(3): 12-19.

Skog, L. E. and L. P. Kvist. 2000. Revision of *Gasteranthus* (Gesneriaceae). *Systematic Botany Monographs* 59: 1-118.

Skog, L. E. and L. P. Kvist. 2002. Novae Gesneriaceae Neotropicarum IX: Cremospermopsis, a New Genus from Colombia. *Novon* 12(2): 262-269.

Smith, J. F. 2000. A Phylogenetic Analysis of Tribes Beslerieae and Napeantheae (Gesneriaceae) and Evolution of Fruit Types: Parsimony and Maximum Likelihood Analyses of *ndhF* Sequences. *Systematic Botany* 25(1): 72-81.

Smith, J. F., S. B. Draper, L. C. Hileman, and D. A. Baum. 1997. Familial Placement of *Cyrtandromoea, Titanotrichum,* and *Sanango,* three Problematic Genera of the Lamiales. *Taxon* 46: 65-74.

Suh, Y., L. B. Thien, H. E. Reeve, and E. A. Zimmer. 1993. Molecular Evolution and Phylogenetic Implications of Internal Transcribed Spacer Sequences of ribosomal DNA in Winteraceae. *American Journal of Botany* 80: 1042-1055.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis using Parsimony (*and other methods).* Version 4. Sunderland: Sinauer Associates.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal Primers for Amplification of three non-coding Regions of Chloroplast DNA. *Plant Molecular Biology* 17: 1105-1110.

Tripp, E. A. and P. S. Manos. 2008. Is Floral Specialization an Evolutionary Dead-End? Pollination System Transitions in *Ruellia* (Acanthaceae). *Evolution* 62(7): 1712-1737.

Wang, C. N., M. Moller, and Q. C. B. Cronk. 2004. Phylogenetic position of *Titanotrichum oldhamii* (Gesneriaceae) Inferred from four Different Gene Regions. *Systematic Botany* 29: 407-418.

Weber, A. 2004. Gesneriaceae. Pp. 63 – 158 in *The families and genera of vascular plants* vol. 7, Dicotyledons, Lamiales (except Acanthaceae including Avicenniaceae), eds. K. Kubitzki and J. W. Kadereit. Berlin: Springer-Verlag.

Wheeler, Q. D. 1995. Systematics, the Scientific Basis for Inventories of Biodiversity. *Biodiversity and Conservation* 4: 476-489.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplifications and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Pp. 315-322 in *PCR protocols: a Guide to Methods and Applications*, eds. M. A. Innis, D. H. Gelfund, J. J. Sninsky, and T. J. White. Academic Press: San Diego.

Wiehler, H. 1975. *Besleria* L. and the Re-establishment of *Gasteranthus* Benth. (Gesneriaceae). *Selbyana* 1(2): 150-156.

Wiehler, H. 1977. New Genera and Species of Gesneriaceae from the Neotropics. *Selbyana* 2(1): 67-132.

Wiehler, H. 1978. The genera *Episcia, Alsobia, Nautilocalxy,* and *Paradrymonia* (Gesneriaceae). *Selbyana* 5: 11-60.

Wiehler, H. 1983. A Synopsis of the Neotropical Gesneriaceae. *Selbyana* 6: 1-219.