The phylogenetics and biogeography of *Leibnitzia* (Asteraceae: Mutiseae): American species in an Asian genus

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By

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Abstract

THE PHYLOGENETICS AND BIOGEOGRAPHY OF *LEIBNITZIA* CASS. (ASTERACEAE: MUTISIEAE): AMERICAN SPECIES IN AN ASIAN GENUS.

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Evolutionary relationships among the seven genera of the *Gerbera* daisy complex (Asteraceae) remain largely untested due to their well-acknowledged need for taxonomic revision. Here I present a phylogenetic analysis of the *Gerbera*-complex that tests the monophyly of one constituent genus, *Leibnitzia* Cass. (6 spp.). Historically *Leibnitzia* comprised four species distributed from the Himalayan Plateau to eastern Asia. Two montane southwestern North America species, *Leibnitzia lyrata* (Sch.Bip.) Nesom and *L. occimadrensis* Nesom, were subsequently placed in *Leibnitzia* based on the similarity of their achene trichomes. The distribution of these two species overlaps with that of *Chaptalia* Vent., a morphologically similar New World genus of the *Gerbera*-complex. Nuclear (ITS) and chloroplast (*trn*L-*rpl*32 intron) DNA sequence data from accessions of *Leibnitzia*, *Chaptalia*, and other *Gerbera*-complex genera were analyzed in order to test the hypothesis that American *Leibnitzia* are more closely related to *Chaptalia* than to

Asian *Leibnitzia*. Our findings confirm the monophyly of *Leibnitzia* and its remarkable biogeographic disjunction. Asian-American disjunctions are typically observed in temperate forest taxa distributed between eastern Asia and eastern North America. *Leibnitzia*, by contrast, occupies open, semi-arid temperate to sub-tropical montane habitat and is distributed widely across Asian and Central America. To our knowledge, this disjunction is unique in the flowering plant family Asteraceae and provides an interesting new example of the Asian-American disjunction pattern.

Introduction

The generic and specific circumscription of taxa within the *Gerbera*-complex (Asteraceae: Mutisieae) is difficult to ascertain due to cryptic morphological differences among taxa and the sheer number of entities involved. Although individual taxa have undergone numerous recircumscriptions (Katinas, 2004), the monophyly of the group as a whole is generally well-supported morphologically and most recently by limited molecular data (Kim, Loockerman, and Jansen, 2002). The generic circumscriptions, however, remain in flux, and large revisions and reorganization of their consituent species remain necessary even among the larger and better-known genera, such as *Gerbera* (Burkart, 1944; Jeffery, 1967; Hansen, 1985, 1988, 1990; Nesom, 1995; Katinas, 2004). Among these poorly characterized lineages, the American species *Leibnitzia lyrata* (Sch.Bip.) Nesom and *Leibnitzia occimadrensis* Nesom stand out as taxa of particular systematic and biogeographical interest.

The Gerbera-*complex*

Genera in the *Gerbera*-complex are distinguished from other members of tribe Mutisieae by their scapose inflorescence and herbaceous habit (Jeffery, 1967; Hansen, 1985). The *Gerbera*-complex comprises roughly 125 species in 7 [6-8] genera with a global distribution. The two largest genera, *Gerbera* L. (ca. 30 spp.) and *Chaptalia* Vent. (ca. 56

spp.), occur predominantly in temperate and tropical regions of Africa and Asia and mountainous, temperate regions of North and South America, respectively. *Leibnitzia* Cass. (6 spp.) is found in mountainous areas of Asia and the Americas (See Fig. 1.1); *Trichocline* Cass. (ca. 23 spp.) in South America; *Uechtritzia* Freyn (3 spp.) in Asia; and both *Perdicium* L. (2 spp.) and *Piloselloides* L. (2 spp.) in southern Africa (Burkart, 1944; Jeffrey, 1967; Nesom, 1983, 1995; Kim, Loockerman, and Jansen, 2002; Katinas, 2004; Kubitzki, Kadereit, and Jeffrey, 2007). (See Appendix A for further descriptions of *Gerbera*-complex distributions.)

Taxonomy of Leibnitzia lyrata *and* Leibnitzia occimadrensis*:*

The species now known as *Leibnitzia lyrata* (Sch. Bip.) Nesom has a convoluted taxonomic history. In 1967 Jeffery noted that several species of *Chaptalia* resembled members of the Asian genus *Leibnitzia* more closely than other *Chaptalias,* and recognized that future study and revisions were necessary. In his subsequent revisions of *Chaptalia*, Guy Nesom concluded that seven species previously placed in *Chaptalia* (*C. alsophila* Greene, *C. confinis* Greene, *C. leucocephala* Greene, *C. monticola* Greene, *C. potosina* Greene, *C. sonchifolia* Greene, and *C. mexicana* Burkart) and two previously placed in *Gerbera* (*G. seemannii* Sch. Bip. and *G. ehrenbergii* Sch. Bip.) represented a single species belonging to the genus *Leibnitzia*, which he published in 1983 as *Leibnitzia seemannii* (Sch. Bip.) Nesom.

The species *Chaptalia lyrata* D. Don was published in 1830, and at the time was an illegitimate homonym of the species *Chaptalia lyrata* (Willd.) Spreng., which had been published in 1826 and is now known as *Leibnitzia anandria* (L.) Turcz. The entity David Don named *C. lyrata* was later published legally as *Gerbera lyrata* by Karl Heinrich 'Bipontinus' Schultz in 1856. After examining photographs of type specimens of *C. lyrata* D. Don and making detailed observations of the specimens' micromorphology, Nesom determined that *C. lyrata* D. Don and *L. seemannii* (Sch. Bip.) Nesom were the same entity. Nesom considered that *C. lyrata* D. Don was the earliest named taxon that represented the species, and he again published the entity as *Leibnitzia lyrata* (D. Don) Nesom, *comb. nov.*. Due to the illegitimate status of the name *Chaptalia lyrata* D. Don, the authority of *L. lyrata* is currently considered to be (Sch. Bip.) Nesom. In 1983, at the same time that he transferred *C. lyrata* to *Leibnitzia* Nesom also described *Leibnitzia occimadrensis* Nesom, a previously undescribed species of American *Leibnitzia*.

Morphology of the American Leibnitzias

Nesom transferred *L. lyrata* to *Leibnitzia* on the basis of achenial trichome morphology, stating that *L. lyrata* has long, sharp, slender achene trichomes typical of *Leibnitzia*, rather than the "short, inflated, or papillose achenial trichomes," with which he characterizes *Chaptalia*. Katinas (2004) does not agree with Nesom that achene vestiture gives *Chaptalia* coherence as a genus, however both Jeffery (1967) and Hansen (1988) supported the transfer. In particular, Jeffery (1967) called achene pubescence and pappus-hair morphology the "most reliable taxonomic character" for delimiting natural groups

within the *Gerbera*-complex, and stated that in *Leibnitzia* these characters are “sharply distinct” from the rest of the complex.

Nesom distinguished *L. occimadrensis* from *L. lyrata* by the morphology of the leaf blade, apex, and petiole; the number and length of the bracts; the number of nerves on the ligules of the cleistogamous heads; the width of the neck and color of the achenes; the location of the bifurcation of the achenial trichomes; and the color and length of the pappus bristles. Jeffery (1967) and Nesom (1983) suggest that *L. occimadrensis* may be more closely related to *L. anandria* than *L. lyrata* is to *L. anandria* on the basis of casual observation; however, it is difficult to compare the morphology of the three species, as the morphological characteristics reported for *L. anandria* by Hansen (1988) do not include comments of the characteristics that distinguish *L. occimadrensis* from *L. lyrata*.

Apart from achenial trichome characters, Nesom reports that *L. lyrata* has a chromosome number of n = 23 (counts were unavailable for *L. occimadrensis*). *Leibnitzia* is accepted as having n = 23 (Nesom, 1983); *Chaptalia* is usually described as n = 24. Nesom (1983) notes that counts of n =16 (irreproducible by him) were previously reported for *L. lyrata* specimens from Mexico by DeJong and Longpre (1963). Aside from these few differences, the morphology of the two genera, including palynological characteristics (Nesom, 1983; Hansen, 1991; Kubitzki, Kadereit, and Jeffrey, 2007), is highly similar or overlapping. Notably, all six species of *Leibnitzia*, and some species of *Chaptalia* are the only Asteraceae taxa that exhibit dimorphic chasmagomous (vernal) and cleistogamous (autumnal) capitula (Burkart, 1944; Nesom, 1983). Nesom regards this shared, unusual

breeding system as a consequence of parallel evolution rather than inheritance from a common ancestor (Nesom, 1983).

Biogeographic Patterns in Leibnitzia:

Leibnitzia lyrata and *L. occimadrensis* are of biogeographic interest because they are two American members of a predominately Asian genus. These two species, which are sympatric in four locations in northwestern Mexico (Nesom, 1983), are a unique example of the Asian–North American biogeographic disjunction pattern. *Leibnitzia lyrata* occupies a range from the southwestern United States through Mexico and as far south as Guatemala, and both American *Leibnitzia* are sympatric with several species of *Chaptalia*. Although *L. anandria* is known to grow at sea level near the Sea of Japan, the four Asian members of *Leibnitzia* occupy mostly high-altitude (upper limits of 3100-5000 m. above sea level vary by species), semi-arid habitats in the Himalayan region (Tibet, China, Nepal, northern India, Kashmir, and northern Pakistan), China, Japan, Korea, Bhutan, Mongolia, Siberia, and the Kyrgyz Republic (Hansen, 1988).

The Asian–North American biogeographic disjunction pattern is well characterized among plant groups (Wen, 1999, 2001; Nie, Wen, and Sun, 2007). To date this pattern has been seen mostly in temperate mesic forest taxa, most likely as a result of the disintegration and fragmentation of the high-latitude temperate forests of the Tertiary period and Pleistocene era, and usually occurs between eastern Asia and eastern North America. A few exceptions to the general pattern have been identified, and include

disjunctions in temperate taxa with a few tropical and subtropical species (e.g., *Magnolia* (Magnoliaceae) and *Osmanthus* (Oleaceae)), eastern Asia/eastern North American/western North American disjuncts (e.g., *Aralia* (Araliaceae)), and broader Asian – North American disjuncts (e.g., *Illicium* (Illiciaceae) and *Itea* (Grossulariaceae)); Wen, 1999, 2001). No other composites exhibiting this type of disjunction pattern have been identified, presumably because of the family's proclivity for occupying open habitats (Funk et al., 2004). *Leibnitzia* occupy rocky, semiarid habitats in high elevation areas of the Himalayas, continental and eastern Asia, and the Americas from the southwestern United States to Guatemala (Nesom, 1983; Hansen, 1988). Due to the difference in habitat, it seems likely that the disjunction in *Leibnitzia* does not derive from the same type of vicariance as other taxa exhibiting the Asian–North American disjunction; rather, it may be the result of long-distance dispersal or migration across the Bering land bridge and south along the Rocky Mountains.

Molecular Systematics

To date, there has been little research done into the molecular systematics of the *Gerbera*-complex. Work by Kim et al. (2002) explored broad phylogenetic patterns within tribe Mutisieae and has confirmed that the *Gerbera*-complex is a well-supported monophyletic group comprising *Chaptalia, Leibnitizia, Gerbera,* and *Piloselloides*. However, given that the study focused on the Mutisieae as a whole rather than on the *Gerbera*-complex specifically, the sample group did not contain enough representatives to thoroughly test the monophyly of the individual genera within the *Gerbera*-complex.

The Kim et al. (2002) study included *L. lyrata* (circumscribed as *Leibnitzia seemanii* Nesom), but no other members of *Leibnitzia* were included in the study. Parsimony analysis of chloroplast *ndh*F sequence data from four representative species of *Chaptalia* (*C. tomentosa, C. escarpa, C. nutans,* and *C. lyratifolia*) in the Kim et al. (2002) study indicated that *Chaptalia* is paraphyletic. Analysis also indicated that *Gerbera* and *Piloselloides* appeared monophyletic and should be merged into one genus sensu Hansen (1990). However, this monophyly was only weakly supported by parsimony bootstrap values (0.53) and not supported by a strict consensus tree, where a polytomy was formed between *Gerbera, Piloselloides, Leibnitzia,* and three of four included species of *Chaptalia*. Furthermore, a single species of each genus (*G. jamesonii* and *P. hirsuta*) was included in the study, leaving the possibility that this apparent monophyly contains two distinct but sister monophyletic clades. Kim et al. (2002) agree that increased taxon sampling and further molecular data is needed before taxonomic and evolutionary relationships within the *Gerbera*-complex can be resolved.

Goals of Study

This study continues the work of Kim et al. (2002) and Nesom (1983) by providing further molecular data regarding the taxonomic and evolutionary relationships of the *Gerbera*-complex. In order to further identify the unclear taxonomic and evolutionary relationships in the *Gerbera*-complex, particularly those of the genus *Leibnitzia,* sequences of nuclear and choroplast DNA were analyzed from herbarium and freshly collected material of representative species. Specific hypotheses tested were: (1)

Leibnitzia lyrata and *L. occimadrensis* are more closely related to Asian species of *Leibnitzia* than other members of the *Gerbera*-complex; (2) *Leibnitzia lyrata* and *L. occimadrensis* are more closely related to each other than to the Asian *Leibnitzia*, indicating one immigation event; and (3) The current circumscription of the genus *Leibnitzia* reflects a natural, monophyletic group.

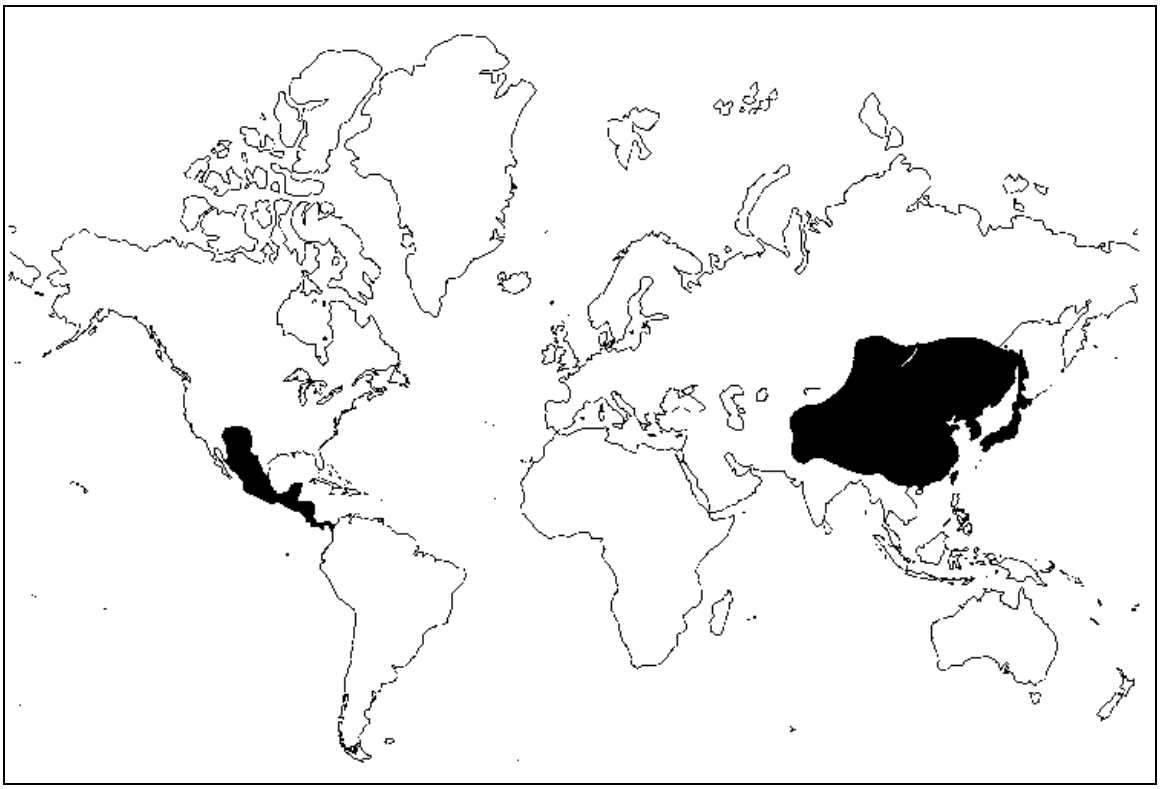

Figure 1.1: Distribution Map of *Leibnitzia*

This map describes the approximate range and distribution of *Leibnitzia* shaded in black. World map outline obtained from www.bristolstories.org/resources.php.

Materials and Methods

Taxon Sampling

Accessions of 19 *Gerbera*-complex taxa comprising five genera (*Leibnitzia* Cass., *Chaptalia* Vent., *Piloselloides* L., *Gerbera* L., and *Trichocline* Cass.) were obtained for study (see Table 1.1). *Leibnitzia* accessions included two Asian species (*L. nepalensis and L. anandria*) and two American species (*L. lyrata* and *L. occimadrensis*). Four of seven *Chaptalia* taxonomic sections were represented by seven species (*C.* sect. *Euchaptalia*: *C. tomentosa, C. pringlei; C.* sect. *Archichaptalia: C. cf. cordata; C.* sect. *Leiberkuhna: C. runcinata, C. mandonii; C.* sect. *Leria: C. similes, C. nutans*). Other *Gerbera*-complex taxa include one species of *Piloselloides* (*P. hirsuta*), one species of *Gerbera* (*G. gossypina*), and four species of *Trichocline* (*T. aurea, T. macrocephala, T. catharinesis, T. speciosa*). Two accessions of *Mutisea orbignyana* Wedd. (sub-tribe Mutisineae) were selected as an out-group following the work of Funk et al. (2002). I attempted to extract DNA from many additional accessions (see Appendix B) to achieve a more extensive sampling but was unable to amplify gene regions from these specimens.

Marker Selection

The internal transcribed spacer (ITS) region of the 18S – 26S nuclear ribosomal subunit was selected as the nuclear marker because the ITS 1 and ITS 2 regions have provided

adequate resolution for closely related taxa in numerous studies of Asteraceae and other families (Baldwin, 1992; Baldwin et al., 1995; Hoggard et al., 2004; Levin et al., 2004). Both spacers amplify well from herbarium material (Baldwin et al., 1995) and occur in high numbers of uniform copies in the genome, further facilitating PCR amplification. Chloroplast sequence data has been key in identifying evolutionarily significant groups in family Asteraceae (Funk et al., 2002). The chloroplast intron situated between the *trn*L (UGA) gene, which encodes for leucine, and the *rpl*32 gene, which encodes for ribosomal protein L32, was selected for use as the chloroplast marker in the present study. Unlike ITS, this marker has not been widely used in phylogenetic studies, even within the Asteraceae family. However, here it provided excellent resolution found lacking in other chloroplast markers due to its highly divergent nature (Timme et al., 2007). Other chloroplast regions (*ndhC-trnV* and *trnY-trnE-rpoB*) shown to be hypervariable in Asteraceae (Timme et al., 2007) failed to amplify as regularly as the *trn*L-*rpl*32 intron and were subsequently not considered for use in this study. The 3' end of the chloroplast gene *ndh*F was originally amplified as a chloroplast marker based on previous studies (Jansen, 1992; Kim, Loockerman, and Jansen 2002), but failed to provide adequate resolution at the generic level as the polytomies present in the Kim et al. (2002) dataset did not resolve with increased taxon sampling. The *trn*L-*trn*F intergenic spacer region was also tested, but also failed to provide adequate resolution.

DNA Extraction, Amplification, and Sequencing

DNAs were extracted from leaf tissue of herbarium specimens and silica-dried samples using a FastDNA® Spin Kit (BIO101 Systems, La Jolla, CA) according to the

manufacturer's instructions. Polymerase Chain Reaction (PCR) was achieved using a 25.0 uL mix of 12.9 uL sterile, de-ionized H_2O, 2.5 uL of 10x buffer (New England Bio Labs), 2.5 uL of 25mM $MgCl_2$, 2.0 uL of 10 mM dNTPs, 1.0 uL each of both the forward and reverse primers (10 uM), 0.5 uL of 10 mg/mL Bovine Serum Albumin (BSA), 0.1 uL of 5 u/uL *Taq* polymerase (New England Bio Labs), and 2.5 uL of template DNA. Amplification of ITS 1, the 5.8S ribosomal subunit, and ITS 2 were completed with ITS1a, ITS2b, ITSp3, and ITSp4 primers (Baldwin, 1992; Baldwin et al., 1995; Hoggard et al., 2004; Levin et al., 2004) and amplified under conditions of 40 cycles of 1 min. at 94°C, 1 min. at 54°C, and 2 min at 72°C followed by a final extension period of 10 min. at 72°C. The *trn*L-*rpl*32 intron was amplified with primers published by Timme et al. (2007) using a touchdown PCR protocol of 6 cycles of 1 min. at 94°C, 1 min. at 58°C minus 1°C for each successive cycle, and 2:30 min. at 72°C followed by 34 cycles annealing at 52°C for 1 min.. To improve or achieve amplification, some DNA samples required additional purification, the addition of a hot-start prior to amplification (3 min. at 95°C and 6 min. at 80°C) or both. The Ultra Clean™ DNA Purification Kit (Mo BIO, San Diego, CA) was used for DNA purification according to the manufacturer's instructions. Primer sequences are listed in Table 1.2.

Amplified PCR products were quantified on 1% agarose gels and cleaned with the exo-sap method (Dugan et al. 2002) to eliminate single-stranded DNA fragments and excess nucleotides. Exo-sap reaction mix comprised 0.5 uL of 10 U/uL of exonuclease I, 1.0 uL of 1 U/uL shrimp alkaline phosphatase (SAP), and 1.5 uL of de-ionized water per 25uL

of PCR product. Exo-sap thermocycler protocol followed that of Dugan et al. (2002): 30 min. at 37°C and 15 min at 80°C. Samples were bidirectionally sequenced by Macrogen USA, Inc. (Bethesda, Maryland, USA).

Sequence Alignment and Phylogenetic Analyses

Bidirectional sequence strands were assembled and edited in Sequencher v. 4.0 (Gene Codes) and aligned and gap coded manually according to the protocol of Ochoterena and Simmons (2000) in MacClade v. 4.0 (Maddison and Maddison 2000). Aligned datasets of ITS and the *trn*L-*rpl*32 intron were tested for contrasting phylogenetic signal (excluding gap characters) using the incongruence length difference test (ILD; Farris et al., 1994) as implemented by PAUP* v. 4.0 beta v. 10 (Swofford, 2002). ILD parameters were set for the partition-homogeneity test with 100 partition-homogeneity replicates, the tree-bisection-reconnection (TBR) branch swapping algorithm with multitrees in effect, and gaps treated as missing data.

Parsimony analysis was conducted in PAUP* for individual and combined datasets using a heuristic search including 1000 random addition replicates, tree-bisection reconnection branch swapping with multitrees in effect, and gaps treated as missing data. Non-parametric bootstrap tests of the data used 1000 pseudoreplicates within PAUP*. Decay indices were determined using TreeRot.v3 (Sorenson and Franzosa, 2007). Bayesian inference of the individual and combined datasets was conducted using the Metropolis Coupled Markov Chain Monte Carlo simulation program, MrBayes v. 3.1.2

(Huelsenbeck and Ronquist, 2001). The best fitting model of sequence evolution for the nuclear and chloroplast datasets were determined using the Akaike model criterion as implemented by MrModeltest v. 2.2 (Nylander and J.A.A., 2004). The model selected for all nucleotide datasets was the general time reversible model (GTR) with a proportion of invariant characters (I) and rate variation as described by the Γ shape parameter (G). The combined, gap coded dataset was run in MrBayes as a partitioned dataset with the GTR + I + G model for both the nuclear and chloroplast sequences and a binary or restriction site model for the gap coded data. The *lset coding=informative* command was used as only parsimony informative characters were scored in the gap coded dataset. Analyses were run for 10 million generations, saving trees every 1000 generations. Preliminary analyses indicated that statitionarity was reached after 1 million generations (average split deviations between parallel runs < 0.01), thus a burnin of 10,000 trees was used for subsequent analyses. Graphs of the cumulative posterior probabilities of the most variable splits among post-burnin trees (AWTY, http://ceb.csit.fsu.edu/awty, Nylander et al., 2008) confirmed stationarity.

Biogeographic Analysis

Biogeographic analysis followed the protocol of Funk et al. (2009). Distributions were mapped onto the tree manually followed by Bremer's 'Ancestral Area' analysis (1992, 1994).

Data were additionally evaluated with Dispersal Vicariance Analysis (DIVA) v. 1.1 to estimate ancestral distribution of clades within the *Gerbera*-complex. The number of

allowed ancestral areas was limited to three with the “maxareas” optimize command (Ronquist, 1996).

Table 1.1: Sampled Taxa and Accession Information.
Continued on page 15.

Sample	Synonyms	Collector No.	Source	Location & Date	GenBank No.
*Leibnitzia lyrata (*Sch.Bip.) Nesom	Syn: *L. seemanii* (Sch. Bip.) Nesom, *Chaptalia seemanii* Sch. Bip.	G. Nesom 24778	ARIZ	Choise Co., Arizona, USA 2001	
Leibnitzia lyrata (Sch.Bip.) Nesom	Syn: *L. seemanii* (Sch. Bip.) Nesom, *Chaptalia seemanii* Sch. Bip.	G. Nesom 3388	ARIZ	Choise Co., Arizona, USA 1990	
Leibnitzia lyrata (Sch.Bip.) Nesom	Syn: *L. seemanii* (Sch. Bip.) Nesom, *Chaptalia seemanii* Sch. Bip.	J.L. Reveal, W.J. Hess 3104	US	Durango, Mexico 1972	
Leibnitzia lyrata (Sch.Bip.) Nesom	Syn: *L. seemanii* (Sch. Bip.) Nesom, *Chaptalia seemanii* Sch. Bip.	J.M Holzinger s.n.	US	SW New Mexico, USA 1911	
Leibnitzia occimadrensis Nesom	n/a	G. Nesom 153	ARIZ	Sonora, Mexico 1992	
Leibnitzia occimadrensis Nesom	n/a	G. Nesom sn	ARIZ	Sonora, Mexico 1992	
Leibnitzia occimadrensis Nesom	n/a	H.S. Gentry 7189	US	Los Pucheros Sierra Surotato, Mexico 1945	
Leibnitzia anandria (L.) Turcz	n/a	Liu 890185	US	China	
Leibnitzia nepalensis (Kunze) Kitamura	Syn: *Gerbera kunzeana* A.Braun & Asch.	MacAnter, Thibet 542	US	TAR, China 2006 or 2007 *leaf sample packet not dated	
Chaptalia mandonii (Sch. Bip.) Burkart	n/a	P.M. Simon 438	US	Buenos Aires, Argentina 2002	
Chaptalia runcinata Kuntze	*Thyrsanthema runcinata* Kuntze	P.M. Simon 415	US	Buenos Aires, Argentina 2000	
Chaptalia nutans (L.) Polak	Syn: *Tussilago nutans* L., *Leria leiocarpa* DC. Prodr. (DC.)	P.M. Simon 477	US	Argentina date unknown	
Chaptalia similis R.E.Fr.	n/a	P.M. Simon 711	US	Unknown	
Chaptalia pringlei Greene	n/a	G. Nesom 4405	US	Tamazulapan, Mexico 1981	

Table 1.1: Sample Taxa and Accession Information, continued from page 14.

Sample	Synonyms	Collector No.	Source	Location & Date	GenBank No.
Chaptalia c.f. *cordata*	indet to sp.	I. Sanchez V., M. Cabanillas S.	F	Cajamarca, Peru 1994	
Chaptalia hintonii Bullock	n/a	G. Nesom 4409	US	Taxco, Mexico 1981	
Chaptalia tomentosa Vent.	n/a	V. Funk 12303	US	SE USA 2002	
Gerbera crocea Kuntze	Syn: *Gerbera burmani* Cass.,		US?		
Gerbera gossypina Beauverd	G. integralis Sond. ex Harv., G. sinuata Spreng.	W. Koelz 4294	US	Punjab, NW India 1933	
Mutisea orbigynana Wedd.	n/a	P. Simon, M. Bonifacino 575	US	Jujuy, Argentina 2001	
Mutisea orbigynana Wedd.	n/a	Marko Lewis 871211	US	La Paz, Bolivia 1987	
Piloselloides hirsuta (Forsk.) C.Jeff.	n/a	M. Koekemoer 2125	US	Mpumalanga, South Africa 2001	
Trichocline aurea (D.Don) Reidre	n/a	F. Hellwig 9094	TEX/LL	Chile 1987	
Trichocline catharensis var. *discolor* Cabr.	n/a	O.S. Ribas, J. Cordeiro, E. Barbosa	TEX/LL	Santa Catarina, Brazil 1998	
Trichocline macrocephala Less.	n/a	E. Barbosa, G. Hatschbach, O.S. Ribas 109	TEX/LL	Parana, Brazil 1998	
Trichocline speciosa Less.	n/a	J.M. Cruz, J. Cordeiro, V. Carre 84	TEX/LL	Parana, Brazil 1999	

Table 1.2: Primer Sequences

Marker	Primers	Sequence
ITS 1	ITS 1a	GGAAGGAGAAGTCGTAACAAGG
	ITS 2b	CTCGATGGAACACGGGATTCTGC
ITS 2	ITS p3	GCATCGATGAAGAACGCAGC
	ITS p4	TCCTCCGCTTCTTGATATGC
trnL-rpl32	trnLretF	TACCGATTTCACCATCGCGG
	rpl32retR	AGGAAAGGATATTGGGCGG

Results

Several accessions collected for inclusion in the study failed to either yield DNA or to amplify despite all attempts, resulting in a more restricted sample group than originally planned. (See Appendix B.) It is probable that future studies could avoid these difficulties with the use of fresh leaf material, as our freshest material easily yielded large amounts of DNA that amplified cleanly with few issues.

Phylogenetic Statistics and Alignment Description

General phylogenetic statistics for each dataset are shown in Table 1.3. The ITS dataset was 705 basepairs long, 26.67% of which were parsimony informative characters. The *trn*L-*rpl*32 dataset was 1074 basepairs long, of which 3.72% were parsimony informative characters. The combined dataset included 1780 basepairs and 54 gap-characters for a total length of 1834 characters, of which 15.16% were parsimony informative. All sequences will be deposited into GenBank at the conclusion of this study.

The nucleotide dataset was straightforward and easy to align, while alignment of the chloroplast dataset was made slightly more difficult by a 375 basepair-long region of

insertions in a few of the sequences. One specimen of *M. orbignyana*[1] contained a 330 basepair-long insertion spanning basepair position (bp) 397 to bp 753 (bp 397-643, 660-669, 672-716, 722-753). *Trichocline* specimens showed similar insertions in this region of the alignment that are comprised mostly of identical, homologous sequences. *Trichocline aurea* had a 276 basepair-long insertion from bp 483 to bp 745, bp 757 to bp 760, and bp 762-772. The rest of *Trichocline* had a 272 basepair-long insertion from bp 483 to bp 643 and from bp 660 to bp 772. These insertions greatly increased the length of the *trn*L-*rpl*32 alignment and required rough positioning in the "Bird's Eye View" data matrix style option in MacClade in order to align these regions with the rest of the study group. Following this approximate positioning, detailed adjustments made in the regular, "Molecular" data matrix style option as necessary. The *trn*L-*rpl*32 alignment was elsewhere characterized by smaller insertions, resulting in window-like gaps in the dataset that also added to the length of the *trn*L-*rpl*32 alignment and subsequent low percentage of parsimony-informative characters. The regular, clean, and, by all appearances, homologous gaps in this dataset in particular suggested the use of gap-coding in the analysis.

Nuclear and Chloroplast Phylogenies

Individual nuclear (Fig. 1.2) and chloroplast (Fig. 1.3) trees are generally characterized by lower support values, but topologies are generally consistent with those obtained from

[1] The presence of this insertion in one specimen of *M. orbignyana* (871211) and not the other (575) raises doubts about the correct determination of one or the other *M. orbignyana* accessions and should be investigated further. The sequences from these accessions will not be published to GenBank until their identifications can be confirmed, but as both taxa were consistent in their outgroup positions, we have determined that this sequence variation has no material affect on the results presented here.

the combined datasets. The nuclear 50% majority rule parsimony tree (Fig. 1.2) is identical to the combined 50% majority rule parsimony tree (Fig. 1.5) in topology, but has lower support values and excludes *L. lyrata* 1911, as nuclear sequences for this accession were not obtained (likely due to age of the leaf material, which was almost 100 years old). The chloroplast 50% majority rule parsimony tree (Fig. 1.3) is unique in that it shows *L. nepalensis* as more closely related to *Gerbera gossypina* (0.74 PP) and *Piloselloides hirsuta* than to the American *Leibnitzia*, albeit with no bootstrap support for either sister group relationship and no Bayesian posterior probability support for the relationship to *Piloselloides.* This unique configuration may be due to the exclusion of *L. anandria* from the dataset, as *trn*L-*rpl*32 sequence data was not obtained for this species. However, Incongruence Length Difference (ILD) tests confirmed that there was no significant difference between the phylogenetic signals of the chloroplast and nuclear datasets ($p = 0.860$). Topological differences were not significantly supported; consequently, the focus of this report will be on results from combined datasets.

Combined Phylogeneies

Several commonalities exist among all resulting trees from parsimony (See figures 1.5, 1.6) and Bayesian analyses (See figure 1.4) of the combined dataset. The monophyly of the *Gerbera*-complex is strongly supported (parsimony bootstrap (PB) = 100%, Bayesian posterior probability (PP) = 1.0). *Trichocline* is established as a monophyletic genus (PB = 100%, PP = 1.0) with a consistent set of nested relationships among its species and is the sister group to the rest of the *Gerbera*-complex. *Gerbera*, *Piloselloidies, Leibnitzia*,

and *Chaptalia* form a well-supported clade (PB = 100%, PP = 1.0) within which species of *Leibnitzia* (PB = 57%, PP = 0.92) and *Chaptalia* (PB = 100%, PP = 1.0) consistently resolve into distinct monophyletic groups.

Phylogenetics of the Gerbera*-complex:*

The relationships among the four genera within the *Gerbera-Piloselloides-Leibnitzia-Chaptalia* clade are less well-resolved than those of the above clades. In the 50% majority rule consensus tree (Fig. 1.5), sister group relationships appear between *Gerbera crocea* and *Piloselloides hirsuta* and between *Leibnitzia* and *Chaptalia. Gerbera gossypina* is inserted as a sister group to the *Leibnitzia-Chaptalia* clade, all of which is then a sister group to the *G. crocea-Piloselloides* clade, thus the *Leibnitzia-Chaptalia* clade is in a more derived position on the tree. In the bootstrap consensus (Fig. 1.5) and Bayesian analysis (Fig. 1.4) the *G. crocea-Piloselloides* sister group maintains moderate to significant support (PB = 67%, PP = 0.99) while the other relationships collapse. In the strict consensus (Fig 1.6) all of these relationships collapse and *Leibnitzia*, *Chaptalia*, *G. gossypina*, and the *G. crocea-Piloselloides* clade form a polytomy.

Phylogenetics of Leibnitzia *and placement of the American species*

Leibnitzia nepalensis consistently appears as the sister group to the rest of the genus with variable support (PB = 57%, PP = 0.92). *Leibnitzia anandria* is the sister group to a monophyletic American clade in all trees (PB = 99%, PP = 1.0). In the parsimony bootstrap consensus and Bayesian trees, *L. lyrata* resolves into a weakly supported

monophyletic group (PB = 60%, PP = 0.65), which is the sister group to a strongly monophyletic *L. occimadrensis* (PB = 100%, PP = 1.0). In the strict consensus *L. lyrata* is not monophyletic and the *L. occimadrensis* clade is nested within a *L. lyrata* polytomy. When gap characters are excluded from the analysis, the topology of the American species and their relationship with *L. anandria* is different (tree not shown). The non-gap coded dataset shows *Leibnitzia lyrata* as paraphyletic, with *Leibnitzia lyrata*1911 inserted as sister to the rest of the American clade, although this could be a consequence of missing nuclear data for this accession. The remainder of the clade forms a polytomy between the other three *L. lyrata* specimens and a monophyletic *L. occimadrensis*. Furthermore, the strict consensus of this dataset collapses the sister group relationship between the American clade and *L. anandria*, forming a polytomy that is punctuated only by the consistently monophyletic *L. occimadrensis*.

Phylogenetics of Chaptalia

A predominant feature of all trees is a moderate to strongly supported (PB = 70%, PP = 0.99) monophyletic clade within *Chaptalia* comprised of *C. mandonii*, *C. nutans*, *C. tomentosa*, and *C. similis*. The *C. similis* clade consists of a sister relationship between *C. mandonii* and *C. nutans* (PB = 52%, PP = 0.63), which is then a sister group of *C. tomentosa* (PB = 97%, PP = 1.0), all of which is a sister group to *C. similis.* In the strict consensus tree the sister relationship between *C. mandonii* and *C. nutans* collapses and forms a polytomy with *C. tomentosa*, however, the sister relationship of *C. similis* to the rest of the clade holds firm. The 50% majority rule tree places *C. cordata* as the sister

group to *C. pringlei* and this pair as the sister group to the *C. similis* clade. However, in the bootstrap and the strict consensus trees the sister relationship between *C. cordata* and *C. pringlei* collapses and these taxa form a polytomy with *C. runcinata* and the *C. similis* clade. Bayesian analysis shows a nested topology with *C. runcinata* as the sister group to the C. *similis* clade (PP = 0.50). *Chaptalia pringlei* is then the sister group to this clade (PP = 0.84) and *C. cordata* is inserted as the sister group to the rest of the genus (PP = 1.0).

Biogeographic Results

Ancestral area analysis results support a South American origin for the Mutisieae, as reported by Funk and Pareno (2008). The analysis indicates a western Asia origin for *Leibnitzia* and a South American origin for *Chaptalia* (refer to Tables 1.4-1.6). DIVA results (Fig. 1.7) support those found by ancestral area analysis.

Table 1.3: Molecular Alignment Statistics

Alignment	**Total Characters**	**# Base Pairs**	**# Non-parsimony informative variable characters**	**# Parsimony informativ characters**
nrDNA *ITS 1, 5.8S gene,* ITS *2*	705	705	63	188 (26.67%)
cpDNA *trnL-rpl32 intron*	1074	1074	59	40 (3.72%)
Combined, gap coded	1834	1780	125	278 (15.16%)

Table 1.4: *Gerbera*-complex Ancestral Area Analysis

Area	**Gains**	**Losses**	**Gains/Losses**	**AA**
South America	6	5	1.20	1.00
Africa	1	4	0.25	0.21
South Asia	2	5	0.40	0.33
West Asia	4	5	0.80	0.67
East Asia	2	7	0.29	0.24
North America	5	10	0.50	0.42
Central America	3	10	0.30	0.25
Caribbean	1	10	0.10	0.08

Table 1.5: *Leibnitzia* Ancestral Area Analysis

Area	**Gains**	**Losses**	**Gains/Losses**	**AA**
West Asia	2	1	2.00	1.00
East Asia	1	2	0.50	0.25
North America	1	3	0.33	0.17
Central America	1	2	0.50	0.25

Table 1.6: *Chaptalia* Ancestral Area Analysis

Area	**Gains**	**Losses**	**Gains/Losses**	**AA**
South America	4	1	4.00	1.00
North America	4	3	1.33	0.33
Central America	3	3	1.00	0.25
Caribbean	1	5	0.20	0.05

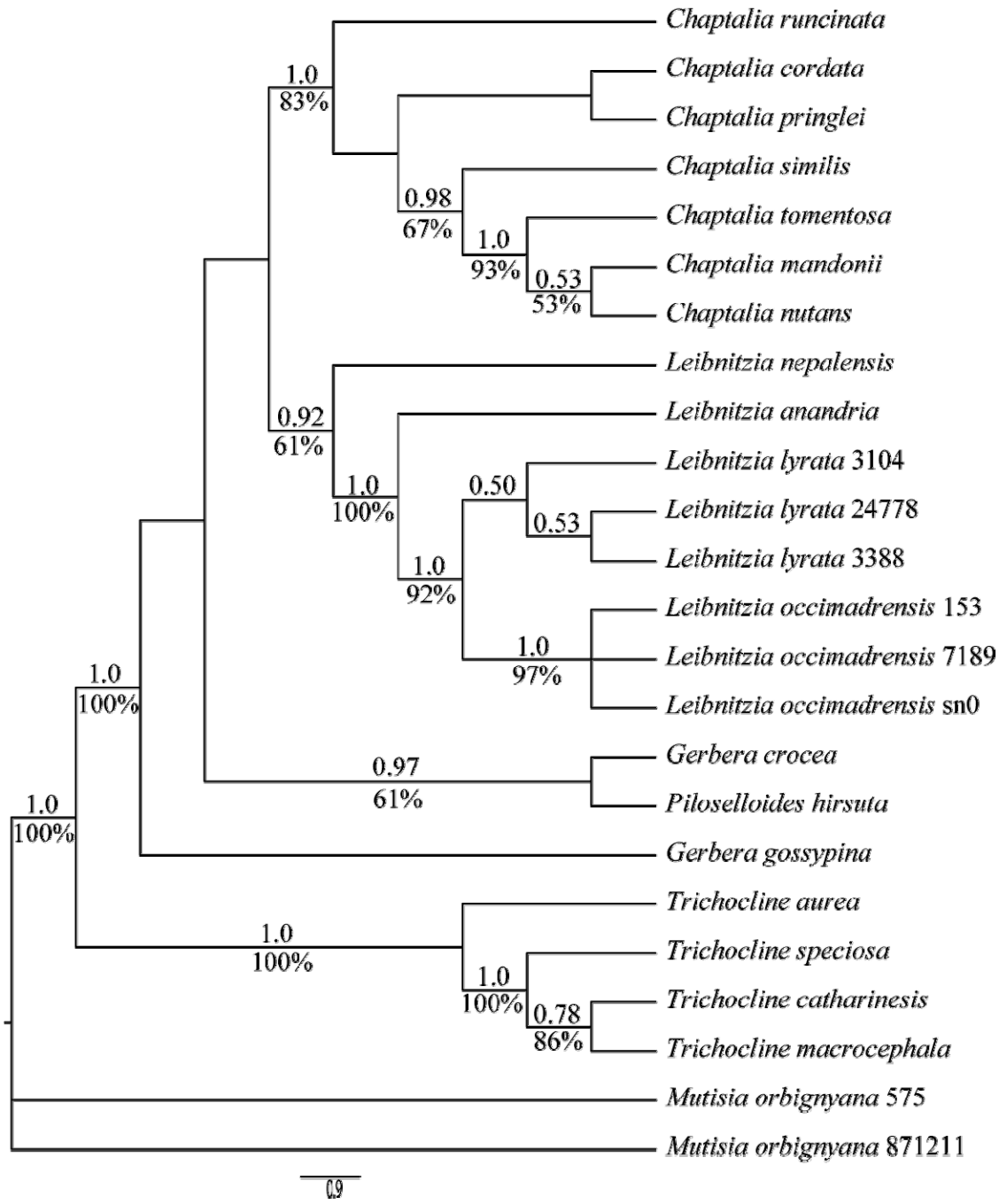

Figure 1.2: Nuclear 50% Majority Rule Tree

50% majority rule tree obtained from parsimony analysis of the gap-coded ITS 1 and 2 dataset. Bayesian posterior probability values appear above branches and bootstrap values over 50% appear below.

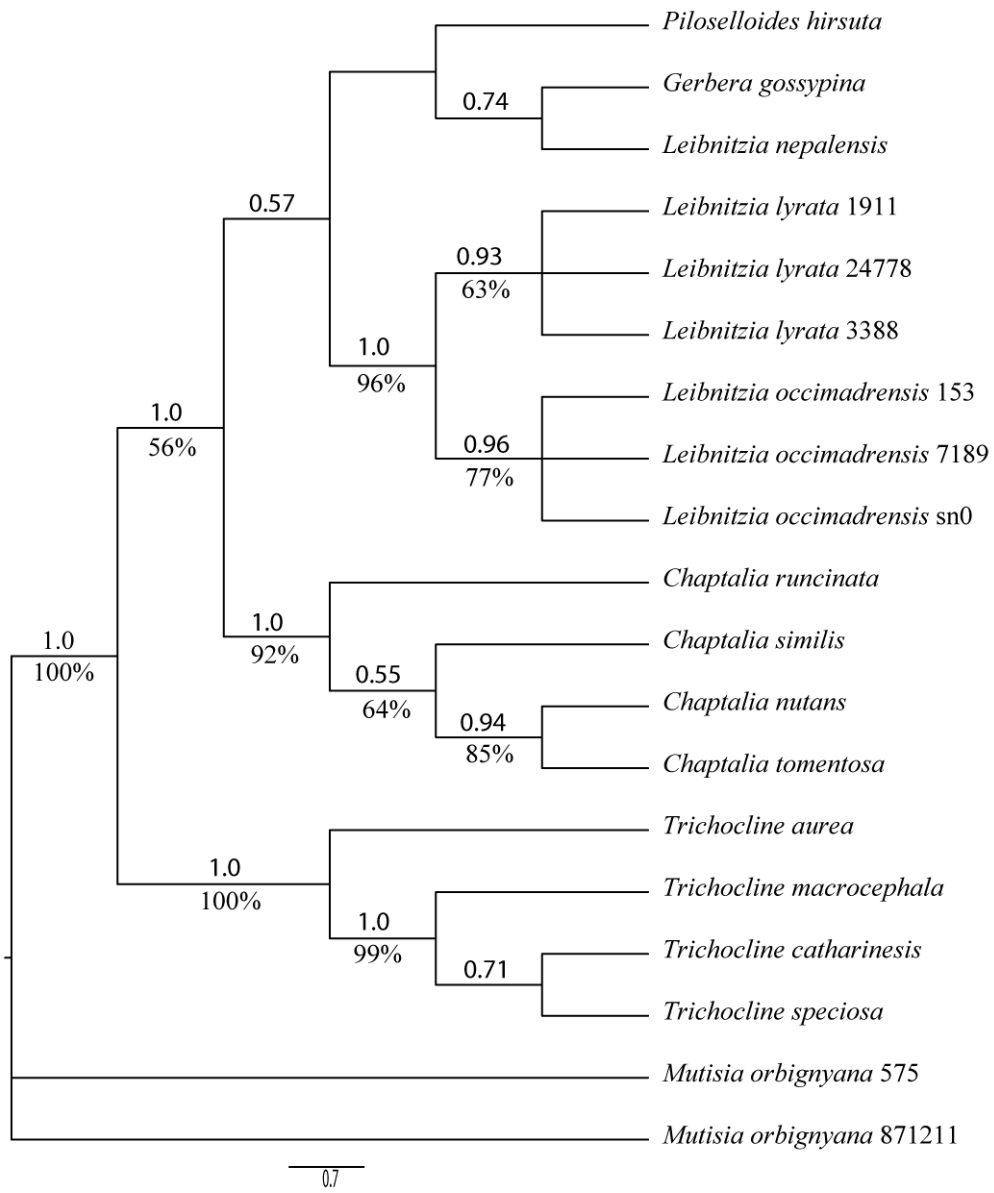

Figure 1.3: Chloroplast 50% Majority Rule Tree

50% majority rule tree obtained from parsimony analysis of the gap-coded *trn*L-*rpl*32 intron dataset. Bayesian posterior probability values appear above branches and bootstrap values over 50% appear below.

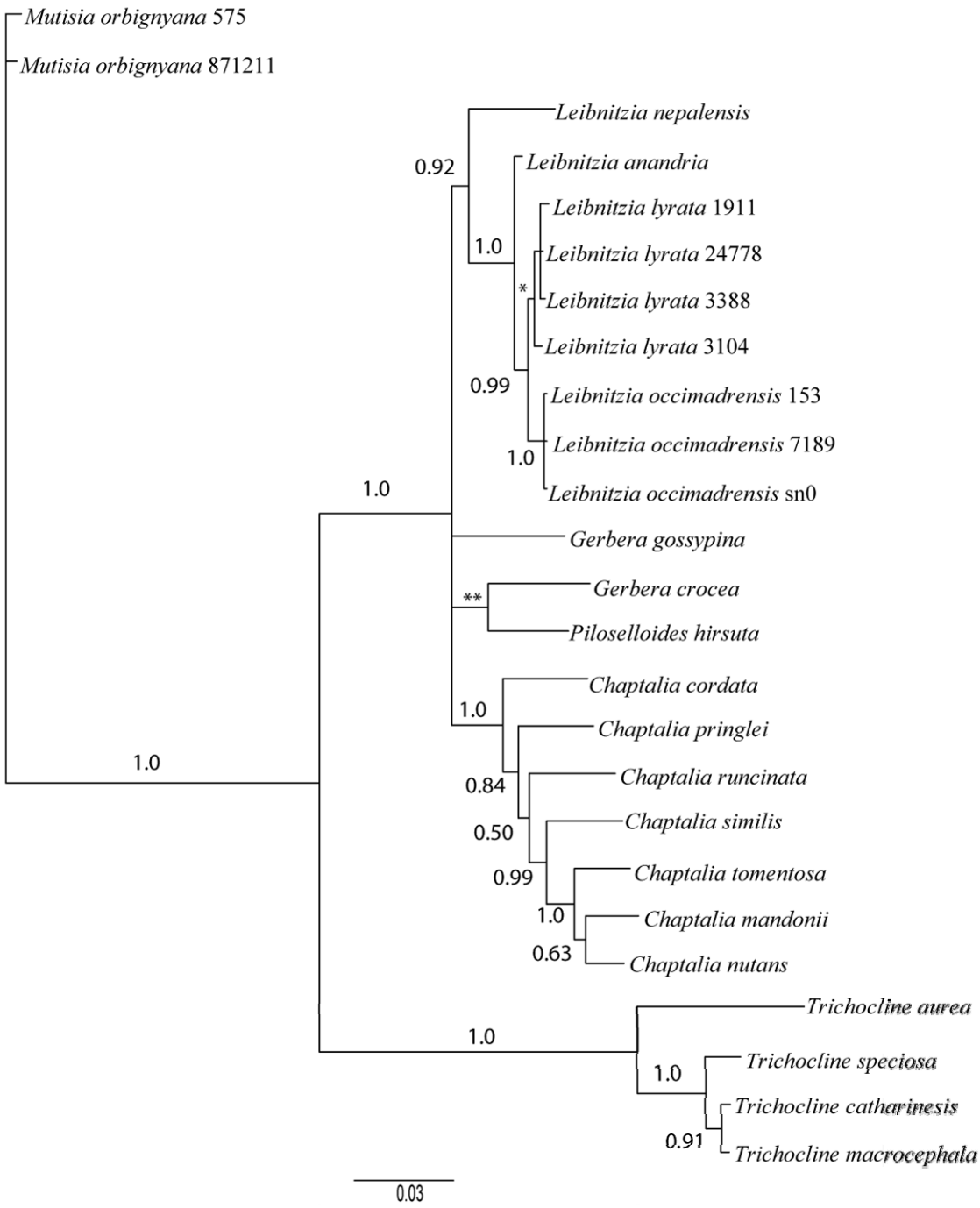

Figure 1.4: Combined Bayesian Tree

Tree obtained from Bayesian analysis of the gap-coded ITS 1 and 2 and *trn*L-*rpl*32 intron dataset showing all Bayesian posterior probabilities. *0.65 **0.99

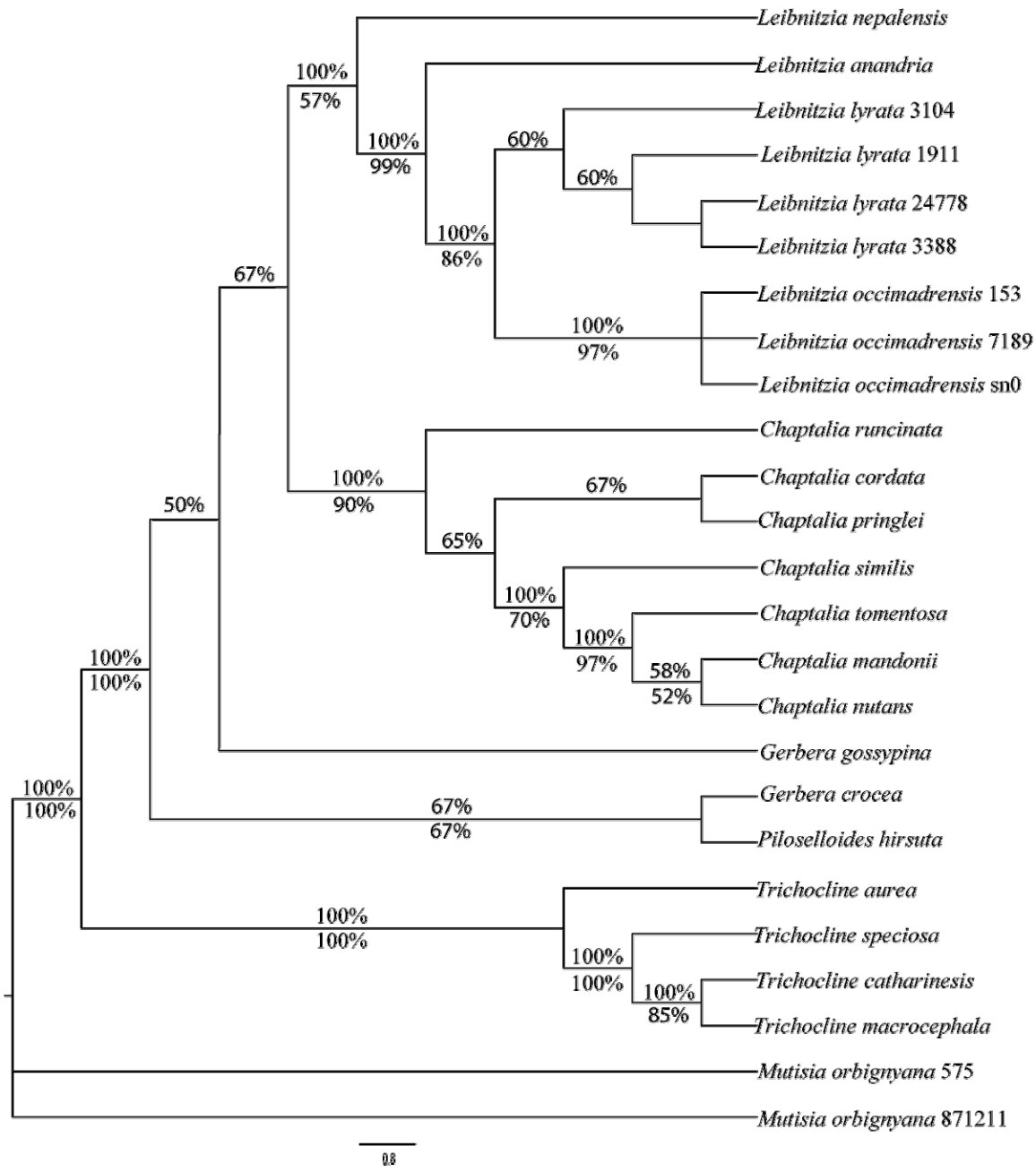

Figure 1.5: Combined 50% Majority Rule Tree

50% majority rule tree obtained from parsimony analysis of the combined, gap-coded ITS 1 and 2 and *trn*L-*rpl*32 intron dataset. Parsimony consensus values appear above branches and bootstrap values over 50% appear below the branches.

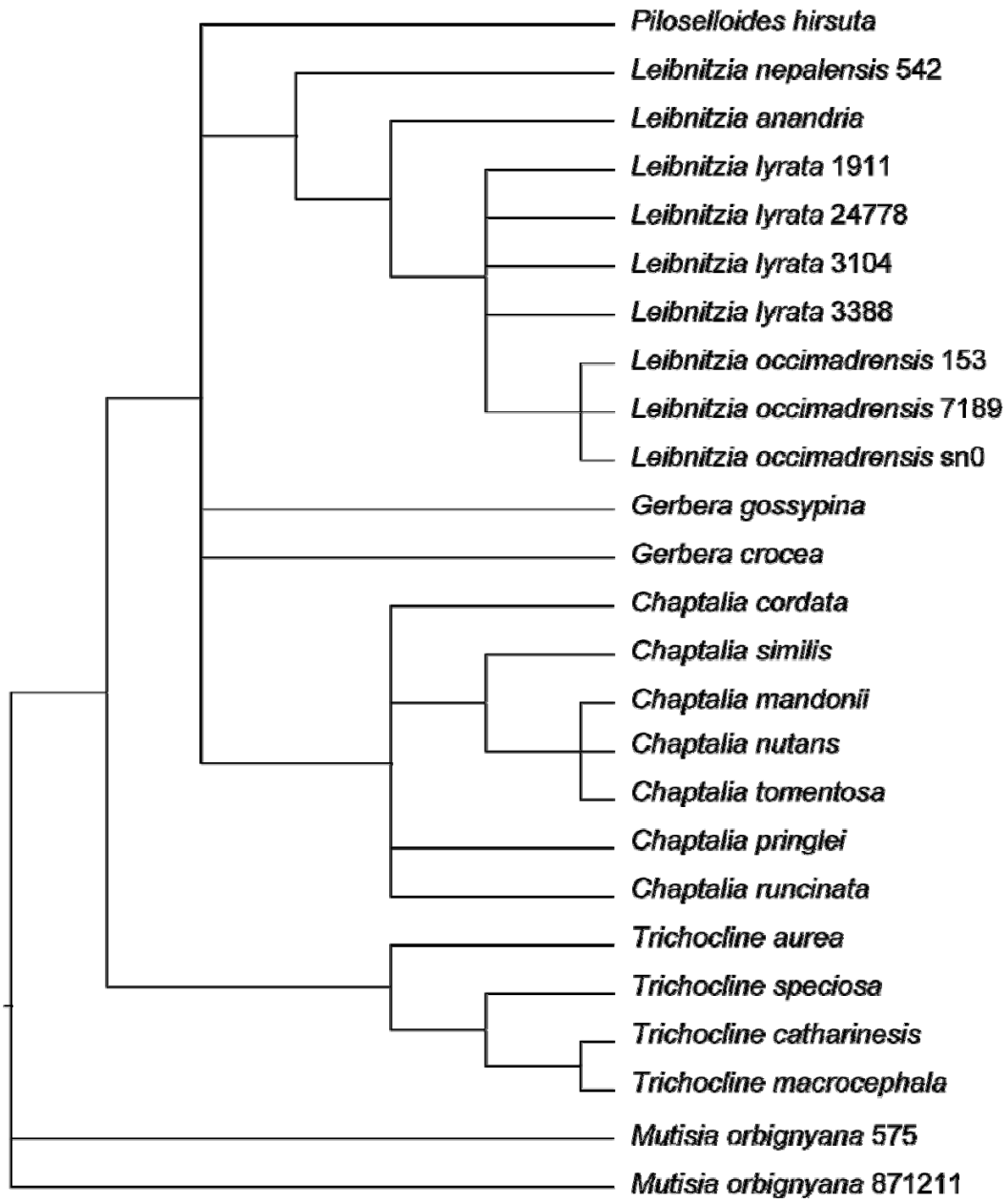

Figure 1.6: Combined Strict Consensus Tree

Strict consensus tree obtained from parsimony analysis of the combined gap-coded ITS 1 and 2 and *trn*L-*rpl*32 intron dataset.

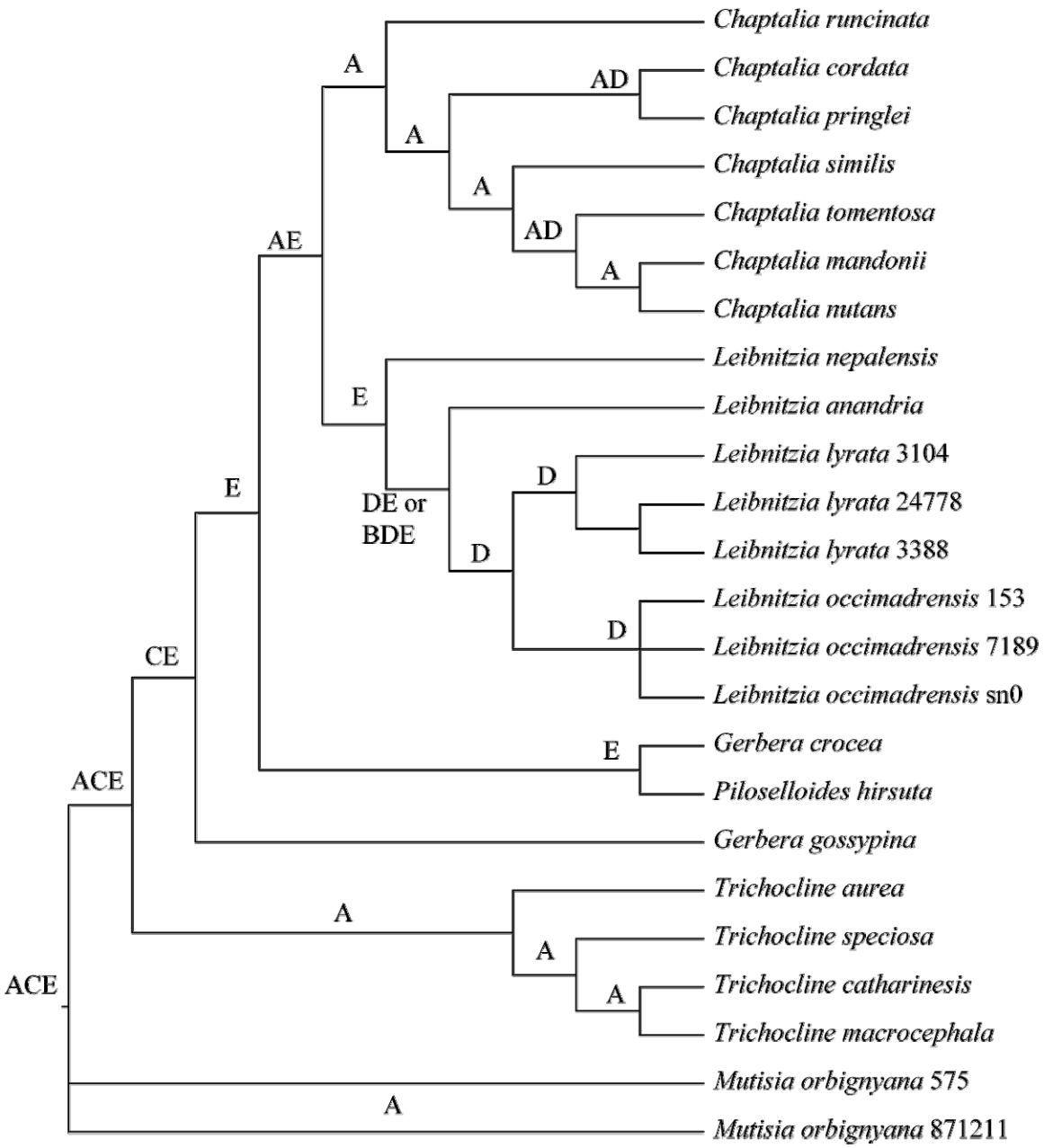

Figure 1.7: DIVA Estimated Ancestral Reconstructions
Geographic regions defined as follows: A = South America, B = East Asia, C = Africa, D = North America, E = West Asia, F = South Asia, G = Central America, H = Caribbean. Ancestral distributions mapped onto the 50% majority rule tree resulting from parsimony analysis of the ITS gap-coded dataset.

Discussion

Phylogenetics of Leibnitzia

The results clearly show that the current circumscription of *Leibnitzia* reflects a natural, monophyletic group whose members arose from a common ancestor. Both *Leibnitzia lyrata* and *L. occimadrensis* fall firmly within this clade with strong support from all datasets. The only moderate support value associated with the monophyly of the *Leibnitzia* clade is the bootstrap support value of 57%. The only exception to the monophyletic results seen elsewhere are the results from the chloroplast dataset, which are likely to have been influenced by the six taxa missing from this dataset, especially *L. anandria* and *Gerbera crocea*. However, the consistency with which this genus resolves into a monophyletic group in all analyses of the combined and nuclear datasets and the consistency of the topology within that group leaves the authors confident in supporting the current taxonomy until further data can be obtained. It is possible that the two species of *Leibnitzia* and additional representatives from *Gerbera*/*Piloselloides* and *Chaptalia* not included in this study could alter these findings, however, even if future studies should dispute the monophly of *Leibnitzia* as it is currently circumscribed, there still appears to be a solid nucleus of *Leibnitzia* species around which the genus could be revised.

Additionally, the support for a monophyletic *Leibnitzia* presented here lends support to Jeffery's (1967) and Nesom's (1983) position that pappus hair morphology and achene pubescence are reliable morphological characters that give coherence to the genus.

Molecular data reported here is again consistent in placing *L. occimadrensis* as most closely related to *L. lyrata* than to any other member of the genus. Jeffery (1967) casually suggested a sister relationship between *L. occimadrensis* and *L. anandria*, which was later mentioned again by Nesom (1983). This proposed sister relationship between *L. occimadrensis* and *L. anandria* is here shown to be mistaken. Wen (1999) pointed out that in most cases of Asian-North American disjunctions between "presumed species pairs," the two species are not in fact each other's closest relatives, as one or both species is likely to have speciated since this initial divergence. While the close relationship between *L. lyrata* and *L. occimadrensis* cannot here be disputed, their resolution from one another is less certain. *Leibnitzia occimadrensis* consistently resolves as a monophyletic group with the highest support values, but in the strict consensus of the gap-coded combined data this clade forms a polytomy with the individual samples of *L. lyrata.* Although the Bayesian and 50% majority rule parsimony analyses of the gap-coded combined dataset (Fig. 1.4 and Fig. 1.5) and the analyses of the nuclear gap-coded dataset (Fig. 1.2) do resolve the two species into sister groups, the *L. lyrata* group commands weak to mild support (PP=0.60, PB=65%, and PP=0.50 respectively) and achieved no bootstrap support in the nuclear only analysis. The questions remaining regarding the American species of *Leibnitzia* deal not with whether they are each others closest

relative, but rather with how closely related they are and whether or not these two taxa constitute status as individual species. Given their sympatric ranges and close relationship, it is conceivable that the two are not reproductively isolated. However, given the coherence and support of *L. occimadrensis*, this is likely a question of semantics that pertains more toward the definition of the term 'species', rather than as to whether these taxa represent the same entity.

Phylogenetics of Chaptalia

Results strongly indicate that *Chaptalia* as a distinct monophyletic genus (BP = 90%, PP = 1.0), although Kim et al. (2002) reported that *Chaptalia* is paraphyletic. These results cannot be otherwise addressed here, as the species that appeared in paraphyly, *Chaptalia lyratifolia*, was not represented in the present study. Given the widely acknowledged need for revision in the *Gerbera*-complex as a whole, the debates over the transfer and recircumscription of individual species of *Chaptalia* (e.g., *Chaptalia hintonii*; Nesom, 2004; Katinas, 2004), and the large number of currently recognized species in *Chaptalia* (ca. 56 spp.) it is reasonable to expect that some species of *Chaptalia* might be placed in another genus within the complex. On the whole, however, the representatives of *Chaptalia* in this study have established a solid nucleus around which future revisions of the genus may be based. Despite suppositions and opinions that *Gerbera* may eventually consume the genus, based on the current data *Chaptalia* appears to be deserving of individual generic status as much as *Leibnitzia*.

The taxonomic sections within *Chaptalia* are not at all supported by the present data. The *C. similis* clade does include both of the representatives of section *Leria* used in this study, which suggests that section *Leria* may have some genetic basis and should perhaps be expanded to include species such as *C. tomentosa* and *C. mandonii*. However, nowhere in any of the results do two members of the same section resolve as most closely related, which is not surprising given the transient nature of these sections since Burkart's review of the genus in 1944. Jeffery (1967) recognizes only four of the seven sections described by Burkart and proposed to combine sections *Leria* and *Lieberkuhna*. Burkart later amended his interpretation of these groups by moving section *Loxodon* Cass. into *Leiberkuhna*. Nesom (1995) accepted Burkart's revised sections with "several caveats" and in 2004 Nesom notes that three sections of *Chaptalia* (*Archichaptalia*, *Leria,* and *Euchaptalia*) overlap in some morphological features such as leaf shape, ray size, and style morphology. Katinas (2004) asserts that traditional sections of *Chaptalia* must be re-evaluated and redefined. Additionally, Nesom reports that *C. runcinata*, a member of section *Leiberkuhna,* is geographically isolated in North America from other members of its section, which are found in South America, although specimens of *C. runcinata* have since been collected in South America (see Table 1.1). Nesom similarly presents *C. pringlei* as being relatively morphologically isolated from other members of section *Euchaptalia*, though he does earlier refer to the section as "clearly monophyletic" (1995). Increased sampling of all *Chaptalia* sections should provide further insight on questions regarding molecular support (or lack thereof) for the current taxonomic divisions in the genus.

Phylogenetics of Gerbera *and* Piloselloides

The phylogenetic relationship between *Gerbera* and *Piloselloides* remain unclear. The first question that must be addressed is whether *Piloselloides* warrants status at the generic level or should be considered part of *Gerbera*. The second question warranting some attention is whether or not *Gerbera* comprises two separate, unrelated evolutionary lineages (regardless of the status of *Piloselloides*). Jeffery first elevated *Piloselloides* to generic status in his 1967 evaluation of the *Gerbera*-complex, but the taxon is still considered a section of *Gerbera* by some. Jeffery's 1967 establishment of *Piloselloides* as a distinct genus was made as a compromise between morphological distinctions of uncertain significance within the genus *Gerbera*, conserving well-known names (including those of horticulturally important species), and taxonomic convenience. Hansen rejected Jeffery's elevation of *Piloselloides* to the genus level in his 1985 revision of the African sections of *Gerbera*, citing a lack of unique morphological characters as his reason. However, Hansen did admit that the achene hairs of *Piloselloides* are a valid character difference, but did not consider this difference to be any greater than that between the achene hairs of *Gerbera*-complex genus *Perdicium* and those of *Gerbera* sect. *Gerbera*. Later in his 1985 revision, Hansen mentioned support for the existence of two groups within the genus based on differences in the number of rays and whether or not the scape is bracteate or ebracteate, and added that this lent support to the idea of "splitting *Gerbera* into two genera". Despite this, Hansen did not consider the creation of two genera from *Gerbera* to be of any practical use nor to more accurately reflect the evolutionary history of *Gerbera*/*Piloselloides*, because he asserted that all of

the African sections of *Gerbera* "must have originated from a common ancestor". The debate regarding the status of *Piloselloides* and the possibility that *Gerbera* comprises two distinct clades cannot be resolved based on any existing study, present results included, and should be investigated with molecular techniques and increased taxon sampling in future studies.

Intergeneric Relationships in the Gerbera*-complex:*

The relationship of *Gerbera* and *Piloselliodes* to the rest of the *Gerbera*-complex remains likewise unresolved. Kim et al. (2002, see Figure 1A) reported a phylogeny for Mutisieae that placed the *Gerbera*/*Piloselloides* clade sister to *Leibnitzia* with mild bootstrap and jackknife support (53 and 56, respectively) and the three genera were then inserted sister to *Chaptalia*. *Gerbera* and *Piloselloides* were thus inserted in the most derived positions on the tree, *Leibnitzia* in a median position, and *Chaptalia* in a position basal to the other genera. Where adequate resolution was obtained on this matter, results here show the opposite relationships with *Leibnitzia* and *Chaptalia* inserted in the most derived positions and *Gerbera*/*Piloselloides* inserted between *Leibnitzia*/*Chaptalia* and *Trichocline*, which is consistently placed in the most basal position within the *Gerbera*-complex. Although not well supported here, analyses of the chloroplast dataset align closely with Kim et al. (2002), nesting *Gerbera* and *Piloselloides* within *Leibnitzia*. We do not believe that the current results warrant questions about the monophyly of *Leibnitzia*, but rather see them as support for the idea that the two groups have recently diverged from a common ancestor. This suggests that *Leibnitzia* is more closely related to

the old world members of the complex, such as *Gerbera* and *Piloselloides*, than to *Chaptalia*, which holds implications for the biogeorgaphic origins and ancestral distribution of *Leibnitzia*. Further information on this point could also help elucidate questions regarding the unusual dimorphic capitula shared between *Leibnitzia* and some species of *Chaptalia*. If *Leibnitzia* proves to be most closely related to *Gerbera*/*Piloselloides*, it would Nesom's position that the breeding system shared between *Leibnitzia* and *Chaptalia* is the result of parallel evolution. If *Leibnitzia* proves to be most closely related to *Chaptalia*, however, the opposite conclusion, that the shared system is a result of inheretance from a common ancestor, would instead be indicated. The intergeneric relationships of the *Gerbera*-complex should be further investigated, as they can offer insight into the biogeographic and evolutionary history of the complex and the migration events that led to the present distributions of *Gerbera*-complex taxa.

Biogeographic Analysis

Ancestral area analysis (Bremer, 1992, 1994) determined a South American origin for the *Gerbera*-complex, which supports the conclusion of Funk et al. (2008) that the basal clades of Asteraceae diversified in South America. *Leibnitzia*'s origin in Asia was supported here, as was *Chaptalia*'s origin in South America. When viewed in conjunction with the phylogenetic results, the biogeography of the *Gerbera*-complex appears to be a microcosm for the biogeography of the family as a whole. Funk et al. (2009) determined that the family originated and initially diversified in southern South America, followed by a dispersal to and explosive radiation in Africa. From Africa there were many

migrations into other areas of the Old World (Eurasia, Asia, Europe, and Australia), which were subsequently followed by many more migrations and radiations in other areas. Chief among these subsequent migrations and radiations is that of Helianthineae *s.l.*, which is hypothesized to have migrated from Asia to North America via the Bering land bridge and then from western North America to have repeatedly migrated into Central and South America.

The *Gerbera*-complex has a similar global biogeography, with representatives living all over the globe. The *Gerbera*-complex originated in South America, with a migration to Africa spawning the massive radiation of *Gerbera*, the most well known genus in the complex. The western Asian origin of *Leibnitzia* indicates that this clade diverged from its closest *Gerbera*-complex relatives in the Old World and that the American species then migrated to North and Central America from Asia, much the same as Helianthineae *s.l.*. The close relationship of *Leibnitzia lyrata* and *L. occimadrensis* indicate one single immigration event from Asia to the New World. *Chaptalia*, on the other hand, logically remained and diversified in the Americas, much like Mutisineae and other basal clades of Asteraceae.

Implications for Future Studies

Chaptalia comprises over 50 species and is widespread in the Americas, thus revising its taxonomy will be challenging. New collections will be quite important because morphological information about some lineages is sparse, particularly for the Caribbean

species, and, as we have found, molecular data are best collected from fresh leaf material of this group. Collecting both spring and fall capitula from the same plants would also provide valuable comparative information that is rarely available on herbarium sheets. We suspect both markers used in the present study, ITS and *trn*L-*rpl*32 intron will continue to provide resolution within the *Chaptalia* clade but may need to be augmented with additional highly variable markers, such as nrDNA ETS, as taxon sampling is increased.

Molecular studies to date have not provided adequate representation from *Gerbera* and *Piloselloides* to answer questions about the legitimacy of these groups as distinct evolutionary lineages, nor questions about the relationships between *Gerbera*-complex genera. The Kim et al. (2002) study includes only one representative of each *Gerbera* and *Piloselloides*, however, the present study does little better with two representatives of *Gerbera* (and only one in the chloroplast dataset). Fresh leaf material from numerous accessions of all 4(5) sections of *Gerbera* and of both species of *Piloselloides* should provide ample molecular data and, with the correct markers, provide adequate resolution to the phylogenetic relationship between these taxa and to other members of the *Gerbera*-complex. Additionally, molecular studies to confirm the inclusion of the Australian species in the *Trichocline* clade would also provide valuable insight.

It should also be noted that the lack of resolution in the choloroplast dataset may hint at a lack of nuclear coalescence at the genomic level in *Leibnitzia* and *Gerbera*/*Piloselloides*

and that the resolution that is seen in this study is the result of the individual loci examined and may not represent species-level relationships accurately. According to the three-times rule of Palumbi et al. (2001), the clonal, uniparental inheritance of organelle DNA results in the coalescence of neutral organelle loci into monophyletic groups approximately three times as quickly as neutral nuclear loci. The three-times rule allows the use of chloroplast DNA to estimate the extent of nuclear loci coalescence into monophyletic (and therefore evolutionarily significant) groups by the estimation of branch lengths, as species with clear coalescence among organellar loci are more likely to have monophyletic nuclear loci. Because the highly variable *trn*L-*rpl*32 marker did not yield adequate resolution regarding the relationship between *Leibnitzia* and *Gerbera*/*Piloselloides*, it remains unclear whether additional nuclear loci would provide increased support for two (or more) separate clades of *Leibnitzia* and *Gerbera*/*Piloselloides*.

If further studies of the *Gerbera*-complex indicate that *Leibnitzia* is most closely related to *Gerbera*/*Piloselloides*, this would indicate a biogeography mirroring the family's South American origins, African dispersal and radiation, subsequent Old World migrations, and, most recently, a single migration from Asia back to the Americas. If, however, future studies indicate that *Leibnitzia* is most closely related to *Chaptalia*, a different biogeography would be indicated, although no less interesting or impressive in its evolutionary circumnavigation of the globe.

Conclusion

The *ITS* and *trn*L-*rpl*32 intron markers have shown to be useful in identifying evolutionary significant units within the cryptic *Gerbera*-complex and are recommended for use in future studies. A foundation for future molecular studies and for any needed revisions of the Gerbera-complex has been established, as Leibnitzia, *Chaptalia*, and *Trichocline* all resolve into well-supported clades, thus providing nuclei around which questionable species can be classified, and the American species of *Leinitzia* are firmly placed within the *Leibnitzia* clade. The placement of *Gerbera* and *Piloselloides* and the status of the latter as a genus are still questionable, but should resolve with further taxon sampling. A revision of *Chaptalia* would be useful in determining the monophyly of the entire genus (rather than the smaller representative group included here) and be invaluable in reevaluating the sections of this genus. Finally, in determining *Leibnitzia*'s closest relationship (to either *Chaptalia* or to *Gerbera* and *Piloselloides*), we can gain information important to inferring the migrations that have led to the current distribution of both *Leibnitzia* and the entire *Gerbera*-complex. This study provides the basis and context for future studies aiming to clarify the placement of questionably circumscribed taxa (e.g., *Chaptalia hintonii*, see Katinas 2004, Nesom 2004) and to provide insight into the biogeography and evolutionary history of the *Gerbera*-complex as a whole.

Appendix A: Genera and Distribution of the *Gerbera*-complex (FGVP_VIII, Hansen 1985, Nesom 1995, Jeffrey 1967, Katinas 2004)

Notes on the distributions of *Perdicium* and *Piloselloides*: *Perdicum* has two species that are endemic to the western Cape area of South Africa. *Piloselloides* comprises two species: one narrowly endemic to the South African forests around Knysna and one widespread taxon distributed across southern Africa, the African tropics, Madagascar, and continental Asia. (Jeffrey, 1967)

Genus	Authority	Size	Distribution
Gerbera	L.	ca. 30 species	Africa: Angola, Eithopia, Kenya, Tanzania, Uganda, Malawi, Mozambique, Madagascar, South Africa, Zimbabwe; Asia: Yemen, China, Nepal, Tibet, Bhutan, India, Pakistan, Kashmir
Chaptalia	Vent.	35-56 species	The Americas: from southern US to southern South America, West Indies
Leibnitzia	Cass.	6 species	Asia: Himalayan region, China, Japan, Russia, Mongolia, Korea, Bhutan, Taiwan; Central America: southwestern US to Guatemala
Piloselloides	L.	2 species	Africa: South Africa through tropical and sub-Saharan eastern Africa, Madagascar, Angola, Nigera, Ghana, Zaire, Guinean Republic; Asia: Yemen, China, Assam, Thailand, Myanmar, Bali
Trichocline	Cass.	22-23 species	South America: Colombia, Peru, Brazil, Chile, Argentina, Paraguay, Uruguay, Bolivia; western Australia
Perdicium	L.	2 species	South Africa
Uechtrizia	Freyn	3 species	Western Asia, China, India, Kashmir

Appendix B: List of Accessions with Irresolvable Issues

Accession	Collector	No.	Year	Source	Notes
Leibnitzia lyrata (Sch.Bip.) Nesom	C.G. Pringle	sn	1902	US	No DNA extracted.
Leibnitzia lyrata (Sch.Bip.) Nesom	J.H. Beaman	3067	1959	US	No DNA extracted.
Possible *Leibnitzia* sp.	Wen	1155	2006 or 2007	US	Specimen unavailable for determination, but initial sequence results indicate that this specimen was not a *Leibnitzia*.
Leibnitzia anandria (L.) Turcz.	T. Makino	182227	unavailable	US	No DNA extracted.
Leibnitzia anandria (L.) Turcz.	H. Ohba et al.	14	unavailable	US	No DNA extracted.
Gerbera nivea Sch.Bip.	J.F. Rock	4863	1922	US	Amplification issues.
Gerbera jamesonii Bolus ex Adlam	Shiu Ying Hu	7630	1969	US	Multiple bands on gel.
Gerbera jamesonii Bolus ex Adlam	H.N. and A.L. Moldenke, M. Jayasuriya	28324	1974	US	Weak, multiple bands.
Piloselloides hirsuta (Forsk.) C.Jeff	C.M. Taylor, R.E. Gereau, and J. Lovett	8399	1989	US	Weak, multiple bands.
Piloselloides hirsuta (Forsk.) C.Jeff	Shiu Ying Hu	11626	1972	US	Weak, multiple bands.
Gerbera gossypina (Royle) Beauv.	Yasmin Nasri	5938	1970	US	Weak, multiple bands.
Chaptalia arechavaletae Arechav.	Simon, Pablo M.	1093	2004	US	Sequencing issues.
Chaptalia denticulata (Baker) Zardini	R.M. Harley	26340	1988	US	Weak, multiple bands.
Chaptalia azuensis Urb. & Fkm	T. Zanoni and J. Pimentel	20868	1982	TEX	No DNA extracted.
Chaptalia dentata Cass	R. Garcia and J. Pimentel	1330	1986	TEX	Weak, multiple bands.
Chaptalia mornicola Zanoni	T. Zanoni and J. Pimentel	27874	1983	TEX	No DNA extracted.
Trichocline macrorhiza Cabr.	A. Schinini, C. Saravia Toledo, and R. Neumann	34626	1998	TEX	Weak, multiple bands.
Mutisia lanata Ruiz & Pavon	Israel G. and Vargas C.	4528	1996	US	No DNA extracted.
Mutisia lanata Ruiz & Pavon	St. G. Beck	17797	1990	US	No DNA extracted.

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Curriculum Vitae

Kristen Baird was born in Virginia Beach, Virginia, on January 7th 1983. Kristen moved with her family to Fairfax, Virginia, in 1985 where Kristen enjoyed her summers playing outside in her yard and nearby parks. Kristen showed a strong interest in biology from a young age and spent significant portions of her free time outside just looking at plants, rocks, animals, and insects while she was growing up. Her fascination of the natural world moved Kristen to study biology at George Mason University, where she completed a Bachelor of Science degree in Biology in 2006. Kristen received departmental honors, graduated *magna cum laude*, and was awarded the 2006 Faculty Award from the Department of Molecular and Microbiology. Unsure of the direction she wanted to take with her career, in the fall of 2005 Kristen read a book that awoke an interest in plant evolution: *The Botany of Desire* by Michael Pollan. Kristen began working towards her Master of Science in Environmental Science and Policy degree at George Mason the next year under the guidance of Dr. Andrea Weeks. During her work on her master's degree Kristen had to opportunity to contract as a visiting scientist for the Botany Department of the National Museum of Natural History at the Smithsonian Institution and also to teach undergraduate biology labs in botany and genetics at George Mason as a Graduate Teaching Assistant. During her time in the classroom Kristen discovered a talent and love for teaching. After finishing her master's degree, Kristen plans to pursue a teaching career so that she can help others learn about the natural world, engage young students in the research process, and motivate students to pursue careers in science. Kristen enjoys learning how to garden, traveling both near to and far from home, and supports Julia Child's assertion that you can never use too much butter while cooking.