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Molecular phylogenetic analysis of *Leibnitzia* Cass. (Asteraceae: Mutisieae: *Gerbera*-complex), an Asian–North American disjunct genus

[1]Kristen E. BAIRD [2]Vicki A. FUNK [2]Jun WEN [1]Andrea WEEKS*

[1](*Department of Environmental Science and Policy, George Mason University,* Fairfax, VA 22030, USA)

[2](*US National Herbarium, Smithsonian Institution,* MRC 166, P.O. Box 371012, Washington, DC 20013-7012, USA)

Abstract *Leibnitzia* comprises six species of perennial herbs that are adapted to high elevation conditions and is one of only two Asteraceae genera known to have an exclusively disjunct distribution spanning central to eastern Asia and North America. Molecular phylogenetic analysis of *Leibnitzia* and other *Gerbera*-complex members indicates that *Leibnitzia* is monophyletic, which is in contrast with our expectation that the American *Leibnitzia* species *L. lyrata* and *L. occimadrensis* would be more closely related to another American member of the *Gerbera*-complex, namely *Chaptalia*. Ancestral area reconstructions show that the historical biogeography of the *Gerbera*-complex mirrors that of the entire Asteraceae, with early diverging lineages located in South America that were followed by transfers to Africa and Eurasia and, most recently, to North America. Intercontinental transfer of *Leibnitzia* appears to have been directed from Asia to North America. Independent calibrations of nuclear (ribosomal DNA internal transcribed spacer region) and chloroplast (*trnL–rpl*32 intron) DNA sequence data using relaxed clock methods and either mean rate or fossil-based priors unanimously support Miocene and younger divergence times for *Gerbera*-complex taxa. The ages are not consistent with most Gondwanan vicariance episodes and, thus, the global distribution of *Gerbera*-complex members must be explained in large part by long-distance dispersal. American species of *Leibnitzia* are estimated to have diverged from their Asian ancestor during the Quaternary (ca. 2 mya) and either migrated overland to North America via Beringia and retreated southwards along high elevation corridors to their present location in southwestern North America or were dispersed long distance.

Key words Beringia, biogeography, *Gerbera*-complex, Mutisieae.

Plant genera distributed between Asia and North America have been the focus of numerous studies aimed at elucidating the age and probable cause of their disjunctions (Wen, 1999, 2001; Nie et al., 2007). Most genera that are recognized as having this biogeographic pattern are native to temperate mesic forests of eastern Asia and eastern North America. Fewer Asian–North American disjuncts occupy (sub-)tropical areas or western North America, and these species, like the others, are typically woody or adapted to forest understories. The emerging consensus from time-calibrated molecular phylogenetic studies suggests these disjunctions arose as a consequence of the fragmentation of the contiguous high-latitude temperate forests during both the early Tertiary and Quaternary periods. Consequently, *Leibnitzia* Cass. (Asteraceae: Mutisieae: *Gerbera*-complex; Table 1), a perennial herb genus adapted to high-elevation conditions in both Asia and North America, stands out as an exception among these disjunct genera.

Leibnitzia is composed of six species that occupy exposed habitats in high-elevation, temperate regions of the Himalayas, central and eastern Asia, and temperate to tropical regions of North America (Nesom, 1983; Hansen, 1988; Fig. 1). The four Asian members of *Leibnitzia*, namely *L. anandria* (L.) Turcz., *L. knorringiana* (B. Fedtsch.) Pobed., *L. nepalensis* (Kunze) Kitam., and *L. ruficoma* (Franch.) Kitam., occupy maximal elevations of 3100–5000 m at temperate latitudes. *Leibnitzia anandria* is the most widespread and occurs in scattered populations across the Himalayan region (Tibet and neighboring regions in China, Bhutan, Nepal, northern India including Kashmir, and northern Pakistan), China, Japan, Korea, Bhutan, Mongolia, and Siberia, and is also known to grow at sea level along the Sea of Japan. The remaining three Asian species are restricted to either the Himalayan region (*L. nepalensis* and *L. ruficoma*) or eastern Kyrgyzstan (*L. knorringiana*). American *Leibnitzia* species occupy lower maximal elevations (2500–3850 m) at both temperate and tropical latitudes. *Leibnitzia lyrata* (Sch. Bip.) G. L.

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* Author for correspondence. E-mail: aweeks3@gmu.edu; Tel.: 1-703-993-3488; Fax: 1-703-993-1066.

Table 1 Membership and distribution of *Gerbera*-complex genera sensu Katinas et al. (2008) and Panero & Funk (2008)

Genus	Size (no. species)	Distribution
Brachyclados D. Don	3	South America
Chaptalia Vent.	20–35*	The Americas: from southern US to southern South America, West Indies
Gerbera L.	29	The Americas: from Mexico to South America Asia: Yemen and countries east of and including the Himalayan plateau to Bali Africa: sub-Saharan Africa and Madagascar
Leibnitzia Cass.	6	North America: from southwestern US to Guatemala Asia: Kyrgyzstan, India, Kashmir region, Pakistan, Russia, Tibet, Bhutan, Nepal, Mongolia, China, Japan, Korea, and Taiwan
Perdicium L.	2	Western Cape of South Africa
Trichocline Cass.	22–23	South America: Colombia, Peru, Brazil, Chile, Argentina, Paraguay, Uruguay, and Bolivia Western Australia
Uechtritzia Freyn.	3	Western Asia, including Afghanistan, Russia, China, India, and Kashmir region

*Katinas et al. (2008) list 68 valid names of *Chaptalia* species but suggest they may represent only 20–35 distinct species.

Nesom ranges from the southwestern US through Mexico, along the Sierra Madre and trans-volcanic mountains of Mexico, to Guatemala. The Mexican endemic *L. occimadrensis* G. L. Nesom is narrowly distributed and is allopatric from *L. lyrata* in northeastern Sierra Madre and Sierra Surutato.

Few composites exhibiting the Asian–North American disjunction pattern have been identified, presumably because the family's proclivity for occupying open habitats excluded them from the widespread high-latitude temperate forests and their episodes of vicariance (Funk et al., 2005, 2009). The most complete biogeographic study of the family to date (Funk et al., 2009) identified only one other genus (*Nabalus* Cass.; Cichorieae) that is distributed exclusively in central to eastern Asia and North America including Mexico, similar to *Leibnitzia*. Three additional genera share this distribution and are present in South America as well (*Anaphalis* DC., *Antennaria* Gaertn., and *Erigeron* L). The disjunct distribution of *Leibnitzia* may be the result of long-distance oceanic dispersal or migration through non-forested, high-latitude land corridors and subsequent retreat to lower latitudes. An alternative and perhaps more probable explanation for the remarkable disjunction of *Leibnitzia* species is taxonomic. The American *Leibnitzia* species *L. lyrata* and *L. occimadrensis* may be part of an American lineage, namely *Chaptalia* Vent. (~20–35 spp.), which is another member of the globally distributed *Gerbera*-complex.

As currently circumscribed, *Leibnitzia* and *Chaptalia* are distinguished by one morphological character: the achene hairs of *Leibnitzia* are slender, long, and

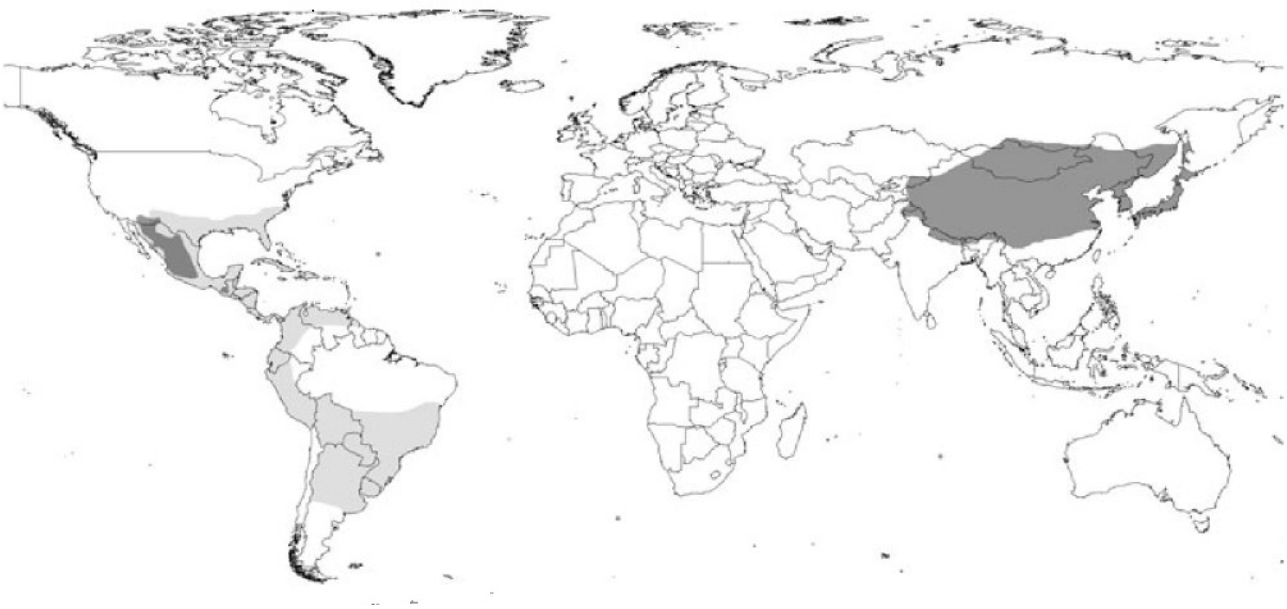

Fig. 1. Distribution of the Asian–North American disjunct genus *Leibnitzia* (dark gray) and fellow *Gerbera*–complex member *Chaptalia* (light gray). Note the overlapping distribution of genera in North America.

sharp, whereas those of *Chaptalia* are short, inflated, or papillose (Nesom, 1983; Hansen, 1991; Hind, 2007). Karyotype information may also distinguish the genera, but data for all species are incomplete. Reports indicate that *Leibnitzia* (including *L. lyrata*) has a haploid count of 23 chromosomes (Nesom, 1983) and *Chaptalia* has 24, although counts of n = 16 for *L. lyrata* specimens from Mexico have occurred (DeJong & Longpre, 1963). Intriguingly, both genera share a reproductive strategy that is unknown among other Asteraceae genera and suggests they may share a recent common ancestor. All six species of *Leibnitzia* and some species of *Chaptalia* exhibit chasmogamous (out-crossing) capitula during spring and cleistogamous (selfing) capitula during autumn (Burkart, 1944; Nesom, 1983). Thus, American *Leibnitzia* species, whose geographic distributions also overlap with those of *Chaptalia* species, could be more closely related to *Chaptalia* than to Asian *Leibnitzia*.

Published molecular phylogenies of the *Gerbera*-complex do not thoroughly test the monophyly of either *Leibnitzia* or *Chaptalia*. Work by Kim et al. (2002) explored broad phylogenetic patterns within tribe Mutisieae and confirmed that the *Gerbera*-complex is a well-supported monophyletic group comprising *Chaptalia*, *Gerbera* L., and *Leibnitzia*. That study focused on the Mutisieae as a whole rather than on the *Gerbera*-complex specifically and the sample group did not contain enough representatives to thoroughly test the monophyly of the individual genera within the *Gerbera*-complex. Kim et al. (2002) included American *L. lyrata* (listed as *L. seemannii* (Sch. Bip.) G. L. Nesom), but no other members of *Leibnitzia*. Their parsimony analysis of chloroplast *ndh*F sequence data from four species of *Chaptalia* (*C. tomentosa* Vent., *C. exscapa* (Pers.) Baker, *C. nutans* (L.) Polák, and *C. lyratifolia* Burkart) indicated that *Chaptalia* is paraphyletic and the strict consensus tree showed a polytomy including *Gerbera*, *Leibnitzia*, and three of the four included species of *Chaptalia*.

The taxonomic history of *L. lyrata* (Sch. Bip.) G. L. Nesom exemplifies the difficulty botanists have experienced in distinguishing lineages of the *Gerbera*-complex. Jeffrey (1967) first noted that several species of American *Chaptalia* resembled members of the Asian genus *Leibnitzia* by their achene trichomes and pappus hair morphology. In his subsequent revisions of *Chaptalia*, Nesom (1983, 1984, 1995) 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.) G. L. Nesom. Nesom (1995) later established that *C. lyrata* D. Don and *L. seemannii* (Sch. Bip.) G. L. Nesom were the same entity and altered the nomenclature to reflect the combination, *Leibnitzia lyrata* (D. Don) G. L. Nesom. It was subsequently determined that *Chaptalia lyrata* D. Don (1830) was an illegitimate homonym of the species *Chaptalia lyrata* (Willd.) Spreng. (1826), which had been published legally as *Gerbera lyrata* by Karl Heinrich 'Bipontinus' Schultz in 1856. Thus, the authority of *Leibnitzia lyrata* (D. Don) G. L. Nesom was altered to *Leibnitzia lyrata* (Sch. Bip.) G. L. Nesom to reflect this information. *Leibnitzia occimadrensis* G. L. Neson was published simultaneously with *Leibnitzia seemannii* (Nesom, 1983) and is distinguished 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 achene trichomes; and the color and length of the pappus bristles.

In order to elucidate the evolution and historical biogeography of the Asian–North American disjunct genus *Leibnitzia*, we reconstructed the phylogeny of *Gerbera*-complex members using DNA sequence data from the nuclear and chloroplast genome. Our objectives were to test the hypotheses that: (i) the American *Leibnitzia* species *L. lyrata* and *L. occimadrensis* are more closely related to American species of *Chaptalia* than to Asian *Leibnitzia*; and (ii) the reconstructed geographic movements of the *Gerbera*-complex, as part of the early diverging Asteraceae lineage Mutisieae, should mirror those of the family as a whole. The latter hypothesis is based on the assumption that overland routes of range expansion or factors that promoted long-distance dispersal would have been equally available to the basal lineages that were extant during the early evolution and radiation of the Asteraceae. Thus, under this assumption, we would expect that early diverging genera of the *Gerbera*-complex would be located in South America, followed by the appearance of taxa in either Africa followed by Asia and, most recently, in North America (Panero & Funk, 2008) or North America followed by appearances in Africa or Asia (Funk et al., 2005, 2009; Panero & Funk, 2008).

1 Material and methods

1.1 Taxon sampling

Accessions of 19 *Gerbera*-complex taxa comprising four genera (*Leibnitzia*, *Chaptalia*, *Gerbera*,

Table 2 Taxon and accession information

Species	Locality	Voucher information	*trn*L–*rpl*32 intron GenBank No.	ITS region GenBank No.
Chaptalia cf. *cordata* Hieron.	Peru, Cajamarca	I. Sanchez V., M. Cabanillas S. s.n. (F)	n/a	GU126770
Chaptalia mandonii (Sch. Bip.) Burkart	Argentina, Buenos Aires	P. M. Simon 438 (US)	n/a	GU126771
Chaptalia nutans (L.) Polák	Argentina	P. M. Simon 477 (US)	GU126751	GU126772
Chaptalia pringlei Greene	Mexico, Tamazulapan	G. Nesom 4405 (US)	n/a	GU126773
Chaptalia runcinata Kuntze	Argentina, Buenos Aires	P. M. Simon 415 (US)	GU126752	GU126774
Chaptalia similis R. E. Fr.	Argentina	P. M. Simon 711 (US)	GU126753	GU126775
Chaptalia tomentosa Vent.	USA, Louisiana	V. Funk 12303 (US)	GU126754	GU126776
Gerbera crocea Kuntze	South Africa	Koekemoer & Funk 1924 (PRE)	n/a	AY504687
Gerbera gossypina Beauverd	India, Punjab	W. Koelz 4294 (US)	GU126755	GU126777
Gerbera piloselloides (L.) Cass.	South Africa, Mpumalanga	M. Koekemoer 2125 (US)	GU126765	GU126788
Leibnitzia anandria (L.) Turcz.	China	Liu 890185 (US)	n/a	GU126778
Leibnitzia lyrata (Sch. Bip.) G. L. Nesom	USA, Arizona	G. Nesom 3388 (ARIZ)	GU126758	GU126781
Leibnitzia lyrata (Sch. Bip.) G. L. Nesom	Mexico, Durango	J. L. Reveal, W. J. Hess 3104 (US)	n/a	GU126780
Leibnitzia lyrata (Sch. Bip.) G. L. Nesom	USA, New Mexico	J. M Holzinger s.n. (US)	GU126756	n/a
Leibnitzia lyrata (Sch. Bip.) G. L. Nesom	USA, Arizona	G. Nesom 24778 (ARIZ)	GU126757	GU126779
Leibnitzia nepalensis (Kunze) Kitam.	China, Xizang	J. Wen et al. 542 (US)	GU126759	GU126782
Leibnitzia occimadrensis G. L. Nesom	Mexico, Sonora	G. Nesom 153 (ARIZ)	GU126760	GU126783
Leibnitzia occimadrensis G. L. Nesom	Mexico, Sonora	G. Nesom s.n. (ARIZ)	GU126762	GU126785
Leibnitzia occimadrensis G. L. Nesom	Mexico, Sonora, Sierra Surotato	H. S. Gentry 7189 (US)	GU126761	GU126784
Mutisia orbignyana Wedd.	Argentina, Jujuy	P. Simon, M. Bonifacino 575 (US)	GU126763	GU126786
Mutisia orbignyana Wedd.	Bolivia, La Paz	Marko Lewis 871211 (US)	GU126764	GU126787
Trichocline aurea (D. Don) Reiche	Chile	F. Hellwig 9094 (TEX/LL)	GU126766	GU126789
Trichocline catharinensis Cabrera var. *discolor* Cabrera	Brazil, Santa Catarina	O. S. Ribas, J. Cordeiro, E. Barbosa s.n. (TEX/LL)	GU126767	GU126790
Trichocline macrocephala Less.	Brazil, Parana	E. Barbosa, G. Hatschbach, O. S. Ribas 109 (TEX/LL)	GU126768	GU126791
Trichocline speciosa Less.	Brazil, Parana	J. M. Cruz, J. Cordeiro, V. Carre 84 (TEX/LL)	GU126769	GU126792

ITS, internal transcribed spacer.

and *Trichocline* Cass.) were obtained for investigation (Table 2). *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* and *C. pringlei*; *C.* sect. *Archichaptalia*: *C.* cf. *cordata*; *C.* sect. *Leiberkuhna*: *C. runcinata* and *C. mandonii*; and *C.* sect. *Leria*: *C. similis* and *C. nutans*). Other *Gerbera*-complex taxa included three species of *Gerbera* (*G. crocea*, *G. gossypina*, and *G. piloselloides*) and four species of *Trichocline* (*T. aurea*, *T. macrocephala*, *T. catharinensis*, and *T. speciosa*). Two accessions of *Mutisia orbignyana* Wedd. (subtribe Mutisineae) were selected as outgroups following the work of Kim et al. (2002) and Panero & Funk (2008).

1.2 Marker selection

The internal transcribed spacer (ITS) region of the 18S-26S nuclear ribosomal subunit was selected as the nuclear marker because the ITS region has provided adequate resolution for closely related taxa in phylogenetic studies of Asteraceae (Baldwin, 1992). This region amplifies well from herbarium material and occurs in high numbers of uniform paralogs in the genome, further facilitating polymerase chain reaction (PCR) amplification (Baldwin et al., 1995). The intron situated between the chloroplast *trnL* (UGA) gene and the chloroplast

ribosomal protein L32 gene (*rpl*32) was selected for use as the chloroplast marker after trial of several other candidate markers. The 3′ end of the chloroplast gene *ndh*F was used in previous studies of Mutisieae phylogeny (Kim et al., 2002) but failed to provide adequate resolution at the generic level. The *trn*L–*trn*F intergenic spacer region was also tested, but also failed to provide adequate resolution. Chloroplast regions *ndh*C–*trn*V and *trn*Y–*trn*E–*rpo*B, which were shown to be as hypervariable the *trn*L–*rpl*32 intron in Asteraceae (Timme et al., 2007), failed to amplify consistently for our accessions.

1.3 DNA extraction, amplification, and sequencing

DNA was extracted from leaf tissue of herbarium specimens and silica-dried samples using a FastDNA Spin Kit (BIO101 Systems, La Jolla, CA, USA) according to the manufacturer's instructions. The PCR was performed using a 25.0 μL mix of 12.9 μL sterile deionized water, 2.5 μL of 10× buffer (New England Bio Labs, Ipswich, MA, USA), 2.5 μL of 25 mmol/L $MgCl_2$, 2.0 μL of 10 mmol/L dNTPs, 1.0 μL each of both the forward and reverse primers (10 μmol/L), 0.5 μL of 10 mg/mL bovine serum albumin (BSA), 0.1 μL of 5 U/μL *Taq* polymerase (New England Bio Labs), and 2.5 μL template DNA. Amplification of ITS1, the 5.8S ribosomal subunit, and ITS2 was completed with ITS1a, ITS2b, ITSp3, and ITSp4 primers (Kim & Jansen, 1994) 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 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 six cycles of 1 min at 94°C, 1 min at 58°C (–1°C for each successive cycle), and 2 min and 30 s at 72°C, followed by 34 cycles of 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, USA) was used for DNA purification according to the manufacturer's instructions.

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. The exo-sap reaction mix comprised 0.5 μL of 10 U/μL exonuclease I, 1.0 μL of 1 U/μL shrimp alkaline phosphatase (SAP), and 1.5 μL deionized water per 25 μL PCR product. The exo-sap thermocycler protocol included 30 min at 37°C and 15 min at 80°C. Samples were bidirectionally sequenced by Macrogen USA (Bethesda, MD, USA) using ABI 3730×1 DNA Analyzers (Applied Biosystems, Foster City, CA, USA).

1.4 Sequence alignment and phylogenetic analyses

Bidirectional sequence strands were assembled and edited in Sequencher v. 4.0 (Gene Codes, Ann Arbor, MI, USA), then aligned and gap coded manually according to the protocol of Ochoterena and Simmons (2000) in MacClade v. 4.0 (Maddison & Maddison, 2003). Aligned datasets of ITS and the *trn*L–*rpl*32 intron were tested for contrasting phylogenetic signals (excluding gap characters) using the incongruence length difference (ILD) test (Farris et al., 1994) as implemented by PAUP* v. 4.0 beta v. 10 (Swofford, 2002). The ILD parameters were set for the partition-homogeneity test with 100 partition-homogeneity replicates, the tree bisection–reconnection (TBR) branch-swapping algorithm with multrees 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, TBR branch swapping with multrees in effect, and gaps treated as missing data. Non-parametric bootstrap tests of the data used 1000 pseudoreplicates within PAUP*. 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 & Ronquist, 2001). The best fitting model of sequence evolution for the nuclear and chloroplast datasets was determined using the Akaike information criterion as implemented by MrModeltest v. 2.2 (J. A. A. Nylander, 2004; program distributed by the author; available from http://www.abc.se/~nylander/, accessed April 2009). The model selected for all nucleotide datasets was the general time reversible (GTR) model 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 = variable* command was used because 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 stationarity was reached after 1 million generations (average split deviations between parallel runs <0.01), thus a burn-in of 10 000 trees was used for subsequent analyses. Graphs of the cumulative posterior probabilities of the most variable splits among post-burn-in trees (AWTY; http://ceb.csit.fsu.edu/awty, accessed April 2009) confirmed stationarity. Maximum likelihood analyses of the combined sequence data without gap codes were

conducted using GARLI v. 0.96b (Zwickl, 2006) using the GTR+I+G model and default run settings. Topological consistency among best-fit trees was determined by comparing results from independent analyses that were started from random taxon additions. Maximum likelihood bootstrap values were compiled from analyses of 1000 pseudoreplicates of the data.

1.5 Biogeographic analysis and divergence time estimation

Distributions of species were partitioned among eight area categories in order to assess the historical biogeography of *Leibnitzia*: North America including Mexico, South America, east Asia, Africa, west Asia, south Asia, Central America, and the Caribbean. Central America and the Caribbean Islands were recognized apart from the American continents because portions of these regions are younger geologically than the surrounding continents and their recognition adds greater resolving power to the biogeographic analysis. Similarly, Asian distributions of species were divided into south (India south of the Himalayan ranges), west (Himalayan ranges and westward, including Arabian Peninsula and Europe), and east (east of the Himalayan ranges, including both southeastern and northeastern regions of the Asian continent) areas to preserve information that could shed light on the movement of lineages in Asia.

Dispersal–vicariance analysis was used to estimate the ancestral distribution of clades within the *Gerbera*-complex. The number of allowed ancestral areas was set to eight (the total number of scored areas) and also limited to three with the "maxareas" optimize command in DIVA 1.1 (F. Ronquist, 1996; computer program and manual available via anonymous FTP from Uppsala University at ftp.uu.se or ftp.systbot.uu.se, accessed April 2009) to counteract DIVA's bias towards favoring large and geologically inaccurate conglomerations of ancestral areas. Distributions on the parsimony-based topology were also analyzed using Bremer's ancestral area analysis (Bremer, 1992) in order to compare them with the results of Funk et al. (2005, 2009).

We then estimated the ages of all clades in order to evaluate biogeographic reconstructions in a temporal framework. Divergence time estimates have the capacity to reject hypothesized routes of range expansion and vicariant episodes during different periods of geologic history, although they cannot confirm them. Clock-like mutation rates of the sequence datasets, which were reduced to one accession per species and excluded gap characters, were tested by scoring the best-fit maximum likelihood topology using PAUP* with and without enforcing the molecular clock and by evaluating the –log likelihood difference with χ^2 tests. We rejected clock-like mutation in both the ITS and combined sequence datasets at the $P < 0.01$ level (d.f. = 18; χ^2 observed value = 126.24 and 112.12, respectively). Consequently, we used the relaxed-clock method of Beast v. 1.4.8 (Drummond & Rambaut, 2007) to estimate tree topology and the age of all divergences simultaneously. We estimated divergence times using three approaches in order to examine the range of possible ages for all nodes. We first applied to the ITS dataset a normally distributed prior on the "mean.rate" parameter using the mean estimated substitution rate for the ITS region derived from 10 different herbaceous plant species (mean = 4.13×10^{-9} substitutions/site per year; SD = 1.81×10^{-9} substitutions/site per year; Kay et al., 2006). In a separate analysis, we then applied to the ITS dataset a prior on the age ("tmrca") of the root of the tree corresponding the minimum age of the Mutisieae lineage as inferred from pollen fossils. Recent reviews of pollen fossils have placed the possible date of divergence for this basal Asteraceae lineage between the Early Miocene (23.3 mya; Graham, 1996) and Middle Eocene (42–46 mya; Scott et al., 2006). The tmrca log normally distributed prior included a zero offset of 20 mya, a log normal mean of 2.22 an SD of 0.52, which resulted in 95% prior distribution of 23.3–45.5 mya. In the third and final analysis, we repeated the fossil-based calibration on the combined sequence dataset. For all three Beast analyses, model parameters included GTR+I+G, unfixed mean substitution rate, and the uncorrelated log normal relaxed clock. The tree prior was set using the Yule process and the MCMC searches were run for 10 000 000 generations logging parameters every 1000 generations. Beast results from each analysis were collated following a burn-in of 1 000 000 generations.

2 Results

GenBank numbers of all DNA sequence data used in analyses are recorded in Table 2. The alignment of the ITS sequences was straightforward and yielded a nuclear dataset of 705 bp and 17 binary gap characters, which, together, comprised 26.67% parsimony informative characters. The alignment of the chloroplast *trn*L–*rpl*32 sequences was made slightly more challenging by the presence of a 375-bp region of insertions in five of the accessions. The specimen of *Mutisia orbignyana* Lewis 871211 contained a series of insertions spanning 397–753 bp and all *Trichocline* specimens showed similar insertions in this region of the alignment. The *trn*L–*rpl*32 alignment yielded a chloroplast dataset containing 1074 bp and 37 binary gap characters (3.72%

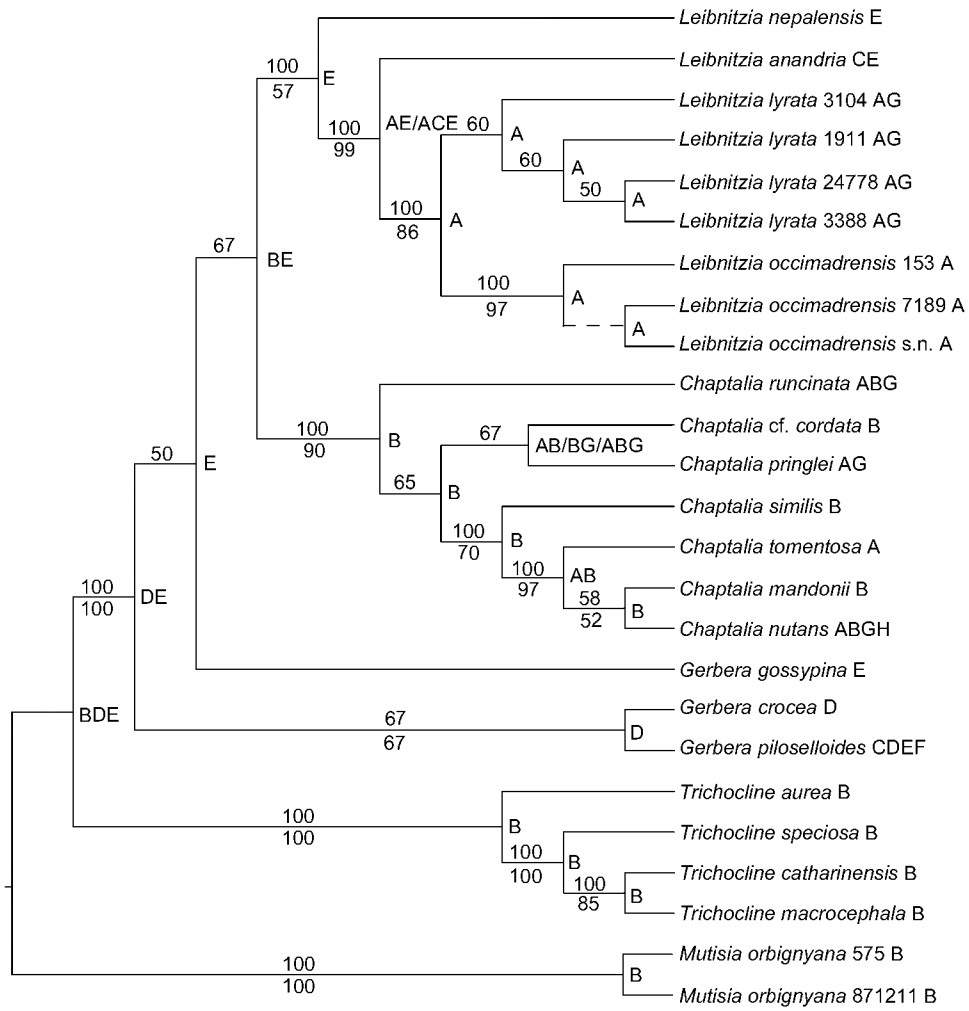

Fig. 2. Phylogeny of *Leibnitzia* obtained from parsimony analysis of the gap-coded nuclear ribosomal DNA internal transcribed spacer (ITS) and chloroplast DNA *trn*L–*rpl*32 intron dataset, 50% majority rule topology. Parsimony consensus values appear above branches and bootstrap values over 50% appear below the branches. DIVA-estimated ancestral reconstructions are shown at the nodes. Geographic areas: A, North America; B, South America; C, east Asia; D, Africa; E, west Asia; F, south Asia; G, Central America; H, Caribbean. The dashed line represents arbitrary bifurcation inserted as required by DIVA.

parsimony informative). The best-fitting model of sequence evolution determined for both datasets was the GTR model with a proportion of invariant characters (I) and rate variation as described by the Γ shape parameter (G).

Incongruence length difference tests confirmed that there was no significant difference between the phylogenetic signals of the chloroplast and nuclear datasets ($P = 0.860$). Slight topological differences between individual phylogenetic analyses of chloroplast and nuclear sequence data (data not shown) did not have minimal bootstrap support (>70%) or significant Bayesian posterior probabilities (≥0.95); thus, we report results from phylogenetic analyses of combined nuclear and chloroplast datasets, which contained 14.5% parsimony informative characters among 1780 bp and 54 gap characters.

Phylogenetic analyses of the sequence data based on parsimony, maximum likelihood, and Bayesian inference were unanimous in their support of the individual monophyly of *Leibnitzia*, *Chaptalia*, and *Trichocline* (Figs. 2, 3) as well as the sister relationship of the clade containing *Leibnitzia*, *Chaptalia*, and *Gerbera* to *Trichocline*. Because our species sampling of *Leibnitzia*, *Chaptalia*, and *Trichocline* is not complete, our results regarding their monophyly must be regarded as preliminary hypotheses. Parsimony analysis of the combined dataset yielded 180 most parsimonious trees with moderate levels of homoplasy (consistency index = 0.696; retention index = 0.795; rescaled consistency

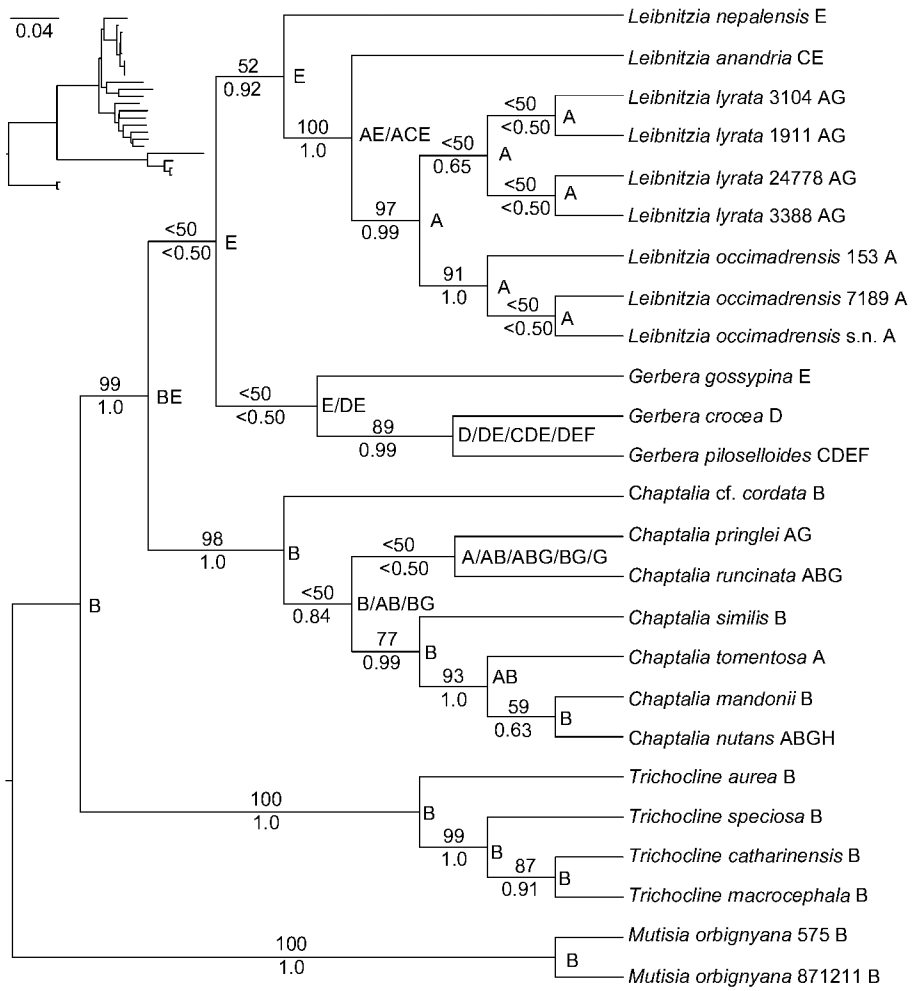

Fig. 3. Phylogeny of *Leibnitzia* obtained from maximum likelihood analysis of the combined nuclear ribosomal DNA internal transcribed spacer (ITS) and chloroplast DNA *trn*L–*rpl*32 intron dataset. Maximum likelihood bootstrap values and Bayesian posterior probabilities are included above and below each branch, respectively. DIVA-estimated ancestral reconstructions are shown at the nodes. Geographic areas: A, North America; B, South America; C, east Asia; D, Africa; E, west Asia; F, south Asia; G, Central America; H, Caribbean. Inset: branch length proportionate topology of best-fit tree in units of expected number of substitutions per site.

index = 0.553). *Leibnitzia* is monophyletic in each equally parsimonious tree, yet received only 57% parsimony bootstrap support (PBS), 52% maximum likelihood bootstrap support (MLBS), and 0.92 Bayesian posterior probability (BPP). *Chaptalia* and *Trichocline* received 90% and 100% PBS, 98% and 100% MLBS, and 1.0 and 1.0 BPP, respectively. The monophyly of the in-group clade sister to *Trichocline* received 100% PBS, 99% MLBS, and 1.0 BPP. Maximum likelihood branch lengths (Fig. 3, inset) show two long branches leading to the clade of *Leibnitzia*, *Chaptalia*, and *Gerbera*, as well as the crown group of *Trichocline* species.

Relationships among the genera of the *Gerbera*-complex clade minus *Trichocline* are less well supported and conflict between parsimony- and likelihood-based phylogenetic analyses. Parsimony analysis (Fig. 2) indicates that *Leibnitzia* is sister to *Chaptalia* (<50% PBS), which, in turn, is derived from a paraphyletic grade of *Gerbera* species (<50% PBS). Likelihood-based analyses (Fig. 3) indicate that *Leibnitzia* is sister to a clade of *Gerbera* species (<50% MLBS, <0.50 BPP), which, in turn, is sister to *Chaptalia*. The maximum likelihood branch length (Fig. 3, inset) at the common node of the *Leibnitzia* and *Gerbera* clades is the shortest branch in the topology that bifurcates clades of species belonging to different genera.

Within the clade of *Leibnitzia* species, *L. nepalensis* is consistently sister to the rest of the species (99% PBS, 100% MLBS, 1.0 BPP) and separated from them by a comparatively long branch. Asian *L. anandria* is sister to the monophyletic American group of species in all trees (86% PBS, 97% MLBS, 0.99 BPP). In

Table 3 Comparison of results from DIVA analysis using unconstrained ancestral areas (maxareas = 8) and constrained ancestral areas (maxareas = 3) on both parsimony- and likelihood-based topologies

Nodes	Parsimony-based tree		Likelihood-based tree	
	maxareas = 8	maxareas = 3	maxareas = 8	maxareas = 3
Gerbera-complex	BCDEF, ABCDEFG	BDE	B, ABCDE, BCDEF, ABCDEF, ABCDEG, BCDEFG, ABCDEFG, ABCDEFGH	B
Gerbera-complex less *Trichocline*	CDEF, ACDEFG, ABCDEFG	DE	BE, ABCDE, ACDEF, BCDEF, ABCDEF, ACDEG, ABCDEG, CDEFG, ACDEFG, BCDEFG, ABCDEFG, ACDEFGH, ABCDEFGH	BE
Leibnitzia+sister clade*	BE, AEG, ABEG	BE	E, ACDE, CDEF, ACDEF, ACDEG, ACDEFG	E
Trichocline	B	B	B	B
Gerbera clade	CDF, CDEF	D	E, DE, DEF, CDEF	E, DE
Chaptalia	B, ABG	B	B, AB, BG, ABG, ABGH	B
Leibnitzia	E	E	E, AE, ACE, AEG, ACEG	E
L. anandria (*L. lyrata*, *L. occimadrensis*)	AE, ACE	AE, ACE	AC, AE, ACE, ACG, AEG, ACEG	AE, ACE
(*L. lyrata*, *L. occimadrensis*)	A	A	A, AG	A

Geographic areas: A, North America; B, South America; C, east Asia; D, Africa; E, west Asia; F, south Asia; G, Central America; H, Caribbean.
*The internal transcribed spacer (ITS) dataset supports *G. gossypina* as a sister; the combined dataset supports the *Gerbera* clade as a sister.

the parsimony and likelihood trees, *L. lyrata* accessions resolve into a weakly supported monophyletic group (<50% PBS, <50% MLBS, 0.65 BPP) sister to a strongly monophyletic group of *L. occimadrensis* accessions (97% PBS, 91% MLBS, 1.0 BPP). When gap characters are excluded from parsimony analysis, the topology of the American species is altered slightly. In the consensus tree (data not shown), *L. lyrata* is paraphyletic with *L. lyrata* Holzinger s.n. ("1911" in Figs. 2, 3) inserted as sister to a polytomy of the other three *L. lyrata* specimens and a monophyletic *L. occimadrensis*. However, maximum likelihood analyses, which also excluded gap characters, support the monophyly of *L. lyrata* accessions. *Leibnitzia lyrata* Holzinger s.n. is the only accession of *Leibnitzia* for which the ITS region could not be amplified and is missing from the nuclear dataset.

Species relationships within the *Chaptalia* clade are mostly parallel among trees derived from different phylogenetic analyses, except for the membership of the early diverging lineages. Parsimony-based analyses support *C. runcinata* as sister to the rest of the species (<50% PBS), whereas likelihood-based analyses support *C.* cf. *cordata* (<50% MLBS, 0.84 BPP). *Chaptalia pringlei* shifts position as sister to either of these two species between analyses, but is without significant statistical support in each case. More strongly supported is the *C. similis* clade (70% PBS, 77% MLBS, 0.99 BPP), whose nested species relationships are consistently supported by all phylogenetic analyses.

Dispersal–variance analyses were conducted on both parsimony and likelihood topologies as a consequence of their differing placement of the Old World species of *Gerbera*. The DIVA analyses that incorporated the maxareas = 8 command resulted in conglomerations of ancestral areas (≤7) for basal nodes that, in all cases, included those resulting from analyses using the maxareas = 3 constraint (Table 3). Because the larger assemblages of ancestral areas from maxareas = 8 analyses often do not have a geological basis and it is physically impossible for an ancestral nodal species to be distributed so widely, we present in more detail the results from the maxareas = 3 analyses that we believe reflects more realistic biogeographic assumptions. Ancestral areas as determined by the maxareas = 3 constraint using the parsimony topology (Fig. 2) from the in-group node to the common ancestor of *Leibnitzia* and its sister *Chaptalia* are as follows: South America–Africa–west Asia to Africa–west Asia to South America–west Asia. On the likelihood tree (Fig. 3), ancestral areas from the in-group node to the common ancestor of *Leibnitzia* and its sister *Gerbera* are as follows: South America to South America–west Asia to west Asia. Both topologies support the reconstructed ancestral area of the *Leibnitzia* crown group node as west Asia and the ancestral area of the *L. anandria*–American *Leibnitzia* node as either west Asia–North America or west Asia–east Asia–North America, regardless of whether the maximum number of ancestral areas in DIVA is constrained to three or allowed to vary up to eight, the total number of areas in the analysis. Bremer ancestral analysis of the parsimony topology (Table 4) supports a South American origin for the *Gerbera*-complex and *Mutisia*, a west Asian origin for *Leibnitzia*, and a South American origin for *Chaptalia*.

Table 4 Bremer ancestral area analysis of the *Gerbera*-complex, *Leibnitzia*, and *Chaptalia* using the parsimony-based topology

Area	Gains	Losses	Gains/ losses	Ancestral area
Gerbera-complex				
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
Leibnitzia				
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
Chaptalia				
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

Results from three divergence–time analyses, including two independent methods of age calibration (Table 5), are broadly similar with the exception of the age of the in-group node for the *Gerbera*-complex. Where mean rate calibration was applied to ITS data, 95% highest posterior distributions (HPD) spanned large intervals of time for all nodes. In the case of the in-group node, this distribution included ages >100 mya. Despite the discrepancies at this basal node, mean and HPD ages for higher-level nodes are overlapping among the three Beast analyses regardless of whether mean rate calibrations or fossil calibrations are used. Crown group radiations of all genera occur within the Miocene, as indicated by mean ages for all analyses. Results from the Beast analysis of the combined sequence dataset and fossil calibration (Fig. 4) indicate that the *Gerbera*-complex radiated during the Late Oligocene (25.76 mya) and the *Leibnitzia–Chaptalia–Gerbera* clade in the Middle Miocene (12.22 mya). *Leibnitzia* diverged from *Gerbera* in the Middle Miocene (10.44 mya) and radiated beginning in the Late Miocene (7.81 mya). The divergence of Asian *L. anandria* from the American *Leibnitzia* species occurred at the end of the Pliocene (1.73 mya). The divergence of the two American *Leibnitzia* species occurred during the Pleistocene (880 000 years ago).

3 Discussion

3.1 Phylogenetic relationships of *Leibnitzia* and other *Gerbera*-complex genera

The results of the present study show that *Leibnitzia* is a monophyletic group, which is contrary to our expectation that the American *Leibnitzia* species *L. lyrata* and *L. occimadrensis* may nest within American *Chaptalia.* Instead, the clade of American *Leibnitzia* species is well nested within *Leibnitzia* and is sister to the widespread Asian species *L. anandria*. Although Nesom (1983) pointed out that *L. lyrata* was more similar morphologically to *L. anandria* than to *L. occimadrensis*, our data suggest that the similarities between these two species are retained ancestral characters rather than synapomorphies. This latter finding is consistent within those of other phylogenetic studies that have tested Asian–North American disjunct "species-pairs". In most cases, the two species are not each other's closest relatives, because one or both of the disjunct lineages is likely to have undergone speciation since the disjunction (Wen, 1999). Including the unsampled endemic Himalayan species *L. ruficoma* and *L. knorringiana* in future phylogenetic analyses would further test our conclusions. However, we expect these species would be closely related to *L. nepalensis,* would break up the long branch leading to the *L. anandria*–American *Leibnitzia* clade, or both.

The relationship of *Leibnitzia* to *Chaptalia* and *Gerbera* remains undetermined because neither

Table 5 Divergence time estimates for nodes within the *Gerbera*-complex phylogeny based on three different Beast analyses of the DNA sequence datasets

Nodes	ITS dataset, mean rate calibration	ITS dataset, fossil calibration	Combined dataset, fossil calibration
Gerbera-complex	50.20, 12.87–110.21	25.73, 17.71–35.69	25.76, 16.99–35.86
Gerbera-complex less *Trichocline*	21.34, 5.93–47.14	11.02, 6.73–15.80	12.22, 7.42–18.03
Leibnitzia+sister clade*	16.52, 4.06–36.85	8.61, 5.07–12.91	10.44, 6.24–15.49
Trichocline	20.04, 4.92–44.88	10.29, 5.92–15.25	9.09, 5.13–13.79
Gerbera clade	13.73, 3.04–31.43	7.02, 3.54–11.47	9.25, 5.34–14.12
Chaptalia	13.74, 3.58–31.14	7.11, 4.18–10.41	8.28, 4.60–12.82
Leibnitzia	11.92, 2.66–26.70	6.12, 3.12–9.71	7.81, 3.82–12.63
L. anandria (*L. lyrata*, *L. occimadrensis*)	2.69, 0.43–6.41	1.40, 0.53–2.47	1.73, 0.58–3.22
(*L. lyrata*, *L. occimadrensis*)	1.43, 0.19–3.55	0.75, 0.20–1.41	0.88, 0.23–1.71

Mean age, 95% highest probability distribution (mya).
*The internal transcribed spacer (ITS) dataset supports *G. gossypina* as a sister; the combined dataset supports the *Gerbera* clade as a sister.

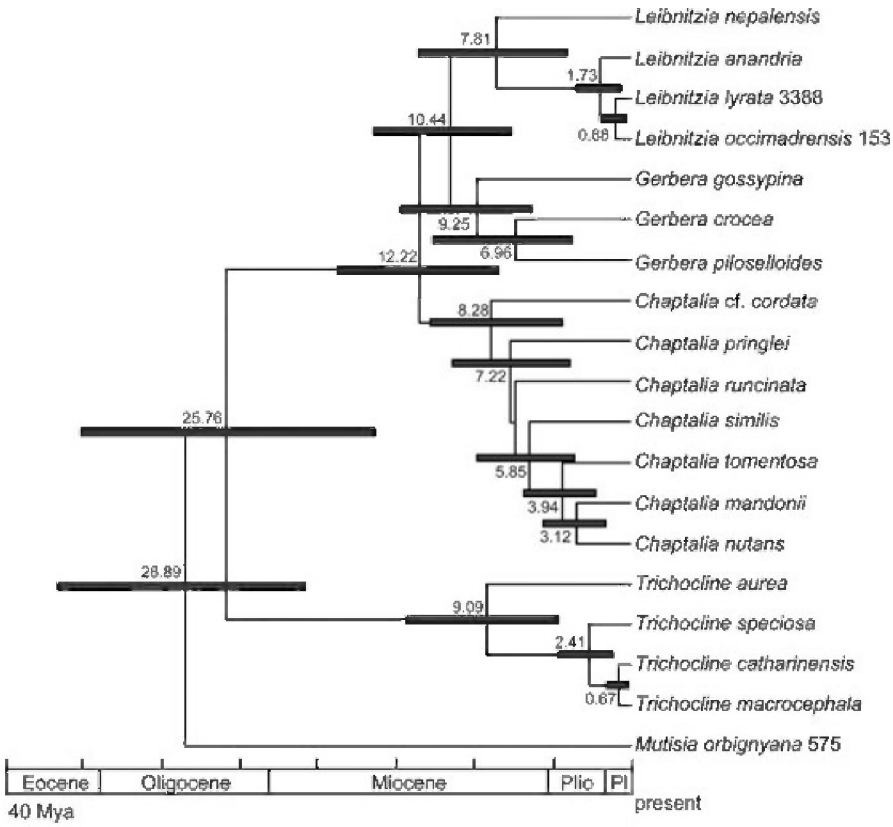

Fig. 4. Chronogram of *Leibnitzia* phylogeny as estimated by Beast analysis of the combined nuclear ribosomal DNA internal transcribed spacer (ITS) and chloroplast DNA *trn*L–*rpl*32 intron dataset using fossil calibration of the root node (23.3–45.5 mya); ≥50% consensus of clade credibility values. Mean ages (mya) are listed by nodes. Node bars represent 95% highest posterior distribution of node age estimates. The scale bar is in units of 5 mya with geological epoch boundaries. Plio, Pliocene; Pl, Pleistocene.

parsimony- nor likelihood-based methods significantly support their differing resolutions of these genera (Figs. 2, 3). Consequently, determining whether the unique breeding system shared by all *Leibnitzia* and some *Chaptalia* species represents a shared ancestral trait or a convergent adaptation remains to be determined. The focus of our study was the placement of the American species of *Leibnitzia*, and fully sampling the *Gerbera*-complex was not our priority. But it is evident that resolving *Leibnitzia*'s sister group in future studies will require both increased numbers of informative characters at this remarkably short node (Fig. 3, inset) and expanding species sampling, particularly of the genus *Gerbera*. Hansen (1990) suggested *Gerbera*, the most widespread genus of the complex, is a polyphyletic assemblage. Although we sampled only three *Gerbera* species, our phylogenies do not reject this hypothesis. Hansen (1985) asserted that African species of *Gerbera* are most closely related to each other. Interestingly, Indian *G. gossypina* is not always sister to the African pair *G. crocea–G. piloselloides* in our analyses. Establishing the major evolutionary lineages of *Gerbera* would also provide information about the biogeographic movements of the genus and, by extension, the complex as a whole.

Our results indicate that detailed phylogenetic analysis of *Chaptalia*, the other possible sister of *Leibnitzia*, is also warranted because none of the three taxonomic sections tested (sect. *Archichaptalia*, *Euchaptalia*, *Leria*) appears to circumscribe monophyletic groups. This finding is not surprising given the historical debate about *Chaptalia*'s infrageneric taxonomy. Jeffrey (1967) recognized only four of the seven sections described by Burkart (1944) and proposed combining sections *Leria* and *Lieberkuhna*. Nesom (1995) accepted Burkart's revised sections with several caveats and later noted that three sections of *Chaptalia* (*Archichaptalia*, *Euchaptalia, Leria*) overlap in some morphological features, such as leaf shape, ray size, and style morphology (Nesom, 2004). Katinas (2004) has asserted that traditional sections of *Chaptalia* must be re-evaluated. Future taxonomic work in *Chaptalia* species would be facilitated with a greater number of serial collections documenting both vernal

and autumnal capitula, particularly of the Caribbean species.

3.2 Historical biogeography of the *Gerbera*-complex and the origin of *Leibnitzia*'s Asian–North American disjunction

Dispersal–vicariance analyses of parsimony and likelihood topologies for the *Gerbera*-complex are unanimous in including South America as an ancestral area among the basal nodes, as are results from Bremer ancestral area analyses. These findings are consistent with the conclusion of Panero & Funk (2008), who hypothesized that the basal clades of Asteraceae arose in South America, transferred to other Southern Hemisphere continents, expanded into Eurasia, and, most recently, invaded and explosively radiated in North America. Intercontinental transfer of *Leibnitzia* appears to have been directed from Asia to North America, which is also consistent with the findings of Funk et al. (2005) and Panero & Funk (2008) that highly derived clades of Asteraceae tend to occupy North America, although exceptions do occur (e.g. *Hecastocleis* A. Gray). The likelihood-based topology of the *Gerbera*-complex most clearly supports the geographic transfers outlined by Panero & Funk (2008). The parsimony-based topology requires a less parsimonious geographic transfer for the common ancestor of *Leibnitzia* and *Chaptalia*, a back-dispersal from west Asia to South America. South America and west Asia were never directly connected after the advent of angiosperms; thus, long-distance dispersal must be inferred. In the likelihood-based topology, both *Trichocline* and *Chaptalia* evolve in South America, then a *Gerbera*–*Leibnitzia* ancestor transfers to west Asia and is followed by *Leibnitzia's* invasion of North America. *Leibnitzia anandria*, the closest Asian relative of American *Leibnitzia*, has a remarkably broad extant distribution in longitude, latitude, and elevation (Fig. 1). It is possible that these ecological traits, such as its climatic adaptability, mode of dispersal, or proclivity for range expansion, were shared by its ancestor and facilitated its transfer to North America.

Despite the differing assumptions of the two methods of divergence time estimation, the ages of higher-level nodes in the *Gerbera*-complex are consistent with the hypothesized Miocene radiation of the Asteraceae (Funk et al., 2005, 2009) and the appearance of most genera by the onset of the Pliocene. However, the ages of all nodes in the *Gerbera*-complex post-date episodes of Gondwanan vicariance that could have could facilitated overland migration among Southern Hemisphere continents. The exception to this pattern is the vicariance of southern South America and the Antarctica Peninsula, which is estimated to have occurred approximately 35 mya (McLoughlin, 2001). Assuming ancestral species were adapted to the cooling temperatures of this region during this time period, long-distance dispersal is still necessary to explain transfer from Antarctica to other continents. Thus, trans-oceanic dispersal is necessary to explain the early migration of *Gerbera*-complex lineages from South America to Africa and Asia with or without invoking Gondwanan vicariance.

The paleobotanical record of Mutisieae suggests transfers of ancestral taxa occurred much earlier than are indicated by our time-calibrated phylogeny of extant *Gerbera*-complex taxa. Earliest fossil evidence of Mutisieae is found in oceanic sediments collected off the coast of south west Africa in which approximately one-quarter of all pollen grains are identified as Mutisieae (Scott et al., 2006). The abundance of Asteraceae pollen in this region suggests basal lineages of Asteraceae arrived in Africa well before its recorded date of 42–46 mya. Future phylogenetic studies that aim to examine basal divergences within the *Gerbera*-complex should include a greater sampling of extant Mutisieae taxa, because these will possibly capture the older, ancestral divergences that are missing from our analyses.

Transfer of *Leibnitzia* from Asia to North America could have been caused by long-distance dispersal, overland migration, or a combination of the two during a period that spans from 6.4 mya to 430 000 years before present. An instance of trans-oceanic dispersal is possible and seems no more unlikely than others that must be invoked to explain the distribution of basal lineages of the Asteraceae. However, the ages of *Leibnitzia*'s transfer are coincident with the Bering land bridge connection between eastern Asia and North America, which was above water continuously from the Paleocene to approximately 5.32 mya (Gladenkov et al., 2002) and then periodically emergent over 100 000-year intervals during the glacial cycles of the Quaternary. The time frame of *Leibnitzia*'s transfer eliminates the possibility of the alternative overland route between Asia and North America, the North Atlantic land bridge that spanned Eurasia and North America during the Paleocene to Middle Eocene (Tiffney & Manchester, 2001). The sister group relationship between the east Asian species (*L. anandria*) and the North American clade seems consistent with the transfer via Beringia.

Paleobotanical data indicate that the Bering land bridge supported a taiga- to tundra-like flora during *Leibnitzia*'s hypothesized migration that is not dissimilar to *Leibnitzia*'s current habitat in high-elevation and high-latitude regions of Asia (Tiffney & Manchester, 2001). It is plausible that an Asian population of *Leibnitzia* expanded its range into North America via the Bering corridor and was subsequently isolated from

Asia by the onset of an interglacial period. Mean ages of divergence times for the Asian and American *Leibnitzia* lineages place the transfer occurring ca. 2 mya, at the onset of the Quaternary glacial cycles. Despite the seeming improbability of successful transfer during these repeated vicariant episodes, time-calibrated molecular phylogeographic studies (Waltari et al., 2007; DeChaine, 2008) repeatedly implicate a trend of west to east transfer of both flora and fauna across Beringia during the Early to Late Pleistocene. Ancestral American populations of *Leibnitzia* may have then migrated southward along the western mountain chains of North America to their present location in Mexico and then speciated in Mexico, possibly in response to glaciation-induced population isolation. However, the absence of American *Leibnitzia* from more northerly latitudes has no immediate explanation. Their absence suggests that *Leibnitzia* was either eliminated from these areas or, alternatively, was dispersed to Mexico from much higher latitudes via long-distance dispersal from either Beringia or Asia.

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References

Baldwin BG. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. Molecular Phylogenetics and Evolution 1: 3–16.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247–277.

Bremer K. 1992. Ancestral areas: A cladistic reinterpretation of the cener of origin concept. Systematic Biology 41: 436–445.

Burkart A. 1944. Estudio del género de Compuestas *Chaptalia* con especial referencia a las especies argentinas. Darwiniana 6: 505–594.

DeChaine EG. 2008. A bridge or a barrier? Beringia's influence on the distribution and diversity of tundra plants. Plant Ecology and Diversity 1: 197–207.

DeJong DCD, Longpre EK. 1963. Chromosome studies in Mexican Compositae. Rhodora 65: 225–240.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214–221.

Dugan KA, Lawrence HS, Hares DR, Fisher CL, Budowle B. 2002. An improved method for post-PCR purification for mtDNA sequence analysis. Journal of Forensic Sciences 47: 1–8.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10: 315–319.

Funk VA, Bayer RJ, Keeley S, Chan R, Watson L, Gemeinholzer B, Schilling EE, Panero JL, Baldwin BG, Garcia-Jacas N, Susanna A, Jansen RK. 2005. Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae. Biologiske Skrifter 55: 343–374.

Funk VA, Anderberg AA, Baldwin BG, Bayer RJ, Bonifacino JM, Breitwieser I, Brouillet L, Carbajal R, Chan R, Coutinho AXP, Crawford DJ, Crisci J, Dillon MO, Freire SE, Galbany-Casals M, Garcia-Jacas N, Gemeinholzer B, Gruenstaeudi M, Hansen HV, Himmelreich S, Kadereit JW, Källersjö, Karaman-Castro V, Karis PO, Katinas L, Keeley SC, Kilian N, Kimball RT, Lowrey TK, Lundberg J, McKenzie RJ, Tadesse M, Mort ME, Nordenstam B, Oberprieler C, Ortiz S, Pelser PB, Randle CP, Robinson H, Roque N, Sancho G, Semple JC, Serrano M, Stuessy TF, Susanna A, Unwin M, Urbatsch L, Urtubey E, Vallés J, Vogt R, Wagstaff S, Ward J, Watson LE. 2009. Compositae metatrees: The next generation. In: Funk VA, Susana A, Stuessy TF, Bayer RJ eds. Systematics, evolution, and biogeography of Compositae. Vienna, Austria: International Association for Plant Taxonomy. 747–777.

Gladenkov AY, Oleinik AE, Marincovich Jr L, Barinov KB. 2002. A refined age for the earliest opening of Bering Strait. Palaeogeography, Palaeoclimatology, Palaeoecology 183: 321–328.

Graham A. 1996. A contribution to the geologic history of the Compositae. In: Hind DJN, Beentje HJ eds. Compositae: Systematics. Proceedings of the International Compositae Conference, Kew, 1994. Kew: Royal Botanic Gardens. 123–140.

Hansen HV. 1985. A taxonomic revision of the genus *Gerbera* (Compositae, Mutisieae) sections *Gerbera*, *Parva*, *Piloselloides* (in Africa), and *Lasiopus*. Opera Botanica 78: 5–35.

Hansen HV. 1988. A taxonomic revision of the genera *Gerbera* sect. *Isanthus*, *Leibnitzia* (in Asia), and *Uechtritzia* (Compositae, Mutisieae). Nordic Journal of Botany- Section of Tropical Taxonomy 8: 61–76.

Hansen HV. 1990. Phylogenetic studies in the *Gerbera*-complex (Compositae, tribe Mutisieae, subtribe Mutisiinae). Nordic Journal of Botany 9: 469–485.

Hansen HV. 1991. Phylogenetic studies in Compositae tribe Mutisieae. Opera Botanica 109: 1–50.

Hind DJN. 2007. V. Tribe Mutisieae. In: Kadereit JW, Jeffrey C eds. The families and genera of vascular plants. Leipzig, Germany: Springer. 8: 90–122.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Jeffrey C. 1967. Notes on Compositae:II. Kew Bulletin 21: 177–223.

Katinas L. 2004. The *Gerbera* complex (Asteraceae: Mutisieae): To split or not to split. Sida 21: 935–940.

Katinas L, Pruski J, Sancho G, Tellería MC. 2008. The subfamily Mutisioideae (Asteraceae). Botanical Review 74: 469–716.

Kay KM, Whittall JB, Hodges SA. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: An approximate molecular clock with life history effects. BMC Evolutionary Biology 6: 36–44.

Kim H-G, Jansen RK. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (Krigia, Asteraceae): Additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. Plant Systematics and Evolution 190: 157–185.

Kim H-G, Loockerman DJ, Jansen RK. 2002. Systematic implications of *ndhF* sequence variation in the Mutisieae (Asteraceae). Systematic Botany 27: 598–609.

Maddison DR, Maddison WP. 2003. MacClade 4: Analysis of phylogeny and character evolution. Version 4.06 ed. Sunderland, Massechusetts: Sinauer Associates.

McLoughlin S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. Australian Journal of Botany 49: 271–300.

Nesom GL. 1983. Biology and taxonomy of American *Leibnitzia* (Asteraceae: Mutisieae). Brittonia 35: 126–139.

Nesom GL. 1984. Taxonomy and distribution of *Chaptalia dentata* and *C. albicans* (Asteraceae: Mutisieae). Brittonia 36: 396–401.

Nesom GL. 1995. Revision of *Chaptalia* (Asteraceae: Mutisieae) from North America and continental Central America. Phytologia 73: 153–188.

Nesom GL. 2004. Response to "The *Gerbera* complex (Asteraceae: Mutisieae): To split or not to split" by Liliana Katinas. Sida 21: 941–942.

Nie Z-L, Wen J, Sun H. 2007. Phylogeny and biogeography of *Sassafras* (Lauraceae) disjunct between eastern Asia and eastern North America. Plant Systematics and Evolution 267: 191–203.

Ochoterena H, Simmons MP. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Botany 49: 369–381.

Panero, JL, Funk VA. 2008. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Molecular Phylogenetics and Evolution 47: 757–782.

Scott L, Cadman A, McMilian I. 2006. Early history of Cainozoic Asteraceae along the Southern African west coast. Review of Palaeobotany and Palynology 142: 47–52.

Swofford DL. 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0 ed. Sunderland, Massechusetts: Sinauer Associates.

Tiffney BH, Manchester SR. 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. International Journal of Plant Science 162 (Suppl.): S3–S17.

Timme RE, Kuehl JV, Boore JL, Jansen RK. 2007. A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: Identification of divergent regions and categorization of shared repeats. American Journal of Botany 94: 302–312.

Waltari E, Hoberg EP, Lessa EP, Cook JA. 2007. Eastward Ho: Phylgeographical perspectives on colonization of hosts and parasites across the Beringian nexus. Journal of Biogeography 34: 561–574.

Wen J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annual Review of Ecology and Systematics 30: 421–455.

Wen J. 2001. Evolution of eastern Asian–eastern North American biogeographic disjunctions: A few additional issues. International Journal of Plant Sciences 162 (Suppl.): S117–S122.

Zwickl D. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Austin: University of Texas at Austin.