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The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed

Jose L. Panero [a,*], V.A. Funk [b]

[a] Section of Integrative Biology, 1 University Station, A6700, 141 Patterson Building, University of Texas, Austin, TX 78712, USA
[b] US National Herbarium, Department of Botany, P.O. Box 37012, Smithsonian Institution MRC 166, Washington, DC 20013-7012, USA

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Abstract

The largest family of flowering plants Asteraceae (Compositae) is found to contain 12 major lineages rather than five as previously suggested. Five of these lineages heretofore had been circumscribed in tribe Mutisieae (Cichorioideae), a taxon shown by earlier molecular studies to be paraphyletic and to include some of the deepest divergences of the family. Combined analyses of 10 chloroplast DNA loci by different phylogenetic methods yielded highly congruent well-resolved trees with 95% of the branches receiving moderate to strong statistical support. Our strategy of sampling genera identified by morphological studies as anomalous, supported by broader character sampling than previous studies, resulted in identification of several novel clades. The generic compositions of subfamilies Carduoideae, Gochnatioideae, Hecastocleidoideae, Mutisioideae, Pertyoideae, Stifftioideae, and Wunderlichioideae are novel in Asteraceae systematics and the taxonomy of the family has been revised to reflect only monophyletic groups. Our results contradict earlier hypotheses that early divergences in the family took place on and spread from the Guayana Highlands (Pantepui Province of northern South America) and raise new hypotheses about how Asteraceae dispersed out of the continent of their origin. Several nodes of this new phylogeny illustrate the vast differential in success of sister lineages suggesting focal points for future study of species diversification. Our results also provide a backbone exemplar of Asteraceae for supertree construction.

Keywords: Asteraceae; Compositae; Mutisieae; Phylogeny; Taxon sampling; Supermatrix; Supertree; Speciation; Sweepstakes dispersal; Guayana Highlands

1. Introduction

Well-resolved and statistically well-supported phylogenies are essential for many kinds of evolutionary studies, but such an estimate of relationships between genera and higher taxa is still needed for the Asteraceae, the largest family of vascular plants. With more than 23,600 species, this family constitutes approximately 8% of all flowering plants and is distributed on all continents of the world except Antarctica (Stevens, 2001 onwards). Asteraceae provides an excellent opportunity to understand adaptation in the recent radiation of a plant group at a global scale. The secondary chemistry, inflorescence morphology, and habit plasticity of Asteraceae are characteristics routinely assumed to be responsible for the worldwide success of the family (Carlquist, 1976; Hendry 1996; Stuessy and Garver, 1996). Polyploidy has also been associated with an increase in speciation rates (Vamosi and Dickinson, 2006) and this phenomenon could be responsible for the large number of species in several clades of the Asteraceae (e.g. Heliantheae alliance). The flowers and stems of many species of Asteraceae are hosts to numerous insect species in parasitic or mutualistic relationships of which only a few have been documented or studied (Ronquist and Liljeblad, 2001; Craig et al., 2007). The specialized inflorescence of Asteraceae, or capitulum, and the secondary pollen presentation mechanism of its flowers have evolved in concert,

* Corresponding author. Fax: +1 512 471 3878.
E-mail address: panero@mail.utexas.edu (J.L. Panero).

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presumably in response to several conspicuous evolutionary pressures including herbivory, pollination and/or fruit dispersal. These interactions have resulted in the evolution of several breeding systems (Ferrer and Good-Avila, 2007) and an astonishing array of morphologies used in classification but still largely unexplained in an evolutionary context. Testing assumptions that specific traits are key innovations (Schluter, 2000) or tracing the transition of character suites that lead to diversification (Donoghue, 2005) requires a phylogenetic model that includes at a minimum all major lineages of the family. Our ability to investigate macroevolutionary patterns and ultimately the processes and timing associated with the worldwide radiation of sunflowers cannot progress without phylogenies that sample sufficient characters and taxa to offer strongly supported hypotheses of relationship and include many more branches of the sunflower tree.

In spite of a long history of taxonomic work and more recently of molecular phylogenetic studies, not all the major lineages of the Asteraceae have been identified, and their relationships are certainly not known with confidence. Both Carlquist (1976) and Wagenitz (1976) reviewed the morphology of the family and concluded that the tribes of the Asteraceae could be grouped into just two main branches, namely subfamilies Cichorioideae and Asteroideae. Subsequent molecular studies based on restriction fragment length polymorphisms of the chloroplast genome identified three genera of Cichorioideae (Mutisieae: Barnadesiinae) that lacked a 22-kb inversion common to all other sunflowers (Jansen and Palmer, 1987). Bremer and Jansen (1992) erected subfamily Barnadesioideae for these and six additional genera establishing a three-subfamily system of classification. Analysis of chloroplast *ndh*F gene sequences (Kim and Jansen, 1995) showed Barnadesioideae as sister to all other sunflowers comprising a monophyletic Asteroideae and a paraphyletic Cichorioideae. Their study recovered a phylogenetic tree placing members of tribe Mutisieae in a paraphyletic grade of three clades diverging early in the family and a paraphyletic tribe Cynareae sister to the rest of Cichorioideae and Asteroideae. These results were used by Bremer (1996) to resurrect subfamily Carduoideae to accommodate the Cynareae lineage at a rank comparable to the Barnadesioideae, Cichorioideae, and Asteroideae. Recognition of Barnadesioideae and Carduoideae implied that the other lineages of the Mutisieae grade would need recognition at the subfamily level as well, once their relationships are clarified (Bremer, 1996). There have been surprisingly few family-wide molecular studies since Kim and Jansen (1995) and these (Bayer and Starr, 1998; Goertzen et al., 2003) have not improved significantly our understanding of relationships among the major lineages of the Asteraceae. Prior to the present study the family has been considered to have five primary branches formally recognized as subfamilies Asteroideae, Barnadesioideae, Carduoideae, Cichorioideae, and tribe Mutisieae (Bremer, 1996). The monophyly of at least one of these, the Mutisieae, cannot be supported (Kim and Jansen, 1995) and the "Mutisieae problem" has been viewed as the key to resolving Asteraceae systematics at higher ranks.

In fact, the monophyly of most tribes, not only of Mutisieae, remains to be tested rigorously with molecular data. Attempts at natural classification of Asteraceae began with Cassini (1819a,b) who placed genera into tribes and associated these tribes based on their collective morphological similarities. Lost in obscurity, then resurrected by Bentham (1873), Cassini's concepts of the tribal lineages of Asteraceae were the focus of modern debate and refinement until the early 1990s (Poljakov, 1967; Carlquist, 1976; Wagenitz, 1976; Cronquist, 1977; Jeffrey, 1978; Robinson, 1983; Thorne, 1983; Bremer, 1987, 1994) and probably the most important tool for understanding lineages before molecular systematics. DNA studies have only begun to test the 17 tribes recognized by Bremer (1994) but have already clarified the limits of the Anthemideae (Watson et al., 2000), Chaenactideae, Eupatorieae, Helenieae, and Madieae (Baldwin and Wessa, 2000). These five tribes appear to be monophyletic. Tribe Cynareae, monophyletic in previous molecular and morphological studies based on broad taxon sampling (Susanna et al., 2006, other references therein), has recently been shown to include the anomalous taxon *Dipterocome* (Anderberg et al. 2007), left as unplaced in Asteroideae by Bremer (1994). A number of studies have identified misplaced genera and refined tribal circumscriptions (*Feddea*, Cariaga et al., 2008; *Gymnarrhena*, *Zoutpansbergia*, *Callilepis*, Anderberg et al., 2005), clarified the phylogenetic position of unplaced genera (*Cratystylis*, Bayer and Cross 2003; *Hoplophyllum*, Karis et al. 2001) or identified genera whose tribal placements are equivocal (*Heterolepis*, *Platycarpha*, Funk et al., 2004; *Jaumea*, Baldwin et al., 2002). In addition to placing anomalous genera, i.e. those without synapomorphies of their presumed tribe, molecular studies have identified genera that are transitional between tribes (*Abrotanella*, Wagstaff et al., 2006) or astonishingly, several transitional genera found to constitute a heterogeneous but monophyletic tribe (Athroismeae, Panero, 2007a). Carlquist (1976) drew attention to both misplaced (anomalous) genera and transitional genera ("non-missing" links) as the major obstacles hindering construction of a new comprehensive classification of Asteraceae. Most of the studies listed above have been unable for the most part to clarify the phylogenetic positions of anomalous or transitional genera.

We initiated this study to construct a robust phylogenetic hypothesis of Asteraceae at the subfamily and tribal levels that would aid in constructing a classification recognizing only monophyletic groups and facilitate future evolutionary studies. In particular, we sought to define a monophyletic Mutisieae, since previous studies had brought the traditional circumscription into question but had not provided the resolution or statistical support needed to make taxonomic changes. Since a number of important taxa had not been sampled, our study includes the key mutisioid genera *Hecastocleis* and *Wunderlichia* as well as representatives of the Guayana Highlands

Mutisieae, some hypothesized to be among the deepest divergences of the tribe (Karis et al., 1992). The Guayana Highlands[1] have a high proportion of endemic taxa and are considered to be an autochthonous plant evolution center for South American plants and mutisioid Asteraceae in particular (Huber, 2005). We have also included *Corymbium*, *Gymnarrhena*, and *Warionia,* identified by Bremer (1994) as of uncertain position in subfamily Cichorioideae. We avoid the lack of resolution and statistical support of previous studies by implementing a multi-gene strategy sampling nucleotide sequences from 10 loci of the plastome (cpDNA), representing more than 13,000 characters, so that we might obtain a fully-resolved and statistically supported phylogenetic tree for Asteraceae. Novel, well-supported groups identified by our studies and lacking taxonomic recognition are formally named in Panero and Funk (2002) and Panero and Funk (2007).

2. Materials and methods

2.1. Taxon sampling

Taxa sampled for this study were chosen to represent all 17 tribes of the family recognized by Bremer (1994) with denser sampling within tribe Mutisieae. Special emphasis was given to include anomalous genera of Cichorioideae unplaced to tribe by Bremer (1994) including *Adenocaulon*, *Corymbium*, *Gymnarrhena*, *Hecastocleis* and *Warionia*. A total of 108 taxa, 56 of these representing the Mutisieae sensu Cabrera (1977) were sampled. The Asteraceae are monophyletic and sister to family Calyceraceae (DeVore and Stuessy, 1995; Kim and Jansen, 1995; Lundberg and Bremer, 2003). We chose *Acicarpha* (Calyceraceae) and *Scaevola* (Goodeniaceae) to serve as the outgroup. Appendix A lists specimens with their collection localities and herbaria where vouchers are deposited with corresponding Genbank accession numbers for the sequences used in this study.

2.2. DNA sampling and sequencing

Total genomic DNA was isolated from field-collected leaves preserved in liquid nitrogen, CTAB solution, or silica using the CTAB method (Doyle and Doyle, 1987) modified to include a 1 volume phenol–chloroform–isoamyl alcohol extraction, resuspending DNA in water–7 M sodium acetate (10:1), precipitating the DNA with 1 volume of ethanol, followed by two 70% ethanol washes. Herbarium samples were isolated using the DNeasy® Plant Mini Kit from Qiagen, Inc. (Qiagen, Valencia, California, USA).

Chloroplast loci sequenced in this study include the genes *mat*K, *ndh*D, *ndh*F, *ndh*I, *rbc*L, *rpo*B, and exon 1 of *rpo*C, as well as the *trn*L–*trn*F and 23S-*trn*A intergenic spacer regions, and the 5′ portion of the *trn*K split intron. The *rbc*L gene was the first and most exploited marker for plant phylogenetic studies and continues to be employed above the genus level (Ritland and Clegg, 1987; Saarela et al., 2007). The *ndh*F gene has been utilized in earlier studies aimed at elucidating the major branches of the Asteraceae (Kim and Jansen, 1995; Jansen and Kim, 1996) and more recently the utility of *mat*K has been shown in numerous intergeneric and infrageneric studies within the family (Susanna et al. 2006; Wagstaff et al., 2006; Watanabe et al., 2006). The genes *ndh*D and *ndh*I are part of the same gene family of *ndh*F, therefore they were explored in our study because of the known phylogenetic utility of the *ndh*F subunit in previous sunflower DNA phylogenetic reconstructions. Preliminary studies of the Heliantheae alliance (Panero et al., unpublished) found that the co-transcribed polymerase genes *rpo*B and *rpo*C also provide many informative characters with low levels of homoplasy. We favor protein coding sequence over noncoding markers for their reduced ambiguity of alignment. However, noncoding data provide informative characters and have been used extensively in Asteraceae systematics, so approximately one-fifth of the characters we analyzed come from noncoding regions. DNA fragments were amplified by Polymerase Chain Reaction (PCR) using primers described in Panero and Crozier (2003) or as otherwise noted.

The *ndh*F gene was amplified in two segments using primers 52 and 1212 (52: 5′-AGG TAA GAT CCG GTG AAT CGG AAA-3′, Jansen, 1992; 1212R: 5′-GGT GGA ATA CCA CAA AGA-3′) and primers 972F and 607 (972F: 5′-GTC TCA ATT GGG TTA TAT GAT G-3′; 607: 5′-ACC AAG TTC AAT GTT AGC GAG ATT AGT C-3′, Jansen, 1992). All primers except 607 were used as sequencing primers also. Primer 1587F (5′-CCA ACC CTT TCT TTC TAT TCC G-3′) was used to sequence the last segment of the gene. The genus *Inula* contains an extra codon in the primer region so primer 1587Inula (5′-CCA ACC CTT TCT TTC TAT TCC TCC G-3′) was used instead.

The *mat*K gene was sequenced in three segments using primers 3914F and 884R (3914F: 5′-TGG GTT GCT AAC TCA ATG G-3′, Johnson and Soltis, 1994; 884R: 5′-TGT CAT AAC CTG CAT TTT CC-3′), primers 816F and 1857R (816F: 5′-ATC TTT CAG GAG TAT ATT TAT G-3′; 1857R: 5′-CCA GAG GCA TAA TTG GAA C-3′), and primers 1755F and trnK2R (1755F: 5′-TCC TAT TTT TAC CTG TGG TCT CA-3′; trnK2R: 5′-AAC TAG TCG GAT GGA GTA G-3′, Johnson and Soltis, 1994). These primers produce a complete sequence

[1] Also known as the Pantepui Province (Huber, 1987), this distinctive phytogeographic region comprises more than 25 Roraima sandstone table mountains (*tepuis*) on the Guayana Shield (Maguire, 1956) between 1500 and 3000 m (Rull, 2004). The Guayana Highlands lie mostly within the Venezuelan Guayana and adjacent northern Brazil and western Guyana, north of the Amazon and south of the Orinoco River (Maguire, 1956). Isolated from surrounding lowlands by steep base slopes and vertical walls, and mostly inaccessible except by helicopter, the tabular summits covered with a diverse and highly endemic flora were described as a fictional "Lost World" by Arthur Conan Doyle in 1912.

of the gene. A partial sequence of the 5′-*trn*K intron as read by primer 884R was also used in the phylogenetic analysis. Primers trnK2R and 3914F were not used in sequencing.

The *rbc*L gene was amplified in two segments using primers rbcL1 and rbcL911R (rbcL1: 5′-ATG TCA CCA CAA ACA GAG ACT AAA GC-3′ Hillis et al., 1996; rbcL911R: 5′-TTT CTT CGC ATG TAC CCG C-3′) and primers rbcL876F and rbcL2 (rbcL876F: 5′-CAG GTG AAA TCA AAG GGC-3′; 5′-CTT TTA GTA AAA GAT TGG GCC GAG-3′; Olmstead et al., 1992). All PCR primers were used as sequencing primers.

The *ndh*D gene was amplified in two segments using primers ndhDF and 732R (ndhDF: 5′-TTC GAC CTT GTC AAC TGC-3′; 732R: 5′-TTG CCG ATT CTA CCC CTA C-3′) and primers ndhDR and 672F (ndhDR: 5′-GAA CTC CTT CTA ACG ACT TAT GC-3′; 672F: 5′-CGG CTA GAA GCA TAC AAG-3′). Primers 732R and 672F were used as sequencing primers.

The *ndh*I gene was amplified using primers ndhGF (5′-CCG ACC CTA GAA AGA CTA AAA G-3′) and primer ndhAexon2R (5′-CGT CCC AAC TTC TTT CAC TG-3′). Primer ndhAexon2R was used as the sequencing primer.

The *trn*L intron and *trn*L–*trn*F spacer were amplified using primers C-Aster (5′-CGA AAT TGG TAG ACG CTA CG-3′) and primer F (5′-ATT TGA ACT GGT GAC ACG AG-3′) (Taberlet et al., 1991). Primer C-Aster was used as the sequencing primer.

The *rpo*B gene was amplified in three sections using primers rpoCF and rpoB1394R (rpoCF: 5′-GAA ACT GAT CCA ATT CGG AG-3′; rpoB1394R: 5′-TGG GGA TAC TCT AAG GAT TCC-3′), rpoB1270F and rpob2503R (rpoB1270F: 5′-TTC GCC ACC AAC TGT AGC AG-3′; rpoB2503R: 5′-TTG TGT AGA GGG AGA TCC G-3′) and rpoB2426and either rpoBR1 or rpoBR2 (rpoB2426: 5′-AAT TGG GAG GGA TTG GTC G-3′; rpoBR1, 5′-CAA GGT TTG ACG GAA GAA C-3′; rpoBR2: 5′-GAT CAA GGT TTG ACG GAA G-3′).

Exon 1 of the rpoC1 gene was amplified and sequenced for this study. Primers rpoC952F and either rpoBSR1 or rpoBSR2 were used as PCR and sequencing primers (rpoC952F: 5′-CCC TCT TTG CCT TCA ATT AC-3′; rpoBSR1: 5′-CGG TTG TTC GTT CGA GAA C-3′; rpoBSR2: 5′-CGA TCT TTA GCT CTG GAA CTG-3′).

The 23S-*trn*A spacer and the *trn*A intron were sequenced using primers 23SF (5′-ATC CAC CGT AAG CCT TTC-3′) and trnIR (5′-ATT GGT TGG ACC GTA GGT GC-3′). Primer 23SF was used to sequence.

The PCR products were cleaned with QIAquick PCR Purification Kit (Qiagen, Inc.) according to manufacturer's protocols. Sequencing was performed at the DNA sequencing facility of the University of Texas using Big Dye terminator chemistry (version 3.0, Applied Biosystems, Foster city, California, USA). Sequence chromatograms were proofread and nucleotides aligned visually using Sequencher (version 4.5, Gene Codes Corp., Ann Arbor, Michigan, USA). Trimmed fragment matrices (contigs) were concatenated in NEXUS format for each data partition. Coding sequence partitions were translated and codon alignment checked, especially for uniformity and homology of stop codons using MacClade 4.05 (Maddison and Maddison, 2002). All character partitions are expected to have the same underlying history because chloroplast genes are linked and non-recombining.

2.3. Phylogenetic analyses

Maximum parsimony analyses of the combined data were performed using PAUP* 4.0b10 (Swofford, 2003). The most parsimonious trees were found by a heuristic search using tree bisection reconnection (TBR), MULPARS, and simple taxon addition. The same analysis was performed with 100 random additions replicates of the data also. All characters in the data matrix were unordered and equally weighted and gaps were treated as missing data. Support for monophyletic groups was assessed using 1000 non-parametric bootstrap replicates (Felsenstein, 1985) using the same settings as the parsimony analysis. Bootstrap proportions >70% are considered well supported (Hillis and Bull, 1993). Tree statistics including consistency index and the retention index were calculated using PAUP*.

Bayesian analysis of the combined data set was accomplished using MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001). A best model of nucleotide evolution was chosen from the 24 models implemented in MrModeltest 2.1 (Nylander, 2004) using Akaike information criterion (Akaike, 1974). Four replicate Metropolis-coupled Markov chain approximations were run for 10 million generations, each starting from random trees. For each replicate run four Markov chains were run, one cold and three heated (temp = 0.5) to facilitate mixing, sampling likelihood values and trees from the cold chain every 100 generations for a total of 100,000 trees. Stationarity of the Markov chain was ascertained by plotting likelihood values against number of generations and the four plots compared for apparent stationarity. Trees sampled before stationarity were discarded as burnin. Following Alfaro et al. (2003) we consider posterior probabilities >0.95 as significant probability for a clade.

2.4. Bayesian hypothesis tests

We used our data to test two a priori hypotheses of Mutisieae. To test the monophyly of the Guayana Highlands genera, including *Stifftia* and *Wunderlichia* as proposed by Jiménez Rodríguez et al. (2004), we first constructed a tree consistent with that hypothesis containing a single resolved node shared by all the Guayana Highlands genera of classical Mutisieae that we sampled including *Stenopadus*, *Stomatochaeta*, *Chimantaea*, *Duidaea*, *Gongylolepis*, *Wunderlichia* and *Stifftia*. We then used this constraint tree to filter all post-burnin trees sampled in one of our Bayesian analyses. The number of trees found to

be consistent with the constraint divided by the total number of trees, yielded a proportion that was converted to a percentage and taken as the probability of monophyly of such a clade given the data and the GTR + I + Γ model used. A similar procedure was used to test the monophyly of Mutisieae including *Stifftia* as found by Kim and Jansen (1995) using a tree constraining the nine genera sampled by Kim and Jansen (*Stifftia*, *Onoseris*, *Trixis*, *Acourtia*, *Perezia*, *Nassauvia*, *Mutisia*, *Adenocaulon*, and *Gerbera*), along with 17 genera not sampled by Kim and Jansen but shown here to be included in our *Mutisia* and *Stifftia* clades namely *Lycoseris*, *Plazia*, *Aphyllocladus*, *Lophopappus*, *Proustia*, *Leucheria*, *Jungia*, *Dolichlasium*, *Pachylaena*, *Trichocline*, *Brachyclados*, *Chaetanthera*, and *Chaptalia* (*Mutisia* clade) and *Gongylolepis*, *Duidaea*, *Hyaloseris* and *Dinoseris* (*Stifftia* clade).

3. Results

3.1. Phylogenetic analyses

Concatenation of all 10 markers resulted in a data matrix containing 13,299 nucleotides for 108 taxa. The data matrix contained 1080 sequences of which more than 1060 were new and contributed to the GenBank database (Appendix A). Nine sequences included in the analyses were obtained from Genbank and correspond to the gene *ndh*F for the genera *Athroisma*, *Barnadesia*, *Blepharispermum*, *Helianthus* and *Tagetes* and the gene *rbc*L for the genera *Barnadesia*, *Dasyphyllum*, *Scaevola*, and *Stokesia* (Appendix A). Missing data amounted to approximately 3.9% of the total. A summary of the Maximum Parsimony statistics for each data partition is presented in Table. 1. Approximately 20% of the 13,299 characters in the data matrix for our taxon sampling were parsimony informative. Among the 10 data partitions, *mat*K had the highest percentage of informative characters with 28% whereas the 23S-*trn*I region had the lowest with only 4% (Table 1).

Maximum parsimony analysis of the concatenated matrix by simple taxon addition produced 72 most parsimonious trees each 11,043 steps in length. The strict consensus tree of these is shown in Fig. 1. The consistency index was 0.4473 (excluding uninformative characters) and Retention Index was 0.6773. Base frequencies for these data were found to be A: 0.3042, C: 0.1748, G: 0.1850, T: 0.3360 and transition rates were calculated as A–C: 1.2814, A–G: 1.8338, A–T 0.2546, C–G: 1.2137, C–T: 1.9020, and G–T: 1.000. Results from the 100 random addition replicates indicate only one tree island was found. The proportion of invariable sites (I) was 0.4189 and the gamma distribution shape parameter was estimated at 0.9259. Based on Akaike criterion a General Time Reversible model with gamma-distributed rates and correcting for a proportion of invariant sites was selected for Bayesian analysis. The Markov chains were determined to have reached stationarity in all replicate runs after 200,000 iterations. A majority rule consensus tree summarizing 98,000 post-burnin samples is shown in Fig. 2.

Table 1
Properties of data partitions used in this study and statistical characteristics resulting from MP analyses

Partition	Aligned length	Informative characters (percentage of total)	Tree length	CI	RI
*mat*K	1610	446 (28%)	1848	0.47	0.62
*ndh*D	1408	264 (19%)	1070	0.45	0.62
*ndh*F	2328	572 (25%)	2586	0.42	0.63
*ndh*I	501	75 (15%)	270	0.49	0.73
*rbc*L	1412	251 (18%)	1217	0.33	0.60
*rpo*B	3151	573 (18%)	2021	0.52	0.53
*rpo*C1exon1	518	71 (14%)	222	0.64	0.86
23S-*trn*A	658	29 (4%)	104	0.85	0.91
*trn*L–*trn*F	1162	234 (20%)	1000	0.53	0.68
5′-*trn*K	551	112 (20%)	464	0.45	0.59
Combined	13,299	2627 (20%)	11,043	0.45	0.68

3.2. Shared insertion/deletions

Several DNA indels were shared by genera that can serve as quick identification for their phylogenetic position among the major clades of Asteraceae. The 9-base pair (bp) deletion in *ndh*F reported by Kim and Jansen (1995) was found in all taxa of the Asteroideae, Cichorioideae, and Corymbioideae (Vernonioid group sensu Bremer, 1996). The *rpoB* gene contains four major indels including: (1) a deletion of 9 bp at aligned position 2082–2090 shared by the Carduoideae–Asteroideae clade; (2) an insertion of 18 bp at aligned position 2408–2425 also shared by the Carduoideae–Asteroideae clade; (3) a 15-bp deletion at aligned position 2385–2399 shared by Hecastocleidoideae–Asteroideae ("Out of South America") clade and; (4) a 6-bp deletion at aligned position 2394–2399 in Wunderlichioideae. These indels have been mapped to the phylogeny presented in Fig. 1.

3.3. Phylogeny

Twelve major lineages of Asteraceae were recovered congruently by both Bayesian and Maximum Parsimony methods (Figs. 1 and 2). Splitting sequentially from the sister family Calyceraceae, these correspond to subfamilies Barnadesioideae, Mutisioideae, Stifftioideae, Wunderlichioideae, Gochnatioideae, Hecastocleidoideae, Carduoideae, Pertyoideae, Gymnarrhenoideae, Cichorioideae, Corymbioideae, and Asteroideae. All 12 lineages are supported by significant (>95%) posterior probabilities (PP) in the Bayesian analyses and moderate to high (>70%) bootstrap proportions (BS) in parsimony analysis with the exception of the Wunderlichioideae clade (52% BS, 91% PP). Relationships among ten of the 12 lineages were also congruent between methods, and supported by significant posterior probabilities and strong bootstrap proportions except for Gochnatioideae (65% BS). However, placement of the Wunderlichioideae and the Stifftioideae lineages was equivocal in Bayesian and parsimony

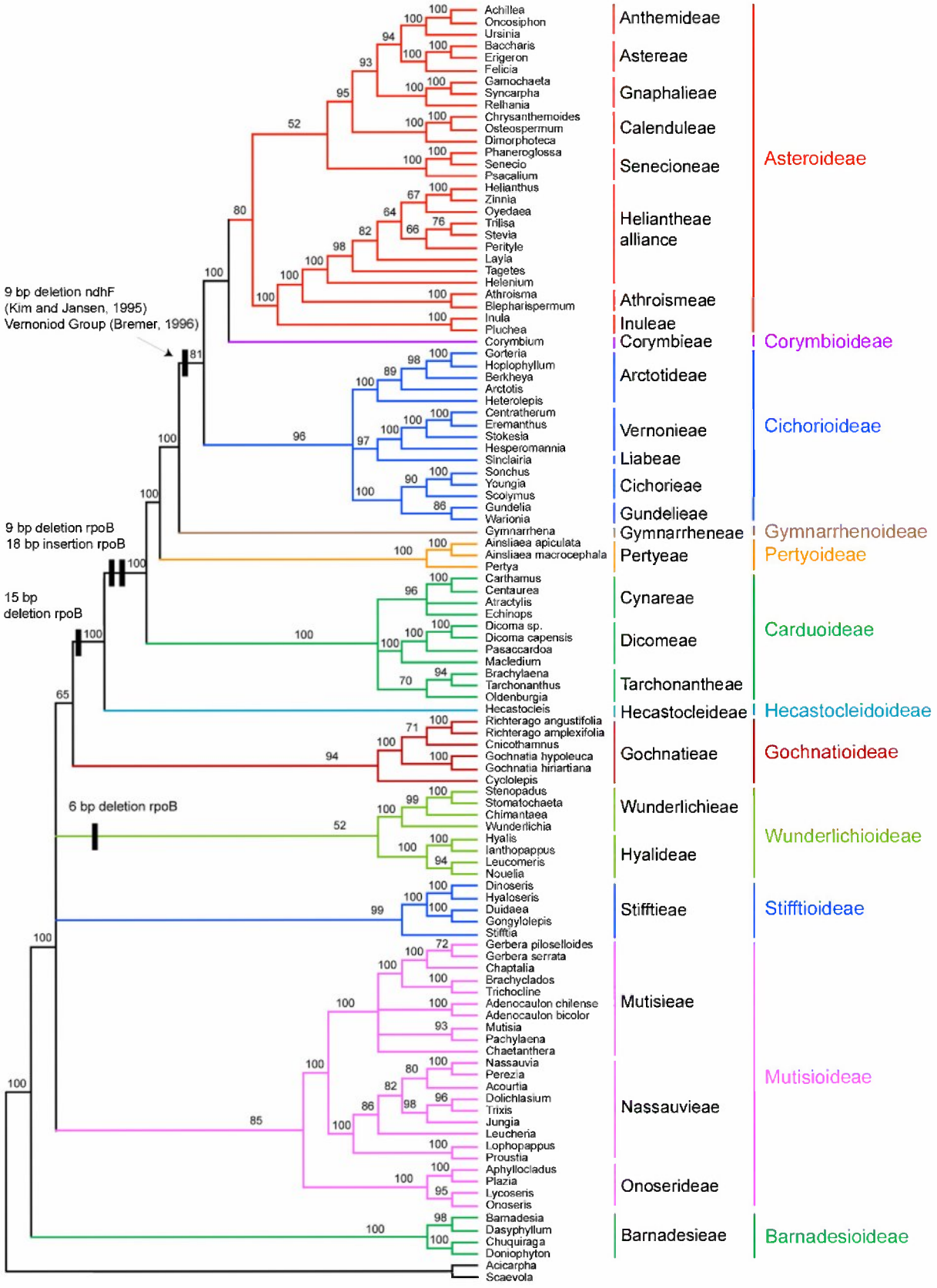

Fig. 1. Strict consensus of 72 most parsimonious trees resulting from Maximum Parsimony analysis of combined data (10 chloroplast loci). Bootstrap proportions shown above the branches. Black bars map major indels discussed in Section 3.2.

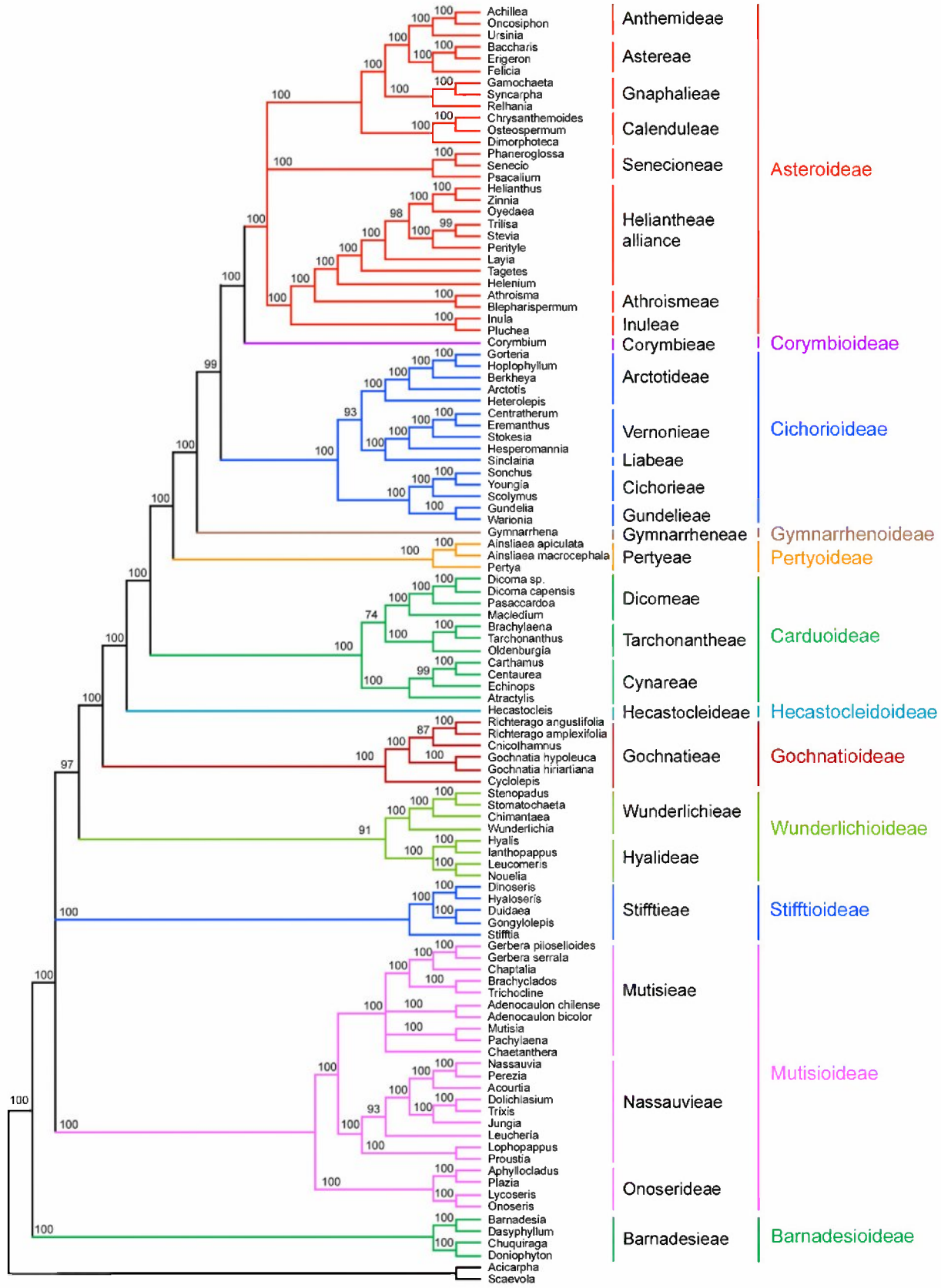

Fig. 2. Majority Rule consensus of 98,000 post-burnin trees obtained from Bayesian analyses of the combined data (10 chloroplast loci) using GTR + I + Γ. Numbers above branches represent posterior probabilities.

methods. In parsimony analysis these two clades were unresolved together with Mutisioideae, whereas in all Bayesian analysis the Wunderlichioideae was placed sister to the Gochnatioideae–Asteroideae clade. The most parsimonious trees placed Wunderlichioideae variously, either as sister to the Gochnatioideae–Asteroideae clade as in Bayesian analyses, or sister to the Mutisioideae, or sister to Stifftioideae and collectively sister to the Mutisioideae. The Stifftioideae were either placed as the next lineage to split after Barnadesioideae or sister to the Mutisioideae.

Not only are the phylogenetic positions of Stifftioideae and Wunderlichioideae novel but their compositions are as well. The taxa endemic to the Guayana Highlands were found not to be monophyletic contrary to previous studies (Maguire, 1956; Pruski 1991; Bremer, 1994). Both the Stifftioideae and Wunderlichioideae contain tropical Brazilian genera sister to clades of Andean, eastern temperate South America and/or Guayana Highlands genera. The Stifftioideae was found to comprise the mostly Brazilian genus *Stifftia* sister to two clades namely, *Hyaloseris* and *Dinoseris* of the central Andes sister to *Gongylolepis* and *Duidaea* of the Guayana Highlands. The Wunderlichioideae was found to contain two clades: Wunderlichieae and Hyalideae both of whom share a 6 bp deletion in *rpo*B unique among Asteraceae. The former holds the Brazilian Planalto endemic genus *Wunderlichia* sister to Guayana Highlands genera *Chimantaea*, *Stenopadus*, and *Stomatochaeta*. The latter clade holds the southern South American genera *Hyalis* and *Ianthopappus*, and the Asian genera *Nouelia* and *Leucomeris*.

The Mutisioideae was found to contain three lineages with Onoserideae sister to the Mutisieae–Nassauvieae clade. Nassauvieae and Mutisieae were found to be monophyletic and *Adenocaulon* was strongly supported as a member of the Mutisieae. Phylogenetic relationships among members of the three tribes were fully resolved except for those within Mutisieae. The scapose inflorescence group of Mutisieae including *Brachyclados*, *Trichocline*, *Chaptalia*, and *Gerbera* was strongly supported as a monophyletic group (100% BS, 100% PP). *Mutisia* is sister to *Pachylaena*. *Lophopappus* and *Proustia* are genera with species having actinomorphic and bilabiate corollas sister to all other genera of Nassauvieae that are characterized by bilabiate corollas. The yellow-corolla Nassauvieae exemplified by *Trixis* were found to be sister to *Nassauvia*, *Perezia*, and *Acourtia*. The Onoserideae comprised two main clades with *Plazia* and *Aphyllocladus* sister to *Onoseris* and *Lycoseris* (Figs. 1 and 2).

The phylogenetic position of the Gochnatioideae was only weakly supported in parsimony analyses (65% BS), but strongly supported in Bayesian analyses (100% PP). *Cnicothamnus* and *Richterago*, the only genera of the subfamily with marginal corollas containing a limb, formed a clade. There is support for the recognition of *Richterago* as distinct from *Gochnatia* although no South American representative of *Gochnatia* was sampled. *Cyclolepis* was found to be the sister taxon to all other genera of the subfamily sampled.

The Carduoideae branch was strongly supported in both analyses (100% BS, 100% PP). The subfamily Carduoideae was found to include not only members of tribe Cynareae but also *Tarchonanthus*, *Brachylaena*, *Dicoma*, *Macledium*, *Pasaccardoa*, and *Oldenburgia*, genera traditionally placed in tribe Mutisieae. The Carduoideae contained three major lineages: tribes Cynareae, Tarchonantheae, and Dicomeae. Resolution among these three tribes was equivocal. The strict consensus of most parsimonious trees collapses these branches to a trichotomy. Bayesian analysis placed Dicomeae as sister to Tarchonantheae with only 74% posterior probability. *Oldenburgia* is supported as a member of Tarchonantheae (70% BS, 100% PP).

The monophyly of the Cichorioideae was strongly supported in both Maximum Parsimony and Bayesian analyses (96% BS, 100% PP). Our studies showed there are three main lineages in the subfamily, Arctotideae, Vernonieae plus Liabeae, and Cichorieae plus Gundelieae. Parsimony analysis failed to resolve the relationships among these three lineages, whereas Bayesian analyses placed the Vernonieae–Liabeae clade as sister to Arctotideae without strong support (93% PP) and collectively sister to the Gundelieae–Cichorieae clade with strong support (100% PP). *Warionia* was strongly placed (86% BS, 100% PP) as sister to *Gundelia* of the Gundelieae (Figs. 1 and 2).

Parsimony and Bayesian analyses strongly support subfamily Asteroideae (80% BS, 100% PP). Both methods also strongly supported a clade consisting of tribe Calenduleae as sister to tribes Gnaphalieae, Anthemideae, and Astereae (95% BS, 100% PP) and another containing tribes Inuleae, Athroismeae, and Heliantheae alliance (100% BS, 100% PP). Parsimony analysis placed Senecioneae sister to the Calenduleae clade but without support (Fig. 1), whereas Bayesian analysis failed to resolve the relationship of the Senecioneae to other tribes of Asteroideae (Fig. 1). The relationships among tribes of the Heliantheae alliance were found to be in agreement with those reported in Panero (2007b).

3.4. Bayesian tests of monophyly

The hypothesis that classical Mutisieae genera of the Guayana Highlands are monophyletic has a 3% posterior probability and is rejected. The monophyly of classical Mutisieae including *Stifftia* (here Mutisioideae including Stifftioideae) has a 26.6% posterior probability and therefore could not be rejected at a 5% significance level. Results from these Bayesian analyses are conditional probabilities, conditioned on the data, the GTR+I+Γ model of nucleotide evolution, and the prior probabilities used.

4. Discussion

Our phylogenetic analyses identified 12 major clades in Asteraceae. Eleven of these lineages are statistically sup-

ported; the exception being Wunderlichioideae with only 91% posterior probability. Only Barnadesioideae, Cichorioideae, and Asteroideae had been identified with strong bootstrap support in previous studies. We recognize all 12 lineages at the subfamily level. Phylogenetic relationships among 10 of the 12 subfamilies are strongly supported by both bootstrap proportions and posterior probabilities and this represents a significant advancement in our understanding of the evolutionary history of Asteraceae. Discussion of the systematic and taxonomic implications of each major clade follows the branching order represented in Figs. 1 and 2. Members of Barnadesioideae are strongly supported as sister to a clade encompassing the 11 other major lineages of the family.

The classical circumscription of tribe Mutisieae (Cabrera, 1977) is herein amended. The lineage containing the type genus *Mutisia* is here recognized as Mutisioideae. The subfamily is much reduced from its classical circumscription with several of its members referred herein to the Stifftioideae, Wunderlichioideae, Gochnatioideae, Hecastocleidoideae, Carduoideae–Dicomeae, Carduoideae–Tarchonantheae, and Pertyoideae.

4.1. Mutisioideae

Mutisioideae was found to contain three main branches: Mutisieae, Nassauvieae, and Onoserideae. Mutisioideae as recognized here contains approximately 44 genera and 630 species. The subfamily is primarily South American with the exception of three derived genera (*Chaptalia*, *Gerbera*, *Trichocline*) and *Adenocaulon* that have attained cosmopolitan distributions except Europe. The subfamily can be characterized as having disc corollas with deeply dissected lobes, some of its members having bilabiate corollas, capitula with imbricate phyllaries, anthers calcarate and caudate with strongly sclerified anther appendages, and styles usually well-exserted from the floret and essentially glabrous. Most species are annual or perennial herbs, although shrubs, small trees and vines are also present. Hybrids of species of the genus *Gerbera* are widely cultivated for their large capitula and vibrant colors.

Many studies have been aimed at clarifying the relationships of classical Mutisieae using detailed morphological features ranging from ligule micromorphology to pollen ultrastructure (Hansen, 1991 and references therein; Zhao et al., 2006). Revisionary or cladistic studies of the group have invariably concluded that Mutisieae are paraphyletic and require dismemberment (Cabrera, 1977; Hansen, 1991; Karis et al., 1992; Bremer, 1994, 1996). However, among these studies there has been no consensus for construction of a stable classification for the group and conclusions about clade circumscription and/or monophyly have often been contradictory.

4.1.1. Mutisieae

Our studies support a reduced tribe Mutisieae to include approximately 14 genera and 200 species with the great majority endemic to South America. The composition of the tribe as supported by our molecular studies roughly corresponds to the circumscription of subtribes Gerberinae and Mutisiinae of Hind (2007) excluding the genera of the Onoserideae (see below). The genera sampled include: *Adenocaulon*, *Brachyclados*, *Chaetanthera*, *Chaptalia*, *Gerbera*, *Mutisia*, *Pachylaena*, and *Trichocline*.

The southern Andean, mostly herbaceous genus *Chaetanthera* has been traditionally allied to *Brachyclados*, *Pachylaena* and *Trichocline* (Cabrera, 1937). Palynological studies by Tellería and Katinas (2004) support this view. Most species of the genus share a translucent wing on the phyllaries and trichomes on the anthers (Hansen, 1991). Chloroplast *ndh*F studies by Kim et al. (2002) revealed *Chaetanthera* as sister to *Duidaea*, a genus endemic to the Guayana Highlands. In contrast, our results show *Chaetanthera* is not closely related to *Duidaea* of the Stifftioideae but firmly nested within Mutisieae, although its relationships within the tribe are equivocal.

Our study shows that *Gerbera* is closely related to *Chaptalia* and collectively sister to *Brachyclados* and *Trichocline* (Gerberinae). This result is consistent with morphological studies (Hind, 2007) and the molecular study of Kim et al. (2002). These four genera for the most part can be characterized by their acaulescent habit and solitary monocephalous inflorescences on long scapes. Their primary distribution is in South America with the genus *Chaptalia* and segregates also present in North America and Asia. Kim et al. (2002) showed *Gerbera* derived from *Chaptalia* making the latter not monophyletic. *Gerbera* is considered to be an Old World endemic (Hansen, 1991). However, other authors believe *Gerbera* to have one or two species in America (*Gerbera hieracioides* (Kunth) Zardini, Zardini, 1974; *Gerbera hintonii* (Bullock) Katinas, Katinas, 1998).

Speculation about the affinities of *Adenocaulon* has been extensive, and historic taxonomic views are summarized in Katinas (2000). *Adenocaulon* contains five species with a disjunct distribution in North America, Mesoamerica, South America, and the Himalayas. It is perhaps the most widespread genus of Mutisioideae. *Adenocaulon* is an anomalous genus that has lost several of the obvious morphological synapomorphies of Mutisieae and possesses characters that point to a relationship to subfamily Asteroideae. These include basally constricted anther appendages, small anthers, and disciform capitula (Katinas, 2000). Katinas concluded that *Adenocaulon* along with *Eriachaenium* should be placed in their own tribe within Cichorioideae. Previous molecular studies have placed the genus in tribe Nassauvieae (Kim et al., 2002), although this position was not supported (24% BS), or in the Mutisieae (Jansen and Kim, 1996). We sampled two American species, *Adenocaulon bicolor* and *Adenocaulon chilense*, and found them to be strongly supported as a lineage within tribe Mutisieae. However, the relationship of *Adenocaulon* to other Mutisieae genera was not resolved (Figs. 1 and 2).

4.1.2. Nassauvieae

Our studies show Nassauvieae to be sister to Mutisieae, with the genera *Lophopappus* and *Proustia* as sister to the other genera of the tribe. Nassauvieae contain 24 genera and approximately 370 species (Panero, 2007c) distributed mainly in South America with the genera *Acourtia* and *Berylsimpsonia*, and some species of *Trixis* endemic to North America. The tribe has been traditionally viewed as a natural group because most of its genera have capitula with only bilabiate corollas and truncate (rarely round) style branch apices, a combination of characteristics distinctive in the family. Crisci (1974) modified the traditional concept of the tribe by adding *Lophopappus* and *Proustia*, which he considered sister genera. These two genera have species with actinomorphic corollas, and rounded styles but share similar pollen morphology with Nassauvieae. Our results are consistent with his circumscription of the tribe based on pollen and floral morphology.

Ours is the first molecular study to provide a robust hypothesis of generic relationships in Nassauvieae. We sampled nine of the 24 genera of the tribe along with *Adenocaulon*. Kim et al. (2002) included seven of the same genera we sampled but did not include *Lophopappus* or *Dolichasium*. They hypothesized relationships based on one of 5056 most parsimonious trees found. In their strict consensus tree (bootstrap values not provided) major clades of *Nassauvia* collapse to a polytomy, though the relationships of *Proustia* with *Trixis*, *Perezia* with *Nassauvia*, and *Triptilion* embedded within *Nassauvia* were maintained, as was the association of *Jungia* with *Leucheria* (shown with 34% bootstrap support on one of the most parsimonious trees). Although we also found *Perezia* and *Nassauvia* to form a clade, our results differ significantly from those reported by Kim et al. (2002). Based on cladistic analyses of morphological characters, Crisci (1980) posited three scenarios for relationships within Nassauvieae depending on whether *Dolichlasium*, *Trixis*, or an hypothetical ancestor was used to polarize characters. Our results do not agree with any of these three scenarios, although we did find *Lophopappus* and *Proustia* to be sister, and these to be sister to the other genera of the tribe. These two genera have floral characteristics seen in the sister tribe Mutisieae, but rare in Nassauvieae. *Leucheria* was found to be sister to a clade containing the two largest genera of the tribe, *Acourtia* and *Trixis*. The *Acourtia* clade also contains *Nassauvia*, and *Perezia*. *Acourtia* was historically included in *Perezia* but later recognized to be distinctive and shown to share features with *Lophopappus* and *Proustia* (Crisci, 1980). The close relationship between *Perezia* and *Nassauvia* found here corroborates that of Kim et al. (2002). The *Trixis* clade also contains *Dolichlasium* and *Jungia*. These three genera have for the most part yellow corollas and tapered cypselae (Bremer, 1994). Since the basal lineages of Nassauvieae, *Lophopappus*, *Proustia*, and *Leucheria*, and most members of the sister tribe Mutisieae, are endemic to the south central Andes, it is reasonable to assume that Nassauvieae originated in dry areas of this region and subsequently expanded to the southern Andes, North America, and Brazil.

4.1.3. Onoserideae

Our study identified a novel clade containing *Aphyllocladus*, *Lycoseris*, *Onoseris*, and *Plazia*. Recent morphological studies of selected members of classical Mutisieae (Tellería and Katinas, 2004) have identified a combination of morphological characteristics that are shared by these four genera and *Gypothamnium* and *Urmenetea*, namely similar tubular corollas and dimorphic pappi of narrowly paleaceous bristles. Several taxonomic and morphological studies of classical Mutisieae are in agreement that *Aphyllocladus*, *Gypothamnium*, and *Plazia* form a natural group based on the shared character of red anther appendages that are distinctive in Mutisioideae (Hansen, 1991; Bremer, 1994; Tellería and Katinas, 2004). We sampled four of the six genera of Onoserideae (Panero and Funk, 2007) and our results show the tribe is biphyletic, with *Onoseris* sister to *Lycoseris* and *Plazia* sister to *Aphyllocladus*. Morphological studies have not allied *Lycoseris* to *Onoseris* but Hansen (1991) noted a similarity (potentially a synapomorphy) between *Lycoseris* and *Onoseris* in their short corolla lobes as compared to other members of Mutisieae. There is general agreement among most workers that *Urmenetea* is closely related to *Onoseris* (Sancho, 2004). Onoserideae as circumscribed here contains the genera *Aphyllocladus*, *Gypothamnium*, *Lycoseris*, *Onoseris*, *Plazia* and *Urmenetea*, comprising 53 species distributed mostly in the dry Andes of northern Chile, Argentina, Bolivia, and southern Peru, with *Onoseris* and *Lycoseris* having a sizeable number of species in mesic or seasonally dry lowland forests of Mesoamerica and South America.

4.2. Stifftioideae

Stifftia, *Gongylolepis*, *Duidaea*, *Hyaloseris* and *Dinoseris* form a clade recognized as subfamily Stifftioideae. This clade is strongly supported in both Parsimony and Bayesian analyses (99% BS, 100 PP) though the relationship of this clade to other subfamilies is equivocal (Figs. 1 and 2). We were unable to statistically reject the hypothesis of *Stifftia* and relatives within Mutisioideae at the 5% level of significance, although the conditional probability that this hypothesis is correct is low (26.6% PP). The common denominator in the taxonomic history of *Stifftia* has been its inclusion, by virtue of its morphology, as a member of the early radiation of the Asteraceae. *Stifftia* has been considered a "primitive genus" in the family because several of its species have large imbricate involucres, long actinomorphic corollas with strongly coiled lobes, and an arborescent habit (Maguire, 1956). Most of its eight species are found in the tropical forest of eastern Brazil with two additional species found in the rainforests of northern Brazil and French Guyana (Hind, 1996; Robinson, 1991). Nonetheless, Maguire (1956) considered *Stifftia* one of the Guayana Highlands genera placed in classical Mutisieae and an early

offshoot of the progenitor of those taxa. He allied *Stifftia* to *Stenopadus*, another genus that figures prominently in discussions about the origins and characteristics of the early sunflowers (Maguire, 1956; Pruski, 1991; Karis et al. 1992; Bremer 1994).

The four genera here found to be strongly supported as sister to *Stifftia* have bilabiate/ligulate corollas. Our results show that *Stifftia* is sister to two clades, one containing *Gongylolepis* and *Duidaea* of the Guayana Highlands and the other *Hyaloseris* and *Dinoseris* of Bolivia and Argentina. Because *Gongylolepis* and *Duidaea* share a similar corolla morphology and other floral characteristics with the Guayana Highlands genera *Achnopogon*, *Glossarion*, *Eurydochus*, *Neblinaea*, and *Quelchia* (Jiménez Rodríguez et al., 2004 and references therein) we consider these genera to belong in subfamily Stifftioideae as well. A potential synapomorphy for the Guayana Highlands genera of Stifftioideae is the presence of laticifers observed by Carlquist (1958) in the genera *Gongylolepis*, *Duidaea*, *Neblinaea*, and *Quelchia*. The inclusion of *Hyaloseris* and *Dinoseris* in the Stifftioideae is strongly supported in our analyses. Cladistic analyses of morphological data by Karis et al. (1992) placed *Hyaloseris* as sister to *Stifftia* and *Gongylolepis*. Hind (2007) included *Hyaloseris* (with *Dinoseris* in synonymy) in his *Stifftia* group along with *Stifftia* and *Wunderlichia*. The seven species of *Hyaloseris* are endemic to the mountains of northern Argentina and southern Bolivia. The ligulate corollas of *Hyaloseris*, *Dinoseris*, and *Glossarion* (Hansen, 1991) are of interest as this unusual corolla morphology may represent a synapomorphy for the group, subsequently lost in the other genera of the Guayana Highlands, or acquired in parallel.

4.3. Wunderlichioideae

Results from Bayesian analyses provide statistical support for the placement of the lineage containing *Wunderlichia* as the next branch to split from Mutisioideae–Stifftioideae, this sister to the rest of the Asteraceae. The subfamily contains two main assemblages whose compositions are novel. One clade contains *Wunderlichia* sister to the Guayana Highlands genera *Chimantaea*, *Stenopadus*, and *Stomatochaeta* hereafter referred to as tribe Wunderlichieae. The sister lineage contains the genera *Hyalis* and *Ianthopappus* sister to *Nouelia* and *Leucomeris* hereafter as tribe Hyalideae.

The geographic distribution of members of subfamily Wunderlichioideae parallels that of Stifftioideae with three allopatric areas of endemism across South America: one in the Andes or temperate eastern South America, one in central Brazil, and one in the Guayana Highlands. *Wunderlichia* is a genus of five or six species of the Planalto of central Brazil having large, homogamous capitula with actinomorphic corollas produced at the end of the dry season on leafless stems. The gross morphology of *Wunderlichia* (tree-like habit, coriaceous, caducous, densely pubescent leaves) is distinctive among genera included in classical Mutisieae, and it appears to be the result of adaptation to the seasonally dry conditions of the Campo Rupestre of central Brazil. In spite of different life form, comparative studies of floral features have suggested a close relationship of *Wunderlichia* to members of the Guayana Highlands genera with actinomorphic corollas represented by *Stenopadus* (Barroso and Maguire, 1973; Carlquist, 1957). However, the close relationship of this group to members of Hyalideae has never been hypothesized.

Relationships within tribe Hyalideae are interesting because of the wide geographical separation of sister clades. *Hyalis* and *Ianthopappus* are genera endemic to subtropical, eastern South America, whereas *Nouelia* and *Leucomeris* are endemic to the mountainous regions of southeast Asia and the foothills of the Himalayas. It is remarkable that sister clades are so distant in their geographic distribution. This amphi-pacific pattern has never been reported for any South American sunflower except for the *Chaptalia/Leibnitzia* and the *Adenocaulon* lineages but these also have species present in North America. *Nouelia* and *Leucomeris* can be added to the list of Asteraceae genera with astonishing sister-taxon disjunctions ranging thousands of kilometers that includes *Abrotanella* (Chile, New Zealand–Tasmania, Wagstaff et al., 2006) and *Hesperomannia* (Africa–Hawaii, Kim et al., 1998) among others. The presence of these genera in Asia either results from vacariance and extinction in North America or very long distance dispersal.

Hyalis, *Ianthopappus*, and *Leucomeris* share with some Gochnatioideae rather short imbricate involucres with corollas and pappi conspicuously exserted beyond the involucre. This characteristic is not present in *Nouelia*, the latter having strongly imbricate involucres, but *Nouelia* shares with *Ianthopappus* and *Hyalis* radiate capitula with bilabiate (3 + 2) corollas. All four genera have leaves with white, pubescent abaxial surfaces. Our studies clearly show that *Leucomeris* does not belong to *Gochnatia* sensu Freire et al. (2002). *Ianthopappus* was recently recognized by Roque and Hind (2001) as a distinctive genus and not closely related to *Richterago* (Gochnatioideae), its original placement. Among classical Mutisieae, only *Ianthopappus* and *Lulia* are restricted to semi-aquatic habitats.

Characters that have been traditionally used to recognize natural groups or maintain certain groups within classical Mutisieae appear labile. Most authors are in agreement that actinomorphic corollas are the ancestral condition in the family (Koch, 1930; Bremer 1994). This assertion is supported by our study and it can now be assured that bilabiate corollas are not ancestral as Jeffrey (1977) asserted, but rather are interpreted as having evolved in parallel in different groups including Mutisioideae, Stifftioideae, Wunderlichioideae, Gochnatioideae, Dicomeae, and Tarchonantheae. Equally important in the circumscription of groups in classical Mutisieae has been the use of anther appendage morphology. For example, the eight genera of the Wunderlichioideae share with some

Gochnatioideae, some Dicomeae, and most members of the Guayana Highlands genera of Stifftioideae apiculate anther appendages, a characteristic shared by these nested lineages and lost in a few species.

Understanding how the Asteraceae first diversified beyond the Andes and Patagonia hinges on reconstructing the historical relationships among the lineages constituting classical Mutisieae. Although our study was unable to ascertain the precise relationship of Stifftioideae to the Mutisioideae, the statistically supported relationships of Stifftioideae and Wunderlichioideae enabled us to falsify an almost universally accepted historical hypothesis concerning the early diversification of sunflowers on the Guayana Highlands, and shed light on the evolution of one aspect of early floral evolution in Asteraceae.

The considerable age of the Guayana Highlands together with the distinctive and apparently primitive morphology of genera traditionally assigned to Mutisieae that inhabit that area have been used to conclude that these genera are monophyletic and the probably most "ancestral" lineage of the family (Maguire, 1956; Maguire and Wurdack, 1957; Huber, 2005). The 10 mutisioid genera of the Guayana Highlands are *Achnopogon*, *Chimantaea*, *Duidaea*, *Eurydochus*, *Glossarion*, *Gongylolepis*, *Neblinaea*, *Quelchia*, *Stenopadus*, and *Stomatochaeta*. After the discovery of Barnadesioideae as the lineage sister to the rest of the family, a monophyletic Guayana Highlands classical Mutisieae (Pruski, 1991; Bremer, 1994) has remained the best hypothesis of the next lineage of the family to diverge (Pruski, 1991; Karis et al., 1992). Implicit in the writings of Maguire (1956) and Maguire and Wurdack (1957) is that they considered all the classical Mutisieae genera of the Guayana Highlands to be monophyletic, arising from a Guayanan progenitor. Following the taxonomy of the time they conveniently positioned genera with bilabiate and actinomorphic corollas into two taxonomic groups, namely Mutisiinae and Gochnatiinae respectively. Maguire's (1956) evolutionary scenario placed the largest mutisioid genera endemic to the Guayana Highlands, *Gongylolepis* and *Stenopadus,* as a bridge between his Mutisiinae and Gochnatiinae. He considered these two genera to be the primitive elements of the group and *Stifftia* and *Moquinia* (now all but the type species transferred to *Gochnatia*) to be extra-Guayana members early diversifying beyond the region. Hansen (1991) provided a potential synapomorphy for the Guayana Highlands Mutisioideae in the blackish color of the stems. He also commented that the black herbage trichomes of these species, once believed to be a synapomorphy for the group, is the result of a fungal infection. Maguire (1956) considered the genus *Stenopadus* with its large capitula and conspicuously imbricate involucres to represent the ancestral lineage of Asteraceae. Karis et al. (1992) identified *Stenopadus* as the first lineage to split from Barnadesioideae, this based on cladistic analysis of morphological data of the Cichorioideae. Bremer (1994) included all Guayana Highlands Mutisioideae in his *Stenopadus* group. Cladistic analyses of the Guayana Highlands genera by Jiménez Rodríguez et al. (2004) support the traditional view that these taxa are monophyletic, but only with the inclusion of *Stifftia* and *Wunderlichia*.

Our results contradict traditional views that the genera of the Guayana Highlands classical Mutisieae are monophyletic. Even though our sampling of the Guayana Highlands taxa was limited to one exemplar each of five of the 10 genera recognized to exist in this area (Hind, 2007), our analyses support two independent introductions into these mountains (Figs. 1 and 2). These two lineages correspond to the two corolla types observed in the group, bilabiate and actinomorphic. The bilabiate corolla genera *Gongylolepis* and *Duidaea* are members of the Stifftioideae whereas the actinomorphic corolla genera *Chimantaea*, *Stenopadus* and *Stomatochaeta* are members of the Wunderlichioideae. Our results show that the progenitors of these taxa arrived in the Guayana Highlands region from the eastern Andes or central/northern Brazil early in the radiation of the family and have diversified there in isolation probably for millions of years. Guayana Highland Asteraceae (Berry and Riina, 2005) belonging to other subfamilies are more recent arrivals. Except for *Chaptalia*, no genera of Mutisioideae occur in the Guayana Highlands area.

4.4. Gochnatioideae

The well-supported clade comprising *Cyclolepis* sister to *Gochnatia*, *Cnicothamnus*, and *Richterago* recovered by our study does not correspond to any previous concept of a lineage containing *Gochnatia*. Cabrera (1977) recognized a subtribe Gochnatiinae with 36 genera distributed worldwide in his treatment of tribe Mutisieae. He believed this subtribe to be the most primitive group in tribe Mutisieae. He characterized most members of the subtribe by having actinomorphic corollas and never truly bilabiate corollas. Hansen (1991) narrowed the concept of the group by removing the African members of the tribe (*Dicoma* and relatives). In the same publication, Hansen considered monophyletic a subset of Gochnatiinae informally labeled as the *Gochnatia* group that also included the genera *Actinoseris*, *Cyclolepis*, *Gochnatia*, *Hyalis*, *Leucomeris*, and *Nouelia*. A cladistic analysis of tribe Mutisieae by Karis et al. (1992) revealed Gochnatiinae as a paraphyletic assemblage. Bremer (1994) believed Gochnatiinae to be polyphyletic and placed all genera of the subtribe Gochnatiinae in subtribe Mutisiinae recognizing this and Nassauviinae as the two main lineages of Mutisioideae (classical Mutisieae). Molecular studies by Kim and Jansen (1995), and Kim et al. (2002) provide evidence for the polyphyletic circumscription of the subtribe as conceived by Cabrera (1977). The *Gochnatia* complex of Freire et al. (2002) included *Gochnatia* and the genera *Ianthopappus*, *Actinoseris*, *Cyclolepis*, *Cnicothamnus, Hyalis*, and *Nouelia*. Their detailed morphological studies characterized this complex by the combination of smooth style branches and apiculate anther appendages and allowed them to exclude *Chucoa*, *Pleiotaxis*, and *Wunderlichia* from it. They

placed *Leucomeris*, *Pentaphorus* and *Richterago* within *Gochnatia*. Hind (2007) recognizes only the genera *Gochnatia*, *Richterago*, and *Pentaphorus* in this lineage.

Our studies support the recognition of the Brazilian endemic genus *Richterago* as circumscribed by Roque and Pirani (2001) including *Actinoseris* as distinctive from *Gochnatia* and sister to *Cnicothamnus*. Most species of *Richterago* form small rosettes with long, scapose inflorescences. The genus is a distinctive element of seepages and edges of intermittent rivers primarily in the sandy mountains of the Serra do Cipo and Diamantina plateau in Minas Gerais, central Brazil, whereas the two species of *Cnicothamnus* are treelets endemic to the moist forests of northern Argentina and southern Bolivia. The genera *Hyalis*, *Ianthopappus*, and *Nouelia* of the *Gochnatia* complex sensu Freire et al. (2002) are herein referred to the Wunderlichioideae.

4.5. Hecastocleidoideae

Hecastocleis, a shrub endemic to the mountains that surround the Mojave Desert of California and Nevada, was found here to form a lineage distinct from Mutisioideae and sister to the Carduoideae–Asteroideae clade. The genus was named by Gray (1882), who believed its closest relative to be the Asian genus *Ainsliaea*. Gray commented on the distinctiveness of the genus in Mutisieae because of its spiny leaves, involucre of aciculate phyllaries, single-flowered capitula, and spiny inflorescence bracts. The corollas are white, and like those of Pertyoideae have five, long, broadly expanded, narrowly triangular lobes. Most corollas are actinomorphic but a few are zygomorphic with equivalent lobe lengths (Jose L. Panero, personal observation). The inflorescence of *Hecastocleis* is distinctive in Asteraceae as its capitula are loosely surrounded by mostly five spiny bracts that enclose up to nine capitula that sit at the end of the shoot axis in an expanded, receptacle-like structure. Accessory flowering branches (paracladia) below these bracts expand after anthesis of the terminal capitula and have a similar arrangement to that of the terminal group. The single-flowered capitula with aciculate phyllaries aggregated in globose secondary heads are reminiscent of some Cynareae. Bremer (1994), echoing the views of Hansen (1991), considered the genus an isolated member of tribe Mutisieae. Hansen (1991) considered the style of *Hecastocleis* similar to that of *Carlina*, the latter a basal lineage of the Cynareae (Susanna et al., 2006). In addition, *Hecastocleis* has several other characteristics rare in Asteraceae, including ring porous wood and imperforate tracheids with prominent bordered piths probably the result of adaptation to seasonally dry habitats (Carlquist, 1957). A similar wood morphology is also present in *Proustia*, and to a lesser degree in *Nouelia*, *Dasyphyllum* (as *Flotovia*), and *Trixis* (Carlquist, 1957). According to Tellería and Katinas (2005; but see also Woodhouse, 1929), *Hecastocleis* along with selected species of *Ainsliaea* share tricolpate pollen as yet unknown in other species of Asteraceae. *Hecastocleis* shares with Pertyoideae distinctive trichomes (Hansen, 1991).

4.6. Carduoideae

With the inclusion of Dicomeae and Tarchonantheae along with the core thistles Cynareae we have expanded the Carduoideae (100% BS, 100% PP). This subfamily was recognized by Bremer (1996) to include only members of tribe Cynareae. Bayesian analyses (Fig. 2) placed Tarchonantheae and Dicomeae as sister and collectively sister to Cynareae but without significant support. Parsimony analysis (Fig. 1) was unable to resolve relationships among these three well-supported lineages. We cannot cite any morphological synapomorphy that defines Carduoideae but nearly all members have papillose styles with papillae mostly confined to a ring below the stigmatic branches, as do some members of the Arctotideae, or as a tuft of trichomes on the abaxial surface of the style branches. *Oldenburgia* and some Dicomeae have apiculate anther appendages similar to those of some Gochnatioideae and Wunderlichieae. The subfamily is dominated by tribe Cynareae which accounts for more than 90% of the species diversity of the group. Some members of Cynareae contain latex, a characteristic found almost exclusively in the Cichorioideae. The highest species and generic diversity of Cynareae is found in Europe and central Asia with very sparse representation in America and Australia.

The distinctiveness of Dicomeae within classical Mutisieae was pointed out by Jeffrey (1967) but it was Hansen (1991) who supported their removal from Mutisieae. The latter worker speculated that the inclusion of Dicomeae in Mutisieae by other workers was based upon the bilabiate corolla of some of its members and the style trichome distribution dissimilar from Cynareae. Morphological studies of 'Mutisieae' endemic to eastern Africa convinced Hansen that the genera *Dicoma*, *Erythrocephalum*, *Pasaccardoa*, and *Pleiotaxis* are not closely related to Mutisieae but rather to Cynareae. Hansen (1991) believed the corolla epidermal trichomes, the conspicuous difference between the narrow tube and broad limb, and style branches with subapical trichomes are not present in any other Mutisieae and are similar to Cynareae. Bremer (1994) followed conclusions from cladistic studies by Karis et al. (1992) that *Dicoma*, *Erythrocephalum*, and *Pleiotaxis* were members of Mutisieae, and he maintained the genera as such under the informal *Dicoma* group. Cladistic studies of the *Dicoma* group by Ortiz (2000) using *Oldenburgia* and *Gochnatia* as outgroups, showed *Erythrocephalum* and *Pleiotaxis* to be sister to a paraphyletic *Dicoma* with the species of *Pasaccardoa* derived from within it. Subsequent studies by Ortiz (2001, 2006) refined the concept of *Dicoma* and the *Dicoma* group to contain also *Cloiselia*, *Dicoma*, *Erythrocephalum*, *Gladiopappus*, *Macledium*, *Pleiotaxis*, and *Pasaccardoa*. Our studies support the conclusions of Hansen (1991) that the *Dicoma* group is a member of subfamily Carduoideae and that the genera we sampled, *Dicoma*, *Macledium*, and

Pasaccardoa form a monophyletic group (Figs. 1 and 2). The relationships of the *Dicoma* clade to other members of the Carduoideae are equivocal based on our data.

Our inclusion of Tarchonantheae in Carduoideae has no historical precedent. The suprageneric taxonomic history of *Tarchonanthus* and *Brachylaena* has been one of controversy. The two genera were initially assigned to the Inuleae then transferred to Mutisieae after pollen studies by Leins (1971) and Skvarla et al. (1977) showed the genera to have anthemoid type pollen. Cabrera (1977) did not include these taxa in his treatment of the tribe, and Hansen (1991) considered the genera not to be Mutisieae. Restriction fragment analyses of the chloroplast DNA by Keeley and Jansen (1991) showed these constitute a clade and one of the early lineages of the family. Their discovery was used to recognize the lineage formally as tribe Tarchonantheae (a later homonym of Tarchonantheae Kostel.). Our studies recovered the *Tarchonanthus–Brachylaena–Oldenburgia* clade with significant statistical support by both phylogenetic methods (100% BS, 100% PP). *Oldenburgia* was revised by Bond (1987) to include four species of shrubs and trees of the Cape region of South Africa. The genus is characterized by its large, radiate capitula and the dense tomentum of their herbage. Bond (1987) believed the genus not to be closely related to the Dicomeae but rather to certain New World Mutisieae including *Chimantaea*, *Cnicothamnus*, and *Wunderlichia*. She believed *Cnicothamnus* in the Gochnatioideae to be the closest relative of *Oldenburgia*. Hansen (1991) maintained *Oldenburgia* in Mutisieae but did not provide any insights about its relationships. Cladistic analysis of Cichorioideae by Karis et al. (1992) placed *Oldenburgia* in a relatively basal position within Mutisieae and Bremer (1994) used these results to conclude that the genus represented an isolated member of the tribe, a survivor from an early diversification of the family. Our results support the close relationship of *Oldenburgia* to the parapatric *Tarchonanthus* and *Brachylaena*. The latter two genera are different from *Oldenburgia* in gross morphology but share with it the arborescent habit and the dense herbage pubescence. Here we expand Tarchonantheae to include *Oldenburgia* and recognize the need for a comprehensive synthetic study of the tribe.

4.7. Pertyoideae

The Pertyoideae represent the most highly nested lineage of classical Mutisieae. The subfamily is strongly supported as sister to Gymnarrhenoideae and the Cichorioideae–Asteroideae clade, and it contains the genera *Ainsliaea*, *Macroclinidium*, *Myripnois*, and *Pertya* (sensu Hind 2007). The only molecular study to include a majority of the species of the genus *Ainsliaea* showed that the monotypic genus *Diaspananthus*, traditionally included in *Ainsliaea* (*Ainsliaea uniflora* Sch. Bip.), is the sister taxon to all the other species sampled (Mitsui et al., 2008). Cladistic analysis of morphological features support as well *A. uniflora* as sister to all other species of the genus (Freire, 2007). According to Jeffrey (2007) the monotypic Himalayan genus *Catamixis* is a member of this group.

The Pertyoideae have approximately 70 species restricted to temperate eastern Asia and the Himalayas (*Pertya* group, Hind, 2007). Most species are hygrophytes that inhabit the understory of temperate forests. According to Hansen (1991) the Pertyoideae (*Ainsliaea* group) has one synapomorphy in the laterally arranged capitula. The capitula are discoid, cylindric or campanulate, and few-flowered. The corollas are distinctive as they are deeply lobed and the lobes in many species are perpendicular to the axis of the capitulum. Some corollas are zygomorphic with one deeper sinus resulting in a corolla that resembles a slightly concave hand (*Pertya*, Koyama, 1975). The genus *Ainsliaea* shares with *Chaptalia* and *Leibnitzia* the formation of cleistogamous and chasmogamous capitula (Freire, 2007). The Pertyoideae share a few morphological characteristics with *Hecastocleis* (see above).

4.8. Gymnarrhenoideae

Gymnarrhena is an excellent example of a "non-missing" link genus, linking Pertyoideae Carduoideae and the Vernonioid group (Cichorioideae, Corymbioideae and Asteroideae, Fig. 1) of Bremer (1996). The genus had been placed in tribe Inuleae because of its habit and near the Cynareae because of its pollen morphology (Leins, 1973; Skvarla et al., 1977) and Bremer (1994) considered the genus as of uncertain position in Cichorioideae. Our analyses do not support its placement in any existing tribe or subfamily. Instead, *Gymnarrhena* was identified as an independent lineage with significant bootstrap support and posterior probability. Furthermore, *Gymnarrhena* lacks the 9-bp deletion in the *ndh*F gene identified by Kim and Jansen (1995) and subsequently used by Bremer (1996) as a molecular characteristic in support of the recognition of the Vernonioid group (Fig. 1). *Gymnarrhena* is an amphicarpic herb of the Mediterranean biome of North Africa and the Middle East. Research on the interesting floral dimorphism associated with the reproductive biology of this unusual plant is summarized by Koller and Roth (1964). The subfamily contains a single genus and species, *Gymnarrhena micrantha* Desf. Our studies support the recognition of *Gymnarrhena* at the subfamily level as sister to the Cichorioideae–Corymbioideae–Asteroideae clade.

4.9. Cichorioideae

Our results and taxonomic concept of Cichorioideae differ slightly from those of Kim and Jansen (1995) and their taxonomic interpretation by Bremer (1996) because we included samples of *Gundelia* and *Warionia*, two previously unplaced or misplaced anomalous genera. Our analyses show that a monophyletic Cichorioideae must include these two genera along with tribes Arctotideae, Cichorieae, Liabeae, and Vernonieae. Recent molecular studies of the subfamily by Karis et al. (2001) identified the genus *Gundelia*

as a member of tribe Cichorieae and placed the anomalous genera *Eremothamnus* and *Hoplophyllum* as members of Arctotideae. Here we confirm the placement of *Hoplophyllum*, and also place *Heterolepis* in Arctotideae. However, we find *Gundelia* together with *Warionia* to be a separate lineage sister to Cichorieae and morphologically different from Cichorieae by its actinomorphic corollas. Therefore, we accept Robinson's (1994) tribe Gundelieae but expand it to include *Warionia*, a large shrub endemic to North Africa. Our study places Gundelieae sister to Cichorieae with strong statistical support but otherwise found tribal relationships within Cichorioideae congruent with the *ndh*F gene phylogeny of Kim and Jansen (1995). Like Kim and Jansen we also do not have support for any relationship of the Cichorieae–Gundelieae clade with other tribes in the subfamily (<50% BS, 93% PP).

Bremer (1994) provided a concise history of the classification and morphology of the group. The subfamily is characterized by the presence of latex as a plesiomorphic condition subsequently lost in most Vernonieae, Arctotideae, and some Liabeae. Members of Cichorieae, Vernonieae, and some Liabeae have tangentially oriented style branches (Robinson, 1984) different from the radially oriented style branches present in most of the other lineages of the family. Cichorieae have ligulate corollas, whereas Arctotideae, Liabeae, and Vernonieae with few exceptions have discoid or radiate capitula. The subfamily contains approximately 2900 species of cosmopolitan distribution (Jeffrey, 2007).

4.10. Corymbioideae

The anomalous genus *Corymbium* was found to be a distinct lineage linking Cichorioideae to the tribes of Asteroideae. A small genus of only nine species of perennial herbs from the Cape region of South Africa (Weitz, 1989; Nordenstam, 2007), *Corymbium* has been traditionally allied to the Vernonieae though several morphological and chemical features suggest otherwise (see Bremer, 1994). Its combination of morphological characteristics distinctive in Asteraceae namely parallel-veined leaves, single flowered capitula, and broad, spreading corolla lobes have made it difficult to place. The style morphology is similar to Vernonieae with long, slender style branches with papillae covering the abaxial surface and distal half of style. The combination of morphological features presented by *Corymbium* precluded Bremer from assigning it to any particular tribe of his Cichorioideae. Results from preliminary molecular studies of *Corymbium* (Jansen and Kim, 1996) suggested placement in Senecioneae. However, our results provide strong support for this lineage outside of both Asteroideae and Cichorioideae.

4.11. Asteroideae

Our study provides strong statistical support for most tribal relationships of the Asteroideae, except the relationship of Senecioneae. The previously unknown positions of Calenduleae and Gnaphalieae were resolved with high statistical support (95% and 93% BS, respectively, 100% PP). Calenduleae was found sister to a clade containing Gnaphalieae as sister to Anthemideae and Astereae. Other tribal relationships found here are congruent with the *ndh*F phylogeny of Kim and Jansen (1995) including the clade containing Inuleae sister to Athroismeae and the tribes of the Heliantheae alliance and a third clade containing members of tribe Senecioneae. The tribal relationships within the Heliantheae alliance are in agreement with recent morphological studies of the group (Panero, 2007b) and molecular phylogenetic results based on denser sampling (Panero, unpublished). As in earlier studies the position of Senecioneae was equivocal in our analyses. Senecioneae was either unresolved in Bayesian analyses or placed as sister to the Calenduleae clade without significant support (52% BS) in Maximum Parsimony analyses. The three main lineages of the Asteroideae identified by previous molecular studies have been recently recognized as supertribes of the subfamily: Asterodae, Helianthodae, and Senecionodae (Robinson 2005). The monophyly of Asteroideae was strongly supported (80% BS, 100% PP) as expected based on earlier morphological and molecular studies.

The Asteroideae are the largest subfamily of the Asteraceae. It contains 1210 genera and approximately 17,000 species or 72% of the diversity of the family (Jeffrey, 2007). According to Bremer (1994), a majority of Asteroideae are characterized by the presence of true ray florets, disc corollas with short lobes, caveate pollen, and style branches with two marginal stigmatic surfaces.

5. Early dispersal of Asteraceae out of South America

Our chloroplast phylogeny confirms again the South American origin of Asteraceae. Of the basal lineages of Asteraceae, Barnadesioideae and Stifftioideae are endemic to South America and Mutisioideae, Wunderlichioideae, and Gochnatioideae are primarily South American. Together these five lineages represent only about 4% of the species diversity of the family whereas the tribal divergences giving rise to approximately 96% of the species of the family occurred outside of South America. The great success of Asteraceae in terms of species diversification appears to have been contingent upon the dispersal of diaspores out of South America and subsequent worldwide expansion. Recently the origin of Asteraceae has been estimated to postdate the breakup of Gondwana in the mid-Eocene to late Paleocene–Selandian (42–47 Ma, Kim et al., 2005; 60 Ma, McKenzie et al., 2006). Thus the early evolution of Asteraceae would have occurred when South America was essentially an isolated land mass connected with North America and Africa only by island chains exposed by fluctuating sea levels (Sclater et al., 1977; Iturralde-Vinent and MacPhee, 1999).

The only speculation as to the route of sunflowers out of the continent of their origin has been that of Bremer (1994) who hypothesized a Pacific-Asian route from South America. His proposal of dispersal to Asia via Hawaii was predicated upon erroneous inferences that the Asian genus *Ainsliaea* (Pertyeae, Pertyoideae) and Hawaiian genus *Hesperomannia* (Vernonieae, Cichorioideae) were early diverging members of the "Mutisieae clade". In light of our phylogeny the North American endemic genus *Hecastocleis* could be interpreted as a North American link to Pertyoideae in Asia; similar floral and pollen morphology may support this. Fossil pollen records confirm the wide distribution of Asteraceae in the Northern Hemisphere on both sides of the Pacific as early as the Eocene (North America—eastern Texas, Elsik and Yancey (2000); northwestern China, Song et al. (1999)). However, to fully explain the radiation of the family out of South America by a North American–Asian route alone (Fig. 3, red arrows) would require that the mostly African Dicomeae–Tarchonantheae lineage be derived from an Asian ancestor (Fig. 3, open circle; most recent common ancestor (MRCA) of Carduoideae and Pertyoideae–Asteroideae). The morphological similarity of *Oldenburgia* (Tarchonantheae) and some Dicomeae to South American Mutisieae suggests a more direct South America–Africa connection. The presence of Pertyoideae in Asia could be postulated alternatively as derived from an African or Eurasian ancestor.

Direct dispersal easterly from South America to Africa or Eurasia (Fig. 3, solid gray arrow) is plausible because the earliest branching clades outside of South America are the monotypic Hecastocleidoideae sister to a clade containing all other Asteraceae whose basal branches are mostly African (Tarchonantheae/Dicomeae, Carduoideae), mostly African/Eurasian (Cichorioideae, Gymnarrhenoideae), and Mediterranean/Central Asian (Cynareae, Carduoideae) as well as the eastern Asian/Himalayan Pertyoideae. Of these lineages the greatest diversity of species by far was attained in Africa, the Mediterranean and Central Asia. The basal lineages of Carduoideae, Cichorioideae and Gymnarrhenoideae are African or Mediterra-

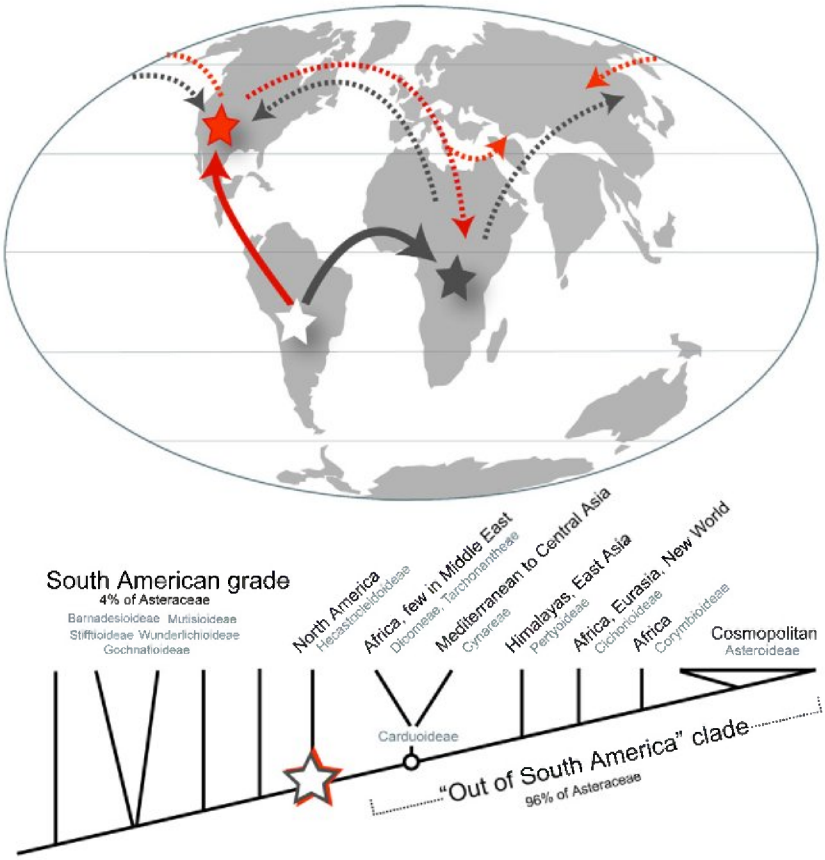

Fig. 3. Alternative "Out of South America" hypotheses. Depending on where the ancestral area of the "Out of South America" clade is reconstructed, three scenarios are postulated. (1) If the ancestral area is African or Eurasian (gray star) then transatlantic dispersal to Africa or Eurasia gave rise to a global expansion of Asteraceae including the Hecastocleidoideae in North America (gray arrows). (2) If the ancestral area is North American (red star) then long distance dispersal or "island-hopping" gave rise to global expansion via northern hemisphere routes (red arrows). (3) If the ancestral area is South American (white star) then two original dispersal events are hypothesized: one that founded a lineage in Africa or Eurasia giving rise to most of Asteraceae diversity, and another to North America that has been far less successful. Stars indicate hypothesized ancestral areas for the MRCA of *Hecastocleis* and the Carduoideae–Asteroideae clade. Solid lines signify first steps of Asteraceae leaving South America and dashed lines migration after initial dispersal event(s). Open circle indicates MRCA of Carduoideae and Pertyoideae–Asteroideae. Base map of middle Eocene continents (~40 Ma) inferred from tectonic plate reconstruction of Lawver et al. (2002).

nean. Samples from Paleocene-Eocene pollen deposits in southwestern Africa have been attributed to classical Mutisieae (Zavada and De Villiers, 2000; De Villiers and Cadman, 2001) or more specifically to a *Dicoma*-like taxon and recently dated to the mid-Eocene, approximately 38 Ma (Scott et al., 2006). With slightly later pollen reported from Egypt (~34–36 Ma; Kedves, 1971) we assume the family was probably widespread on the African continent by the Late Eocene. Sweepstakes dispersal across the Atlantic has been concluded to explain taxonomic similarities between South America and Africa in studies of several angiosperm groups using molecular phylogenies calibrated by fossil evidence (Pennington and Dick, 2004; Lavin et al., 2004), or to explain Eocene to Miocene pollen of South American angiosperms in Africa (Morley, 2003). Of 110 extant genera with species on both sides of the Atlantic, wind dispersal specifically has been invoked to explain the presence of a few South American taxa in Africa (Renner, 2004).

Sweepstakes dispersal has been the mode commonly invoked to explain the presence of Asteraceae outside South America (Raven and Axelrod, 1974; Stuessy et al., 1996). The earliest successful colonizations of areas outside South America by Asteraceae may well have been the result of two long distance dispersal events, or possibly stepping-stone migration, across oceanic barriers to North America (Fig. 3, solid red arrow) and Africa or Eurasia, most probably Africa (Fig. 3, solid gray arrow). If true, the African dispersal gave rise to the explosive radiation of Asteraceae across the world resulting in the largest family of flowering plants, whereas the dispersal to North America was much less successful and today is represented only by a single species, *Hecastocleis shockleyi*. *Hecastocleis* illustrates that all sweepstakes winners may not be equally successful in terms of speciation and adaptation after colonization depending on where and when they arrive, and reinforces that long distance dispersal is a continuous process only rarely rewarded.

After the first steps out or South America several permutations of Northern hemisphere dispersal could be postulated. *Hecastocleis* could represent an important "non-missing link" lineage that gave rise to worldwide diversity via the Bering land bridge or the North American land bridge, or both. Alternatively, *Hecastocleis* could be the only relict of an Asteraceae lineage that reached North America from the Old World by either route. The importance of the North American land bridge to explain North American–African disjunctions has gained recent attention (Davis et al., 2002 and other references therein). However, we note that despite abundant sampling the earliest fossil evidence of Asteraceae in Europe dates only from the late Oligocene or early Miocene (Graham, 1996), later than in either North America or Africa.

The high number of Asteraceae species of different lineages sympatric in most biomes of the world is indicative of a complex biogeographical history that began with first steps out of South America. Rigorous testing of alternative hypotheses, including those proposed here of dual dispersals out of south America and more complex scenarios that invoke land bridge migrations and longer routes between the New and Old Worlds, depends upon reconstructing ancestral areas (sensu Bremer, 1993) and dating the major divergences of the family described here, and will appear in future studies.

6. Phylogenetic reconstruction of closely spaced cladogenesis

The historical difficulty in solving the relationships of the deep divergences of Asteraceae represented by members of classical Mutisieae is probably due to closely spaced cladogenic events. Informative characters supporting these branches are relatively few. By increasing the number of characters sampled approximately sixfold over earlier single marker studies and extending taxon sampling to include a good representation of the deep divergences of the family, we were able to amplify the phylogenetic signal to resolve most branches with strong statistical support. As data partitions were successively concatenated, preliminary analyses yielded increasing resolution and support for branches as we had expected. Future expansion of this data matrix will likely allow the placements of Stifftioideae and Senecioneae to be resolved also. Although systematic bias can also be amplified as more data are added as in the case of the long-branch attraction problem (Felsenstein, 1978), our phylogeny appears robust to different phylogenetic methods. Both maximum parsimony and the general time reversible modeled Bayesian method yielded highly congruent topologies.

7. Comparison with phylogenetic hypothesis from the nuclear compartment

We would like to compare our plastome phylogeny with an estimate based on data obtained from the nuclear compartment. Consensus among independent studies would corroborate findings, however we expect that the evolutionary histories of the two compartments differ somewhat since modes of inheritance differ and the nuclear genome is subject to significant recombination in contrast to the plastid genome (Clegg and Zurawski, 1992). Incongruence between phylogenetic estimates could also be useful to discover reticulation, incomplete lineage sorting, and evidence of horizontal gene transfer. Asteraceae provides well-known and important examples of hybridization in the evolution of plants (Rieseberg et al., 2003; Comes and Abbott, 2001; Francisco-Ortega et al., 1996; Schilling and Panero, 1996) and we like to investigate the extent to which reticulation may have played a role in the evolution of the major lineages of sunflowers.

Only one study of Asteraceae has sampled the nuclear compartment across the entire family, a combined analyses of the internal transcribed spacers of the 18S–5.8S–26S nuclear ribosomal cistron (ITS1 and ITS2) by Goertzen et al. (2003). Maximum parsimony analysis of 288 taxa

resulted in more than 34,000 trees, the strict consensus of which was summarized as a tribal phylogeny (17 terminals). The generic composition of tribal clades (one MP tree shown) is highly congruent past chloroplast studies and our results. However, relationships between tribal clades are highly incongruent with both. Goertzen et al. (2003) nonetheless characterize their ITS results as having considerable topological congruence of major lineages of the family with chloroplast studies when bootstrap values are considered. To support this assertion they compare a mostly unresolved 50% bootstrap consensus ITS tree with a mostly resolved *ndh*F tree for comparable analyses of a reduced taxon set (82-taxon matrices). Since meaningful discussion of tribal relationships based on the bootstrap consensus is ineffectual, the authors shift their comparison of ITS with chloroplast results to a strict consensus overview (Fig. 2, Goertzen et al., 2003) that shows resolved tribal relationships, and we will compare our results to that tree also. Goertzen et al. cite the monophyly of Asteroideae, paraphyly of Cichorioideae (defined to include Mutisieae) and the position of classical Mutisieae as congruent with chloroplast studies. They also cite examples of incongruence with past studies including the phylogenetic positions of tribes Arctotideae, Cichorieae, Cynareae, Liabeae, and Vernonieae. Comparing their results to those of Kim and Jansen (1995) further incongruences include the position of Anthemideae, the sister taxon relationship of Calenduleae to Senecioneae, the sister taxon relationship of the previous to Inuleae and Plucheeae, and the sister taxon relationship of Astereae to Gnaphalieae.

Our results are congruent with the ITS strict consensus topology in only three respects: (1) placing the Mutisioideae as the next lineage to split above Barnadesioideae, (2) placing Heliantheae Alliance sister to Athroismeae, and (3) placing Inuleae sister to Plucheeae (now Inuleae, Anderberg et al., 2005). The ITS topology showing Cichorieae as sister to the other tribes of Asteraceae except Barnadesioideae and Mutisieae is incongruent with our results and other studies based on sequence data of chloroplast markers (Kim and Jansen, 1995; Karis et al., 2001). Cynareae placed as sister to Arctotideae and Liabeae in the ITS study is also incongruent with our results as well as those of past sequence studies based on the *ndh*F gene. The tribal relationships within Asteroideae reconstructed in the ITS study are vastly in disagreement with our and other published results based on chloroplast sequence data (Kim and Jansen, 1995; Kim et al., 2005).

Goertzen et al. (2003) trace the topological incongruence between their ITS and earlier chloroplast results to unspecified analytical or biological phenomena. Comparing the 82-taxon ITS and chloroplast *ndh*F trees in that study, the *ndh*F alone had far more power to resolve relationships among the tribal lineages than did the ITS; the relatively lower number of informative characters ITS provides for resolving these relationships (380 ITS vs. 465 *ndh*F) and higher homoplasy (homoplasy index: 0.88 ITS vs. 0.61 *ndh*F) resulted in low bootstrap values supporting tribal splits in the ITS trees. Their study highlights that when ITS is the only marker used, it appears to be more appropriate for phylogenetic comparisons at lower taxonomic levels rather than addressing deep divergences in Asteraceae. This conclusion resonates with that of Bailey et al. (2006) who found ITS provided only limited resolution of deeper nodes of Brassicaceae.

If the evolutionary histories of the nuclear and chloroplast compartments in Asteraceae truly differ so extensively at the tribal relationships it would be remarkable, and could suggest an even greater role of hybridization in generating Asteraceae diversity. However, this interpretation of the incongruence between these nuclear and plastome topologies is confounded by other factors that can lead to conflicting phylogenetic signal, namely sampling error and homoplasy arising from the assessment of sequence orthology as well as nucleotide substitution saturation. Our chloroplast and the ITS study differ significantly in both dimensions of the data matrices. Overall more than twice as many taxa were sampled in the ITS study, while sampling of the basal lineages of Mutisioideae, Stifftioideae, Wunderlichioideae, Gochnatioideae, and Hecastocleidoideae is nearly four times greater in our study (43 in the chloroplast study compared to 11 in the ITS study). Increased taxon sampling is thought to reduce phylogenetic error (Wheeler, 1992; Graybeal, 1998; Zwickl and Hillis, 2002; Pollock et al., 2002) primarily through the subdivision of long branches (Lyons-Weiler and Hoelzer, 1997; Purvis and Quicke, 1997; Poe and Swofford, 1999). When long-branch attraction (Felsenstein, 1978) is a problem, accuracy is apparently facilitated when taxa are added at deeper nodes near the base of long branches rather than at the tips of long branches (Geuten et al., 2007; Graybeal, 1998; Poe, 2003), but the existence of long-branch problems in either of these Asteraceae data sets has not been demonstrated. Taxon sampling differences must account for some different placements but may not fully explain incongruence in tribal relationships in so many branches across the tree. Hypothesizing positional homology of nucleotides from hypervariable spacer data does present a challenge for alignment of more divergent taxa (Baldwin et al., 1995; Kim and Jansen, 1995). In the ITS study, samples were first aligned manually by roughly tribal groups, then tribal groups aligned with the aid of 80% consensus sequences based on each group. The high correspondence of generic composition of each tribal lineage contrasts starkly with the low correspondence of tribal relationships between the ITS and chloroplast studies; it is not clear what effect the primary assessment of homology may have had on these ITS results. Also unexplored is the possibility of unidentified divergent paralogues among the ITS sequences assembled from various sources that could mislead phylogenetic inference (Sanderson and Doyle, 1992; Buckler et al., 1997; Bailey et al., 2006). Alvarez and Wendel (2003) recently reviewed some characteristics of nrITS and advocate the alternative use of low copy nuclear genes for plant phylogenetic studies. A well-resolved statistically

supported nuclear phylogeny based on balanced taxon sampling is still needed to compare with chloroplast results to understand the organismal phylogeny of Asteraceae.

8. Conclusions

With this study a few more branches of the sunflower tree of life have been identified and a new paradigm has been established in Asteraceae systematics. We now know with confidence that the family comprises many more major lineages than previously thought. The 'Mutisieae problem' has been mostly solved as the component lineages have been identified and recognized as new subfamilies of the Asteraceae. However, the position of Stifftioideae is still equivocal and the phylogenetic relationships among the three main lineages of the Cichorioideae are still problematic. Our results support the close relationship of Liabeae and Vernonieae, but failed to identify the relationship of this lineage to either Arctotideae or the Cichorieae-Gundelieae clades. Equivocal too are the phylogenetic position of Senecioneae within Asteroideae and some of the tribal relationships of the Heliantheae alliance.

Our phylogeny clearly shows that from its South American origin the great diversity of the family was obtained in the Old World with the subsequent reintroductions of these lineages into the New World and Australia. Although the Stifftioideae and Wunderlichioideae are considered to be the most characteristic members of the Guayana flora (Berry and Riina, 2005) the Guayana Highlands did not give rise to any major lineages of the Asteraceae. The phylogenetic position of the North American endemic *Hecastocleis* sister to the Carduoideae–Asteroideae clade precipitates new hypotheses about how sunflowers may have first expanded beyond the continent of their origin, and we suggest that dual long distance dispersals to North America and to Africa met with dual fates. The capacity of Asteraceae to disperse long distances and establish successfully in other habitats is demonstrated again with one new example revealed here: *Nouelia* and *Leucomeris* are Asian genera whose sister taxa are endemic to South America.

Several lineages of the Asteraceae have experienced significant cladogenesis resulting in the formation of thousands of species. The first step in recognizing a pattern attributable to adaptive radiation is the differential diversification among lineages that results from differences in extinction and speciation rates (Guyer and Slowinski, 1993). We have identified several lineages whose sister taxa contain significantly larger numbers of species and including: Cyclolepis/Gochnatioideae (1:74 spp.); Gundelieae/Cichorieae (2:1500 spp.); Liabeae/Vernonieae (190:1000 spp.); Hecastocleis/Carduoideae–Asteroideae clade (1:22,000 spp.); Gymnarrhena/Cichorioideae, Asteroideae, and Corymbioideae (1:20,000 spp.); Corymbium/Asteroideae (9:17,000 spp.); Calenduleae/Anthemideae, Astereae, and Anthemideae (120:6200 spp.). The relatively recent origin of the family and the extraordinary cladogenesis of some of its more derived lineages suggest the family may contain groups (e.g. Astereae) with some of the fastest diversification rates in the flowering plants. With a strong phylogenetic framework and denser taxon sampling future studies aimed at comparing salient features of the morphology, chemistry, breeding systems, pollination, and herbivory of the taxa contained in these lineages may document traits responsible for the extraordinary global diversification of Asteraceae.

Even denser taxon sampling is still needed to address important macroevolutionary questions in Asteraceae. Our study demonstrates that despite the large size of the family more inclusive taxonomic and character sampling is profitable. We have demonstrated the utility of five chloroplast markers new to Asteraceae studies, with primer sequences and protocols for these markers tested across all tribes of the family. With the increasing ease of gathering sequence data these sequences provide an infrastructure on which even larger supermatrices can be built in the near future. Our hypothesis of phylogenetic relationships among the major clades of Asteraceae, based on preliminary results of this study, has already been utilized as the backbone of a metatree (or informal supertree sensu Bininda-Emonds, 2004) that summarizes phylogenetic hypotheses of many independent studies at various taxonomic levels across the family (Funk et al., 2005). Source trees based on molecular studies of Asteraceae have typically sampled within existing taxonomic categories with little or no taxon overlap among studies. As the present study points out, existing suprageneric taxa in Asteraceae may or may not be monophyletic. Formal supertree methods using source trees available prior to our study could not have identified the lineages found here. Furthermore, there is no reason to expect that our study has uncovered all the major lineages of the family. A continually updated metatree for the family can be obtained at http://www.tolweb.org/asteraceae.

The surprising number of new major lineages found in this study results from sampling genera identified by morphological studies as anomalous, in the context of denser taxon and broader character sampling than in previous studies. Our strategy could work well for the design of supermatrix studies aimed at building a backbone for bigger trees at the family and ordinal levels. Empirical studies have demonstrated the value of a multigene approach to building backbone phylogenies for large flowering plant families (Bailey et al., 2006; Potter et al., 2007) and have stressed the importance of broad taxon sampling. Sampling anomalous and transitional genera in the context of dense and balanced sampling should also be considered a priority, as some of these underrepresented taxa may constitute novel lineages. Although the explosive radiation of Asteraceae is not as recent as previously thought (Kim et al., 2005), Carlquist's (1976) expectation that transitional genera should be extant has been validated by this molecular study.

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Appendix A. Appendix

Voucher information and GenBank Accession numbers for sequences used in this study. Voucher information listed in the following order: taxon name, collection, country of origin, herbarium. Genbank numbers listed in the following order: *trn*K intron and *mat*K, *ndh*D, *ndh*I, *ndh*F, *rbc*L, *rpo*B, *rpo*C1 exon1, 23S-*trn*A spacer, *trn*L intron–*trn*L-F spacer (in some taxa 2 Genbank numbers comprise this region). ND, missing sequence.

Achillea millefolium L., Panero 2002-55, USA, TEX. EU385315, EU385219, EU243242, EU385124, EU384938, EU385410, EU385506, EU243147, EU385030. *Acicarpha spathulata* R. Br., Salgado 7660, Brazil, TEX. EU385316, EU385220, EU243243, EU385125, EU384939, EU385411, EU385507, EU243148, EU385031. *Acourtia turbinata* (La Llave & Lex.) DC., Panero 2891, Mexico, TEX. EU385317, EU385221, EU243244, EU385126, EU384940, EU385412, EU385508, EU243149, EU385032. *Adenocaulon chilense* Less., Simon 382, Argentina, US. EU385319, EU383223, EU243246, EU385128, EU384942, EU385414, EU385510, EU243151, EU385034. *Adenocaulon bicolor* Hook., Twisselmann 7661, USA, TEX. EU385320, EU385224, EU243247, EU385129, EU384943, EU385415, EU385511, EU243152, EU385035. *Ainsliaea apiculata* Sch. Bip ex. Zoll., Ohtsuka s.n., Japan, no voucher. EU385321, EU385225, EU243248, EU385130, EU384944, EU385416, EU385512, EU243153, EU385036. *Ainsliaea macrocephala* (Mattf.) Y.Q. Tseng, Bartholomew and Bufford 6167, Taiwan, US. EU385322, EU385226, EU243249, EU385131, EU384945, EU385417, ND, EU243154, EU385037. *Aphyllocladus spartioides* Wedd., Simon 508, Argentina, US. EU385323, EU385227, EU243250, EU385132, EU384946, EU385418, EU385513, EU243155, EU385038. *Arctotis hirsuta* (Harv.) P. Beauv., Panero 2002-61, cultivated, seed source: Kirstenboch Botanical Garden, South Africa, TEX. EU385224, EU385228, EU243251, EU385133, EU384947, EU385419, EU385514, EU243156, EU385039. *Athroisma gracile* (Oliv.) Mattf. ssp. *psyllioides* (Oliv.) T. Eriksson, Eriksson, Kalema, and Leliyo 559, Tanzania, TEX. AY215765, AF384437, AF383757, L39455, AY215085, AY213763, EU385515, AY216277, AY216019/AY216144. *Atractylis cancellata* L., Panero 7098, Spain, TEX. EU385325, EU385229, EU243252, EU385134, EU384948, EU385420, EU385516, EU243157, EU385040. *Baccharis neglecta* Britton ex Britton and A. Br., Panero 2002-31, USA, TEX. EU385326, EU385230, EU243253, EU385135, EU384949, EU385421, EU385517, EU243158, EU385041. *Barnadesia spinosa* L. f., Panero and Crozier 8492, Argentina, TEX. EU385327, EU385231, EU243254, L39394 (*Barnadesia caryophylla* (Vell.) S.F. Blake), AY874427 (*Barnadesia caryophylla*), EU385422, EU385518, EU243159, EU385042. *Berkheya purpurea* (DC.) Mast., Panero 2002-49, cultivated, seed source: Kirstenboch Botanical Garden, South Africa, TEX. EU385328, EU385232, EU243255, EU385136, EU384950, EU385423, EU385519, EU243160, EU385043. *Blepharispermum zanguebaricum* Oliv. and Hiern., T. Eriksson 604, Kenya, TEX. AY215768, AF384440, AF383760, L39456, AY215088, AY213766, ND, AY216280, AY216022/AY216147. *Brachyclados caespitosus* (Phil.) Speg., Bonifacino 459, Argentina, US. EU385329, EU385233, EU243256, EU385137, EU384951, EU385424, EU385520, EU243161, EU385044. *Brachylaena elliptica* (Thunb.) DC., Koekemoer and Funk 1971, South Africa, US. EU385330, EU385234, EU243257, EU385138, EU384952, EU385425, EU385521, EU243162, EU385045. *Carthamus tinctorius* L., al-Hosseini s.n., Iran, US. EU385331, EU385235, EU243258, EU385139, EU384953, EU385426, EU385522, EU243163, EU385046. *Centaurea melitensis* L., Panero 2002-48, USA, TEX. EU385332, EU385236, EU243259, EU385140, EU384954, EU385427, EU385523, EU243164, EU385047. *Centratherum punctatum* Cass., Panero 2002-53, USA cultivated, TEX. EU385333, EU385237, EU243260, EU385141, EU384955, EU385428, EU385524, EU243165, EU384048. *Chaetanthera pentacaenoides* (Phil.) Hauman, Bonifacino 293, Argentina, US. EU385334, EU385238, EU243261, EU385142, EU384956, EU385429, EU385525, EU243166, EU384049. *Chaptalia nutans* (L.) Pol., Panero 2002-19, USA, TEX. EU385335, EU385239, EU243262, EU385143, EU384957, EU385430, EU385526, EU243167, EU385050. *Chimantaea humilis* Maguire, Steyermark and Wurdack, Weitzman et al. 412, Venezuela, US. EU385336, EU385240, EU243263, EU385144, EU384958, EU385431, EU385527, EU243168, EU385051. *Chrysanthemoides monilifera* (L.) Norl., Panero 2002-5, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385337, EU385241, EU243264, EU385145,

EU384959, EU385432, EU385528, EU243169, EU385052. *Chuquiraga spinosa* Less., Simon 522, Argentina, US. EU385338, EU385242, EU243265, EU385146, EU384960, EU385433, EU385529, EU243170, EU385053. *Cnicothamnus lorentzii* Griseb., Panero 1934, Argentina, TENN. EU385339, EU385243, EU243266, EU385147, EU384961, EU385434, EU385530, EU243171, EU385054. *Corymbium glabrum* L., Moffett 8764, South Africa, TEX. EU385340, EU385244, EU243267, EU385148, EU384962, EU385435, EU385531, EU243172, EU385055. *Cyclolepis genistoides* D. Don, Bonifacino 3, Argentina, US. EU385341, EU385245, EU243268, EU385149, EU384963, EU385436, EU385532, EU243173, EU385056. *Dasyphyllum reticulatum* (DC.) Cabrera, Roque, Funk & Kim 485, Brazil, US. EU385342, EU385246, EU243269, EU385150, AY874428 (*Dasyphyllum argenteum* Kunth. in H.B.K.), EU385437, EU385533, EU243174, EU385057. *Dicoma capensis* Less., Trinder-Smith 349, South Africa, US. EU385344, EU385247, ND, EU385152, EU384965, EU385439, EU385534, EU243176, EU385059. *Dicoma sp.*, Funk 1960, South Africa, US. EU385343, EU385248, EU243270, EU385151, EU384964, EU385438, ND, EU243175, EU385058. *Dimorphoteca sinuata* DC., Panero 2002-3, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385345, EU385249, EU243271, EU385153, EU384966, EU385440, EU385535, EU243177, EU385060. *Dinoseris salicifolia* Griseb., Simon 330, Argentina, US. EU385346, EU385250, EU243272, EU385154, EU384967, EU385441, EU385536, EU243178, EU385061. *Dolichlasium lagascae* D. Don, Simon 811, Argentina, US. EU385347, EU385251, EU243273, EU385155, EU384968, EU385442, EU385537, EU243179, EU385062. *Doniophyton anomalum* (D. Don) Kurtz, Bonifacino 96, Argentina, US. EU385348, EU385252, EU243274, EU385156, EU384969, EU385443, EU385538, EU243180, EU385063. *Duidaea pinifolia* S. F. Blake, V. A. Funk 8010, Venezuela, US. EU385349, EU385253, EU243275, EU385157, EU384970, EU385444, EU385539, EU243181, EU385064. *Echinops ritro* L., Panero 2002-71. cultivated, TEX. EU385350, EU385254, EU243276, EU385158, EU384971, EU385445, EU385540, EU243182, EU385065. *Eremanthus erythropappus* (DC.) MacLeish, Acosta 1661, Brazil, TEX. EU385351, EU385255, EU243277, EU385159, EU384972, EU385446, EU385541, EU243183, EU385066. *Erigeron tenuis* Torr. and Gray, Panero 2002-25, USA, TEX. EU385352, EU385256, EU243278, EU385160, EU384973, EU385447, EU385542, EU243184, EU385067. *Felicia heterophylla* (Cass.) Grau, Panero 2002-1, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385353, EU385257, EU243279, EU385161, EU384974, EU385448, EU385543, EU243185, EU385068. *Gamochaeta pensylvanica* (Willd.) Cabr., Panero 2003-27, USA, TEX. EU385354, EU385260, EU243282, EU385162, EU384977, EU385449, EU385544, EU243188, EU385070. *Gerbera serrata* (Thunb.) Druce, Koekemoer 2001, South Africa, US. EU385356, EU385258, EU243281, EU385164, EU384976, EU385451, ND, EU243187, EU385069. *Gerbera piloselloides* (L.) Cass., Koekemoer and Funk, 1972, South Africa, US. EU385355, EU384259, EU243280, EU385163, EU384975, EU385450, EU385545, EU243186, ND. *Gochnatia hiriartiana* Medrano, Villaseñor and Medina, Panero MEX-2, Mexico, TEX. EU385358, EU385262, EU243284, EU385166, EU384979, EU385453, ND, EU243190, EU385072. *Gochnatia hypoleuca* (DC.) A. Gray, Panero MEX-1, Mexico, TEX. EU385357, EU385261, EU243283, EU385165, EU384978, EU385452, EU385546, EU243189, EU385071. *Gongylolepis benthamiana* R. H. Schomb., Berry 6564, Venezuela, US. EU385359, EU385263, EU243285, EU385167, EU384980, EU385454, EU385547, EU243191, EU385073. *Gorteria diffusa* Thunb, Koekemoer and. Funk 1945, South Africa, US. EU385360, EU385264, EU243286, EU385168, EU384981, EU385455, EU385548, EU243192, EU385074. *Gundelia tournefortii* L., al-Hosseini s.n., Iran, US. EU385361, EU385265, EU243287, EU385169, EU384982, EU385456, EU385549, EU243193, EU385075. *Gymnarrhena micrantha* Desf., Mandeville 157, Saudi Arabia, US. EU385362, EU385266, EU243288, EU385170, EU384983, EU385457, EU385550, EU243194, EU385076. *Hecastocleis shockleyi* A. Gray, Panero and Crozier 8157, USA, TEX. EU385363, EU385267, EU243289, EU385171, EU384984, EU385458, EU385551, EU243195, EU385077. *Helenium bigelovii* A. Gray, Baldwin 681, USA, DAV. AY215804, AF384475, AF383795, AF384730, AY215123, AY213801, EU385552 (*Helenium amarum* (Raf.) H. Rock), AY216315, AY216057/AY216182. *Helianthus annuus* L., Mammoth Russian, grown from seed, no voucher. AY215805, AF384476, AF383796, L39383, AY215124, AY213802, EU385553, AY216316, AY216058/AY216183. *Hesperomannia arbuscula* Hillebr., Chin 11a, USA, no voucher. EU385364, EU385268, EU243290, EU385172, EU384985, EU385459, EU385554, EU243196, EU385078. *Heterolepis aliena* (L. f.) Druce, Panero 2002-35, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385365, EU385269, EU243291, EU385173, EU384986, EU385460, EU385555, EU243197, EU385079. *Hoplophyllum spinosum* DC., Koekemoer 2045, South Africa, US. EU385366, EU385270, EU243292, EU385174, EU384987, EU385461, EU385556, EU243198, EU385080. *Hyalis argentea* D. Don ex Hook. and Arn., Simon 657, Argentina, US. EU385367, EU385271, EU243293, EU385175, EU384988, EU385462, EU385557, EU243199, EU385081. *Hyaloseris rubicunda* Griseb., Simon 716, Argentina, US. EU385368, EU385272, EU243294, EU385176, EU384989, EU385463, EU385558, EU243200, EU385082. *Ianthopappus corymbosus* (Less.) Roque and D.J.N. Hind, Roque, Funk & Kim 462, Brazil, US. EU385369, EU385273, EU243295, EU385177, EU384990, EU385464, EU385559, EU243201, EU385083. *Inula britannica* L., Santos and Francisco ACC55-98, cultivated at TEX, ORT. AY215812, AF384483, AF383803, AF384737, AY215130, AY213809, EU385560, AY216323, AY216065/AY216190. *Jungia polita* Griseb.,

Simon 292, Argentina, US. EU385370, EU385274, EU243296, EU385178, EU384991, EU385465, EU385561, EU243202, EU385084. *Layia heterotricha* (DC.) Hook. and Arn., Baldwin 794, USA, JEPS. AY215818, AF384489, AF383809, AF384742, AY215136, AY213815, EU385562 (*Layia platyglossa* A. Gray), AY216328, AY216071/AY216196. *Leucheria thermarum* (Phil.) Phil., Simon 383, Chile, US. EU385371, EU385275, EU243297, EU385179, EU384992, EU385466, EU385563, EU243203, EU385085. *Leucomeris spectabilis* D. Don, Nicolson 3254, Nepal, US. EU385372, EU385276, EU243298, EU385180, EU384993, EU385467, EU385564, EU243204, EU385086. *Lophopappus cuneatus* R.E. Fr., Simon 563, Argentina, US. EU385374, EU385278, EU243300, EU385182, EU384995, EU385469, EU385566, EU243206, EU385088. *Lycoseris crocata* (Bertol.) S. F. Blake, Funk 12019, Colombia, US. ND, EU385279, EU243301, EU385183, EU384996, EU385470, EU385567, EU243207, EU385089. *Macledium zeyheri* (Sond.) S. Ortiz, Panero 2002-47, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385375, EU385280, EU243302, EU385184, EU384997, EU385471, EU385568, EU243208, EU385090. *Mutisia retrorsa* Cav., Bonifacino 148, Argentina, US. EU385376, EU385281, EU243303, EU385185, EU384998, EU385472, EU385569, EU243209, EU385091. *Nassauvia pygmaea* (Cass.) Hook. f., Bonifacino 179, Argentina, US. EU385377, EU385282, EU243304, EU385186, EU384999, EU385473, EU385570, EU243210, EU385092. *Nouelia insignis* Franch., Rock 8534, China, US. EU385378, EU385283, EU243305, EU385187, EU385000, EU385474, EU385571, EU243211, EU385093. *Oldenburgia grandis* (Thunb.) Baill., Trinder-Smith s. n., South Africa, US. EU385379, EU385284, EU243306, EU385188, EU385001, EU385475, EU385572, EU243212, EU385094. *Oncosiphon grandiflorum* (Thunb.) M. Kallersjo, Panero 2002-6, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385380, EU385285, EU243307, EU385189, EU385002, EU385476, EU385573, EU243213, EU385095. *Onoseris hastata* Wedd., Horn 1756, South America, cultivated, US. EU383381, EU385286, EU243308, EU385190, EU385003, EU385477, EU385574, EU243214, EU385096. *Osteospermum asperulum* (DC.) Norl., Funk 12264, South Africa, cultivated, US. EU383382, EU385287, EU243309, EU385191, EU385004, EU385478, EU385575, EU243215, EU385097. *Oyedaea verbesinoides* DC., Panero 2609, Venezuela, TEX. AY215835, AF384507, AF383827, AF384758, AY215153, AY213829, EU385576, AY216345, AY216088/AY216213. *Pachylaena atriplicifolia* D. Don ex Hook. & Arn., Simon 684, Argentina, US. EU383383, EU385288, EU243310, EU385192, EU385005, EU385479, EU385577, EU243216, EU385098. *Pasaccardoa grantii* (Benth. ex Oliv.) Kuntze, Bamps 8573, Zaire, US. EU383384, EU385289, EU243311, EU385193, EU385006, EU385480, ND, ND, EU385099. *Perezia purpurata* Wedd., Simon 594, Argentina, US. EU383385, EU385290, EU243312, EU385194, EU385007, EU385481, EU385578, EU243217, EU385100. *Perityle lindheimeri* (A. Gray) Shinners, Smith 617, USA, TEX. AY215839, AF384510, AF383831, AF384761, AY215157, AY213832, EU385579, AY216349, AY216092/AY216217. *Pertya scandens* Sch. Bip., Ohtsuka s.n., Japan, no voucher. EU383386, EU385291, EU243313, EU385195, EU385008, EU385482, EU385580, EU243218, EU385101. *Phanerogloss bolusii* (Oliv.) B. Nordenstam, Watson and Panero 94-62, South Africa, TEX. AY215843, AF384514, AF383835, AF384765, AY215161, AY213836, EU385581, AY216353, AY216096/AY216221. *Platycarpha carlinioides* Oliv. & Hiern, Seydel 3549, Namibia, US. EU383387, EU385292, EU243314, EU385196, EU385009, EU385483, ND, ND, EU385102. *Plazia daphnoides* Wedd., Simon 536, Argentina, US. EU383388, EU385293, EU243315, EU385197, EU385010, EU385484, EU385582, EU243219, EU385103. *Pluchea carolinensis* (Jacq.) G. Don, Panero s. n., Mexico, TEX. EU385389, EU385294, EU243316, EU385198, EU385011, EU385485, EU385583, EU243220, EU385104. *Proustia cuneifolia* D. Don, Simon 511, Argentina, US. EU385390, EU385295, EU243317, EU385199, EU385012, EU385486, EU385584, EU243221, EU385105. *Psacalium paucicapitatum* (Rob. and Greenm.) H. Robinson and Brettell, Panero 2476, Mexico, TEX. EU385391, EU385296, ND, EU385200, EU385013, EU385487, EU385585, EU243222, EU385106. *Relhania calycina* (Rob. and Greenm.) H. Robinson and Brettell, Watson and Panero 94-96, South Africa, TEX. EU385392, EU385297, EU243318, EU385201, EU385014, EU385488, ND, EU243223, EU385107. *Richterago amplexifolia* (Gardner) Kuntze, Roque, Funk and Kim 476, Brazil, US. EU385393, EU385298, EU243319, EU385202, EU385015, EU385489, EU385586, EU243224, EU385108. *Richterago angustifolia* (Gardner) Roque, Roque, Funk and Kim 489, Brazil, US. EU385318, EU385222, EU243245, EU385127, EU384941, EU385313, EU385509, EU243150, EU385033. *Scaevola aemula* R. Br., Panero 2002-24, Australia, cultivated, TEX. EU383394, EU385299, ND, EU385203, L13932, EU385490, EU385587, EU243225, EU385109. *Scolymus maculatus* L., Panero 6993, Spain, TEX. EU385395, EU385300, EU243320, EU385204, EU385016, EU385491, EU385588, EU243226, EU385110. *Senecio polypodioides* Greene, McDonald 2964, Mexico, TEX. EU385396, EU385301, EU243321, EU385205, EU385017, EU385492, EU385589, EU243227, EU385111. *Sinclairia palmeri* (A. Gray) B.L. Turner, Panero 7457, Mexico, TEX. EU385373, EU385277, EU243299, EU385181, EU384994, EU385468, EU385565, EU243205, EU385087. *Sonchus oleraceus* L., Panero 2002-80, USA, TEX. EU385397, EU385302, EU243322, EU385206, EU385018, EU385493, EU385590, EU243228, EU385112. *Stenopadus talaumifolius* S. F. Blake, Clarke 5459, Venezuela, US. EU385398, EU385303, EU243323, EU385207, EU385019, EU385494, EU385591, EU243229, EU385113. *Stevia rebaudiana* Bertoni, Schilling 02-24, Grown from seed, TENN. AY215865, AF384534, AF383856, AF384787, AY215182,

AY213856, ND, AY216374, AY216117/AY216242. *Stifftia chrysantha* Mikan, Serra 235, Brazil, TEX. EU385399, EU385304, EU243324, EU385208, EU385020, EU385495, EU385592, EU243230, EU385114. *Stokesia laevis* (Hill) Greene, Tripple Brook Farm Nursery, USA, TEX. EU385400, EU385305, EU243325, EU385209, L13076, EU385496, EU385593, EU243231, EU385115. *Stomatochaeta condensata* (Baker) Maguire and Wurdack, Berry 6574B, Venezuela, US. EU385401, EU385306, EU243326, EU385210, EU385021, EU385497, EU385594, EU243232, EU385116. *Syncarpha vestita* (L.) B. Nord., Watson & Panero 94-18, South Africa, TEX. EU385402, EU385307, EU243327, EU385211, EU385022, EU385498, EU385595, EU243233, EU385117. *Tagetes erecta* L., Soule 3004, Guatemala, TEX. AY215867, AF384536, AF383858, L39466, AY215184, AY213858, EU385596, AY216376, AY216119/AY216244. *Tarchonanthus camphoratus* L., Koekemoer and Funk 1967, South Africa, US. EU385403, EU385308, EU243328, EU385212, EU385023, EU385499, ND, EU243234, EU385118. *Trichocline boecheri* Cabrera, Bonifacino 142, Argentina, US. EU385404, EU385309, EU243329, EU385213, EU385024, EU385500, EU385597, EU243235, EU385119. *Trilisa paniculata* (Walter ex J. F. Gmel.) Cass., Cox 5466, USA, TENN. ND, AF384491, AF383811, AF384744, AY215138, AY213816, ND, AY216330, AY216073/AY216198. *Trixis divaricata* (Kunth) Spreng., Santos 2659, Brazil, TEX. EU385405, EU385310, EU243330, EU385214, EU385025, EU385501, EU385598, EU243236, EU385120. *Ursinia speciosa* DC., Panero 2002-4, cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. EU385406, EU385311, EU243331, EU385215, EU385026, EU385502, EU385599, EU243237, EU385121. *Warionia saharae* Bentham ex Coss., Lippat 25346, Morocco, US. EU385407, EU385312, EU243332, EU385216, EU385027, EU385503, EU385600, ND, AY702089/AY702090. *Wunderlichia mirabilis* Riedel, Roque, Funk & Kim 466, Brazil, US. EU385408, EU385313, EU243333, EU385217, EU385028, EU385504, EU385601, EU243238, EU385122. *Youngia japonica* (L.) DC., Panero 2002-92, USA, TEX. EU385409, EU385314, EU243334, EU385218, EU385029, EU385505, EU385602, EU243239, EU385123. *Zinnia juniperifolia* (DC.) A. Gray, Panero 2184, Mexico, TEX. AY215883, AF384552, AF383874, AF384805, AY215200, AY213874, EU385603, AY216392, AY216135/AY216260.

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