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Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae

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ABSTRACT

A backbone phylogeny that fully resolves all subfamily and deeper nodes of Asteraceae was constructed using 14 chloroplast DNA loci. The recently named genus *Famatinanthus* was found to be sister to the Mutisioideae–Asteroideae clade that represents more than 99% of Asteraceae and was found to have the two chloroplast inversions present in all Asteraceae except the nine genera of Barnadesioideae. A monotypic subfamily Famatinanthoideae and tribe Famatinantheae are named herein as new. Relationships among the basal lineages of the family were resolved with strong support in the Bayesian analysis as (Barnadesioideae (Famatinanthoideae (Mutisioideae (Stifftioideae (Wunderlichioideae–Asteroideae))))). Ancestral state reconstruction of ten morphological characters at the root node of the Asteraceae showed that the ancestral sunflower would have had a woody habit, alternate leaves, solitary capitulescences, epaleate receptacles, smooth styles, smooth to microechinate pollen surface sculpturing, white to yellow corollas, and insect-mediated pollination. Herbaceous habit, echinate pollen surface, pubescent styles, and cymose capitulescences were reconstructed for backbone nodes of the phylogeny corresponding to clades that evolved shortly after Asteraceae dispersed out of South America. No support was found for discoid capitula, multiseriate involucres or bird pollination as the ancestral character condition for any node. Using this more resolved phylogenetic tree, the recently described *Raiguenrayun cura* + *Mutisiapollis telleriae* fossil should be associated to a more derived node than previously suggested when time calibrating phylogenies of Asteraceae.

1. Introduction

The early evolution of Asteraceae (sunflower or daisy family) is obscured by the still incomplete phylogenetic resolution at deep nodes of the family. Although the split of subfamily Barnadesioideae (91 species) from the Mutisioideae–Asteroideae clade (>25,000 species) is well documented (Jansen and Palmer, 1987; Kim and Jansen, 1995; Goertzen et al., 2003; Kim et al., 2005; Panero and Funk, 2008) the branching order of earliest divergences of the larger clade remains unclear. The best sampled and resolved phylogenetic trees of basal Asteraceae are based on chloroplast DNA (Panero and Funk, 2008) and these form the 'backbone' topology of metatree constructions that have been used recently to study the origin and divergence time of the family (Funk et al., 2009; Torices, 2010; Barreda et al., 2012). Unfortunately, these trees lack resolution of important relationships among basal Mutisioideae, Stifftioideae and Wunderlichioideae–Asteroideae clades, and lack statistical support for the placement of Wunderlichioideae as sister to the Gochnatioideae–Asteroideae clade. Resolving these relationships remains a prominent objective of molecular systematics of Asteraceae. A reliable phylogeny would allow us to more accurately reconstruct the ancestral features of sunflowers and trace character transitions of interest, for instance, those that may have promoted the diversification of the family.

Characteristics of an ancestral sunflower at the root node of Asteraceae have been hypothesized previously based on phylogenies in which basal lineages of the family were sparsely sampled or incompletely resolved. Based on outgroup comparisons the earliest Asteraceae are thought to have been woody plants with

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alternate phyllotaxy and an indeterminate inflorescence arranged in solitary capitula (Bremer, 1994), had entire leaf margins (Bremer, 1994), dry, papillose stigmas, pollen with three cells at time of dispersal, a pollen kit that facilitates pollen presentation to pollinators and used inulin instead of starch as a storage polysaccharide (Lundberg, 2009). Bremer (1994) also optimized several characters on a rooted cladogram in which Barnadesioideae, Mutisieae sensu Cabrera (1977; a polytomy comprising species of Mutisioideae, Stifftioideae, Wunderlichioideae, Pertyoideae, Gochnatioideae, Hecastocleidoideae, Dicomeae, Tarchonantheae and Oldenburgieae), and Carduoideae represented three most basal branches of Asteraceae. He concluded that yellowish, actinomorphic corollas, discoid capitula with multiple rows of bracts or phyllaries, thick, glabrous or minutely papillose styles, tailed anthers, and dry fruits with a pappus of scabrid bristles were also plesiomorphic states. Bremer's reconstruction of an ancestral flower with fused anthers surrounding a style that pushes pollen shed into the anther tube suggests that animal-mediated pollination was the ancestral condition in Asteraceae. This secondary pollen presentation is present in nearly all extant Asteraceae (a few wind pollinated species have variously free anthers), Calyceraceae, and Goodeniaceae (Erbar and Leins, 1995; Lane, 1996; Jeffrey, 2007). Parallel evolution of hummingbird pollination derived from insect pollination has been shown for a few Barnadesioideae genera including *Barnadesia*, *Chuquiraga*, and probably *Arnaldoa* (Gruenstaeudl et al., 2009). However, Barreda et al. (2012) claim indirect fossil evidence for bird pollination at the root node of the family. Their conclusion is based on interpretation of ligulate corollas in *Raiguenrayun cura*, a recently-described macrofossil co-fossilized with *Mutisiapollis telleriae* pollen, and on the phylogenetic placement of these fossil taxa. Although *Raiguenrayun cura* lacks diagnostic floral features to associate it with any particular extant basal Asteraceae lineage, *Mutisiapollis telleriae* is similar to extant pollen of five clades in Stifftioideae, Wunderlichioideae, Gochnatioideae and Carduoideae and is dissimilar to Mutisioideae (Barreda et al., 2012). Barreda et al. concluded that the fossil pair belongs to a lineage that diverged between the Mutisioideae, Stifftioideae, Wunderlichioideae–Asteroideae trichotomy and the Barnadesioideae–Asteroideae nodes. Resolving relationships among basal Asteraceae would allow more accurate placement of *Mutisiapollis telleriae* and help refine ancestral character state reconstructions and thus our understanding of root node morphological features and early transitions in Asteraceae.

Sampling morphologically anomalous taxa has been useful in dividing stems in phylogenetic trees and discovering new lineages in Asteraceae and in other large groups when complete taxon sampling is prohibitive (Panero and Funk, 2008; Rydin et al., 2008). In Asteraceae anomalous taxa with long histories of taxonomic uncertainty in their placement tend to be small (*Corymbium*) or monotypic genera (*Cavea*, *Feddea*, *Gymnarrhena*, *Hecastocleis*, *Dipterocome*). *Cavea* and *Dipterocome* belong to lineages for which they mostly lack the morphological synapomorphies of their taxonomic groups (Anderberg et al., 2007; Anderberg and Ohlson, 2012) but *Corymbium*, *Feddea*, *Gymnarrhena*, and *Hecastocleis* were each found to be sister to large radiations (Cariaga et al., 2008; Panero and Funk, 2008). An anomalous member of Mutisioideae was recently identified that has never been sampled in a molecular phylogenetic study; *Aphyllocladus decussatus*, a sunflower previously known only from the type collection gathered more than 125 years ago, was recently rediscovered in the wild and recollected (Freire et al., 2014). Having new specimens of this taxon enabled Freire et al. to reevaluate its morphology and conclude that it represents a new genus of Asteraceae, *Famatinanthus*. The authors tentatively placed the new genus in Mutisioideae tribe Onoserideae, but observed that *F. decussatus* also shares multiple characteristics with several other basal lineages of Asteraceae including Gochnatioideae, Stifftioideae and Wunderlichioideae. At the same time, *Famatinanthus* is distinctive among other genera of basal Asteraceae for its decussate, opposite leaves and solitary, broadly campanulate, radiate capitula. This combination of features makes *Famatinanthus* anomalous as a member of any tribe or subfamily of basal Asteraceae based on morphology (Fig. 1). If not found to be within a Mutisioideae clade, *Famatinanthus* could be sister to Mutisioideae or to a larger clade, and sampling it might help resolve our estimation of basal relationships. If we can resolve the basal topology of Asteraceae with confidence, *Famatinanthus* also has the potential to improve our ancestral reconstructions because of its unique combination of morphological characteristics and thus advance our understanding of the early radiation of the family in South America.

To resolve the backbone phylogeny of Asteraceae and the phylogenetic placement of *Famatinanthus* we construct a data matrix for representatives of all subfamilies of Asteraceae including a dense sampling from the basal lineages of the family. The data matrix includes eight cpDNA loci used in Panero and Funk (2008) and an additional six chloroplast genes. The resulting phylogenetic tree is used to calculate proportional likelihoods of binary character states of several morphological traits traditionally assigned to the root node of Asteraceae, including features of the capitulum, habit and pollination syndrome. All genera of Asteraceae for which bird pollination has been recorded are sampled in order to examine the likelihood of bird pollination at the root node and early radiation of Asteraceae. In addition, we test whether *Famatinanthus* has the two chloroplast DNA inversions that characterize all species of Asteraceae except those belonging to subfamily Barnadesioideae (Kim et al., 2005).

2. Materials and methods

Taxonomic sampling—a list of taxa sampled for this study with corresponding voucher information is provided in Appendix A. Included were representatives of all 12 subfamilies of Asteraceae identified by Panero and Funk (2008) and all the tribes belonging to the five basalmost subfamilies of the family. These tribes were sampled broadly to include the range of morphological characteristics diagnostic for the tribe to which they belong. *Menyanthes* (Menyanthaceae) served as outgroup.

Matrix assembly—total genomic DNA was isolated using the DNeasy® Plant Mini Kit (Qiagen Sciences, Germantown, MD, USA) from field-collected leaves preserved in silica and leaves removed from herbarium specimens. DNA fragments were amplified by using the Polymerase Chain Reaction (PCR) in 50 μl reactions as described by Panero and Crozier (2003). Sanger DNA sequencing was performed at the University of Texas DNA Sequencing Facility using an AB3730xl sequencer. We sampled about 12% of the Asteraceae chloroplast to gain enough informative characters to resolve all nodes of interest and favored coding regions in order to facilitate unambiguous alignment. Sampled loci included the genes *acc*D, *atp*B, *mat*K, *ndh*C, *ndh*D, *ndh*I, *ndh*J, *ndh*K, *ndh*F, *rbc*L, *rpo*B and *rpo*C, but also the *trn*L and *trn*F introns, and the *trn*L–*trn*F, *ndh*C–*ndh*K and *ndh*K–*ndh*J intergenic spacer regions. Summary statistics for each partition are presented in supplemental Table S1. The concatenated data matrix contained a total of 18,996 characters. The *mat*K, *ndh*D, *ndh*F, *ndh*I, *rbc*L *rpo*B genes and the *trn*L intron and *trn*L-F intergenic spacer region were amplified using the protocols of Panero and Crozier (2003) and primers as specified in Panero and Funk (2008). The *acc*D, *atp*B, *ndh*JKC, and the *rpo*C1 genes were amplified using the following primers:

The *acc*D gene was amplified in two sections using primers rbcL1581F and accD1091R (rbcL1581F: 5′-TGG AGT CCT GAA CTA CGT G-3′; accD1091R: 5′-GCG TAT TCA ATC AAA CGG-3′). The

Fig. 1. Representative species of the basal lineages of the Asteraceae. 1. Barnadesioideae: A. *Barnadesia odorata*. Famatinanthoideae: B and C. *Famatinanthus decussatus*, capitulum; habit. Mutisioideae: D. Tribe Onoserideae: *Aphyllocladus ephedroides*. E. Tribe Mutisieae: *Mutisia acuminata*. F. Tribe Nassauvieae: *Leucheria purpurea*. Wunderlichioideae: G. Tribe Hyalideae: *Hyalis argentea*. Gochnatioideae: H. *Cnicothamnus lorentzii*. Stifftioideae: I. *Hyaloseris andrade-limae*. Carduoideae: J. Tribe Dicomeae: *Macledium zeyheri*. (Photo credits. A. Lone Aagesen. B. Gloria E. Barboza. C. Juan J. Cantero. D,G,H. Fernando O. Zuloaga. E. Julian A. Greppi. F. Victor Sotelo. I. C. Aguirre. J. Jose L. Panero).

internal primer accD556R (5-′CTG GAA TTG TCA CTA CCA C-3′) was used to sequence the 5′ end of the gene. The 3′ end of the gene was amplified by using the primers accD912F and accD1481R (accD912F: 5′-TTT CAT TCG GAG GAG GAG CC-3′; accD1481R: 5′-AGC GTG GAG CTG AAA TAA C-3′). For the *acc*D gene, primers accD1091R, accD556R and accD912F were used as sequencing primers.

The *atp*B gene was amplified in two sections using primers atpEF and atpBmiddleR (atpEF: 5′-GCT GTG GCA ATA GGA GCG TGA TTT-3′; atpBmiddleR: 5′-TTG ACT GCC CTA ACT ATG GCG GAA-3′) and primers rbcL1RC and atpBmiddleF (rbcL1RC: 5′-GCT TTA GTC TCT GTT TGT GGT GAC AT-3′; atpBmiddleF: 5′-TAA CCC ACA GCG GAA GGC ATT CTA-3′). Primers atpBmiddleR and atpB-middleF were used as sequencing primers.

The *ndh*C, *ndh*J, and *ndh*K genes were amplified in two sections using primers ndhC1 and ndhK600 (ndhC1: 5′-ATG TTT CTG CTT TAC GAA TAT GA-3′; ndhK600: 5′-GAT GGC GGT TGA TAA AGG-3′) and primers ndhJend and ndhK700 (ndhJend: 5′-TCA

ATG AGC ATC TTG TAT TTC AT; ndhK700: 5′-CCT GTG GAT GTC TAT TTG CC-3′). The four primers were used as sequencing primers.

The *rpo*C1 gene was amplified in two sections using primers rpoCexon2 and either rpoBSR1 or rpoBSR2 (rpoCexon2: 5′-ATC GAC CCG TTT ACC AAG CAG AGT-3′; rpoBSR1: 5′-CGG TTG TTC GTT CGA GAA C-3′; rpoBSR2: 5′-CGA TCT TTA GCT CTG GAA CTG-3′) and primers rpoC1Exon2R and rpoC2F (rpoC1Exon2R: 5′-GAT GAG GAT AAA CTA GTG ACC TCG G-3′; rpoC2F: 5′-TTG TGA AAG ACC AGA TTG GCC CGT-3′). The four primers were used as sequencing primers.

Sequence alignments were performed with MUSCLE (Edgar, 2004) using the EMBL-EBI portal (http://www.ebi.ac.uk/Tools/msa/muscle/) followed by minor manual adjustments. Genbank accession numbers are provided in Appendix A.

Phylogenetic analyses—Bayesian analyses using MrBayes 3.2.2 (Ronquist and Huelsenbeck, 2003) and Maximum Likelihood (ML) analyses using GARLI (Zwickl, 2006) were performed in the CIPRES Science Gateway portal (http://www.phylo.org/sub_sections/portal/; Miller et al., 2010). The model of nucleotide evolution was evaluated for each locus using jModelTest 2.1 (Darriba et al., 2012) under the Akaike information criterion (Akaike, 1974). GTR + G best fit the *atp*B and *acc*D partitions and TVM + G best fit the other nine partitions. GARLI accepts a maximum of five data partitions, so for the ML analysis we modeled two data partitions by grouping chloroplast loci under the corresponding model found using jModelTest. In Bayesian analysis we used the GTR + G model for all partitions because the TVM model is similar but not recognized by Mr. Bayes 3.2.2. Posterior distributions were estimated using Markov Chain Monte Carlo (MCMC) sampling every 5000 steps for 10 million MCMC steps with two runs and four chains. The first 10% of steps of each analysis was eliminated as burn-in and the runs combined. Stationarity of the Markov chains was ascertained by plotting likelihood values in Tracer v1.5 (Rambaut and Drummond, 2007). A maximum clade credibility (MCC) tree was constructed in TreeAnnotator v1.4.8 (Drummond and Rambaut, 2007) depicting the maximum sum of posterior clade probabilities for the trees produced by the Bayesian analysis. The MCC tree was visualized in FigTree v1.4.1 (http://www.tree.bio.ed.ac.uk/software/figtree/). A majority rule tree was produced in PAUP* 4.0b10 (Swofford, 2003) from the 100 bootstrap trees file produced by GARLI. We also performed ML and Bayesian analyses as described above with *Famatinanthus* removed from the data matrix to observe the contribution of this genus in the resolution of basal lineages of Asteraceae.

Gene order assessment—to test if *Famatinanthus* has the pair of chloroplast inversions found in all Asteraceae except taxa of subfamily Barnadesioideae, we amplified DNA fragments using primers P1, P2, P3, P4, P5 and P6 as described by Kim et al. (2005); in taxa without the inversions, the combinations P1/P2, P3/P4 and P5/P6 should result in 3 DNA fragments amplified, whereas the combinations P1/P4, P5/P3 and P2/P6 should produce amplifications in species having the double inversion.

Ancestral character state reconstruction—we reconstructed ancestral character states for each of the following 10 binary traits either cited by Bremer (1994) or Funk et al. (2009) that exhibit variability among the taxa of Asteraceae: habit (herbaceous/woody), pollination syndrome (insect pollinated/bird pollinated), capitulum composition (discoid/not discoid), leaf phyllotaxy (opposite/alternate), pollen surface sculpturing (smooth-microechinate/echinate-lophate), receptacular bracts (present/absent), involucre morphology (few-seriate 1–5 series of phyllaries/multiseriate 6 or more series of phyllaries), corolla color (white, cream-colored or yellow/orange, red, pink or blue), style surface (smooth to papillose/pubescent), capitulescence (solitary/cymose). We did not reconstruct those characteristics of the ancestral sunflower cited by Funk et al. (2009) that are present in all species of the family (filaments free, five anthers and floral corolla lobes, pollen grains with three cells at time of dispersal, indeterminate capitula, inferior ovary and basal ovule, fruit an achene); we assume these originated along the stem between Calyceraceae and Asteraceae. Genera with capitula matching the bird pollination syndrome in Asteraceae of long, red or orange corollas on pendulous or long pedunculate capitula (Cronk and Ojeda, 2008), but lacking literature reports of bird visitation (e.g., *Hyaloseris* and *Stifftia*) were marked as equivocal in the analysis. Genera that include bird-pollinated species were marked as being bird-pollinated even if we did not sample the species upon which the report is based. We used a stochastic ML Markov *k*-state one-parameter model (Lewis, 2001) with equal probability for any character state change as implemented in Mesquite 2.75 (Maddison and Maddison, 2011) to model character states likelihood proportions on the highest likelihood tree found in our GARLI analysis. The farthest outgroup taxon *Menyanthes* was not considered in the reconstruction of ancestral characters.

3. Results

Our Maximum Likelihood (ML) majority rule and Bayesian maximum clade credibility trees share the same topology except for the position of the genus *Macledium* (Fig. 2). Bayesian and ML analyses placed *Famatinanthus* sister to the Mutisioideae–Asteroideae clade that contains all other species of Asteraceae except those in subfamily Barnadesioideae (Fig. 2); this was strongly supported with a posterior probability (PP) of 1.00 and 100% bootstrap (BS). *Famatinanthus* was found to have both cpDNA inversions present in all Asteraceae except in taxa of subfamily Barnadesioideae. That is, primer combinations P1/P2, P3/P4 and P5/P6 produced no amplifications, whereas primer combinations P1/P4, P5/P3 and P2/P6 produced strong amplification bands.

Bayesian posterior probabilities of 0.95–1.00 were found for relationships among the 12 subfamilies of Asteraceae, with the Wunderlichioideae–Asteroideae clade nested within the Stifftioideae–Asteroideae clade and this within the Mutisioideae–Asteroideae clade (Fig. 2). However, the relationships among subfamilies Stifftioideae, Wunderlichioideae and Gochnatioideae had only weak to moderate support in the ML analysis (Fig. 2). ML analyses using the same parameters but excluding *Famatinanthus* resulted in the identical topology with equivalent support for the Mutisioideae–Asteroideae clade but weaker support (79% BS vs. 91% BS) for the Stifftioideae–Asteroideae clade and stronger support (90% BS vs. 82% BS) for the Mutisioideae clade (Fig. S1). Bayesian analyses showed similar moderate to strong supports for the Stifftioideae–Asteroideae clade (0.95 PP if *Famatinanthus* present, 0.87 PP if *Famatinanthus* is absent, see Fig. S1). ML and Bayesian analyses show strong support (100% BS, 1.00 PP) for the sister relationship of *Schlechtendalia* to the clade containing *Dasyphyllum*, *Barnadesia* and *Huarpea*.

Most other clades were strongly supported with posterior probabilities of 1.00 (Fig. 2) except the *Mutisia–Pachylaena* clade (66% BS, 0.93 PP), *Phaneroglossa* sister to *Felicia* and *Artemisia* (69% BS, 0.97 PP). Although strongly supported in the Bayesian analysis (1.00 PP) the Mutisioideae and Wunderlichioideae clades had only weak support in the ML analysis (82% and 71% BS respectively, see Fig. 2). The relationship of Asteroideae supertribe Senecionodae, represented in our study by *Phaneroglossa*, was weakly support as sister to the clade containing *Felicia* and *Artemisia* of the Asterodae (69% BS, 0.97 PP). The monophyly of the Carduoideae was strongly supported (100% BS, 1.00 PP), but tribal relationships within the subfamily lacked support with *Macledium* (Dicomeae) sister to *Oldenburgia* (Oldenburgieae, no support in the ML analysis, 0.46 PP) and *Brachylaena* (Tarchonantheae) sister to *Macledium* and *Oldenburgia* (no support in the ML analysis, 0.43 PP; Fig. 2).

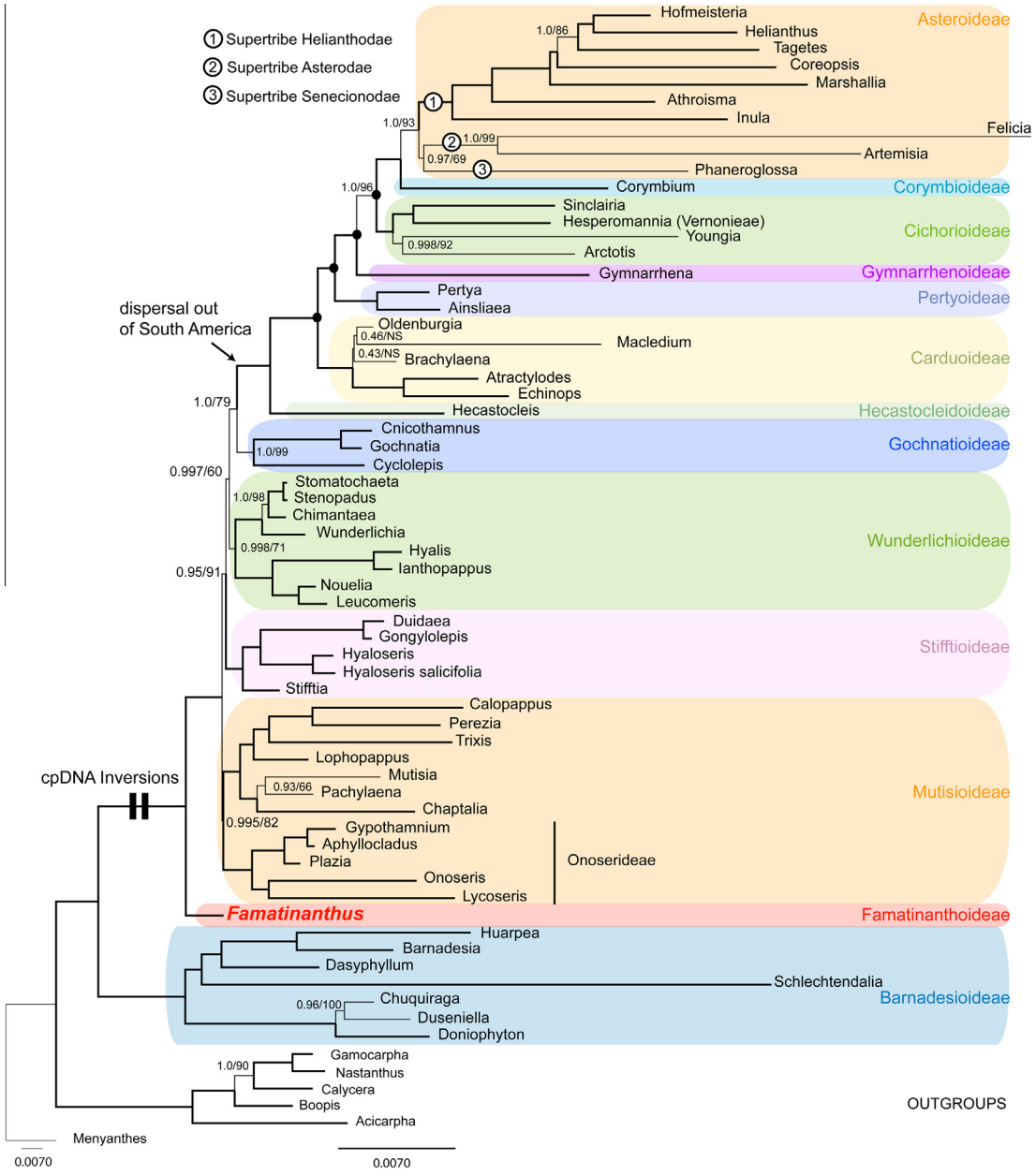

Fig. 2. Resolved backbone splits including *Famatinanthus* sister to all Asteraceae except Barnadesioideae. Maximum clade credibility tree resulting from Bayesian analysis of 14 cpDNA loci is shown with branches having 1.0 posterior probabilities and 100% ML bootstrap proportions indicated by thickened lines. All branches with less than 1.0 PP/100% BS support are labeled with corresponding values. Arrow locates the phylogenetic position of intercontinental dispersal out of South America that coincides with or precedes shifts from woody to herbaceous habit, solitary to cymose inflorescences, smooth to papillose style pubescence, and smooth to microechinate/echinate pollen (closed circles).

Proportional likelihoods for ancestral character states reconstructed at the root node of Asteraceae were: habit (woody 0.84; herbaceous 0.16), leaf phyllotaxy (alternate 0.99; opposite 0.01), capitulescence (solitary 0.98; cymose 0.02), pollen surface sculpturing (smooth or microechinate 0.996; echinate or lophate 0.004), receptacle morphology (epaleate 0.999; paleate 0.001), involucre morphology (few-seriate 0.50; multi-seriate 0.50), pollination (bird-mediated 0.01; insect-mediated 0.99), style surface (smooth or papillose 0.999; with trichomes 0.001), corolla color (white to yellow 0.92; orange, red, pink or blue 0.08), capitulum (not discoid 0.52; discoid 0.48). Capitulum and involucre morphology were quite variable across the phylogeny with most internal nodes having similar proportional likelihoods as at the root node of the family (Fig. 3).

For four characters proportional likelihoods indicated state shifts at the earliest backbone nodes following dispersal of Asteraceae from South America in ancestors assumed to have evolved on the African continent. Woody habit was reconstructed as the most likely condition at most of the nodes of the backbone, but the herbaceous state as most likely at the root node of the Cichorioideae–Asteroideae (0.899, Fig. 3) and internal nodes of the Corymbioideae and Asteroideae (Fig. 3). Similarly, microechinate or smooth pollen was reconstructed with proportional likelihoods above 0.90 for most nodes of the backbone phylogeny, but echinate pollen was reconstructed as the most likely state at the root node of the Gymnarrhenoideae–Asteroideae and other internal nodes of this clade (0.97, Fig. 3). Reconstructions resulted in smooth or papillose style as the most likely state at the Barnadesioideae–Carduoideae node and other internal nodes included therein, but pubescent styles were most likely at the Pertyoideae–Asteroideae node and nodes downstream (0.98, Fig. 3). Solitary capitula were reconstructed as the most likely condition at most of

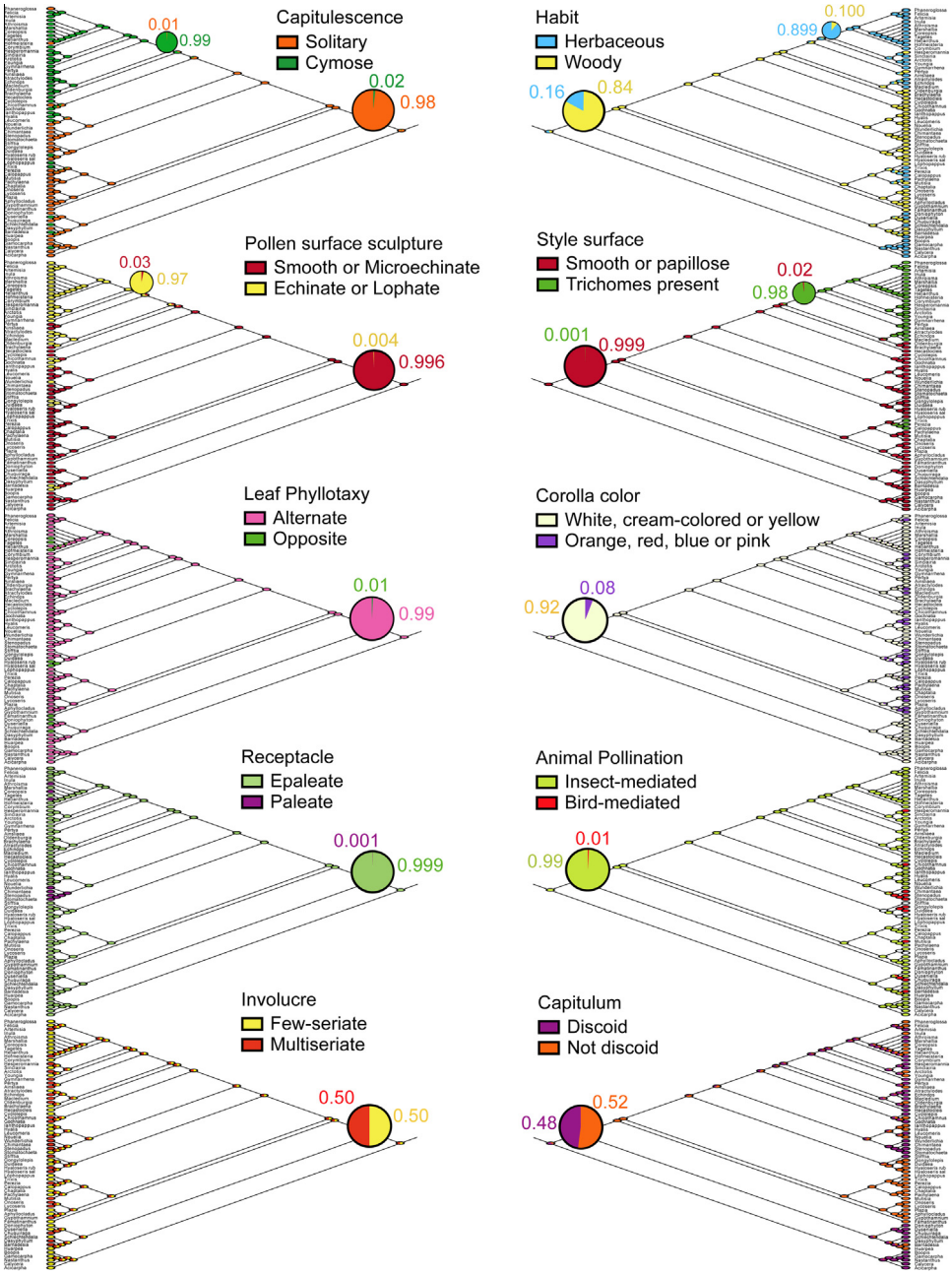

Fig. 3. Maximum Likelihood ancestral character state reconstruction for selected morphological features of Asteraceae. Proportional likelihoods for selected nodes of the phylogeny indicated at node. Maximum Likelihood reconstructions performed on ML tree under a Markov *k*-state one-parameter model. Characters lacking information are colored gray.

the nodes of the backbone, but cymose capitulescences as most likely at the root node of the Carduoideae–Asteroideae (0.99, Fig. 3) and internal nodes included therein.

The majority of internal nodes of the phylogeny were reconstructed as having corollas that were either white, creamy white or yellow, with colors such as red, orange, pink or blue found in a few members of the family reconstructed as derived (Fig. 3).

4. Discussion

4.1. Asteraceae phylogeny

These results provide the first comprehensive and fully resolved phylogenetic hypothesis of deep divergences in Asteraceae. The basal polytomy comprised of Mutisioideae, Stifftioideae and the Wunderlichioideae–Asteroideae clade and the more derived Senecionodae–Helianthodae polytomy found by Panero and Funk (2008) are solved here (Fig. 2). Not surprisingly, because our data matrix included seven of the same chloroplast loci, our phylogeny is completely congruent with that of Panero and Funk (2008) and similarly incongruent with results from a nrITS alignment by Goertzen et al. (2003). However, our phylogeny is also congruent with the phylogenetic analysis of genomic data from the nuclear compartment found by Mandel et al. (2014) with the sole exception of their placement of Vernonieae sister to the Cichorioideae–Asteroideae clade. Except for *Fulcaldea* (Barnadesioideae) and *Gerbera* (Mutisioideae) Mandel et al. did not sample any other members of basal Asteraceae. The well-resolved and statistically supported phylogeny presented here includes exemplars of all

known lineages at the subfamily and higher nodes and offers a better backbone for supertree construction of Asteraceae than any previously available.

By sampling the new genus *Famatinanthus* we recovered a node along the stem between Barnadesioideae and the rest of Asteraceae (Fig. 2) not present in any previous phylogenetic study. Narrowly-distributed monotypic *Famatinanthus* represents an early diverging lineage of Asteraceae sister to 25,000+ species that are broadly distributed globally. Such large clade imbalance in species numbers recalls *Hecastocleis*, sister to more than 24,000 species of Asteraceae (see Fig. 2), also monotypic with anomalous morphology and restricted distribution, and coincidentally also adapted to dry rocky slopes and vertical canyon walls. As with *Hecastocleis*, we lack a fossil record to know if *Famatinanthus* represents a taxon that has undergone gradual anagenic change or is the sole survivor of a larger radiation that has been losing species towards the present. Because *Famatinanthus* contains the pair of chloroplast inversions characteristic of all Asteraceae except Barnadesioideae, the origin of these mutations that Kim et al. (2005) speculates must have occurred either simultaneously or in rapid succession must be constrained to a shorter interval than previously thought (Fig. 2). The phylogenetic position of *Famatinanthus* discovered here underscores the importance of specimen reserves and wild plant conservation, and highlights the continued opportunity for discovery even where taxa are assumed to be well known.

Novel nodes recovered by our phylogenetic analyses allow more accurate placement of fossil calibrations among basal nodes in divergence time studies of Asteraceae. For example, because ecaveate echinate pollen is confined in basal Asteraceae to Stifftioideae, Wunderlichioideae, Gochnatioideae and Carduoideae (Barreda et al., 2012) the fossil echinate pollen grain *Mutisiapollis telleriae* probably represents a member of the stem group Stifftioideae–Asteroideae. The *Mutisiapollis telleriae* pollen grain, and by extension the *Raiguenrayun cura* macrofossil, can now be associated to the Mutisioideae–Asteroideae node (Fig. 2), downstream from the root node of Asteraceae where it has been placed previously (Beaulieu et al., 2013) as suggested by Barreda et al. (2012).

4.2. Ancestral character state reconstruction in Asteraceae

Five of the character states that we reconstructed for the root node of Asteraceae are in agreement with Bremer's (1994) hypothesis of a taxon that was probably woody, had white, cream-colored or yellow corollas, solitary capitula, epaleate receptacles, and alternate leaves (Fig. 3). Of these, alternate phyllotaxy, epaleate receptacles, and white, cream-colored or yellow corolla color were reconstructed at all internal nodes and are interpreted as symplesiomorphic for each of the clades shown in our phylogeny. Habit, capitulescence structure, pollen sculpturing and style morphology transitioned from root node ancestral states to alternate states for the earliest nodes of Asteraceae that evolved following dispersal to Africa (Carduoideae–Asteroideae, Fig. 3). For example, woody habit was reconstructed for most basal nodes of the family but an herbaceous habit had a significant likelihood (0.90) at the Cichorioideae–Asteroideae node and other nodes contained within this clade (Fig. 3). Solitary capitula were reconstructed for all nodes of the phylogeny subtending subfamilies that originated in South America (Barnadesioideae, Famatinanthoideae, Mutisioideae, Stifftioideae, Wunderlichioideae, and Gochnatioideae) but cymose capitulescences reconstructed with a significant likelihood (0.99) at the Carduoideae–Asteroideae and more derived nodes. A similar pattern is found in the distributions of echinate pollen and style pubescence whose proportional likelihoods were significant at the Gymnarrhenoideae–Asteroideae clade and Pertyoideae–Asteroideae clade respectively (Fig. 3). Because the Carduoideae–Asteroideae clade contains more than 90% of the species of the family, echinate pollen, herbaceous habit, pubescent styles and cymose capitulescences are derived conditions for the majority of the species of Asteraceae. These shifts appear to coincide more or less with the radiation of the family shortly after its dispersal out of South America (see Panero and Funk, 2008; Beaulieu et al., 2013). An herbaceous habit and cymose capitulescences are indicative of shorter generation times and larger seed sets respectively that may have contributed to the family's early success in colonizing large areas of the Old World.

A surprising result was the lack of significant support found for discoid capitula as the ancestral condition at the Asteraceae root node. Discoid capitula have been considered to be the ancestral condition in the family (Bremer, 1994; Jeffrey, 2007; Funk et al., 2009) because all Calyceraceae and most Barnadesioideae have only discoid capitula. Based on the phylogenetic placement of *Raiguenrayun cura* near the root node of Asteraceae, a position not supported by our phylogeny, Barreda et al. (2012) hypothesized a radiate capitulum. Nonetheless, our equivocal result (Fig. 3) does not exclude the possibility that non-discoid capitula could have been present in the ancestor. Radiate (having ray florets with outer lip expanded) would be the most probable non-discoid capitulum type to be associated with the root node ancestor because disciform types are not found and ligulate capitula are found only rarely in basal Asteraceae. Pozner et al. (2012) showed that extant Asteraceae have a capacity not present in Calyceraceae to produce a combination of cymose and racemose reduced inflorescences that presumably can lead to multiple floral type capitula. Although observations of floral primordia in Asteraceae are limited, this floral flexibility that Pozner suggests may contribute to the family's success in diversification might have been present in the ancestor of all extant Asteraceae.

Bird pollination was not supported at the root node by our data. Instead, insect-mediated pollination appears to be the ancestral condition in Asteraceae and this was reconstructed for all internal nodes of the phylogeny as well (Fig. 3). Our conclusion is consistent with that of Gruenstaeudl et al. (2009) for the root node of Barnadesioideae, and similar to other angiosperm families in which bird pollination is in a majority of cases derived from insect-mediated pollination systems (Cronk and Ojeda, 2008). Previous authors have concluded based on few pollination studies of Asteraceae that the family is mostly pollinated by solitary bees (Lane, 1996; Jeffrey, 2007). Bird pollination is rare in Asteraceae (Lane, 1996), absent in Calyceraceae, and rare in Goodeniaceae (a family sister to Calyceraceae and Asteraceae) where it is only found in a few derived species (Jabaily et al., 2012). Furthermore, the most important bird pollinators of New World plants, the hummingbirds, are estimated to have a mean crown age of about 22 My and have diverged from their sister group, northern hemisphere swifts, approximately 42 Ma (McGuire et al., 2014). Therefore, the taxon represented by the 47.5 My old fossil *Raiguenrayun cura* was probably not coeval with hummingbirds and its fossilized corolla characteristics not the result of coevolution with hummingbirds as suggested by Barreda et al. (2012).

4.3. Subfamily Famatinanthoideae and tribe Famatinantheae

Our chloroplast phylogeny does not support the inclusion of *Famatinanthus* in a monophyletic Onoserideae, Mutisioideae, or any other tribe and subfamily (Fig. 2), thus we formally recognize a new tribe and subfamily of Asteraceae. It is the second monospecific subfamily of Asteraceae after Hecastocleidoideae.

Famatinanthoideae S.E. Freire, Ariza, and Panero, subfam. nov. TYPE: *Famatinanthus* Ariza and S.E. Freire, Syst. Bot. 39, 353, 2014.

Distinctive from other subfamilies of Asteraceae by having a combination of characters including shortly petiolate, clasping, opposite leaves arranged decussately along the stem, solitary,

erect, homogamous, radiate, campanulate, few-flowered capitula, involucre of small phyllaries in three series, ray florets with bilabiate corollas, disc florets with deeply 5-lobed corollas, tailed anthers with sclerified appendages, and papillose styles.

Famatinantheae S.E. Freire, Ariza, and Panero, trib. nov. TYPE: *Famatinanthus* Ariza and S.E. Freire, Syst. Bot. 39, 353, 2014.

Tribe of Asteraceae distinctive from other tribes of the family by a combination of characters including microechinate pollen, opposite, decussate leaves with thick clasping petioles, erect, solitary, homogamous, radiate, few-flowered capitula, each with few phyllaries and broadly campanulate at anthesis, corollas light white to light yellow in color, pappus elements of different lengths, barbellate.

Long-lived shrubs 0.5–1.5 m tall, stems terete, black, with secretory cavities. Leaves opposite, petioles clasping at base, ovate, margins entire, bases connate, sparsely pubescent on both surfaces, glandular trichomes sunken, appearing as dots, eglandular trichomes erect, T-shaped. Capitula, solitary, radiate, homogamous, receptacle epaleate, involucre obovate when immature and campanulate in anthesis and fruiting, phyllaries herbaceous in 3 shallowly graduated series, flowers 10–11, corollas light white to light yellow, ray flowers fertile, corolla bilabiate, outer lip 3-toothed, inner lip deeply 2-lobed, disc flowers, fertile, corolla tubulose, 5-lobed, lobes deeply coiled, anthers 5, yellow-orange, tails pilose, apical appendages apiculate, sclerified, styles shortly bifid, yellow, lobes rounded at apices, dorsally papillose beyond point of bifurcation, stigmatic surface continuous. Pollen prolate-spheroidal, tricolporate, tectum microechinate-rugulate. Achene obovate, green, brown when mature, setuliferous, with twin hairs, pappus of multiple, dimorphic, barbellate bristles in 2–3 series, apices subplumose, tightly wrapped around corolla, spreading to patent in mature fruits.*Famatinanthus* is endemic to the Sierra del Famatina in northwest Argentina, a side arm of the Andean cordillera capped by one of the tallest mountains of South America, Cerro General Belgrano or Famatina (6250 m). Several populations are found in nearby localities at elevations ranging from 1800 to 2700 m. It grows in sparsely vegetated areas, along with other shrubs with a similar open habit including *Larrea* and *Gochnatia* (Freire et al., 2014). According to Aves Argentinas (http://www.avesargentinas.org.ar/12/noticia.php?id=494#_ftn1) the Sierra del Famatina contains an endemic daisy shrub (*Baccharis famatinensis*), two small reptiles (*Liolaemus famatinae*, *Phymaturus mallimacci*), four subspecies of birds (*Asthenes modesta serrana*, *Cinclodes fuscus riojanus*, *Upucerthia ruficauda famatinae*, *Upucerthia validirostris riojanus*), and two taxa of rodents related to chinchillas, *Abrocoma famatina* and *Lagidium viscacia famatinae*. Floristic surveys by two of us (Barboza and Cantero, in progress) add an additional 22 plant species endemic to La Rioja province and found in Sierra del Famatina belonging to families Alliaceae, Amaryllidaceae, Asteraceae, Brassicaceae, Cactaceae, Convolvulaceae, Fabaceae, Malvaceae, Pteridaceae, Violaceae to this list.*Famatinanthus* has morphological characteristics unique to basal Asteraceae and probably the family. Developing achenes are photosynthetic and tightly appressed to each other blocking access to the receptacle by herbivores. The involucre is very short and the achenes overtop the phyllaries during anthesis. During anthesis the ovaries are much larger than when they are released, as achenes, from the capitulum, broadly separating the corollas and pappus elements from each other (Fig. 1B). The pappus bristles are tightly wrapped around the corollas allowing sunlight to strike the achene shoulders and sides and are not protective of the developing achenes, whereas the pappus of Asteraceae typically serves a defensive role in protecting the developing achenes from herbivory (Stuessy and Garver, 1996). The pappus elements become increasingly patent during achene maturation to the time of eventual dispersal from the capitulum. We speculate that photosynthetic achenes probably contribute their carbohydrate production to their own development and that of the corolla nectar and pollen. This would facilitate the maintenance of such a large capitulum despite small leaf surface area for photosynthesis in this xeric species.

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Appendix A

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: *acc*D, *atp*B, *mat*K, *ndh*D, *ndh*F, *ndh*I, *ndh*JKC, *rbc*L, *rpo*B, *rpo*C1exon1, *rpo*C1exon2, *trn*L intron-*trn*L-F spacer (in some taxa 2 Genbank numbers comprise this region). NS, missing sequence.

Acicarpha spathulata R. Br., Salgado 7660, Brazil, TEX. KM192035, KM191983, EU385317, EU385220, EU385125, EU243243, KM191818, EU384939, EU385411, EU385507, KM191922, EU385031. *Ainsliaea apiculata* Sch. Bip ex. Zoll., Japan, no voucher. KM192036, KM191984, EU385321, EU385225, EU385130, EU243248, KM191819, EU384944, EU385416, EU385512, KM191923, EU385036. *Aphyllocladus spartioides* Wedd., Panero and Crozier 8500, Argentina, SI, TEX. KM192037, KM191985, EU385323, EU385227, EU385132, EU243250, KM191820, EU384946, EU385418, EU385513, KM191924, EU385038. *Arctotis hirsuta* (Harv.) P. Beauv., Panero 2002-61, Cultivated, seed source: Kirstenboch Botanical Garden, South Africa, TEX. KM192038, KM191986, EU385324, EU385228, EU385133, EU243251, KM191821, EU384947, EU385419, EU385514, KM191925, EU385039. *Artemisia frigida* Willd., NM1, China, no voucher. NC020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607, NC 020607. *Athroisma gracile* (Oliv.) Mattf. ssp. *psyllioides* (Oliv.) T. Eriksson, Eriksson, Kalema, and Leliyo 559, Tanzania, TEX. AY214947, KJ434416, AY215765, AF384437, L39455, AF383757, AY217218, AY215085, AY213763, EU385515, KM191926, AY216019/AY216144. *Atractylodes japonica* Koidz., cultivated Asiatica Nursery, no voucher, TEX. KM192039, KM191987, KM192111, KM191890, KM192100, KM191868, KM191822, KM192089, KM191879, KM191912, KM191927, KM191901. *Barnadesia odorata* Griseb., Panero and Crozier 8492, Argentina, TEX. KM192040, KM191988, EU385327, EU385231, NS, EU243254, KM191823, EU841102, EU385422, EU385518, KM191928, EU345042. *Boopis anthemoides* Juss., Simon 258, Argentina, WU. NS, NS, EU841363, NS, NS, NS, NS, EU841136, NS, NS, NS, EU841095/EU547627. *Brachylaena elliptica* (Thunb.) DC., Koekemoer and Funk 1971, South Africa, US. KM192041, KM191989, EU385330, EU385234, EU385138, EU243357, KM191824, EU384952, EU385425, EU385521, KM191929, EU385045. *Calopappus acerosus* Meyen, Panero and Crozier 8457, Chile, CONC, TEX. KM192042, KM191990, KM192112, KM191891, KM192101, KM191869, KM191825, KM192090, KM191880, KM191913,

KM191930, KM191902. *Calycera crassifolia* Hicken., Panero and Crozier 8392, Argentina, SI, TEX. KM192043, KM191991, KM192113, KM191892, KM192102, KM191870, KM191826, KM192091, KM191881, KM191914, KM191931, KM191903. *Chaptalia nutans* (L.) Pol., Panero 2002-19, USA, TEX. KM192044, KM191992, EU385335, EU385239, EU385143, EU243262, KM191827, EU384957, EU385430, EU385526, KM191932, EU385050. *Chimantaea humilis* Maguire, Steyermark & Wurdack, Weitzman et al. 412, Venezuela, US. KM192045, KM191993, EU385336, EU385240, EU385144, EU243263, KM191828, EU384958, EU385431, EU385527, KM191933, EU385051. *Chuquiraga spinosa* Less., Simon 522, Argentina, US. KM192046, KM191994, EU385338, EU385242, EU385146, EU243265, KM191829, EU384960, EU385433, EU385529, KM191934, EU385053. *Cnicothamnus lorentzii* Griseb., Panero 1934, Argentina, TENN. KM192047, KM191995, EU385339, EU385243, EU385147, EU243266, KM191830, EU384961, EU385434, EU385530, KM191935, EU385054. *Coreopsis petrophiloides* B. L. Rob. & Greenm., Panero 3047, Mexico, TEX. AY214958, KJ434420, AY215776, AF384448, AF384704, AF383768, AY217229, AY215096, AY213774, AY213774, KM191936, AY216030/AY216155. *Corymbium glabrum* L., Moffett 8764, South Africa, US. KM192048, KM191996, EU385340, EU385344, EU385148, EU243267, KM191831, EU384962, EU385435, EU385531, KM191937, EU385055. *Cyclolepis genistoides* D. Don, Panero and Crozier 8387, Argentina, SI, TEX. KM192049, KM191997, EU385341, EU385345, EU385149, EU243268, KM191832, EU384963, EU385436, EU385532, KM191938, EU385056. *Dasyphyllum reticulatum* (DC.) Cabrera, Roque, Funk and Kim 485, Brazil, US. KM192050, KM191998, EU385342, EU385246, EU385150, EU243269, KM191833, AY874428, EU385437, EU385533, KM191939, EU385057. *Doniophyton anomalum* (D. Don) Kurtz, Bonifacino 96, Argentina, US. KM192052, KM192000, EU385348, EU385252, EU385156, EU243274, KM191835, EU384969, EU385443, EU385538, KM191941, EU385063. *Duidaea pinifolia* S.F. Blake, V.A. Funk 8010, Venezuela, US. KM192053, KM192001, EU385349, EU385253, EU385157, EU243275, KM191836, EU384970, EU385444, EU385539, KM191942, EU385064. *Duseniella patagonica* (O. Hoffm.) K. Schum., Urtubey 149-2006, Argentina, LP. NS, NS, NS, NS, NS, NS, NS, NS, NS, NS, NS, EU547652. *Echinops ritro* L., Panero 2002-71. Cultivated, TEX. KM192054, KM192002, EU385350, EU385254, EU385158, EU243276, KM191837, EU384971, EU385445, EU385540, KM191943, EU385065. *Famatinanthus decussatus* Ariza and S.E. Freire, Barboza 3307, Argentina, SI. KM192055, KM192003, KM192114, KM191893, KM192103, KM191871, KM191838, KM192092, KM191882, NS, KM191944, KM191904. *Felicia heterophylla* (Cass.) Grau, Panero 2002-1. Cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. KM192056, KM192004, EU385353, EU385257, EU385161, EU243279, KM191839, EU384974, EU385448, EU385543, KM191945, EU385068. *Gamocarpha alpina* (Poepp. ex Less.) H.V. Hansen, Panero and Crozier 8414, Chile, CONC, TEX. KM192057, KM192005, KM192115, KM191894, KM192104, KM191872, KM191840, KM192093, KM191883, KM191915, KM191946, KM191905. *Gochnatia hypoleuca* (DC.) A. Gray, Panero MEX-1, Mexico, TEX. KM192058, KM192006, EU385357, EU385261, EU385165, EU243283, KM191841, EU384978, EU385452, EU385546, KM191947, EU385071. *Gongylolepis benthamiana* R. H. Schomb., Berry 6564, Venezuela, US. KM192059, NS, EU385359, EU385263, EU385167, EU243285, NS, EU384980, EU385454, EU385547, KM191948, EU385073. *Gymnarrhena micrantha* Desf., Mandeville 157, Saudi Arabia, US. KM192060, KM192007, EU385362, EU385266, EU385170, EU243288, KM191842, EU384983, EU385457, EU385550, KM191949, EU385076. *Gypothamnium pinifolium* Phil., Panero and Crozier 8437, Chile, CONC, TEX. KM192061, KM192008, KM192116, KM191895, KM192105, KM191873, KM191843, KM192094, KM191884, KM191916, KM191950, KM191906. *Hecastocleis shockleyi* A. Gray, Panero and Crozier 8157, USA, TEX. KM192062, KM192009, EU385363, EU385267, EU385171, EU243289, KM191844, EU384984, EU385458, EU385551, KM191951, EU385077. *Helianthus annuus* L., Panero 2002-27, USA, TEX. AY214986, KJ434427, AY215805, AF384476, L39383, AF383796, AY217256, AY215124, AY213802, EU385553, KM191952, AY216058/AY216183. *Hesperomannia arbuscula* Hillebr., cultivated, University of Hawaii, USA, no voucher. KM192063, KM192010, EU385364, EU385268, EU385172, EU243290, KM191845, EU384985, EU385459, EU385554, KM191953, EU385078. *Hofmeisteria fasciculata* Brandegee, Panero 2817, Mexico, TEX. AY214987, KM192011, AY215806, AF384477, AF384731, AF383797, AY217257, AY215125, AY213803, AY213803, KM191954, AY216059/AY216184. *Huarpea andina* Cabrera, Dalmasso s.n., Argentina, LP. NS, NS, EU841349, NS, NS, NS, NS, EU841124, NS, NS, NS, EU841084/EU547653. *Hyalis argentea* D. Don ex Hook. & Arn., Panero and Crozier 8388, Argentina, TEX. KM192064, KM192012, EU385367, EU385271, EU385175, EU243293, KM191846, EU384988, EU385462, EU385557, KM191955, EU385081. *Hyaloseris salicifolia* (Griseb.) Hieron., Simon 330, Argentina, US. KM192051, KM191999, EU385346, EU385250, EU385154, EU243272, KM191834, EU384967, EU385441, EU385536, KM191940, EU385061. *Hyaloseris rubicunda* Griseb., Simon 716, Argentina, US. KM192065, KM192013, EU385368, EU385272, EU385176, EU243294, KM191847, EU384989, EU385463, EU385558, KM191956, EU385082. *Ianthopappus corymbosus* (Less.) Roque & D.J.N. Hind, Panero and Crozier 8530, Argentina, SI, TEX. KM192066, KM192014, EU385369, EU385273, EU385177, EU243295, KM191848, EU384990, EU385464, EU385559, KM191957, EU385083. *Inula britannica* L., Santos and Francisco ACC55-98, Cultivated, ORT. AY214993, KJ434428, AY215812, AF384483, AF384737, AF383803, AY217263, AY215130, AY213809, AY213809, KM191958, AY216065/AY216190. *Leucomeris spectabilis* D. Don, Nicolson 3254, Nepal, US. KM192067, KM192015, EU385372, EU385276, EU385180, EU243298, KM191849, EU384993, EU385467, EU385564, KM191959, EU385086. *Lophopappus cuneatus* R.E. Fr., Panero and Crozier 8508, Argentina, SI, TEX. KM192069, KM192017, EU385374, EU385278, EU385182, EU243300, KM191851, EU384995, EU385469, EU385566, KM191961, EU385088. *Lycoseris latifolia* (D. Don) Benth., Panero 2626, Venezuela, MY. KM192070, KM192018, KM192117, KM191896, KM192106, KM191874, KM191852, KM192095, KM191885, KM191917, KM191962, KM191907. *Macledium zeyheri* (Sond.) S. Ortiz, Panero 2002-47. Cultivated, seed source: Kirstenbosch Botanical Garden, South Africa, TEX. KM192071, KM192019, EU385375, EU385280, EU385184, EU243302, KM191853, EU384997, EU385471, EU385568, KM191963, EU385090. *Marshallia caespitosa* Nutt. Ex. DC., Panero 7426, USA, TEX. AY215006, KJ434430, AY215824, AF384496, L39458, AF383816, AY217274, AY215142, AY213820, AY213820, KM191964, AY216077/AY216202. *Menyanthes trifoliata* L., Panero 2011-1, cultivated, TEX. KM192072, KM192020, KM192118, KM191897, KM192107, KM191875, KM191854, KM192096, KM191886, KM191918, KM191965, KM191908. *Mutisia retrorsa* Cav., Bonifacino 148, Argentina, US. KM192073, NS, EU385376, EU385281, EU385185, EU243303, NS, EU384998, EU385472, EU385569, NS, EU385091. *Nastanthus caespitosus* (Phil.) Reiche, Panero and Crozier 8431, Chile, CONC, TEX. KM192074, KM192021, KM192119, KM191898, KM192108, KM191876, KM191855, KM192097, KM191887, KM191919, KM191966, KM191909. *Nouelia insignis* Franch., Rock 8534, China, US. KM192075, KM192022, EU385378, EU385283, EU385187, EU243305, KM191856, EU385000, EU385474, EU385571,

KM191967, EU385093. *Oldenburgia grandis* (Thunb.) Baill., B & T World Seeds, South Africa, no voucher. KM192076, KM192023, EU385379, EU385284, EU385188, EU243306, KM191857, EU385001, EU385475, EU385572, KM191968, EU385094. *Onoseris hastata* Wedd., cultivated, Fairchild Gardens, no voucher. KM192077, KM192024, EU385381, EU385286, EU385190, EU243308, KM191858, EU385003, EU385477, EU385574, KM191969, EU385096. *Pachylaena atriplicifolia* D. Don ex H. & A., Panero and Crozier 8477A, Argentina, SI, TEX. KM192078, KM192025, EU385383, EU385288, EU385192, EU243310, KM191859, EU385005, EU385479, EU385577, KM191970, EU385098. *Perezia recurvata* Less., Panero and Crozier 8399, Argentina, SI, TEX. KM192079, KM192026, KM192120, KM191899, KM192109, KM191877, KM191860, KM192098, KM191888, KM191920, KM191971, KM191910. *Pertya scandens* Sch. Bip., Japan, no voucher. KM192080, KM192027, EU385386, EU385291, EU385195, EU243313, KM191861, EU385008, EU385482, EU385580, KM191972, EU385101. *Phaneroglossa bolusii* (Oliv.) B. Nordenstam, Watson and Panero 94-62, South Africa, TEX. AY215025, KM192028, AY215843, AF384514, AF384765, AF383835, AY217293, AY215161, AY213836, AY213836, KM191973, AY216096/AY216221. *Plazia daphnoides* Wedd., Panero and Crozier 8439, Chile, CONC, TEX. KM192081, KM192029, EU385388, EU385293, EU385197, EU243315, KM191862, EU385010, EU385484, EU385582, KM191974, EU385103. *Schlechtendalia luzulifolia* Less., Panero and Crozier 8535, Argentina, TEX, SI. KM192082, KM192030, KM192121, KM191900, KM192110, KM191878, KM191863, KM192099, KM191889, KM191921, KM191975, KM191911. *Sinclairia palmeri* (A. Gray) B.L. Turner, Panero 7457, Mexico, TEX. KM192068, KM192016, EU385373, EU385277, EU385181, EU243299, KM191850, EU384994, EU385468, EU385565, KM191960, EU385087. *Stenopadus talaumifolius* S. F. Blake, Clarke 5459, Venezuela, US. KM192083, NS, EU385398, EU385303, EU385207, EU243323, NS, EU385019, EU385494, EU385591, KM191976, EU385113. *Stifftia chrysantha* Mikan, Serra 235, Brazil, TEX. KM192084, KM192031, EU385399, EU385304, EU385208, EU243324, KM191864, EU385020, EU385495, EU385592, KM191977, EU385114. *Stomatochaeta condensata* (Baker) Maguire & Wurdack, Berry 6574B, Venezuela, US. KM192085, NS, EU385401, EU385306, EU385210, EU243326, NS, EU385021, EU385497, EU385594, KM191978, EU385115. *Tagetes erecta* L., Soule 3004, Guatemala, TEX. AY215049, KJ434436, AY215867, AF384536, L39466, AF383858, AY217315, AY215184, AY213858, AY213858, KM191979, AY216119/AY216244. *Trixis divaricata* (Kunth) Spreng., Santos 2659, Brazil, TEX. KM192086, KM192032, EU385405, EU385310, EU385214, EU243330, KM191865, EU385025, EU385501, EU385598, KM191980, EU385120. *Wunderlichia mirabilis* Riedel, Roque, Funk and Kim 466, Brazil, US. KM192087, KM192033, EU385408, EU385313, EU385217, EU243333, KM191866, EU385028, EU385504, EU385601, KM191981, EU385122. *Youngia japonica* (L.) DC., Panero 2002-92, USA, TEX. KM192088, KM192034, EU385409, EU385314, EU385218, EU243334, KM191867, EU385029, EU385505, EU385602, KM191982, EU385123.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.07.012.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Control 19, 716–723.

Anderberg, A.A., Ohlson, J.I., 2012. The genus *Cavea*, an addition to the tribe Gymnarrheneae (Asteraceae: Gymnarrhenoideae). Compos. Newslett. 50, 46–55.

Anderberg, A.A., Ghahremaninejad, F., Kallersjo, M., 2007. The enigmatic genus *Dipterocome*. Compos. Newslett. 45, 23–25.

Barreda, V.D., Palazzesi, L., Katinas, L., Crisci, J.V., Telleria, M.C., Bremer, K., Passala, M.G., Bechis, F., Corsolini, R., 2012. An extinct Eocene taxon of the daisy family (Asteraceae): evolutionary, ecological and biogeographical implications. Ann. Bot. 109, 127–134.

Beaulieu, J.M., Tank, D.C., Donoghue, M.J., 2013. A southern hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. BMC Evol. Biol. 13, 80.

Bremer, K., 1994. Asteraceae: Cladistics and Classification. Timber Press, Portland, USA.

Cabrera, A.L., 1977. Mutisieae-systematic review. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The Biology and Chemistry of the Compositae, vol. 2. Academic Press, London, pp. 1039–1066.

Cariaga, K.A., Pruski, J.F., Oviedo, R., Anderberg, A.A., Lewis, C.E., Francisco-Ortega, J., 2008. Phylogeny and systematic position of *Feddea* (Asteraceae: Feddeeae): a taxonomically enigmatic and critically endangered genus endemic to Cuba. Syst. Bot. 33, 193–202.

Cronk, Q., Ojeda, I., 2008. Bird-pollinated flowers in an evolutionary and molecular context. J. Exp. Bot. 59, 715–727.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32, 1792–1797.

Erbar, C., Leins, P., 1995. Portioned pollen release and the syndromes of secondary pollen presentation in the Campanulales–Asterales complex. Flora 190, 323–338.

Freire, S.E., Barboza, G.E., Cantero, J.J., Ariza Espinar, L., 2014. *Famatinanthus*, a new Andean genus segregated from *Aphyllocladus* (Asteraceae). Syst. Bot. 39, 349–360.

Funk, V.A., Susanna, A., Stuessy, T., Bayer, R., 2009. Systematics, Evolution, and Biogeography of the Compositae. IAPT, Vienna.

Goertzen, L.R., Cannone, J.J., Gutell, R.R., Jansen, R.K., 2003. ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny of the Asteraceae. Mol. Phylogenet. Evol. 29, 216–234.

Gruenstaeudl, M., Urtubey, E., Jansen, R.K., Samuel, R., Barfuss, M.H.J., Stuessy, T.F., 2009. Phylogeny of Barnadesioideae (Asteraceae) inferred from DNA sequence data and morphology. Mol. Phylogenet. Evol. 51, 572–587.

Jabaily, R.S., Shepherd, K.A., Gustafsson, M.H.G., Sage, L.W., Krauss, S.L., Howarth, D.G., Motley, T.J., 2012. Systematics of the Austral-Pacific family Goodeniaceae: establishing a taxonomic and evolutionary framework. Taxon 61, 419–436.

Jansen, R.K., Palmer, J.D., 1987. A chloroplast DNA inversion marks an ancient evolutionary split in the sunflower family Asteraceae. Proc. Natl. Acad. Sci. USA 84, 5818–5822.

Jeffrey, C., 2007. Compositae: introduction with key to tribes. In: Kadereit, J.W., Jeffrey, C. (Eds.), Families and Genera of Vascular Plants, Flowering Plants, Eudicots, Asterales, vol. VIII. Springer-Verlag, Berlin, pp. 61–87.

Kim, K.-J., Jansen, R.K., 1995. *Ndh*F sequence evolution and the major clades in the sunflower family. Proc. Natl. Acad. Sci. USA 92, 10379–10383.

Kim, H.G., Choi, K.S., Jansen, R.K., 2005. Two chloroplast DNA inversions originated simultaneously during the early evolution of the sunflower family (Asteraceae). Mol. Biol. Evol. 22, 1–10.

Lane, M.A., 1996. Pollination biology of compositae. In: Caligari, P.D.S., Hind, D.J.N. (Eds.), Compositae: Biology and Utilization. Proceedings of the International Compositae Conference, Kew, vol. 2, 1994. Royal Botanic Gardens, Kew, pp. 61–80.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.

Lundberg, J., 2009. Asteraceae and relationships within Asterales. In: Funk, V.A., Susanna, A., Stuessy, T., Bayer, R. (Eds.), Systematics, Evolution, and Biogeography of the Compositae. IAPT, Vienna, pp. 157–169.

Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75 "http://mesquiteproject.org" <http://mesquiteproject.org>.

Mandel, J.R., Dikow, R.B., Funk, V.A., Masalia, R.R., Staton, S.E., Kozik, A., Michelmore, R.W., Rieseberg, L.H., Burke, J.M., 2014. A target enrichment method for gathering phylogenetic information from hundreds of loci: an example from the compositae. Appl. Plant Sci. 2 (2), 1300085. http://dx.doi.org/10.3732/apps.1300085.

McGuire, J.A., Witt, C.C., Remsen Jr., J.V., Corl, A., Rabosky, D.L., Altshuler, D.L., Dudley, R., 2014. Molecular phylogenetics and the diversification of hummingbirds. Curr. Biol. 24, 910–916.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November, 2010, New Orleans, LA, pp. 1–8.

Panero, J.L., Crozier, B.S., 2003. Primers for PCR amplification of Asteraceae chloroplast DNA. Lundellia 6, 1–9.

Panero, J.L., Funk, V.A., 2008. The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47, 757–782.

Pozner, R., Zanotti, C., Johnson, L.A., 2012. Evolutionary origin of the Asteraceae capitulum: insights from Calyceraceae. Am. J. Bot. 99, 1–13.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. Available at: <http://tree.bio.ed.ac.uk/software/tracer/>.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rydin, C., Razafimandimbison, S.G., Bremer, B., 2008. Rare and enigmatic genera (*Dunnia*, *Schizocolea*, *Colletoecema*), sisters to species-rich clades: phylogeny and aspects of conservation biology in the coffee family. Mol. Phylogenet. Evol. 48, 74–83.
Stuessy, T.F., Garver, D., 1996. The defensive role of pappus in heads of Compositae. In: Caligari, P.D.S., Hind, D.J.N. (Eds.), Compositae: Biology and Utilization. Proceedings of the International Compositae Conference, Kew, vol. 2, 1994. Royal Botanic Gardens, Kew, pp. 81–91.
Swofford, D.L., 2003. PAUP*: Phylogenetic Analysis using Parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts, USA.
Torices, R., 2010. Adding time-calibrated branch lengths to the Asteraceae supertree. J. Syst. Evol. 48, 271–278.
Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, The University of Texas at Austin.