



Supplementary Information for

A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae

Jennifer R. Mandel¹, Rebecca B. Dikow², Carolina M. Siniscalchi¹, Ramhari Thapa¹, Linda E. Watson³, and Vicki A. Funk⁴

¹ Department of Biological Sciences, University of Memphis, Memphis, TN 38152, USA,
ORCID: 0000-0003-3539-2991 jmandel@memphis.edu

² Data Science Lab, Office of the Chief Information Officer, Smithsonian Institution,
Washington DC 20024, USA

³ Department of Plant Biology, Ecology, and Evolution, Oklahoma State University,
Stillwater, OK 74078-3013, USA ORCID: 0000-0001-6098-779X

⁴ Department of Botany, National Museum of Natural History, Smithsonian Institution,
Washington DC 20013-7012, USA ORCID: 0000-0002-7975-1450

Email: jmandel@memphis.edu

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Additional Methods

Taxon Sampling, Library Preparation, and Sequencing. For this study, we employed the Hyb-Seq method using Asteraceae-specific probes (baits) for target enrichment of approximately 1000 nuclear loci (1–3). We sampled a total of 256 species from 13 subfamilies, ~45 tribes, and 207 genera from across Asteraceae including one species from the sister family Calyceraceae and two from the closely related Goodeniaceae (Dataset S2). Sequence capture was performed on 238 samples using the CompCOS probes: myBaits COS Compositae/Asteraceae 1Kv1 (Arbor Biosciences). Data for 18 of the 256 species were provided by the 1000 Plant Transcriptomes (1KP; onekp.org) initiative (4–7). At the tribal level, we are missing only two small tribes consisting of 4 species total, Feddeeeae (1 species) and Polymnieae (3 species). Samples were provided by the 1KP project (onekp.org), the Compositae Genome Project, Eric Carpenter, Mark Chase, Michael Deyholos, Benoît Loeuille, Jim Leebens-Mack, Rachel Jabaily, Norbert Kilian, Marinda Koekemoer, Rowan Sage, Ed Schilling, Douglas Soltis, C. Neal Stewart Jr., and Gane Ka-Shu Wong.

For 238 species, tissues samples were either freshly collected (dried in silica gel) or obtained from herbarium material, and for some samples previously extracted DNA was provided by colleagues. DNA extraction methods followed either the CTAB protocol (8), the Qiagen DNeasy plant mini kit, or the SP Omega Biotek Plant Kit. DNA quality was assessed using a Nanodrop 2000 spectrophotometer and quantified using the Qubit 2.0 or 3.0 Fluorometer. DNA samples with 260/280 ratios below 1.5 (measured on the Nanodrop) were cleaned with Omega Biotek E.Z.N.A. Cycle Pure Kit and DNA samples that were too dilute for library preparation were concentrated by vacuum centrifugation.

Up to 1 µg of genomic DNA in 65 µL water was sonicated using either the QSonica Q800R2 or Covaris instruments, targeting a fragment size of 400–500 bp. Individually-indexed sequencing libraries were constructed using the NEBNext Ultra (original or II) DNA Library Prep Kit and their quantity was assessed using the Qubit. Libraries were pooled (four per pool, ~500 ng total). Sequence capture was performed using the CompCOS probes: myBaits COS Compositae/Asteraceae 1Kv1 (Arbor Biosciences) as

described in (1), following the manufacturer protocols. The resulting captured, pooled libraries were checked for quality using an Agilent 2100 Bioanalyzer and quantified using the Qubit. Paired-end Illumina sequencing was carried out on either the Illumina MiSeq or HiSeq 2000.

Data Processing and Alignment. Data were processed following the bioinformatic workflow and methods of (1, 2) with minor modifications detailed below (scripts and associated files available at github.com/Smithsonian/Compositae-COS-workflow). Transcriptomes were assembled *de novo* using Trinity v2.5.1 (9), with the default normalization. The resulting contig file was incorporated into the PHYLUCE pipeline with the remaining files. Reads from the Illumina sequencing were cleaned and trimmed using Trimmomatic 0.36 (10) with a sliding window of 20 bp, retained only if they had a quality score of Q20 or greater, and assembled into contigs *de novo* using the SPAdes assembler (v. 3; 11). Contigs of each taxon were analyzed in the PHYLUCE pipeline (v. 1.5.0; 12, 13), which generates orthology predictions across loci and taxa in a conservative manner, specifically, when two or more contigs from a single taxon match a targeted locus, that locus is removed for that taxon. Nucleotide alignments on individual loci were carried out in MAFFT (v. 7.029b; 14).

Phylogenomic Analyses. We generated phylogenetic trees based on a concatenated data matrix of all loci using a maximum likelihood (ML) approach with RAxML (v. 8.2.7; 15) and Bayesian estimation with ExaBayes (16). For the ML analysis, we used PartitionFinder2 (17) with the rcluster search option and AICc criterion to identify the best nucleotide substitution model for our individual loci. For the majority of loci, GTR+I+G was the best model, and we therefore applied this model to the entire concatenated matrix. These RAxML analyses included 1000 rapid bootstrap replicates using the best tree search. Bayesian estimation was carried out using ExaBayes (4 chains with 10 million generations each, GTR model, discarding 25% of the sampled trees as burnin) to create a consensus tree. In addition to the concatenated approaches, we analyzed our data using a pseudo-coalescence method as employed in the software program ASTRAL-II (hereafter ASTRAL, v4.10.2, 18) to compute a consensus “species” tree based on individual gene trees. To

prepare the data for ASTRAL, gene trees were generated based on the individual loci recovered from PHYLUCE using best models from PartitionFinder2 in RAxML with 1000 bootstrap replicates each, which were then input into ASTRAL using the default parameters.

Divergence Time Analyses. We generated a time-calibrated ML phylogeny using the program RelTime which is suitable for large datasets (19–21). Local clocks were used for each lineage and the GTR model was employed with a gamma distributed model and five discrete gamma categories. The “Use all sites” option was employed for the analyses. For calibration points (*SI Appendix*, Table S1) to constrain nodes in the ML phylogeny, we utilized either seven or eight fossils by either excluding or including a putative (and controversial) fossil pollen sample from Antarctica, as variously included in other studies (22–24). We tested different calibration scenarios for the minimum and maximum ages of the root of the family, different age constraints of a key macrofossil, and the inclusion/exclusion of some taxa on particularly long branches at the base of the tree to assess the effect of different combinations (*SI Appendix*, Table S1). The presented chronogram (also used for biogeographic and diversification rate analyses) is based upon setting a maximum age for the root of the family to 91.5 MYA, which represents the Apiales/Asterales split that was used in previous studies (22, 23). Goodeniaceae was removed as an outgroup following Kumar et al. (25) and Mello et al. (26); *Chuquiraga* and *Fulcadea* (Barnadesioideae: Asteraceae) also were excluded because their inclusion during preliminary runs resulted in unreasonably old age estimates (*SI Appendix*, Table S1).

Historical Biogeography. Ancestral ranges were estimated using ML implemented in BioGeoBEARS (27, 28) on a dated phylogeny pruned to include only one species per genus. Six models were explored including dispersal–extinction–cladogenesis (DEC), the likelihood version of DIVA (DIVALIKE), and the BayArea likelihood version of the range evolution model (BAYAREALIKE), each run with and without the j parameter which incorporates founder-events into the model and allows for one of the descendants to jump to a new range outside the ancestral range without requiring anagenetic dispersal (*SI Appendix*, Table S2). All terminals were coded as present or absent in 10 broad geographic

areas based on present day distributions of each genus following (29) including: 1) southern South America and the southern Andes region, 2) north and central Andes region, 3) Brazil including Guiana Shield, 4) southern and central Africa, 5) northern Africa-Mediterranean, 6) Europe-Eurasia, 7) Asia, 8) southeast Asia to New Guinea and Australia, 9) North America (including Hawaii and Mexico), and 10) MesoAmerica-Caribbean (*SI Appendix*, Table S5). We also carried out biogeographical stochastic mapping (BSM) to estimate the number of dispersal events between each of the defined geographic regions based on ancestral-area estimation in BioGeoBEARS (30). This resulted in the mean and standard deviations for anagenetic dispersal or range expansions ($A \rightarrow A+B$, i.e., from geographic area A expanding to geographic areas A and B), extinctions ($A+B \rightarrow A$), cladogenetic (speciation) range expansions involving sympatry ($A \rightarrow A, A$) or jump-dispersal events (founder-events) ($A \rightarrow A, B$) from 50 stochastic maps following (31).

Diversification Analyses. Diversification rates were estimated with the software MEDUSA: Modeling Evolutionary Diversification Using Stepwise Akaike Information Criterion (AIC) (32) using the dated ultrametric tree described above and pruned to the level of tribe. Species richness data detailing the number of species per tribe were taken from Funk et al. (33) (*SI Appendix*, Table S6). The mixed model of either birth-death or Yule and the corrected AIC criteria were used in the analyses.

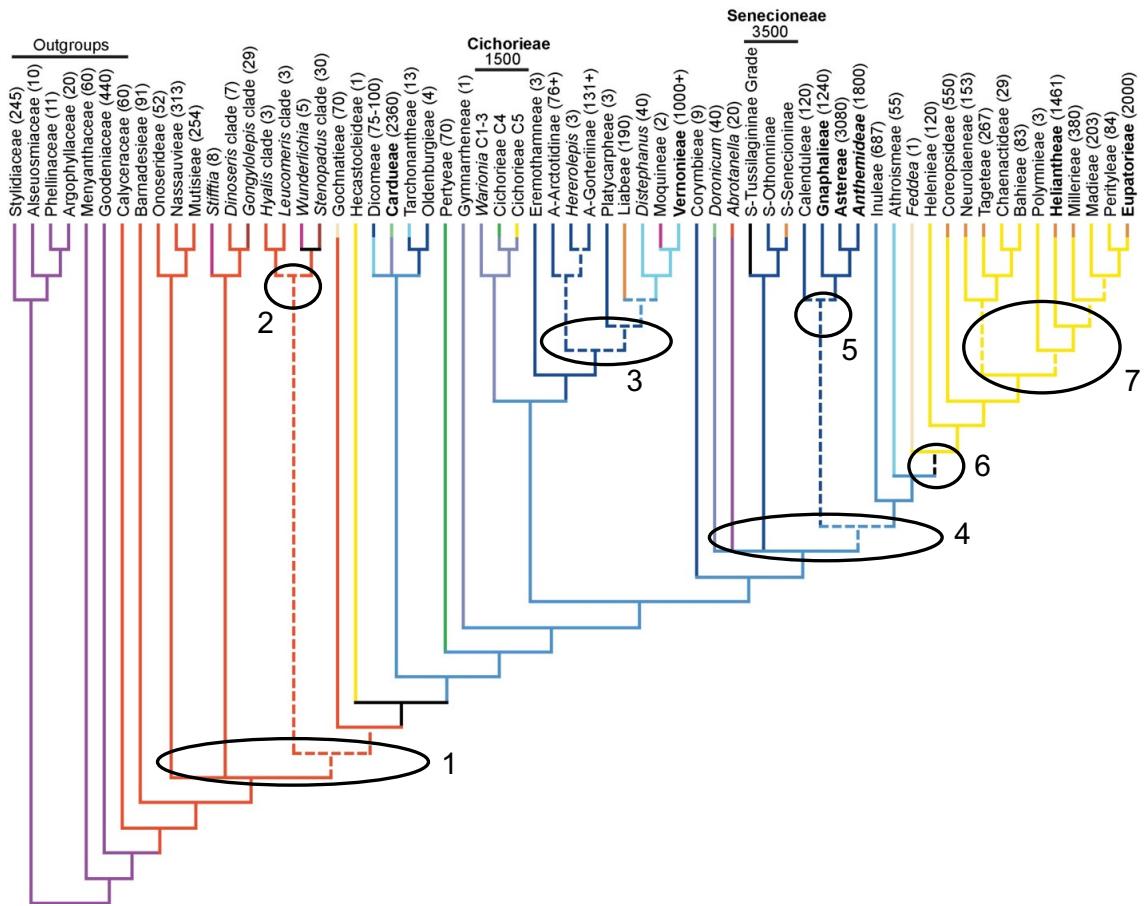


Fig. S1. Redrawn plastid supertree (metatree) of Funk et al. 2009 with unresolved nodes indicated as dashed lines; red circles indicate seven unresolved nodes discussed in Funk et al. 2009.

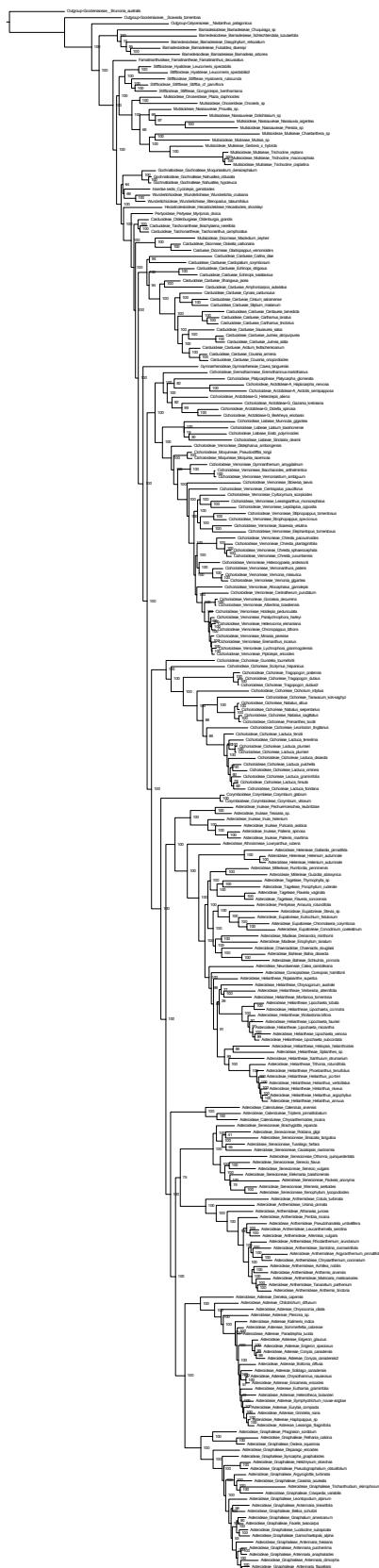


Fig. S2. Maximum likelihood tree of Asteraceae with all sampled species displayed.

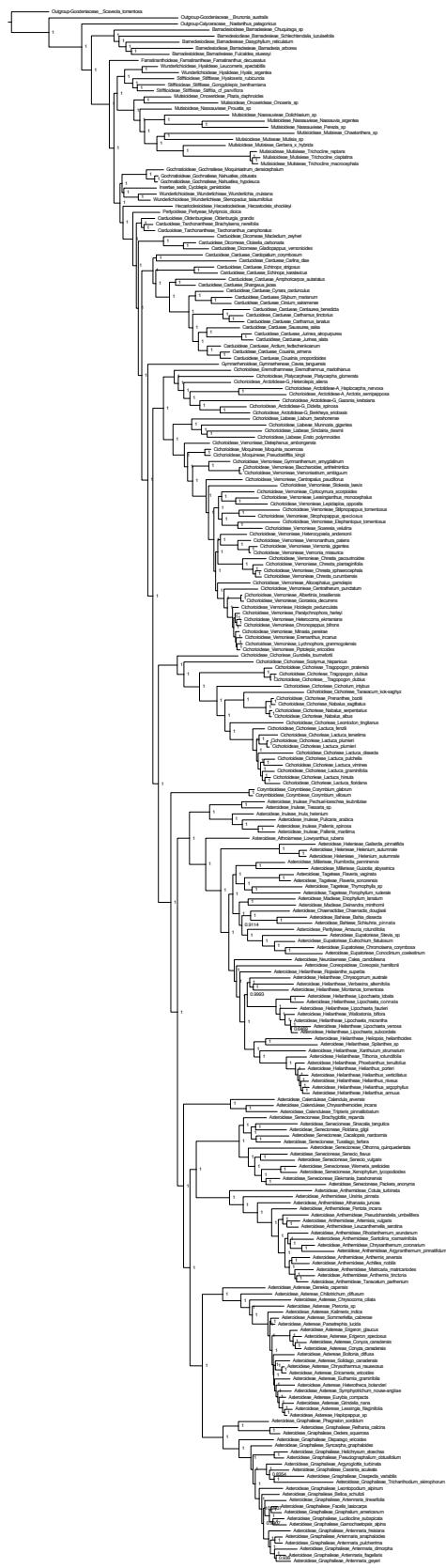


Fig. S3 Bayesian tree of Asteraceae with all sampled species displayed.

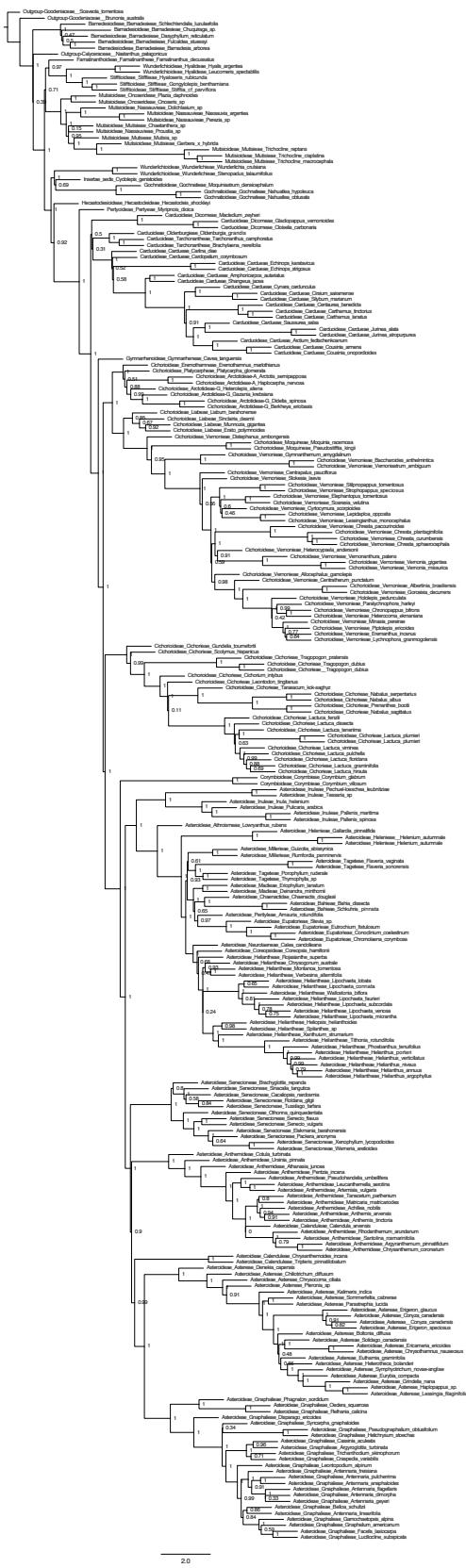


Fig. S4 ASTRAL tree of Asteraceae with all sampled species displayed.

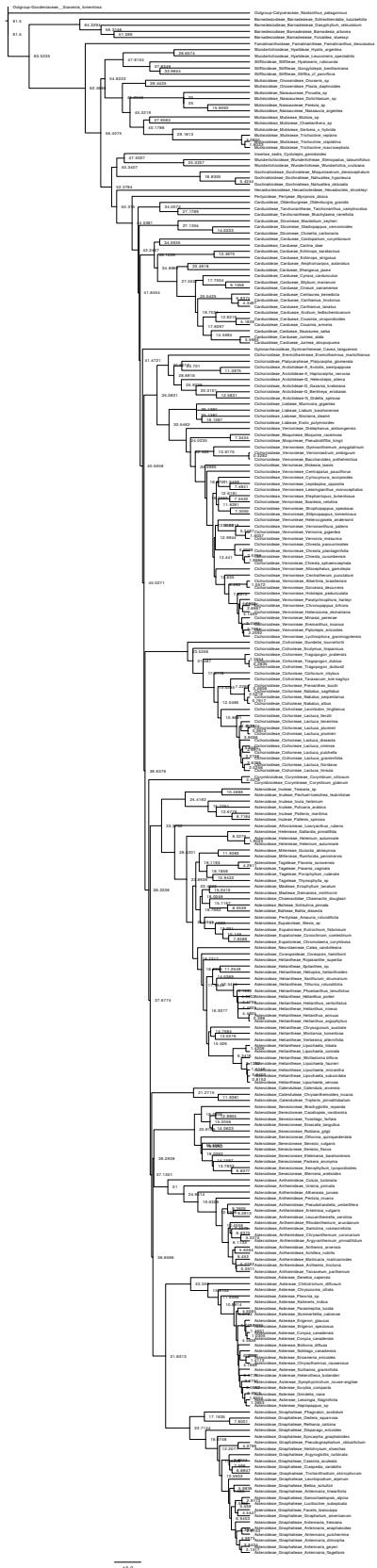


Fig. S5 Chronogram of Asteraceae with all sampled species displayed.



Fig. S6 Chronogram with confidence intervals of Asteraceae with all sampled species displayed.

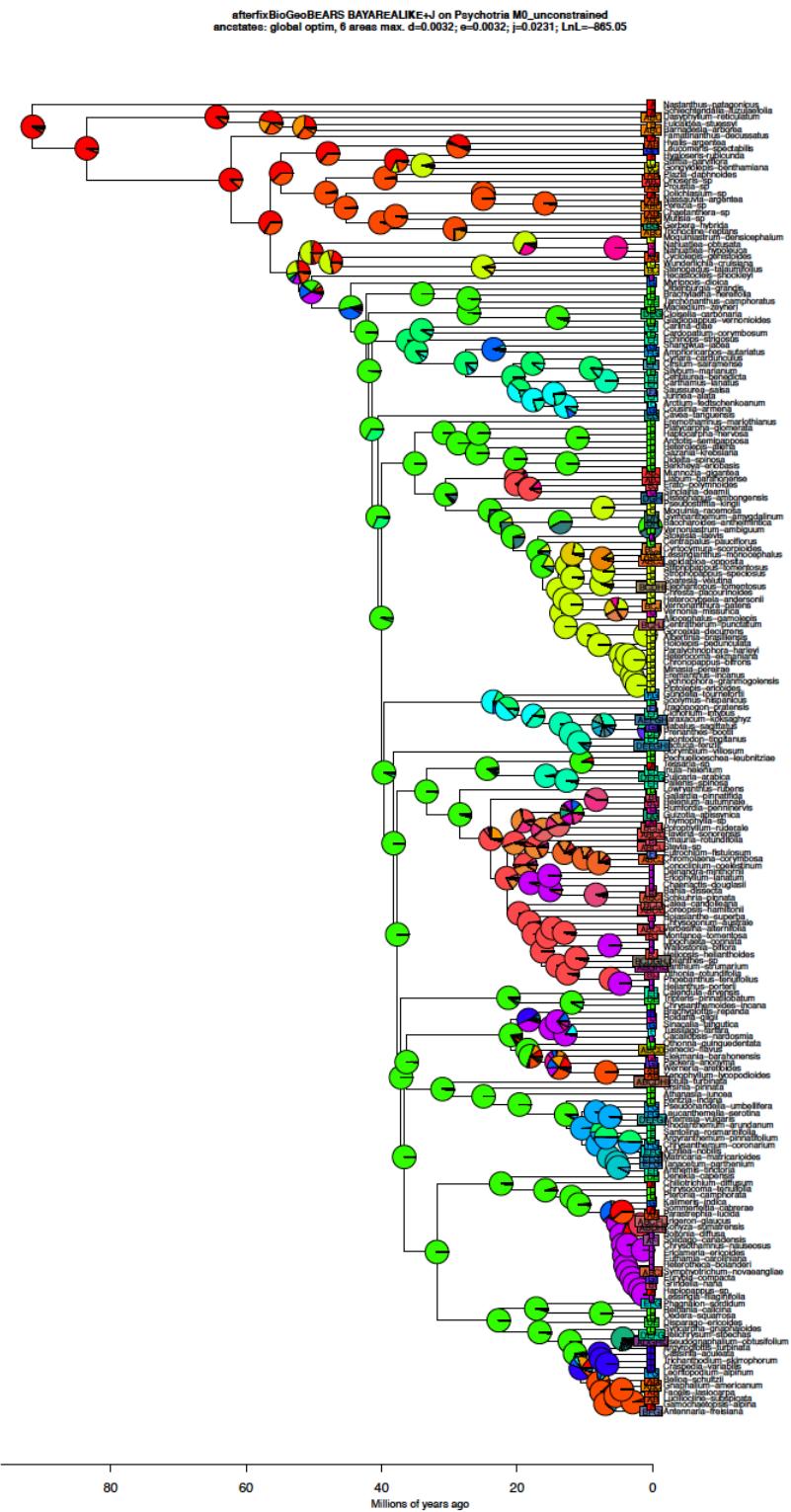


Fig. S7 Most likely ancestral range distributions of Asteraceae estimated using BAYAREA+J (raw plot from BioGeoBears).

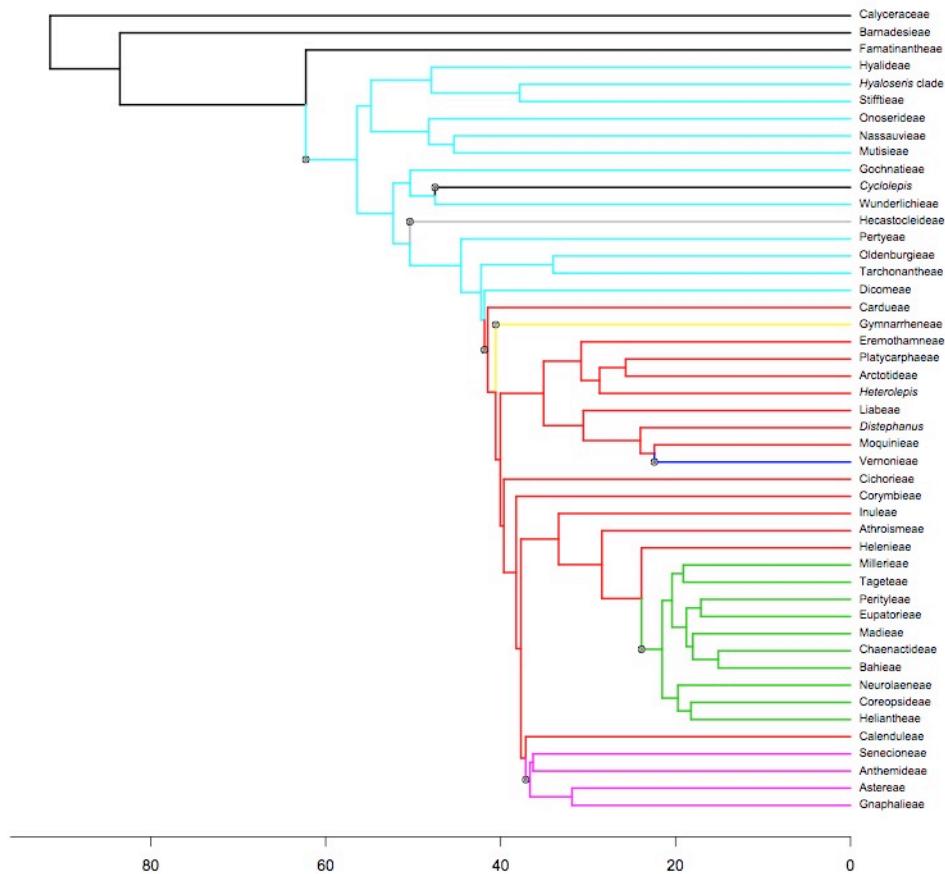


Fig. S8 Tribe-level phylogeny with diversification rate shifts as identified by MEDUSA.

Table S1. Fossil and calibration point information used for RelTime dating analyses.

Fossil name	Clade	Type	Date	Reference		
<i>Raiquenrayun cura</i> + <i>Mutisiapollis telleriae</i>	Asteroideae-Mutisiodeae clade	inflorescences/attac	47.5	34, 24, 35		
<i>Artemisia</i>	<i>Artemisia-Chrysanthemum</i> node	pollen	31	36, 37		
<i>Huanopolis crisci</i>	<i>Nassauvieae Calopappus- Nassauvia-Triptilian</i>	pollen	25.5	38, 39		
<i>Quilembaypolis gameroi</i>	Barnadesioideae	pollen	23	40, 41		
<i>Cichorium intybus</i> type	core-Cichorieae	pollen	22	42-44		
<i>Psilatricopites protrudens</i>	<i>Calyceraceae Gamacarpha-Nastanthus</i> node	pollen	22	40, 41		
<i>Sonchus oleraceus</i> type	stem <i>Sonchus</i>	pollen	5.4	43, 44		
<i>Centauria</i> type	Carduoideae	pollen	4.6	45		
<i>Tubulifloridites lillei</i> Type A	Calyceraceae-Asteraceae	pollen	72.1	24		
Different calibration scenarios						
	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	
Max Age	100	100	91.5	91.5	91.5	
Include <i>Tubulifloridites lillei</i> Type A?	no	yes	no	no	yes	
Constrain Mutiseae node, 47-60?	no	yes	no	yes	yes	
Include <i>Chuquiraga</i> ?	no	no	no	no	no	
Ages of different calibration scenarios						
	Scenario 1	Scenario 2	Scenario 3	Scenario 4*	Scenario 5	
Age Estimates						
Split or Node						
Barnadesioideae - rest of family	91.7	90.5	84.3	83.5	85.3	87.1
Mutiseae - rest of family	67.5	58.6	63.4	56.4	57	60.6
Cardueae - rest of family	46.7	42.9	44.9	41.4	41.7	43.5
Corymbium - rest of family	41.5	38.9	40.2	38.2	38.4	39.4
						1.39
Comparison to other studies						
	Barreda et al. (24)	Huang et al. (23)	Panero and Crozier (22)	This study		
Age Estimates						
Split or Node						
Barnadesioideae - rest of family	85.9	72	65	83.5		
Mutiseae - rest of family	53	62	55	56.4		
Cardueae - rest of family	48	54	49.5	41.4		
Corymbium - rest of family	-	-	44	38.2		

Table S2. Biogeographic models tested in this study with estimated parameters and values and range probabilities for all nodes from BioGeoBears analyses.

Biogeographical models tested in this study with estimated parameters and AIC values.																
alg	null	LnLall	LnLnull	DFall	DFnnull	DF	Dstatistic	pval	test	tall	AIC1	AIC2	AICwt1	AICwt2	AICweight_r	AICweight_ratio_model2
DEC+j	DEC	-909	-921.6	3	2	1	25.1	5.40E-07	chi-squared	one-tailed	1824	1847	1	9.60E-06	103661	9.60E-06
DIVALIKE+j	DIVALIKE	-918.7	-929.8	3	2	1	22.17	2.50E-06	chi-squared	one-tailed	1843	1864	1	4.20E-05	23957	4.20E-05
BAYAREALIKE+j	BAYAREALIKE	-866.4	-901.4	3	2	1	70.07	5.70F-17	chi-squared	one-tailed	1739	1807	1	1.70E-15	6.05E+14	1.70E-15
Model																
DEC	LnL	numparams	d	e	j		AICc	AICc_wt								
DEC	-921.6	2	0.0052	0.001	0		1847	3.00E-24								
DEC+j	-909	3	0.0048	1.00E-12	0.012		1824	3.00E-19								
DIVALIKE	-929.8	2	0.0058	0.001	0		1864	8.40E-28								
DIVALIKE+j	-918.7	3	0.0053	1.00E-12	0.0096		1843	1.90E-23								
BAYAREALIKE	-901.4	2	0.0035	0.035	0		1807	1.70E-15								
BAYAREALIKE+j	-866.4	3	0.0031	0.0034	0.023		1739	1								
Model																
DEC	AICc	numparams	rtips	deltaAICc	rel_likes											
DEC	1847	2	12	108	3.5326E-24											
DEC+j	1824	3	12	85	3.4873E-19											
DIVALIKE	1864	2	12	125	7.1878E-28											
DIVALIKE+j	1843	3	12	104	2.6103E-23											
BAYAREALIKE	1807	2	12	68	1.7139E-15											
BAYAREALIKE+j	1739	3	12	0	1											

Table S3. Stochastic mapping statistics and data from BioGeoBears results.

Total dispersal means (from column A to row 3)														
	A	B	C	D	E	F	G	H	I	J	Sums			
A	.	6.62	6.1	1.56	0.18	0.98	2.02	1.3	3.58	2.34	24.68	A = Southern South America		
B	7.02	.	7.32	1.48	0.16	0.96	2.1	1.98	8.12	3.86	33	B = North & Central -Andes		
C	2.86	5.64	.	1.2	0.08	0.26	0.44	1.4	3.1	3.1	18.08	C = Brazil Guiana Shield		
D	3.98	2.78	2.92	.	8.16	3.84	6.46	4.6	3.02	1.6	37.36	D = South & Central -Africa		
E	0.48	0.02	0.04	1.64	.	6.26	3.94	0.74	2.46	0.1	15.68	E = North Africa-Mediterranean		
F	0.84	0.4	0.18	1.88	6.02	.	4.52	0.98	4.52	0.22	19.56	F = Europe-Eurasia		
G	1	0.68	0.26	2.52	3.36	3.9	.	1.68	3.3	0.46	17.16	G = Asia		
H	0.6	0.92	0.72	0.72	0.1	1.46	.	1.06	0.44	6.12		H = South East Asia to Australia		
I	4.58	4.24	2.64	1.32	0.36	3.28	3.8	1.46	.	2.94	24.62	I = North America		
J	3	2.14	2.6	0.68	0.16	0.36	1.2	1.04	7.7	.	18.88	J = Meso-Carib		
Sums	24.36	23.44	22.78	13	18.58	19.94	25.94	15.18	36.86	15.06				
Anagenetic dispersal means												0		
	A	B	C	D	E	F	G	H	I	J	Sums			
A	.	5.98	4.5	0.66	0.16	0.58	0.52	0.8	2.12	1.74	17.06			
B	6.68	.	6.96	1	0.14	0.54	0.86	1.58	4.62	3.48	25.86			
C	2.48	5.24	.	0.66	0.06	0.14	0.38	1.28	1.9	2.24	14.38			
D	1.2	1.1	1.14	.	5.44	1.32	4.24	3.64	1.3	0.38	19.76			
E	0.46	0.02	0.04	1	.	5.14	2.42	0.74	2.18	0.08	12.08			
F	0.68	0.32	0.16	1.66	4.14	.	3.42	0.78	3.94	0.2	15.3			
G	0.62	0.3	0.2	1.88	2.2	3.68	.	1.48	2.82	0.26	13.44			
H	0.54	0.64	0.52	0.64	0.08	0.04	1.24	.	0.62	0.4	4.72			
I	3.1	4.08	2.34	0.8	0.34	2.24	2.68	1.08	.	2.64	19.3			
J	2.74	1.98	2.3	0.46	0.12	0.22	0.8	0.98	4.66	.	14.26			
Sums	18.5	19.66	18.16	8.76	12.68	13.9	16.56	12.36	24.16	11.42				
Founder dispersal means														
	A	B	C	D	E	F	G	H	I	J	Sums			
A	.	0.64	1.6	0.9	0.02	0.4	1.5	0.5	1.46	0.6	7.62			
B	0.34	.	0.36	0.48	0.02	0.42	1.24	0.4	3.5	0.38	7.14			
C	0.38	0.4	.	0.54	0.02	0.12	0.06	0.12	1.2	0.86	3.7			
D	2.78	1.68	1.78	.	2.72	2.52	2.22	0.96	1.72	1.22	17.6			
E	0.02	.	.	0.64	.	1.12	1.52	.	0.28	0.02	3.6			
F	0.16	0.08	0.02	0.22	1.88	.	1.1	0.2	0.58	0.02	4.26			
G	0.38	0.38	0.06	0.64	1.16	0.22	.	0.2	0.48	0.2	3.72			
H	0.06	0.28	0.2	0.08	0.02	0.06	0.22	.	0.44	0.04	1.4			
I	1.48	0.16	0.3	0.52	0.02	1.04	1.12	0.38	.	0.3	5.32			
J	0.26	0.16	0.3	0.22	0.04	0.14	0.4	0.06	3.04	.	4.62			
Sums	5.86	3.78	4.62	4.24	5.9	6.04	9.38	2.82	12.7	3.64				
	founder	a	d	e	subset	vicariance	sympatry	ALL_disp	ana_disp	all_ana	all_clado	total_events		
means	58.98	0	156.2	0	0	0	0	147	215.1	156.2	206	362.2		
std devs	2.84	0	4.81	0	0	0	0	2.84	4.72	4.81	0	4.81		
sums	2949	0	7808	0	0	0	0	7351	10757	7808	10300	18108		

Table S4. Results of MEDUSA diversification analyses.

	Model.ID	Shift.Node	Cut.At	Model	Ln.Lik.part	r	epsilon	r.low	r.high
1	1	48	node	yule	-21.1013	0.0464486	NA	0.0350422	0.0634364
2	2	57	stem	yule	-123.1464	0.171198	NA	0.1555977	0.1900465
3	3	70	stem	yule	-87.52522	0.358459	NA	0.3261214	0.398265
4	4	21	stem	yule	-8.312887	0.326149	NA	0.2600769	0.4538123
5	5	51	stem	yule	-100.8984	0.102519	NA	0.0902122	0.1174788
6	6	64	stem	yule	-41.15462	0.231712	NA	0.2062134	0.2671626
7	7	29	stem	yule	-1.386294	0.017087	NA	0	0.0805475
8	8	35	stem	yule	0	0	NA	0	0.0381142
9	9	37	stem	yule	0	0	NA	0	0.0404179

Table S5. Biogeographic areas assigned for BioGeoBears analyses

Table S6. Species Richness data for MEDUSA diversification analyses.

Richness data for MEDUSA		
Tribe	taxon	n.taxa
Eremothamneae	Eremothamnus-marlothianus	3
Chaenactidae	Chaenactis-douglasii	29
Platycarphaeae	Platycarpha-glomerata	3
Calenduleae	Calendula-arvensis	120
Calyceraceae-Outgroup	Nastanthus-patagonicus	50
Gymnarrheneae	Cavea-tangensis	2
Vernonieae	Gymnanthemum-amygdalinum	1500
Wunderlichiae	Wunderlichia-crulsiana	35
Tarchonantheae	Tarchonanthus-camphoratus	13
Oldenburgieae	Oldenburgia-grandis	4
Cardueae	Carlina-diae	2500
Perityleae	Amauria-rotundifolia	84
Moquineae	Moquinia-racemosa	2
Neurolaenaeae	Calea-candolleana	153
Hyaloseris clade	Hyaloseris-rubicunda	7
Astereae	Denekia-capensis	3080
Onoserideae	Onoseris-sp	52
incertae sedis	Cyclolepis-genistoides	1
Bahieae	Bahia-dissecta	83
Senecioneae	Brachyglottis-repanda	3500
Famatinantheae	Famatinanthus-decussatus	1
Dicomeae	Macledium-zeyheri	100
Mutisieae	Mutisia-sp	254
Gnaphalieae	Phagnalon-sordidum	1240
Hecastocleideae	Hecastocleis-shockleyi	1
Inuleae	Tessaria-sp	687
Athroismeae	Lowryanthus-rubens	55
Pertyeae	Myriophyllum-dioica	70
Madieae	Deinandra-minthornii	203
Coreopsidæae	Coreopsis-hamiltonii	550
Stifttieae	Gongylolepis-benthamania	37
Milleriaeae	Guizotia-abyssinica	380
Heliantheae	Rojasianthe-superba	1461
Helenieae	Helenium-autumnale	120
Eupatorieae	Eutrochium-fistulosum	2200
Cichorieae	Gundelia-tournefortii	1100
Heterolepis	Heterolepis-aliena	131
Nassauvieae	Proustia-sp	313
Arctotideae	Arctotis-semipapposa	76
Hyalideae	Leucomeris-spectabilis	6
Gochnatiaeae	Moquiniastrum-densicephalus	70
Anthemideae	Cotula-turbinata	1800
Tageteae	Thymophylla-sp	267
Barnadesiaeae	Schlechtendalia-luzulaefolia	91
Vernonieae-Distephanus	Distephanus-ambongensis	40
Liabaeae	Liabum-barahonense	174
Corymbiaeae	Corymbium-villosum	9

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