TITLE

Macroevolutionary dynamics in the early diversification of Asteraceae

ABSTRACT

Spatial and temporal differences in ecological opportunity can result in disparity of net species diversification rates and consequently uneven distribution of taxon richness across the tree of life. The largest eudicotyledonous plant family Asteraceae has a global distribution and at least 460 times more species than its South American endemic sister family Calyceraceae. In this study, diversification rate dynamics across Asteraceae are examined in light of the several hypothesized causes for the family's evolutionary success that could be responsible for rate change. The innovations of racemose capitulum and pappus, and a whole genome duplication event occurred near the origin of the family, yet we found the basal lineages of Asteraceae that evolved in South America share background diversification rates with Calyceraceae and their Australasian sister Goodeniaceae. Instead we found diversification rates increased gradually from the origin of Asteraceae approximately 69.5 Ma in the late Cretaceous through the Early Eocene Climatic Optimum at least. In contrast to earlier studies, significant rate shifts were not strongly correlated with intercontinental dispersals or polyploidization. The difference is due primarily to sampling more backbone nodes, as well as calibrations placed internally in Asteraceae that resulted in earlier divergence times than those found in most previous relaxed clock studies. Two clades identified as having transformed rate processes are the Vernonioid Clade and a clade within the Heliantheae alliance characterized by phytomelanic fruit (PF Clade) that represents an American radiation. In Africa, subfamilies Carduoideae, Pertyoideae, Gymnarrhenoideae, Cichorioideae, Corymbioideae, and Asteroideae diverged in a relatively short span of only 6.5 million years during the Middle Eocene.

KEY WORDS: Adaptive radiation, Cretaceous-Tertiary extinction, diversification rate, global biodiversity, key innovation, polyploidy.

1. Introduction

A fundamental and intriguing open question about biodiversity is why some lineages proliferate many species and others do not. Observations of disparity in taxon richness, ubiquitous across all branches of life (Harmon 2012), underpin the study of mechanisms that promote species diversification. Only two eudicot plant families, Asteraceae and Fabaceae, and one monocot family, Orchidaceae, have more than 19,500 species each. The five next largest families contain between 5,000-13,200 species, but more than 400 flowering plant families have less than 5,000 species each. Asteraceae is counted as the largest eudicotyledonous and second largest angiosperm family with nearly 23,600 species (Stevens, 2001 onwards) representing more than 9% of angiosperms. Yet Asteraceae's sister clade Calyceraceae has only 54 species (Zavala-Gallo et al., 2010). Subfamily-level differences in diversity and multiple instances of sister-clade imbalance are apparent across the reconstructed Asteraceae phylogeny backbone (Panero et al., 2014). As in several other eukaryotes (Rabosky et al 2012), the oldest lineages of Asteraceae are not the most diverse; the more species-rich clades of Asteraceae originated later in the evolution of the family. Understanding the processes that result in asymmetrical diversification is central to evolutionary biology (Heard and Hauser, 1995; Phillimore et al., 2006; Wiens, 2011; Rabosky et al., 2012) yet the tempo of divergence is poorly known for many important groups including most families of flowering plants.

Macroevolutionary rates are not constant across the tree of life (Alfaro et al., 2009; Purvis et al., 2011; Smith et al., 2011) and rate variation is thought to reflect differences in ecological opportunity in space and time (Glor 2010; Yoder et al., 2010) or variation in lineage-specific traits that directly affects speciation rate (Coyne and Orr, 2004; Wagner et al. 2012). Increases in net diversification rate, that is the excess of speciation rate above extinction rate, can be driven by the evolution of key innovations, extinction of antagonists or dispersal to new habitats (Simpson 1953) resulting in greater ecological opportunity (Matschiner et al., 2011; Etienne and Haegeman, 2012). Key innovations that facilitate the use of new or underutilized resources in the absence of competing organisms may result in adaptive radiations (Simpson 1953; Heard and Hauser, 1995). Key innovations have been hypothesized for plants and animals (Farrell, 1991; Hodges and Arnold, 1995; Losos, 2009; Salzburger, 2009; Matschiner et al., 2011; Rainford et al., 2014) but it has proven difficult to find such key single traits in most groups despite wide interest to do so (Glor, 2010). Independent of key innovation, colonization of new geographical areas can also result in ecological opportunity (Heard and Hauser, 1995, Antonelli and Sanmartin, 2011; Vamosi and Vamosi, 2011; Wiens, 2011; Gillespie et al., 2012). Though best documented in islands (Gillespie, 2004; Grant and Grant 2008; Lerner et al., 2011; Garcia-Verdugo et al., 2014) there are also examples of continental radiations (Moore and Donoghue 2007; Schenk et al., 2013). Whole genome duplications have also been invoked to explain increases in diversification rates, but most studies show that crown group radiations immediately downstream from genome duplications are probably the result of ecological opportunity (i.e., resources rarely used by competing taxa, Schluter, 2000) rather than whole genome duplications alone (Schranz et al., 2012). Decreasing net rates of diversification are often attributed to niche space competition and usually attributed to a decrease in ecological opportunity towards the present (Levinton, 1979; Schluter, 2000; Ricklefs, 2010; Etienne and Haegeman, 2012, Rabosky and Hurlbert, 2015), but empirical studies have rarely shown niche space to be completely filled (Cornell, 2013; Harmon and Harrison, 2015). Biotic or abiotic environmental change, as well as incomplete species level sampling, especially of incipient or cryptic species, could be responsible for this pattern instead (Purvis et al., 2009; Moen and Morlon, 2014). Net differences in diversification rate are manifested in the uneven distribution of species richness (Wiens, 2011).

Classic explanations for the megadiversity of Asteraceae generally invoke adaptive radiation with one or more morphological, cellular, or physiological characteristics presumed to have allowed sunflowers to invade new adaptive zones. The condensed compound inflorescence characteristic of all Asteraceae, the capitulum, is widely regarded by plant systematists as key to the evolutionary success of the Asteraceae (Cronquist, 1977; Jeffrey, 2009). Capitula or capitula-like inflorescences are present in a few other families of flowering plants (eg. Apiaceae, Calyceraceae, Dipsacaceae, Goodeniaceae, Proteaceae, etc.) but are not as diverse as in Asteraceae and do not exhibit the integration of floral function typical of Asteraceae. For example, the peripheral corollas of Asteraceae capitula are the first to open and do not wither until all disc florets are past anthesis (Weberling, 1989). The capitulum of Asteraceae is a compound structure in which the outer bracts produce a cup-shaped involucre that protects all the flowers of the inflorescence from herbivory and the modified calyx of each flower, the pappus, further protects each flower by encircling it with bristles. The pappus also aids in dispersal. This dual functionality advantageous in escaping herbivory and dispersal has been viewed by some as another reason for the success of the family (Stuessy and Garver, 1996). Because of the tight arrangement of flowers in the capitulum and the differential developmental origin of the peripheral versus the central flowers (Pozner, et al., 2012), there are multiple combinations of floral gender and corolla shapes in the capitula of Asteraceae. Combinations diagnostic of large taxonomic groups may be adaptive but this has not been studied. A large array of bitter and toxic compounds produced by Asteraceae are known to confer chemical defense against herbivory, and the secondary chemistry of the Asteraceae has been viewed as another reason for the success of

the family (Cronquist 1977; Harborne et al., 1977; Jeffrey, 2007). The majority of these compounds are found in subfamily Asteroideae (Calabria et al., 2009) that diverged relatively late in the history of the family. The presence of water-soluble polymers of fructose (fructans and inulins) in Asteraceae is associated with the ability to withstand lower temperatures and drought (Livingston et al., 2009; Krasensky and Jonak, 2012) and proposed to explain the success of the family in seasonally dry and colder environments (Hendry, 1996). However, fructans are also present in closely related families Calyceraceae, Goodeniaceae, Menyanthaceae that have not radiated as extensively as sunflowers.

Ecological opportunity may also be precipitated by gene redundancy as a result of whole genome duplication (WGD) or polyploidy (Stephens, 1951; Wendel, 2000; Otto 2007; Parisod et al., 2010; te Beest et al., 2012) and post-polyploidy genome reduction processes termed *diploidization* (Ohno 1970; Werth and Windham, 1991; Leitch and Bennett, 2004; Edger and Pires, 2009). Polyploidy is widespread in eukaryotes and especially significant in angiosperm genomes where it has been noted in several large clades (Blanc and Wolfe, 2004; Cui et al., 2006; Paterson et al., 2004; Barker et al., 2008, Barker et al., 2009; Tang et al., 2010, Sato et al., 2012). Phylogenetically-nested genome duplications have been found early in the evolution of seed plants and again in angiosperms (Jiao, 2011) and eudicots (Jaillon et al., 2007) including Asteraceae. Within Asteraceae, the genome duplication shared by samples from subfamilies Carduoideae, Cichorioideae, Mutisioideae and Asteroideae is inferred near the origin of the family (Barker et al., 2008) and another duplication along the branch leading to the Heliantheae alliance crown node is inferred based on chromosome numbers (Baldwin et al., 2002; Semple and Watanabe, 2009) and gene duplications in *Helianthus* (Barker et al., 2008). A causal link between polyploidy and high diversification in Asteraceae has been suggested (Schranz et al., 2012; Tank et al., 2015). However, even though 2-4% of angiosperm speciation events are thought to involve polyploidy (Otto and Whitton, 2000), studies of diverse groups have not shown a tight correspondence of polyploidization and diversification (Wood et al., 2009; Mayrose et al., 2011; Arrigo and Barker, 2012). Nonetheless, polyploidy is a source of genetic novelty that can provide new pathways to evolutionary success through subfunctionalization and neofunctionalization arising from divergent resolution of gene paralogs or alterations to gene expression (Comai, 2005, Otto, 2007; Edger and Pires, 2009). For example, in *Helianthus* and Asteraceae of some other tribes, multiple CYCLOIDEA-like paralogs have been independently co-opted to specify larger zygomorphic corollas that might be advantageous in attracting pollinators (Chapman et al., 2012), but it is not known whether this change in floral morphology is correlated with higher rates of diversification.

Asteraceae's seed dispersal apparatus, the pappus, is thought to have contributed to its rapid radiation by facilitating transoceanic dispersal (Carlquist, 1976). Fossils record the family's global expansion from its presumed origin in South America. The earliest pollen unequivocally identified as Asteraceae is from Paleocene to mid Eocene of South America (Barreda et al., 2012a; Macphail et al., 2013), the early Eocene of Antarctica (Pross et al., 2012; Contreras et al., 2013), and the Eocene or Paleocene of southern Africa (Zavada and de Villiers, 2000; de Villiers and Cadman, 2001; Scott et al., 2006; Zavada and Lowrey, 2010). Asteraceae pollen becomes increasingly common and abundant from the Miocene to the present on all continents where Asteraceae is extant (Germeraad et al., 1968; Raven and Axelrod, 1974; Turner, 1977; Muller, 1981; Graham 1996). The increase of Asteraceae pollen in strata of the lower Miocene of tropical regions across the world is associated to the dispersal ability of Asteraceae seeds (Germeraad et al., 1968). The pappus evolved early in the evolution of the family, before the crown radiation. However, if traits that facilitate long distance dispersal promoted allopatric speciation, we would expect that increased diversification rate would follow major intercontinental dispersals of Asteraceae.

Purported causes of great species richness in Asteraceae have not yet been considered in light of a robust phylogeny that includes most major lineages of the family. Two previous studies that employed the constant rate birth-death model of Alfaro et al. (2009) to identify the location of rate shifts sampled very few Asteraceae. In a broad study of angiosperms, Tank et al. (2015) used the time-calibrated phylogeny of Zanne et al. (2014) but collapsed the Asteraceae clade assigning all species richness to a single familial exemplar. In that study, the rate increase found along the basal stem leading to this collapsed clade is associated to the earliest genome duplication in Asteraceae. Beaulieu and Donoghue (2013) drew trees from a previous divergence time study based on the taxon sample of Tank and Donoghue (2010) whose 9 Asteraceae are the same sampled by Zanne et al. Their assignment of species richness to terminals in the phylogeny is not explicit, and the resulting two rate shifts in Asteraceae are depicted on a phylogenetic tree of Campanulids that includes a tremendously larger taxon sample of Asteraceae. The largest shift, reported on the branch leading to the *Out-of South America* clade sensu Panero and Funk (2008), is associated to the origin and radiation of that clade. Since neither *Hecastocleis* nor any member of Gochnatioideae, Stiffioideae or Wunderlichioideae were sampled, we can't know whether this shift is actually synchronous with the dispersal of Asteraceae out of South America or well before, nearer the divergence of *Gerbera* (Mutisioideae). The smaller shift appears to be earlier but not to coincide with the location of the shift in Tank et al. A more focused study of Asteraceae rate dynamics is needed with expanded taxon sampling that allows for more distributed assignment of species richness data to terminal nodes.

Here we sampled all major clades of Asteraceae sensu Panero et al. (2014) to reconstruct evolutionary rates and explore in more detail how changes in rate process correlate with changes in genotype, phenotype, intercontinental dispersals, and major geoclimatic historical events that have been associated with divergences of major taxonomic groups. To do this we constructed and time-calibrated a cpDNA phylogeny using a Bayesian relaxed-clock method employing nine fossil and geological calibrations and a birth-death branching process prior. Then we accounted for unsampled species by tallying and assigning species richness sums to 65 (58 Asteraceae) terminals. In order to ascertain whether and how many discrete shifts in rate process could best explain our tree, and identify the locations of rate shifts, we used Bayesian mixture modeling and inference outlined by Rabosky (2014) that simultaneously allows rate regimes to vary through time and across regions of the tree topology. Comparing our results with the location of events along the backbone divergences of Asteraceae that have been proposed as drivers of macroevolutionary change provides a more nuanced view of sunflower evolution than past, more broadly focused studies offer.

2. Materials and methods

2.1. Taxon Sampling

The 94-taxon data matrix contains 85 Asteraceae species sampled from the 13 subfamilies of the family. This sample includes the basalmost divergences of each subfamily and all taxa associated with pollen fossils used in calibrating the divergence time analysis (see below). Sampling of outgroups was informed by a previous study of Campanulids (Beaulieu et al., 2013) and included nine taxa in families Apiaceae, Araliaceae, Calyceraceae, Campanulaceae, Goodeniaceae, and Menyanthaceae.

Asteraceae species richness was tallied using taxonomic and phylogenetic studies of the family (Anderberg et al., 2007; Panero et al., 2014) supplemented by species described since 2007 that are catalogued in the taxonomic index IPNI (International Plant Names Index, see <http://ipni.org>, last accessed November, 2014) to produce a list of all the genera in the family, the number of species in each genus and their continental distribution. Total species richness for tribe Cichorieae was obtained from the International Cichorieae Network (<http://wp6-cichorieae.e-taxonomy.eu/blog/home>). Species richness for tribes Astereae, Anthemideae, Cardueae, Cichorieae, Gnaphalieae and Vernonieae was difficult to ascertain because some of their genera lack revisions or include asexual species in their counts. We chose not to include agamospermous species of *Hieracium* in the total number of species of Cichorioideae. We used a seven continent division of the planet's landmasses (<https://en.wikipedia.org/wiki/Continent>) with a Pacific region including islands far from continental regions substituting for Antarctica. Antarctica has no native species of Asteraceae but two introduced species native to southern South America have been recorded there in the past (Lewis Smith and Richardson, 2011).

2.2. Character sampling and Sequencing

We sequenced 11 chloroplast genes and one noncoding region for all ingroup species included in the study, of which 7 loci for 55 of the sampled species were published in an earlier study (Panero & Funk, 2008). Outgroup taxa sequences were obtained from Genbank (Appendix A). The 11 genes included *atpB* (1581 sites), *matK* (1612 sites), the three proximal *ndh* exons J, K and C and the short intergenic spacers between these three genes (1763 sites), *ndhD* (1516 sites), *ndhF* (2257 sites), *ndhI* (507 sites), *rbcL* (1404 sites), *rpoB* (3178 sites), *rpoC1* (2271 sites) and the *trnL* intron and *trnL-trnF* spacer region (1229 sites). Asteraceae taxonomy followed Anderberg et al., 2007. Our data matrix is freely available through Dryad (<http://dx.doi.org/>)

Total DNA was from herbarium and wild collected specimens preserved in silica gel or frozen in liquid nitrogen in the field isolated using the CTAB extraction method (Doyle and Doyle, 1987). DNA was further cleaned either by using high-speed centrifugation (65K revolutions/min) with DNA extract suspended in a CsCl gradient (1g CsCl/ml) or phenol-chloroform-isoamyl alcohol (25-24-1) solution followed by two 70% ETOH washes. DNA was also isolated from silica samples and herbarium specimens using the DNeasy Plant Kit (QIAGEN, Hilden, Germany).

Polymerase chain reactions were performed with 2 units of Taq polymerase, 0.2 M Tris-HCl, 8 mM (NH₄)₂SO₄, 0.2 mM dNTPs, 5 mM MgCl₂, 20 µM of primers, target DNA and water to a volume of 50µl. PCR amplification protocols for all markers included the following steps: 95 °C for 4 min, 50 °C for 45 s, 72 °C for 1 min followed by 36 cycles of 95 °C for 1 min, 50 °C for 45 s, 72 °C for 1 min with a 2 s extension per cycle and a final extension of 72 °C for 8 min (Lopes Rivera et al., 2016). Primer sequences and combinations for amplification of *atpB*, *ndhJKC* and *rpoC1* are included in Panero et al. (2014), for *matK*, *ndhD*, *ndhF*, *ndhI*, *rbcL*, *rpoB* in Panero and Crozier (2003) and Panero and Funk (2008) and for the *trnL* intron and *trnL-trnF* spacer region in Taberlet (1991). PCRs were cleaned with the QIAquick PCR purification kit (QIAGEN) following manufacturers specifications. Cleaned PCR products were sequenced at the University of Texas Sequencing facility using an ABI 3730 xl sequencer.

Sequences were edited using Sequencher v 4.9 (Gene Codes Corporation, Ann Arbor, USA) and assembled into primer-based matrices. Matrices were exported as Nexus interleaved files and concatenated in Paup* (Swofford, 2003) then imported into Mesquite (Maddison and Maddison, 2015) to produce phylip and nbrf files. Alignment of the nbrf files was performed at

the European Bioinformatics Institute web site (<http://www.ebi.ac.uk/Tools/msa/mafft>) using the program MAFFT (Kato and Standley, 2013). The aligned matrices were imported into Mesquite, edited and subsequently exported as simplified Nexus interleaved files and concatenated in Paup*. The concatenated matrix of 17,319 sites was exported as a simplified Nexus sequential file to use in divergence time analyses.

2.3. Phylogenetic and Divergence Time Inference

For the combined data set, the model of molecular evolution was evaluated under the Akaike Information Criteria (AIC) using the program jModeltest 2 (Guindon and Gascuel, 2003; Darriba et al., 2012). The GTR + I + G model had the highest likelihood and was used for the Maximum Likelihood (ML) analysis. The ML phylogenetic analysis was performed using the RAXML-HP BlackBox (7.7.1; <http://embnet.vital-it.ch/raxml-bb/index.php>) portal using rapid bootstrap (Stamatakis, et al., 2008).

BEAST v1.8.0 (Drummond et al., 2012) was used to infer the phylogeny and divergence times for taxa in Asteraceae and six closely related families. The analysis was performed at the CIPRES Science Gateway (<http://www.phylo.org/>, Miller et al., 2015) assuming a Bayesian relaxed molecular clock. Molecular rates were allowed to vary among lineages around an average value, by enforcing an uncorrelated lognormal clock of evolutionary rates. A birth-death branch process prior was used in the analysis. A general time reversible model with a gamma distributed substitution rates among sites (Lanave et al., 1984; Tavaré, 1986) allowing four single-nucleotide substitution categories was applied to the concatenated data matrix.

After reviewing the fossil and systematic literature of Asterales, nine absolute time constraints were chosen that could be assigned with confidence to specific nodes of the phylogeny:

1. An external calibration for the root node between Asterales and Apiales was set as the average of the mean ages of confidence intervals on this node found in previous studies (Magallón and Castillo, 2009; Smith et al., 2010; Beaulieu et al., 2013) and a lognormal prior implemented with an offset of 91.5 Ma and a mean of 1.0 and standard deviation of 0.7.
2. The oldest fossil pollen attributable to Calyceraceae and dated to approximately 21-22 Ma, *Psilatricolporites protrudens*, (Barreda et al., 2010; Palazzesi et al., 2010) was used to model a lognormal prior with an offset of 22 Ma and mean and standard deviation of 1.3 and to constrain the *Gamocarpha-Nastanthus* node.
3. Fossil pollen associated to extant members of Asteraceae subfamily Barnadesioideae and dated to approximately 23 Ma, *Quilembaypollis gamerroi*, (Barreda et al., 2010; Palazzesi et al., 2009) was used to model a lognormal prior with an offset of 23 Ma and mean of 1.0 and a standard deviation of 1.9 to constrain the root node of *Barnadesioideae*.
4. Fossil pollen associated to extant members of Asteraceae tribe Nassauvieae and dated to approximately 25.5 Ma, *Huanilipollis criscii*, (Barreda et al., 2008; Heredia et al., 2012) was used to model a lognormal prior with an offset of 25.5 Ma and a mean and standard deviation of 0.2 to constrain the node containing *Calopappus*, *Nassauvia* and *Triptilion*.
5. We followed Panero et al. (2014) to constrain the Mutisioideae-Asteroideae clade using the fossil pollen *Mutisiapollis telleriae* co-fossilized with the macrofossil *Raiguenrayn cura* and dated to 47.5 Ma (Barreda et al., 2012a) from central Patagonia middle Eocene Rio Pichileufu paleoflora (Wilf et al., 2005). We modeled an exponential prior distribution with an offset of 47.5 Ma and a mean of 1.9. The mean value was chosen to contain in the 95% confidence interval of the distribution assuming a soft maximum constraint of 53.2 Ma that includes the age of a pollen grain of *Tubulifloridites* sp. belonging to Asteraceae and having well-

developed spines as in *Mutisiapollis telleriae* collected in Antarctica and magneto- and bio-stratigraphically dated to approximately 53 Ma (Pross et al., 2012; Contreras et al., 2013).

6. *Centaurea* type fossil pollen from the Pliocene (Popescu, 2002) was used to model a prior using a lognormal distribution with an offset of 4.6 Ma and mean and standard deviation of 0.5 to constrain the *Centaurea-Carthus* (Carduoideae) node.

7. *Cichorium intybus* type fossil pollen from the early Miocene (Tremetsberger et al., 2012) was used to model a prior using a lognormal distribution with an offset of 23.5 Ma and mean and standard deviation of 0.75 to constrain the Cichorieae node (*Gundelia*, *Sonchus* and *Youngia*).

8. The oldest *Artemisia* fossil pollen has been recorded from the Eocene-Oligocene boundary of northwestern China (Wang, 2004; Hobbs and Baldwin, 2013). We modeled this prior using a lognormal distribution with an offset of 31 Ma and a mean and standard deviation of 0.5 to constrain the node *Artemisia-Chrysanthemum*.

9. The high elevation Páramo habitat of northern South America has resulted from orogeny that has been dated to the last 2-4 Ma (Hughes and Eastwood, 2006; Sklenar et al., 2011). The *Espeletia* clade of tribe Millerieae is endemic to the Páramo habitat and adjacent high elevation Andean forests. We modeled a lognormal prior on the root node of the *Espeletia* clade (*Carramboa*, *Espeletia*, *Libanothamnus*) with an offset of 3 Ma, a standard deviation of 0.4 and a mean of 0.3.

Tubulifloridites lilliei type A pollen fossils are of dubious value in calibrating phylogenetic trees in Asterales and were not used in this study. *Tubulifloridites lilliei* circumscribes several fossilized pollen morphotypes that have been associated to angiosperms, possibly Asteraceae if exine characteristics are overlooked (Dettmann and Jarzen, 1988) or families outside of Asterales (Ranunculaceae as *Tricolporites lilliei*, Dettmann 1994; Euphorbiaceae as *Tensucolpites lilliei*, Macphail et al., 2014). When considering *Tubulifloridites lilliei*, Zavada and de Villiers (2000) and Zavada and Lowrey (2010) find these tricolporate, psilate to microechinate pollen grains with longitudinal endoapertures could represent the ancestral pollen type of Asteraceae but are difficult to separate from those of other families. This combination is found in Calyceraceae where it is hypothesized to be ancestral (*Gamocarpha* type, DeVore et al., 2007), and Goodeniaceae (Gustafsson et al., 1997) as well as in Asteraceae in *Chuquiraga*, *Doniophyton* and some species of *Dasyphyllum* of the Barnadesioideae. *T. lilliei* type A pollen grains are ascribed to extant *Dasyphyllum* based on the optimal tree resulting from maximum parsimony analysis of 26 pollen morphological characters, but trees one step longer place type A pollen in Barnadesioideae, Calyceraceae, Goodeniaceae or the Asteraceae-Calyceraceae stem, and two-step longer trees place type A grains in Goodeniaceae, Menyanthaceae, Alseuosmiaceae, Campanulaceae or stems leading to more inclusive clades (Barreda et al., 2015). Barreda et al. concede the phylogenetic uncertainty underlying their identification of type A fossils when employ them at several nodes outside Asteraceae to calibrate chronograms.

Unequivocal attribution of *T. lilliei* type A to Asteraceae by Barreda et al. is rejected here. Fewer than half of trees resulting from bootstrap resampling of the Barreda et al. data matrix (5,000 replicates) in PAUP* place *T. lilliei* in or sister to Asteraceae (Panero, 2016). Encoding and scoring of some characters appears biased. The presence/degree of depth of intercolpar concavities, and columellae visibility/number of exine layers are probably not independent characters and the use of both results in unjustified character weighting (Panero, 2016). Intercolpar concavities in *T. lilliei* type A are described as “sometimes absent,” but are scored as “present” rather than “present or absent.” The homology of intercolpar concavities shown in the type A fossils and that of extant taxa has been challenged (Panero, 2016). Whereas concavities in Barnadesioideae, Calyceraceae, and Goodeniaceae are symmetrically distributed

on the intercolpar regions with equivalent degrees of definition, concavities of *T. lilliei* type A are found in different areas in different pollen grains and are of various sizes and shapes (see Barreda et al., 2015, Figure 4); these concavities appear to result from mechanical forces during fossilization and/or sample retrieval or preparation (Panero, 2016). The same ectexine features produce different synapomorphies when encoded as visible under light microscopy vs. transmission electron microscopy (Panero, 2016). Tellería et al. (2015) describe all species of *Dasyphyllum* as having ectexines with two layers as in most other Barnadesioideae but with the much smaller second layer not distinguishable under light microscopy. Encoding both number and visibility of columellae observed under light microscopy results in *Dasyphyllum* sharing synapomorphies with *T. lilliei* type A rather than other Barnadesioideae (Barreda et al., 2015) and these characters are not independent (Panero, 2016). Encoding $<1\mu\text{m}$, $1-3\mu\text{m}$, and $>3\mu\text{m}$ categories for exine thickness combined with the choice of *Dasyphyllum inerme* and *D. velutinum* exemplars favors *Dasyphyllum* – *T. lilliei* type A relatedness also (Panero, 2016). Type A fossils' exine width ranges from $1.2-2\mu\text{m}$ (Barreda et al., 2015), smaller than recorded Barnadesioideae (Urtubey and Tellería, 1988, Tellería et al., 2015) but typical of Calyceraceae (DeVore et al., 2007), whereas *Dasyphyllum* has exine layers that are mostly around $3\mu\text{m}$ (Urtubey and Tellería, 1988) with *D. velutinum* as small as around $2.5\mu\text{m}$ (Tellería et al., 2015). The fossil would not share a synapomorphy with Barnadesioideae had the cut-off between larger categories been $2\mu\text{m}$.

Pollen fossils from marine sediments that might otherwise be considered to calibrate the Carduoideae-Asteroidae node and attributed to two species, *Tubulifloridites antipodica* and *T. viteauensis*, were not employed because of uncertainty in their ages (Zavada and de Villiers, 2000; Scott et al., 2006). The same species found in river sediments north of Koinaas unfortunately have not been precisely-enough dated (de Villiers and Cadman, 2001) to be used to calibrate the Carduoideae-Asteroidae node.

The MCMC chain was run in two separate analyses for 570 million posterior iterations sampling every 20,000 posterior iterations. LogCombiner v1.8 (Drummond et al., 2012) was used to combine the log and tree files with the initial 20 million iterations of each analysis discarded (burn-in) from the text file before combining. Tracer v1.6 (Rambaut et al., 2014) was used to assess Effective Sample Sizes (ESS) with values above 200 considered for optimal convergence and tree likelihood stationarity. Tracer automatically removed the first 10% of the log statistics from the combined sample, so we correspondingly discarded the first 10% of trees. A maximum clade credibility (MCC) tree was constructed in TreeAnnotator v1.8.0 (Drummond et al., 2012) depicting the maximum sum of posterior clade probabilities. The MCC Tree was visualized in FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) including mean ages and a time scale and bars for the 95% highest posterior densities for all branches with > 0.95 posterior probabilities.

2.4. Rate shifts in diversification

Net rates of diversification, extinction and speciation through time were modeled across all branches of the phylogeny using BAMM version 2.1 (Rabosky, 2014; Rabosky, et al., 2014; <http://bamm-project.org/>). BAMM uses rjMCMC to move between diversification models containing multiple processes and to sample these hierarchically based on the frequency of their posterior probabilities. Because the number of processes governing a tree is not predetermined, transitions between processes are modeled using a compound Poisson prior to calculate the probability of the number and distribution of processes in the phylogeny.

The time calibrated phylogeny in Newick format resulting from the divergence time inference using BEAST was pruned in R (R Development Core Team, 2014) to 65 terminal taxa to facilitate unambiguous assignment of species richness. Asteraceae diversity not sampled in this study was assigned to each tip of the phylogeny based on previous taxonomic and phylogenetic studies of the family (Anderberg et al., 2007; Panero and Funk, 2008) to produce a timetree with total species diversity for each lineage of the pruned phylogeny (Supplemental material S1). The proportions of sample taxa to total diversity for each terminal of the phylogeny were tabulated and input into the BAMM control file under the sampleProbsFilename parameter. Producing a fully reconciled chronogram of all Asteraceae at the species or even genus level using all available sequence data and clade constraints based on taxonomic information was not computationally feasible because we employ multiple internal calibrations. The BAMM control file setting the parameters of the MCMC search and prior simulations used default values except for the initial lambda prior on the root process that was set to 0.32 and a Poisson rate prior set to 1 to minimize the number of shifts under the prior alone. The control file was run in the Unix platform and the MCMC chain set to run for 50 million generations recording results every 10,000th generation for a total of 5,000 samples from the posterior.

The BAMM output was analyzed using BAMMtools (Rabosky et al., 2014, 2015) and the APE package (Paradis et al., 2004; Popescu et al., 2012) in R. After a burn-in of 10%, MCMC stationarity and (ESS) were assessed in R using CODA (Plummer et al., 2006). Because the probability of no shift was zero, BAMMtools was unable to use Bayes factors to calculate number of shifts so instead prior and posterior marginal probabilities were compared to assess the probability of rate shift models containing different numbers of shifts from 0 to 7. We also calculated a mean phylorate tree in which mean diversification rates of segments of each branch of the phylogeny are depicted as colors providing a visual summary of rates across the entire posterior. To ascertain distinct rate shift configurations best supported by the data, we excluded configurations with non-core shifts in which branches associated with rate shifts are not supported by at least Bayes Factor 100. These configurations were then ranked by frequency in posterior probability and the set of most probable configurations that sum to at least 0.95 makes up the 95% credible shift set. We also calculated and plotted the marginal probability that a shift may have occurred on a given branch and compared this to a phylogeny with branches rescaled to marginal odds ratios. To visualize cohorts of taxa that share macroevolutionary rate regimes we constructed a correlation matrix of pairwise probabilities in BAMMtools. Lastly, rates of speciation and extinction were calculated for several clades of interest.

To more directly compare our results with those of recently published studies that explore diversification rates in Asteraceae we also analyzed the same data using MEDUSA (Alfaro et al., 2009) version TurboMEDUSA 0.93-4-33 (Brown et al., 2012) with the packages GEIGER (Harmon et al., 2008) and LASER (Rabosky, 2006) in R (R Core Development Team, 2014). MEDUSA optimizes birth-death model size by stepwise AIC scores using branch lengths obtained from a time-calibrated phylogeny and taxonomic richness likelihoods (Alfaro et al., 2009; Santini et al., 2009). MEDUSA does not calculate rates changes in diversification across branches but rather macroevolutionary parameters are fixed through time within each rate domain found. Similar to the methodology employed for the BAMM analysis, the BEAST time-tree resulting from the divergence time inference was pruned to 65 terminal taxa to facilitate unambiguous assignment of species richness. Asteraceae diversity not sampled in this study was assigned to each tip of the phylogeny. The tree and text file containing Asteraceae diversity were input into R.

3. Results

3.1. Taxonomic and geographical distribution of Asteraceae species richness.

Asteraceae species richness (25,037 spp.) and taxonomic diversity is distributed unevenly across continents (Fig. 1). South America has the highest number of species of Asteraceae with 6,316 followed by Asia (6,016 spp.), North America (5,404 spp.), Africa (4,631 spp.), Europe (2,283 spp.), Oceania (1,444 spp.), and the Pacific island region (174 spp.). The largest taxonomic component of the species richness of South America belongs to supertribe Helianthodae with 2,535 species but it is in North America where the largest concentration of species of this group is found (3,159 spp.). Helianthodae species richness is significantly lower in other continents of the world (Fig. 1). There is an eightfold difference between the native South American species that belong to subfamilies that evolved in South America (659 spp.) versus those that belong to subfamilies that originated outside the continent (5,657 spp.). The species richness of supertribes Asteroideae and Senecionodae is more evenly distributed across continents than that of Helianthodae, with South America having the largest number of species of Senecionodae (1,214 spp.) and Africa the highest number of Asteroideae (1,900 spp.). Species of Asteroideae are the largest group in Oceania with more than 78% of the Asteraceae diversity of the region. The diversity of Cichorioideae is mostly found in South America, Africa, Asia and Europe whereas a large majority of the species richness of Carduoideae is mostly found in Asia (Fig. 1).

3.2. Phylogenetic relationships

The maximum clade credibility (MCC) tree estimated in BEAST (Fig. 2) resolved all nodes of the phylogeny with posterior probabilities >0.95, except the following: Stiffthioideae as sister to the clade Wunderlichioideae-Asteroideae (0.76), Arctotideae sister to Cichorieae (0.84), Asteroideae sister to Helianthodae (0.74), and *Carramboea* sister to *Espeletia* (0.34). The topology is congruent with the most recent molecular phylogenies of the family except for the relationships of the supertribes of Asteroideae that differs from Panero et al. (2014) and Mandel et al. (2014) in having Senecionodae sister to the Asteroideae–Helianthodae clade and Vernoniaceae as part of Cichorioideae. Our phylogeny differs from the phylogeny of Liu et al. (2015) based on nuclear markers in the relationships among the tribes of the Heliantheae alliance and in Anthemideae being sister to Senecioneae rather than Astereae. Results from the ML analysis produced a topology identical to the MCC tree with a majority of nodes having moderate to strong bootstrap support (70-100%) (Fig. S1). Nodes with bootstrap values of 54% or below include: Arctotideae sister to Cichorieae (53%), Asteroideae sister to Helianthodae (46%), Madieae sister to Milleriaceae (54%) and Heliantheae sister to Eupatorieae-Perityleae (52%).

3.3. Divergence time estimates

All operators and priors for our BEAST analysis exceeded an effective sample size (ESS) of 200. Divergence of Goodeniaceae from the Calyceraceae-Asteraceae clade occurred about 75.2 Ma (63.6-86.9 Ma 95% highest posterior density (HPD)). Asteraceae is estimated to have diverged from its closest relative, Calyceraceae, 69.5 Ma (59-80.1 Ma 95% HPD) in the late Cretaceous near the Cretaceous-Paleocene boundary (Fig. 2). Estimated crown node ages of the two families differ by approximately 25 million years, with extant Calyceraceae originating approximately 39.2 Ma (24.9-57 95% HPD) in the late Eocene and Asteraceae estimated at 64.75 Ma (55.1-74.4 95% HPD) in the early Paleocene. We recovered stem ages for all 13 subfamilies and crown ages for nine of these including the three main groups (supertribes) of Asteroideae (Fig. S2, Table S1). The four subfamilies lacking crown age values are the monogeneric Famatinanthoideae, Hecastocleidoideae and Corymbioideae, and the two species (one sampled)

of the Gymnarrhenoideae (Fig. S2, Table S1). The 13 subfamilies of Asteraceae originated in the Paleocene and Eocene between 64.75-43.2 Ma (Fig. 1).

3.4. Shifts in diversification rate process

The MCMC chain of the BAMM analysis converged with values much higher than 200 (ESS log-likelihood = 1575; ESS number of shifts = 509). Evidence for multiple rate processes was overwhelming, with the two-shift model receiving the highest marginal probability of 0.56 (Fig. S3). The mean marginal densities of diversification rates plotted for branch segments across the phylogeny (phylorate plot) are shown in Figure 3. A set of nine distinct rate shift configurations accounted for 95.6% of the data, with two-shift configurations (3-rate domains) found in 69.9%, single-shift configurations in 23.8% and no rate shift (single rate process) in 3% of the posterior filtered by BF 100 (Fig. S5A). In this set a total of six *core* shifts were observed more frequently than expected under the prior probability alone. The configuration with highest posterior probability (*Maximum a posteriori probability* (MAP) configuration) located core shifts at the Phytomelanic Fruit (PF) Clade and at the Vernonioid Clade ($f=0.4$; Fig. 3, Fig. S5A). A core shift associated with the PF Clade was present in 66.4% of credible shift configurations including the three most frequent, and was the most frequently sampled among the single-shift configurations [PF Clade (0.11), Vernonioid Clade (0.091), AAD Clade (0.027)]. Configurations that did not include a PF Clade shift showed a core shift downstream at the 9-tribes Clades or were single-shift or single rate process configurations (Fig. S5A). Besides the MAP configuration, the PF Clade shift was also found in combinations with either a shift at the AAD ($f=0.11$) or Gymnarrhenoideae-rest of Asteraceae Clade ($f=0.044$). Marginal probabilities of a shift occurring on stems subtending these core shifts are shown in Figure S5B; marginal odds ratios of stems leading to Vernonioid, PC, AAD, 9-tribes, and Gymnarrhenoideae-rest of Asteraceae Clade shifts were 3712, 1579, 265, 164, and 114 respectively (Fig. S6). A visualization of the pairwise probability that any two terminals in the phylogeny share a macroevolutionary rate process (speciation regime) is provided in Figure S4 and shows three main rate domains. Speciation and extinction rates calculated for rate shifts in the two top MAP configuration and Asteraceae are included in Table S2.

MEDUSA found a 5-rate model (Fig. S7) best fit the maximum clade credibility tree ($-\ln[L]=547.5$, $K=14$). A low background diversification rate ($r=0.054$ lineages/Ma) with a death rate of 90% of the birth rate ($\epsilon=0.9$) was calculated for the outgroup families Menyanthaceae, Goodeniaceae and Calyceraceae and nine subfamilies of Asteraceae (Barnadesioideae, Famatinanthoideae, Mutisioideae, Stiffthioideae, Wunderlichioideae, Gochnatioideae, Hecastocleidoideae, Carduoideae, Pertyoideae and Gymnarrhenoideae). A rate increase ($r=0.15$) with a relatively high extinction rate ($\epsilon=0.81$) was estimated for the clade Cichorioideae-Asteroideae. The clade including the tribes Perityleae, Heliantheae, Eupatorieae, Millerieae and Madieae (PHEMM clade) was identified as having a higher rate of diversification than the background rate with a relatively high diversification rate ($r=0.39$) and a low extinction rate ($\epsilon=0.52$). The Carduoideae clade was estimated to have the second highest rate of diversification in the family ($r=0.32$) and a moderate extinction rate ($\epsilon=0.61$). The only rate decrease found in the Asteraceae corresponds to subfamily Corymbioideae with an $r=0.023$ and a medium extinction rate ($\epsilon=0.69$).

4. Discussion

4.1. Origins of Asteraceae and Calyceraceae

Our mean estimate (69.5 Ma HPD) places the divergence of Asteraceae and Calyceraceae shortly before the Chicxulub bolide impacted North America 66 Ma at a time of significant rapid temperature oscillations (Barrera and Savin, 1999; Renne et al., 2013). The extraterrestrial strike exacerbated biotic turnover causing extinction in vegetation assemblages across the world (Vajda and McLoughlin, 2007) and permanently changed the early Paleocene plant communities of Patagonia (Barreda et al., 2012b) where Asteraceae presumably began to evolve (DeVore and Stuessy, 1995; Stuessy, 2010). If our estimate is correct, these conditions likely caused the extinguishing of early transition forms between Calyceraceae and Asteraceae, resulting in the discontinuities in ovary position, chemistry, and inflorescence morphology (Pozner et al., 2012) that makes polarizing morphological characters difficult (Carlquist and DeVore 1998) and have led to Asteraceae being viewed historically as a distinctive or ‘natural’ family (Cronquist, 1977; Stuessy, 2010).

The stem and crown node ages of Asteraceae estimated here bracket the 64.9 Ma (62.1-67.6, 95% HPD) mean age for the genome duplication near the crown node of Asteraceae estimated by Fawcett et al. (2009). The Fawcett et al. estimate cannot be reconciled with any previously estimated dates for the divergence of Asteraceae and Calyceraceae, and the younger mean estimate of 58.32 Ma (55.64-60.04) by Vanneste et al. (2014) also predates all previous mean estimates for divergence of these families except those of Barreda et al. (2015). Both these WGD studies sample only *Lactuca sativa* to represent the family and thus employ no internal calibration. Our stem node estimate of 69.5 Ma (59-80.1, 95% HPD) is also significantly earlier than other estimates of 44 +/-3 Ma (Wikström et al., 2001), 48 Ma (Kim et al., 2005), 51 Ma (Bremer et al., 2004), 55.8 Ma (Barres et al., 2013), 53 (Beaulieu et al., 2013) and 54.4 (49-61, 95% HPD (Jabaily et al., 2014), 49.3 (47.7-53.8, 95% HPD, Magallón et al., 2015), 48.8 Ma (36.5-53.9 min-max, Tank et al., 2015) and 56.8 (55.8-58.4, 95% HPD, Park and Potter, 2015). The preferred estimate of Barreda et al. (2015) ~89 Ma relies on a *Tubulifloridites lilliei* type A fossil calibration placed at the *Barnadesia-Dasyphyllum* common ancestor. This calibration accounts for the nearly 20-million year older estimate for the origin of Asteraceae than ours. The large difference between ours and younger relaxed-clock age estimates results primarily from calibrations placed on backbone nodes of the Asteraceae in our study, particularly the constraint on the Mutisioideae-Asteroideae root node using *Raiguenrayun cura* + *Mutisiapollis telleriae* as suggested by Panero et al. (2014) rather than placing it on the Asteraceae root node as originally suggested by Barreda et al. (2012a). This calibration is clearly most influential on our estimation of the origins of Asteraceae subfamilies Famatinanthoideae through Carduoideae between ~60-49 Ma in the Paleogene. Using *M. telleriae* placed as we did, Barreda et al. (2015, Fig. 5) found the same range for major divergences from Famatinanthoideae through Carduoideae but they only signify the importance of their *T. lilliei* type A fossil calibration, a calibration that was not employed here. Denser taxon sampling in ours than in all earlier timed phylogeny studies allowed us to place internal calibrations on nodes in Barnadesieae, Cardueae, Cichorieae, Millerieae, Anthemideae, Nassauvieae, and family Calyceraceae that contributed to older ages at the root nodes for these tribes and collectively pushed the ages of backbone nodes and origin of Asteraceae and Calyceraceae toward earlier dates. An estimated Cretaceous origin for Asteraceae precludes us from providing support to the idea that whole genome duplication occurred specifically after the extraterrestrial strike as proposed by Fawcett et al. (2009).

Any discrete rate shift (core shift) during the first nearly 20 million years of Asteraceae evolution is clearly rejected given our data (Fig. 3; Figs. S5, S6, S7), thus we cannot provide support for any key innovation hypothesis tied to the basal stem. Although clade imbalance near the origin of Asteraceae is arresting, it's not surprising to find significant rate increases downstream rather than early in the family's evolution. Only Barnadesioideae and

Famatinanthoideae, collectively 93 species, break the long stem between the origin of Asteraceae and the clade containing more than 99.6% of the family richness (Figs 1, 2). The long basal branches in Asteraceae are emblematic of a pattern found in many other plant groups where long branches have been interpreted as the signature of significant extinction (Crisp and Cook, 2009). Extinction can confound attempts to reconstruct diversity dynamics by sampling only extant taxa (see review in Morlon, 2014) as illustrated by molecular phylogenies from extant taxa that show increasing patterns of diversification in contrast to the fluctuating patterns observed from the fossil record (Ricklefs, 2007; Quental and Marshall, 2010). The fossil record of early Asteraceae is poor and almost entirely composed of dispersed pollen deposited in the Eocene. No macrofossils unequivocally attributed to the Asteraceae stem provide a window on taxon richness and extinction early in the family's evolution. Theoretically, extinction rates can be accurately estimated from extant taxa alone (Nee et al., 1994; Morlon, 2011) but declining diversity, such as mass extinction, presents a particular challenge to modeling diversification dynamics (Quental and Marshall, 2010). Allowing rates to vary across lineages under a diversity-dependent scenario using the BAMM model should better accommodate changes in pattern through time and thus provide a better assessment of extinction and speciation in Asteraceae than models that preclude temporal variation in rates. Consistent with the fossil record, Asteraceae diversification rates within this study increased gradually throughout the Paleocene and across the Early Eocene Climatic Optimum (EECO) prior to intercontinental dispersal.

4.2. Significant rate increases and Asteraceae global expansion

Two increases in net diversification that each follow transoceanic dispersal and genomic reduction after WGD best explain the 460:1 species richness asymmetry between Asteraceae and Calyceraceae given our data (Fig. 3; Fig. S5A, MAP configuration; Fig. S3). The earlier core shift at the crown node of the Vernonioid Clade (Vernonioid Group of Bremer, 1996) temporally follows diploidization of the genome (to $x=10$) and the Eocene dispersal of Asteraceae from South America to Africa. Spiny pollen (Karis et al., 1992) and rigid sweeping stylar hairs indicative of a transition in secondary pollen presentation (Leins and Erbar, 2010) are synapomorphies for the Vernonioid Clade but their possible significance as regards to species radiation has not been explored. The other significant increase present in the best rate configuration is nested within the Vernonioid Clade along the branch leading to the Phytomelanic Fruit (PF) Clade (Phytomelanic Cypselae Clade of Panero and Funk (2008)¹). This shift follows a more recent polyploidization of the genome and the Oligocene dispersal of Asteraceae from Africa to North America 32.2 – 27.6 Ma. The increase coincides with the canalization of phytomelanic fruit in this lineage (Fig. 3). The PF Clade comprises more than 98% of the Heliantheae alliance whose species are distributed mostly in the Americas, nearly all of which have black fruits resulting from phytomelanin deposition (Panero, 2007). Several studies have shown that the phytomelanin layer confers protection from herbivory by certain insect larvae and resistance to desiccation (reviewed in Pandey et al., 2014). Other morphological features prevalent in the clade such as the presence of receptacular bracts that wrap tightly around the fruit, corolla shape, corolla color, or floral reproductive structures cannot be considered synapomorphies and associated to this rate increase. The PF Clade diversified in the late Oligocene through the Miocene (Fig. 2) during times of increasingly drier and colder climate compared to the Eocene (Zachos et al., 2001; Hansen 2013) and many species are found in dry areas. However, a significant proportion of its species, particularly of tribes Eupatorieae, Millerieae and Heliantheae, are also found in mesic montane areas. Notably their radiation in

¹ Leins and Erbar (2010) argue convincingly that the fruit of Asteraceae is neither cypselae nor achene but rather a nut.

paramos and cloud forests where few other species of Asteraceae are found constitutes examples of broad niche shifts. Our finding could be interpreted as the detection of phytomelanization as a key innovation (sensu Heard and Hauser, 1995) in the rapid continental radiation of the PF Clade in the Western Hemisphere.

None of our data were explained by any significant change in rate process prior to Asteraceae dispersal from South America, but neither was rate change strongly correlated with dispersal. Our Bayesian approach and BAMMtools utility allowed us to quantify uncertainty in reconstructing rate dynamics and examine the equivocal location of rate shifts (Fig. S5A). The only alternative to the PF Clade shift in other two-shift configurations was located at the crown node of one of the subclades comprising nine tribes belonging to the Heliantheae alliance (Perityle-Neurolaeneae; see Fig. 2), but this was sampled 4-5 times less frequently. Nothing in the literature of Asteraceae suggests any feature common to this clade. Two alternative locations to the Vernonioid Clade shift were found with much less frequently in the Bayesian posterior. One alternate is located upstream of the Vernonioid Clade at the root node of another unnamed clade that we call the African Asteraceae Dispersion (AAD) Clade because its component subfamilies are thought to have diverged in Africa but the extant diversity of each is much greater on other continents (see Fig. 1). Whereas the AAD Clade radiated worldwide giving rise to more than 96% of Asteraceae species richness, its North American sister Hecastocleidoideae is monotypic. The AAD Clade plus Hecastocleidoideae together constitute the Out-of-South America (OOSA) Clade. Although they share a common polyploid ancestor and histories that include transoceanic dispersal from South America, the evolutionary trajectories of these were clearly influenced by unique histories of dysploidy and establishment on different continents probably at different times. Only about 17% of our data could be explained by shift configurations that include a core shift at the AAD Clade synchronous with dispersal of Asteraceae to Africa. Even more rarely sampled in the posterior was the earlier shift located at the Gymnarrhenioideae-rest-of-Asteraceae clade (Fig. S5A) only in combination with the PF Clade shift. Interestingly, we found no core increase in the net rate of diversification associated with the Asteroideae Clade that contains more than 70% of all of Asteraceae species from which more than 90% of all chemical structures of Asteraceae have been characterized (Calabria, et al., 2009) (Fig. 2). None of the core shifts identified by BAMM corresponds with any hypothesized transition traditionally invoked to explain unusual Asteraceae diversity.

Significant rate increases were not synchronous with genome duplication but did coincide with, or follow, diploidization. A haploid chromosome number of 27 in basal subfamilies Barnadesioideae through Gochnatioideae relative to the $x=9$ base number in Calyceraceae and Goodeniaceae (Semple and Watanabe, 2009; *Famatinanthus* not yet counted) suggests a hexaploid increase along the basal stem of Asteraceae, although only tetraploidy near the origin of the family has been inferred from expressed sequence tags (Barker et al., 2008). WGD near the origin implies an abrupt undocumented dysploid series to $x=10$ in an ancestor of the AAD Clade (Semple and Watanabe, 2009) and the maintenance of a polyploid karyotype for some time. Assuming early genome duplication, at least 15 - 20 million years separate duplication and the earliest rate shift, with subsequent diploidization beginning during the Early Eocene Climatic Optimum and coinciding with dispersal from South America (Fig. 3). A more recent WGD that Barker et al. observed in *Helianthus* is reconstructed as having occurred along the stem of the Heliantheae alliance (Baldwin et al., 2002; Semple and Watanabe, 2009) where a dysploid loss of one chromosome is also thought to have occurred ($n = 20$ to $n = 19$; Semple and Watanabe, 2009). Chromosome number reduction has progressed in many clades of all tribes of the Heliantheae alliance, sometimes reaching numbers similar to the hypothesized base number of the ancestor of the alliance, or lower. This trend has been documented in many groups of the alliance (Robinson, 1981) and may be ubiquitous in the family as it is a general trend in polyploids

(Leitch and Bennett, 2004). Several studies suggest that the process of diploidization may start immediately after genome duplication (Edger and Pires, 2009) and differential loss of duplicated genes can lead to reproductive isolation between subpopulations (Werth and Windham, 1991; Lynch and Forge, 2000; Jiao and Paterson, 2014). Some argue that this may increase speciation rate temporarily and over time this could lead to species radiation (McGrath et al., 2014).

The lack of tight correspondence between genome duplication or dispersal events and shifts in diversification rate process in Asteraceae contrasts with recent studies. Precise location of rate shifts is known to be highly dependent on the level of phylogenetic resolution (Purvis et al., 1995) and spurious identification of rate shifts at more inclusive nodes when more nested nodes are not included in an analysis is known as ‘trickle down’ (Moore et al., 2004). Tank et al. (2015) found a rate increase in Asteraceae to be synchronous with WGD, but that perfect correspondence was practically assured when the Asteraceae clade was collapsed and the entire diversity of the family assigned to a single exemplar. A strong rate increase leading to the OOSA clade reported by Beaulieu and Donoghue (2013) was also not seen in our study; increases were instead distributed to younger clades (Figs. 3, S5, S7). Our placement of the *Mutisiapollis* (*Raiguenrayun*) fossil calibration on the Mutisioideae-Asteroideae node rather than the Barnadesioideae split as in their study contributed to our older ages of backbone nodes downstream of our calibration, but the difference probably also reflects the apportioning of diversity to more terminals in our study. Incomplete taxon sampling is a caveat of the present study as well. Although we account for all species numerically, bias toward earlier divergences arises from non-random sampling that we’ve necessarily accepted in order to ensure sampling the complete backbone of Asteraceae to address questions of interest. The lack of correlation found here between rates and polyploidy or dispersal should be robust to future more comprehensive taxon sampling because bias toward non-random sampling is expected to push the location of rate shifts towards deeper nodes and we have sampled all the backbone nodes of Asteraceae. However, in future more-densely sampled studies we expect the rate increases found on branches leading to the Vernonioid and Heliantheae alliance clades could trickle-down toward terminals in several species rich tribes of Cichorioideae and Asteroideae and additional rate changes identified. We might also expect to see rate increases downstream from the PF Clade, perhaps in clades that dispersed to South America and radiated extensively such as *Aldama*, *Wedelia*, and *Verbesina* in the Heliantheae, *Ageratina*, *Chromolaena*, *Mikania* and *Stevia* in Eupatorieae, or *Espeletia* in the Millerieae. If so, diversification rate shifts would be more distant from major intercontinental dispersals and asynchronous with innovations defining broad clades. Our goal here was to investigate the pattern of diversification at higher taxonomic levels to address traditional hypotheses and recent interest in the role of dispersal and polyploidy in structuring extant Asteraceae diversity.

5. Conclusions

Asteraceae originated near the end of the Cretaceous Period. The family is substantially younger than Orchidaceae though similar in number of species, and younger but more species rich than the other mega-diverse eudicot family Fabaceae. Extant diversity is distributed unevenly both taxonomically and geographically. From this distribution it is evident, arguably, that intercontinental dispersal was prerequisite to the family attaining its unusual richness.

Key morphological innovations, genome duplication and dispersal events thought to drive the success of Asteraceae precede but are decoupled from significant shifts of diversification rate. Core shifts follow WGD and transoceanic dispersal facilitated by pappus and capitulum features that evolved at least 15-20 million years before the earliest tipping-point

where diversification rate discretely changes. These shifts presumably occurred as each ancestral species was either undergoing diploidization or had reached an effectively diploid state. Based on reconstructed base chromosome numbers of major clades, it appears that polyploidy has punctuated the evolutionary history of Asteraceae triggering diploidization in virtually all lineages of the family, but much genomic data are still needed to precisely locate the phylogenetic positions of genome duplication/restructuring events throughout the family. This area of research deserves much more attention if we are to pursue how diploidization leading to increased speciation or decreased extinction rate might produce increased diversification long-term.

It appears that both taxon specific attributes and response to changed biotic and abiotic conditions contributed to ecological opportunity that triggered continental radiations. Our first glimpse of rate dynamics in Asteraceae using the shift framework of BAMM resulted in unexpected taxonomic locations of rate increases, and at least one new hypothesis for proximate causes of large species numbers in Asteraceae. We identified a sunflower radiation of 5,700 mostly American species, the PF Clade, as potentially arising from colonization of new habitat and genome restructuring that might have contributed to increased speciation rate and the evolution of seed phytomelanization that may contribute to decreased extinction rate. This radiation is surprisingly recent, within the last 25 Ma. But we caution that with more comprehensive sampling, locations of core rate shifts could trickle-down to more downstream nodes. The apparent rate increase in Africa with the Vernonioid or AAD Clade could signal rate change that actually occurs farther downstream, perhaps associated with clades diverging in Asia. In future, additional data from tribes of the Heliantheae alliance, Astereae, Anthemideae, Gnaphalieae, Senecioneae, and Vernonieae will help us to understand the Vernonioid Clade's ~21,000 species rich diversity. The limited and uneven taxonomic depth of available sequence data and phylogenetic information remains an impediment to precise and complete identification of immediate causes of rate shifts in Asteraceae and the origins of this biodiversity.

Figure Captions

Fig. 1. Global distribution of Asteraceae species richness by continent and lineage. Circles are proportional to species number on continents of same color for each subfamily shown to the left (total number per subfamily in parentheses). Open circles represent species richness of the islands of the Pacific Ocean. Note the small contribution of basal lineages that diverged before transcontinental dispersal of Asteraceae out of South America (names in red).

Fig. 2. Estimated divergence times for Asteraceae and most-closely related families. Asteraceae subfamilies diverged between 64.75 and 43.25 Ma with those originating outside South America (Hecastocleidoideae through Asteroideae) all within a short time interval in the Eocene between 51.72-43.25 Ma. Green bars indicate confidence intervals on nodes of the MCC tree produced using BEAST that are supported by >95% posterior probability. Boxed numbers refer to absolute time constraints numbered Section 2.3.

Fig. 3. Net diversification rate dynamics in early Asteraceae in relation to putative drivers of rate increase. The time-calibrated MCC tree shown in Fig. 2 has been pruned to apportion the 25,037 Asteraceae species diversity to 58 terminals. Branch color reflects the mean of the marginal posterior density of net diversification rates for each segment of the phylogeny as summarized in BAMM. Two red circles indicate the most probable rate shift configuration found using BAMM. The earlier shift along the stem of the Vernonioid Clade follows intercontinental dispersal to

Africa and diploidization well after the earliest Asteraceae WGD (dotted line = location deduced by Barker et al., 2008). The PF Clade shift coincides with acquisition of fruit phytomelanin and presumed chromosome loss following dispersal to North America and a later WGD.

Reconstructed base chromosome numbers to the right of clade names follow Semple and Watanabe (2009) except for basal subfamilies Barnadesioideae through Gochnatioideae where we presume a common hexaploid shift from an ancestral $x=9$ state. Low diversification and long stems during the early evolution of Asteraceae contrasts with higher rates and short stems approximately 15 Ma after Chicxulub bolide struck Earth. Polyploidization and putative key innovations such as racemose capitula and fruit pappi, absent in Calyceraceae, do not coincide with rate change. Deep ocean water temperatures (red curve) used as proxy for mean global temperatures through time are adapted from Zachos et al., 2001, where temperatures since the early Oligocene are affected by polar glaciation and less certain. Rates toward the present should be interpreted with caution for Cichorioideae, Corymbioideae, Asteroideae and Goodeniaceae that are proportionally less well sampled.

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Acknowledgements

We thank two anonymous reviewers for critical reading of the manuscript and the curators of herbaria mentioned in Appendix A for use of tissue samples for molecular studies. We also thank Cecilia Ezcurra of BCRU and Fernando O. Zuloaga of SI as well as Clodomiro Marticorena and Alicia Marticorena of CONC for their logistical support and hospitality during our visit to Argentina and Chile. We are also grateful to the staff of CONC and SI for help drying and packing collections. We thank Steve Wagstaff for DNA of *Abrotanella rostrata* and Carlos Anderson for help with BAMM. We also thank Lineth Contreras for providing us with a photo of the fossil pollen grain of *Tubulifloridites* sp. We thank Mark Miller for help with the BEAST analyses in CIPRES. This study was supported by a grant from the National Science Foundation (DEB 0344116).

Appendix A.

Genbank accession numbers for sequences used in this study. Genbank numbers listed in the following order: taxon name plus authority, *atpB*, *matK*, *ndhD*, *ndhF*, *ndhI ndhJKC*, *rbcL*, *rpoB*, *rpoC1*exon1, *rpoC1*exon2, *trnL* intron-*trnL*-F spacer (in some taxa two Genbank numbers comprise this region). NS, missing sequence.

Abrotanella rostrata Swenson, KT344985, KT345015, KT345051, KT345061, KT345070, KT345025, KT345079, KT345089, KT345099, KT345121, KT345157. *Abrotanella trichoachaenia* Cabrera, KT344986, KT345016, KT345052, KT345062, KT345071, KT345026, KT345080, KT345090, KT345100, KT345122, KT345158. *Achillea millefolium* L., KT344987, EU385315, EU385219, EU385124, EU243242, KT345027, EU384938, EU385410, EU385506, KT345123, EU385030. *Acicarpa spathulata* R. Br., KM191983, EU385317, EU385220, EU385125, EU243243, KM191818, EU384939, EU385411, EU385507, KM191922, EU385031. *Ainsliaea apiculata* Sch. Bip ex. Zoll., KM191984, EU385321, EU385225, EU385130, EU243248, KM191819, EU384944, EU385416, EU385512, KM191923, EU385036. *Aphyllocladus spartioides* Wedd., KM191985, EU385323, EU385227, EU385132, EU243250, KM191820, EU384946, EU385418, EU385513, KM191924, EU385038. *Arctotis hirsuta* (Harv.) P. Beauv., KM191986, EU385324, EU385228, EU385133, EU243251, KM191821, EU384947, EU385419, EU385514, KM191925, EU385039. *Artemisia frigida* Willd., NC020607, NC020607, NC020607, NC020607, NC020607, NC020607, NC020607, N 020607, NC020607, NC020607. *Athroisma gracile* (Oliv.) Mattf. ssp. *psyllioides* (Oliv.) T. Eriksson, KJ434416, AY215765, AF384437, L39455, AF383757, AY217218, AY215085, AY213763, EU385515, KM191926, AY216019/AY216144. *Atractylodes lancea* Thunb., KM191987, KM192111, KM191890, KM192100, KM191868, KM191822, KM192089, KM191879, KM191912, KM191927, KM191901. *Baccharis neglecta* Britton ex Britton & A. Br., KT344988, EU385326, EU385230, EU385135, EU243253, KT345028, EU384949, EU385421, EU385517, KT345124, EU385041. *Bahia absinthifolia* Benth., KJ434417, AY215766, AF384438, AF405267, AF383758, AY217219, AY215086, AY213764, KT345101, KT345125, AY216020/AY216145. *Barnadesia odorata* Griseb., KM191988, EU385327, EU385231, L39394 (*Barnadesia caryophylla*), EU243254, KM191823, EU841102, EU385422, EU385518,

KM191928, EU345042. *Berkheya purpurea* (DC.) Mast., KT344989, EU385328, EU385232, EU385136, EU243255, KT345029, EU384950, EU385423, EU385519, KT345126, EU385043. *Brachyclados caespitosus* (Phil.) Speg., KT344990, EU385329, EU385233, EU385137, EU243256, KT345030, EU384951, EU385424, EU385520, KT345127, EU385044. *Brachyglottis greyi* (Hook. f.) B. Nord., KT344991, KT345017, KT345053, KT345063, KT345072, KT345031, KT345081, KT345091, KT345102, KT345128, KT345159. *Brachylaena elliptica* (Thunb.) DC., KM191989, EU385330, EU385234, EU385138, EU243357, KM191824, EU384952, EU385425, EU385521, KM191929, EU385045. *Calea megacephala* B. L. Rob. & Greenm., KT344992, AY215769, AF384441, AF384697, AF383761, AY217222, AY215089, AY213767, KT345103, KT345129, AY216023. *Callilepis caerulea* Hutch., KT344993, KT345019, KT345054, KT345064, KT345073, KT345032, KT345082, KT345092, KT345104, KT345156, KT345160. *Callilepis laureola* DC., KT344994, KT345018, KT345055, KT345065, KT345074, KT345033, KT345083, KT345093, KT345105, KT345130, KT345161. *Calopappus acerosus* Meyen, KM191990, KM192112, KM191891, KM192101, KM191869, KM191825, KM192090, KM191880, KM191913, KM191930, KM191902. *Calycera crassifolia* Hicken., KM191991, KM192113, KM191892, KM192102, KM191870, KM191826, KM192091, KM191881, KM191914, KM191931, KM191903. *Carramboa trujillensis* (Cuatrec.) Cuatrec., KJ434418, KJ434451, KJ434448, KJ434445, KJ434463, KJ434442, KJ434457, KJ434460, KJ434454, KJ434454, KJ434439. *Carthamus tinctorius* L., KT344995, EU385331, EU385235, EU385139, EU243258, KT345034, EU384953, EU385426, EU385522, KT345131, EU385046. *Centaurea melitensis* L., KT344996, EU385332, EU385236, EU385140, EU243259, KT345035, EU385954, EU385427, EU385523, KT345132, EU385047. *Centipeda elatinoides* (Less.) O. Hoffm., KJ434419, KJ434452, KJ434449, KJ434446, KJ434464, KJ434443, KJ434458, KJ434461, KJ434455, KJ434455, KJ434440. *Centratherum punctatum* Cass., KT344997, EU385333, EU385237, EU385141, EU243260, KT345036, EU384955, EU385428, EU385524, KT345133, EU385048. *Chaenactis macrantha* D. C. Eaton, KT344998, KT345020, KT345056, KT345066, KT345075, KT345037, KT345084, KT345094, KT345106, KT345134, KT345162. *Chimantaea humilis* Maguire, Steyermark & Wurdack, KM191993, EU385336, EU385240, EU385144, EU243263, KM191828, EU384958, EU385431, EU385527, KM191933, EU385051. *Chrysanthemoides monilifera* (L.) Norl., KT344999, EU385337, EU385241, EU385145, EU243264, KT345038, EU384959, EU385432, EU385528, KT345135, EU385052. *Chrysanthemum indicum* L., JN867592, JN867592, JN867592, JN867592, JN867592, JN867592, JN867592, JN867592, JN867592, JN867592. *Chuquiraga spinosa* Less., KT026444, EU385338, EU385242, EU385146, EU243265, KT026447, EU384960, EU385433, EU385529, KT026450, EU385053. *Cnicothamnus lorentzii* Griseb., KM191995, EU385339, EU385243, EU385147, EU243266, KM191830, EU384961, EU385434, EU385530, KM191935, EU385054. *Coreopsis petrophiloides* B. L. Rob. & Greenm., KJ434420, AY215776, AF384448, AF384704, AF383768, AY217229, AY215096, AY213774, KT345107, KM191936, AY216030/AY216155. *Corymbium glabrum* L., KM191996, EU385340, EU385344, EU385148, EU243267, KM191831, EU384962, EU385435, EU385531, KM191937, EU385055. *Cyclolepis genistoides* D. Don, KM191997, EU385341, EU385345, EU385149, EU243268, KM191832, EU384963, EU385436, EU385532, KM191938, EU385056. *Dahlia coccinea* Cav., KJ434421, AY215779, AF384450, AF384706, AF383770, AY217231, AY215098, AY213776, KT345108, KT345136, AY216032/AY216157. *Daucus carota* L., NC008325, NC008325, NC008325, NC008325, NC008325, NC008325, NC008325. *Dicoma anomala* Sond., KT345000, KT345021, KT345057, KT345067, KT345076, KT345039, KT345085, KT345095, KT345109, KT345137, KT345163. *Doniophyton anomalum* (D. Don) Kurtz, KM192000, EU385348, EU385252, EU385156, EU243274, KM191835, EU384969, EU385443, EU385538, KM191941, EU385063. *Duidaia pinifolia* S. F. Blake, KM192001, EU385349, EU385253, EU385157, EU243275, KM191836, EU384970, EU385444, EU385539, KM191942, EU385064. *Echinops ritro* L., KM192002, EU385350, EU385254, EU385158,

EU243276, KM191837, EU384971, EU385445, EU385540, KM191943, EU385065. *Espeletia schultzei* (Cuatrec.) Cuatrec., KJ434422, KJ434453, KJ434450, KJ434447, KJ434465, KJ43444, KJ434459, KJ434462, KJ434456, KJ434456, KJ434441. *Eutetras palmeri* A. Gray, KJ434423, AY215797, AF384468, AF384724, AF383788, AY217248, AY215116, AY213794, KT345110, KT345138, AY216050/AY216175. *Famatinanthus decussatus* S.E.Freire & Ariza, KM192003, KM192114, KM191893, KM192103, KM191871, KM191838, KM192092, KM191882, NS, KM191944, KM191904. *Felicia heterophylla* (Cass.) Grau, KM192004, EU385353, EU385257, EU385161, EU243279, KM191839, EU384974, EU385448, EU385543, KM191945, EU385068. *Galeana pratensis* Rydb., KJ434424, AY215800, AF384471, AF384726, AF383791, AY217251, AY215119, AY213797, KT345111, KT345139, AY216053AY216178. *Gamocarpha alpina* (Poepp. ex Less.) H. V. Hansen, KM192005, KM192115, KM191894, KM192104, KM191872, KM191840, KM192093, KM191883, KM191915, KM191946, KM191905. *Gamochaeta pensylvanica* (Willd.) Cabrera, KT345001, EU385354, EU385260, EU385162, EU243282, KT345040, EU384977, EU385449, EU385554, KT345140, EU385070. *Gochnatia hypoleuca* (DC.) A. Gray, KM192006, EU385357, EU385261, EU385165, EU243283, KM191841, EU384978, EU385452, EU385546, KM191947, EU385071. *Gundelia tournefortii* L., KT345002, EU385361, EU385265, EU385169, EU243287, KT345041, EU384982, EU385456, EU385549, KT345141, EU385075. *Gymnarrhena micrantha* Desf., KM192007, EU385362, EU385266, EU385170, EU243288, KM191842, EU384983, EU385457, EU385550, KM191949, EU385076. *Hecastocleis shockleyi* A. Gray, KM192009, EU385363, EU385267, EU385171, EU243289, KM191844, EU384984, EU385458, EU385551, KM191951, EU385077. *Helenium bigelovii* A. Gray, KT345003, AY215804, AF384475, AF384730, AF383795, AY217255, AY215123, AY213801, EU385552 (*H. amarum*), KT345142, AY216057. *Helianthus annuus* L., KJ434427, AY215805, AF384476, L39383, AF383796, AY217256, AY215124, AY213802, EU385553, KM191952, AY216058/AY216183. *Hesperomannia arbuscula* Hillebr., KM192010, EU385364, EU385268, EU385172, EU243290, KM191845, EU384985, EU385459, EU385554, KM191953, EU385078. *Hofmeisteria fasciculata* Brandege, KM192011, AY215806, AF384477, AF384731, AF383797, AY217257, AY215125, AY213803, KT345112, KM191954, AY216059/AY216184. *Hyalis argentea* D. Don ex Hook. & Arn., KM192012, EU385367, EU385271, EU385175, EU243293, KM191846, EU384988, EU385462, EU385557, KM191955, EU385081. *Hyaloseris salicifolia* (Griseb.) Hieron., KM191999, EU385346, EU385250, EU385154, EU243272, KM191834, EU384967, EU385441, EU385536, KM191940, EU385061. *Ianthopappus corymbosus* (Less.) Roque & D.J.N. Hind, KM192014, EU385369, EU385273, EU385177, EU243295, KM191848, EU384990, EU385464, EU385559, KM191957, EU385083. *Inula britannica* L., KJ434428, AY215812, AF384483, AF384737, AF383803, AY217263, AY215130, AY213809, EU385560, KM191958, AY216065/AY216190. *Layia heterotricha* (DC.) Hook. & Arn., KJ434429, AY215818, AF384489, AF384742, AF383809, AY217269, AY215136, AY213815, EU385562, KT345143, AY216071/AY216196. *Liabellum palmeri* (A. Gray) Rydb., KM192016, EU385373, EU385277, EU385181, EU243299, KM191850, EU384994, EU385468, EU385565, KM191960, EU385087. *Libanothamnus occultus* (S. F. Blake) Cuatrec., KT345004, KT345022, KT345058, KT345068, KT345077, KT345042, KT345086, KT345096, KT345113, KT345144, KT345164. *Macledium zeyheri* (Sond.) S. Ortiz, KM192019, EU385375, EU385280, EU385184, EU243302, KM191853, EU384997, EU385471, EU385568, KM191963, EU385090. *Marshallia caespitosa* Nutt. Ex. DC., KJ434430, AY215824, AF384496, L39458, AF383816, AY217274, AY215142, AY213820, KT345114, KM191964, AY216077/AY216202. *Menyanthes trifoliata* L., KM192020, KM192118, KM191897, KM192107, KM191875, KM191854, KM192096, KM191886, KM191918, KM191965, KM191908. *Montanoa revealii* H. Rob., KJ434433, AY215830, AF384502, AF384753, AF383822, AY217280, AY215148, AY213825, KT345115, KT345145, AY216083/AY216208. *Mutisia retrorsa* Cav., KT026446, EU385376, EU385281, EU385185, EU243303, KT026449, EU384998, EU385472, EU385569, KT026452, EU385091. *Nassauvia*

[illegible]

Fig.1

Fig.2

Fig.3