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Biogeography and diversification of Brassicales: A 103 million year tale

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ABSTRACT

Brassicales is a diverse order perhaps most famous because it houses Brassicaceae and, its premier member, *Arabidopsis thaliana*. This widely distributed and species-rich lineage has been overlooked as a promising system to investigate patterns of disjunct distributions and diversification rates. We analyzed plastid and mitochondrial sequence data from five gene regions (>8000 bp) across 151 taxa to: (1) produce a chronogram for major lineages in Brassicales, including Brassicaceae and *Arabidopsis*, based on greater taxon sampling across the order and previously overlooked fossil evidence, (2) examine biogeographical ancestral range estimations and disjunct distributions in BioGeoBEARS, and (3) determine where shifts in species diversification occur using BAMM. The evolution and radiation of the Brassicales began 103 Mya and was linked to a series of inter-continental vicariant, long-distance dispersal, and land bridge migration events. North America appears to be a significant area for early stem lineages in the order. Shifts to Australia then African are evident at nodes near the core Brassicales, which diverged 68.5 Mya (HPD = 75.6–62.0). This estimated age combined with fossil evidence, indicates that some New World clades embedded amongst Old World relatives (e.g., New World capparoids) are the result of different long distance dispersal events, whereas others may be best explained by land bridge migration (e.g., *Forchhammeria*). Based on these analyses, the Brassicaceae crown group diverged in Europe/Northern Africa in the Eocene, circa 43.4 Mya (HPD = 46.6–40.3) and *Arabidopsis* separated from close congeners circa 10.4 Mya. These ages fall between divergent dates that were previously published, suggesting we are slowly converging on a robust age estimate for the family. Three significant shifts in species diversification are observed in the order: (1) 58 Mya at the crown of Capparaceae, Cleomaceae and Brassicaceae, (2) 38 Mya at the crown of Resedaceae + *Stixis* clade, and (3) 21 Mya at the crown of the tribes Brassiceae and Sisymbrieae within Brassicaceae.

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1. Introduction

Brassicales is a morphologically diverse lineage of ~4700 species placed in 18 families (APGIII, 2009) and united by the presence of mustard oils or glucosinolates (Table 1; Rodman et al., 1993, 1994, 1996, 1998). Variation in growth form, habitat occupied, floral and fruit features is so pronounced that these families were traditionally placed in seven orders across three different subclasses (e.g., Cronquist, 1981). Due to the evolution of this "mustard oil bomb" (Lüthy and Matile, 1984; Stauber et al., 2012) which involves glucosinolates and the accompanying hydrolytic enzyme myrosinase often compartmentalized in special myrosin cells, the Brassicales have been a model group to evaluate the interplay of biochemical processes, natural selection involving deterring of herbivory, and myrosinase gene family evolution (Bekaert et al., 2012; Benderoth et al., 2006; Edger et al., 2015; Ehrlich and Raven, 1964; Hofberger et al., 2013; Prasad et al., 2012; Wheat et al., 2007; Wittstock et al., 2004). Because the largest family in the order, Brassicaceae, houses *Arabidopsis thaliana*, many studies focused on understanding the broader phylogenetic surroundings of this model plant (Hall et al., 2004; Rodman et al., 1993, 1996; Su et al., 2012). Examining the evolutionary development of flowers, for example, extends beyond studying *Arabidopsis*, and now involves explicit attempts to include the floral diversity portrayed by Brassicales (Bhide et al., 2014; Cheng et al., 2013; Patchell et al., 2011). Other economically important members are also in the order, such as papaya (*Carica papaya*, Caricaceae), mustard (*Brassica* and *Sinapis*, Brassicaceae), broccoli/cabbage/kale (*Brassica oleracea*, Brassicaceae), canola (*Brassica rapa*,

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Table 1
Members of the Brassicales including number of species, geographical range and sampling for this study.

Family	Genera/species (# in current study)	Geographical distribution
Akaniaceae	2/2 (2/2)	*Akania* – Australia *Bretschneidera* – Asia
Bataceae	1/2 (1/1)	Tropical America, Austral-Asia
Borthwickiaceae	1/1 (1/1)	China
Brassicaceae	338/3700 (26/35)	Cosmopolitan (mostly temperate)
Capparaceae	25/450 (23/49)	Cosmopolitan (mostly tropical)
Caricaceae	4/34 (1/1)	Tropical Africa and America
Cleomaceae	10/300 (8/29)	Cosmopolitan
Emblingiaceae	1/1 (1/1)	Southwest Australia
Forchhammeria	1/10 (1/3)	North and Central America
Gyrostemonaceae	5/18 (2/3)	Australia
Koeberliniaceae	1/1 (1/1)	North, Central, and South America
Limnanthaceae	2/10 (2/2)	North America
Moringaceae	1/13 (1/1)	Africa, Madagascar, and Asia
Pentadiplandraceae	1/1 (1/1)	Western Africa
Resedaceae	6/85 (5/8)	North Africa, Eurasia, North America
Salvadoraceae	3/12 (0/0)	Africa to Southeast Asia
Setchellanthaceae	1/1 (1/1)	North America
Stixis	1/7 (1/3)	India and Malaysia
Tirania	1/1 (1/1)	Southern Vietnam
Tovariaceae	1/2 (1/1)	Tropical America
Tropaeolaceae	3/92 (1/1)	North and South America

Brassicaceae), horseradish (*Armoracia rusticana*, Brassicaceae), caper (*Capparis spinosa*, Capparaceae), and moringa (*Moringa oleifera*, Moringaceae). Genomic resources abound with over 20 published genomes from the Brassicales (Arabidopsis Genome Initiative, 2000; Cheng et al., 2013; Dassanayake et al., 2011; Dorn et al., 2014; Edger et al., 2015; Haudry et al., 2013; Kagale et al., 2014a; Ming et al., 2008; Moghe et al., 2014; Slotte et al., 2013; Wang et al., 2011). The presence of at least three ancient whole genome duplications (WGD: reviewed in Barker et al., 2009; Franzke et al., 2011; Schranz and Mitchell-Olds, 2006; Schranz et al., 2006; Vision et al., 2000) makes the order a powerful system to investigate the role of WGD in angiosperm evolution (Schranz et al., 2012a,b).

Issues inherent in understanding the diversification of this order in form, time, and space are seen at many nodes in the emerging phylogenetic framework of Brassicales (Edger et al., 2015; Hall et al., 2004; Su et al., 2012). The woody Australasian Akaniaceae are sister to the mainly fleshy and scandent South American Tropaeolaceae, and these two families are then sister to all other families. The largely African Moringaceae (deciduous trees, shrubs, succulents) and South American Caricaceae (small trees or vines) are sister families and diverge next. The remainder of Brassicales is comprised of a group of five small families with uncertain relationships and a well-supported clade referred to as the "core Brassicales" (Hall et al., 2002, 2004; Rodman et al., 1996). The former includes the monotypic Setchellanthaceae (shrub from Mexico), the herbaceous Limnanthaceae from North America, the ditypic shrubby Koeberliniaceae from Mexico (and apparently having lost glucosinolates), and the closely related Bataceae (halophytic subshrubs) and Salvadoraceae (Old World shrubs or trees).

The core Brassicales contains two well-supported clades (Table 1; Hall et al., 2004; Su et al., 2012). The first comprises Capparaceae, Cleomaceae, and Brassicaceae, with the vast majority of species (~94%) within Brassicales placed in these three closely related families (Beilstein et al., 2010, and cited by others unfortunately restricted the term "core Brassicales" to only these three families). The second clade (hereafter referred to as "GRFT") comprises Gyrostemonaceae, Borthwickiaceae, Resedaceae, and three anomalous genera, *Forchhammeria*, *Stixis*, and *Tirania*, currently without family designation (Table 1; Hall et al., 2004; Su et al., 2012). The placements of African Pentadiplandraceae and Neotropical Tovariaceae relative to these two clades are less clear (Hall et al., 2004; Su et al., 2012). The Australian endemic Emblingiaceae is sister to all other core Brassicales.

Despite the presence of several species-rich clades within the Brassicales, explicit tests examining shifts in species diversification and/or extinction rates within the order have been limited in the context of a well-sampled phylogeny with appropriate fossil-based chronology and use of rate shift models that are allowed to vary through time in a diversity-dependent manner. Species diversification within Brassicales has been examined in the context of glucosinolate evolution and the corresponding coevolution of pierid cabbage butterflies, WGD, and the Cretaceous–Paleogene (K–Pg) event (previously referred to as the K–T event) (Beilstein et al., 2010; Benderoth et al., 2006; Edger et al., 2015; Prasad et al., 2012; Wheat et al., 2007), but many of these analyses used only limited taxon sampling and placeholders for most families. For example, the most recent study to date examining shifts in species diversification within Brassicales sampled 18 accessions from 14 of the 18 families, although this genomic approach did analyze 75,000 molecular characters (Edger et al., 2015). Moreover, the timing of such diversification events, whole genome duplications, glucosinolate evolution, the origin of *Arabidopsis thaliana* and close relatives is controversial because widely divergent dates exist for estimated ages within the order, especially Brassicaceae (Beilstein et al., 2010; Couvreur et al., 2010; Edger et al., 2015; Huang et al., 2016; Hohmann et al., 2015; Franzke et al., 2009, 2011).

No large-scale analysis of biogeographical relationships in the context of an age-structured phylogeny has been done, despite the fact that the apparent mid-Cretaceous age of the order (Bell et al., 2010; Magallón and Castillo, 2009; Magallón et al., 2015) and the widely distributed or strongly disjunct geographic patterns make this lineage an ideal system to investigate these phenomena. Of particular importance in Brassicales is the presence of multiple, intercontinental disjunctions, a pattern of intense biogeographical interest that has invoked vicariance or dispersal explanations (e.g., Antonelli et al., 2015; Axelrod, 1970; Berger et al., 2016; Buerki et al., 2011; Clayton et al., 2009; Cracraft, 1988; Crisp et al., 2011; Donoghue and Edwards, 2014; Givnish and Renner, 2004; Raven and Axelrod, 1974; Sanmartín and Ronquist, 2004; Spalink et al., in press, 2016; Sytsma et al., 2004, 2014). Within Brassicales, biogeographical analyses have been conducted within the amphi-Atlantic Caricaceae (Carvalho and Renner, 2012), cosmopolitan-distributed Cleomaceae (Feodorova et al., 2010), mainly Old World Resedaceae (Martín-Bravo et al., 2007, 2009), and Mexican Setchellanthaceae (Hernández-Hernández et al., 2013). These studies revealed complex patterns mostly driven by long-distance dispersal, which is consistent with some tropical amphi-Atlantic families (e.g., Bromeliaceae, Rapateaceae, Vochysiaceae; Berger et al., 2016; Givnish et al., 2000, 2004; Sytsma et al., 2004). However, other disjunct patterns in Brassicales may be explained by vicariance or by, often overlooked, intercontinental migration via four Northern Hemisphere land bridges (Brikiatis, 2014; Tiffney, 1985; Tiffney and Manchester, 2001; Wen, 1999). The more southerly Thulean North Atlantic Land Bridge (NALB) has been demonstrated to be important for other tropical families such as the Malpighiaceae, Fabaceae, and Rubiaceae (Davis et al., 2004; Christenhusz and Chase, 2013; Lavin and Luckow, 1993; Smedmark et al., 2010). Additional examinations in the Brassicales with multiple fossil calibrations are needed to identify whether long distance dispersal is the primary driving force in forming disjunct biogeographical patterns in the order.

In addition, we focus on two separate intercontinental disjunctions in the order that have yet to be investigated. First,

Forchhammeria is distributed in North and Central America and molecular data reveal this genus is more closely related to taxa with Old World affinities (Hall et al., 2004). In fact, *Forchhammeria* is placed in a polytomy of the GRFT clade that includes Asiatic *Stixis* plus *Tirania* and Resedaceae (Hall et al., 2004). Resedaceae is primarily distributed in Europe, Middle East and Africa, although one taxon (*Oligomeris linifolia*) is found in southwest North America (Martín-Bravo et al., 2007). This New World disjunct is derived within Resedaceae and its distribution appears to be the result of a long-distance dispersal event (Martín-Bravo et al., 2007, 2009). Second, within the Capparaceae, there is a strongly supported clade that encompasses multiple taxa endemic to Central and South America, including *Atamisquea*, *Belencita*, *Capparis* (in part: New World species only; recently segregated into numerous genera: Cornejo and Iltis, 2006, 2008a,b,c, 2010; Iltis and Cornejo, 2007, 2011), and *Steriphoma* (Hall, 2008; Hall et al., 2002). This New World capparoids group is found in a polytomy with two clades, one comprised of species found in Africa and the second with members widely distributed in Africa, Australia, Asia, Europe, and Madagascar (Hall, 2008; Hall et al., 2002). Thus, both focal clades represent New World clades with relatives in either Asia or Africa.

Finally, investigations of the age within Brassicales and especially Brassicaceae reveal substantial conflict in estimates depending on the study. The Brassicaceae originated 54–15 Mya depending on the analysis, a range broad enough to encompass diversification either during a cooling or warming event (reviewed in Couvreur et al., 2010; Franzke et al., 2011). A major challenge in dating Brassicaceae has been the lack of fossils for the family, resulting in some age estimates being based on synonymous substitution rates (Kagale et al., 2014b; Koch et al., 2000, 2001; Schranz and Mitchell-Olds, 2006), nodes calibrated from independent molecular-clock studies (Franzke et al., 2009), or fossil-calibrated approaches using fossils from other clades within Brassicales (Beilstein et al., 2010; Couvreur et al., 2010; Edger et al., 2015), from outside Brassicales altogether (Hohmann et al., 2015), or from a combination of within and outside Brassicales (Huang et al., 2016). Even the latter fossil-calibrated studies provide varying estimates for the crown age of Brassicaceae based on different taxa and gene coverage: ~54.3 Mya (Beilstein et al., 2010), ~42 Mya (Huang et al., 2016), ~38 Mya (Couvreur et al., 2010; Huang et al., 2016), ~32 Mya (Edger et al., 2015; Hohmann et al., 2015). Because our taxon sampling is extensive across the entire order, we are able to include previously overlooked fossils in Cleomaceae in addition to two fossil calibrations near the root of the order and one in crown group of Brassicaceae. Importantly, this approach enables us to examine how different taxon sampling and fossil calibrations may impact age estimates of Brassicaceae and the divergence of *Arabidopsis thaliana*, ages of much interest as they are applied in many other research endeavors.

Our goals here are: (1) Produce a well-sampled chronogram for the Brassicales using fossil calibrated relaxed molecular clock dating and to estimate ages of lineages within Brassicales, with emphasis on core Brassicales and *Arabidopsis*, as well as ages of WGD events within the order. (2) Reconstruct ancestral ranges within the order using more explicit likelihood approaches now available (BioGeoBEARS; Matzke, 2013), and specifically investigate alternative hypotheses explaining two disjunct distributions in the order, *Forchhammeria* and New World capparoids. And, (3) determine where significant shifts in species diversification (and extinction) have occurred, using for the first time in Brassicales an approach (BAMM – Bayesian Analysis of Macroevolutionary Mixtures; Rabosky, 2014) where rate shifts are allowed to vary through time in a diversity-dependent manner. To resolve relationships in the order, a necessary framework for determining ages of relevant clades, reconstructing biogeographic history, and measuring rates of diversification, we sample extensively across the order and include DNA sequence information (~8000 bp) for five genes from the plastid and mitochondrial genomes.

2. Materials and methods

2.1. Taxon sampling

We sampled 145 taxa of Brassicales, representing 18 of the 19 members of the order (Table 1; see Supplementary Information Table S1). The small family Salvadoraceae was the only lineage not included for the molecular phylogenetic analysis, but it was later added for both the biogeographic and diversification analyses. Because generic boundaries are problematic in both Capparaceae and Cleomaceae (Feodorova et al., 2010; Hall, 2008; Hall et al., 2002; Patchell et al., 2011, 2014), our strategy emphasized sampling identified major clades, not genera. In total, 48 and 28 species were included from Capparaceae and Cleomaceae, respectively. Thirty-five members of Brassicaceae were sampled, including *Aethionema*, which is sister to the rest of the family (Beilstein et al., 2006; Hall et al., 2002). The supermatrix tree of Brassicaceae (Couvreur et al., 2010) was used to sample widely across major lineages. A recent study in the Cornales (Xiang et al., 2011) demonstrated that Bayesian approaches for molecular dating are not sensitive to taxon sampling, at least for determining the age of nodes between families, which is the emphasis of this study. Thus, this sampling was sufficient to determine the age of Brassicaceae. Six representatives of Malvales were designated as outgroups because this order is sister to Brassicales (APGIII, 2009; Soltis et al., 2011; Worberg et al., 2009).

2.2. DNA extraction, amplification, and sequencing

Five coding regions were investigated: three cpDNA (*ndhF*, *matK*, and *rbcL*) and two mtDNA (*rps3*, and *matR*). We built upon our previously published cpDNA data sets (Hall, 2008; Hall et al., 2002, 2004; Rodman et al., 1998), generated two new mtDNA data sets, and included sequences and genes isolated from plastomes available on GenBank. Sequencing of *ndhF*, *matK*, and *rbcL* was conducted following our previous studies in Brassicales (Hall, 2008; Hall et al., 2002, 2004). A gene embedded in the intron of two *trnK* exons, *matK* sequences were generated using primers from (Koch et al., 2001). The 3′ half of *ndhF* was amplified and sequenced from primers previously described (Beilstein et al., 2006; Olmstead et al., 1993). Gene amplification and sequencing for *rbcL* followed the protocol of Conti et al. (1997). The *rps3* region was amplified in two reactions using primers F1/R1.5 and F2/R1 and was sequenced using a combination of primers: F1, F3, F2, R1.5, R12, and R1 (Davis et al., 2007; Wurdack and Davis, 2009). The *matR* region was amplified using primers 26F and 1858R and sequenced with primers 26F, 1002R, 879F, and 1858R (Davis and Wurdack, 2004). All primer sequences are provided in Supplementary Information Table S2. A total of 245 new sequences were generated for this study: 10 *ndhF*, 6 *matK*, 80 *rbcL*, 83 *rps3*, and 66 *matR* sequences (see Supplementary Information Table S1).

All PCR products were cleaned with QIAquick PCR purification columns (Qiagen, Inc.). Both strands were cycle sequenced using BigDye then cleaned with Performa DTR V3 96-well Short Plate Kit (Edge BioSystems, Gaithersburg, MD, USA), and sequenced using an ABI-3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Chromatograms were edited and initially aligned using Sequencher 4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA).

2.3. Phylogenetic analysis

Sequences were codon aligned using the *Arabidopsis thaliana* sequence information in MacClade v4.08 (Maddison and Maddison, 2000) and Mesquite v2.5 (Maddison and Maddison, 2008). As previous studies demonstrated that excluding or including the noncoding regions of the *matK*/*trnK* does not affect either topology or support (Hall, 2008), non-coding regions were excluded from all analyses. In addition, the ends of aligned sequences were trimmed so that regions with high number of missing data for the majority of taxa were excluded from analyses.

Initial phylogenetic analyses were conducted on individual data sets to assess congruence between gene regions. Specifically, maximum parsimony (MP) bootstrap analyses (BS) were run in PAUP* v4.0b10 (Swofford, 2000) for 1000 replicates with simple addition, tree-bisection and rearrangement (TBR), and examining maximum 1000 trees per replicate. Topologies from individual analyses were considered similar based on visual comparison of clades with greater than 70% MP bootstrap values (Mason-Gamer and Kellogg, 1996; Seelanan et al., 1997), a useful approach for determining areas of incongruence between data sets (e.g., Daru et al., 2013; Merckx et al., 2013; Scheunert and Heubl, 2014). Because no branches with support of 70% BS or greater were in conflict with each other across all individual data sets, detailed analyses were only conducted on the following combined data sets: (1) cpDNA (*ndhF*, *matK*, and *rbcL*), including the 108 taxa that have sequences for all three genes, (2) all five genes (cpDNA plus the *matR* and *rps3* data sets), including all 151 taxa such that some sequences are missing for some taxa (Table 1). Uncertainty of analyses due to inclusion of partial sequence data is not expected to obscure relationships between taxa (Burleigh et al., 2009; Galtier and Daubin, 2008; Sanderson et al., 2010; Wiens, 2006).

Bayesian inference (BI) analyses were conducted in MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). Models of molecular evolution for the individual data sets were determined using the Akaike Information Criterion (AIC) as implemented in MrModeltest v2 (Nylander, 2004). Model parameters were estimated in all analyses. Each Markov chain Monte Carlo (MCMC) run for the Bayesian analyses included two independent runs of 2 million generations, with each run comprised eight separate chains per run and temperature of 0.1 (default is 0.2). We used default priors and sampled trees every 100 generations. Runs were stopped when the average standard deviation of split frequencies reached 0.01. We also verified that Potential Scale Reduction Factor (PSRF) values were close to 1.0 and effective sample size (ESS) values were >200 as assessed in Tracer v1.5 (Rambaut and Drummond, 2009). We applied a burnin of 25%.

2.4. Evaluation of fossil calibrations

For the evaluation of fossil calibrations, we followed the specimen-based approach for assessing paleontological data described by Parham et al. (2012). Using their suggested best practices, we accepted four fossils from Brassicales for node calibrations in our molecular dating analyses (Table 2). See Supplementary Information Table S3 for detailed evaluation of fossil information, including (1) citation of museum specimens, (2) apomorphy-based diagnosis for phylogenetic placements, (3) reconciliation of morphological and molecular data, (4) locality and stratigraphy of fossils, (5) referenced stratigraphic age, and (6) additional remarks. These fossils are placed on both deep and derived positions within the Brassicales (Supplementary Information Fig. S1). First, *Thlaspi primaevum* (Becker, 1961) was assigned to the *Thlaspi-Alliaria* node within Brassicaceae. This winged fruit fossil has been used in several molecular dating analyses (Beilstein et al., 2010; Huang et al., 2016; Koch, 2012), although both its generic affinity and age boundary have been questioned (Franzke et al., 2011). We consider the generic inclusion of *T. primaevum* strongly supported by fruit morphology and wing venation (Becker, 1961; Manchester and O'Leary, 2010) and distinctive concentrically striated seeds (Beilstein et al., 2010). This calibration is placed at the *Thlaspi-Alliaria* node following the presumed origin of striated seeds (Beilstein et al., 2010), which are present in both genera. Second, we assigned *Palaeocleome lakensis* (Chandler, 1962) to the stem node of Cleomaceae. This fossil was not assessed by Beilstein et al.'s (2010) influential review of brassicalean fossils and has yet to be used in any molecular-based dating analysis. These camplyotropous seeds have invaginated seeds clefts with extended radicular claws and tuberculed testa, which epitomize the unique seed structure of Cleomaceae (Iltis et al., 2011; Supplementary Information Table S3). Third, *Akania* leaf fossils, a set of fossils that are without controversy, were assigned to the *Akania-Bretschneidera* node (Gandolfo et al., 1988; Iglesias et al., 2007; Romero and Hickey, 1976). Fourth, the charcoalified flower *Dressiantha bicarpellata* was assigned as an earlier diverging lineage of Brassicales (Gandolfo et al., 1998). The precise placement of this fossil is uncertain, but it appears to have brassicalean affinities (Beilstein et al., 2010; Ronse De Craene and Haston, 2006).

2.5. Divergence date estimation

To account for rate variation among lineages, divergence date estimates were assessed under a relaxed molecular clock using Bayesian methods (Drummond et al., 2012). We implemented an uncorrelated lognormal (UCLN) model of rate evolution in the program BEAST v1.7.5 to simultaneously estimate a phylogeny and divergence times. Fossil calibrations were constrained as lognormal distributions where the 95% upper limit was equal to the fossil's stratigraphic age plus 10%, to account for the reasonable assumption that the node a fossil calibrates is older than the fossil's first recorded appearance (Ho and Phillips, 2009). To explore the influence of our fossil calibrations on age estimates, we conducted six separate analyses testing the inclusion of various fossil combinations (Table 2). Numerous preliminary runs determined the parameters essential to yield an effectively sampled and time-conscious search.

XML files were created in BEAUti v1.7.5 (part of the BEAST distribution) as a partitioned five-gene data set, with independent nucleotide substitution models estimated under GTR + Γ for *matR* and GTR + I + Γ for *ndhF*, *matK*, *rbcL*, and *rsp3*. Divided into plastid and mitochondrial regions, two UCLN relaxed clock models were used to estimate the rate of molecular evolution and rate variation parameters. UCLN mean rate priors were set as uniform distributions from 0 to 1.0×10^{100} (effectively positive infinity), with starting values specified at 8.3×10^{-4} for cpDNA and 2.0×10^{-4} for mtDNA. A single-tree model was evaluated under the Yule process of speciation (Yule, 1925) that started from a randomly generated tree. The crown and stem (root) of the Brassicales were given height priors with a uniform distribution from 0 to 125 Mya, such that those nodes could not be older than the earliest recorded evidence of eudicot fossils (Brenner, 1996; Sun et al., 2011). For each of our six analyses (Table 2), we initiated two independent MCMC runs for 100 million generations, sampling every 10,000 steps. Runs were assessed for convergence and ESS > 200 using Tracer v1.5 (Rambaut and Drummond, 2009). If convergence and ESS were satisfied then runs were combined after a 10% (10 million generation) burn-in using LogCombiner v1.7.5, and summarized in TreeAnnotator v1.7.5 (both part of the BEAST distribution) to produce a maximum clade credibility tree with mean ages.

Table 2
Fossil calibration table, with analysis sets (use indicated by '✠'). The tmrca (time [since] most recent common ancestor) priors were designed so each node's 95% highest posterior density (HPD) is constrained to a lognormal distribution with an upper limit equal the fossil's age +10% Mya (million years). Prior values are provided as directly entered into BEAUti v1.7.5.

	Fossil taxon, minimum age	Node calibrated	95% HPD upper limit	Lognormal tmrca prior	Analysis set					
					1	2	3	4	5	6
1	*Thlaspi primaevum* 30.8 Mya	*Thlaspi/Alliaria*	33.88 Mya	log(mean) = 0.304 log(stdv) = 0.5 offset = 30.8	✠	✠	–	✠	✠	–
2	*Palaeocleome lakensis* 47.8 Mya	Cleomaceae/Brassicaceae	52.58 Mya	log(mean) = 0.743 log(stdv) = 0.5 offset = 47.8	✠	✠	✠	✠	–	–
3	*Akania* sp. 61.7 Mya	*Akania/Bretschneidera*	67.87 Mya	log(mean) = 0.998 log(stdv) = 0.5 offset = 61.7	✠	✠	✠	–	✠	✠
4	*Dressiantha bicarpellata* 89.8 Mya	Caricaceae + Moringaceae/remaining Brassicales	98.78 Mya	log(mean) = 1.373 log(stdv) = 0.5 offset = 89.8	✠	–	✠	✠	✠	✠

2.6. Reconstructing biogeographical history

Ancestral range estimation (ARE) was conducted to estimate possible historical patterns of geographical distribution across Brassicales. To improve lineage sampling for biogeographical analyses, we added two tips to the maximum credibility tree from BEAST using the bind.tip function of the *phytools* package (Revell, 2012) for R v3.1.1 (R Development Core Team, 2014). *Salvadora* (Salvadoraceae) was placed sister to Bataceae (Rodman et al., 1996, 1998). *Hesperis* (Brassicaceae) was added as a placeholder for one of the major unsampled clades (Lineage III) in Brassicaceae and placed as in Couvreur et al. (2010). Biogeographic distributions of species were compiled from literature (e.g., Elffers et al., 1964; Hall, 2008; Hewson, 1982, 1985; Kubitzki and Bayers, 2003; Martín-Bravo et al., 2009; Rollins, 1993) and web-based resources (Global Biodiversity Information Facility, www.gbif.org; Stevens, 2001 onwards). For these analyses, we categorized seven major areas of distribution: (1) North America, including Mexico, (2) Central and South America, (3) Africa (south of the Saharo-Arabian area), (4) Madagascar, (5) Europe (west of the Urals and Caucasus), Mediterranean Africa, Saharo-Arabian, Irano-Turanian, (6) Asia (east of the Urals, Caucasus, and Irano-Turanian) east to Wallace Line, and (7) Australia west to Wallace Line and east through Polynesia. Tip taxa representing a more diverse and widespread lineage were coded to cover the maximal distribution of the lineage, unless the lineage more recently extends into an area (e.g., *Forchhammeria* into Central America). The inclusion of only six placeholders (four families: Bixaceae, Dipterocarpaceae, Malvaceae, Thymelaeaceae) for the outgroup Malvales presents difficulty in ARE. Relationships within the order are still unresolved, and thus biogeographical patterns remain uncertain. Additionally, except for Dipterocarpaceae, the other families sampled are widespread making biogeographical scoring difficult for these placeholders. Due to the challenges in scoring the under-sampled outgroup order for geographic areas, we restricted ARE to the Brassicales.

Ancestral range estimation for the Brassicales included the nested DEC and DECJ models in BioGeoBEARS (Matzke, 2013, 2014) with R v3.1.1 (R Development Core Team, 2014). The Dispersal-Extinction Cladogenesis (DEC) ML approach is based on that used in LaGrange (Ree and Smith, 2008), but the DECJ model further incorporates cladogenetic "founder-events" with the J parameter for "jump-dispersals." We allowed the inferred ancestors to occupy up to four areas (sampled tips only occupy up to three areas). Dispersal probabilities between pairs of areas were specified for four separate time slices based on known geological events similarly analyzed elsewhere (e.g., Berger et al., 2016; Buerki et al., 2011; Drew and Sytsma, 2012; Sessa et al., 2012; Spalink et al., in press; Sulman et al., 2013). The resulting ML score for the more parameter-rich DECJ model was tested for significance against the resulting ML score of the DEC model.

2.7. Identifying rate shifts in species diversification

We used the program BAMM (Bayesian Analysis of Macroevolutionary Mixtures; Rabosky, 2014; Rabosky et al., 2014) to estimate rates of speciation (λ), extinction (μ), and net diversification (r) within Brassicales with a specific focus to identify nodes exhibiting significant rate shifts in speciation. BAMM accounts for incomplete sampling by analyzing the proportion of tips sampled for a given clade. Tips were assigned to the smallest possible taxonomic unit, which ranged from the genus to family level. Following methods described for the biogeographical analyses, we added *Salvadora* to the BEAST tree. To more proportionally sample within the main lineages of Brassicaceae described by Couvreur et al. (2010), we also used the bind tip function in *phytools* to place eight other genera of Brassicaceae (*Anchonium*, *Braya*, *Calepina*, *Clausia*, *Crambe*, *Eruca*, *Hesperis*, *Parrya*). Due to the present lack of phylogenetic detail or resolution within the three large families, Cleomaceae, Capparaceae, and Brassicaceae were only partitioned into two, five, and four terminals, respectively. Species numbers were obtained from published sources as described above and a total of 4743 species were estimated for Brassicales, updating previous numbers (Edger et al., 2015; Stevens, 2001; Soltis et al., 2011). Supplementary Information Table S4 provides the list of 32 terminals used in the BAMM analysis, sampled accessions belonging to each, and the proportion sampled for each terminal.

Priors for BAMM were obtained with BAMMtools v2.0.2 (Rabosky, 2014) by providing the BEAST maximum clade credibility tree and total species numbers across Brassicales. Two independent MCMC chains of 100 million generations were run in BAMM and convergence was assessed by computing the ESS of log likelihoods, as well as the number of shift events present in each sample using the R package coda v0.16-1 (Plummer et al., 2006). After removing 10% of trees as burn-in, we analyzed the BAMM output using BAMMtools and computed the 95% credible rate shift configurations using Bayes factors. We also estimated the best shift configuration with the highest maximum a posteriori probability. Rates-through-time plots were generated for λ, μ, and r for all Brassicales, for just clades identified as having significant rate shifts in speciation, and for all Brassicales minus these identified clades.

3. Results

3.1. Phylogenetic analysis

The cpDNA and mtDNA combined matrix was aligned to 8159 characters, 4670 bp of cpDNA and 3486 bp of mtDNA (Table 3). Topologies are consistent across analyses and data sets. Overall relationships across the order are well supported, with relationships amongst a few lineages remaining elusive (Fig. 1). For example, there is no support for positions of Setchellanthaceae and Limnanthaceae (Fig. 1). Core Brassicales are monophyletic (100% PP, 92% MP BS) with Emblingiaceae sister to remaining members of this clade (100% PP, 82% MP BS). Within the core Brassicales, the GRFT clade is monophyletic (100% PP, 99% MP BS) with Gyrostemonaceae strongly supported as sister to remaining GRFT members (100% PP, 84% MP BS). Within the GRFT clade Resedaceae, *Forchhammeria*, *Stixis*, and *Tirania* form a clade with relationships amongst them poorly supported except a sister relationship between the latter two genera.

All families with more than one taxon sampled are strongly supported as monophyletic (100% PP and >95% MP BS), as are the orphan genera for which multiple species were included (Fig. 1). Within the monophyletic Capparaceae (100% PP, 91% MP BS), *Crateva* and *Euadenia* are sister to remaining members of family. Within the remainder of the family, a paraphyletic assemblage of all Old World (African, Asian, European) genera and species of *Capparis* exists although some branches are weakly supported. However, a strongly supported New World clade is derived out of this Old World assemblage and includes all of the recently segregated New World capparoid genera (Fig. 1). Thus, the capparoid clade exhibits strong biogeographic signal. Cleomaceae, like Capparaceae, is also monophyletic (100% PP, 98% MP BS) with *Cleome droserifolia* plus *Cleomella* sister to remaining members of the family. Brassicaceae is strongly supported as monophyletic (100% PP and MP BS) with the tribe Aethionemeae sister to remaining members of the family (100% PP and MP BS). Lineage I is monophyletic although with no branch support. Expanded Lineage II is not monophyletic with current sampling as one member of Lineage III (*Lobularia*) is in a polytomy with taxa from Expanded Lineage II.

3.2. Molecular dating

Overall, divergence date estimates were robust to which fossil calibrations were included (Table 2, Supplementary Information Table S5). The average difference of median dates across 39 nodes was 3.4 Myr (Supplementary Information Table S5), including the three nodes that varied substantially across calibrations (see below). This consistency is important to highlight, especially when considering our use of more contentious fossils: controversial *Thlaspi* and relatively untested *Palaeocleome*. However, exclusion of these two fossils either individually or in combination did not result in substantial differences in age estimates (range from 0 to 13.7 Myr; Supplementary Information Table S5). To illustrate this point, we compared the dates using all four calibrations to those excluding both *Thlaspi* and *Palaeocleome*, which resulted in similar estimates of key nodes: core Brassicales (75.6 – **68.5** – 62.0 versus 72.1 – **64.4** – 56.1; median age bolded, range is the 95% highest posterior density [HPD]), crown age of Capparaceae (57.8 – **49.5** – 39.2 versus 55.8 – **46.0** – 36.1), crown age of Cleomaceae (48.7 – **43.2** – 37.3 versus 47.1 – **39.8** – 32.7), and crown age of Brassicaceae (46.6 – **43.4** – 40.3 versus 44.1 – **37.7** – 31.4).

Only three nodes varied in divergence times across analyses, using the criterion of age estimates falling outside the HPD (Supplementary Information Table S5). When *Akania* was not included as a calibration, the estimated ages of Akaniaceae and Caricaceae plus Moringaceae were dramatically younger. When all fossils were included, Akaniaceae was estimated at an age of 75 Mya (HPD = 86.6–66.1) compared to 35.7 Mya (HPD = 59.5–16.1) when the fossil *Akania* was removed from analysis. A comparable shift was seen in Caricaceae plus Moringaceae lineage with an estimated age of 64.1 Mya (HPD = 66.3–62.4) when all four fossils were included and of 11.6 Mya (HPD = 21.0–4.3) without the *Akania* calibration. Estimates of clade age were younger for the *Thlaspi-Alliaria* split (Brassicaceae) when the *T. primaevum* fossil was excluded as a calibration. When *T. primaevum* was included, the *Thlaspi-Alliaria* split was estimated at 31.8 Mya (HPD = 32.7–31.1). In contrast, when this fossil was excluded either in combination with *Palaeocleome* or by itself, the age of this node was estimated younger at 18.1 Mya (HPD = 24.9–10.8) and 18.8 Mya (HPD = 26.0–10.7), respectively.

Here we present results from the BEAST analysis that included all four fossil calibrations (Fig. 2; Supplementary Information Table S3) given that node estimates were consistent within 5 (most) to 10 Myr across median dates. The supplemental material provides information on estimated ages for 43 nodes across the Brassicales and across analyses with different calibrations (Supplementary Information Table S5 and Fig. S1). The crown age of Brassicales is estimated to have diverged in the Early Cretaceous (102.8 Mya, HPD = 112.6–94.4) and the core Brassicales diverged at 68.5 Mya (HPD = 75.6–62.0). The crown age of the GRFT clade diverged in the Eocene at 53.5 Mya (HPD = 62.8–44.3), whereas the split between *Forchhammeria* and Resedaceae occurred at 36.7 Mya (HPD = 45.9–28.6). Capparaceae, excluding *Crateva* plus *Euadenia*, diverged in the late Eocene at 34.8 Mya (HPD = 42.3–28.0). New World capparoids diverged in the Oligocene at 24.0 Mya (HPD = 29.6–18.8). Brassicaceae crown group diverged in the Eocene at 43.4 Mya (HPD = 46.6–40.3). *Arabidopsis thaliana* diverged from other species of *Arabidopsis* during the late Miocene at 10.4 Mya (HPD = 15.7–5.5).

3.3. Reconstructing biogeographical history

In BioGeoBEARS, significant improvement in the likelihood score of the model was seen when the "jump dispersal" parameter was added (DECJ) vs. without (DEC), as indicated by a likelihood ratio test (DEC LnL −357.349, DECJ LnL = −346.920, df = 1, $P = e-5$). Because of this significant improvement in model likelihood with the jump dispersal parameter, only ancestral range estimation under the DECJ model is presented. The resulting parameters of the DECj models included: anagenetic dispersal rate $d = 0.0097$; extinction rate $e = 0$; and cladogenetic dispersal rate $j = 0.0639$. ARE on the Brassicales BEAST chronogram using BioGeoBEARS is shown in Fig. 3, which depicts area or combined areas with highest probability shown for each node and corner. Area probabilities of many basal or backbone nodes/corners are not strong, in which case pies are added showing proportion of probable areas (Fig. 3). The BEAST chronogram with pies illustrated for all nodes/corners is shown in Supplementary Information Fig. S2.

Brassicales have a complex biogeographical history as revealed by ARE (Fig. 3; Supplementary Information Fig. S2). Biogeographical history at the crown of the Brassicales is unresolved but likely involved North America, Africa, Australia, and perhaps South America. All four of these continental areas play important roles in subsequent but early diversification of the order. North America appears to be a significant area during the Late Cretaceous (~95–70 Mya) especially in the stem lineages of Setchellanthaceae, Limnanthaceae, Bataceae, and Koeberliniaceae. A shift to Australia (~80–75 Mya) and then Africa (~70–65 Mya) are evident at nodes near the core Brassicales, although ARE is not definitive at these nodes. Australia is significant for the early diverging lineage of the core Brassicales, Emblingiaceae. Within the core Brassicales,

Table 3
Data set characteristics.

Characteristic	*ndhF*	*matK*	*rbcL*	*rps3*	*matR*	cpDNA	mtDNA	cpDNA + mtDNA
Taxa number	142	145	148	107	76	140	111	151
Genome	cpDNA	cpDNA	cpDNA	mtDNA	mtDNA	n/a	n/a	n/a
Aligned length (bp)[a]	1185	2077	1408	1670	1816	4670	3486	8156
Parsimony informative characters (%)	512 (43.2%)	912 (43.9%)	350 (24.9%)	307 (18.4%)	146 (8.0%)	1774 (38.0%)	453 (13.0%)	2227 (27.3%)
AIC selected model	GTR + I + Γ	TVM + I + Γ	GTR + I + Γ	GTR + I + Γ	TPM1uf + Γ	n/a	n/a	n/a
Model implemented	GTR + I + Γ	GTR + I + Γ	GTR + I + Γ	GTR + I + Γ	GTR + Γ	n/a	n/a	Partitioned by gene

[a] Excluding trimmed edges and the non-coding region of *matK*/*trnK*.

ARE of the stem lineages leading up to the GRFT clade is complex with Tovariaceae, Pentadiplandraceae, and Gyrostemonaceae each restricted to the New World, Africa, and Australia, respectively. Australia is resolved as the ARE for the crowns of the GRFT clade (~53 Mya) and the first diverging Gyrostemonaceae, but with an immediate dispersal event to Asia in the early Eocene (~53–48 Mya) for the remainder of the GRFT clade. During the Oligocene, migration events from Asia to the Northern Hemisphere likely occurred twice, once to North American *Forchhammeria* and once to European Resedaceae.

The lineage leading to three largest families, Capparaceae, Cleomaceae, and Brassicaceae was likely African during the Paleocene (Fig. 3). Capparaceae has a strong African ancestry with two subsequent dispersals between 34 and 22 Mya (Oligocene–Miocene) to Australasia and to the New World. Concerning the latter, the phylogenetic and biogeographical results show clearly for the first time a strongly supported North and South American clade of New World capparoids (including *Anisocapparis*, *Atamisquea*, *Belencita*, *Calanthea*, *Capparicordis*, *Capparidastrum*, *Colicodendron*, *Cynophalla*, *Monilicarpa*, *Morisonia*, *Preslianthus*, *Quadrella*, and *Steriphoma*) arising out of an Old World paraphyletic clade (ancestrally Africa) of the remaining *Capparis* and all other genera of the Capparaceae. Within the Old World Capparaceae, three recent (late Miocene) dispersals from Africa to Madagascar are seen.

In contrast, there was a shift in the Eocene (~51 Mya) from Africa to Europe (and North Africa) in the lineage leading to Brassicaceae plus Cleomaceae (Fig. 3). The Brassicaceae retained the Europe/North Africa distribution for much of its diversification, although repeated migrations to North America and Asia occurred subsequently. Early diversification within Cleomaceae included migrations from Europe to North America in the mid-Eocene, and later radiations from Europe to both Africa and to South America/North America in the Oligocene.

3.4. Species diversification in Brassicales

BAMMtools indicated a conservative poissonRatePrior of 1.0 thus favoring fewer distinct evolutionary regimes on the tree. Convergence of the MCMC chains in the BAMM analyses was seen after discarding the burn-in and ESS were >900 for both the number of shifts and log likelihoods. BAMM analyses supported a diversity-dependent speciation process across Brassicales with a net diversification rate (**r = 0.09 species/Myr**; 95% quantile = 0.08–0.10), speciation rate (**λ = 0.23 species/Myr**; 95% quantile = 0.16–0.32), and extinction rate (**μ = 0.14 species/Myr**; 95% quantile = 0.06–0.24). The 95% credible set of rate shift configurations sampled with BAMM included 94 distinct ones of which the configuration with the highest probability (0.44) exhibited three shifts (Fig. 4A). The next most probable (0.12) configuration had three shifts, but with the third shift along a nearby branch relative to that seen in the most probable configuration set (Fig. 4A). The three shifts detected within Brassicales were (**shift 1**) along the stem leading to the crown clade of Capparaceae, Cleomaceae, and Brassicaceae in the early Eocene (~62.4 Mya); (**shift 2**) along the stem leading to the crown GRFT clade in the early Eocene (~57.7 Mya); and (**shift 3**) within the Brassicaceae near the crown of the tribes Brassiceae and Sisymbrieae of "Lineage 2" of Couvreur et al. (2010) near the Oligocene–Miocene border (~21.1 Mya). Each of the three shifts occurred in different biogeographical areas: Africa, Australia (and/or Asia), and Europe, respectively (see Fig. 3). Bayes factor evidence for rate shifts on each specific branch was strongest for shift 1 (bf = 47,000) and considerably less for shifts 2 (bf = 453) and shift 3 (bf = 459). Although less likely, alternative placements of shift configurations were sometimes in other, but nearby, branches (see Fig. 4A). Examination of the first nine distinct configurations (accounting for 83.5% probability), shows that shift 1 is found in all nine, shift 2 is found in eight of the nine and sometimes on nearby branches, and shift 3 is found in seven of the nine and sometimes on nearby branches.

Rate-through-time plots for λ, μ, and r across the 103 Myr history of the Brassicales are shown in Fig. 4B. Speciation rate decreased in the order until the early Eocene (~62 Mya) when the first of the three rate shifts occurred. Extinction rate showed no increase until the early Eocene and then showed steady increase. Net diversification, therefore, mirrored speciation rates through time but with a flatter rate increase since the Eocene due to increased extinction. The contribution of the three largest families (Capparaceae, Cleomaceae, and Brassicaceae), the first significant rate shift within the order, is shown in Fig. 4C. Most of the speciation rate increase in the Brassicales over the last ~62 Myr is attributed to this one clade (average λ = 0.30 species/Myr). The rate-through-time plot of speciation in Brassicales remains almost flat over the last ~62 Myr (average λ = 0.18 species/Myr) when removing the contribution of this large clade.

4. Discussion

4.1. Brassicales: the emerging story of phylogenetic relationships, vicariance, dispersal, and diversification across the continents

Our results, which are based on the largest assemblage of taxa, genes, and fossil priors for the order Brassicales, provide strong evidence for phylogenetic relationships among and within 17 of the 18 families. They also provide the temporal framework for identifying key biogeographical shifts in distribution and diversification rates. Thus, we fulfilled all three of our initial goals: (1) Generate a well-sampled chronogram for the Brassicales using rigorous dating approaches and multiple fossils, with emphasis on core Brassicales and *Arabidopsis*. (2) Reconstruct ancestral areas using explicit likelihood approaches for the first time to determine early biogeographical shifts in the order and specifically support hypotheses explaining two disjunct distributions involving *Forchhammeria* and New World capparoids. And, (3) identify where changes in diversification rates occur in Brassicales using an approach where rate shifts are allowed to vary through time in a diversity-dependent manner. For each of these objectives, the results provide clarification on these long-standing issues or support new conclusions on the evolutionary history, biogeographical vicariance vs. dispersal, and rates of species diversification of this remarkable lineage.

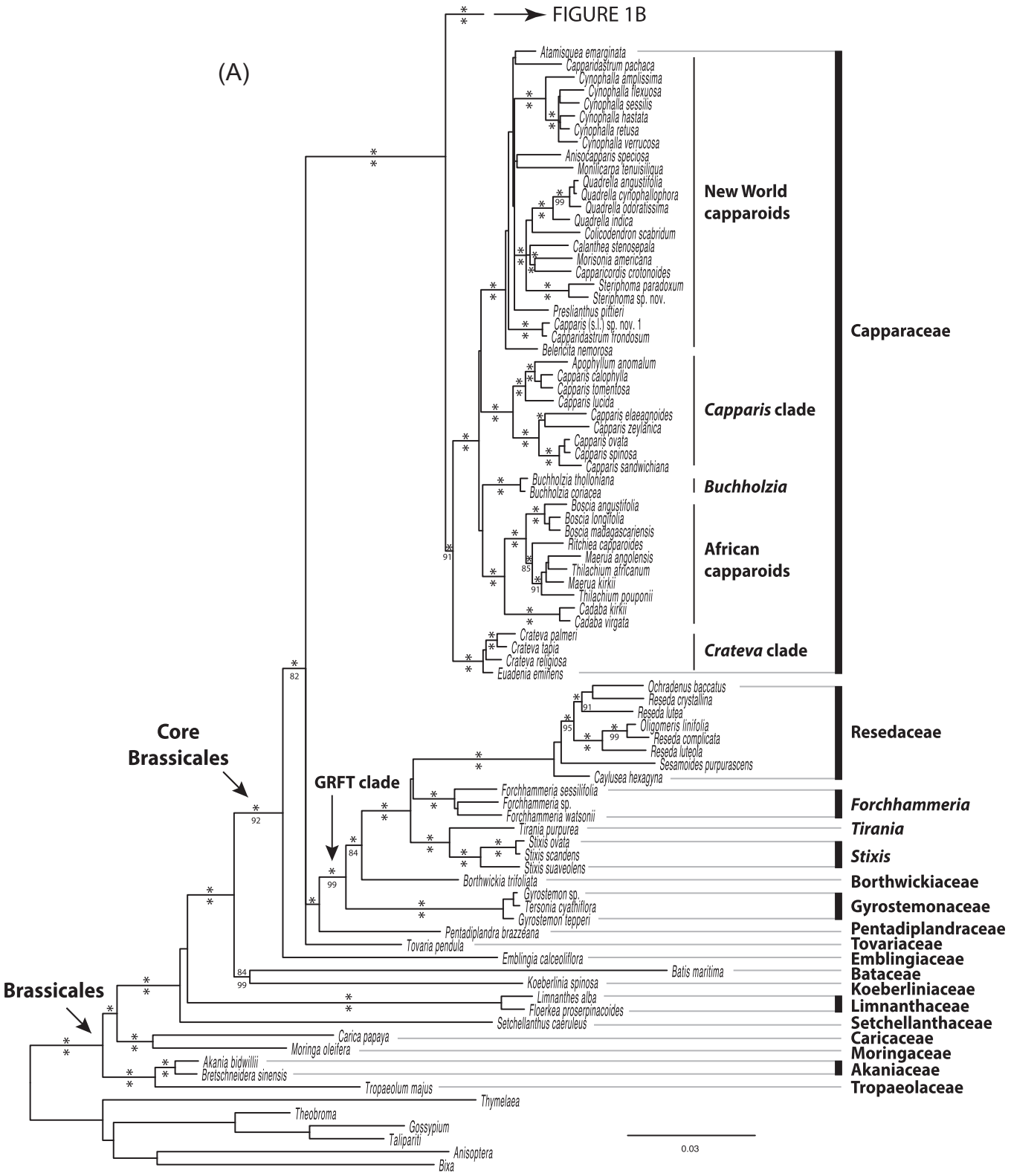

Fig. 1. Bayesian 50% majority-rule consensus tree of Brassicales inferred from cpDNA (*ndhF*, *matK*, *rbcL*) and mtDNA (*matR*, *rps3*) sequence data. Posterior probabilities (PP) and maximum parsimony bootstrap (MP BS) values are indicated above and below branches, respectively. Asterisks (∗) represent 100% PP or MP BS. Families or genera are indicated at right.

4.2. Phylogenetic relationships within Brassicales

Increased character and taxonomic sampling resulted in a well-supported phylogeny of Brassicales that is consistent with previous studies (Fig. 1; Edger et al., 2015; Hall et al., 2004; Rodman et al., 1993, 1994, 1996, 1998; Su et al., 2012). The most recent phylogenomic study had a final alignment of nearly 2.5 million bp (74,579 informative characters) and had 100% support for all nodes except

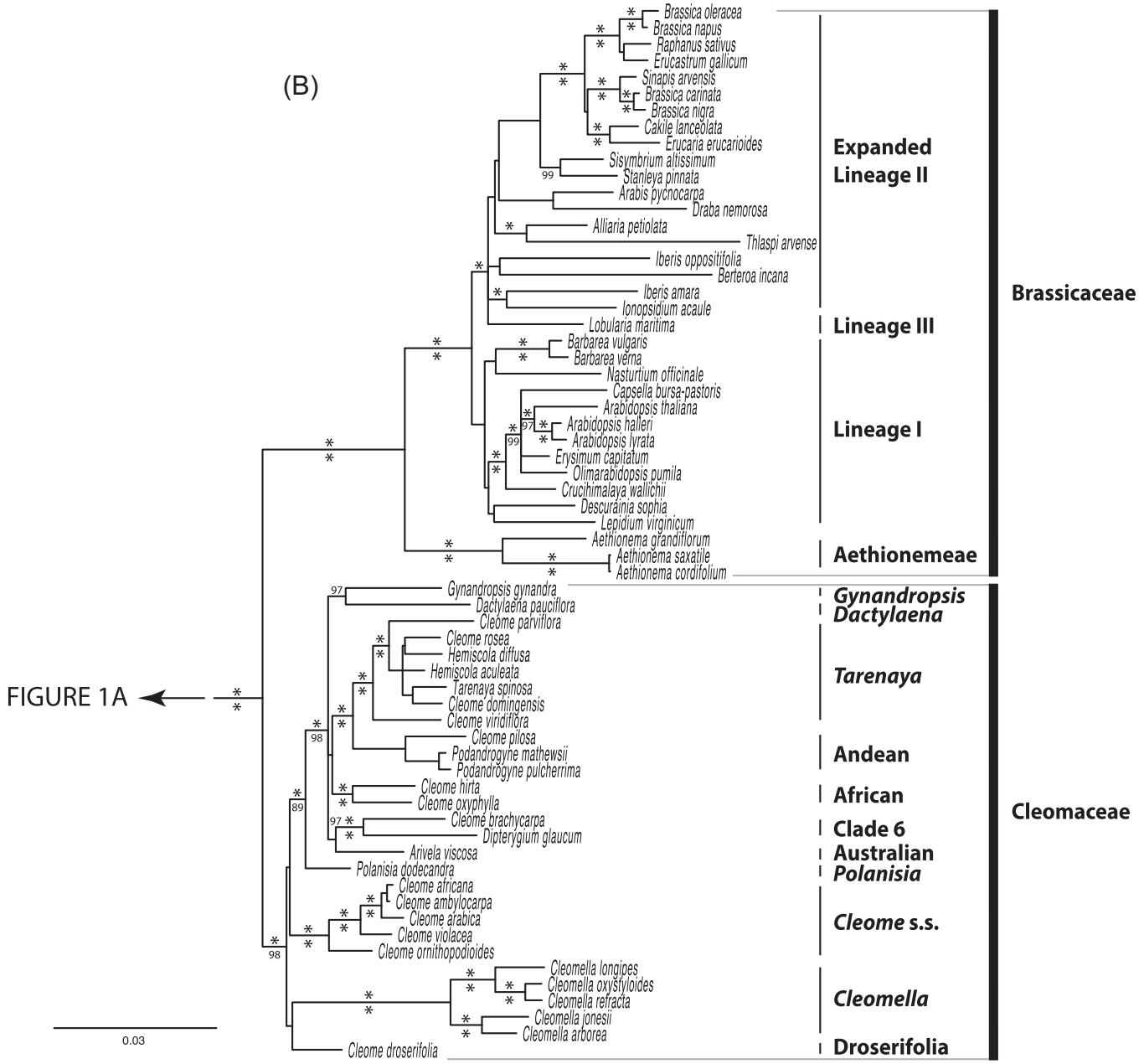

Fig. 1 (*continued*)

one (Edger et al., 2015). However, the trade-off was that one species was sampled from each of 13 families and six from the Brassicaceae. Our more densely taxon sampled study, but with less gene coverage, provided very similar results to that of Edger et al. (2015) and builds on smaller studies (Hall et al., 2004; Rodman et al., 1996, 1998; Su et al., 2012). The emerging phylogenetic framework for Brassicales indicates that Akaniaceae plus Tropaeolaceae is sister all other Brassicales, with Moringaceae plus Caricaceae as the next diverging lineage. The weakest set of relationships among families in Brassicales involves the placement of Setchellanthaceae and the sister relationship of Bataceae and Koeberliniaceae. Edger et al. (2015) did not sample Setchellanthaceae, and the relationship of the two latter families was the only unsupported branch in their tree despite massive amounts of genomic evidence.

The monophyly of the core Brassicales remains well supported, as does the placement of Emblingiaceae as sister to all other members. A few relationships remain elusive within the core Brassicales, especially the unclear placement of Tovariaceae and Pentadiplandraceae. Our BI analyses suggest a sister relationship of African endemic Pentadiplandraceae with the GRFT clade, which is consistent with previous Bayesian analyses of cpDNA (Martín-Bravo et al., 2009; Su et al., 2012). This placement of Pentadiplandraceae is also well supported by Edger et al. (2015), but they did not include Borthwickiaceae, *Stixis*, Tovariaceae, or *Tirania*. Relationships within the GRFT clade are largely resolved with Australian Gyrostemonaceae sister to all others, followed then by divergence of Asian Borthwickiaceae. The only lack of support within the GRFT clade involves the placement of North American *Forchhammeria* relative to the Asian clade of *Stixis* and *Tirania* and to European Resedaceae. A sister relationship between *Forchhammeria* and Resedaceae was strongly supported with cpDNA data in a previous analysis that did not sample *Borthwickia*, *Stixis*,

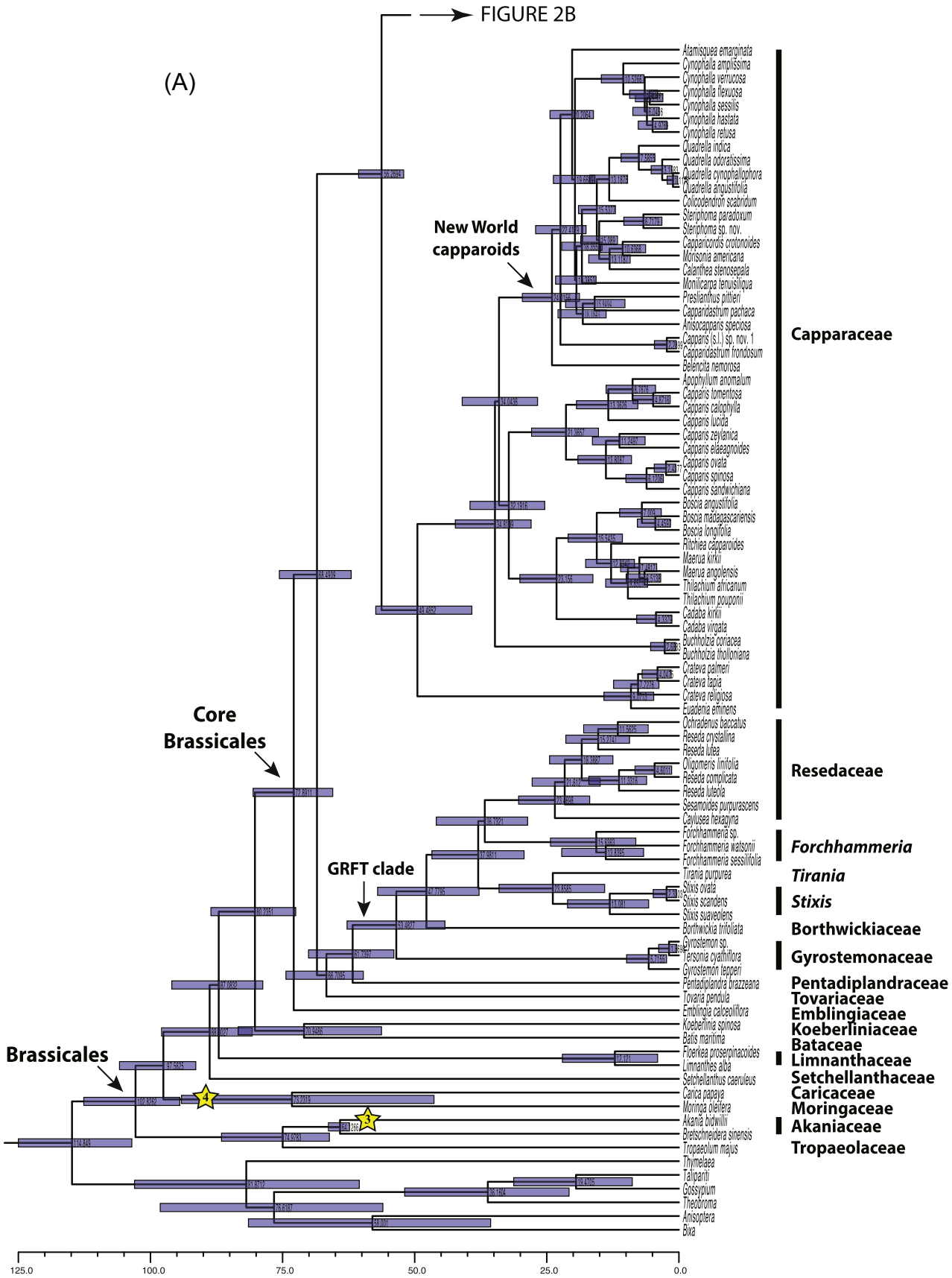

Fig. 2. BEAST chronogram of Brassicales inferred from combined cpDNA (*ndhF*, *matK*, *rbcL*) and mtDNA (*matR*, *rps3*) sequence data and four fossil calibrations. Supplemental Information Table S5 provides estimated ages across analyses with different combination of fossil calibrations. Numbers indicate mean divergence times with bars representing the 95% confidence intervals. Families or genera are indicated at right.

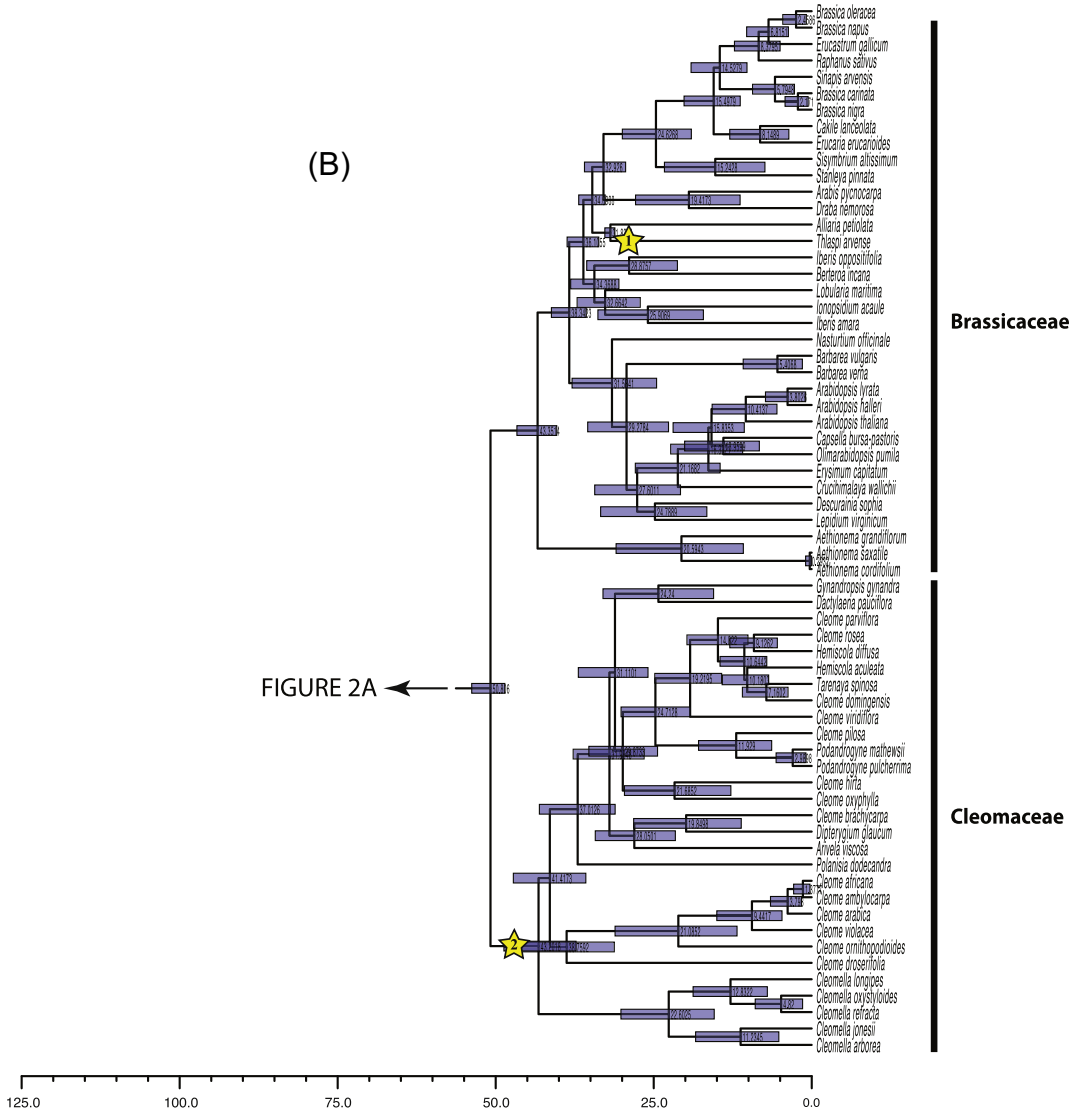

Fig. 2 (*continued*)

and *Tirania*, but included more species of Resedaceae (Martín-Bravo et al., 2009). However, support for this relationship decreases when these enigmatic genera are included (Fig. 1; Su et al., 2012).

While our analyses confirm the monophyly of Brassicaceae, Cleomaceae, and Capparaceae (Al-Shehbaz et al., 2006; Beilstein et al., 2006; Hall, 2008; Hall et al., 2002), some relationships within these large families were not well supported or resolved. Relationships within Brassicaceae and Cleomaceae have been challenging to assess, although recent progress has been made in elucidating infrafamilial relationships (Al-Shehbaz, 2012; Couvreur et al., 2010; Feodorova et al., 2010; Hall, 2008; Huang et al., 2016; Patchell et al., 2014). Moreover, our sampling focused on major and early-diverging lineages in order to assess biogeographical patterns. Despite this limited sampling, our topologies are consistent with published phylogenies overall. In contrast, our sampling of Capparaceae is the same as the largest taxonomic sampling of the family to date (Hall, 2008), both of which represent about 11% of the described species. Recent efforts by Cornejo and Iltis (2006, 2008a,b,c, 2010; Iltis and Cornejo, 2007, 2011) to revise the New World species of *Capparis* s.l. into distinct genera are mostly supported by our sampling, with the exception of *Capparidastrum*, which is polyphyletic. With these taxonomic changes, *Capparis* s.s. is now an exclusively Old World genus that is nearly monophyletic with the Australian shrub genus *Apophyllum* embedded within *Capparis*.

4.3. Origin, biogeography, and diversification of Brassicales – an 103 Myr tale

4.3.1. Early historical biogeography of Brassicales – vicariance and long distance dispersal

The biogeographical scenario presented here is the first for the entire Brassicales. Using four fossils, we show that the crown Brassicales diverged in the Early Cretaceous (~103 Mya, HPD = 112.6–94.4). Other fossil calibrated chronograms produced a range of ages, both older and younger, for the crown of the Brassicales: 112 Mya (BEAST, Beilstein et al., 2010), 92 Mya (BEAST, Edger et al., 2015), 90 Mya (Penalized likelihood, Magallón et al., 2015), 82 Mya (BEAST, Magallón et al., 2015). The influence of root priors in fossil-calibrated chronogram can be significant (e.g., Sytsma et al., 2014). We note that our methods allowed considerable relaxation in the root date by providing a uniform distribution between 0 and 125 Mya (i.e., allowing the four fossil priors to drive the root

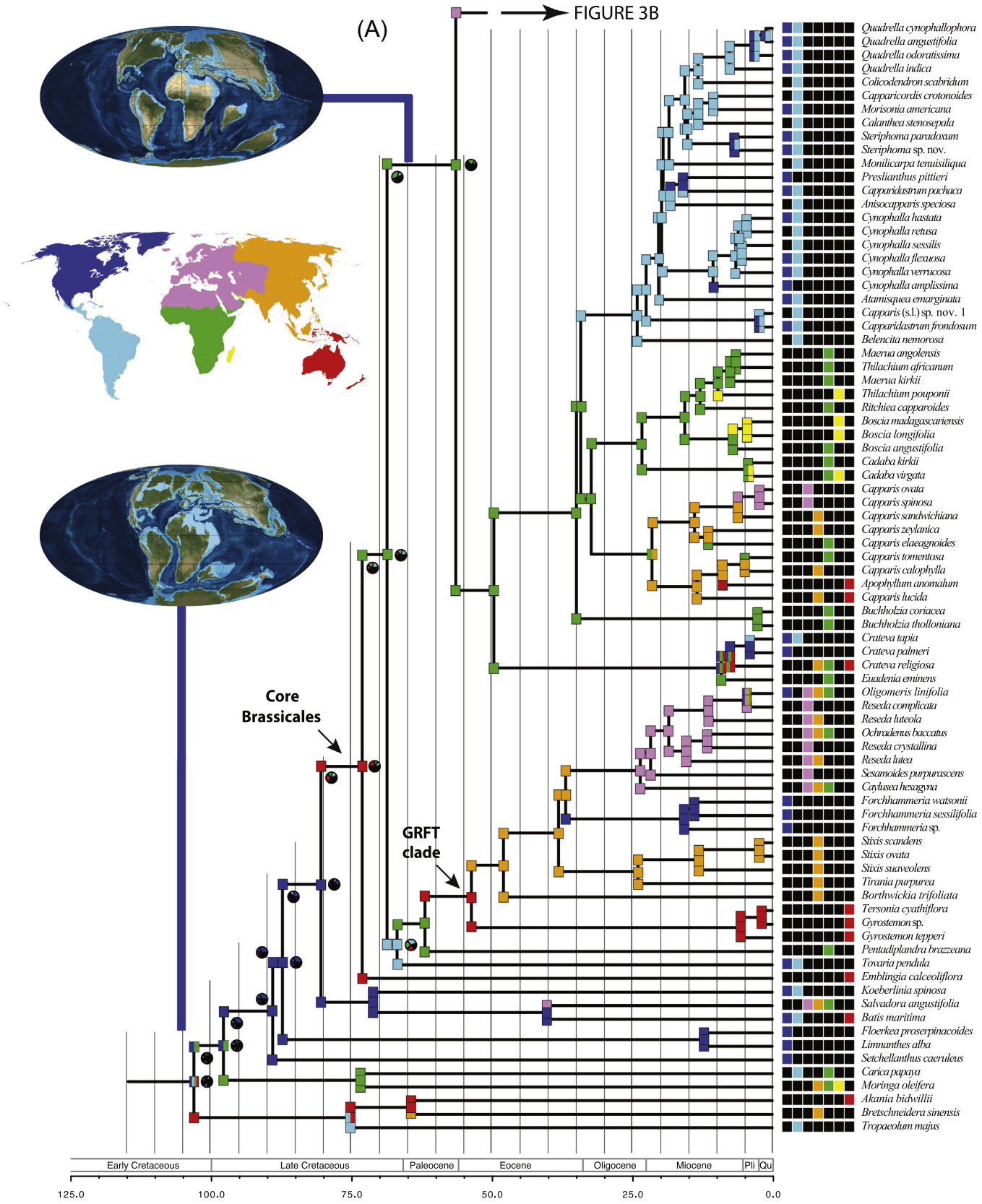

Fig. 3. Ancestral range estimation (ARE) on the Brassicales BEAST chronogram with BioGeoBEARS (DEC+J model). Areas of tip species are shown left of taxa names, color-coded for the seven biogeographical areas shown on the map inset. Boxes at each node and corner are color coded for the area (or combined area, up to four allowed) with the highest ML probability. Supplementary Information Fig. S2 provides all ARE per node and corner with pies designating probability of each area (or combined area). Such pies are placed in this figure at nodes or corners (mainly near the base or backbone of the chronogram). Inset globes depict continental positions at the beginning of the Late Cretaceous, the K–Pg boundary, and early Miocene (modified after Scotese, 2001).

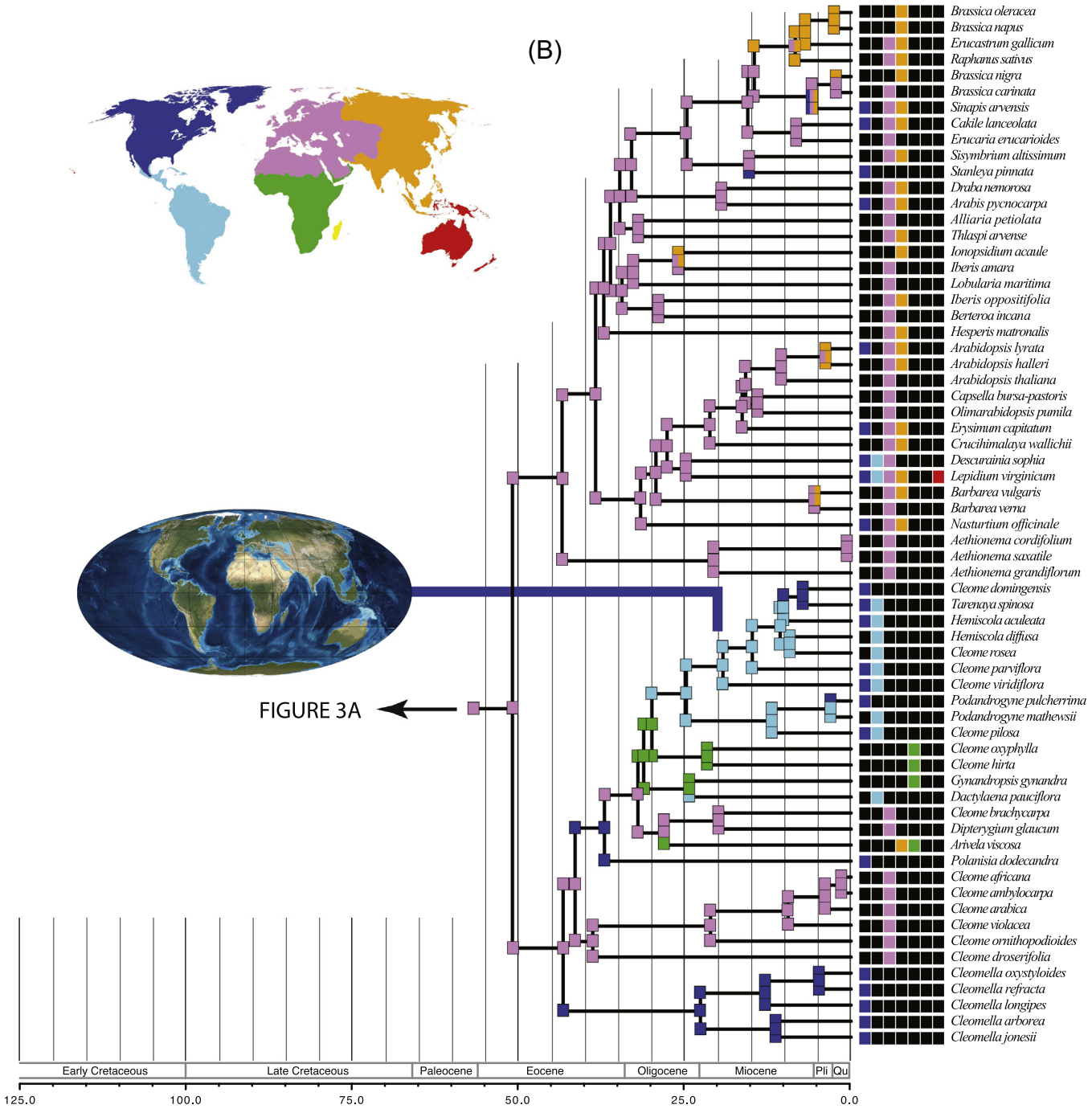

Fig. 3 (*continued*)

date), with the resulting root (=stem of Brassicales) following BEAST analysis receiving an age of 114 Mya. Most of the stem and crown dates of the order and families obtained with our four fossil calibrations fall within the 95% credibility ages obtained in other recent BEAST analyses (Beilstein et al., 2010; Edger et al., 2015; Magallón et al., 2015). Thus, we argue that the downstream biogeographical and diversification analyses using this Brassicales chronogram have a strong phylogenetic and temporal framework from which to interpret those results.

BioGeoBEARS analyses indicate that the crown of the order most likely occurred in a widespread area comprising North and South America, Africa, and Australia during the Early Cretaceous (Fig. 3A), although other somewhat less likely ARE are possible (Supplementary Information Fig. S2). The occurrence of the oldest (conservatively placed at ~90 Mya), putative fossil of Brassicales, *Dressiantha bicarpellata* (Gandolfo et al., 1998), from New Jersey, USA supports this biogeographical reconstruction. By 80 Mya the core Brassicales and all other early diverging lineages comprising the remaining nine families had separated. Although early diverging events in Brassicales are younger than age estimates for the initial breakup of Gondwana (180–150 Mya; McLoughlin, 2001; Scotese et al., 1988), it appears that continental connections persisted in both tropical (e.g., South America and Africa) and temperate settings (South America, Antarctica, Australia) to allow for continued floristic exchange into the Late Cretaceous or Mid-Tertiary, respectively (Hallam, 1994; Pitman et al., 1993;

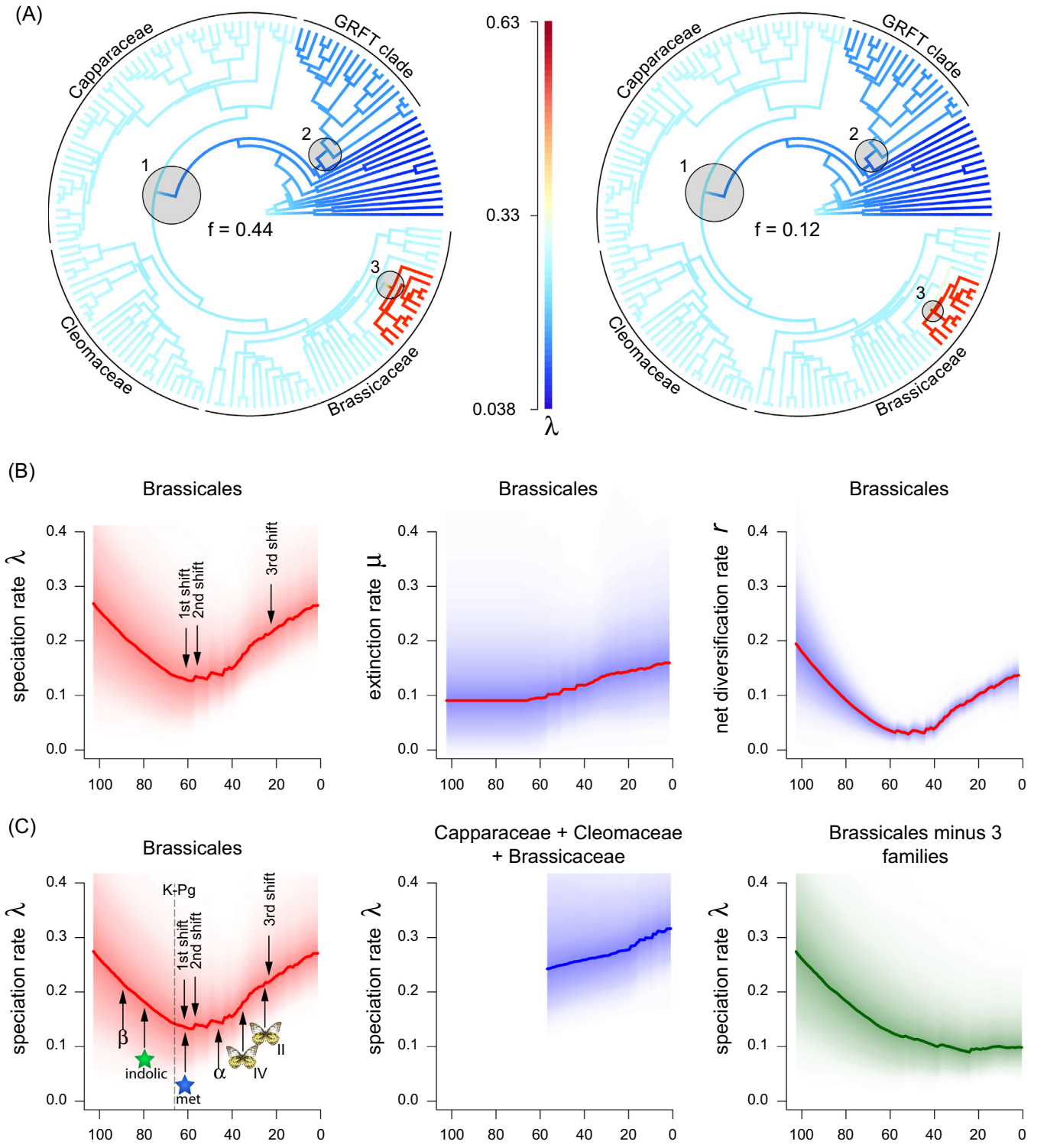

Fig. 4. BAMM analysis of rate shifts and diversification within Brassicales. (A) Rate shift configurations with the two highest posterior probabilities (out of 92) from the 95% credible set. Both show three significant rate shifts in speciation (λ) – color coded by λ: 1. Stem node of Capparaceae + Cleomaceae + Brassicaceae; 2. Stem node of GRFT clade; 3. Crown of or within tribes Brassiceae and Sisymbrieae. (B) Rates-through-time analysis of speciation (λ), extinction (μ), and net diversification (r) in all Brassicales across 103 Myr. (C) Rates-through-time analysis of speciation in all Brassicales, in clade of Capparaceae + Cleomaceae + Brassicaceae, and in all Brassicales minus this clade. Various events discussed in the context of species rate shifts in Brassicales and of the Cretaceous–Paleogene mass extinction event (K–Pg) indicated by arrows: α = *At*-α WGD, β = *At*-β WGD, star indolic = origin of indolic glucosinolates, star met = origin of methionine-derived glucosinolates, butterflies = Pierinae butterfly species radiations of Lineages II (Anthocharidini – Brassicaceae feeders) and IV (Pierina – Capparaceae/Cleomaceae feeders) (see Edger et al., 2015, for details and timing of all these events).

Raven and Axelrod, 1974). Thus, the early diversification of Brassicales may be explained with vicariant scenarios. For example, the earliest divergence in the order involved the separation of Tropaeolaceae plus Akaniaceae from the ancestor of all other Brasssicales. The subsequent divergence of South American Tropaeolaceae and Australian (*Akania*) + Asian (*Bretschneidera*)

Akaniaceae at ~75 Mya in the Late Cretaceous presumably involved an initially widespread and connected Southern Hemisphere distribution judging from the ~62–47 Mya fossils of *Akania* in Argentina (Gandolfo et al., 1988; Iglesias et al., 2007; Romero and Hickey, 1976; Wilf et al., 2005). Recent paleographical models indicate that South America and the Antarctic Peninsula and Australia and Eastern Antarctica were connected until ~57 Mya (Reguero et al., 2014) and ~45 Mya (White et al., 2013), respectively. Subsequent vicariance and extinction in the Southern Hemisphere followed by a Paleocene long-distance dispersal event from Australia to Asia is thus a likely biogeographical scenario for this early diverging clade in Brassicales.

4.3.2. Long-distance dispersals and land-bridge migrations explain biogeographical patterns in the core Brassicales

The crown of the core Brassicales underwent a rapid and dramatic diversification in the Late Cretaceous (~73 Mya). By the Cretaceous–Paleogene boundary (~67 Mya) at least five clades had diversified: the Australian Emblingiaceae, the New World Tovariaceae, the African Pentadiplandraceae, the diverse GRFT clade (Australian ancestrally), and the super-diverse clade of Capparaceae, Cleomaceae, and Brassicaceae (African ancestrally) (Fig. 3). Although Australia is the most likely ARE for the crown of the core Brassicales (Fig. 3A), it is not surprising given this rapid and worldwide radiation that support exists for alternative ARE (Fig. 3A, Supplementary Information Fig. S2). Likewise, the GRFT clade radiation exhibits a complex biogeographic signal. Australia is resolved as the ARE for the crowns of the GRFT clade (~53 Mya) and the first diverging Gyrostemonaceae, but an immediate dispersal event to Asia in the early Eocene (~53–48 Mya) is evident for the remainder of the GRFT clade. Vicariance is an unlikely scenario for the rapid diversification seen in the core Brassicales. Instead, repeated long-distance dispersals both across the Atlantic and Indian Oceans likely occurred during the early Paleogene. Spalink et al. (in press) presented an intriguing hypothesis that the time period from the early Paleocene to the Eocene was a unique moment in paleogeographic history in the Old World. They suggested that the Indian subcontinent, beginning its rapid movement from Gondwana in the Paleocene and colliding with Asia by the mid-Eocene (DeCelles et al., 2014), was a major landmass in the middle of the Indian Ocean and served as an intermediary dispersal point between Australia, Africa, and Eurasia. Similar to the Cyperaceae they examined, many of these inter-continental dispersal events seen in the Brassicales during the early Paleogene may have been facilitated by a congested Indian Ocean.

Within the GRFT clade, two late Eocene to Oligocene migration events occurred from Asia to other areas of the Northern Hemisphere. One migration was westward and gave rise to the radiation of Resedaceae in Europe and the Mediterranean region. This migration was likely facilitated by the closure of the Turgai Straits in the Oligocene allowing movement between central Asia and Europe (Brikiatis, 2014; Milne, 2006; Tiffney, 1985). The second migration gave rise to *Forchhammeria* in Central America and was probably facilitated by the Thulean NALB. Although both the NALB and the Bering Land Bridge (BLB) were active early in the Paleocene as tropical connections to North America (Brikiatis, 2014), during the Oligocene the NALB had a warmer climate whereas BLB was more suitable for temperate elements (Tiffney, 1985; Tiffney and Manchester, 2001; Wen, 1999).

The interplay of long-distance dispersal and migration across newly formed bridge connections is also seen in Capparaceae and Cleomaceae. Capparaceae has an African ancestry with two subsequent dispersals between 34 and 22 Mya (Oligocene–Miocene) to Asia (and later to Australia and to Europe) and the New World (Fig. 3A). The first dispersal from Africa to Asia is synchronous with the migration of the GRFT clade to Europe and thus both might be explained by the closure of the Turgai Straits and the beginning of more or less overland connections between SE Europe, NE Africa and Asia. However, the shift from Africa to the Neotropics was likely the result of a single, long-distance dispersal and rendered pantropical *Capparis* s.l. broadly paraphyletic (Fig. 3A). Like most Capparaceae, the fruits of New World capparoids are fleshy (Iltis et al., 2011) with variable dehiscence, whereas Old World *Capparis* tend to be indehiscent berries consistent with mostly bird or perhaps mammal dispersal (Jacobs, 1960, 1965). Thus, a long distance event is consistent with many New World tropical groups whose distributions appear to be the result of long distance dispersal events, even when fruits morphology suggests otherwise (Christenhusz and Chase, 2013). If so, *Capparis* s.l. can be added to the growing list of disjunct genera that have dispersed from Africa westward to South America (Renner, 2004). However, we cannot exclude the possibility that this disjunct pattern was due to an over-land migration from Africa to South America via the NALB, as biota were able to cross over the NALB between 40 and 30 Mya (Milne, 2006). A "*Capparis*" wood fossil that was described from Germany and dated to 17–16.3 Mya (Selmeier, 2005) might support an over-land migration. Because this wood fossil was only directly compared to Old World *Capparis*, not to New World taxa, the placement of this fossil in our phylogeny is challenging. This fossil has affinities to Old World *Capparis*, and given its age, may be more consistent with African/Asian distributions exhibited within the Old World *Capparis* clade (Fig. 3A).

The repeated dispersals from mainland Africa to Madagascar are a noteworthy result in the ARE of the African lineages of Capparaceae (Fig. 3A). A minimum of three separate dispersals over the Mozambique Channel, all within the last 10 Myr (late Miocene to early Pliocene), is seen in *Boscia*, *Cadaba* and *Thilachium*. These dates are consistent with a meta-analysis of endemic genera to Madagascar that supports an Eocene/Oligocene onset for the origin of the Madagascan endemic flora and with the majority arising in the Miocene or more recently (Buerki et al., 2013). Repeated dispersals from Africa to Madagascar within a recent clade (4X) are also seen in *Rinorea* (Violaceae) (Velzen et al., 2015).

Finally, Cleomaceae exhibit several long-distance dispersal and migration events. A short dispersal event over water from Africa to Europe in the lineage leading to Brassicaceae plus Cleomaceae occurred ~51 Mya in the early Eocene. At this time, the African (Nubia) plate was approaching Eurasia but the Neotethys Sea separating the two would still be open until the Oligocene (Fig. 3A; McClusky et al., 2003). The crown of Cleomaceae (Fig. 3B) shows a mid-Eocene (~43 Mya) separation of a North American lineage that later radiates in primarily arid, western North America at the Oligocene–Miocene border (the "western North American cleomoids"; Riser et al., 2013; Roalson et al., 2015). This migration from Europe to North America would have been facilitated by the NALB, as with other lineages of the core Brassicales. That these cleomoids took refuge in southern portions of North America (as with *Forchhammeria*, see above) is not unusual, as it conforms to a general pattern of taxa that disperse during the Paleogene via the NALB before the terminal Eocene–Oligocene cooling event (Couvreur et al., 2010; Morley, 2003; Tiffney and Manchester, 2001). The broadly paraphyletic *Cleome* and associated genera, forming the remainder of the family, exhibit striking disjunct patterns across Europe, Africa, North and South America (Fig. 3B). A minimum of two dispersal events back to Africa is seen during the closure of the Neotethys Sea in the Oligocene. A minimum of two dispersal events to the New World also occurs in the Oligocene. These rapid radiations of lineages and corresponding areas seen during the Oligocene in the *Cleome* clade preclude precise interpretation of ARE and migration or dispersal routes (Fig. 3B).

A larger sampling of taxa and gene regions in the Cleomaceae is warranted to address whether, for example, the American lineages

in the *Tarenaya* and Andean clades are derived from Africa or Europe and via over-Atlantic dispersal or migration across the NALB. Feodorova et al. (2010) presented a DEC biogeographical analysis with better sampling of the family (87 spp.). However, comparisons with their results are difficult for two reasons. First, their chronograms were not fossil-calibrated but relied on previous estimates based on synonymous substitution rates, and thus the crown radiation was considerably younger than we find (~18 vs. 43 Mya). Second, their trees lacked resolution in early diverging nodes so they evaluated two quite different topologies in DEC. Their biogeographic results suggested either central Asia (coded in our analysis as Europe) or North America as the ARE for the crown node. Among multiple Cleomaceous seed fossils found in England from the early Eocene (~47 Mya), *Burtonella emarginata* exhibits strong affinities with the western North American cleomoid clade (Chandler, 1962; Patchell et al., 2014). The location and affinity of this fossil, together with our BioGeoBEARS results, support an Old World origin of Cleomaceae, with a NALB migration hypothesis explaining the disjunct origin of the early diverging western North American cleomoids (*Cleomella*).

4.3.3. Brassicaceae originated in the Eocene and Arabidopsis diverged in the late Miocene

Data presented here support Brassicaceae originating in the Eocene in Europe (west of the Urals and Caucasus), Mediterranean Africa, Saharo-Arabian, and Irano-Turanian region (Franzke et al., 2011) with an estimated crown age of 43.4 Mya (HPD = 46.6–40.3; Fig. 2). Our date is younger by 11 Myr than analyses of Beilstein et al. (2010), which placed the family at 54.3 Mya (HPD = 64.2–45.2). In contrast, our estimate is approximately 5 Myr older than Couvreur et al.'s (2010) date of 37.6 Mya (HPD = 49.4–24.2) and 11 Myr older than Edger et al.'s (2015) date of 31.8 Mya (HPD = 41.9–16.8). A recent study also compared age estimates of Brassicaceae with and without including *Thlaspi primaevum* as a fossil calibration, although they had limited taxonomic sampling in the Brassicales (only two species outside of Brassicaceae; Huang et al., 2016). When Huang et al. (2016) included *T. primaevum*, our date is within a million year difference of their estimate of 42.0 (HPD = 42.7–41.4) and approximately 5 Myr older (37.1; HPD = 37.8–36.3) when the fossil was excluded. Importantly, our age of Brassicaceae is robust across all our permutations of fossil calibrations. When different fossils were excluded from analyses, our crown age of the family ranged from 45.9 to 37.7 Mya (Supplementary Information Table S5). Despite concern that the *Thlaspi* fossil calibration might be driving ages in the Brassicaceae (Franzke et al., 2011), this fossil appears to have only immediate effects on the node with the prior and not ages of other nodes in the family (see also Beilstein et al., 2010) or minimal impact on age estimates (Huang et al., 2016).

Brassicaceae thus evolved from a common ancestor with Cleomaceae somewhere in Europe, Mediterranean Africa, Saharo-Arabian, and Irano-Turanian during the Eocene. During this time, warm and humid climatic conditions prevailed worldwide (Zachos et al., 2001) and tropical rain forests extended well into Europe and the Mediterranean region (Morley, 2003). Much of the subsequent diversification within Brassicaceae, including the crown of *Aethionema*, occurred during the more drastic cooling of the Eocene–Oligocene border and later epochs (Couvreur et al., 2010). In Europe and the Mediterranean region, the tropical flora changed dramatically to one characterized by deciduousness and arid adaptations to cooler and drier climates (Zachos et al., 2001; Morley, 2003). Although our taxa sampling within Brassicaceae is not as dense as in recent family analyses (Couvreur et al., 2010; Huang et al., 2016) and our gene sampling for this across-Brassicales survey is not intended to fully resolve relationships within the family, several results are highlighted. Within the Brassicaceae, the clade comprising the tribes Brassiceae and Sisymbrieae is relatively old at ~24 Mya, the three diploid crop *Brassica* species are not closely related and separated 15 Mya. These dates are consistent with the recent findings of Beilstein et al. (2010) and Huang et al. (2016) for the Brassiceae and Arias et al. (2014) for *Brassica*.

Lastly, our results provide clarification on the long-standing debate on diversification of *Arabidopsis thaliana*. As summarized by Beilstein et al. (2010), this and other dates within Brassicaceae have long been used to understand the pace of molecular evolution in this model plant system, changes in signal transduction and gene expression, the age of whole genome duplications, and coevolution of the pierid butterflies specializing on mustard-oil producing plants. A major challenge for obtaining this date was the general lack of fossils for the family, resulting in continued reliance on synonymous substitution rates to estimate such ages (Kagale et al., 2014b; Koch et al., 2000, 2001). These latter dates are recent, 5.8–3.0 Mya, and indicate rapid evolution of *Arabidopsis* in a period of cooling climatic conditions. More recent attempts to date the *Arabidopsis* crown group utilized fossils from within Brassicales (Beilstein et al., 2010; Edger et al., 2015). These two studies place the crown of *Arabidopsis* at 13.0 Mya (HPD = 17.9–8.0) and 13.8 Mya (HPD = 20.9–6.6), respectively. Our results using four fossils place the crown group of *Arabidopsis* slightly younger in the late Miocene at 10.4 Mya (HPD = 15.7–5.5), although the 95% confidence intervals of all three ages overlap widely. Thus, *Arabidopsis* evolved more slowly than assumed previously when basing dates on synonymous substitution rates, and diversified during a climatic period of global warmth as opposed to more cool and arid conditions of the later Pliocene and Quaternary.

4.3.4. Rates of diversification within Brassicales and identification of three significant rate shifts

The order Brassicales is emerging as a model lineage to address the nature of processes leading to significant shifts in diversification and extinction (Beilstein et al., 2010; Couvreur et al., 2010; Edger et al., 2015; Willis et al., 2014). Brassicales is an ideal candidate for such studies considering its origin in the Early Cretaceous, widespread biogeographical distribution influenced by complex interplay between vicariance, long-distance dispersal, and land bridge migrations, radiations in diverse tropical and temperate biomes, considerable morphological diversity in both vegetative and reproductive features, and the presence of both species-rich and depauperate clades, WGD events, co-evolution with pierid butterflies and concurrent innovations in mustard oil biochemistry, and a now well-supported and dated phylogeny. However, important issues in detecting significant rate shifts include phylogenetic scale, sampling density, phylogenetic uncertainty, assumptions used in the method to detect shifts, and correlation and/or causality of biotic or niche attributes driving any detected rate shift (Berger et al., 2016; Spalink et al., in press, 2016; Rose et al., 2016).

We present here the first broad sampling of the Brassicales implementing the program BAMM that allows rate shifts to vary through time in a diversity-dependent manner (Rabosky, 2014). Our analysis of species diversification in Brassicales provides a baseline rate of net diversification for the order that is average for angiosperms (see Fig. 4B; $r = 0.09$ species/Myr; 95% quantile = 0.08–0.10). This rate is similar to that estimated by Magallón and Sanderson (2001) using different approaches for the order and angiosperms generally ($r = 0.07$–0.09 species/Myr). Unlike previous attempts to quantify net diversification in the Brassicales (Beilstein et al., 2010; Edger et al., 2015) in which extinction is not modeled specifically, the BAMM analysis provides rates of speciation and extinction with 95% confidence intervals: speciation rate ($\lambda = 0.23$ species/Myr; 95% quantile = 0.16–0.32), and extinction rate ($\mu = 0.14$ species/Myr; 95% quantile = 0.06–0.24).

The rates-through-time analysis indicates that the extinction rate in Brassicales increased steadily since ~60 Mya, whereas speciation rate declined during the first 40 Myr history of the order and then rapidly increased at ~60 Mya (Fig. 4B).

Three different significant shifts in accelerated rates of speciation are shown for the first time in Brassicales. The strongest shift in terms of Bayes factor evidence (bf = 47,000) is placed at ~62 Mya probably in Africa in the early Eocene on the stem leading to the three large families – Capparaceae, Cleomaceae, and Brassicaceae (Fig. 4A and B). This branch is the longest (68.5–56.3 Mya) of the three branches exhibiting shifts and thus it is not surprising that BAMM strongly detects a shift at this specific branch versus neighboring branches; i.e., a significant shift in speciation rate might not be pinpointed to a specific branch with high support if it is nested within a clade of short branches (Rabosky, 2014; Rabosky et al., 2014). The descendants of this shift contributed to the bulk of the speciation rate evident across Brassicales (Fig. 4C). Using different approaches (LASER; Rabosky, 2006) to estimate shifts in diversification in a supermatrix tree of Brassicales, Beilstein et al. (2010) detected only one shift within the order, a 2- to 10-fold shift in speciation rate at this same node. Likewise, Edger et al. (2015) examining 15 representative taxa of Brassicales and the program MEDUSA (Alfaro et al., 2009), which assumes a constant-rate diversification process using stepwise AIC, unlike BAMM, detected two rate shifts with the most significant placed at this crown node of Capparaceae, Cleomaceae, and Brassicaceae.

The second shift in speciation rate placed along the stem leading to the crown GRFT clade has not been documented before (Fig. 4). This shift, like the first, occurred in the early Eocene (~58 Mya) along a branch (62–53 Mya) in the core Brassicales after the divergence of Emblingiaceae, Tovariaceae, and Pentadiplandraceae. Although biogeographical reconstruction at this node is not strong (Fig. 3A; Supplementary Information Fig. S2), the shift likely occurred shortly after dispersal from Africa to Australia and was then followed by dispersals and radiations into tropical and temperate Asia (*Borthwickia*, *Stixis*, *Tirania*), Europe (Resedaceae), and the Americas (*Forchhammeria*) (Fig. 3A). Bayes factor support is considerably less for this branch (bf = 453) and alternative shift configurations place it one or two nodes further back (i.e., including African *Pentadiplandra* or/and American *Tovaria*).

The third shift in speciation rate occurs within the Brassicaceae, near the crown of the tribes Brassiceae and Sisymbrieae, and at the Oligocene–Miocene border (~21 Mya) (Fig. 4). Although this rate shift is the most dramatic of the three (Fig. 4A), Bayes factor support for this specific branch is similar to the second shift (bf = 459) and alternative configuration shifts place this third shift in nearby branches (see Fig. 4A). Thus, there is a marked acceleration in speciation rate but its exact placement is uncertain with our data. The MEDUSA analysis of Edger et al. (2015) placed the second of its two rate shifts at the crown of the core Brassicaceae after *Aethionema* diverged. However, their MEDUSA tree has only two terminals for Brassicaceae and thus it is not possible to further compare their placement with our more taxon-rich BAMM shift placement within the core Brassicaceae. The supermatrix study on Brassicaceae (placeholders for 207 genera) by Couvreur et al. (2010) used LASER to estimate lineage-through-time plots within the family. They uncovered one significant acceleration in diversification earlier in the family between 32 and 22 Mya, a time frame just preceding our dated rate shift in Brassicaceae, with a subsequent slow down of diversification.

We note several possible limitations and assumptions in how diversification analyses are conducted in the context of identifying rate shifts and, thus caution, interpretation of the third shift in Brassicales. BAMM is an important improvement over previous methods in estimating diversification rates and shifts in these rates for two reasons: it allows rate shifts to vary through time in a diversity-dependent manner and it can utilize phylogenetic structure present within identified terminals (i.e., sampling can be greater than identified terminals). As phylogenetic structure within Brassicaceae is poorly supported beyond the recognition of the major lineages (e.g., Couvreur et al., 2010), we were conservative in identifying only four terminals within the family although each terminal had multiple accessions retained in the tree (Supplementary Information Table S4). We explored the placement of this third shift in several different sampling regimes of the number of Brassicaceae accessions retained per terminal (data not shown). In all cases, this third shift is placed near or at the clade including the tribe Brassiceae (as seen in two shift configurations in Fig. 4A). Many diversification analyses do not account for phylogenetic uncertainty, instead use only one resulting BEAST tree (but see Couvreur et al., 2010, as an exception). Phylogenetic uncertainty in some parts of the Brassicales tree prior to divergence of the core Brassicales and in the Brassicaceae suggests caution in interpretation of downstream analyses.

4.3.5. Rate shifts in diversification within Brassicales are correlated with multiple events – but what drives these shifts?

A number of different explanations have been proposed for the sudden shifts of speciation at the crown clade of Capparaceae, Cleomaceae, and Brassicaceae and within the crown of Brassicaceae (Beilstein et al., 2010; Couvreur et al., 2010; Edger et al., 2015; Willis et al., 2014). Specific events that may have triggered the first shift in diversification at the crown of the three large families include the (1) Cretaceous–Paleogene (K–Pg) extinction event, (2) whole genome duplications, (3) pierid butterfly/Brassicales coevolution involving feeding shifts of pierids onto specific clades within Brassicales, and (4) subsequent mustard-oil glucosinolate innovations by these host clades in response. Our chronogram dated with four fossils provides the most taxon-sampled temporal framework to date to examine such correlations. We overlaid the timing of these four types of events onto the rate-through-time plot of speciation in Brassicales throughout its 103 Myr history (Fig. 4C). Beilstein et al. (2010) specifically argued that the K–Pg extinction event was an important factor in the evolution of Brassicales, noting the similarity of the crown date they estimate for the three families (71.3 Mya) to the K–Pg border (66 Mya). Our date for the stem branch leading to this node is even closer (68–56 Mya) to the K–Pg and suggests, if causative, a delayed response in crown node speciation to the event. Indeed our second rate shift seen in the GRFT clade occurs soon after the K–Pg event as well (62–53 Mya). Similarly, in a BAMM diversification analysis of Myrtales, all three identified rate shifts occur on branches that span the K–Pg boundary (Berger et al., 2016). Plant fossil evidence is mounting for the profound impact that this climatic perturbation had on vegetation (McElwain and Punyasena, 2007).

Genome-wide data demonstrate that an increasingly large number of WGD events in plants are time-correlated with the K–Pg boundary event (Vanneste et al., 2014). Beilstein et al. (2010) further argued that two WGD events in Brassicales (*At*-β and *At*-α WGD) are linked with diversification in Brassicaceae and with survival of the K–Pg mass extinction event. The phylogenomic approach of Edger et al. (2015) placed the earlier *At*-β WGD after divergence of Caricaceae + Moringaceae but before the divergence of Limnanthaceae (they did not sample Setchellanthaceae). Thus using our chronogram, we estimate the *At*-β WGD occurred between 97.6 and 87.1 Mya (see Fig. 4C). Edger et al. (2015) conclusively placed the later WGD on the stem leading to the crown of Brassicaceae, and thus we estimate the *At*-α WGD occurred between 50.8 and 43.4 Mya (Fig. 4C). Using this range of dates for the *At*-α WGD and its K_s value = 0.34, we also estimate that the *Cs*-α WGD in *Tarenaya spinosa* (K_s value = 0.20) occurred between 29.4 and 25.5 Mya in the Oligocene (Barker et al., 2009).

This duplication would be placed on the branch leading to the largely Neotropical "*Cleome*" clade coming out of Africa (Fig. 3B), and we predict that all members of this clade should possess both the *At*-β and *Cs*-α WGD. Thus, placements within Brassicales of the first (crown of Capparaceae, Cleomaceae, and Brassicaceae) and second (crown of GRFT clade) significant shifts in speciation rate are consistent with a link between genome doubling (the earlier *At*-β WGD) and survival across the K–Pg extinction event. As Beilstein et al. (2010) summarized, this causal link is an attractive hypothesis as WGD may result in the colonization of new habitats, major ecological transitions, and the generation of morphological novelty.

Edger et al. (2015) further provide evidence of correlation of their two identified rate shifts in Brassicales to the co-diversification of pierid butterflies and host clades of Brassicales. In the context of our Brassicales chronogram, their scenario is updated to involve several linked and causal events (see Fig. 4C). (1) Brassicales arise ~103 Mya with ability to only synthesize glucosinolates from phenylalanine and branched chain amino acids; (2) Indolic glucosinolates evolve in Brassicales ~80 Mya, followed by the diversification ~70 Mya of Pierinae (the Brassicales-feeding Pieridae that detoxify indolic glucosinolates); (3) Methionine-derived glucosinolates evolve ~68–56 Mya in the stem of the Capparaceae, Cleomaceae, and Brassicaceae clade, which exhibits a simultaneous significant shift in speciation rate; (4) *At*-α WGD occurs prior to the crown diversification of Brassicaceae ~50–43 Mya; (5) Significant shift in speciation rate occurs ~35 Mya in the Pierina butterfly clade – Capparaceae/Cleomaceae feeders; (6) Significant shift in speciation rate occurs ~22 Mya in the Anthocharidini butterfly clade – Brassicaceae feeders, and concurrently by a significant shift in speciation rate ~21 Mya within Brassicaceae.

The emerging story of the evolution and radiation of the Brassicales over 103 Myr thus appears linked to a series of intercontinental vicariant, long-distance dispersal, and land bridge migration events. Across this temporal and biogeographical stage, the order diversified into a set of lineages which entered into a wide range of ecological conditions and evolved dramatically in habit, floral and fruit features – changes so intense that the close relationships of most families were not evident until the advent of molecular systematics. Several shifts in speciation rates in Brassicales were triggered by a cascading set of events involving co-diversification of pierid butterflies and glucosinolate machinery of host plants. These events in turn were likely spurred on by the genetic and ecological opportunities provided by whole genome duplications and the K–Pg mass extinction.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.02.021.

References

Alfaro, M.E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D.L., Carnevale, G., Harmon, L.J., 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. USA 106, 13410–13414.

Al-Shehbaz, I.A., 2012. A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon 61, 931–954.

Al-Shehbaz, I.A., Beilstein, M.A., Kellogg, E.A., 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Syst. Evol. 259, 89–120.

Antonelli, A., Zizka, A., Silvestro, D., Scharn, R., Cascales-Miñana, B., Bacon, C.D., 2015. An engine for global plant diversity: highest evolutionary turnover and emigration in the American tropics. Front. Genet. 6, 130.

APGIII, 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161, 105–121.

Arabidopsis Genome Initiative, 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408, 796–815.

Arias, T., Beilstein, M.A., Tang, M., McKain, M.R., Pires, J.C., 2014. Diversification times among *Brassica* (Brassicaceae) crops suggest hybrid formation after 20 million years of divergence. Am. J. Bot. 101, 86–91.

Axelrod, D.I., 1970. Mesozoic paleogeography and early angiosperm history. Bot. Rev. 36, 277–319.

Barker, M.S., Vogel, H., Schranz, M.E., 2009. Paleopolyploidy in the Brassicales: analyses of the *Cleome* transcriptome elucidate the history of genome duplications in *Arabidopsis* and other Brassicales. Genome Biol. Evol. 1, 391–399.

Becker, H.F., 1961. Oligocene plants from the Upper Ruby River Basin, southwestern Montana. Geol. Soc. Am. Mem. 82, 1–122.

Beilstein, M.A., Al-Shehbaz, I.A., Kellogg, E.A., 2006. Brassicaceae phylogeny and trichome evolution. Am. J. Bot. 93, 607–619.

Beilstein, M.A., Nagalingum, N.S., Clements, M.D., Manchester, S.R., Mathews, S., 2010. Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. 107, 18724–18728.

Bekaert, M., Edger, P.P., Hudson, C.M., Pires, J.C., Conant, G.C., 2012. Metabolic and evolutionary costs of herbivory defense: systems biology of glucosinolate synthesis. New Phytol. 196, 596–605.

Bell, C.D., Soltis, D.E., Soltis, P.S., 2010. The age and diversification of the angiosperms re-revisited. Am. J. Bot. 97, 1296–1303.

Benderoth, M., Textor, S., Windsor, A.J., Mitchell-Olds, T., Gershenzon, J., Kroymann, J., 2006. Positive selection driving diversification in plant secondary metabolism. Proc. Natl. Acad. Sci. 103, 9118–9123.

Berger, B.A., Kreibel, R., Spalink, D., Sytsma, K.J., 2016. Divergence times, historical biogeography, and shifts in speciation rates of Myrtales. Mol. Phylogenet. Evol. 95, 116–136.

Bhide, A., Schliesky, S., Reich, M., Weber, A.P., Becker, A., 2014. Analysis of the floral transcriptome of *Tarenaya hassleriana* (Cleomaceae), a member of the sister group to the Brassicaceae: towards understanding the base of morphological diversity in Brassicales. BMC Genom. 15, 1–16.

Brenner, G.J., 1996. Evidence for the earliest stage of angiosperm pollen evolution: a paleoequatorial section from Israel. In: Taylor, D.W., Hickey, L.J. (Eds.), Flowering Plant Origin, Evolution and Phylogeny. Chapman and Hall, New York, pp. 91–115.

Brikiatis, L., 2014. The De Geer, Thulean and Beringia routes: key concepts for understanding early Cenozoic biogeography. J. Biogeogr. 41, 1036–1054.

Buerki, S., Devey, D.S., Callmander, M.W., Phillipson, P.B., Forest, F., 2013. Spatio-temporal history of the endemic genera of Madagascar. Bot. J. Linn. Soc. 171, 304–329.

Buerki, S., Forest, F., Alvarez, N., 2011. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. J. Biogeogr. 38, 531–550.

Burleigh, J.G., Hilu, K.W., Soltis, D.E., 2009. Inferring phylogenies with incomplete data sets: a 5-gene, 567-taxon analysis of angiosperms. BMC Evol. Biol. 9, 61.

Carvalho, F.A., Renner, S.S., 2012. A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. Mol. Phylogenet. Evol. 65, 46–53.

Chandler, M.E.J., 1962. The Lower Tertiary Floras of Southern England II: Flora of the Pipe-Clay Series of Dorset (Lower Bagshot). British Museum (Natural History), London.

Cheng, S., van den Bergh, E., Zeng, P., Zhong, X., Xu, J., Liu, X., Hofberger, J., Bruijn, S., Bhide, A.S., Bian, C., Chen, J., Fan, G., Kaufmann, K., Hall, J.C., Becker, A., Shi, C., Zheng, Z., Li, W., Lv, M., Tao, Y., Xu, X., Wang, J., Zou, H., Quan, Z., Hibberd, J., Lim, B.L., Zhu, X., Zhang, G., Schranz, M.E., 2013. Insights into crucifer evolution by sequencing the sister-lineage species *Tarenaya hassleriana*. Plant Cell 25, 2813–2830.

Christenhusz, M.J.M., Chase, M.W., 2013. Biogeographical patterns of plants in the Neotropics – dispersal rather than plate tectonics is most explanatory. Bot. J. Linn. Soc. 171, 277–286.

Clayton, J.W., Soltis, P.S., Soltis, D.E., 2009. Recent long-distance dispersal overshadows ancient biogeographical patterns in a pantropical angiosperm family (Simaroubaceae, Sapindales). Syst. Biol. 58, 395–410.

Conti, E., Litt, A., Wilson, P.G., Graham, S.A., Briggs, B.G., Johnson, L.A., Sytsma, K., 1997. Interfamilial relationships in Myrtales: molecular phylogeny and patterns of morphological evolution. Syst. Bot. 22, 629–647.

Cornejo, X., Iltis, H.H., 2006. New combinations in Capparaceae *sensu stricto* for flora of Ecuador. Harvard Pap. Bot. 11, 17–18.

Cornejo, X., Iltis, H.H., 2008a. New combinations in South American Capparaceae. Harvard Pap. Bot. 13, 117–120.
Cornejo, X., Iltis, H.H., 2008b. *Anisocapparis* y *Monilicarpa*: dos nuevos géneros de Capparaceae de América del Sur. J. Bot. Res. Inst. Texas 2, 61–74.
Cornejo, X., Iltis, H.H., 2008c. A revision of *Colicodendron* Mart. (Capparaceae s.s.). J. Bot. Res. Inst. Texas 2, 75–93.
Cornejo, X., Iltis, H.H., 2010. Studies in the Capparaceae XXIX: synopsis of *Quadrella*, a Mesoamerican and West Indies genus. J. Bot. Res. Inst. Texas 4, 117–132.
Couvreur, T.L.P., Franzke, A., Al-Shehbaz, I.A., Bakker, F.T., Koch, M.A., Mummenhoff, K., 2010. Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). Mol. Biol. Evol. 27, 55–71.
Cracraft, J., 1988. Deep-history biogeography: retrieving the historical pattern of evolving continental biotas. Syst. Zool. 37, 221–236.
Crisp, M.D., Trewick, S.A., Cook, L.G., 2011. Hypothesis testing in biogeography. Trends Ecol. Evol. 26, 66–72.
Cronquist, A., 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.
Daru, B.H., Manning, J.C., Boatwright, J.S., Maurin, O., Maclean, N., Schaefer, H., Kuzmina, M., van der Bank, M., 2013. Molecular and morphological analysis of subfamily Alooideae (Asphodelaceae) and the inclusion of *Chortolirion* in *Aloe*. Taxon 62, 62–76.
Dassanayake, M., Oh, D.H., Haas, J.S., Hernandez, A., Hong, H., Ali, S., Yun, D.J., Bressan, R.A., Zhu, J.K., Bohnert, H.J., Cheeseman, J.M., 2011. The genome of the extremophile crucifer *Thellungiella parvula*. Nat. Genet. 43, 913–918.
Davis, C.C., Fritsch, P.W., Bell, C.D., Mathews, S., 2004. High-latitude Tertiary migrations of an exclusively tropical clade: evidence from Malpighiaceae. Int. J. Plant Sci. 165, S107–S121.
Davis, C.C., Latvis, M., Nickrent, D.L., Wurdack, K.J., Baum, D.A., 2007. Floral gigantism in Rafflesiaceae. Science 315, 1812.
Davis, C.C., Wurdack, K.J., 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. Science 305, 676–678.
DeCelles, P.G., Kapp, P., Gehrels, G.E., Ding, L., 2014. Paleocene–Eocene foreland basin evolution in the Himalaya of southern Tibet and Nepal: implications for the age of initial India-Asia collision. Tectonics 33, 824–849.
Donoghue, M.J., Edwards, E.J., 2014. Biome shifts and niche evolution in plants. Annu. Rev. Ecol. Evol. Syst. 45, 547–572.
Dorn, K.M., Fankhauser, J.D., Wyse, D.L., Marks, M.D., 2014. A draft genome of field pennycress (*Thlaspi arvense*) provides tools for the domestication of a new winter biofuel crop. DNA Res. 22, 121–131.
Drew, B.T., Sytsma, K.J., 2012. Phylogenetics, biogeography, and staminal evolution in the tribe Mentheae (Lamiaceae). Am. J. Bot. 99, 933–953.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Edger, P.P., Heidel-Fischer, H.M., Bekaert, M., Rota, J., Glöckner, G., Platts, A.E., Heckel, D.G., Der, J., Wafula, E., Tang, M., Hofberger, J.A., Smithson, A., Hall, J.C., Blanchette, M., Bureau, T.E., Wright, S.I., dePamphilis, C.W., Schranz, M.E., Barker, M.S., Conant, G.C., Wahlberg, N., Vogel, H., Pires, J.C., Wheat, C.W., 2015. The butterfly plant arms-race escalated by gene and genome duplications. Proc. Natl. Acad. Sci. 112, 8362–8366.
Ehrlich, P.R., Raven, P.H., 1964. Butterflies and plants: a study in coevolution. Evolution 18, 586–608.
Elffers, J., Graham, A., DeWolf, G.P., 1964. Capparidaceae. In: Hubbard, C.E., Milne-Redhead, E. (Eds.), Flora of Tropical East Africa. Crown Agents for Oversea Governments and Administrations, London, UK, pp. 1–88.
Feodorova, T.A., Voznesenskaya, E.V., Edwards, G.E., Roalson, E.H., 2010. Biogeographic patterns of diversification and the origins of C_4 in *Cleome* (Cleomaceae). Syst. Bot. 35, 811–826.
Franzke, A., German, D., Al-Shehbaz, I.A., Mummenhoff, K., 2009. *Arabidopsis* family ties: molecular phylogeny and age estimates in Brassicaceae. Taxon 58, 425–437.
Franzke, A., Lysak, M.A., Al-Shehbaz, I.A., Koch, M.A., Mummenhoff, K., 2011. Cabbage family affairs: the evolutionary history of Brassicaceae. Trends Plant Sci. 16, 108–116.
Galtier, N., Daubin, V., 2008. Dealing with incongruence in phylogenomic analyses. Philos. Trans. Roy. Soc. B 363, 4023–4029.
Gandolfo, M.A., Dibbern, M.C., Romero, E.J., 1988. *Akania patagonica* n. sp. and additional material on *Akania americana* Romero & Hickey (Akaniaceae), from Paleocene sediments of Patagonia. Bull. Torrey Bot. Club 115, 83–88.
Gandolfo, M.A., Nixon, K.C., Crepet, W.L., 1998. A new fossil flower from the Turonian of New Jersey: *Dressiantha bicarpellata* gen. et sp. nov. (Capparales). Am. J. Bot. 85, 964–974.
Givnish, T.J., Evans, T.M., Zjhra, M.L., Patterson, T.B., Berry, P.E., Sytsma, K.J., 2000. Molecular evolution, adaptive radiation, and geographic diversification in the amphiatlantic family Rapateaceae: evidence from *ndhF* sequences and morphology. Evolution 54, 1915–1937.
Givnish, T.J., Millam, K.C., Evans, T.M., Hall, J.C., Pires, J.C., Berry, P.E., Sytsma, K.J., 2004. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American–African disjunctions in Rapateaceae and Bromeliaceae based on *ndhF* sequence data. Int. J. Plant Sci. 165, S35–S54.
Givnish, T.J., Renner, S.S., 2004. Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreotropics, and transoceanic dispersal. Int. J. Plant Sci. 165, S1–S6.
Hall, J.C., 2008. Systematics of Capparaceae and Cleomaceae: an evaluation of the generic delimitations of *Capparis* and *Cleome* using plastid DNA sequence data. Botany 86, 682–696.
Hall, J.C., Iltis, H.H., Sytsma, K.J., 2004. Molecular phylogenetics of core Brassicales, placement of orphan genera *Emblingia*, *Forchhammeria*, *Tirania*, and character evolution. Syst. Bot. 29, 654–669.
Hall, J.C., Sytsma, K.J., Iltis, H.H., 2002. Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. Am. J. Bot. 89, 1826–1842.
Hallam, A., 1994. An Outline of Phanerozoic Biogeography. Oxford Univ. Press, Oxford.
Haudry, A., Platts, A.E., Vello, E., Hoen, D.R., Leclercq, M., Williamson, R.J., Forczek, E., Joly-Lopez, Z., Steffen, J.G., Hazzouri, K.M., Dewar, K., Stinchcombe, J.R., Schoen, D.J., Wang, X.W., Schmutz, J., Town, C.D., Edger, P.P., Pires, J.C., Schumaker, K.S., Jarvis, D.E., Mandakova, T., Lysak, M.A., van den Bergh, E., Schranz, M.E., Harrison, P.M., Moses, A.M., Bureau, T.E., Wright, S.I., Blanchette, M., 2013. An atlas of over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions. Nat. Gen. 45, 891–898.
Huang, C., Sun, R., Hu, Y., Zeng, L., Zhang, N., Cai, L., Zhang, Q., Koch, M.A., Al-Shehbaz, A., Edger, P.P., Pires, J.C., Tan, D., Zhong, Y., Ma, H., 2016. Resolution of Brassicaceae phylogeny using nuclear genes uncovers nested raditions and supports convergent morphological evolution. Mol. Biol. Evol. 33, 394–412.
Hernández-Hernández, T., Colorado, W.B., Sosa, V., 2013. Molecular evidence for the origin and evolutionary history of the rare American desert monotypic family Setchellanthaceae. Org. Divers. Evol. 13, 485–496.
Hewson, H.J., 1982. Capparaceae. In: George, A.S. (Ed.), Flora of Australia, vol. 8. Australian Government Publishing Service, Canberra, pp. 207–230.
Hewson, H.J., 1985. Akaniaceae. In: George, A.S. (Ed.), Flora of Australia, vol. 25. Australian Government Publishing Service, Canberra, pp. 2–4.
Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58, 367–380.
Hofberger, J.A., Lyons, E., Edger, P.P., Pires, J.C., Schranz, E.M., 2013. Whole genome and tandem duplicate retention facilitated glucosinolate pathway diversification in the mustard family. Genome Biol. Evol. 5, 2155–2173.
Hohmann, N., Wolf, E.M., Lysak, M.A., Koch, M.A., 2015. A time-calibrated road map of Brassicaceae species radiation and evolutionary history. Plant Cell 27, 2770–2784.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Iglesias, A., Wilf, P., Johnson, K.R., Zamuner, A.B., Cuneo, N.R., Matheos, S.D., Singer, B.S., 2007. A Paleocene lowland macroflora from Patagonia reveals significantly greater richness than North American analogs. Geology 35, 947–950.
Iltis, H.H., Cornejo, X., 2007. Studies in the Capparaceae XXX: *Capparicordis*, a new genus from the neotropics. Brittonia 59, 245–254.
Iltis, H.H., Cornejo, X., 2011. Two new genera and three new combinations in Neotropical Capparaceae. Harvard Pap. Bot. 16, 65–75.
Iltis, H.H., Hall, J.C., Cochrane, T.S., Sytsma, K.J., 2011. Studies in the Cleomaceae I: on the separate recognition of Capparaceae, Cleomaceae, and Brassicaceae. Ann. Missouri Bot. Gard. 98, 28–36.
Jacobs, M., 1960. Capparidaceae. In: Van Steens, C.G.G.J. (Ed.), Flora Malesiana. Wolters-Noordhoff Publishing, Goningen, pp. 61–105.
Jacobs, M., 1965. The genus *Capparis* (Capparaceae) from the Indus to the Pacific. Blumea XII, 385–541.
Kagale, S., Koh, C.S., Nixon, J., Bollina, V., Clarke, W.E., Tuteja, R., Spillane, C., Robinson, S.J., Links, M.G., Clarke, C., Higgins, E.E., Huebert, T., Sharpe, A.G., Parkin, I.A.P., 2014a. The emerging biofuel crop *Camelina* sativa retains a highly undifferentiated hexaploid genome structure. Nat. Commun. 5, 3706.
Kagale, S., Robinson, S.J., Nixon, J., Xiao, R., Huebert, T., Condie, J., Kessler, D., Clarke, W.E., Edger, P.P., Links, M.G., Sharpe, A.G., Parkin, I.A.P., 2014b. Polyploid evolution of the Brassicaceae during the Cenozoic era. Plant Cell 26, 2777–2791.
Koch, M.A., 2012. Mid-Miocene divergence of *Ionopsidium* and *Cochlearia* and its impact on the systematics and biogeography of the tribe Cochlearieae (Brassicaceae). Taxon 61, 76–92.
Koch, M.A., Haubold, B., Mitchell-Olds, T., 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). Mol. Biol. Evol. 17, 1483–1498.
Koch, M.A., Haubold, B., Mitchell-Olds, T., 2001. Molecular systematics of the Brassicaceae: evidence from coding plastidic *matK* and nuclear *Chs* sequences. Am. J. Bot. 88, 534–544.
Kubitzki, K., Bayers, C. (Eds.), 2003. The Families and Genera of Vascular Plants, vol. 5. Springer-Verlag, Berlin, Heidelberg.
Lavin, M., Luckow, M., 1993. Origins and relationships of tropical North-America in the context of the Boreotropics Hypothesis. Am. J. Bot. 80, 1–14.
Lüthy, B., Matile, P.H., 1984. The mustard oil bomb: rectified analysis of the subcellular organisation of the myrosinase system. Biochem. Physiol. Pfl. 179, 5–12.
Maddison, D.R., Maddison, W.P., 2000. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer Associates, Sunderland, Massachusetts.
Maddison, W.P, Maddison, D.R., 2008. Mesquite: a Modular System for Evolutionary Analysis. Version 2.5. <http://mesquiteproject.org>.
Magallón, S., Castillo, A., 2009. Angiosperm diversification through time. Am. J. Bot. 96, 349–365.
Magallón, S., Sanderson, M.J., 2001. Absolute diversification rates in angiosperm clades. Evolution 55, 1762–1780.
Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L.L., Hernández-Hernández, T., 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. New Phytol. http://dx.doi.org/10.1111/nph.13264.

Manchester, S.R., O'Leary, E.L., 2010. Phylogenetic distribution and identification of fin-winged fruits. Bot. Rev. 76, 1–82.
Martín-Bravo, S., Meimberg, H., Luceno, M., Markl, W., Valcarcel, V., Brauchler, C., Vargas, P., Heubl, G., 2007. Molecular systematics and biogeography of Resedaceae based on ITS and *trnL-F* sequences. Mol. Phylogenet. Evol. 44, 1105–1120.
Martín-Bravo, S., Vargas, P., Luceno, M., 2009. Is *Oligomeris* (Resedaceae) indigenous to North America? Molecular evidence for a natural colonization from the Old World. Am. J. Bot. 96, 507–518.
Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. 45, 524–545.
Matzke, N.J., 2013. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 4, 242–247.
Matzke, N.J., 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. Syst. Biol. http://dx.doi.org/10.1093/sysbio/syu056 (Available online).
McClusky, S., Reilinger, R., Mahmoud, S., Ben Sari, D., Tealeb, A., 2003. GPS constraints on Africa (Nubia) and Arabia plate motions. Geophys. J. Int. 155, 126–138.
McElwain, J., Punyasena, S., 2007. Mass extinction events and the plant fossil record. TREE 22, 548–557.
McLoughlin, S., 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. Aust. J. Bot. 49, 271–300.
Merckx, V., Kissling, J., Hentrich, H., Janssens, S.B., Mennes, C.B., Specht, C.D., Smets, E.F., 2013. Phylogenetic relationships of the mycoheterotrophic genus *Voyria* and the implications for the biogeographic history of Gentianaceae. Am. J. Bot. 100, 712–721.
Milne, R.I., 2006. Northern hemisphere plant disjunctions: a window on Tertiary land bridges and climate change? Ann. Bot. 98, 465–472.
Ming, R., Hou, S.B., Feng, Y., Yu, Q.Y., Dionne-Laporte, A., Saw, J.H., Senin, P., Wang, W., Ly, B.V., Lewis, K.L.T., Salzberg, S.L., Feng, L., Jones, M.R., Skelton, R.L., Murray, J.E., Chen, C.X., Qian, W.B., Shen, J.G., Du, P., Eustice, M., Tong, E., Tang, H.B., Lyons, E., Paull, R.E., Michael, T.P., Wall, K., Rice, D.W., Albert, H., Wang, M. L., Zhu, Y.J., Schatz, M., Nagarajan, N., Acob, R.A., Guan, P.Z., Blas, A., Wai, C.M., Ackerman, C.M., Ren, Y., Liu, C., Wang, J.M., Wang, J.P., Na, J.K., Shakirov, E.V., Haas, B., Thimmapuram, J., Nelson, D., Wang, X.Y., Bowers, J.E., Gschwend, A.R., Delcher, A.L., Singh, R., Suzuki, J.Y., Tripathi, S., Neupane, K., Wei, H.R., Irikura, B., Paidi, M., Jiang, N., Zhang, W.L., Presting, G., Windsor, A., Navajas-Perez, R., Torres, M.J., Feltus, F.A., Porter, B., Li, Y.J., Burroughs, A.M., Luo, M.C., Liu, L., Christopher, D.A., Mount, S.M., Moore, P.H., Sugimura, T., Jiang, J.M., Schuler, M. A., Friedman, V., Mitchell-Olds, T., Shippen, D.E., dePamphilis, C.W., Palmer, J.D., Freeling, M., Paterson, A.H., Gonsalves, D., Wang, L., Alam, M., 2008. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). Nature 452, 991–997.
Moghe, G.D., Hufnagel, D.E., Tang, H.B., Xiao, Y.L., Dworkin, I., Town, C.D., Conner, J. K., Shiu, S.H., 2014. Consequences of whole-genome triplication as revealed by comparative genomic analyses of the wild radish *Raphanus raphanistrum* and three other Brassicaceae species. Plant Cell 26, 1925–1937.
Morley, R.J., 2003. Interplate dispersal paths for megathermal angiosperms. Perspect. Plant Ecol. Evol. Syst. 6, 5–20.
Nylander, J.A.A., 2004. MrModeltest v2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University.
Olmstead, R.G., Sweere, J.A., Wolfe, K.H., 1993. Ninety extra nucleotides in the *ndhF* gene of tobacco chloroplast DNA: a summary of revisions to the 1986 genome sequence. Plant Mol. Biol. 22, 1191–1193.
Parham, J.F., Donoghue, P.C.J., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J. G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patané, J.S.L., Smith, N.D., Tarver, J.E., van Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs, L., Makovicky, P.J., Müller, J., Smith, K.T., Theodor, J.M., Warnock, R.C.M., Benton, M. J., 2012. Best practices for justifying fossil calibrations. Syst. Biol. 61, 346–359.
Patchell, M.J., Bolton, M.C., Mankowski, P., Hall, J.C., 2011. Comparative floral development in Cleomaceae reveals two distinct pathways leading to monosymmetry. Int. J. Plant Sci. 172, 352–365.
Patchell, M.J., Roalson, E.H., Hall, J.C., 2014. Resolved phylogeny of Cleomaceae based on all three genomes. Taxon 63, 315–328.
Pitman III, W.C., Cande, S., LaBrecque, J., Pindell, J., 1993. Fragmentation of Gondwana: the separation of Africa from South America. In: Goldblatt, P. (Ed.), Biological Relationships between Africa and South America. Yale University Press, New Haven, Connecticut, pp. 15–34.
Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: convergence diagnosis and output analysis for MCMC. R News 6, 7–11.
Prasad, K.V.S.K., Song, B.-H., Olson-Manning, C., Anderson, J.T., Lee, C.-R., Schranz, M. E., Windsor, A.J., Clauss, M.J., Manzaneda, A.J., Naqvi, I., Reichelt, M., Gershenzon, J., Rupasinghe, S.G., Schuler, M.A., Mitchell-Olds, T., 2012. A gain-of-function polymorphism controlling complex traits and fitness in nature. Science 337, 1081–1084.
R Development Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org/>.
Rabosky, D.L., 2006. LASER: a maximum likelihood toolkit for detecting temporal shits in diversification rates from molecular phylogenies. Evol. Bioinform. Online 2, 257–260.
Rabosky, D.L., 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS ONE 9, e89543.
Rabosky, D.L., Grundler, M., Anderson, C., Title, P., Shi, J.J., Brown, J.W., Huang, H., Larson, J.G., 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Meth. Ecol. Evol. 5, 701–707.
Rambaut, A., Drummond, A.J., 2009. Tracer v.1.5. <http://tree.bio.ed.ac.uk/software/tracer/%3E>.
Raven, P.H., Axelrod, D.I., 1974. Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 61, 539–673.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
Reguero, M.A., Gelfo, J.N., López, G.M., Bond, M., Abello, A., Santillana, S.N., Marenssi, S.A., 2014. Final Gondwana breakup: the Paleogene South American native ungulates and the demise of the South America–Antarctica land connection. Glob. Planet. Change. http://dx.doi.org/10.1016/j.gloplacha.2014.07.016.
Renner, S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. Int. J. Plant Sci. 165, S23–S33.
Revell, L.J., 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Meth. Ecol. Evol. 3, 217–223.
Riser II, J.P., Cardinal-McTeague, W.M., Hall, J.C., Hahn, W.J., Sytsma, K.J., Roalson, E. H., 2013. Phylogenetic relationships among the North American cleomoids (Cleomaceae): a test of Iltis' reduction series. Am. J. Bot. 100, 2102–2111.
Roalson, E.H., Hall, J.C., Riser II, J.P., Cardinal-McTeague, W.M., Cochrane, T.S., Sytsma, K.J., 2015. A revision of generic boundaries and nomenclature in the North American cleomoid clade (Cleomaceae). Phytotaxa 205, 129–144.
Rodman, J.E., Karol, K.G., Price, R.A., Conti, E., Sytsma, K.J., 1994. Nucleotide sequences of *rbcL* confirm the capparalean affinity of the Australian endemic Gyrostemonaceae. Aust. Syst. Bot. 7, 57–69.
Rodman, J.E., Karol, K.G., Price, R.A., Sytsma, K.J., 1996. Molecules, morphology, and Dahlgren's expanded order Capparales. Syst. Bot. 21, 289–307.
Rodman, J.E., Price, R.A., Karol, K., Conti, E., Sytsma, K.J., Palmer, J.D., 1993. Nucleotide sequences of the *rbcL* gene indicate monophyly of mustard oil plants. Ann. Missouri Bot. Gard. 80, 686–699.
Rodman, J.E., Soltis, P.S., Soltis, D.E., Sytsma, K.J., Karol, K.G., 1998. Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. Am. J. Bot. 85, 997–1006.
Rollins, R.C., 1993. The Cruciferae of Continental North America: Systematics of the Mustard Family from the Arctic to Panama. Stanford University Press, Stanford, California, USA.
Romero, E.J., Hickey, L.J., 1976. A fossil leaf of Akaniaceae from Paleocene beds in Argentina. Bull. Torrey Bot. Club 103, 126–131.
Ronse De Craene, L.P., Haston, E., 2006. The systematic relationships of glucosinolate-producing plants and related families: a cladistic investigation based on morphological and molecular characters. Bot. J. Linn. Soc. 151, 453–494.
Rose, J.P., Kriebel, R., Sytsma, K.J., 2016. Shape analysis of moss (Bryophyta) sporophytes: insights into land plant evolution. Am. J. Bot. http://dx.doi.org/10.3732/ajb.1500394 [Epub ahead of print].
Sanderson, M.J., McMahon, M.M., Steel, M., 2010. Phylogenomics with incomplete taxon coverage: the limits to inference. BMC Evol. Biol. 10, 155.
Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst. Biol. 53, 216–243.
Scheunert, A., Heubl, G., 2014. Diversification of *Scrophularia* (Scrophulariaceae) in the Western Mediterranean and Macaronesia – phylogenetic relationships, reticulate evolution and biogeographic patterns. Mol. Phylogenet. Evol. 70, 296–313.
Schranz, M.E., Edger, P.P., Pires, J.C., van Dam, N.M., Wheat, C.W., 2012a. Comparative genomics in the Brassicales: ancient genome duplications, glucosinolate diversification and Pierinae herbivore radiation. In: Edwards, D., Batley, J., Parkin, I., Kole, C. (Eds.), Genetic, Genomics and Breeding in Crop Plants: Brassica oilseeds. CRC Press, pp. 206–218.
Schranz, M.E., Lysak, M.A., Mitchell-Olds, T., 2006. The ABC's of comparative genomics in the Brassicaceae: building blocks of crucifer genomes. Trends Plant Sci. 11, 535–542.
Schranz, M.E., Mitchell-Olds, T., 2006. Independent ancient polyploidy events in the sister families Brassicaceae and Cleomaceae. Plant Cell 18, 1152–1165.
Schranz, M.E., Mohammadin, S., Edger, P.P., 2012b. Ancient whole genome duplications, novelty and diversification: the WGD radiation lag-time model. Curr. Opin. Plant Biol. 15, 147–153.
Scotese, C.R., 2001. Atlas of earth history. PALEOMAP project. Arlington (TX). Website <http://www.scotese.com> [accessed 15 November 2010].
Scotese, C.R., Gahagan, L.M., Larson, R.L., 1988. Plate tectonic reconstructions of the Cretaceous and Cenozoic ocean basins. Tectonophysics 155, 27–48.
Seelanan, T., Schnabel, A., Wendel, J.F., 1997. Congruence and consensus in the cotton tribe (Malvaceae). Syst. Bot. 22, 259–290.
Selmeier, A., 2005. *Capparidoxylon holleisii* nov. spec., a silicified *Capparis* (Capparaceae) wood with insect coprolites from the Neogene of southern Germany. Zitteliana 45, 199–209.
Sessa, E.B., Zimmer, E.A., Givnish, T.J., 2012. Phylogeny, divergence times, and historical biogeography of New World *Dryopteris* (Dryopteridaceae). Am. J. Bot. 99, 730–750.
Slotte, T., Hazzouri, K.M., Agren, J.A., Koenig, D., Maumus, F., Guo, Y.L., Steige, K., Platts, A.E., Escobar, J.S., Newman, L.K., Wang, W., Mandakova, T., Vello, E., Smith, L.M., Henz, S.R., Steffen, J., Takuno, S., Brandvain, Y., Coop, G., Andolfatto, P., Hu, T.T., Blanchette, M., Clark, R.M., Quesneville, H., Nordborg, M., Gaut, B.S., Lysak, M.A., Jenkins, J., Grimwood, J., Chapman, J., Prochnik, S., Shu, S.Q., Rokhsar, D., Schmutz, J., Weigel, D., Wright, S.I., 2013. The *Capsella rubella*

genome and the genomic consequences of rapid mating system evolution. Nat. Genet. 45, 831–835.
Smedmark, J.E.E., Eriksson, T., Bremer, B., 2010. Divergence time uncertainty and historical biogeography reconstruction – an example from Urophylleae (Rubiaceae). J. Biogeogr. 37, 2260–2274.
Soltis, D.E., Smith, S.A., Cellinese, N., Wurdack, K.J., Tank, D.C., Brockington, S.F., Refulio-Rodriguez, N.F., Walker, J.B., Moore, M.J., Carlsward, B.S., Bell, C.D., Latvis, M., Crawley, S., Black, C., Diouf, D., Xi, Z., Gitzendanner, M.A., Sytsma, K.J., Qui, Y.-L., Hilu, K.W., Davis, C.C., Sanderson, M.J., Olmstead, R.G., Judd, W.S., Donoghue, M.J., Soltis, P.S., 2011. Angiosperm phylogeny: 17 genes, 640 taxa. Am. J. Bot. 98, 704–730.
Spalink, D., Drew, B.T., Pace, M.C., Zaborsky, J.G., Starr, J.R., Cameron, K.M., Givnish, T.J., Sytsma, K.J., 2016a. Conquering then dividing: cosmopolitan sedges (Cyperaceae) and the area-richness correlation in plants. J. Biogeogr. (in press).
Spalink, D., Drew, B.T., Pace, M.C., Zaborsky, J.G., Li, P., Cameron, K.M., Givnish, T.J., Sytsma, K.J., 2016. Evolution of geographical place and niche space: patterns of diversification in the North American sedge (Cyperaceae) flora. Mol. Phylogenet. Evol. 95, 183–195.
Stauber, E.J., Kuczka, P., van Ohlen, M., Vogt, B., Janowitz, T., Piotrowski, M., Beuerle, T., Wittstock, U., 2012. Turning the 'Mustard Oil Bomb' into a 'Cyanide Bomb': aromatic glucosinolate metabolism in a specialist insect herbivore. PLoS ONE 7, e35545.
Stevens, P.F., 2001 onwards. Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since]. <http://www.mobot.org/MOBOT/research/APweb/>.
Su, J.X., Wang, W., Zhang, L.B., Chen, Z.D., 2012. Phylogenetic placement of two enigmatic genera, *Borthwickia* and *Stixis*, based on molecular and pollen data, and the description of a new family of Brassicales, Borthwickiaceae. Taxon 61, 601–611.
Sulman, J., Drew, B.T., Drummond, C., Hayasaka, E., Sytsma, K.J., 2013. Systematics, biogeography, and character evolution of *Sparganium* (Typhaceae): diversification of a widespread, aquatic lineage. Am. J. Bot. 100, 2023–2039.
Sun, G., Dilcher, D.L., Wang, H., Chen, Z., 2011. A eudicot from the Early Cretaceous of China. Nature 471, 625–628.
Swofford, D.L., 2000. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
Sytsma, K.J., Litt, A., Zjhra, M.L., Pires, J.C., Nepokroeff, M., Conti, E., Walker, J., Wilson, P.G., 2004. Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the Southern Hemisphere. Int. J. Plant Sci. 165, S85–S105.
Sytsma, K.J., Spalink, D., Berger, B., 2014. Calibrated chronograms, fossils, outgroup relationships, and root priors: re-examining the historical biogeography of Geraniales. Biol. J. Linn. Soc. 113, 29–49.
Tiffney, B.H., 1985. The Eocene North Atlantic Land Bridge: its importance in Tertiary and modern phytogeography of the northern hemisphere. J. Arnold Arboretum 66, 243–273.
Tiffney, B.H., Manchester, S.R., 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. Int. J. Plant Sci. 162, S3–S17.
Vanneste, K., Baele, G., Maere, S., Van de Peer, Y., 2014. Analysis of 41 plant genomes supports a wave of successful genome duplications in association with the Cretaceous–Paleogene boundary. Genome Res. 24, 1334–1347.
Velzen, R., Wahlert, G.A., Sosef, M., Onstein, R.E., 2015. Phylogenetics of African *Rinorea* (Violaceae): elucidating infrageneric relationships using plastid and nuclear DNA sequences. Syst. Bot. 40, 174–184.
Vision, T.J., Brown, D.G., Tanksley, S.D., 2000. The origins of genomic duplications in *Arabidopsis*. Science 290, 2114–2117.
Wang, X.W., Wang, H.Z., Wang, J., Sun, R.F., Wu, J., Liu, S.Y., Bai, Y.Q., Mun, J.H., Bancroft, I., Cheng, F., Huang, S.W., Li, X.X., Hua, W., Wang, J.Y., Wang, X.Y., Freeling, M., Pires, J.C., Paterson, A.H., Chalhoub, B., Wang, B., Hayward, A., Sharpe, A.G., Park, B.S., Weisshaar, B., Liu, B.H., Li, B., Liu, B., Tong, C.B., Song, C., Duran, C., Peng, C.F., Geng, C.Y., Koh, C.S., Lin, C.Y., Edwards, D., Mu, D.S., Shen, D., Soumpourou, E., Li, F., Fraser, F., Conant, G., Lassalle, G., King, G.J., Bonnema, G., Tang, H.B., Wang, H.P., Belcram, H., Zhou, H.L., Hirakawa, H., Abe, H., Guo, H., Wang, H., Jin, H.Z., Parkin, I.A.P., Batley, J., Kim, J.S., Just, J., Li, J.W., Xu, J.H., Deng, J., Kim, J.A., Li, J.P., Yu, J.Y., Meng, J.L., Wang, J.P., Min, J.M., Poulain, J., Wang, J., Hatakeyama, K., Wu, K., Wang, L., Fang, L., Trick, M., Links, M.G., Zhao, M.X., Jin, M.N., Ramchiary, N., Drou, N., Berkman, P.J., Cai, Q.L., Huang, Q.F., Li, R.Q., Tabata, S., Cheng, S.F., Zhang, S., Zhang, S.J., Huang, S.M., Sato, S., Sun, S.L., Kwon, S.J., Choi, S.R., Lee, T.H., Fan, W., Zhao, X., Tan, X., Xu, X., Wang, Y., Qiu, Y., Yin, Y., Li, Y.R., Du, Y.C., Liao, Y.C., Lim, Y., Narusaka, Y., Wang, Y.P., Wang, Z.Y., Li, Z.Y., Wang, Z.W., Xiong, Z.Y., Zhang, Z.H., 2011. The genome of the mesopolyploid crop species *Brassica rapa*. Nat. Genet. 43, 1035–1040.
Wen, J., 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annu. Rev. Ecol. Syst. 30, 421–455.
Wheat, C.W., Vogel, H., Wittstock, U., Braby, M.F., Underwood, D., Mitchell-Olds, T., 2007. The genetic basis of a plant-insect coevolutionary key innovation. Proc. Natl. Acad. Sci. 104, 20427–20431.
White, L.T., Gibson, G.M., Lister, G.S., 2013. A reassessment of paleogeographic reconstructions of eastern Gondwana: bringing geology back into the equation. Gondwana Res. 24, 984–998.
Wiens, J.J., 2006. Missing data and the design of phylogenetic analyses. J. Biomed. Inform. 39, 34–42.
Wilf, P., Johnson, K.R., Cúneo, N.R., Smith, M.E., Singer, B.S., Gandolfo, M.A., 2005. Eocene plant diversity at Laguna del Hunco and Río Pichileufú, Patagonia, Argentina. Am. Nat. 165, 634–650.
Willis, C.G., Hall, J.C., Rubio de Casas, R., Wang, T.Y., Donohue, K., 2014. Diversification and the evolution of dispersal ability in the tribe Brassiceae (Brassicaceae). Ann. Bot. 114, 1675–1686.
Wittstock, U., Agerbirk, N., Stauber, E.J., Olsen, C.E., Hippler, M., Mitchell-Olds, T., Gershenson, J., Vogel, H., 2004. Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc. Natl. Acad. Sci. 101, 4859–4864.
Worberg, A., Alford, M.H., Quandt, D., Borsch, T., 2009. Huerteales sister to Brassicales plus Malvales, and newly circumscribed to include *Dipentodon*, *Gerrardina*, *Huertea*, *Perrottetia*, and *Tapiscia*. Taxon 58, 468–478.
Wurdack, K.J., Davis, C.C., 2009. Malpighiales phylogenetics: gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. Am. J. Bot. 96, 1551–1570.
Xiang, Q.Y., Thomas, D.T., Xiang, Q.P., 2011. Resolving and dating the phylogeny of Cornales – effects of taxon sampling, data partitions, and fossil calibrations. Mol. Phylogenet. Evol. 59, 123–138.
Yule, G.U., 1925. A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis, F.R.S. Philos. Trans. Roy. Soc. B 213, 21–87.
Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693.