Using a supermatrix approach to explore phylogenetic relationships, divergence times, and historical biogeography of Saxifragales

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Abstract

The Saxifragales is a morphologically and ecologically diverse clade of flowering plants with a cosmopolitan distribution. Although families and genera within the order exhibit classic biogeographical disjunctions, no studies to date have rigorously examined and described its historical biogeography. Here, we analyze the historical biogeography of Saxifragales by first generating a new chronogram for the order using a supermatrix approach, based on 61 loci from 251 representative members of Saxifragales. Our results suggest that Saxifragales originated in the early Albian ~107 Ma and diversified rapidly in the next 15 Ma, with all stem lineages of extant families present by the Campanian at ~75 Ma. The ancestral geographic range of the order is unclear, but ancestral range reconstructions point to an east Asian origin as the most tenable hypothesis. Ancestral ranges of the Haloragaceae/Crassulaceae clade suggest a strong signal for an Australasia origin for all families, and Saxifragaceae shows strong signal for bidirectional movement across the Asian-Alaskan land bridge during the Upper Cretaceous. Disjunct distributions are best explained by long-distance dispersal rather than vicariance, but we demonstrate that timing and directionality within particular disjunctions are similar within Saxifragales and consistent with results from distantly related angiosperm clades.

Keywords

Saxifragales, long-distance dispersal, vicariance, disjunction, Bering land bridge

1. Introduction

Disjunct distributions of closely related organisms are one of the most curious types of spatial patterns in organismal biology (Darwin, 1859; Wallace, 1880; Raven, 1963, 1972; Wen

1999; Simpson et al., 2017). Disjunctions occur when populations or lineages within or between taxa are discontinuous and can be regional (e.g., on either side of a mountain), intracontinental (e.g. on opposite sides of a continent), intercontinental (e.g. on continents which are not presently contiguous), or global (e.g., amphitropical disjunctions). Of these types of disjunctions, intercontinental and global disjunctions have served as classic examples of the phenomenon and are perhaps the best studied, with some of the most notable examples in plants being those between western Africa and South America (Givnish et al., 2004; Renner, 2004), and between eastern Asia and North America (Xiang et al., 1998, Wen, 1999, 2001; Donoghue & Smith, 2004; Wen et al., 2010, 2016). Disjunctions may arise through processes such as vicariance via geological processes, long-distance dispersal (i.e. founder event) from propagule movement, and/or short distance dispersal(s) followed by local extinction. Given that similar intercontinental disjunctions are also found across distantly related organisms, it has been argued that these patterns might be best explained as a result of vicariance; however analyses using modern phylogenetic hypotheses well-calibrated with carefully scrutinized fossils across a diversity of plant lineages instead suggest that most of these patterns are better explained by long distance dispersal alone or long distance dispersal in concert with vicariance (Givnish et al., 2004; de Queiroz, 2005; Barker et al., 2007; Clayton et al., 2009; Dupin et al., 2017; Ruhfel et al., 2016; Drew et al., 2017; Simpson et al. 2017).

The angiosperm order Saxifragales consists of 15 families that encompass about 2,500 species (APG IV, 2016), and are sister to rosids (Soltis et al., 2011; APG IV, 2016). Saxifragales are diverse ecologically and morphologically, and also possesses an intriguing extant geographical distribution that features several different types of intercontinental disjunctions. Although Saxifragales are most species-rich in the Northern Hemisphere, several families within the order are either more diverse or currently restricted to the Southern Hemisphere, with some narrowly endemic and ostensibly relictual in the Southern Hemisphere (e.g. Aphanopetalaceae and Tetracarpaeaceae).

The diverse morphology and distribution within Saxifragales, along with a lack of clear synapomorphies, has caused considerable taxonomic uncertainty regarding relationships amongst the constituent families in the past, but during the past 25 years major progress has been made in understanding relationships within Saxifragales and in circumscribing families within the order (Morgan & Soltis, 1993; Soltis et al., 1990,2000, 2013; Soltis & Soltis, 1997; Hoot et al., 1999; Fishbein et al., 2001). With the recent addition of the previously controversially placed holoparasitic Cynomoriaceae in Saxifragales (Nickrent et al., 2005; Jian et al., 2008; Zhang et al., 2011; Bellot et al., 2016; Folk et al., 2019, 2021), the order now seems taxonomically stable at the familial level. However, the exact placement of Cynomoriaceae within Saxifragales is still unclear (APG IV, 2016; Bellot et al., 2016; Folk et al., 2019). Some of these studies have also shown weak support for the placement of Peridiscaceae, an enigmatic family atypical for the order due to the presence of large seeds and an exclusively tropical distribution (Davis & Chase, 2004; Soltis et al., 2007), but which is possibly sister to the remainder of the order (Soltis et al., 2007; Jian et al., 2008; Folk et al., 2019). Excluding Peridiscaceae, Saxifragales can be grouped into three major clades. A clade of largely woody plants comprised of families Altingiaceae, Cercidiphyllaceae, Daphniphyllaceae, Hamamelidaceae, and Paeoniaceae (which are non-woody) sister to the rest of the families within this clade (Moore et al., 2011; Soltis et al., 2011; but see Folk et al., 2019, 2021), hereafter referred to as the PWC (Paeoniaceae + woody clade). The PWC is sister to the Core Saxifragales (Jian et al., 2008), which contains the remaining two major subclades. The first of these subclades is comprised of Aphanopetalaceae, Crassulaceae, Haloragaceae, Penthoraceae, and Tetracarpaeaceae (Morgan & Soltis, 1993) and has been referred to as the Haloragaceae/Crassulaceae clade (Jian et al., 2008). The second subclade includes Grossulariaceae, Iteaceae, and Saxifragaceae (Soltis et al., 2007), commonly called the Saxifragaceae Alliance (Fishbein et al., 2001; Soltis et al., 2007; Jian et al., 2008; Stubbs et al., 2020).

As with several other angiosperm orders such as Caryophyllales (Cuénoud et al., 2002; Brockington et al., 2009; Walker et al., 2018), Ericales (Schönenberger et al., 2005; Rose et al., 2018), Lamiales (Olmstead et al., 2001; Schäferhoff et al., 2011; Refulio-Rodriguez & Olmstead, 2014), and Malpighiales (Wurdack & Davis, 2009; Xi et al., 2012), Saxifragales appears to have undergone an ancient and rapid diversification (Fishbein et al., 2001; Fishbein & Soltis, 2004; Jian et al., 2008). As a result, it has proven challenging to resolve deep-level relationships within the order (Magallón et al., 1999; Moore et al., 2007, 2010; Wang et al., 2009; Smith et al., 2010; Soltis et al., 2011; Folk et al., 2019). Based upon fossil evidence, Saxifragales clearly began to diversify at least 89.5 million years ago (Ma; Magallón et al., 1999; Wikström et al., 2001) but crown age estimates from molecular dating have varied widely from 83-118 Ma (Hermsen et al., 2006; Jian et al., 2008; Magallón et al., 2015; Tank et al., 2015; Folk et al., 2019).

Biogeographically, Saxifragales exhibit several different, classic, and/or striking inter- and intracontinental disjunction patterns including eastern North America/eastern Asia (Hamamelidaceae: *Hamamelis*, Penthoraceae: *Penthorum*), South America/western Africa (Peridiscaceae), eastern/western Australia (Aphanopetalaceae), eastern North America/eastern Asia/South Africa (Hamamelidaceae, Iteaceae), and western North America/Mediterranean/eastern Asia (Paeoniaceae), making it an ideal clade to examine the timing and drivers (long-distance dispersal vs. vicariance) of these disjunctions. Despite the striking number and types of disjunctions within the order, previous phylogenetic studies that have included or focused on Saxifragales have not rigorously explored or clarified divergence times and biogeographic history of the entire order using broad taxon and gene sampling (but see Folk et al., 2021). Here, we employ a supermatrix alignment consisting of plastid (cpDNA), nuclear ribosomal (nrDNA), and mitochondrial DNA (mtDNA) to elucidate intra-ordinal phylogenetic relationships within Saxifragales. We use this supermatrix in conjunction with multiple fossil calibration points to produce a robust chronogram to examine divergence times of major lineages of Saxifragales and make ancestral range estimations. Specifically, we focus on the following questions: (1) Is Peridiscaceae sister to the remaining Saxifragales? (2) Is the current distribution of Peridiscaceae a result of vicariance or long-distance dispersal? (3) What is the geographic origin of Saxifragales and are Australasian families Aphanopetalaceae and Tetracarpaeaceae relictual? and (4) Are there similarities in timing and underlying biogeographical process across lineages of Saxifragales with similar disjunct distributions?

2. Methods

2.1. Taxon sampling and supermatrix assembly

Initially, we compiled a supermatrix that consisted of 918 taxa. We iteratively reduced our sampling to 351 samples. This sampling strategy largely eliminated taxa that were redundant geographically (e.g., Halogoraceae, South African Crassulaceae), and was employed so the dataset would be small enough to make analyses using BEAST (Drummond & Rambaut, 2007) tractable. Our final dataset of 351 taxa included 251 within Saxifragales and an additional 100 taxa to serve as an outgroup. The high proportion of outgroup taxa relative to the ingroup served the dual purposes of providing calibration points outside the order as well as providing a broad dating framework. The outgroup consisted of representatives from the orders Ranunculales (Berberidaceae [1 accession], Eupteleaceae [1 accession], Lardizabalaceae [1 accession], Menispermaceae [1 accession], Papaveraceae [1 accession], Ranunculaceae [1 accession]), Proteales (Nelumbonaceae [2 accessions], Platanaceae [3 accessions], Proteaceae [36 accessions] Sabiaceae [4 accessions]), Trochodendrales (Trochodendraceae, 2 accessions), Buxales (Buxaceae, 7 accessions), Gunnerales (Gunneraceae [6 accessions], Myrothamnaceae [2 accessions]), Dilleniales (Dilleniaceae, 14 accessions), and Vitales (Vitaceae, 18 accessions). Ranunculales served as the ultimate outgroup for rooting purposes. The 251 accessions within Saxifragales represented all families within Saxifragales with the exception of Cynomoriaceae. Species level sampling within Saxifragales was chosen to encompass as much geographic breadth as possible. Our intrafamilial sampling was as follows: Altingiaceae (11 species), Aphanopetalaceae (2 species), Cercidiphyllaceae (2 species), Crassulaceae (60 species), Daphniphyllaceae (3 species), Grossulariaceae (8 species), Haloragaceae (29 species), Hamamelidaceae (35 species), Iteaceae (including Pterostemonaceae, 5 species), Paeoniaceae (12 species), Penthoraceae (2 species), Peridiscaceae (3 species), Saxifragaceae (78 species), and Tetracarpaeaceae (1 species).

The supermatrix dataset was compiled from multiple sources. The supermatrix of Soltis et al. (2013) provided the bulk of our sampling. This was augmented by samples from Deng et al. (2015) and 1 KP data (Matasci et al., 2014). The dataset was enlarged through GenBank searches based on gene regions added post-2012 (i.e., after the submission of Soltis et al. [2013]). Finally, plastome data from 13 early-diverging eudicots were included for outgroup taxa (Sun et al., 2016). When multiple species were found for the same gene region on GenBank, we included only the one with the longest sequence. In total, we assembled a data matrix of 68 gene regions that represented all three plant genomes, chloroplast (63 gene regions; 56,970 nucleotides), nuclear (3 regions; 6,973 nucleotides) and mitochondrial (2 regions; 3,190 nucleotides; Appendix 1). The external transcribed spacer (ETS) and *trnL-trnF* regions were difficult to align across different families, so family-specific alignment blocks were created for each region (ETS– Crassulaceae, Grossulariaceae, Hamamelidaceae, Saxifragaceae; *trnL-trnF*– Crassulaceae, Saxifragaceae; Appendix 1). Each gene region was compiled and aligned in Mesquite v. 3.61 (Maddison & Maddison, 2019). Preliminary trees to confirm the orthology of gene regions were computed using maximum likelihood as implemented RAxML (Stamatakis, 2014) using the Black Box tool on CIPRES (Miller et al., 2010). The concatenated supermatrix consisted of 67,133 aligned nucleotides. After removing 519 missing data characters (missing across all taxa), and 780 characters due to ambiguous alignment, the final supermatrix was composed of 65,834 characters.

2.2. Phylogeny and divergence time estimation

Phylogeny and divergence times were estimated simultaneously in BEAST v. 1.8.4 (Drummond & Rambaut, 2007) on the concatenated, unpartitioned supermatrix with indels treated as missing data. We used an uncorrelated relaxed lognormal clock and a birth-death branching process under the GTR + I + G model of sequence evolution as suggested by JModeltest2 (Darriba et al., 2012). We used six fossil calibration points based on their justification in Magallón et al. (2015), Hermsen (2013; Iteaceae), and Manchester (2013; Vitaceae), five of which were within Saxifragales and one within Vitales. Dates were based on the 2019-05 version of ChronostratChart (Cohen et al., 2019). Fossil calibrations were given a truncated lognormal prior with mean = 2 (mean = 2.5 for stem of Cercidiphyllaceae) and standard deviation = 1 with an offset corresponding to the age estimate of the fossil, and a maximum date of 133.4 Ma (see below). Fossil priors constrained the stem of Altingiaceae with an offset of 89.8 Ma, stem of Cercidiphyllaceae with an offset of 56.0 Ma, stem of Haloragaceae with an offset of 72.1 Ma, stem of Iteaceae with an offset of 89.8 Ma, and crown Vitaceae to 66.0 Ma. Magallón et al. (2015) constrained crown Hamamelidoideae with an offset of 83.6 Ma, but we more conservatively placed their fossil on crown Hamamelidaceae with the offset of 83.6 Ma. Lastly, we constrained the root (eudicot crown) with a uniform prior from 129.7–133.4 Ma based on a secondary date from Magallón et al. (2015) with minima and maxima corresponding to the 95% highest posterior density (HPD) for this node. We ran 12 MCMC chains for 3.5×10^8 generations each with sampling every 10,000 generations. Convergence was assessed using TRACER v. 1.7.1 (Rambaut et al., 2014). Runs were combined using LogCombiner v. 1.8.4 after excluding samples from each run as a burn-in as appropriate following assessment using TRACER, with the burn-in varying from 4.0×10^7 to 2.0×10^8 generations. The posterior distribution of trees was summarized as a maximum clade credibility (MCC) tree in TreeAnnotator v. 1.8.4 (Drummond et al., 2012).

2.3. Ancestral range estimation

We determined the geography of extant tips using Tropicos (https://www.tropicos.org/home), Flora of China (http://www.efloras.org/flora_page.aspx?flora_id=2), GBIF (https://www.gbif.org/), Flora of Australia (https://www.environment.gov.au/science/abrs/online-resources/flora-of-australia-online), and Flora of North America (http://www.efloras.org/flora_page.aspx?flora_id=1). GBIF records were checked as necessary following established protocols to ensure accurate species distributions from this data depository (Maldonado et al., 2015; Spalink et al., 2016a, b). Terminals were initially scored for presence/absence in the following 12 regions delimited on the location of present geographic barriers and previous tectonic activity: (1) Northwestern North America west of the Rocky Mountains and north of Mexico, (2) eastern North America east of the Rocky Mountains and north of Mexico including Greenland, (3) southwestern North America including Mexico and Central America, (4) western South America including the Andes, (5) Guiana Shield, (6) southeastern South America, (7) Europe to the Russian border including the Mediterranean, (8) northern Africa including the Sahara and the Middle East, (9) southern Africa south of the Sahara Desert including tropical west Africa, east Africa, South Africa, and Madagascar, (10) northern Asia west of the Caucasus, north of the Himalaya and north of the Indo-China peninsula, (11) southern, tropical Asia including India, and Papua New Guinea, and (12) Australasia. Given the paucity of occupancy in areas (5) and (6), we combined these two areas into one (eastern South America) for downstream analyses for a total of 11 areas.

Ancestral range estimation (ARE) was conducted in BioGeoBEARS v. 1.1.2 (Matzke, 2012, 2013) on the MCC tree with outgroups removed, given the cosmopolitan distribution of all rosids, the sister clade of Saxifragales. To better model geological history, we conducted a time-stratified analysis with dispersal multipliers. We implemented the Dispersal-Extinction-Cladogenesis (DEC) model (Ree & Smith, 2008), testing between models with and without founder events (jump dispersal, j parameter in the BioGeoBEARS super model). Recent criticism of this parameter and other aspects of the model (Ree & Sanmartín, 2018) have been countered and thus remain as valid (Klaus & Matzke, 2019; Matzke, 2021). The significance of the fit of the DEC and DECj models were tested using a likelihood ratio test. We allowed a maximum range size of up to four areas (the maximum found in any extant tip). Dispersal probabilities between pairs of areas were specified for the following five separate time slices (see below) based on known geological events affecting geographic distributions within Saxifragales and that have been similarly modeled in DEC and DECj analyses in other ancient and widespread lineages (e.g., Buerki et al., 2011; Berger et al., 2016; Cardinal-McTeague et al., 2016; Spalink et al., 2016a, b; Rose et al., 2018). Dispersal probabilities among areas were specified for five separate time slices: 0–10, 10–35, 25–65, 65–90, 90–113 Ma, with dispersal multipliers modified in each time slice based on adjacency of landmasses at each time. These time slices allowed for testing of the importance of continental vicariance and collision as well as the role of possible Northern Hemisphere land bridges (Tiffney, 1985, 2000; Tiffney & Manchester, 2001; Graham, 2011). Additionally, we conducted biogeographical stochastic mapping (Matzke, 2016; Dupin et al., 2017) in BioGeoBEARS with 100 stochastic maps under both DEC and DECj models to examine the timing, type, and number of biogeographical events, and the number of each class of event (e.g., vicariance, sympatry, subset-sympatry, and jump dispersals) given the DEC or DECj model was summarized using the "count_ana_clado_events" function in BioGeoBEARS . Details regarding model development, temporal stratification, and dispersal probabilities among the geographical regions through time, as well as alignment and BEAST files, are provided in the supplementary material available on Dryad (https://doi.org/10.5061/dryad.ffbg79cvg).

3. Results

3.1. Phylogenetic inference

Our analysis of the supermatrix of 351 taxa and 65,834 aligned bp resulted in a well-supported and resolved phylogenetic hypothesis of Saxifragales, with all major backbone nodes having posterior probabilities (PP) > 0.99 and generally 1.0. All families were recovered as monophyletic with PP = 1.0 (Fig.1). Our topology recovers the three major clades consistently recovered within Saxifragales as monophyletic, all with PP = 1.0: Peridiscaceae, the PWC, and Core Saxifragales. We also recover the two subclades of Core Saxifragales with PP = 1.0: the Haloragaceae/Crassulaceae clade and the Saxifragaceae Alliance. Our tree resolves backbone Saxifragales, strongly suggesting that Peridiscaceae is sister to the remainder of the order (PP > 0.99).

Within the PWC, Paeoniaceae is sister to Altingiaceae + Cercidiphyllaceae + Daphniphyllaceae + Hamamelidaceae (PP = 1.0), Altingiaceae is sister to Cercidiphyllaceae + Daphniphyllaceae + Hamamelidaceae (PP > 0.99), and Cercidiphyllaceae is sister to Daphniphyllaceae (PP = 0.86). Within the Haloragaceae/Crassulaceae clade, Crassulaceae is sister to the remaining families (PP = 1.0), Aphanopetalaceae is sister to Haloragaceae + Penthoraceae + Tetracarpaeaceae (PP = 0.96), and Tetracarpaeaceae is sister to Haloragaceae + Penthoraceae (PP = 1.0). Finally, within the Saxifragaceae Alliance, we recover Iteaceae (including Pterostemonaceae) as monophyletic (PP = 1.0) and sister to Saxifragaceae + Grossulariaceae (PP = 1.0).

3.2. Major divergence times

Crown Saxifragales dates to ~107 Ma (95% HPD = 100.87–112.30 Ma) and diversified rapidly, with the crown ages of the three major Saxifragales clades dating > 94 Ma (Figure 1). All stem lineages of extant families were dated to the Upper Cretaceous at ~75 Ma (youngest stem ages are Haloragaceae and Penthoraceae), although the crown ages for most families were much younger. The stem age of Australasian Aphanopetalaceae was ~87 Ma, while the crown of the family is relatively recent at ~13 Ma (95% HPD = 1.90–28.98 Ma). The stem age of the similarly distributed Tetracarpaeaceae dates to ~83 Ma. Crown Iteaceae, excluding Pterostemonaceae which marks the divergence of *Choristylis* Harv. and *Itea* L.*,* dates to ~34 Ma (HPD = 14.45-55.80 Ma). Within Peridiscaceae *Soyauxia* Oliv. diverged from *Peridiscus* Benth. ~29 Ma, although there is a wide error bar around this age estimate (95% HPD = 8.16–52.85 Ma).

3.3 Ancestral range estimation

The BioGeoBEARS analysis selected the DECj (LnL = -604.2, d = 4.3×10^{-3}, e = 1.0×10^{-8}, j = 0.064) model over the DEC model (LnL = -660.4, d = 6.7×10^{-3}, e = 6.1×10^{-4}) (D = 112.3, df = 1, P = 3.1×10^{-26}), so we report the results of DECj only (Figure 2; S1). The biogeographic origin of Saxifragales, the order excluding Peridiscaceae, and the Core Saxifragales is ambiguously reconstructed, with the area with the highest probability for all three nodes being both highly polymorphic and discontinuous (joint Central America + southern Africa + east Asia + Oceania) but with low probability (P = 0.24, 0.20, and 0.13 respectively). Most other major nodes show high probabilities of one or more areas: crown Peridiscaceae is reconstructed as found in southern Africa (P = 0.80) and crown *Peridiscus* + *Soyauxia* is reconstructed as joint southern Africa and South America (P = 0.50) with stem *Peridiscus* originating in South America.

In the PWC all nodes representing super-familial ancestors are clearly reconstructed as found in northern Asia, excepting stem and crown Hamamelidaceae which were reconstructed as originating in southern Asia (crown P = 0.61). In addition, most family stems or crowns in the PWC are Asian, and particularly northern Asian, in origin, with the exception of stem and to a lesser extent the crown Altingiaceae, with an inferred European origin (P = 0.56).

Crown Haloragaceae/Crassulaceae clade is ambiguously reconstructed as originating in Australasia (P = 0.28), but with a clear signal for an Australasian origin of all super-familial ancestors in the Haloragaceae/Crassulaceae Clade excluding Crassulaceae (P = 0.98). Crown Crassulaceae itself is ambiguously reconstructed as originating in southern Africa (P = 0.18).

Finally, the biogeographic history of the deepest nodes of the Saxifragaceae Alliance are ambiguously reconstructed, with the highest ancestral ranges of the crown of the clade being either northern Asia (P = 0.30) or northwestern North America (P = 0.11). The stem and crown of the Saxifragaceae Alliance excluding Iteaceae + Pterostemonaceae arose in northern Asia (crown P = 0.41), with stem and crown Grossulariaceae + Saxifragaceae arising in either northwestern North America or northern Asia (crown P = 0.42 and 0.38, respectively), with a clearer signal of a northern Asian origin of stem and crown Saxifragaceae (crown P = 0.54) but with some probability for northwestern North America (P = 0.25) or joint origin in northern Asia + northwestern North America (P = 0.17). There is extensive movement from northern Asia to northwestern North America along the backbone of Saxifragaceae, with movement back to northern Asia within the last 35 Ma. The distribution of Iteaceae + Pterostemonaceae is best explained as long distance dispersal from northern Asia to Central America when the lineage diverged from Grossulariaceae + Saxifragaceae, followed by dispersal to eastern North America at the divergence of stem Iteaceae, and finally with long distance dispersal from eastern North America to Africa giving rise to *Choristylis*.

3.4. Biogeographical stochastic mapping

Stochastic mapping of ancestral ranges indicates that there is an average of 327.0 biogeographical events across our representative sample of Saxifragales. Of these, a mean of 136.5 (42%) are dispersal events. An average of 59.5 (44%) of all dispersal events are cladogenetic dispersal (founder events, parameter *j*) and the remaining 56% of dispersal events are anagenetic dispersal (parameter *d*). An average of 17.7 (5.4%) of all events are vicariant events (parameter *v*).

Most cladogenetic dispersal and vicariance events occur before the early Eocene, with the vast majority of all cladogenetic events occurring 65–105 Ma (Figure 3). Most cladogenetic events since the Eocene are reconstructed as being jump dispersal events. Of 21 notable infrafamilial disjunctions highlighted in Table 1, most are best explained by jump dispersal rather than vicariance. The timing and directionality within disjunction types is variable, but some temporal and directional similarities emerge, notably multiple jump dispersals from east Asia to western North America 15-25 Ma, jump dispersals from Europe to North America 6-9 Ma, and jump dispersal from Asia to Eastern North America 3–18 Ma (Table 1).

4. Discussion

Our results represent one of the most comprehensive phylogenetic analyses of Saxifragales and is the most comprehensive study to date to provide both divergence times with an estimate of statistical uncertainty surrounding node ages and a formal analysis and discussion of the biogeographic history of the order to clarify the causes of disjunct geographic distributions. This dataset has provided a new, well-resolved phylogenetic framework to clarify the 107 Ma history of the order.

4.1. Progress towards a robust phylogenetic hypothesis of Saxifragales

4.1.1. Ancient Saxifragales divergences

Until recently, the ordinal and family placement of genera now included in Peridiscaceae was controversial (Davis & Chase, 2004; Wurdack & Davis, 2009). Subsequently, phylogenetic studies have suggested that Peridiscaceae is sister to the rest of Saxifragales, but generally without strong statistical support (Soltis et al., 2007, 2013). However, Jian et al. (2008), who used ~50kb for 25 Saxifragales, recovered Peridiscaceae as sister to the remaining Saxifragales with high statistical support. More recently, Folk et al. (2019), using 301 protein-coding nuclear loci, placed Peridiscaceae as sister to the PWC in a concatenated analysis with poor support, and recovered Peridiscaceae in a polytomy with the PWC and Core Saxifragales in a coalescent analysis. This polytomy was also found in the dated tree of Folk et al. (2021). Our results are more consistent with the results of Jian et al. (2008), but it remains unclear whether there is a lack of phylogenetic information about this relationship present in the dataset of Folk et al. (2019, 2021) or the sister relationship of Peridiscaceae/PWC represents a true topological conflict between datasets (primarily nuclear data in Folk et al. [2019, 2021] and primarily chloroplast data in Jian et al. [2008] and the dataset presented in this paper). One major difference between this paper and that of Folk et al. (2019, 2021) is the number of outgroup taxa used in each study. We included 100 outgroup taxa (251 ingroup) here whereas Folk et al. (2019, 2021) included 14 (627 ingroup). The different taxon sampling in outgroups may influence the different placement of Peridiscaceae in the two studies. Apart from the placement of Peridiscaceae, the monophyly of and relationships among the major clades of Saxifragales are largely congruent with those of previous studies (Jian et al., 2008, Soltis et al., 2013; Folk et al., 2019, 2021).

4.1.2. PWC (*Altingiaceae, Cercidiphyllaceae, Daphniphyllaceae, Hamamelidaceae, and Paeoniaceae clade*)

Familial relationships within the PWC are identical to those in Soltis et al. (2013) and Jian et al. (2008). However, those previous studies and this study differ considerably from relationships recovered by Folk et al. (2019) in both the concatenated and coalescent analyses, where Cercidiphyllaceae is sister to Altingiaceae + Hamamelidaceae with strong support in the concatenated analysis and nested within Altingiaceae or forming a polytomy with Hamamelidaceae and Altingiaceae in the coalescent analysis. While our data are uncertain about the exact relationships of Cercidiphyllaceae, Daphniphyllaceae, and Hamamelidaceae (Figure 1), they clearly contradict the placement of Altingiaceae in Folk et al. (2019). Again, the source of this discordance remains unclear, but merits further study. Relationships within the PWC families are consistent with relationships around supported nodes in previous studies focusing on intrafamilial relationships: Altingiaceae (Ickert-Bond & Wen, 2006; with the exception that Indochinese *Altingia* appear nested in the East Asian clade), Paeoniaceae (Sang et al., 1997), and Hamamelidaceae (Shi et al., 1998; Xie et al., 2010) with the exception of the placement of *Mytilaria*, which differs in its phylogenetic placement between nuclear ribosomal and plastid datasets (Xie et al., 2010): here it falls in the position suggested based on plastid data in Xie et al. (2010) and 301 nuclear loci in Folk et al. (2019).

4.1.3. Haloragaceae/Crassulaceae clade

Relationships among families in this clade are generally consistent with previously published studies, with Crassulaceae sister to a clade consisting of Aphanopetalaceae + Tetracarpaeaceae + Haloragaceae + Penthoraceae (Jian et al., 2008; Soltis et al., 2013; Folk et al., 2019). However, while we find continued support for Aphanopetalaceae as sister to Tetracarpaeaceae + Haloragaceae + Penthoraceae, we find less than complete statistical support for this relationship (PP = 0.96; Figure 1), which might merit further study. Indeed, Folk et al. (2019, 2021) recovered Tetracarpaeaceae as sister to Aphanopetalaceae + Haloragaceae + Penthoraceae, suggesting potential conflict between chloroplast and nuclear data. In addition, Folk et al. (2019) recovered Crassulaceae as sister to the Saxifragaceae Alliance (Grossulariaceae, Iteaceae, Saxifragaceae; see below) in their coalescent analysis, as opposed to sister to Aphanopetalaceae + Tetracarpaeaceae + Haloragaceae + Penthoraceae as recovered in Jian et al. (2008), Soltis et al. (2013), and the concatenated analyses of Folk et al. (2019, 2021). Infrafamilial relationships are in in agreement with focused studies in Crassulaceae (Mort et al., 2001; Carrillo-Reyes et al., 2009) and Haloragaceae (Moody & Les, 2007; Chen et al., 2014) in places where relationships are supported in these previous studies.

4.1.4. Saxifragaceae Alliance

As with other clades, major relationships in the Saxifragaceae Alliance in this study confirm those recovered in previous studies (Jian et al., 2008; Soltis et al., 2013; Folk et al., 2019).

Grossulariaceae, containing only the genus *Ribes* L., has been poorly studied in a molecular phylogenetic context. The most recent study on Grossulariaceae phylogenetic relationships is from Schultheis & Donoghue (2004), but this studied failed to find any support for resolution below the sectional level. By contrast, many more studies have focused on relationships, divergence times, and biogeography of Saxifragaceae. The topology of Saxifragaceae recovered in this study is consistent with that recovered by previous phylogenetic studies if the family (Xiang et al., 2012; Soltis et al., 2013; Deng et al., 2015), especially with the most recent of the studies (Deng et al., 2015). In particular, the phylogenetic placement of *Saniculiphyllum* with the Heucheroid clade was unclear in Xiang et al. (2012) while Deng et al. (2015) and Folk et al. (2019) placed *Saniculiphyllum* sister to the *Boykinia* group.

4.2. Biogeography of Saxifragales

Our divergence time estimates, while varying widely from those of Soltis et al. (2013), are largely in line with those of Folk et al. (2019), with some exceptions which are discussed below (Figure 4). The similarity of our age estimates to those of Folk et al. (2019; Figure 4) are not surprising given that their ages are based on a combined penalized likelihood/BEAST “congruification” analysis using secondary calibrations from node ages from Magallón et al. (2015), from which our fossil priors were derived, while Soltis et al. (2013) used penalized likelihood with four constraints clustered in the PWC. Not surprisingly then, most crown ages older in Soltis et al. (2013) versus this study are in the PWC, while most node ages younger in Soltis et al. (2013) versus this study are in the Core Saxifragales, particularly Crassulaceae and Saxifragaceae (Figure 4). Compared with Folk et al. (2019), the greatest outlying ages are found in Peridiscaceae and the PWC (Altingiaceae, Cercidiphyllaceae, and Hamamelidaceae). In all cases, equivalent node ages are younger in Folk et al. (2019). The discordances undoubtedly reflect topological differences between studies (see sections 4.1.1 and 4.1.2). Striking nodal divergence times between Folk et al. (2019) and this study are also found within Hamamelidaceae, all of which are again younger in Folk et al. (2019). This may be due to our conservative placement of the Hamamelidaceae prior on crown Hamamelidaceae instead of crown Hamamelidoideae. The divergence times for the Saxifragaceae alliance and crown Saxifragaceae we estimated are similar to Folk et al. (2019, 2021) but over 30 MY older than the estimates from Deng et al. (2015). This major discrepancy is likely due to both a paucity of outgroup sampling (only two species of *Liquidambar*) as well as an inadequate calibration strategy by Deng et al. (2015). Nevertheless, apart from these few exceptions, the nearly equivalent ages across different datasets point to a growing consensus on divergence times within the order when similar or identical calibrations are used, especially once there is a clear consensus on interfamilial relationships in the PWC (see section 4.1.2).

4.2.1. Biogeographic origins

Our ancestral range estimation accounts for the extant ranges of 115 of the 121 recognized genera in the order: either directly coded or represented by placeholders for their larger clade. The exact origin of the order remains unclear (Figure 2), as the most probable joint ancestral area does not seem plausible in light of area adjacency at the time of the crown divergence of Saxifragales (joint Central America + southern Africa + south Asia + Australasian distribution at ~107 Ma). There are at least two issues playing a role in confounding a clearer reconstruction of the ancestral range of crown Saxifragales, Core Saxifragales, and to a lesser extent the Saxifragaceae Alliance. First, a lack of suitable outgroups that help polarize the states in an adequate fashion. This is a general problem in studies of ancestral state reconstruction (Omland 1999), but even more so at the phylogenetic scale investigated here, where orders are sister to large, polymorphic clades that possess all (or most) available character states which, in the case of Saxifragales, are all rosids. Second, the distribution of Cynomoriaceae, the sole member of Saxifragales excluded from this study, may have important implications for the historical biogeography of the order, or at least the Core Saxifragales and possibly the Saxifragaceae Alliance. Cynomoriaceae is an African/Eurasian family that ranges from the Mediterranean to central Asia. Folk et al. (2019) clarified its phylogenetic placement to some extent, with the family certainly closely related to Core Saxifragales but less certainly placed within or around the clade as either sister to the Haloragaceae/Crassulaceae clade with 62% bootstrap support in the concatenated analysis of 301 nuclear loci or sister to all Core Saxifragales in the coalescent analysis of the same dataset with nearly full support. In either placement of Cynomoriaceae, given the ancestral area of the PWC, as well as some signal in the ambiguous reconstructions of the deepest nodes of the Saxifragaceae Alliance, the plurality of the evidence argues for a Eurasian, and probably northern Asian origin of Saxifragales in whole or in large part. The deepest backbone divergences of Saxifragales are therefore best explained by diversification and stasis within a geographic realm, rather than explained by dispersal across landmasses (Figure 2).

Based on our stochastic mapping, movement outside of east Asia is best explained by founder events rather than vicariance sensu the BioGeoBEARS model, where vicariance is a cladogenetic event where a widespread ancestor diverges into two lineages that each occupies a subset of the wider range. In our analyses, as many as 77% of the cladogenetic events occurring > 50 Ma (excluding the oldest event at the root) are reconstructed as jump dispersal (founder) events (Figure 3). While uncertainty about ancestral range of major nodes of Saxifragales prevents us from making solid conclusions about the biogeographic processes at play, we argue that the origin of stem Peridiscaceae represents long distance dispersal from east Asia to west Africa ~107 Ma. Likewise, the origin of stem Haloragaceae/Crassulaceae clade in Australasia ~102 Ma most likely represents a long-distance dispersal event from east Asia, when these areas were even more separated than they are presently. Excluding Crassulaceae, our inference of historical biogeography in the clade is nearly identical to that of Chen et al. (2014) using the DEC model and nearly identical divergence time estimates for Haloragaceae, with reconstructions of deeper nodes not reported. The Australasian Aphanopetalaceae and Tetracarpaeaceae therefore clearly represent relictual derivatives from an Australasian ancestor (Figure 2). Given their nearly identical distributions as coded in this study, any uncertainly about their relationships with Haloragaceae + Penthoraceae should not have any major effect on the ancestral ranges inferred at these nodes. Similarly, Deng et al. (2015) studied the historical biogeography of Saxifragaceae although they only used Grossulariaceae as an outgroup in their ancestral range estimation. Again, despite young ages in Deng et al. (2015) relative to this study, they found Saxifragaceae + Grossulariaceae to be of Asian origin, with an either northern Asian or western North American origin along the backbone of Saxifragaceae, as we find in this study but with a slightly more predominant Asian signal. Folk et al. (2021; Fig. S1 & S2), however, found Saxifragaceae + Grossulariaceae to be of North American origin, although this reconstruction was somewhat equivocal. Also, the geographic coding strategy used by Folk et al. (2021) differed from ours, which makes direct comparisons tricky. While our reconstruction is clear that these backbone nodes in Saxifragaceae must be one area or another and not a joint area, nearly equiprobable reconstructions of either area make it difficult to infer directionality, if any to movement between the two regions, although the results of stochastic mapping suggests that transitions to western North America from northern Asia are more common than the reverse (mean number of transitions 12.68 and 5.90, respectively).

Finally, uncertainty about the biogeographic history of the deepest nodes of the Saxifragaceae Alliance are confounded in two ways. The first is the distribution of *Pterostemon*, a clade with an extant distribution restricted to Mexico (Figure 2). This southwestern North American distribution as scored in our study is very rare in the order, not a range currently occupied by any close relatives, and is a distribution that is especially rare for such an ancient lineage (stem age ~61 Ma). Second, poor understanding of relationships in Grossulariaceae (see section 4.1.4) with apparent conflicting signal for northern Asia and northwestern North America at its crown, exerts some influence on the ancestral range of Grossulariaceae + Saxifragaceae. Nevertheless, a single area of origin for this clade is strongly favored, with a slightly greater chance of a northern Asian origin (Figure S1).

4.2.2. Patterns within disjunct distributions

The inter and intrafamilial geographic diversity of Saxifragales makes it an interesting group in which to examine the timing and processes behind these events. In particular, the extant distributions of several families represent recurrent patterns that have fascinated biogeographers. Molecular analyses suggest that long distance dispersal is more likely to explain these patterns as opposed to vicariance via continental drift from both divergence times and formal analyses of historical biogeography. Similarly, our analyses suggest that these strikingly disjunct distributions are the result of long-distance dispersal rather than vicariance. However, known divergence times coupled with an inference of the underlying directionality are in general agreement within relatively distantly related clades in Saxifragales, as well as with distantly related angiosperm lineages examined in other studies (Table 1). Although our sampling towards the tips is not exhaustive in many cases, our analyses still shed light on the minimum divergence times for these events, and still inform the likely type and directionality of these events.

In particular, the origin of South American Peridiscaceae is the result of a vicariance event from west Africa ~29 Ma following trans-Atlantic anagenic dispersal along stem *Peridiscus*/*Soyauxia* after the origin of the stem lineage ~53 Ma. While it is difficult to estimate the exact timing of the dispersal event along the branch, such a dispersal window lines up with dispersal ages inferred from trans-Atlantic lineages within Annonaceae (~38 Ma for the divergence of *Cymbopetalum* Benth./*Trigynaea* Schltdl. from *Mkilua* Verdc. [Thomas et al., 2015]), Lecythidaceae (24.4 Ma for the divergence of *Asteranthos* Desf. from African Scytopetaloideae [Rose et al., 2018]), Proteaceae (43.8 Ma for the divergence of *Brabejum* L./*Panopsis* Salisb. ex Knight [Barker et al., 2007]), and Vitaceae (30–36 Ma for the divergence of members of *Cissus* L. s.l. [Nie et al., 2012; Liu et al., 2013]).

Our observed occurrences of amphitropical disjunctions are from across wide ranges of divergence times and only one corresponds to the classic disjunction of North America/South America (*Cascadia nuttallii* (Small) A.M. Johnson/*Saxifragodes albowiana* (Kurtz ex Albov) D.M. Moore). This disjunction is not an artifact of taxon sampling, as these are the only two extant members of the *Cascadia* A.M. Johnson clade. Deng et al. (2015) reconstructed this node as representing a vicariance event following long distance dispersal. The crown age estimate for this clade by Deng et al. (2015) is much younger than ours (35.6; 95% HPD = 15.4–55.6) at 19.1 Ma (95% HPD = 11.0–27.9) but in either case, the timing of dispersal to South America is unusually old compared to other lineages (e.g., Wen & Ickert-Bond, 2009; Simpson et al., 2017), suggesting repeated occurrences of North American/South American connectivity throughout the Tertiary.

Asian-North American disjunctions have probably been the best studied of all types of disjunctions, and the existing data has been reviewed multiple times. Within Saxifragales, 11 of the 20 infrafamilial disjunctions highlighted in Table 1 involve eastern Asia and North or Central America, with all but one jump dispersal event involving movement from Asia to the New World. The three European/North American disjunctions also involve close ancestors with east Asian affinities. Again, all inferred cladogenetic dispersal involves movement to the New World. Wen et al. (2010) surveyed the literature at the time and found that of eastern Asian-North American disjuncts, there was a strong signal of Old World to New World movement (62–70% of events) and strong signal of Beringian rather than North Atlantic migration (56–79% of events). Within Saxifragales, the vast majority of events we highlight show movement from the Old World to New World and also show a Beringian rather than North Atlantic route, highlighting the importance of Beringian land bridges in the assembly of north temperate floras (Wen et al., 2016). In terms of timing, Wen et al. (2010, 2016) reported these disjunctions occurring in a wide time frame from 89 Ma to the present but with most events < 20 Ma. Similarly, our results fall within this range, especially in the 5–20 Ma range (Table 1). Such frequent movement has been attributed to the presence of land bridges across both Beringia and the North Atlantic, although the strong directionality to this movement has yet to be explained. Long distance dispersal via air or water is also a possibility, but the dry capsular or follicular fruit of most Saxifragales (and all of those illustrated in Table 1) argues against such means of colonization.

Conclusions

This study clarifies the historical biogeography of Saxifragales with strong and clear support. While the ancestral range of the crown of the order is unclear based on our analyses, the preponderance of evidence clearly points to an east Asian origin, especially when the extant distribution of Cynomoriaceae is considered, as well as an important Australasian (but not Gondwanan) element which gave rise to multiple extant families. Both long distance dispersal and vicariance have played a role in shaping the extant distribution of Saxifragales, but our analyses clearly show that long-distance dispersal has been a more prevalent force, even at deeper nodes. Recent disjunctions are overwhelmingly explained by long-distance dispersal and highlight the importance of land bridges during the Neogene. These land bridges strongly favored dispersal from east Asia to North America and shaped the flora of north temperate regions.

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Table 1. Summary of the type, timing, and directionality of 20 selected intrafamilial, intercontinental disjunctions in Saxifragales. Disjunctions are sorted by general pattern and the event type is as reconstructed on the moist likely ancestral state reconstruction. Taxa listed reflect the most recent common ancestor (MRCA) node only and the disjunction may involve

more than two species. Note that due to extensive interchange, Saxifragaceae disjunctions between Asia and Western North America are curtailed to show the older disjunctions only. An asterisk (*) indicates nodes lacking a highest posterior density (HPD) of node ages because it is present in the maximum clade credibility tree but found in < 50% of posterior trees.

Disjunction (MRCA)	**Family**	**Age/HPD (Ma)**	**Event Type and Direction**
W. N. America/Asia			
Astilbe rivularis/ *Saxifragopsis fragarioides*	Saxifragaceae	14.9 (4.1-26.3)	jump dispersal (Asia to N. America)
Leptarrhena pyrolifolia/ *Tanakaea radicans*	Saxifragaceae	20.0 (5.5-35.1)	jump dispersal (Asia to N. America)
Paeonia anomala/ *P. brownii*	Paeoniaceae	20.0 (11.0-29.5)	jump dispersal (Asia to N. America)
Saxifraga mertensiana/ *S. stolonifera*	Saxifragaceae	26.4 (7.6-46.5)	jump dispersal (Asia to N. America)
E. N. America/Asia			
Astilbe biternata/ *A. chinensis*	Saxifragaceae	3.4*	jump dispersal (Asia to E. N. America)
Chrysosplenium iowense/ *C. lanuginosum*	Saxifragaceae	18.6 (7.5-29.4)	jump dispersal (Asia to N. America)
Hamamelis mollis/ *H. virginiana*	Hamamelidaceae	3.6 (0.6-6.2)	jump dispersal (E. N. America to Asia)
Itea virginica/ *I. yunnanensis*	Iteaceae	12.4 (4.2-21.1)	vicariance
Penthorum chinense/ *P. sedoides*	Penthoraceae	7.0 (1.0-14.4)	jump dispersal (Asia to E. N. America
Central America/Asia			
Loropetalum chinense/ *Matudaea trinervia*	Hamamelidaceae	12.5 (5.4-19.0)	jump dispersal (Asia to Central America)
Molinadendron guatemalense/ *Sinowilsonia henryi*	Hamamelidaceae	8.5 (2.7-13.8)	jump dispersal (Asia to Central America)
Europe/E. N. America			
Chrysosplenium americanum/ *C. oppositifolium*	Saxifragaceae	9.1 (2.2-16.4)	jump dispersal (Europe to E. N. America)
Fothergilla major/ *Parrotiopsis jacquemontiana*	Hamamelidaceae	6.0 (1.3-10.3)	jump dispersal (Europe to E. N. America)

Liquidambar orientalis/ *L. styraciflua*	Altingiaceae	7.1 (2.0-11.7)	jump dispersal (Europe to E. N. America)
Amphitropical			
Cascadia nuttallii/ *Saxifragodes albowiana*	Saxifragaceae	35.6 (15.4-55.6)	jump dispersal (W. N. America to S. America)
Saxifraga balfourii/ *S. bicuspidata*	Saxifragaceae	52.5 (39.8-64.7)	jump dispersal (Asia to Andes)
Saxifraga cymbalaria/ *S. moschata*	Saxifragaceae	5.2*	jump dispersal (Europe to S. America)
Gondwanan/Miscellaneous			
Dicoryphe stipulacea/ *Noahdendron nicholasii*	Hamamelidaceae	9.5 (3.5-15.1)	jump dispersal (Africa to Australasia)
Choristylis rhamnoides/ *Itea virginica*	Iteaceae	34.3 (14.5-55.8)	jump dispersal (E. N. America to Africa)
Peridiscus lucidus/ *Soyauxia talbotii*	Peridiscaceae	29.1 (8.2-52.9)	vicariance following anagenetic dispersal

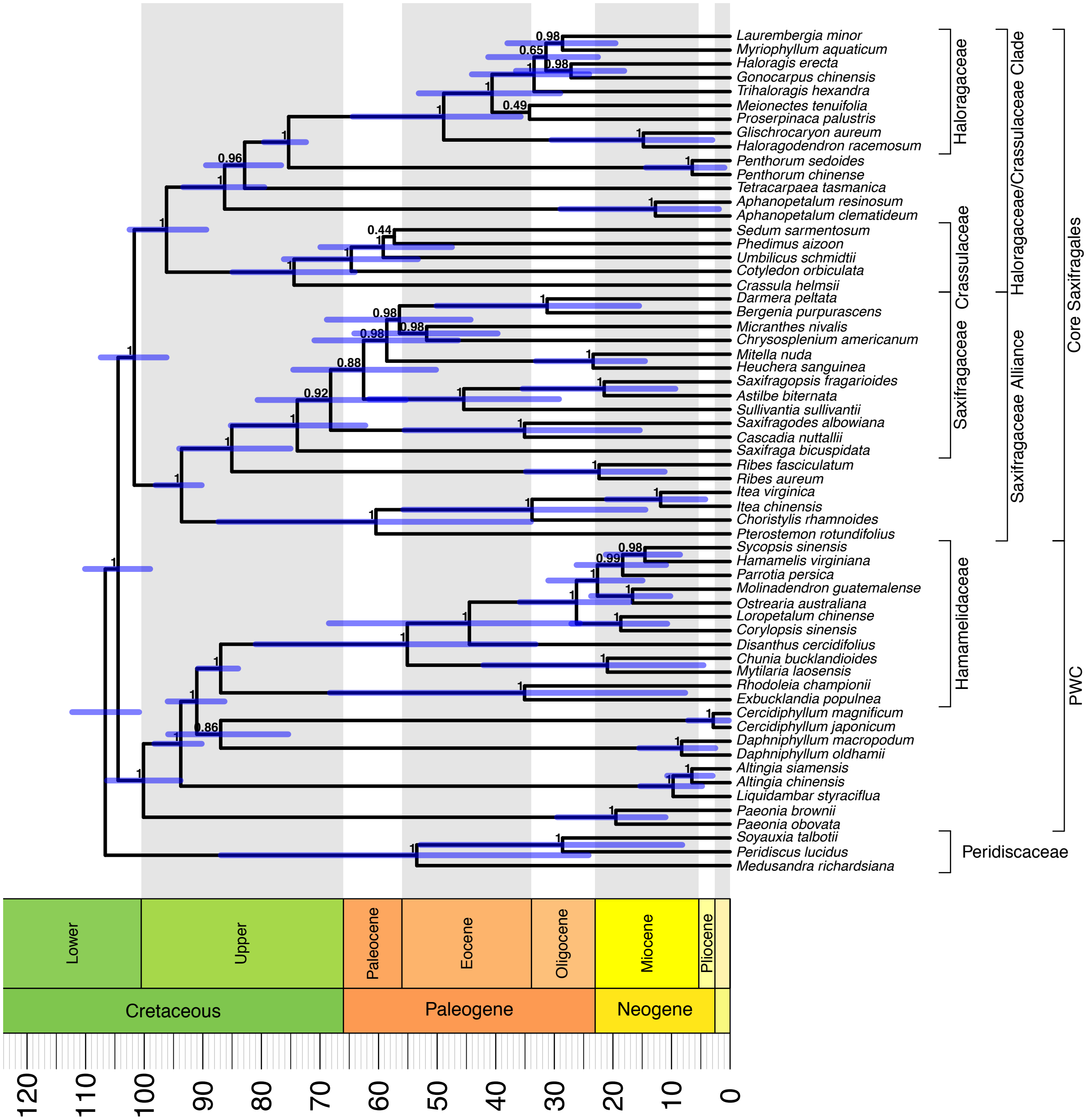

Figure 1. BEAST chronogram of Saxifragales pruned to 61 tips out of a total of 251 ingroup taxa selected to represent major interfamilial, familial, and infrafamilial crown nodes. Major clades discussed in the text are indicated to right of the tree. Numbers above branches represent posterior probabilities and the blue bars around each node represent the 95% highest posterior density of node ages.

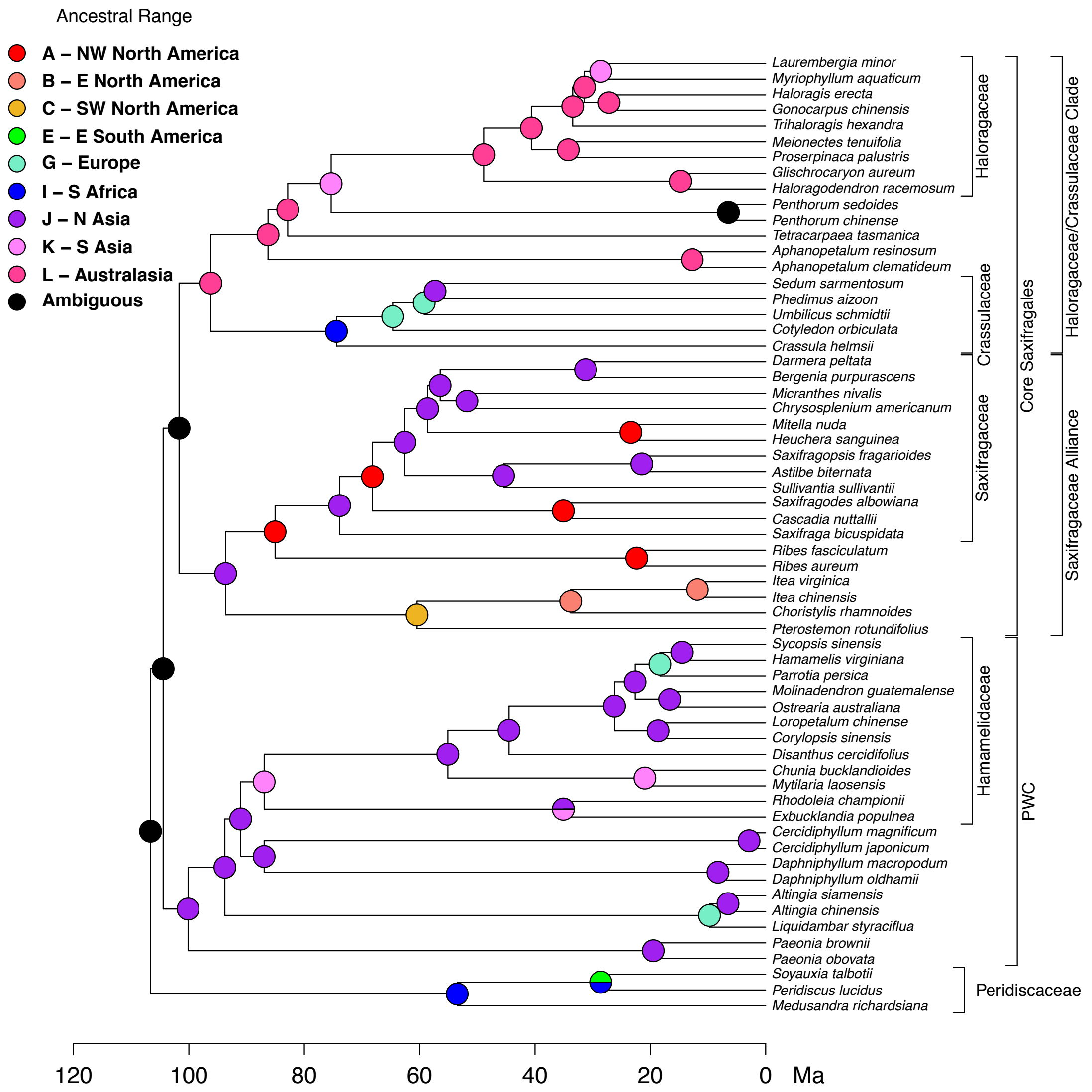

Figure 2. Ancestral range estimation (ARE) of Saxifragales under the DECj model pruned to 61 tips out of a total of 251 ingroup taxa selected to represent major interfamilial, familial, and infrafamilial crown nodes. Major clades discussed in the text are indicated to right of the tree. Node pies represent the most probable ancestral range of each node. In pies with multiple colors, the ancestral range is inferred to include all areas indicated in the pie. Note that due to pruning, biogeographic transitions between nodes closer to the tips do not necessarily accurately reflect the historical biogeography. See Figure S1 for the complete ARE.

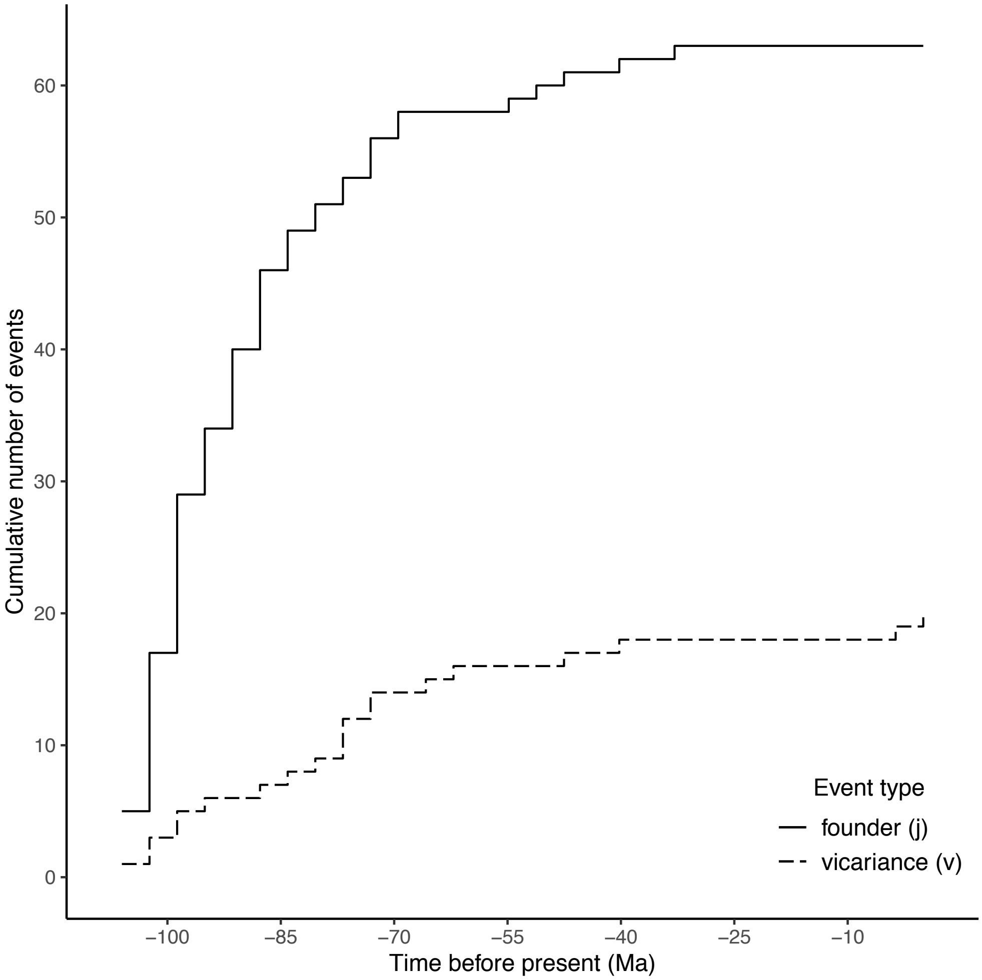

Figure 3. Cumulative distribution of cladogenetic dispersal (founder) and vicariant events in the Saxifragales through time as inferred with biogeographical stochastic mapping.

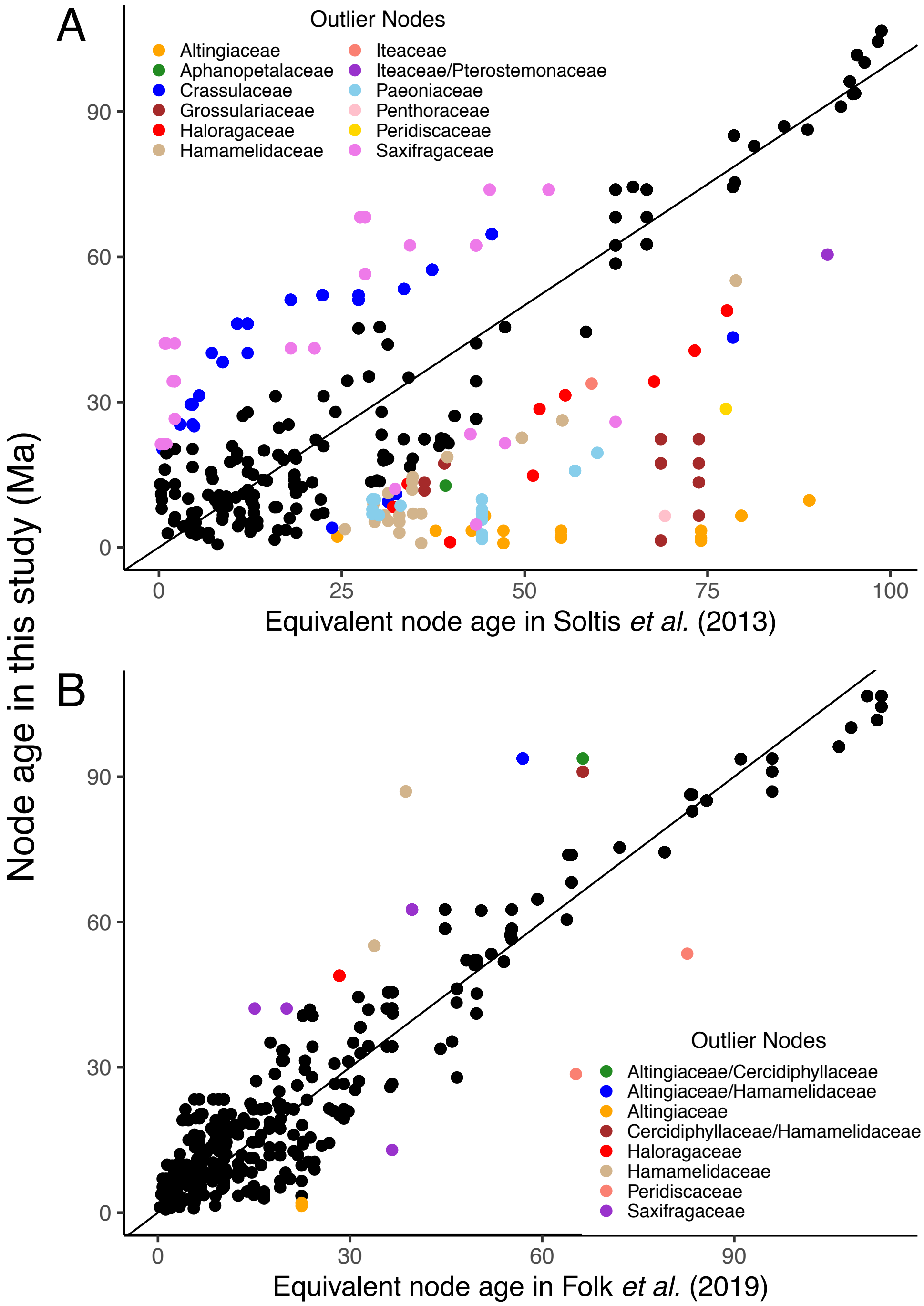

Figure 4. Age comparisons between this study and the two largest and most recent prior analyses of Saxifragales: (A) Soltis et al. (2013) and (B) Folk et al. (2019). Points represent crown ages of equivalent common ancestor nodes for a pair of tips in each study. The diagonal line represents where points should fall if common ancestor nodes are equal in age.