bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

# Dynamics of evolution in Irano-Anatolian and Caucasus biodiversity hotspots: Evolutionary radiation and its drivers in *Gypsophila* (Caryophyllaceae)

**Hossein Madhani[1,2], Richard K. Rabeler[3], Guenther Heubl[4], Navid Madhani[5], and Shahin Zarre[1]**

[1]Department of Plant Science, Center of Excellence in Phylogeny of Living Organisms, School of Biology, College of Science, University of Tehran, PO Box 14155-6455 Tehran, Iran; [2]School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV 89119, U.S.A.; [3]University of Michigan Herbarium-EEB, 3600 Varsity Drive, Ann Arbor, Michigan 48108-2228, U.S.A.; [4]Biodiversity Research – Systematic Botany, Department of Biology I, Ludwig–Maximilians Universität München, Menzinger Str. 67, D-80638 München, Germany and GeoBio Center LMU; [5]Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, PO Box 14155-6655 Tehran, Iran.

Author for correspondence:
*Hossein Madhani*
*Email: hossein.madhani@gmail.com*

## Summary

- Irano-Anatolian and Caucasus biodiversity hotspots, characterized by a high degree of alpine endemism and extremely dynamic geographical history, are among the 36 globally defined biodiversity hotspots. However, the dynamics of evolution and the cofactors of diversification have not been investigated in these regions.
- We explore the evolutionary dynamics of *Gypsophila* (Caryophylleae: Caryophyllaceae), a large and diverse genus with a high degree of endemism in the Irano-Anatolian and Caucasus biodiversity hotspots. We investigate the diversification rate and its biotic and abiotic cofactors within the Caryophylleae tribe, with a special focus on *Gypsophila*.
- We identified a shift in the diversification rate of *Gypsophila* that started about 3 million years ago and was influenced by both biotic and abiotic forces. The results suggest that the diversity inside *Gypsophila* evolved due to evolutionary radiation that was triggered by both paleoenvironmental factors and acquiring morphological novelties.
- The result of this study demonstrates a highly dynamic evolutionary history across the Caryophylleae clade and *Gypsophila*, which is consistent with the extensive fluctuation in the geological and climatological history of Irano-Anatolian and Caucasus biodiversity hotspots. This study significantly improves our understanding of the dynamics of evolution in the Irano-Anatolian and Caucasus biodiversity hotspots and the impact of environmental changes on the rate of diversification.

**Keywords:** Biodiversity hotspot, Caryophylleae, Caucasus biodiversity hotspot, diversification rate, evolutionary radiation, *Gypsophila,* Irano-Anatolian biodiversity hotspot.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

## Introduction

Finding the relation between clade age and species richness is a fundamental step in understanding the dynamics of diversification and its cofactors (Rabosky et al. 2012). Heterogeneous patterns of diversity among different taxa and in different environments is a long-standing question in evolutionary biology. Studies on the dynamics of evolution show episodes of increase in diversification rate in all major lineages in the Tree of Life and evolutionary radiation is known to play a major role in the emergence of species-rich clades (Simões et al. 2016; Wiens 2017; Naciri and Linder 2020). During the last two decades, studies on evolutionary dynamics in species-rich lineages of several biodiversity hotspots around the world increased our knowledge about the role of evolutionary radiations and their drivers in shaping the biodiversity of these endangered geographical regions (Table S1). Of the 36 global biodiversity hotspots (Mittermeier et al. 2011; Noss et al. 2015), many of them have been subjects of diversification dynamics studies and reports of evolutionary radiations, indicating their status as species-rich and endangered areas (Table S1). However, diversification rates and biotic and abiotic factors associated with changes in speciation and extinction rates are poorly studied in some of the biodiversity hotspots, such as Irano-Anatolian and Caucasus biodiversity hotspots, largely due to insufficient sampling of endemic species and a dearth of resolved phylogenies. Given that these biodiversity hotspots are both biologically rich and under severe threat, it is crucial to study the dynamics and cofactors of speciation and extinction as the ongoing climate change accelerates extensive extinction in these endangered regions (Malcolm et al. 2006; Fonseca 2009; Le Roux et al. 2019).

## Irano-Anatolian and Caucasus biodiversity hotspots

Home to about 12,400 plant species including over 30% endemics, the Irano-Anatolian and Caucasus biodiversity hotspots are classified among the 36 global biodiversity hotspots (Mittermeier et al. 2011). These two adjacent hotspots are predominantly mountainous areas in South-West (SW) Asia and part of the Irano-Turanian (IT) floristic region, one of the richest floristic regions in the world with a dramatic geological and climatic history (Zohary 1973; Takhtajan et al. 1986; Davis et al. 1994; Djamali et al. 2012b; Manafzadeh et al. 2017). Alpine and sub-alpine ecosystems followed by vast deserts such as Dasht-e Kavir and Dasht-e Lut contribute chiefly to the natural landscape of the Irano-Anatolian and Caucasus biodiversity hotspots. The area geographically covers the Turkish-Iranian plateau, characterized by several

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

major mountain ranges, i.e. Taurus Mountains, Armenian Highlands, Zagros Mountains, Alborz Mountains, and Kopet-Dag mountain range, all recognized as the main centers of biodiversity in the IT region (Zazanashvili et al. 2004; Zazanashvili 2009; Djamali et al. 2012a; Manafzadeh et al. 2014; Paroly 2015; Manafzadeh et al. 2017; Noroozi et al. 2018).

Historic geological and climatological events like orogenic activities, mountain uplifts, and climate fluctuations have been shown to change the dynamics of diversification, dispersal, and consequently the patterns of biodiversity (Hughes and Eastwood 2006; Antonelli et al. 2009, 2018; Rahbek et al. 2019). Two main strong tectonic activities linked with the India–Asia collision in the East, and the Arabia–Eurasia collision in the West, influenced the geography, topography, and climate history of the IT region from the early Eocene to the current time (Hsü et al. 1977; Zachos et al. 2001; van Dam 2006; Zachos et al. 2008; Allen and Armstrong 2008; Ballato et al. 2010; Djamali et al. 2012b; Manafzadeh et al. 2017). The first major tectonic activity in the India–Asia collision started in the early Eocene with the disappearance of the Tethys Ocean and promoted the emergence of the western parts of the IT region by the end of Eocene and early Oligocene (Báldi 1980; Şengör and Yilmaz 1981; Rögl 1997, 1999; Meulenkamp and Sissingh 2003; Popov et al. 2006). By the end of the Oligocene, the India–Asia collision resulted in the formation of the Tibetan plateau, and after that, the Arabia–Eurasia collision caused the uplift of very young altitudes in the Irano-Anatolian plateau, and Caucasus mountains (Şengör and Kidd 1979; Şengör et al. 1985; Pearce et al. 1990; Keskin 2003; Vincent et al. 2007; Dilek et al. 2010; Gavillot et al. 2010; Hatzfeld and Molnar 2010; Yin 2010; Mouthereau et al. 2012). These extensive geological activities along with climatic fluctuations such as cooling and aridification episodes during the Eocene-Oligocene transition, warming phases in the late Oligocene and the Middle Miocene, the late Miocene cooling, aridification events in the Pliocene, the Messinian Salinity Crisis, and the Quaternary glacial stages, all had major impacts on the patterns of biodiversity in the area (Hsü et al. 1977; Zachos et al. 2001; van Dam 2006; Zachos et al. 2008; Allen and Armstrong 2008; Ballato et al. 2010; Djamali et al. 2012b; Manafzadeh et al. 2017). The orogenic activities and the subsequent climate changes promoted a high degree of fragmentation and isolation in the alpine flora, which caused high levels of alpine endemism in these regions (Noroozi et al. 2008; Manafzadeh et al. 2017).

Topology-driven isolation in island-like systems, such as mountains -so-called ‘sky islands’, lakes, and valleys, increases the speciation rate which results in highly endemic biodiversity

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

(Gillespie and Roderick 2014; Steinbauer et al. 2016; Antonelli et al. 2018; Rahbek et al. 2019). An increasing body of research corroborates that the exceptional diversity and unique species endemic to alpine habitats evolved rapidly and relatively recently due to evolutionary radiation (Hughes and Atchison 2015). This phenomenon has been reported across various global mountain systems, including the Andes Mountains in South America (Hughes and Eastwood 2006; Madriñán et al. 2013; Nürk et al. 2013; Lagomarsino et al. 2016; Pérez-Escobar et al. 2017), Rockies and Sierras mountains in Western North America (Wolfe et al. 2006; Tank and Olmstead 2008; Drummond et al. 2012), New Zealand and New Guinea mountains (Winkworth et al. 2005; Brown et al. 2006; Joly et al. 2014), European alpine systems (Comes and Kadereit 2003; Kadereit et al. 2004; Roquet et al. 2013), Eastern African highlands (Knox and Palmer 1995; Gehrke and Linder 2009; Linder 2014), alpine habitats of Himalaya mountains and Tibetan Plateau (Liu et al. 2006; Jabbour and Renner 2012; Sun et al. 2012; Favre et al. 2015; Wen et al. 2014; Zhang et al. 2014; Xing and Ree 2017), and Irano-Turanian altitudes (López-Vinyallonga et al. 2009; Moharrek et al. 2019). Considering the relatively young age of elevations in the Irano-Anatolian and Caucasus biodiversity hotspots and their highly dynamic recent geological climatological history, and based on evidence from Andean uplift, we posit that highly diverse taxa, endemic to the mountain habitats of Irano-Anatolian and Caucasus hotspots, diverged recently due to shift in diversification rate. Here, we use *Gypsophila*, a highly diverse alpine genus of which almost half of its species (~70 spp) are endemic to alpine and subalpine habitats of the Irano-Anatolian and Caucasus biodiversity hotspots, as a model to test this hypothesis and explore the change in diversification rate and its cofactors within these two biodiversity hotspots.

**The Genus *Gypsophila* L.**

This group is one of the large and heterogeneous genera in the family Caryophyllaceae Lam. & DC, including approximately 150 species (Barkoudah 1962; Hernández-Ledesma et al. 2015; Madhani et al. 2018) of annual or perennial herbaceous/woody, creeping, or cushion-forming plants. Members of the genus inhabit primarily the mountainous steppes in the Holarctic region, but most of the species are restricted to the high-elevation habitats of western parts of the IT region in the Irano-Anatolian and Caucasus biodiversity hotspots (Barkoudah 1962; Rechinger 1988; Huber-Morath 1967). This high degree of endemism along with the remarkable morphological diversity and resistance ability of *Gypsophila* members to harsh environments imply the

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

evolutionary adaptation of *Gypsophila* spp. to the alpine habitats of these two biodiversity hotspots. Molecular phylogenetic studies during the last decade resolved the problematic taxonomic boundaries of *Gypsophila* within the tribe Caryophylleae, elucidated phylogenetic relationships and limits of genera in this group (Greenberg and Donoghue 2011; Pirani et al. 2014; Madhani et al. 2018; Noroozi et al. 2020; Pirani et al. 2020; Fassou et al. 2022), and paved the way for exploring the dynamics of diversification and its cofactors inside *Gypsophila* and within Caryophylleae.

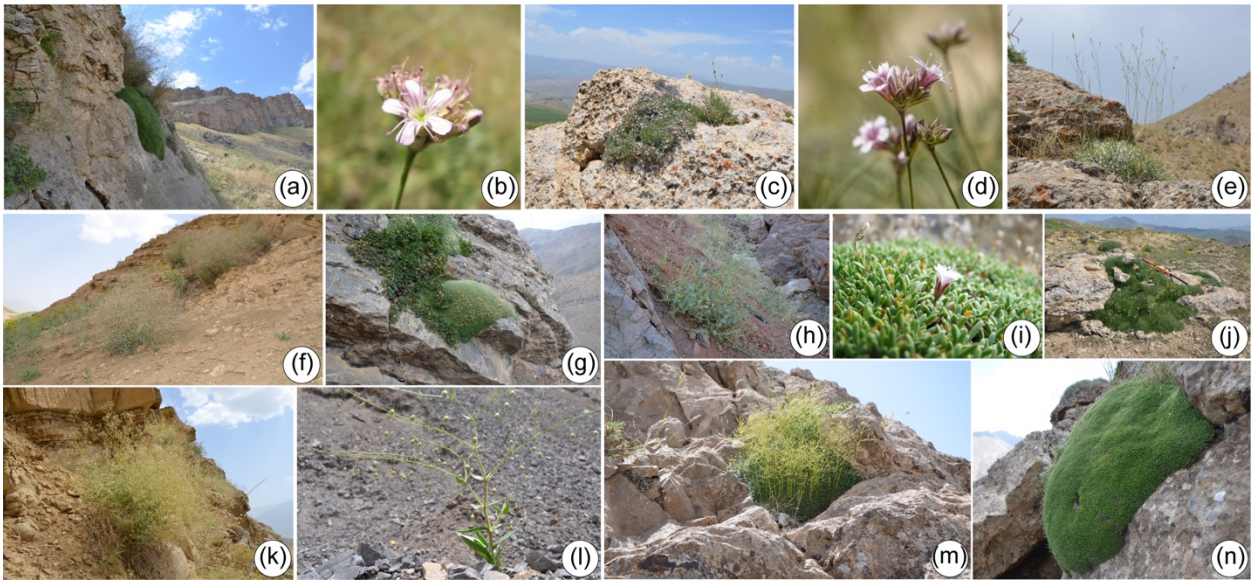


**Figure 1.** Diversity in *Gypsophila* in its natural habitats in high altitudes of Irano-Anatolian biodiversity hotspot. (a) *Gypsophila pulvinaris* (b) *Gypsophila caricifolia* (c) *Gypsophila wilhelminae* (d and j) *Gypsophila bazorganica* (e) *Gypsophila graminifolia* (f) *Gypsophila leioclada* (g) *Gypsophila aretioides* (h) *Gypsophila ruscifolia* (i and n) *Gypsophila saponarioides* (k) *Gypsophila virgata* (l) *Gypsophila pilosa* (m) *Gypsophila acantholimoides*. (all photos by H. and N. Madhani)

The main goals of the present study are: 1) to clarify the evolutionary history within *Gypsophila* using phylogenetic reconstruction and molecular dating, 2) to investigate tempo and rates of diversification within Caryophylleae and *Gypsophila*, 3) to assess the impacts of biotic and abiotic factors on diversification rates in *Gypsophila*, and 4) to reconstruct the ancestral ranges of biogeographic pathways within Caryophylleae and its major clades.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

## Materials and Methods

### Taxon sampling, DNA extraction, PCR, and sequencing

About half of *Gypsophila* species were sampled, including representatives from all previously recognized sections, and all morphological and geographical diversity inside the genus, except for its Australian representative, *G. australis* (Schltdl.) A.Gray. We generated new DNA sequences for 40 species of *Gypsophila*. Along with our previously sequenced accessions for *Gypsophila* (Madhani et al. 2018) and those from GenBank, we included the molecular data of 73 species of *Gypsophila* in our study. We also added some members of all other genera within Caryophylleae in our study with a special focus on *Dianthus*, which is one of the fastest evolutionary radiations in terrestrial habitats reported to-date (Valente et al. 2010). Altogether we prepared three datasets: the internal transcribed spacer (ITS) region of the ribosomal cistron (consisting of ITS1, the intervening 5.8S gene, and ITS2) for 184 accessions of Caryophylleae including 72 species of *Gypsophila*; the *rps16* intron for 105 accessions from Caryophylleae including 55 species of *Gypsophila*; and the *mat*K gene for 146 accessions from all 11 tribes and main genera of the family Caryophyllaceae including 25 species of *Gypsophila*, 37 species of *Dianthus*, mostly sequenced by Greenberg and Donoghue (2011), and 22 species of *Saponaria*. All DNA extractions, PCR amplification, and sequencing were conducted as described previously in Madhani et al. (2018). Information on voucher specimens and GenBank accession numbers are provided in Supporting Information Table S2.

### Sequence alignment and phylogenetic reconstruction

First, we edited sequences in Geneious v.8.0.5 (Kearse et al. 2012) and performed the multiple sequence alignments using MAFFT v.7 with default parameters (Katoh and Standley 2013). The results were manually corrected using Mesquite v.3.7 (Maddison and Maddison 2021). We used Bayesian inference (BI) and maximum parsimony (MP) to reconstruct the phylogenetic history of the group. The general time-reversible model with gamma-shape rate variation and a proportion of invariable sites (GTR + I + G) is estimated as the optimal substitution model for *mat*K and ITS datasets, and GTR + G is estimated as the best model for *rps16* using the Akaike information criterion (AIC) implemented in jModelTest v.2.1.6 (Darriba et al. 2012). We used MrBayes v.3.2.7a (Ronquist and Huelsenbeck 2003) with the default of three "heated" and one "cold" chain and 40,000,000 generations, trees were sampled every 1000 generations and 0.25 of pre-

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

stationarity MCMC samples were discarded as burn-in, which include 10000 samples in each run. The convergence between runs and ESS values was assessed using TRACER v.1.7.2 (Rambaut et al. 2018). Finally, we summarized the remaining trees in a 50% majority-rule consensus tree for each dataset. To reconstruct phylogenies by the MP method we used PAUP* v.4.0a168 (Swofford 1993) with the following parameters: all characters unordered and equally weighted, heuristic search with random sequence addition, tree-bisection-reconnection branch swapping, 100 random-addition-sequence replicates, MAXTREES option set to 10000, and resulting trees were summarized in a majority rule consensus tree. For bootstrapping we used the maximum likelihood method as implemented in RAxML-HPC2 on XSEDE v.8 (Stamatakis 2014) with the GTRCAT model, 1000 bootstrap replicates. All phylogenetic analyses were done under the CIPRES server (Miller et al. 2010).

**Missing species survey**

To account for missing species of *Gypsophila* in our analyses and have a more precise estimation of diversification rates associated with each studied biotic (life strategy, calyx shape, life form) and abiotic (habitat and elevation) traits, we performed a comprehensive taxonomic survey on all accepted species names of *Gypsophila* (Table S26). This taxonomic survey includes four years of fieldwork during the flowering and fruiting seasons of *Gypsophila* in high-elevation habitats of Zagros and Alborz mountains and examining more than 1000 herbarium sheets. All missing species are assigned to a particular trait group by examining protologues, monographs, and floras, such as Barkoudah (1962), Flora of Turkey (Huber-Morath, 1967), and Flora Iranica (Rechinger 1988). We also searched major available online resources (Tropicos, http://www. tropicos.org/; JSTOR Global Plants, https://plants.jstor.org; Global Biodiversity Information Facility - GBIF, www.gbif.org; and Integrated Digitized Biocollections - iDigBio, /www.idigbio.org), as well as websites of several individual herbaria (BM, BR, E, G, GH, K, KEW, L, LINN, OS, P, US, WU: herbarium abbreviations follow Thiers 2023+). Information about all accepted species names currently recognized in *Gypsophila* is presented in Table S26.

**Molecular dating**

The *mat*K tree was used to infer the age of Caryophylleae. To calibrate the *mat*K tree, we used the only studied fossil of the family, *Caryophylloflora paleogenica* G. J. Jord. & Macphail, which is

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

inferred as the sister to either one or both of the subfamilies Alsinoideae and Caryophylloideae (Jordan and Macphail 2003). The age of the strata that the fossil has been found is inferred as middle to late Eocene, 48.6–33.9 Ma (http://www.paleodb.org). The monophyly enforced to Caryophyloideae and Alsinoideae subfamilies with lognormal distribution, mean = 0.0, offset = 33.9, and SD = 1.37 in BEAST v.1.10.4 (Suchard et al. 2018), which sets a hard minimum boundary for the age of the group and allows the maximum ages in prior distribution to extend beyond the maximum age of the strata (Ho and Phillips 2009; Frajman et al. 2009). We selected GTR + I + G as the best-fitting model for the *mat*K dataset and a relaxed clock for preliminary analysis, as the coefficient of variation frequency is not abutting against zero, and also the standard deviation of the uncorrelated lognormal relaxed clock did not include 0, thus there is among branch rate heterogeneity and a strict molecular clock could be rejected for the *mat*K dataset (Drummond et al. 2007). The BEAST analyses were run for both fossilized birth-death and Calibrated Yule models as the tree priors to infer the divergent time of Caryophylleae and the main genera inside it. All input XML files for BEAST analyses were prepared using BEAUti v.1.10.4 and all dating analyses were performed in the CIPRES server (Miller et al. 2010).

The inferred age for Caryophylleae from fossil calibration of the *mat*K analysis was used as the secondary calibration point to calibrate *rps16* and ITS phylogenies. So, we set a normal distribution with mean=24.68 and SD=3.4 for the Caryophylleae clade, which corresponds to 95% highest posterior density intervals (HPDs) of the age of Caryophylleae (30.90–17.99 Ma). The preliminary BEAST analyses for ITS and *rps16* using an uncorrelated relaxed lognormal clock, rejected a strict clock, as the coefficient of variation frequency did not include 0 (Drummond et al. 2007), so we used a relaxed clock log-normal for the clock model in all subsequent analyses of *rps16* and ITS datasets. A Calibrated Yule tree prior was used for both datasets and the analyses were repeated with a Birth-Death tree prior. Independent runs for each dataset with different tree priors were performed for 40 million generations, sampling every 1000 generations. We assessed the accuracy of chain convergence and adequacy of effective sample size (ESS) by inspection of posterior estimates for different parameters and MCMC sampling of the log file for each run in TRACER v.1.7.2 (Rambaut et al. 2018). The first 20% of the samples in each run were discarded and tree files combined by LOGCOMBINER v.1.10.4 (Drummond and Rambaut 2007), and the maximum clade credibility trees were generated using TREEANNOTATOR v.1.10.4. We used the R package STRAP (Bell and Lloyd 2015), and ver. ICS2013 (Cohen et al. 2013) of the

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

international chronostratigraphic chart to plot the maximum clade credibility trees against stratigraphy.

**Net diversification rate**

We measured net diversification rates for major clades inside Caryophylleae based on the maximum clade credibility trees obtained from different analyses on ITS, *mat*K, and *rps16* datasets. We estimated net diversification rates in stem and crown groups using the Magallón & Sanderson method (Magallón and Sanderson 2001) implemented in the R package GEIGER (Harmon et al. 2008).

**Time-dependent diversification**

To infer the diversification dynamics of the Caryophylleae clade, and to identify shifts in the diversification rates, we used a time-dependent model implemented in BAMM v.2.5.0 (Rabosky 2014). We accounted for incomplete taxon sampling by assigning a sampling fraction of major clades in Caryophylleae for each dataset. We generated priors for BAMM using the setBAMMpriors function, implemented in the R package BAMMtools v.2.1.10 (Rabosky et al. 2014) based on the maximum clade credibility tree from the BEAST analysis. Four independent MCMC chains were run in BAMM for 40 million generations and sampled every 10000. We set the prior for the expected number of shifts based on Bayes factor calculations (Mitchell and Rabosky 2017), and used ESS values (> 200) to assess the convergence of the runs using R package coda v.0.19 (Plummer et al. 2006). Then we used R-package BAMMtools v.2.1.10 (Rabosky et al. 2014) to identify the credible sets of speciation rate shift and single best shift configuration, plot rate through time curves, calculate speciation, extinction, and net diversification rates through time for Caryophylleae and its major clades. Further, to test whether (and how) diversification rates varied through time (Morlon et al. 2011), we fit likelihood models of time-dependent birth-death functions using the *fit_bd* function implemented in RPANDA v.2.1 (Morlon et al. 2016). We fitted 12 different birth-death models to the Caryophylleae, *Gypsophila*, *Dianthus*, *Saponaria*, and *Acanthophyllum* phylogenies for the three datasets (Tables S10-S12). The best model for each clade within three datasets was chosen via the corrected Akaike Information Criterion (AICc) and Likelihood Ratio Test (LRT), significant at P-value < 0.05.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

**Trait-dependent diversification**

To test character-associated diversification inside *Gypsophila* we used BiSSE (Maddison et al. 2007) models implemented in the R package diversitree 0.9-16 (FitzJohn 2012). We estimated six rate parameters of the BiSSE analysis for five binary traits including, 1) life strategy: annual vs. perennial, 2) habitat: non-montane vs. montane, 3) life form: herbaceous vs. woody, 4) calyx shape: tubiform vs. campanulate/turbinate, and 5) elevation: low vs. high. We estimated BiSSE parameters in these five binary traits by eight different models, each constrained for one or more of the six parameters in the BiSSE analyses (see Tables S4-S8). These six parameters include speciation rates of lineages in states 0 and 1 ($\lambda_0$ and $\lambda_1$, respectively), extinction rates of lineages in states 0 and 1 ($\mu_0$ and $\mu_1$, respectively), the transition rate from 0 to 1 ($q_{01}$), and transition rate from 1 to 0 ($q_{10}$). To account for incomplete sampling, the proportion of sampled species in state 0 and state 1 were calculated based on the missing species survey (Table S26) and specified for each trait as the sampling fractions. We used AIC and likelihood ratio test by anova function in R to find the best-fitting model for each binary trait. The Chi-squared values (ChiSq) and their significance (Pr) were calculated by comparing each model to the null model (minimal model), in which all rates for state 0 are equal to the rates for state 1 ($\lambda_0=\lambda_1$; $\mu_0=\mu_1$; $q_{01}=q_{10}$). We ran the MCMC analysis for 100,000 steps using an exponential prior for the best-fitting model, applied a burn-in for the first 10% of the steps, and examined the posterior probability of the six rates of BiSSE analysis in the five binary traits.

To test whether high species richness and endemism in Irano-Anatolian and Caucasus hotspots are driven by spacial asymmetries in speciation, extinction, and dispersal rate or not, we used GeoSSE analysis (Goldberg et al. 2011), an extension of BiSSE which also implemented in the R package diversitree 0.9-16 (FitzJohn 2012). We estimated the seven rate parameters of the GeoSSE analysis, including speciation and extinction rates within the two biodiversity hotspots ($\lambda_A$ and $\mu_A$, respectively), speciation and extinction rates outside the two biodiversity hotspots ($\lambda_B$ and $\mu_B$, respectively), speciation rate between inside and outside of the two hotspots ($\lambda_{AB}$), dispersal from Irano-Anatolian and Caucasus hotspots to rest of the world (range expansion) ($d_A$), and dispersal rate into Irano-Anatolian and Caucasus hotspots from outside (range contraction) ($d_B$). We defined 14 models by setting different constraints on these parameters and found the best fitting model using AIC and likelihood ratio test (see Table S9). We ran the MCMC analysis for 100,000 steps using an exponential prior for the best-fitting model, applied a burn-in for the first 10% of the

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

steps, and examined the posterior probability of the seven rates of the GeoSSE analysis.

**Paleoenvironment-depended diversification**

To test the association between paleo-environmental changes and diversification rates of Caryophylleae, *Gypsophila,* and *Dianthus* we used a birth-death model with speciation and extinction varying as a function of temperature changes through geological time (Condamine et al. 2013) using *fit_env* function implemented in R package RPANDA (Morlon et al. 2016). We fit 12 different birth-death models (Tables S13-S15). To give the value of the paleoenvironmental variable at each time point, based on deep-sea oxygen isotope records (Zachos et al. 2008), we interpolate a smooth line during each birth–death modeling process using the R-package PSPLINE (Ramsey and Ripley 2013). Then the speciation and/or extinction rates were estimated as a function of these values along the dated phylogenies according to the parameters of each model and the best-fitted model identified based on AICc and LRT, like the time-dependent analyses of the RPANDA package (see above).

**Ancestral area reconstruction**

To reconstruct the historical distribution of Caryophylleae we used RASP v.4 (Yu et al. 2020). We divided the range of Caryophylleae into five areas, based on the extant distribution of the majority of the tribe and the floristic regions it covers. These areas are western Asia (A), central Asia (B), eastern Asia (C), Europe except the Mediterranean part (D), and the Mediterranean region including North Africa (E). We removed the outgroup from the maximum clade credibility trees generated in BEAST analyses of the three datasets and used them as the input phylogenies in the historical distribution reconstruction analysis by RASP. To compare different historical biogeography models, we fit the data to all six models implemented in the BioGeoBEARS package in R (Matzke 2014) including DEC, DEC + j, DIVALIKE, DIVALIKE + j, BAYAREALIKE, and BAYAREALIKE + j. The ancestral distributions at all nodes were reconstructed using the Bayesian Binary Method (BBM: Ronquist 2004; Yu et al. 2015), BAYAREALIKE + j (Matzke 2014), and Dispersal- Extinction-Cladogenesis model (DEC: Ree and Smith 2008) implemented in RASP v.4. For the BBM analysis, chains were run for 5,000,000 generations, and states were sampled every 1000 generations. F81+G (estimated Felsenstein 1981 + gamma) was used with null root distribution, and the maximum number of possible ancestral areas was set to five (all

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

possible states) for all analyses.

## 3. Results

Detailed information on three datasets, including numbers of terminals, variable sites, and parsimony informative sites, is presented in Table 1.

**Table 1.** Alignment characteristics of the three genomic regions (loci) used in the present study.

| | *mat*K | *rps16* | ITS |
|---|---|---|---|
| Total number of terminals | 146 | 105 | 184 |
| No. of Caryophylleae taxa | 101 | 101 | 180 |
| No. of *Gypsophila* taxa | 25 | 55 | 71 |
| No. of *Dianthus* taxa | 38 | 10 | 40 |
| No. of *Saponaria* taxa | 22 | 7 | 24 |
| No. of *Acanthophyllum* taxa | 6 | 10 | 14 |
| No. of *Bolanthus* taxa | 0 | 8 | 15 |
| Alignment length [bp] | 931 | 993 | 698 |
| Conserved characters [bp] | 342 | 554 | 240 |
| Parsimony-uninformative characters [bp] | 152 | 180 | 108 |
| Parsimony-informative characters [bp] | 437 | 259 | 350 |
| Parsimony-informative characters [%] | 46.9 | 36.1 | 50.1 |

### 3.1. Phylogeny inference

The results of phylogenetic reconstructions of the three datasets using MP (Figs S1-S3), BI (Figs S4-S6), and RAxML (S7-S9) in the present study support the classification system for Caryophylleae in Madhani et al. (2018), and later revisions on the status of *Graecobolanthus* (Zografidis et al. 2020), boundaries of *Acanthophyllum* (Pirani et al. 2020), and the newly introduced genus *Yazdana* A.Pirani & Noroozi, (Noroozi et al. 2020). According to the gained phylogenies, *Gypsophila* includes generic names *Ankyropetalum*, *Vaccaria*, *Bolbosaponaria* (p.p. excl. type), *Dichoglottis*, *Pseudosaponaria*, and one species of *Diaphanoptera* (Barkoudah 1962; Madhani et al. 2018). The results of phylogenetic analysis on *rps16* and ITS loci indicate two well-supported clades within *Gypsophila*. The first clade includes the majority of species of *Gypsophila*, as well as the type species of the genus, *G. repens* L.; we call this clade, **Eugypsophila** (Figs S2, S3, S5, and S6). Most of the Eugypsophila clade members show the general morphology that we know for *Gypsophila* (baby's-breath), which is a perennial or annual herb with many-flowered lax

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

thyrse or panicle inflorescences, sepals scariose at margin and connate at base, and a capsule overtopping the calyx. The second clade, which is the smaller one, comprises plants that are highly adapted to alpine habitats with a pulvinate or dense caespitose life form, tiny succulent or spiny leaves, and usually small few-(uni-)flowered raceme-like monochasia, we call this group the **Pulvinate** clade (Figs S1-S9). This clade is most clearly evident in the MrBayes analyses (Figs S4-S6). These two clades which are basically sister, include almost all *Gypsophila* species which we call the **core *Gypsophila*** thereafter (Figs S2-S3, S5-S6, S8-S9). Although we included representatives of all sections of *Gypsophila* recognized by Barkoudah (1962), our results did not support recognizing any additional infrageneric groups.

**Divergence time estimation**

The results of the divergence time estimation using fossil calibration on the *mat*K dataset are presented in Table 2 and Fig S10. The results suggest that Caryophylleae started to diverge from the early Oligocene to the early Miocene (31.02–18.04 Ma); these results are similar to those reported by Xue et al. (2023). The ages inferred for four large genera inside Caryophylleae, including *Dianthus*, *Gypsophila*, *Acanthophyllum*, and *Saponaria* show that the divergence of their common ancestors started during the late Miocene to early Pliocene (Table 2). Our estimates for the age of the *Dianthus* clade, the largest genus inside Caryophylleae, are consistent with the previous study on the diversification dynamics of the genus (Valente et al. 2010). The estimated age for the crown group of *Gypsophila*, the second largest genus in the tribe with about 150 accepted species, is 5.31 Ma with SD=1.32 (95% HPD interval: 3.02-7.95), which is surprisingly young given the species richness and morphological diversity of the clade (Table 2). The results of secondary calibration on ITS and *rps16* datasets are presented in Figs S11 and S12, and Table S3. The estimates for divergence time by secondary calibration are older and the credible intervals (CIs) are wider compared to fossil calibration, which is not surprising as wider uncertainty and deviation from primary calibration are usually associated with secondary calibrations (Schenk 2016).

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

**Table 2.** Age estimates for major clades in the Caryophylleae clade using fossil calibration of the *mat*K dataset by BEAST; Net diversification rate estimates using Magallon & Sanderson (2001) method under low (ε= 0), medium (ε= 0.5), and high (ε= 0.9) extinction rates (ε) implemented in geiger package in R; speciation and extinction rates estimated by BAMM.

| Major clades | Ages estimates & 95% HPD (Ma) | Net diversification rate (r) Magallon & Sanderson (2001) | | | BAMM analysis | |
|---|---|---|---|---|---|---|
| | | r (ε= 0) | r (ε= 0.5) | r (ε= 0.9) | Speciation rate mean (95% HPD) | Extinction rate mean (95% HPD) |
| Caryophylleae | 24.68 (18.04–31.02) | 0.23 | 0.22 | 0.17 | 0.98 (0.74–1.41) | 0.42 (0.12–0.98) |
| *Gypsophila*-Stem group n = 183 | 14.85 (9.14–20.90) | 0.35 | 0.31 | 0.20 | 0.96 (0.64–1.56) | 0.42 (0.05–1.18) |
| *Gypsophila*-Crown group n = 150 | 5.31 (3.02–7.95) | 0.81 | 0.76 | 0.51 | 1.54 (0.95–2.87) | 0.71 (0.06–2.33) |
| Core *Gypsophila* n = 114 | 2.38 (1.26–3.75) | 1.78 | 1.66 | 1.08 | 2.34 (1.45–3.67) | 1.003 (0.04–2.87) |
| *Dianthus*-Stem group (*Dianthus* + *Petrorhagia*) | 6.92 (4.23–9.74) | 0.82 | 0.72 | 0.50 | 2.46 (1.70–3.98) | 0.89 (0.10–2.85) |
| *Dianthus*-Crown + *Velezia* n = 300 | 3.14 (1.83–4.59) | 1.59 | 1.50 | 1.07 | 3.07 (2.1–5.0) | 1.11 (0.10–3.67) |
| *Saponaria* n = 30 | 5.87 (3.45–8.55) | 0.46 | 0.42 | 0.22 | 0.52 (0.31–0.92) | 0.19 (0.01–0.72) |
| *Acanthophyllum* n = 95 | 6.38 (3.71–9.35) | 0.60 | 0.56 | 0.36 | 0.56 (0.3–1.2) | 0.21 (0.004–0.85) |

## Diversification analyses

The results of time-dependent diversification analysis in BAMM strongly reject constant diversification rate among the markers used (Bayes factors = 13152, 1142, and 16672 for *mat*K, *rps16*, and ITS datasets, respectively, Tables S20-S22). Instead, at least six distinct credible shifts in speciation rate across the Caryophylleae clade were identified using the three datasets (Figs S13-S15). The best-fitting number of shifts calculated using the stepwise Bayes factor procedure (Mitchell and Rabosky 2017) shows five, six, and eight shifts for ITS, *mat*K, and *rps16* datasets, respectively (Tables S23-S25). The best rate shift configurations of the BAMM analysis of all datasets identified at least one shift in the speciation rate of *Dianthus* (consistent with Valente et al. 2010) and another in *Gypsophila* (Fig S16-S18), and also a shift in *Acanthophyllum* in the *rps16* dataset (Fig S18). The best speciation rate shift configuration within *Gypsophila* was detected at the common ancestor of core *Gypsophila* in the BAMM analyses of all datasets. The rate through time plots also indicate that speciation, extinction, and net diversification rates were almost

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

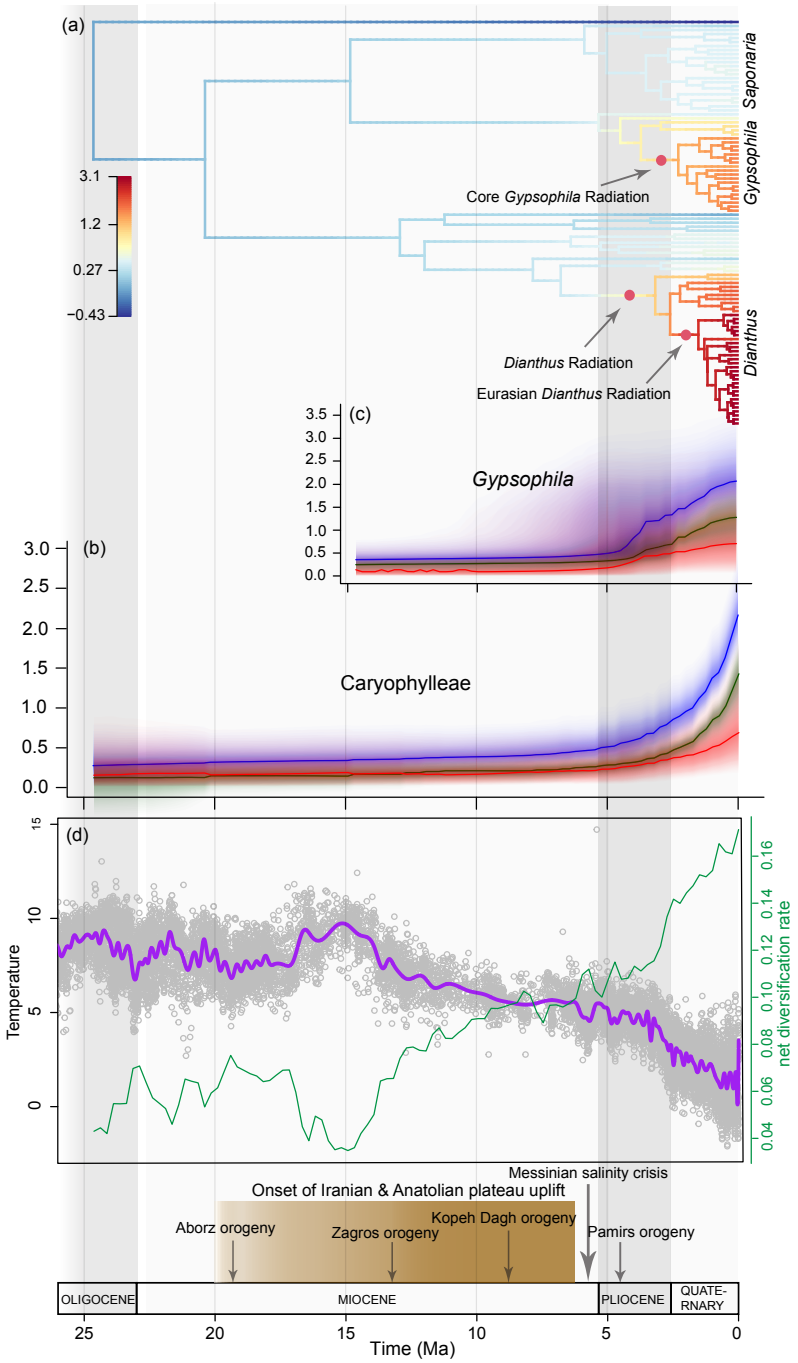


**Figure 2.** Diversification of Caryophylleae. (a) Net diversification rate plot (phylorate) of the *mat*K dataset showing the best configuration shift identified by BAMM. Evolutionary rate through time for Caryophylleae (b) and *Gypsophila* (c), Speciation (blue line), extinction (red line), and net diversification rates (dark green line) shown with color density shading indicate confidence interval. (d) The global temperature through time (purple line) and net diversification rate through time (green line) of the best-fitting model of the temperature-dependent diversification analysis estimated by RPANDA.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

constant following the divergence of the most recent common ancestor (MRCA) of the Caryophylleae clade in the late Oligocene until early Pliocene (~ 25-5 Ma, Fig 2b), just before the two identified shifts in speciation occurred in *Gypsophila* and *Dianthus* (Fig 2c), but all rates start to increase in early Pliocene and continue increasing until now (Fig 2b, and c). Congruent with the identified shifts in these clades, estimates of net diversification rate using the Magallón and Sanderson method also show extremely high rates of diversification in these large clades inside Caryophylleae (Tables 2 and S3). The diversification rates using the Magallón and Sanderson method calculated 1.08 and 1.78 events $Myr^{-1}$ per lineage for core *Gypsophila* under high (e = 0.90) and low (e = 0) extinction fractions, respectively (Table 2), which is among the fastest diversification rates in the Tree of Life. Consistent with these calculations, speciation, and extinction rates calculated using BAMM analysis also support very high speciation and extinction rate in all major clades within Caryophylleae (Tables 2 and S3).

Additionally, from the 12 models analyzed for each major clade across all datasets by RPANDA (resulting in 220 models in total), the best-fitting likelihood model of time-dependent functions indicates a constant, yet high, speciation and extinction rate over time in the whole Caryophylleae clade (Table S10); however, the second (model 6) and third (model 5) best models — that also have significant p-values in the Likelihood Ratio Test (LRT) — indicate exponential and linear change of speciation over time with a constant extinction rate. The best-fitting models for *Dianthus* and *Gypsophila* suggest that speciation rates vary and exhibit an exponential increase over time in all datasets (Tables S10-S12), with the exception of *Gypsophila* in the ITS dataset, where the best model indicates a constant, yet high, speciation and extinction rate over time. Interestingly, the second-best model for this dataset, which also exhibits a significant p-value (4e-04) in the LRT test, shows an exponential increase over time for *Gypsophila* (Tables S12). In the most probable models for *Saponaria* within the ITS and *rps16* datasets, the speciation rate remains constant over time (as shown in Tables S11 and S12). In the *Acanthophyllum* clade, the optimal model in the ITS dataset indicates a constant speciation rate throughout all times (Table S12). However, in the *rps16* dataset, the speciation rate aligns with the BAMM results, showing an exponential increase over time (Table S11).

The temperature-dependent analysis rejected the null hypothesis of no influence from temperature on the speciation rate of Caryophylleae, *Dianthus*, *Gypsophila*, and *Acanthophyllum* clades in most datasets (Tables S13-S15). However, for *Gypsophila* in the *mat*K dataset and *Acanthophyllum* in

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

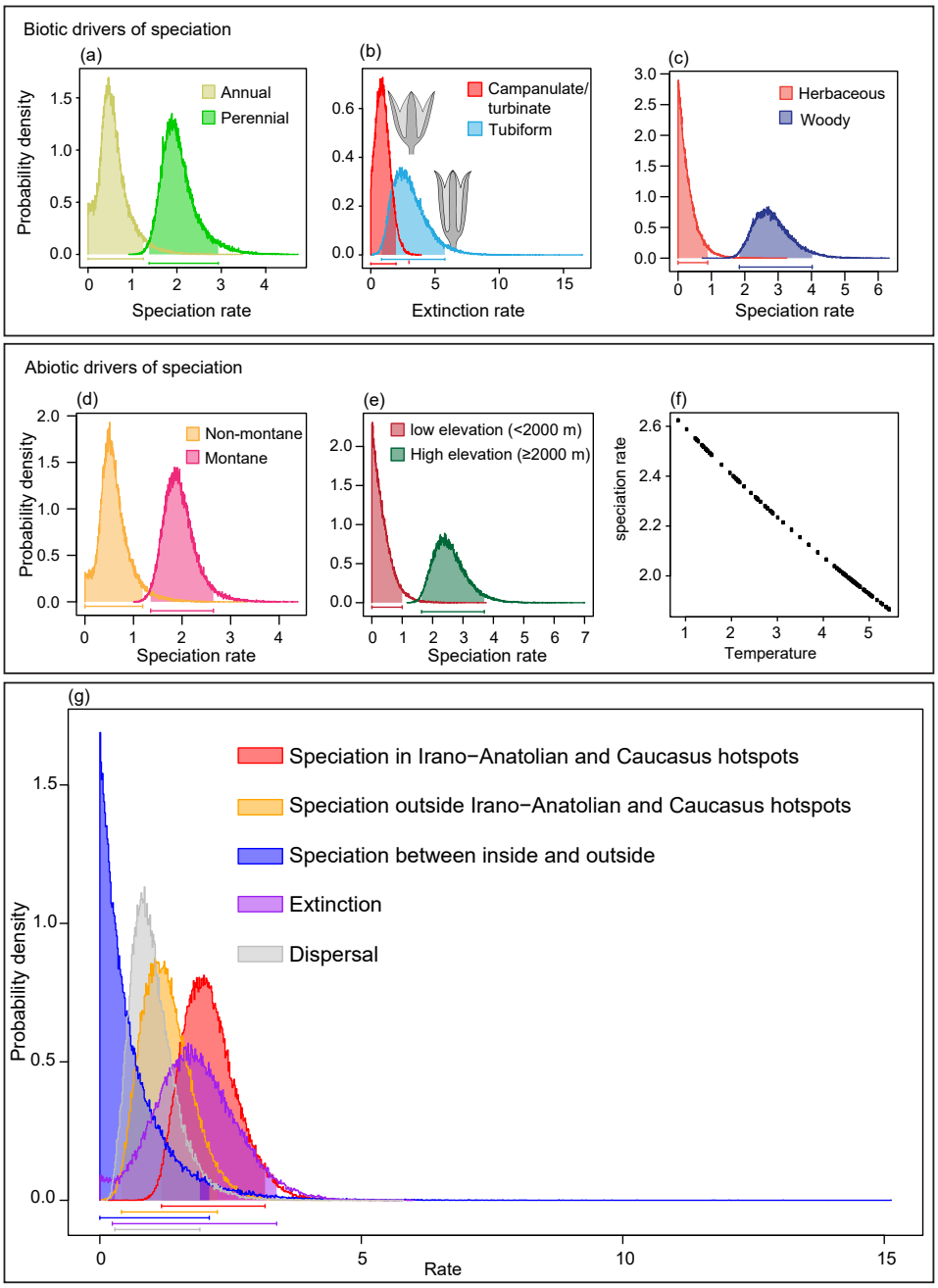


**Figure 3.** Biotic and abiotic drivers of diversification in *Gypsophila*. Posterior probability plots of speciation/extinction rates of binary traits analyzed by BiSSE: (a) Life strategy (annual vs perennial). (b) Calyx shape (tubiform vs. campanulate/turbinate; illustrated by Shirin Sabeti). (c) Life form (herbaceous vs. woody). (d) Habitat (non-montane vs. montane). (e) Elevation (high elevation vs. low elevation). (f) Speciation rate plot estimated as a function of temperature for the best-fitting model of temperature-dependent analysis of RPANDA for the *Gypsophila* clade. (g) Posterior probability plot of speciation extinction and dispersal rates per area estimated by GeoSSE.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

the ITS dataset, the best models indicate a constant speciation and extinction rate unaffected by temperature fluctuations (Table S13). Interestingly, the second best model for *Gypsophila* within the *mat*K dataset, with a significant p value of 1.7e-04, points towards an exponential correlation between speciation rate and temperature (model 4, Table S13). In contrast, the optimal likelihood models for *Saponaria* show no connection between historical temperature shifts and the diversification rate in both the *rps16* and ITS datasets (Tables S14 and S15).

The best-fitting models derived from the BiSSE analyses for the five binary traits in *Gypsophila* demonstrate higher speciation rates in perennial species compared to annual, montane species compared to non-montane, woody species compared to herbaceous, and species at high elevations compared to those at low elevations (Fig 3a, c-e; Supplementary Tables S4-S6 and S8). According to the best-fitting model for calyx morphology, species with tubiform calyces exhibit higher extinction rates compared to those with campanulate calyces (Fig 3b; Table S7). Lastly, the GeoSSE analysis reveals higher speciation rates within the Irano-Anatolian and Caucasus hotspots, compared to areas outside these two biodiversity hotspots (Fig 3g; Table S9).

**Ancestral biogeography reconstruction**

For the biogeographic reconstruction analyses, the BAYAREALIKE + j model was identified as the best-fitting model for all datasets (Table S16), however, the BBM model results in higher probabilities for the estimated ancestral ranges and probably better explains the geographical history of the group (Tables S17-S19). Based on the BBM analyses of the ITS and *rps16* datasets (Tables S18 and S19), as well as the BAYAREALIKE + j estimates for ITS (Table S19), western Asia and the Mediterranean region (AE) were inferred as the ancestral area for *Gypsophila* (Fig 4, Tables S17-S19), while the analysis of BBM and BAYAREALIKE + j for *mat*K and *rps16*, respectively, estimated western Asia (80.12%, Table S18) as the ancestral distribution of the MRCA of the genus (Fig 4, Tables S17-S19). Overall, our analysis suggests that dispersal and expansion were more frequent than vicariance in explaining the current geographical distribution of *Gypsophila* (Tables S18 and S19) and Caryophylleae (Table S17).

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

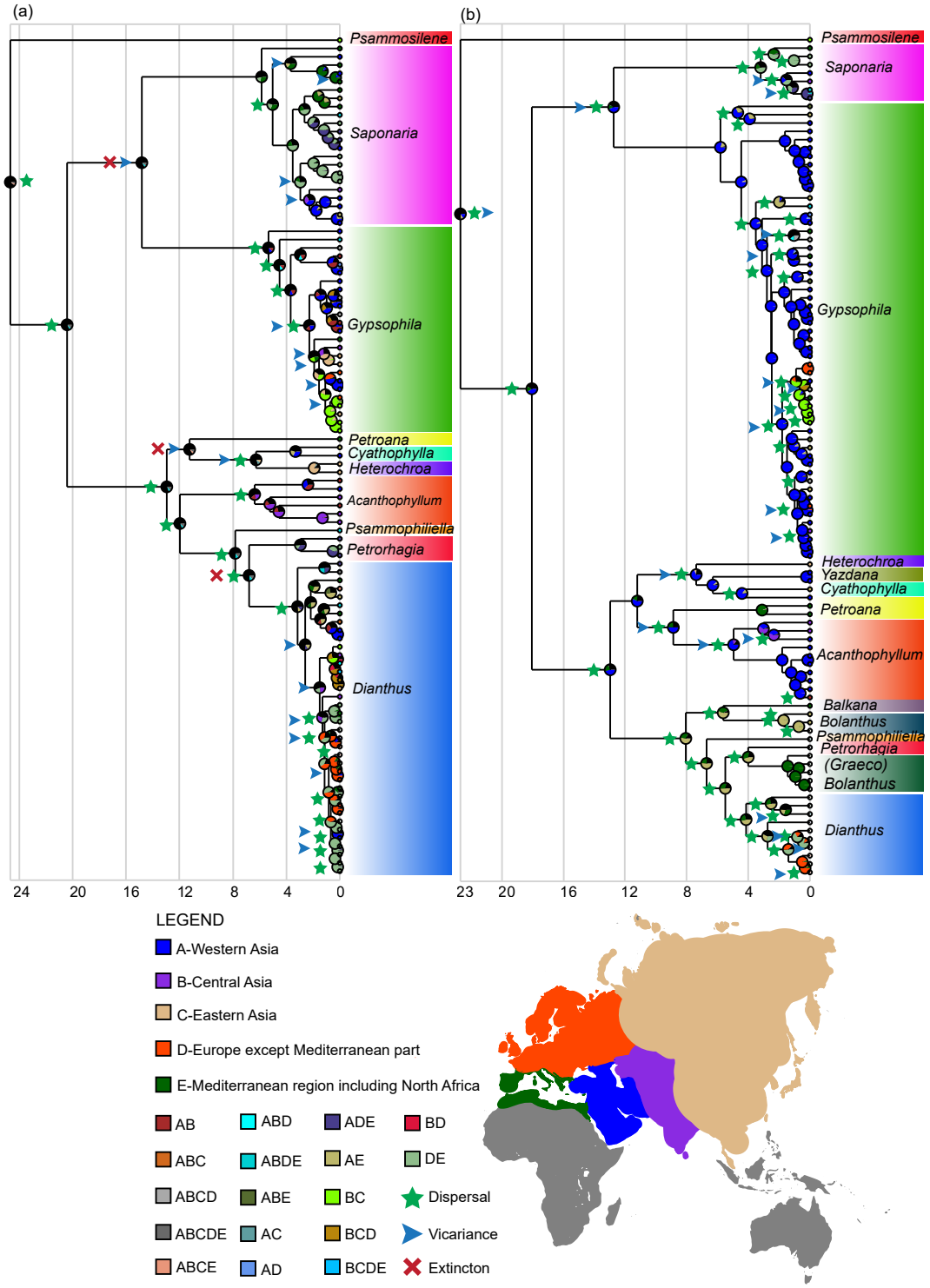


**Fig. 4** Ancestral area reconstruction of Caryophylleae. The reconstructed ancestral area of the best-fitting model (BAYAREALIKE + j) performed in RASP for the *mat*K (a) and *rps16* (b) datasets. The position of identified dispersal, vicariance, and extinction events indicated by green star, blue arrowhead, and red crosses, respectively.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

## Discussion

Irano-Anatolian and Caucasus biodiversity hotspots are known for their highly endemic alpine and subalpine flora which correlates with a high degree of topological isolation caused by the recent uplift in the various mountain systems in these regions, such as the Turkish-Iranian plateau plateau and its major mountainous components, i.e., Zagros, Alborz, Caucasus, and Kopet-Dag mountains. Although some studies attempted to assess the huge diversity within some endemic taxa in these regions in terms of molecular clock, biogeography, and dispersal patterns (Ghane-Ameleh et al. 2021; Noroozi et al. 2018), our study is the first to address the issue of mega-diversity in the endemic taxa of Irano-Anatolian and Caucasus biodiversity hotspots through phylogeny, molecular clock, and statistical analyses of diversification.

### Evolutionary radiation in *Gypsophila*

Our study suggests that the majority of the diversity within *Gypsophila* was shaped recently and represent an evolutionary radiation. Within the large and diverse clade of the Carnation tribe (Caryophylleae), at least three distinct significant shifts to increase the diversification rate were identified using BAMM analysis, two in *Dianthus* and one in *Gypsophila* (Fig 2 and S13-S18), which are consistent with the estimated young ages for both clades based on molecular clock analyses (Tables 2 and S3). The two shifts in diversification rate within *Dianthus* clades confirm the evolutionary radiation reported in this genus and its Eurasian clade by Valente et al (2010). The shift in diversification rate detected within *Gypsophila*, in the core *Gypsophila*, by BAMM analysis (Fig 2 and S13-S18) is aligned with the surprisingly young age of the crown group of *Gypsophila* (5.31 Ma in the fossil calibration, see Tables 2 and S3), and the secondary calibration of the *rps16* datasets (Table S3). These findings are supported by the best-fitting time-dependent functions selected in RPANDA, which show an exponential increase in speciation rates of *Gypsophila* over time (Tables S10-S12). Our calculation for the net diversification and speciation rates in core *Gypsophila* illuminates one of the fastest evolutionary radiations in the Tree of Life (95% HPD interval of speciation rate: 1.45–3.67 $Myr^{-1}$ per lineage, Table 2).

### The impact of orogeny and climate change on species diversification in *Gypsophila*

The beginning of the rise in speciation, extinction, and diversification rates within *Gypsophila*, as depicted in the rate through time plot (Fig 2c), coincided with the final phases of the uplift of the

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

major mountain systems in the Irano-Anatolian and Caucasus biodiversity hotspots (Fig 2d). During this period, new alpine environments emerged, creating diverse ecological opportunities that facilitated diversification. Through its impact on aspects such as productivity, the divergence of climatic niches, body size, and metabolic rates, temperature plays a crucial role in shaping biological processes (Condamine et al. 2019). This is particularly significant when considering the influence of temperature fluctuations on the rate of speciation. Our paleotemperature-dependency analysis provides evidence of influences of past climate changes on diversification rates across Caryophylleae and its major clades (Tables S13-S15). One interesting finding of this study is the negative correlation between speciation rate in *Gypsophila* and temperature in both *rps16* and ITS datasets (Tables S14-S15; Fig 3f). The best fitting paleo-environmental dependant model in the *mat*K dataset however, suggest no correlation between speciation and extinction rates with temperature, but interestingly, the second best model in this analysis, which turns out to also have a significant p-value in the likelihood ratio test, also shows an negative correlation of speciation rate with past temperature changes (Model 4, Table S13).

**Evidence suggests an alpine radiation in *Gypsophila***

Our trait-dependent analyses suggest a significant role of species morphology and ecological traits in shaping diversification rates (Fig 3; Tables S4-S8). Consistent with findings from other alpine radiations such as *Lupinus* (Drummond et al. 2012) we discern a higher diversification rate in perennial species than in annual species, with the speciation rate being quintupled in perennials (Fig 3a; Table S5). Our analysis also uncovers a notably higher speciation rate in woody species compared to their herbaceous counterparts (Fig 3c; Table S6). Such findings corroborate with the adaptation of *Gypsophila* species to the harsh alpine habitats of the Irano-Anatolian and Caucasus hotspots—environments previously unfavorable to ancestral annual and herbaceous species. From an ecological perspective, speciation rate in montane ecosystems is found to be four times faster than in non-montane ecosystems (Fig 3d; Table S4). Congruent with this finding, high elevation habitats exhibit a significantly higher speciation rate than low elevation habitats (Fig 3e; Table S8). These observations lead us to conclude that the evolutionary radiation within *Gypsophila* is closely tied to morphological and ecological adaptations to the challenging montane environments of the Irano-Anatolian and Caucasus biodiversity hotspots. Moreover, our findings indicate that

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

the extinction rate is higher in species with a tubiform calyx, commonly found in annual and low elevation species, compared to those with a campanulate/turbinate shape, typically observed in high elevation species (Fig 3b; Table S7). In addition to this, our geographic analysis of the diversification rate within *Gypsophila* points to a higher speciation rate within the aforementioned biodiversity hotspots compared to the rest distribution range of the genus (Fig 3g, Table S9).

**The Messinian salinity crisis and the origin of Gypsophily**

Our ancestral biogeography reconstruction suggests that the Mediterranean region and western Asia served as the ancestral range for *Gypsophila*. This is corroborated by the substantial species diversity of *Gypsophila* in these regions, largely attributable to the complex topography, varied climate, and the rich geological history inherent in these areas. Gypsophily, that can be defined as the ability of members of the *Gypsophila* species to thrive in calcareous-rich soil, is a significant ecological adaptation that likely developed in response to the environmental conditions presented by the Messinian Salinity Crisis (MSC). This dramatic event, which occurred in the Mediterranean Sea around 5.96 to 5.33 million years ago during the late Miocene epoch (Hsü et al. 1973), was characterized by a significant reduction or complete evaporation of the Mediterranean Sea, leading to the deposition of massive salt and gypsum layers throughout the basin. resulting in widespread gypsum deposits across the Mediterranean region and Europe (Hsü et al. 1977; Hsü et al. 1973). We posit that this crisis may have functioned as an evolutionary catalyst, fostering gypsophily in the *Gypsophila* common ancestor. The advent and subsequent diversification of *Gypsophila* in these regions, followed by its dispersion to the young high-altitude habitats of western Asia and the Caucasus, are likely tied to the species' acquisition of limestone-dwelling abilities during the Messinian Salinity Crisis. This capability facilitated the geographic expansion and subsequent speciation of *Gypsophila*, intertwined with other historical phenomena such as tectonic activities, orogeny, and climate variations.

In conclusion, our study provides valuable insights into the phylogenetic relationships, diversification patterns, and biogeographic history of *Gypsophila* and the tribe Caryophylleae. The rapid diversification of *Gypsophila*, particularly in the Irano-Anatolian and Caucasus biodiversity hotspots, highlights the importance of these regions for the evolution and diversification of this genus. Further research is needed to investigate the specific environmental factors and underlying genomics architecture that have driven the diversification of *Gypsophila* and to explore its

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

potential role as a model system for understanding the evolution of plant diversity in these biodiversity hotspots.

## Acknowledgments

Our sincere appreciation goes to the curators of the herbaria B, G, LE, M, MSB, TUH, W, and WU for their invaluable support and for providing us with the chance to examine specimens of this plant group and to collect samples for DNA extraction.

## Author contributions

HM: Specimen study, plant collection, laboratory procedures, phylogenetic and statistical analyses, manuscript preparation. RR: Nomenclatural research, manuscript revision, comments on phylogenetic trees. GH: Providing laboratory and technical facilities, providing some plant materials. NM: plant collection, manuscript preparation, statistical analyses. SZ: Specimen study, plant collection, Manuscript revision, providing sequences. — HM, https://orcid.org/0000-0003-0360-9527, hossein.madhani@gmail.com; RR, https://orcid.org/0000-0002-6765-0353, rabeler@umich.edu); BO, https://orcid.org/0000-0002-6104-4264, bengt.oxelman@bioenv.gu.se; GH, heubl@lrz.uni-muenchen.de; NM, https://orcid.org/0009-0008-4812-3209, navid.madhani@gmail.com; SZ, https://orcid.org/0000-0001-9159-1800, zarre@khayam.ut.ac.ir)

## References

Aguirre-Santoro J, Salinas NR, Michelangeli FA. 2019. The influence of floral variation and geographic disjunction on the evolutionary dynamics of Ronnbergia and Wittmackia (Bromeliaceae: Bromelioideae). Botanical journal of the Linnean Society. *Linnean Society of London* 192: 609–624.

Allen MB, Armstrong HA. 2008. Arabia–Eurasia collision and the forcing of mid-Cenozoic global cooling. *Palaeogeography, palaeoclimatology, palaeoecology* 265: 52–58.

Anthony F, Diniz LEC, Combes M-C, Lashermes P. 2010. Adaptive radiation in Coffea subgenus Coffea L. (Rubiaceae) in Africa and Madagascar. *Plant systematics and evolution* 285: 51–64.

Antonelli A, Kissling WD, Flantua SGA, Bermúdez MA, Mulch A, Muellner-Riehl AN, Kreft H, Linder HP, Badgley C, Fjeldså J, et al. 2018. Geological and climatic influences on mountain biodiversity. *Nature geoscience* 11: 718–725.

Antonelli A, Nylander JAA, Persson C, Sanmartín I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America* 106: 9749–9754.

Báldi T. 1980. The early history of the Paratethys. Bulletin of the Hungarian Geological Society.

Baldwin BG, Sanderson MJ. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences of the United States of America* 95: 9402–9406.

Ballato P, Mulch A, Landgraf A, Strecker MR, Dalconi MC, Friedrich A, Tabatabaei SH. 2010. Middle to Late Miocene Middle Eastern Climate from Stable Oxygen and Carbon Isotope Data, Southern Alborz Mountains, N Iran. *Earth and Planetary Science Letters* 300 (1): 125–138.

Barkoudah YI. 1962. A revision of *Gypsophila*, *Bolanthus*, *Ankyropetalum* and *Phryna*. *Wentia* 9: 1-203.

Bell MA, Lloyd GT. 2015. Strap: An R Package for Plotting Phylogenies against Stratigraphy and Assessing Their Stratigraphic Congruence. *Palaeontology* 58 (2): 379–389.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Brennan IG, Oliver PM. 2017. Mass Turnover and Recovery Dynamics of a Diverse Australian Continental Radiation. *Evolution; International Journal of Organic Evolution* 71 (5): 1352–1365.

Brown GK, Nelson G, Ladiges PY. 2006. Historical Biogeography of Rhododendron Section Vireya and the Malesian Archipelago. *Journal of Biogeography* 33 (11): 1929–1944.

Calonje M, Meerow AW, Griffith MP, Salas-Leiva D, Vovides AP, Coiro M, Francisco-Ortega J. 2019. A Time-Calibrated Species Tree Phylogeny of the New World Cycad Genus Zamia L. (Zamiaceae, Cycadales). *International Journal of Plant Sciences* 180 (4): 286–314.

Calsbeek R, Thompson JN, Richardson JE. 2003. Patterns of Molecular Evolution and Diversification in a Biodiversity Hotspot: The California Floristic Province. *Molecular Ecology* 12 (4): 1021–1029.

Cohen KM, Finney SC, Gibbard PL, Fan J-X. 2013. The ICS International Chronostratigraphic Chart. *Episodes* 36 (3): 199–204.

Comes HP, Kadereit JW. 2003.Spatial and Temporal Patterns in the Evolution of the Flora of the European Alpine System. *Taxon* 52 (3): 451–462.

Condamine FL, Rolland J, Morlon H. 2013. Macroevolutionary Perspectives to Environmental Change. *Ecology Letters* 16 Suppl 1 (May): 72–85.

———. 2019. Assessing the Causes of Diversification Slowdowns: Temperature-Dependent and Diversity-Dependent Models Receive Equivalent Support. *Ecology Letters* 22 (11): 1900–1912.

Cox SC, Prys-Jones RP, Habel JC, Amakobe BA, Day JJ. 2014. Niche Divergence Promotes Rapid Diversification of East African Sky Island White-Eyes (Aves: Zosteropidae). *Molecular Ecology* 23 (16): 4103–4118.

van Dam JA. 2006. Geographic and Temporal Patterns in the Late Neogene (12–3 Ma) Aridification of Europe: The Use of Small Mammals as Paleoprecipitation Proxies. *Palaeogeography, Palaeoclimatology, Palaeoecology* 238 (1): 190–218.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: More Models, New Heuristics and Parallel Computing. *Nature Methods* 9 (8): 772.

Davis SD, Heywood VH, Hamilton AC. 1994. *Centres of Plant Diversity: Asia, Australasia, and the Pacific*. Vol. 2. World Conservation Union.

Dilek Y, Imamverdiyev N, Altunkaynak Ş. 2010. Geochemistry and Tectonics of Cenozoic Volcanism in the Lesser Caucasus (Azerbaijan) and the Peri-Arabian Region: Collision-Induced Mantle Dynamics and Its Magmatic Fingerprint. *International Geology Review* 52 (4-6): 536–578.

Djamali M, Baumel A, Brewer S, Jackson ST, Kadereit JW, López-Vinyallonga S, Mehregan I, Shabanian E, Simakova A. 2012a. Ecological Implications of Cousinia Cass. (Asteraceae) Persistence through the Last Two Glacial–interglacial Cycles in the Continental Middle East for the Irano-Turanian Flora. *Review of Palaeobotany and Palynology* 172 (February): 10–20.

Djamali M, Brewer S, Breckle SW, Jackson ST. 2012b. Climatic Determinism in Phytogeographic Regionalization: A Test from the Irano-Turanian Region, SW and Central Asia. *Flora - Morphology, Distribution, Functional Ecology of Plants* 207 (4): 237–349.

Drummond CS, Eastwood RJ, Miotto STS, Hughes CE. 2012. Multiple continental radiations and correlates of diversification in Lupinus (Leguminosae): testing for key innovation with incomplete taxon sampling. *Systematic biology* 61: 443–460.

Drummond AJ, Ho SYW, Rawlence N, Rambaut A. 2007. A rough guide to BEAST 1.4. Available from https://www.researchgate.net/publication/228988177_A_Rough_Guide_to_beast_14

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology* 7: 214.

Ebersbach J, Muellner-Riehl AN, Michalak I, Tkach N, Hoffmann MH, Röser M, Sun H, Favre A. 2017a In and out of the Qinghai-Tibet Plateau: Divergence Time Estimation and Historical Biogeography of the Large Arctic-Alpine genus *Saxifraga* L. *Journal of Biogeography* 44 (4): 900–910.

Ebersbach J, Schnitzler J, Favre A, Muellner-Riehl AN. 2017b. Evolutionary Radiations in the Species-Rich Mountain Genus Saxifraga L. *BMC Evolutionary Biology* 17 (1): 1–13.

Esselstyn JA, Timm RM, Brown RM. 2009. Do Geological or Climatic Processes Drive Speciation in

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Dynamic Archipelagos? The Tempo and Mode of Diversification in Southeast Asian Shrews. *Evolution; International Journal of Organic Evolution* 63 (10): 2595–2610.

Fassou G, Korotkova N, Nersesyan A, Koch MA, Dimopoulos P, Borsch T. 2022. Taxonomy of *Dianthus* (Caryophyllaceae) – overall phylogenetic relationships and assessment of species diversity based on a first comprehensive checklist of the genus. *PhytoKeys* 196: 91–214.

Favre A, Päckert M, Pauls SU, Jähnig SC, Uhl D, Michalak I, Muellner-Riehl AN. 2015. The Role of the Uplift of the Qinghai-Tibetan Plateau for the Evolution of Tibetan Biotas. *Biological Reviews of the Cambridge Philosophical Society* 90 (1): 236–253.

FitzJohn RG. 2012. Diversitree: Comparative Phylogenetic Analyses of Diversification in R. *Methods in Ecology and Evolution* 3(6): 1084–1092.

Fonseca CR. 2009. The Silent Mass Extinction of Insect Herbivores in Biodiversity Hotspots. *Conservation Biology: The Journal of the Society for Conservation Biology* 23 (6): 1507–1515.

Frajman B, Eggens F, Oxelman B. 2009. Hybrid Origins and Homoploid Reticulate Evolution within Heliosperma (Sileneae, Caryophyllaceae)--a Multigene Phylogenetic Approach with Relative Dating. *Systematic Biology* 58 (3): 328–345.

Gavillot Y, Axen GJ, Stockli DF, Horton BK, Fakhari MD. 2010. Timing of Thrust Activity in the High Zagros Fold-Thrust Belt, Iran, from (U-Th)/He Thermochronometry. *Tectonics* 29 (4).

Gehrke B, Linder HP. 2009. The Scramble for Africa: Pan-Temperate Elements on the African High Mountains. *Proceedings. Biological Sciences / The Royal Society* 276 (1667): 2657–2665.

Ghane-Ameleh S, Khosravi M, Saberi-Pirooz R, Ebrahimi E, Aghbolaghi MA, Ahmadzadeh F. 2021. Mid-Pleistocene Transition as a Trigger for Diversification in the Irano-Anatolian Region: Evidence Revealed by Phylogeography and Distribution Pattern of the Eastern Three-Lined Lizard. *Global Ecology and Conservation* 31: e01839.

Gillespie RG, Roderick GK. 2014. Evolution: Geology and Climate Drive Diversification. *Nature* 509 (7500): 297–298.

Goldberg EE, Lancaster LT, Ree RH. 2011. Phylogenetic Inference of Reciprocal Effects between Geographic Range Evolution and Diversification. *Systematic Biology* 60 (4): 451–465.

Greenberg AK, Donoghue MJ. 2011. Molecular Systematics and Character Evolution in Caryophyllaceae. *Taxon* 60 (6): 1637–1652.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008. GEIGER: Investigating Evolutionary Radiations. *Bioinformatics* 24 (1): 129–131.

Hatzfeld D, Molnar P. 2010. Comparisons of the Kinematics and Deep Structures of the Zagros and Himalaya and of the Iranian and Tibetan Plateaus and Geodynamic Implications. *Reviews of Geophysics* 48 (2).

Hernández-Ledesma P, Berendsohn WG, Borsch T, Von Mering S, Akhani H, Arias S, Castañeda-Noa I, Eggli U, Eriksson R, Flores-Olvera H, et al. 2015. A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. Willdenowia 45: 281-383.

Ho SYW, Phillips MJ. 2009.. Accounting for Calibration Uncertainty in Phylogenetic Estimation of Evolutionary Divergence Times. *Systematic Biology* 58 (3): 367–380.

Hsü KJ, Montadert L, Bernoulli D, Cita MB, Erickson A, Garrison RE, Kidd RB, Mèlierés F, Müller C, Wright R. 1977. History of the Mediterranean Salinity Crisis. *Nature* 267 (5610): 399–403.

Hsü KJ, Ryan WBF, Cita MB. 1973. Late Miocene Desiccation of the Mediterranean. *Nature* 242 (5395): 240–244.

Huber-Morath. A. 1967. *Gypsophila* L. in P.H. Davis. ed. 1967. Flora of Turkey and the East Aegean Islands, Vol. 2. Edinburgh University Press.

Hughes CE, Atchison GW. 2015. The Ubiquity of Alpine Plant Radiations: From the Andes to the Hengduan Mountains. *The New Phytologist* 207 (2): 275–282.

Hughes C, Eastwood R. 2006. Island Radiation on a Continental Scale: Exceptional Rates of Plant Diversification after Uplift of the Andes. Proceedings of the National Academy of Sciences of the United States of America 103 (27): 10334–10339.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Jabbour F, Renner SS. 2012. A Phylogeny of Delphinieae (Ranunculaceae) Shows That Aconitum Is Nested within Delphinium and That Late Miocene Transitions to Long Life Cycles in the Himalayas and Southwest China Coincide with Bursts in Diversification. *Molecular Phylogenetics and Evolution* 62 (3): 928–942.

Janssens SB, Vandelook F, De Langhe E, Verstraete B, Smets E, Vandenhouwe I, Swennen R. 2016. Evolutionary Dynamics and Biogeography of Musaceae Reveal a Correlation between the Diversification of the Banana Family and the Geological and Climatic History of Southeast Asia. *The New Phytologist* 210 (4): 1453–1465.

Joly S, Heenan PB, Lockhart PJ. 2009. A Pleistocene Inter-Tribal Allopolyploidization Event Precedes the Species Radiation of Pachycladon (Brassicaceae) in New Zealand. *Molecular Phylogenetics and Evolution* 51 (2): 365–372.

———. 2014. Species Radiation by Niche Shifts in New Zealand's Rockcresses (Pachycladon, Brassicaceae). *Systematic Biology* 63 (2): 192–202.

Jordan GJ, Macphail MK. 2003. A Middle-Late Eocene Inflorescence of Caryophyllaceae from Tasmania, Australia. *American Journal of Botany* 90 (5): 761–768.

Kadereit JW, Griebeler EM, Comes HP. 2004. Quaternary Diversification in European Alpine Plants: Pattern and Process. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359 (1442): 265–274.

Katoh K, Standley DM. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30 (4): 772–780.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. *Bioinformatics* 28 (12): 1647–1649.

Keskin M. 2003. Magma Generation by Slab Steepening and Breakoff beneath a Subduction-Accretion Complex: An Alternative Model for Collision-Related Volcanism in Eastern Anatolia, Turkey. *Geophysical Research Letters* 30 (24).

Klak C, Reeves G, Hedderson T. 2004. Unmatched Tempo of Evolution in Southern African Semi-Desert Ice Plants. *Nature* 427 (6969): 63–65.

Knox EB, Palmer JD. 1995. Chloroplast DNA Variation and the Recent Radiation of the Giant Senecios (Asteraceae) on the Tall Mountains of Eastern Africa. *Proceedings of the National Academy of Sciences of the United States of America* 92 (22): 10349–10353.

Lagomarsino LP, Condamine FL, Antonelli A, Mulch A, Davis CC. 2016. The Abiotic and Biotic Drivers of Rapid Diversification in Andean Bellflowers (Campanulaceae). *The New Phytologist* 210 (4): 1430–1442.

Le Roux JJ, Hui C, Castillo ML, Iriondo JM, Keet J-H, Khapugin AA, Médail F, Rejmánek M, Theron G, Yannelli FA, et al. 2019. Recent Anthropogenic Plant Extinctions Differ in Biodiversity Hotspots and Coldspots. *Current Biology: CB* 29 (17): 2912–2818.

Linder HP. 2003. The Radiation of the Cape Flora, Southern Africa. *Biological Reviews of the Cambridge Philosophical Society* 78 (4): 597–638.

Linder HP, Hardy CR. 2004. Evolution of the Species-Rich Cape Flora. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359 (1450): 1623–1632.

Linder HP. 2014. The Evolution of African Plant Diversity. *Frontiers in Ecology and Evolution* 2.

Liu J-Q, Wang Y-J, Wang A-L, Hideaki O, Abbott RJ. 2006. Radiation and Diversification within the Ligularia–Cremanthodium–Parasenecio Complex (Asteraceae) Triggered by Uplift of the Qinghai-Tibetan Plateau. *Molecular Phylogenetics and Evolution* 38 (1): 31–49.

López-Vinyallonga S, Mehregan I, Garcia-Jacas N, Tscherneva O, Susanna A, Kadereit JW. 2009. Phylogeny and evolution of the *Arctium-Cousinia* complex (Compositae, Cardueae-Carduinae). *Taxon*, 58(1): 153-171.

Maddison, WP, Midford PE, Otto SP. 2007. Estimating a Binary Character's Effect on Speciation and Extinction. *Systematic Biology* 56 (5): 701–710.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Maddison WP, Maddison DR. 2021. Mesquite: A Modular System for Evolutionary Analysis. Version 3.31. 2017.

Madhani H, Rabeler R, Pirani A, Oxelman B, Heubl G, Zarre S. 2018. Untangling Phylogenetic Patterns and Taxonomic Confusion in Tribe Caryophylleae (Caryophyllaceae) with Special Focus on Generic Boundaries. *Taxon* 67 (1): 83–112.

Madriñán S, Cortés AJ, Richardson JE. 2013. Páramo Is the World's Fastest Evolving and Coolest Biodiversity Hotspot. *Frontiers in Genetics* 4 (October): 192.

Magallón S, Sanderson MJ. 2001. Absolute Diversification Rates in Angiosperm Clades. *Evolution; International Journal of Organic Evolution* 55 (9): 1762–1780.

Malcolm JR, Liu C, Neilson RP, Hansen L, Hannah L. 2006. Global Warming and Extinctions of Endemic Species from Biodiversity Hotspots. *Conservation Biology: The Journal of the Society for Conservation Biology* 20 (2): 538–548.

Manafzadeh S, Salvo G, Conti E. 2014. A Tale of Migrations from East to West: The Irano-Turanian Floristic Region as a Source of Mediterranean Xerophytes. *Journal of Biogeography* 41 (2): 366–379.

Manafzadeh S, Staedler YM, Conti E. 2017. Visions of the Past and Dreams of the Future in the Orient: The Irano-Turanian Region from Classical Botany to Evolutionary Studies. *Biological Reviews of the Cambridge Philosophical Society* 92 (3): 1365–1388.

Matzke NJ. 2014. Model Selection in Historical Biogeography Reveals That Founder-Event Speciation Is a Crucial Process in Island Clades. *Systematic Biology* 63 (6): 951–970.

McCullough JM, Oliveros CH, Benz BW, Zenil-Ferguson R, Cracraft J, Moyle RG, Andersen MJ. 2022. Wallacean and Melanesian Islands Promote Higher Rates of Diversification within the Global Passerine Radiation Corvides. *Systematic Biology* 71 (6): 1423–1439.

Meseguer AS, Aldasoro JJ, Sanmartín I. 2013. Bayesian Inference of Phylogeny, Morphology and Range Evolution Reveals a Complex Evolutionary History in St. John's Wort (Hypericum). *Molecular Phylogenetics and Evolution* 67 (2): 379–403.

Meulenkamp JE, Sissingh W. 2003. Tertiary Palaeogeography and Tectonostratigraphic Evolution of the Northern and Southern Peri-Tethys Platforms and the Intermediate Domains of the African–Eurasian Convergent Plate Boundary Zone. *Palaeogeography, Palaeoclimatology, Palaeoecology* 196 (1-2): 209–228.

Miller, M. A., Pfeiffer, W., & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 2010 gateway computing environments workshop (GCE):1-8. Ieee.

Mitchell JS, Rabosky DL. 2017. Bayesian Model Selection with BAMM: Effects of the Model Prior on the Inferred Number of Diversification Shifts. *Methods in Ecology and Evolution / British Ecological Society* 8 (1): 37–46.

Mittermeier RA, Turner WR, Larsen FW, Brooks TM, Gascon C. 2011. Global Biodiversity Conservation: The Critical Role of Hotspots. In: Zachos FE, Habel JC, eds. Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas. Berlin, Heidelberg: Springer Berlin Heidelberg, 3–22.

Moharrek F, Sanmartín I, Kazempour-Osaloo S, Nieto Feliner G. 2019. Morphological Innovations and Vast Extensions of Mountain Habitats Triggered Rapid Diversification Within the Species-Rich Irano-Turanian Genus Acantholimon (Plumbaginaceae). *Frontiers in Genetics* 9: 698.

Moore W, Robertson JA. 2014.. Explosive Adaptive Radiation and Extreme Phenotypic Diversity within Ant-Nest Beetles. *Current Biology: CB* 24 (20): 2435–2439.

Morlon, Hélène, Eric Lewitus, Fabien L. Condamine, Marc Manceau, Julien Clavel, and Jonathan Drury. 2016. RPANDA: An R Package for Macroevolutionary Analyses on Phylogenetic Trees. *Methods in Ecology and Evolution / British Ecological Society* 7 (5): 589–97.

Morlon H, Parsons TL, Plotkin JB. 2011. Reconciling Molecular Phylogenies with the Fossil Record. *Proceedings of the National Academy of Sciences of the United States of America* 108 (39): 16327–16332.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Mouthereau F, Lacombe O, Vergés J. 2012. Building the Zagros Collisional Orogen: Timing, Strain Distribution and the Dynamics of Arabia/Eurasia Plate Convergence. *Tectonophysics* 532-535 (April): 27–60.

Naciri Y, Linder HP. 2020. The genetics of evolutionary radiations. *Biological reviews of the Cambridge Philosophical Society* 95: 1055–1072.

Nge FJ, Biffin E, Thiele KR, Waycott M. 2021. Reticulate Evolution, Ancient Chloroplast Haplotypes, and Rapid Radiation of the Australian Plant Genus Adenanthos (Proteaceae). *Frontiers in Ecology and Evolution* 8.

Noroozi J, Akhani H, Breckle S-W. 2008. Biodiversity and phytogeography of the alpine flora of Iran. *Biodiversity and conservation* 17: 493–521.

Noroozi J, Pirani A, Moazzeni H, Mahmoodi M, Zare G, Noormohammadi A, Barfuss MHJ, Suen M, Schneeweiss GM. 2020. The new locally endemic genus *Yazdana* (Caryophyllaceae) and patterns of endemism highlight the high conservation priority of the poorly studied Shirkuh Mountains (central Iran). *Journal of systematics and evolution* 58: 339–353.

Noroozi J, Talebi A, Doostmohammadi M, Rumpf SB, Linder HP, Schneeweiss GM. 2018. Hotspots within a global biodiversity hotspot - areas of endemism are associated with high mountain ranges. *Scientific reports* 8: 10345.

Noss RF, Platt WJ, Sorrie BA, Weakley AS, Means DB, Costanza J, Peet RK. 2015. How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. *Diversity & distributions* 21: 236–244.

Nürk N, Scheriau C, Madriñán S. 2013. Explosive radiation in high Andean Hypericum—rates of diversification among New World lineages. *Frontiers in genetics* 4.

Paroly G. 2015. The High-Mountain Flora and Vegetation of the Western and Central Taurus Mts. (Turkey) in the Times of Climate Change. In: Öztürk, M., Hakeem, K., Faridah-Hanum, I., Efe, R. (eds) Climate Change Impacts on High-Altitude Ecosystems. Springer, Cham.

Pearce JA, Bender JF, De Long SE, Kidd WSF, Low PJ, Güner Y, Saroglu F, Yilmaz Y, Moorbath S, Mitchell JG. 1990. Genesis of collision volcanism in Eastern Anatolia, Turkey. *Journal of Volcanology and Geothermal Research* 44: 189–229.

Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke NJ, Silvestro D, Antonelli A. 2017. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *The New phytologist* 215: 891–905.

Pirani A, Moazzeni H, Zarre S, Rabeler RK, Oxelman B, Pavlenko AV, Kovalchuk A. 2020. Phylogeny of *Acanthophyllums*.l. revisited: An update on generic concept and sectional classification. *Taxon* 69: 500–514.

Pirani A, Zarre S, Pfeil BE, Bertrand YJK, Assadi M, Oxelman B. 2014. Molecular phylogeny of *Acanthophyllum* (Caryophyllaceae: Caryophylleae), with emphasis on infrageneric classification. *Taxon* 63: 592–607.

Plummer M, Best N, Cowles K, Vines K. 2006. CODA: convergence diagnosis and output analysis for MCMC. *R News* 6: 7–11.

Popov SV, Shcherba IG, Ilyina LB, Nevesskaya LA, Paramonova NP, Khondkarian SO, Magyar I. 2006. Late Miocene to Pliocene palaeogeography of the Paratethys and its relation to the Mediterranean. *Palaeogeography, palaeoclimatology, palaeoecology* 238: 91–106.

Rabosky DL. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PloS one* 9: e89543.

Rabosky DL, Grundler M, Anderson C. 2014. BAMM tools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in ecology and evolution / British Ecological Society*.

Rabosky DL, Slater GJ, Alfaro ME. 2012. Clade age and species richness are decoupled across the eukaryotic tree of life. *PLoS biology* 10: e1001381.

Rahbek C, Borregaard MK, Antonelli A, Colwell RK, Holt BG, Nogues-Bravo D, Rasmussen CMØ,

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Richardson K, Rosing MT, Whittaker RJ, et al. 2019. Building mountain biodiversity: Geological and evolutionary processes. *Science* 365: 1114–1119.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic biology* 67: 901–904.

Ramsey, Ripley. 2013. pspline: Penalized smoothing splines. *R package version*.

Rechinger, K.H. 1988. Flora Iranica: Caryophyllaceae, Vol. 163. *Graz: Akademische Druck-U. Verlagsanstalt*.

Ree RH, Smith SA. 2008. Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. *Systematic Biology* 57 (1): 4–14.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001. Rapid Diversification of a Species-Rich Genus of Neotropical Rain Forest Trees. *Science* 293 (5538): 2242–45.

Rivera VL, Panero JL, Schilling EE, Crozier BS, Moraes MD. 2016 Origins and Recent Radiation of Brazilian Eupatorieae (Asteraceae) in the Eastern Cerrado and Atlantic Forest. *Molecular Phylogenetics and Evolution* 97 (April): 90–100.

Rögl F. 1997.Palaeogeographic Considerations for Mediterranean and Paratethys Seaways (Oligocene to Miocene). *Annalen Des Naturhistorischen Museums in Wien. Serie A. Mineralogie Und Petrographie, Geologie Und Palaeontologie, Anthropologie Und Praehistorie* 99: 279–310.

———. 1999. Mediterranean and Paratethys. Facts and Hypotheses of an Oligocene to Miocene Paleogeography (short Overview).

Ronquist F. 2004 Bayesian Inference of Character Evolution. *Trends in Ecology & Evolution* 19 (9): 475–481.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. *Bioinformatics* 19 (12): 1572–1574.

Roquet C, Boucher FC, Thuiller W, Lavergne S. 2013. Replicated Radiations of the Alpine Genus Androsace (Primulaceae) Driven by Range Expansion and Convergent Key Innovations. *Journal of Biogeography* 40 (10): 1874–1886.

Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC. 2009. Amazonian Amphibian Diversity Is Primarily Derived from Late Miocene Andean Lineages. *PLoS Biology* 7 (3): e56.

Schenk JJ. 2016. Consequences of Secondary Calibrations on Divergence Time Estimates. *PloS One* 11 (1): e0148228.

Şengör AMC, Yilmaz Y. 1981. Tethyan Evolution of Turkey: A Plate Tectonic Approach. *Tectonophysics* 75 (3): 181–241.

Şengör AMC, Görür N, Şaroğlu F. 1985. Strike-Slip Faulting and Related Basin Formation in Zones of Tectonic Escape: Turkey as a Case Study.

Şengör AMC, Kidd WSF. 1979. Post-Collisional Tectonics of the Turkish-Iranian Plateau and a Comparison with Tibet. *Tectonophysics* 55 (3): 361–376.

Simões M, Breitkreuz L, Alvarado M, Baca S, Cooper JC, Heins L, Herzog K, Lieberman BS. 2016. The Evolving Theory of Evolutionary Radiations. *Trends in Ecology & Evolution* 31 (1): 27–34.

Smíd J, Carranza S, Kratochvíl L, Gvoždík V, Nasher AK, Moravec J. 2013. Out of Arabia: A Complex Biogeographic History of Multiple Vicariance and Dispersal Events in the Gecko Genus Hemidactylus (Reptilia: Gekkonidae). *PloS One* 8 (5): e64018.

Stamatakis A. 2014. RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 30 (9): 1312–1313.

Steinbauer MJ, Field R, Grytnes J-A, Trigas P, Ah-Peng C, Attorre F, Birks HJB, Borges PAV, Cardoso P, Chou C-H, et al. 2016. Topography-Driven Isolation, Speciation and a Global Increase of Endemism with Elevation. *Global Ecology and Biogeography: A Journal of Macroecology* 25 (9): 1097–1107.

Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. 2018. Bayesian Phylogenetic and Phylodynamic Data Integration Using BEAST 1.10. *Virus Evolution* 4 (1): vey016.

Sun Y, Wang A, Wan D, Wang Q, Liu J. 2012. Rapid Radiation of Rheum (Polygonaceae) and Parallel

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Evolution of Morphological Traits. *Molecular Phylogenetics and Evolution* 63 (1): 150–158.
Swofford DL. 1993. PAUP: Phylogenetic Analysis Using Parsimony, Ver. 3.1. 1. Illinois Natural History Survey, Champaign. https://scholar.google.ca/scholar?cluster=7826873793355939830&hl=en&as_sdt=0,5&sciodt=0,5.
Takhtajan, AL, Crovello TJ, Cronquist A. 1986. *Floristic Regions of the World*. University of California Press.
Tank DC, Olmstead RG. 2008. From Annuals to Perennials: Phylogeny of Subtribe Castillejinae (Orobanchaceae). *American Journal of Botany* 95 (5): 608–625.
Thiers BM. 2023. *Index Herbariorum.* https://sweetgum.nybg.org/science/ih/
Tolley KA, Chase BM, Forest F. 2008. Speciation and Radiations Track Climate Transitions since the Miocene Climatic Optimum: A Case Study of Southern African Chameleons. *Journal of Biogeography* 35 (8): 1402–14.
Toussaint EFA, Hendrich L, Shaverdo H, Balke M. 2015. Mosaic Patterns of Diversification Dynamics Following the Colonization of Melanesian Islands. *Scientific Reports* 5 (November): 16016.
Valente LM, Savolainen V, Vargas P. 2010. Unparalleled Rates of Species Diversification in Europe. *Proceedings. Biological Sciences / The Royal Society* 277 (1687): 1489–1496.
Van Bocxlaer I, Biju SD, Loader SP, Bossuyt F. 2009. Toad Radiation Reveals into-India Dispersal as a Source of Endemism in the Western Ghats-Sri Lanka Biodiversity Hotspot. *BMC Evolutionary Biology* 9 (June): 131.
Vincent SJ, Morton AC, Carter A, Gibbs S, Barabadze TG. 2007. Oligocene Uplift of the Western Greater Caucasus: An Effect of Initial Arabia?Eurasia Collision. *Terra Nova* 19 (2): 160–66.
Weir JT. 2006. Divergent Timing and Patterns of Species Accumulation in Lowland and Highland Neotropical Birds. *Evolution; International Journal of Organic Evolution* 60 (4): 842–55.
Wen J, Zhang J-Q, Nie Z-L, Zhong Y, Sun H. 2014. Evolutionary Diversifications of Plants on the Qinghai-Tibetan Plateau. *Frontiers in Genetics* 5 (February): 4.
Wiens, John J. 2017. What Explains Patterns of Biodiversity across the Tree of Life?: New Research Is Revealing the Causes of the Dramatic Variation in Species Numbers across Branches of the Tree of Life. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 39 (3). https://doi.org/10.1002/bies.201600128.
Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ. 2005. Evolution of the New Zealand Mountain Flora: Origins, Diversification and Dispersal. *Organisms, Diversity & Evolution* 5 (3): 237–47.
Wolfe AD, Randle CP, Datwyler SL, Morawetz JJ, Arguedas N, Diaz J. 2006. Phylogeny, Taxonomic Affinities, and Biogeography of Penstemon (Plantaginaceae) Based on ITS and cpDNA Sequence Data. *American Journal of Botany* 93 (11): 1699–1713.
Wood PL Jr, Heinicke MP, Jackman TR, Bauer AM. 2012. Phylogeny of Bent-Toed Geckos (Cyrtodactylus) Reveals a West to East Pattern of Diversification. *Molecular Phylogenetics and Evolution* 65 (3): 992–1003.
Xing Y, Ree RH. 2017. Uplift-Driven Diversification in the Hengduan Mountains, a Temperate Biodiversity Hotspot. *Proceedings of the National Academy of Sciences of the United States of America* 114 (17): E3444–E3451.
Xue B, Song Z, Cai J, Ma Z, Huang J, Li , and Yao G. 2023. Phylogenetic analysis and temporal diversification of the tribe Alsineae (Caryophyllaceae) with the description of three new genera, Hesperostellaria, Reniostellaria and Torreyostellaria. Front. Plant Sci. 14:1127443.
Yamada T, Sugiyama T, Tamaki N, Kawakita A, Kato M. 2009. Adaptive radiation of gobies in the interstitial habitats of gravel beaches accompanied by body elongation and excessive vertebral segmentation. *BMC evolutionary biology* 9: 145.
Ye X-Y, Ma P-F, Yang G-Q, Guo C, Zhang Y-X, Chen Y-M, Guo Z-H, Li D-Z. 2019. Rapid diversification of alpine bamboos associated with the uplift of the Hengduan Mountains. *Journal of biogeography* 46: 2678–2689.
Yin A. 2010. Cenozoic tectonic evolution of Asia: A preliminary synthesis. *Tectonophysics* 488: 293–

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.24.568494; this version posted November 25, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

325.
Yu Y, Blair C, He X. 2020. RASP 4: Ancestral State Reconstruction Tool for Multiple Genes and Characters. *Molecular biology and evolution* 37: 604–606.
Yu Y, Harris AJ, Blair C, He X. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular phylogenetics and evolution* 87: 46–49.
Zachos JC, Dickens GR, Zeebe RE. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* 451: 279–283.
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.
Zazanashvili N, Sanadiradze G, Bukhnikashvili A, Kandaurov A, Tarkhnishvili D, Mittermaier RA, Gil PG, Hoffmann M, Pilgrim J, Brooks T, Mittermaier CG. 2004. Hotspots revisited, Earth's biologically richest and most endangered terrestrial ecoregions. 148-153
Zazanashvili, N., 2009. The Caucasus Hotspot. Status and protection of globally threatened species in the Caucasus. 15.
Zhang J-Q, Meng S-Y, Allen GA, Wen J, Rao G-Y. 2014. Rapid radiation and dispersal out of the Qinghai-Tibetan Plateau of an alpine plant lineage Rhodiola (Crassulaceae). *Molecular phylogenetics and evolution* 77: 147–158.
Zografidis A, Fragman-Sapir O, Strid A, Dimopoulos P. 2020. Notes on the generic name Graecobolanthus (Caryophylleae, Caryophyllaceae). *Taxon* 69: 992–997.
Zohary M. 1973. Geobotanical foundations of the Middle East. Fischer.