

1 **Insights into the drivers of radiating diversification in biodiversity**  
2 **hotspots using *Saussurea* (Asteraceae) as a case**

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4 Xu Zhang<sup>1,2,3\*</sup>, Jacob B. Landis<sup>4,5\*</sup>, Yanxia Sun<sup>1,2</sup>, Huajie Zhang<sup>1</sup>, Tao Feng<sup>1</sup>, Nan Lin<sup>1</sup>, Bashir B.  
5 Tihamiyu<sup>1</sup>, Xianhan Huang<sup>6</sup>, Tao Deng<sup>6</sup>, Hengchang Wang<sup>1,2</sup>, Hang Sun<sup>6</sup>

6 <sup>1</sup>CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical  
7 Garden, Chinese Academy of Sciences, Wuhan 430074, Hubei, China;

8 <sup>2</sup>Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Wuhan  
9 430074, Hubei, China;

10 <sup>3</sup>University of Chinese Academy of Sciences, Beijing, 100049 China;

11 <sup>4</sup> School of Integrative Plant Science, Section of Plant Biology and the L.H. Bailey Hortorium, Cornell  
12 University, Ithaca, NY 14850, USA;

13 <sup>5</sup> BTI Computational Biology Center, Boyce Thompson Institute, Ithaca, NY 14853, USA;

14 <sup>6</sup> Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany,  
15 Chinese Academy of Sciences, Kunming, Yunnan, 650201 China

16 \*These authors contributed equally to this work.

17

18 Authors for correspondence:

19 Tao Deng

20 Email: [dengtao@mail.kib.ac.cn](mailto:dengtao@mail.kib.ac.cn)

21

22 Hengchang Wang

23 Email: [hcwang@wbgcas.cn](mailto:hcwang@wbgcas.cn)

24

25 Hang Sun

26 Email: [sunhang@mail.kib.ac.cn](mailto:sunhang@mail.kib.ac.cn)

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## 30 **Abstract**

31 • The Qinghai-Tibet Plateau (QTP) encompasses areas with a remarkably high degree  
32 of biodiversity, harboring exceptional species-rich radiations. How these radiations  
33 formed by interacting with geology, climate and ecology remains seldom examined.

34 • We investigate the roles of abiotic (environmental) and biotic (species-intrinsic)  
35 factors in driving radiating diversification of *Saussurea* (Asteraceae) by deploying a  
36 number of time-dependent, paleoenvironment-dependent and trait-dependent models, as  
37 well as ecological distribution data.

38 • We show that three main clades of *Saussurea* begin to diversify in the Miocene  
39 almost simultaneously, with increasing diversification rates toward the present and  
40 negative dependence to paleotemperature. Acceleration in diversification rates are  
41 correlated with adaptive traits, as well climate lability, niche breadth and species range.

42 • We conclude that fluctuation of paleoclimate along with complex QTP environments  
43 provided opportunities for increased diversification rates of *Saussurea* with diverse  
44 adaptive traits, highlighting the importance of combinations of clade-specific traits and  
45 ecological niches in driving rapid radiation.

46 **Key words:** radiating diversification, *Saussurea*, the Qinghai-Tibet Plateau, biodiversity  
47 hotspots, adaptive traits, diversification rates, ecological niche.

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## 49 **Introduction:**

50 The diversification pattern of species-rich rapid radiations reflects the evolutionary  
51 dynamics of biodiversity hotspots (Linder & Verboom, 2015). Understanding how these  
52 radiating lineages formed in response to historical process can advance our knowledge of  
53 adaptive evolution and enhance our ability to predict the threats to biodiversity posed by  
54 global warming (Ding *et al.*, 2020). Mountainous regions represent just one-eighth of  
55 terrestrial land surface but are home to one-third of all species and exceptional species-  
56 rich radiations (Antonelli, 2015; Schwery *et al.*, 2015; Antonelli *et al.*, 2018). Particularly  
57 enigmatic is the Qinghai-Tibet Plateau (QTP) region, also known as the “Third Pole,”  
58 characterized by a complex geographical history and encompassing areas of remarkably  
59 high degree of biodiversity (Favre *et al.*, 2015; Xing & Ree, 2017; Chen *et al.*, 2018;  
60 Ding *et al.*, 2020; Spicer *et al.*, 2020). The QTP stands out as the earth’s highest and  
61 largest plateau, and includes the Himalaya and Hengduan Mountains which are listed as  
62 two of the 36 hotspots of biodiversity in the world (Myers *et al.*, 2000; Li *et al.*, 2014;  
63 Wen *et al.*, 2014; Favre *et al.*, 2015). The presence of steep environmental gradients in  
64 temperature and precipitation create abundant micro-habitats providing a variety of  
65 ecological niches essential for evolutionary radiations on the QTP (Mosbrugger *et al.*,  
66 2018; Muellner-Riehl *et al.*, 2019). While a plethora of studies have suggested that  
67 diversification of plants on the QTP have evolved in association with plateau uplifting  
68 processes (reviewed by Wen *et al.*, 2014), how such high species diversity form in such a  
69 short period of geologic time, and the interactions with geography, climate and ecology,  
70 remain seldom examined.

71 Evolutionary and diversification patterns of plants are often correlated with  
72 environmental abiotic forces, such as abrupt changes in climate or geological tectonic  
73 events that drive speciation and extinction rates, and/or species-intrinsic/biotic factors,

74 such as interactions among species and key innovation traits (Drummond *et al.*, 2012;  
75 Hughes & Atchison, 2015; Condamine *et al.*, 2018; Muellner-Riehl *et al.*, 2019; Nürk *et*  
76 *al.*, 2019). There is a gap in our current understanding of radiating diversification drivers  
77 in the flora of the QTP, with previous studies mostly providing only a temporal  
78 (molecular dating) framework associating rapid radiations with the time span of plateau  
79 uplifting (e.g. Wang *et al.*, 2009; Zhang *et al.*, 2014; Xu *et al.*, 2019). Employing models  
80 assuming continuous variation in diversification rates over time that depend on  
81 paleoenvironmental variables is essential to precisely determine how diversification rates  
82 are affected by abiotic environmental changes (Condamine *et al.*, 2013; Sun *et al.*, 2020).  
83 In addition to abiotic factors, diversification shifts are often correlated with the evolution  
84 of certain functional traits (Hughes & Atchison, 2015). Examples include geophytism in  
85 monocots leading to higher rates of diversification (Howard *et al.*, 2020),  
86 polyploidization promoting species diversification of *Allium* (Han *et al.*, 2020), and  
87 pollinator shifts, fruit types as well as elevational changes in the Andean bellflowers  
88 (Lagomarsino *et al.*, 2016). Furthermore, the inclusion of ecological niche data is also  
89 crucial, because this reflects the interplay between historical processes and species  
90 intrinsic factors (Lavergne *et al.*, 2010; Folk *et al.*, 2019; Muellner-Riehl *et al.*, 2019).

91 Here, we address the knowledge gap of rapid diversification by examining the roles  
92 of abiotic (environmental) and biotic (species-intrinsic) factors in driving radiating  
93 diversification of the species-rich genus *Saussurea* DC. (Asteraceae). *Saussurea* is one of  
94 the most diverse genera in Asteraceae, serving as an ideal study system for investigating  
95 the evolutionary patterns of a rapid radiation. The genus comprises approximately 400  
96 species that are distributed in Asia, Europe and North America, with the highest diversity  
97 in the QTP (Wang *et al.*, 2009; Shi & Raab-Straube, 2011; Chen, 2015; Zhang, *et al.*,  
98 2019a). Uncertainty in the number of species has largely been attributed to the complex  
99 taxonomy of related QTP taxa (Chen & Yuan, 2015), indicative of a recent radiation.

100 *Saussurea* exhibits extraordinary morphological diversity. For example, the most  
101 impressive species groups are the ‘snowball plants’ or ‘snow rabbits’, *S.* subg.  
102 *Eriocoryne*, with a thick woolly indumentum (densely haired), and the so-called  
103 ‘greenhouse plants’ or ‘snow lotuses’, *S.* subg. *Amphilaena*, in which the synflorescence  
104 is hidden by semi-transparent, white, yellowish or purple leafy bracts (Shi & Raab-  
105 Straube, 2011; Chen, 2015). *Saussurea* is present in virtually all possible habitats of the  
106 QTP, including steppes, moist forests, cold and dry alpine meadows, and scree slopes  
107 above 5,000 m, demonstrating a highly adaptive capability (Shi & Raab-Straube, 2011).  
108 Previous studies suggested that attractive morphological traits were the result of  
109 convergent adaptation to diverse environments in the QTP (Kita *et al.*, 2004; Wang *et al.*,  
110 2009; Zhang, *et al.*, 2019a), yet their contributions to the high-level diversity of  
111 *Saussurea* are still elusive. While biogeographic analysis inferred that *Saussurea* arose  
112 during the Miocene in the Hengduan Mountains (Xu *et al.*, 2019), limited information  
113 about macro-evolutionary patterns related to historical climate and geologic processes  
114 were provided due to the lack of modeling diversification rates.

115 A robust phylogenetic framework is the basis for large-scale analyses of evolutionary  
116 patterns (Koenen *et al.*, 2020), yet previous studies mainly relied on fragment DNA  
117 markers (e.g. Han *et al.*, 2020; Howard *et al.*, 2020; Sun *et al.*, 2020), which have been  
118 revealed to provide insufficient phylogenetic signals and always yield parallel  
119 relationships for phylogenies of rapid radiations (Whitfield & Lockhart, 2007; Wang *et*  
120 *al.*, 2009). In the present study, we reconstructed a robust time-calibrated phylogeny of  
121 *Saussurea* using 226 complete plastomes to explore the role played by abiotic and biotic  
122 factors in this rapidly radiating clade. If evolutionary dynamics are driven primarily by  
123 abrupt abiotic perturbations, we would expect diversification rate shifts following major  
124 climate changes that extirpated certain lineages while favoring the radiation of others. In  
125 contrast, if biotic factors or interactions among species are the dominant drivers of

126 evolution, we would expect diversification shifts to be correlated with the evolution of  
127 functional traits and/or the colonization of new habitats (Condamine *et al.*, 2018). While  
128 in a joint-effect scenario, diversification rates may vary continuously through time and  
129 paleoenvironments may shift with some clade-specific traits. We could hypothesize that  
130 fluctuations of terrestrial and climatic systems provide vast ecological opportunities,  
131 which are seized by lineages with ample adaptive traits and promote rapid radiating,  
132 emphasizing the decisive role of morphological diversity/plasticity and ecological niche  
133 availability. To test these hypotheses, we deployed a number of time-dependent,  
134 paleoenvironment-dependent and trait-dependent models, as well as ecological  
135 distribution data. Our study is designed to address the effects of paleoenvironmental and  
136 biological drivers on radiating diversification in the biodiversity hotspots, while  
137 providing a compelling example of the pivotal roles of morphological diversity and  
138 ecological niche.

## 139 **Materials and Methods**

### 140 **Plastome Sampling, Sequencing and Assembly**

141 To build a dated phylogeny of the genus *Saussurea*, we newly sequenced plastomes for  
142 63 species and downloaded 163 additional plastomes from GenBank (accessed 29  
143 November 2019); collectively these species included 199 taxa of *Saussurea* and 27  
144 outgroup taxa. Collection details of the specimens were provided in Supporting  
145 Information Table S1. Total genomic DNA of was extracted from silica-gel dried leaves  
146 with a modified hexadecyltrimethylammonium bromide (CTAB) method (Yang *et al.*,  
147 2014). Purified DNA was fragmented and used to construct short-insert (500 bp) libraries  
148 per the manufacturer's instructions (Illumina). Libraries were quantified using an Agilent  
149 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and were then  
150 sequenced on an Illumina HiSeq 4000 platform at Novogene Co., Ltd. in Kunming,

151 Yunnan, China. Raw reads were directly assembled with the organellar assembler  
152 NOVOPLASTY v.2.7.2 (Dierckxsens *et al.*, 2017), using a seed-and-extend algorithm  
153 employing the plastome sequence of *Saussurea japonica* (Genbank accession:  
154 MH926107.1) as the seed input, and all other parameters kept at default settings.  
155 Assembled plastome sequences were initially annotated using Plastid Genome Annotator  
156 (PGA) (Qu *et al.*, 2019), and then manually checked in GENEIOUS v.9.0.5 (Kearse *et*  
157 *al.*, 2012).

### 158 **Estimates of Divergence Times**

159 Our prior study suggested that including noncoding regions in phylogenetic analysis can  
160 maximize the power to resolve relationships of *Saussurea* (Zhang, *et al.*, 2019a). Whole  
161 plastome sequences of 226 samples containing one inverted repeat region were aligned  
162 using MAFFT v.7.22 (Kato & Standley, 2013). Poorly aligned regions were removed  
163 with TRIMAL v.1.2 (Capella-Gutiérrez *et al.*, 2009) using the command ‘-automated1’.  
164 Age estimates were obtained using Markov Chain Monte Carlo (MCMC) analysis in  
165 BEAST v.1.10.4 (Suchard *et al.*, 2018). We used a GTR + I +  $\Gamma$  nucleotide substitution  
166 model, uncorrelated relaxed lognormal clock and a birth-death model for the tree prior  
167 (Suchard *et al.*, 2018). The MCMC analysis was run for 500 million generations,  
168 sampling every 10,000 generations, resulting in 50,000 samples in the posterior  
169 distribution of which the first 10,000 samples were discarded as burn-in. Convergence  
170 and performance of the MCMC runs were checked using TRACER v.1.6 (Rambaut *et al.*,  
171 2018). A maximum clade credibility (MCC) tree was then reconstructed in  
172 TREEANNOTATOR v.1.8.4 (Rambaut & Drummond, 2010), with median age and 95%  
173 height posterior density (HPD) annotated. Two high confident fossil calibrations with  
174 lognormal distributions were assigned: (A) The crown age of *Carduus-Cirsium* group  
175 was set to a minimum age of 14 million years ago (Mya) based on the Middle Miocene  
176 achenes identified as *Cirsium* (Mai, 1995; Barres *et al.*, 2013); (B) The split of *Centaurea*

177 and *Carthamus* was calibrated with a minimum age of 5 Mya, based on the records of  
178 pollen and achenes for *Centaurea* dating from the Early Pliocene (Popescu, 2002).  
179 Additionally, the crown age of Cardueae was set to 39.2 Mya as a secondary calibration  
180 with a normal distribution based on the estimation by Barres *et al.* (2013).

### 181 **Estimates of Diversification rate**

182 We explored the diversification dynamics of *Saussurea* using BAMM 2.5.0 (Rabosky,  
183 2014), which employs a reversible-jump MCMC to sample a large number of possible  
184 diversification regimes from a given time-calibrated phylogeny. We pruned the MCC tree  
185 for BAMM analysis and retained only one sample of each species. Prior values were  
186 selected using the ‘setBAMMpriors’ function in the R package BAMMtools v.2.1.7 (R  
187 Core Team, 2014; Rabosky *et al.*, 2014). Due to the controversial species number in  
188 *Saussurea*, the incomplete taxon sampling was appropriately set as 0.5 for all following  
189 analyses. The MCMC was run for 500 million generations and sampled every 50,000  
190 generations. Post-run analyses were performed using the BAMMtools, with an initial  
191 10% of the MCMC run discarded as burn-in, and the remaining data assessed for  
192 convergence and ESS values > 200. Rates-through-time plots were generated using  
193 ‘PlotRateThroughTime’ function for the entire genus as well as three clades. Speciation  
194 rates of *Saussurea* species were obtained using the ‘getTipRates’ function. Considering  
195 recent criticism relating to the statistical methods for lineage specific diversification  
196 models like BAMM (Moore *et al.*, 2016; but also see Rabosky *et al.*, 2017), we also  
197 employed the semiparametric DR statistic to calculate speciation rates, following the  
198 method described in Jetz *et al.* (2012) and Sun *et al.* (2020). Analysis of variance  
199 (ANOVA) was performed to determine whether differences among three phylogenetic  
200 clades and among four traditional subgenera were significant. In addition, we used TESS  
201 v.2.1 (Höhna *et al.*, 2016) in R to detect the abrupt changes in speciation and extinction  
202 rates, applying the R-scripts of Condamine *et al.* (2018).



## 203 **Paleoenvironment dependent analyses**

204 To quantify the effects of past environmental conditions on *Saussurea* diversification, we  
205 used RPANDA v1.9 (Condamine *et al.*, 2013) to fit a series of time- and temperature-  
206 dependent likelihood diversification birth-death (BD) models, following the methodology  
207 of Condamine *et al.* (2018). Briefly, seven models were tested: BD model with constant  $\lambda$   
208 (speciation rate) and  $\mu$  (extinction rate) (i); BD model with  $\lambda$  dependent to time (ii) and  
209 environment (iii) exponentially, and constant  $\mu$ ; BD model with constant  $\lambda$ , and  $\mu$   
210 dependent to time (iv) and environment (v) exponentially; and BD model with  $\lambda$  and  $\mu$   
211 dependent to time (vi) and environment (vii) exponentially. Thus, we can obtain the  
212 equations:  $\lambda(E) = \lambda_0 \times e^{\alpha E}$  and  $\mu(E) = \mu_0 \times e^{\beta E}$ , in which  $\lambda_0$  and  $\mu_0$  are the speciation and  
213 extinction rates for a given environmental variable. The values of  $\alpha$  and  $\beta$  are the rates of  
214 change according to the environment, and positive values for them mean a positive effect  
215 of the environment on speciation or extinction (Condamine *et al.*, 2013). We used  
216 paleotemperature over the last 12 million years (Myrs) (retrieved from Zachos *et al.*,  
217 2008) as environmental variables, and randomly sampled 500 trees from the BEAST  
218 posterior distribution (outgroups removed) to accommodate phylogenetic and dating  
219 uncertainties. The R package PSPLINE v.1.0 (Ben & Roberto, 2008) was used to  
220 visualize the speciation rates varying with paleoenvironmental variables.

## 221 **Trait dependent analyses**

222 Nine characters were selected and coded based on descriptions in eFloras  
223 (<http://www.efloras.org/>), herbarium specimens and taxonomic literature, or were  
224 manually checked directly using online herbarium specimens from the Chinese Virtual  
225 Herbarium (<http://www.cvh.ac.cn/>), JSTOR (<https://plants.jstor.org/>), and field collections  
226 (Supporting Information Table S2). These characters included four binary morphological  
227 traits: stemless (0) vs. cauliferous (1), stem glabrous (0) vs. densely haired (1), the

228 absence (0) vs. presence (1) of leafy bracts, and capitula solitary (0) vs. numerous (1);  
229 four multistate morphological traits: leaf margin entire (1) vs. pinnately lobed (2) vs. both  
230 types (3), leaves glabrous (1) vs. sparsely haired (2) vs. densely haired (3), phyllary in <5  
231 (1) vs. 5 (2) vs. 6 (3) vs. >6 (4) rows, and phyllary glabrous (1) vs. sparsely haired (2) vs.  
232 densely haired (3) vs. appendage (4); as well as the geographical habitats: widespread (0)  
233 vs. alpine (1) vs. lowland (2).

234 The diversification rate shifts of binary traits were investigated using the hidden state  
235 speciation and extinction (HiSSE) model, which allows us to demonstrate hypotheses  
236 related to both the effects of the observed traits as well as incorporate unmeasured factors  
237 (Beaulieu & O'Meara, 2016). As described in Beaulieu and O'Meara (2016), 25 models  
238 were tested in the R package HISSE v.1.9.10: a full HiSSE model allowing all states to  
239 vary independently; four binary state speciation and extinction (BiSSE)-like models that  
240 excluded hidden states or constrained specific parameters of  $\lambda$ ,  $\mu$ , and transition rates ( $q$ );  
241 four null HiSSE models with various character-independent diversification (CID) forms;  
242 and 16 models assuming a hidden state associated with both observed character states  
243 with a variety of constrained values for  $\lambda$ ,  $\mu$ , and  $q$  (Supporting Information Table S3).  
244 The best-fitting model was selected based on likelihood-ratio tests under a Chi-square  
245 distribution and Akaike's information criterion (AIC) (Akaike, 1974). We also used a  
246 nonparametric FiSSE model (Fast, intuitive SSE model; Rabosky & Goldberg, 2017)  
247 serving as a complement to measure the robustness of our results. For multistate traits,  
248 MuSSE analyses were performed in the R package DIVERSITREE v.0.9.10 (FitzJohn,  
249 2012) by fitting four distinct models with subsequent ANOVA testing: a null model with  
250 fully constrained variables; a full model allowing all variables to change independently; a  
251 model constraining each  $\mu$  to be equal (free  $\lambda$ ); and a model constraining the  $\lambda$  values for  
252 each state to be equal (free  $\mu$ ). Further estimates for the parameters of  $\lambda$ ,  $\mu$ , and net  
253 diversification rates ( $\lambda - \mu$ ) for each state were obtained in a Bayesian approach by

254 incorporating a MCMC analysis with an exponential prior with 5,000 generations. A  
255 GeoHiSSE analysis was used to test hypotheses about range-dependent diversification  
256 processes (Caetano *et al.*, 2018), implemented in the HiSSE package. Two models with a  
257 range-independent diversification process and two other models in which the range have  
258 an effect on the diversification rate of the lineages were deployed and compared, as  
259 described in Caetano *et al.* (2018).

## 260 **Ecological distribution data and association with diversification rates**

261 We used the ‘occ\_search’ function of RGBIF v.1.3.0 (Chamberlain & Boettiger, 2017) to  
262 retrieve GPS coordinates for *Saussurea* species from GBIF on October 28, 2020. We  
263 extracted only data records that were georeferenced and excluded any coordinates with  
264 zero and/or integer latitude and longitude. We then removed geographic outliers defined  
265 as boxplot outliers of species occurrences in R. Range size of each species was estimated  
266 by applying a five kilometer buffer around each locality point using the ‘gBuffer’  
267 function of RGEOS v.0.5-5 (<https://CRAN.R-project.org/package=rgeos>) following the  
268 descriptions of Testo *et al.* (2019). Range size data were log-transformed before analysis  
269 to overcome their skewed distribution (Testo *et al.*, 2019). We extracted the values of 19  
270 bioclimatic variables (from 1970 to 2000) from WorldClim (<http://worldclim.org>) using  
271 RASTER v.2.6-6 (<https://CRAN.R-project.org/package=raster>), and calculated a mean  
272 value for each species. Highly correlated variables were identified with a Pearson’s  
273 correlation coefficient  $> 0.75$ , and were removed. The remaining eight most predicative  
274 bioclimatic variables were: annual temperature (BIO1), mean diurnal range (BIO2),  
275 isothermality (BIO3), max temperature of warmest month (BIO5), annual precipitation  
276 (BIO12), precipitation seasonality (BIO15), precipitation of warmest quarter (BIO18) and  
277 precipitation of coldest quarter (BIO19). The main variation of bioclimatic variables  
278 representing climate lability was estimated by extracting the first two principal  
279 components (PC1 and PC2) from a PCA in R. To calculate the ecological niche breadth,

280 we first estimated environmental niche models (ENM) in the R package ENMTOOLS  
281 v.1.0.2 (Warren *et al.*, 2010), and then measured the spatial heterogeneity of the  
282 distribution of suitability scores using Levins' B metrics (Levins, 1968) ('raster.breadth'  
283 function).

284 To demonstrate whether ecological factors drove rapid diversification of *Saussurea*  
285 species, multiple QuaSSE tests were performed under different models using  
286 DIVERSITREE. Five models with increasing complexity were constructed to fit the  
287 changes in speciation rates with climate lability (PCs of bioclimatic variables), niche  
288 breadth and species range size. Moreover, we used the *ES-sim* tests (Harvey & Rabosky,  
289 2018) to crosscheck the correlation pattern revealed by QuaSSE. In addition to the default  
290 inverse equal splits statistic (Harvey & Rabosky, 2018), the DR statistic was also used as  
291 a reliable estimator to investigate correlation between speciation rate and continuous  
292 ecological factors using the R-scripts retrieved from Sun *et al.* (2020).

### 293 **Data availability**

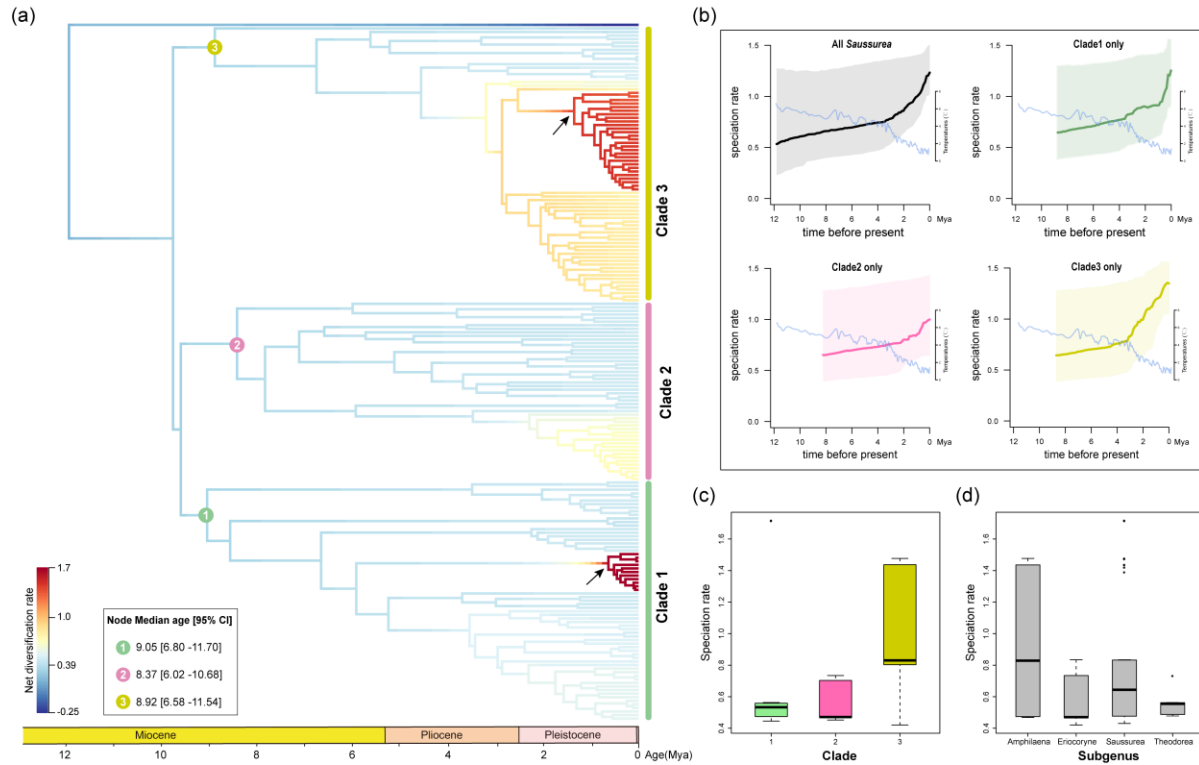
294 All newly sequenced plastomes were deposited in the National Center for Biotechnology  
295 Information (NCBI) database with accession numbers provided in Supporting  
296 Information Table S1. R scripts used in this study are available on GitHub  
297 (<https://github.com/ZhangXu-CAS/Saussurea-diversification.git>).

## 298 **Results**

### 299 **Divergence time and diversification rate**

300 Our phylogeny resolved a median stem age of ca. 11.79 Mya (95% HPD, 8.38–15.35  
301 Mya) for *Saussurea*, with three clades beginning to diversify in parallel during the  
302 Miocene (ca. 9.05 Mya, ca. 8.37 Mya and ca. 8.92 Mya, respectively; Figs 1a, Supporting  
303 Information Figs S1, S2), suggesting a rapid radiation in this period. Our tree topology  
304 showed that four traditional morphology-based subgenera of *Saussurea* are paraphyletic,  
305 indicating adaptive traits have occurred multiple times. BAMM analysis revealed a  
306 scenario in which two shifts in net diversification rates occurred within *Saussurea* with  
307 high posterior probability (Figs 1a, Supporting Information Figs S3). Rates-through-time  
308 plots showed that while slightly offset in timing, diversification rates of the three clades  
309 accelerated during the Pliocene (Figs 1a, 1b), when the temperature dropped sharply.  
310 BAMM tip rates showed that clade-3 (0.981 events Myr<sup>-1</sup> per lineage) had significantly  
311 higher mean speciation rate than clade-2 (0.560 events Myr<sup>-1</sup> per lineage) and clade-1  
312 (0.708 events Myr<sup>-1</sup> per lineage) ( $p < 0.001$ , Supporting Information Tables S4, S5).  
313 Among four morphological-based subgenera, speciation rates of *S. subg. Amphilaena*  
314 (0.945 events Myr<sup>-1</sup> per lineage) was highest ( $p < 0.001$ , Fig 1c, Supporting Information  
315 Tables S4, S5). While DR statistic revealed no significant difference among three main  
316 clades ( $p = 0.099$ ), and *S. subg. Saussurea* (1.106 events Myr<sup>-1</sup> per lineage) have the  
317 highest mean speciation rate ( $p = 0.022$ , Supporting Information Fig S4, Tables S4, S6).  
318 TESS analysis suggested that speciation and extinction shifts had higher posterior  
319 probability during the Pleistocene, consistent with the BAMM results (Supporting  
320 Information Fig S5).

321

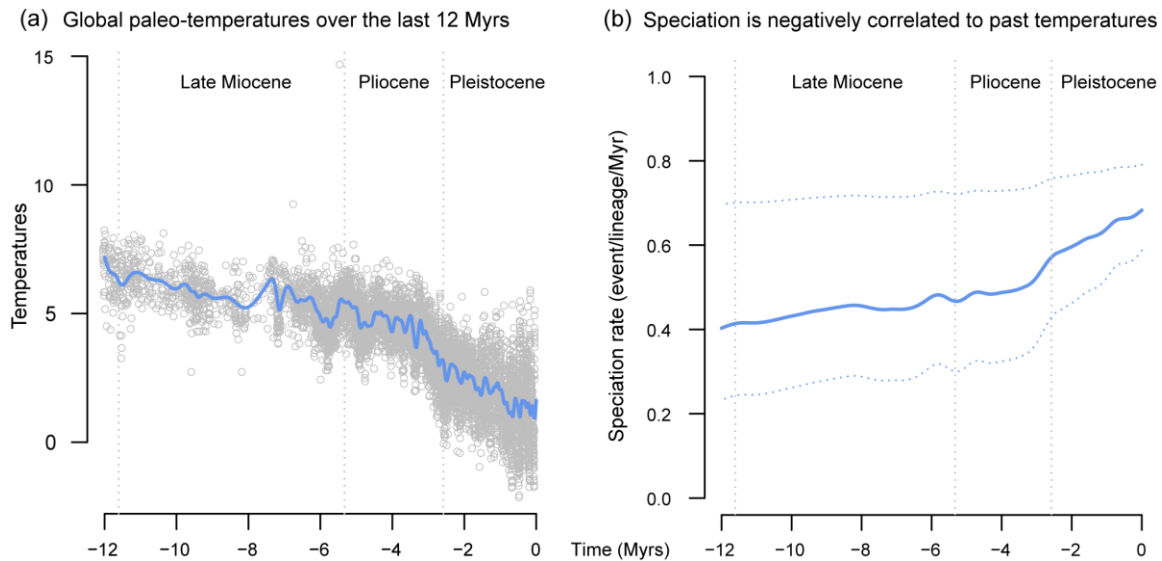


322 **Fig. 1** Diversification dynamics of *Saussurea* inferred from BMM analysis. (a) BMM identified  
 323 two shifts in diversification rates (represented by arrows). The time of three clades beginning to  
 324 diversify is provided. (b) Rates-through-time plots of all *Saussurea* species and three main clades  
 325 separately, with trends in global climate change over 12 million years (Zachos *et al.* 2008) depicted.  
 326 (c-d) BMM tip rates of three clades and four morphology-based subgenera of *Saussurea*,  
 327 respectively.

### 328 **Paleoenvironment dependent diversification**

329 We used a maximum-likelihood framework to illustrate diversification dynamics  
 330 dependent to paleoenvironment based on BD models to gain insight into the role of  
 331 historical processes on diversification. Out of seven models, a model with temperature-  
 332 dependent speciation fit the data best (Table 1). The best-fit model further indicated a  
 333 negative dependence ( $\alpha < 0$ ) between past temperature and speciation rate for *Saussurea*,  
 334 while extinction rate remained constant, suggesting extinction was likely not affected by  
 335 temperature fluctuations. RPANDA results demonstrated a diversification regime in  
 336 which diversification rates had opposite responses to changes of temperature over time,

337 and accelerated sharply in the Pleistocene and increased toward the present (Fig. 2),  
338 consistent with the conclusion of rates-through-time in BAMM analysis (Fig. 1b).

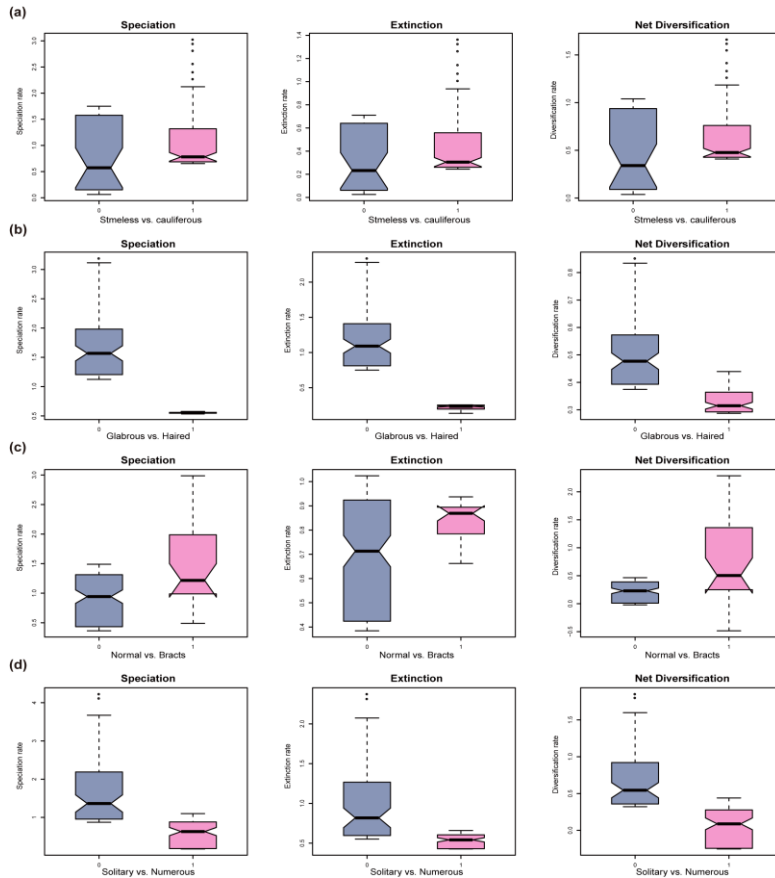


339 **Fig. 2** Paleoenvironment-dependent diversification processes in *Saussurea*. The best-fit  
340 paleoenvironment-dependent model implemented in RPANDA shows negative dependence  
341 between paleotemperatures (a) and speciation rate (b).

### 342 **Trait dependent diversification**

343 We investigated eight morphological characters and geographical habitat that serve as a  
344 proxy for the effect of adaptive traits on diversification rate, to understand the role of trait  
345 innovations in the rapid radiation of *Saussurea*. For all four binary traits, the best model  
346 of the 25 models tested was the full HiSSE model with unique speciation, extinction and  
347 transition rates between the two character states observed and the hidden states  
348 (Supporting Information Tables S7). We then calculated mean speciation, extinction and  
349 net diversification rates values from the model-averaged marginal ancestral state  
350 reconstruction for each extant species in our tree. The results suggested that species with  
351 cauliferous plant, glabrous stem, leafy bracts and solitary capitula have higher mean  
352 speciation, extinction and net diversification (Table 2, Fig. 3). While the full HiSSE  
353 model showed observed differences in diversification rates between the states of these

354 traits, it also indicated some unobserved traits drive the diversification. The  
 355 complementary results from our FiSSE analysis supported the tendency of speciation rate  
 356 revealed by HiSSE, but the only significant differences were between solitary capitula  
 357 and numerous capitula (Table 2;  $p = 0.024$ ).

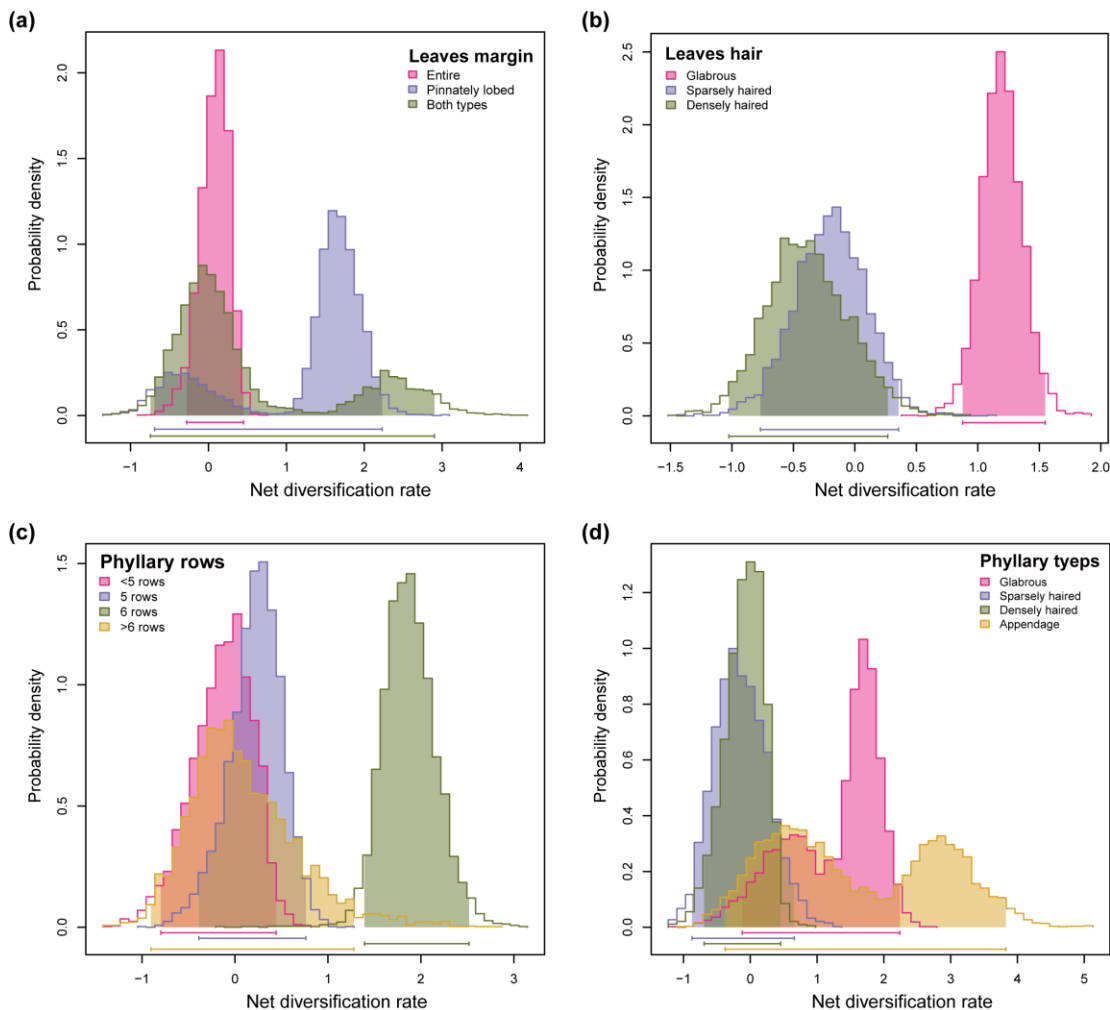


**Fig. 3** Binary trait dependent diversification of *Saussurea* inferred from HiSSE analysis. Speciation, extinction and net diversification rates are calculated by the model-averaged marginal ancestral state reconstruction for four binary traits: (a) stemless (0) vs. cauliferous (1), (b) stem glabrous (0) vs. densely haired (1), (c) the absence (0) vs. presence (1) of leafy bracts, and (d) capitula solitary (0) vs. numerous (1).

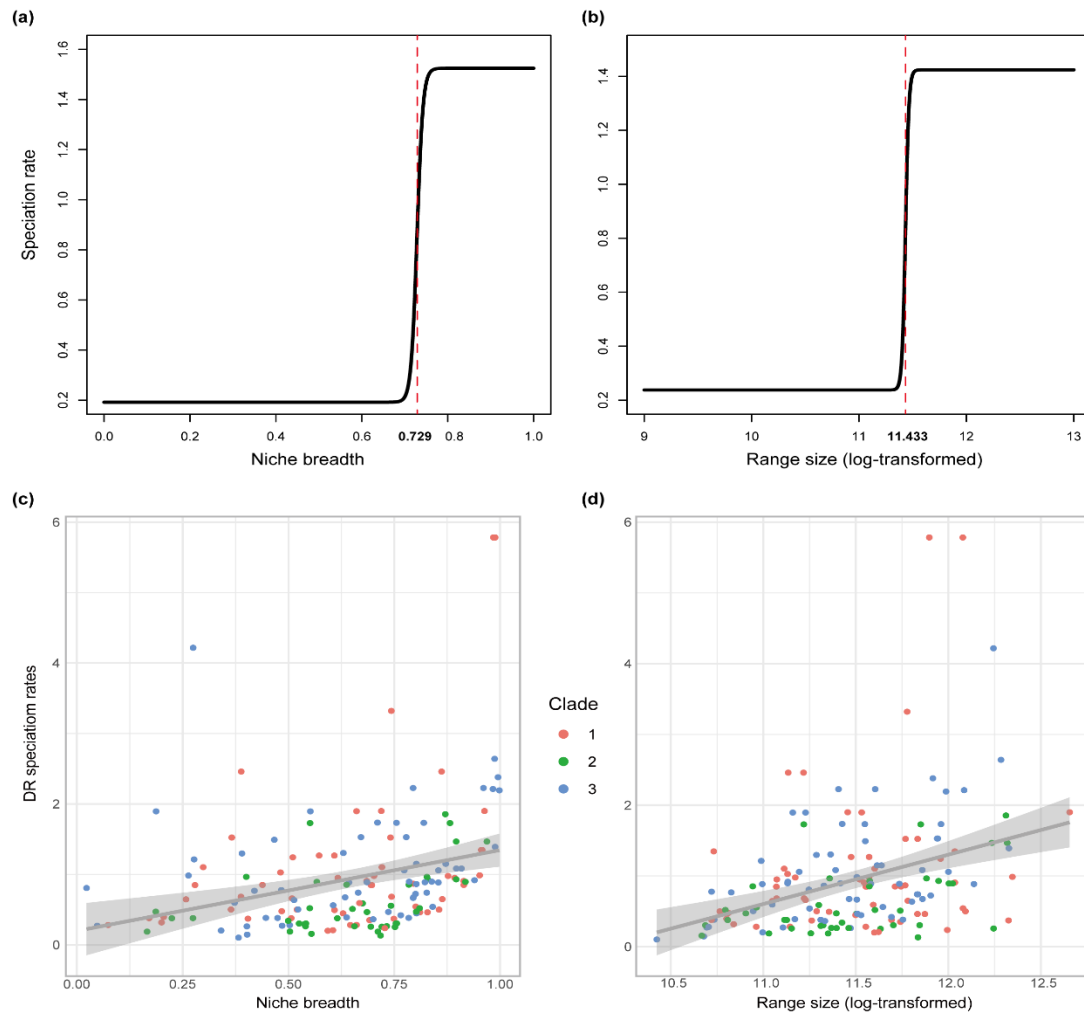
374 In the MuSSE analyses, ANOVA calculations all preferred models constraining each  
 375  $\mu$  to be equal and allowing  $\lambda$  to vary (free  $\lambda$ ), compared with either null models and full  
 376 models (Supporting Information Table S8). The best-fitting model was then used as the  
 377 starting point for a MCMC run of 5,000 generations to estimate the marginal distributions  
 378 of rates for each traits using a state-dependent model (Fig. 4). Since all the models  
 379 preferred constrained  $\mu$  values, all of the estimated probability densities of  $\mu$  overlapped  
 380 (data not shown). The reconstructions of probability density of the net diversification  
 381 rates ( $\lambda - \mu$ ) showed that some traits, i.e. leaf margin and phyllary types, have an overlap



382 in net diversification rates among examined character states (Fig. 4a, d). The results  
383 suggest that species with glabrous leaves have higher net diversification rates than  
384 sparsely or densely haired species, consistent with higher mean rates for glabrous stem in  
385 the HiSSE analysis. For the phyllary character, the glabrous state also showed higher net  
386 diversification rates than sparsely or densely haired states despite some overlapping (Fig.  
387 4d), and the rates of phyllary with six rows are higher than the remaining character states  
388 (Fig. 4c). The GeoHiSSE analysis suggested a model with one hidden area and no range-  
389 dependent diversification was the best fitting model (Supporting Information Table S9).  
390 From the result, we can see that species with lowland habitats have a substantially higher  
391 speciation, extinction and net diversification rates in comparison with both alpine and  
392 widespread distributions (Supporting Information Fig. S6).



393 **Fig. 4** Multistate trait dependent diversification of *Saussurea* estimated from MuSSE analysis.  
394 Marginal distributions of net diversification rates are estimated by the MCMC run of 5, 000  
395 generations for four multistate traits: (a) leaves margin entire (1) vs. pinnately lobed (2) vs. both types  
396 (3), (b) leaves glabrous (1) vs. sparsely haired (2) vs. densely haired (3), (c) phyllary in <5 (1) vs. 5 (2)  
397 vs. 6 (3) vs. >6 (4) rows, and (d) phyllary glabrous (1) vs. sparsely haired (2) vs. densely haired (3) vs.  
398 appendage (4).



399 **Fig. 5** Speciation rates of *Saussurea* correlated with ecological factors based on the QuaSSE best-  
400 fitted model and *ES-sim* tests. Both (a) niche breadth and (b) species range size (log-transformed)  
401 show positive sigmoidal curves in QuaSSE analysis with the midpoints (represented by the red dashed  
402 line) of 0.729 and 11.433 on the x-axis respectively. *EM-sim* tests show significant positive  
403 relationships between DR speciation rates and (c) niche breadth and (d) species range size. Species  
404 from three clades are in different colors.

## 405 **Ecological drivers of diversification**

406 By correlating climate lability (PCs of bioclimatic variables), niche breadth and species  
407 range size with speciation rates (Supporting Information Table S10), we explored the role  
408 of ecological opportunities created by complex QTP environments in driving  
409 diversification of *Saussurea*. The first two PCs of bioclimatic variables explained 75.7%  
410 of the total climate variation in *Saussurea* (Supporting Information Fig. S7a). Among the  
411 eight retained bioclimatic variables, the precipitation of warmest quarter (BIO18) had the  
412 largest contribution to first two PCs, followed by the annual precipitation (BIO12) and  
413 the mean diurnal range (BIO2) (Supporting Information Fig. S7b). Under the QuaSSE  
414 analyses, PC1 of the climate variables showed a significant positive linear ( $\text{l.m} = 0.330$ ,  
415  $\text{AIC} = 1240.548$ ,  $p\text{-value} = 0.005^{**}$ ) relationships with speciation rate, while climate  
416 PC2 preferred a constant model ( $\text{AIC} = 1183.524$ ,  $p\text{-value} = 0.953$ ); both niche breadth  
417 ( $\text{AIC} = 529.532$ ,  $p\text{-value} < 0.000^{**}$ ) and species range size ( $\text{AIC} = 700.671$ ,  $p\text{-value} <$   
418  $0.000^{**}$ ) showed a significant positive sigmoidal (with drift) relationships with  
419 speciation rate (Supporting Information Table S11). Under the best sigmoidal models, the  
420 speciation rates of *Saussurea* kept a stable low state until the niche breadth and  
421 distribution range reached at 0.729 and 11.433 (log-transformed), respectively  
422 (midpoints; Fig. 5a, 5b). Under the *EM-sim* tests, both the DR statistic and the default  
423 inverse equal splits statistic revealed the same correlation pattern, in which niche breadth  
424 ( $\rho = 0.363$  and  $0.387$ ,  $p = 0.027$  and  $0.019$ ) and range size ( $\rho = 0.399$  and  $0.411$ ,  $p =$   
425  $0.018$  and  $0.011$ ) showed significant positive relationship with speciation rates (Fig. 5c,  
426 5d), while the correlation between speciation rates and climate lability (climate PC1:  $\rho =$   
427  $0.170$  and  $0.188$ ,  $p = 0.359$  and  $0.335$ ; climate PC2:  $\rho = 0.098$  and  $0.095$ ,  $p = 0.649$  and  
428  $0.635$ ) was not significant (Table 3).

## 429 **Discussion**

430 Our results demonstrate rapid diversification of *Saussurea* occurred in parallel during the  
431 Miocene, a period with extensive tectonic movement and climatic fluctuation on the QTP.  
432 A recent paper by (Louca & Pennell, 2020) raised limitations of macroevolutionary  
433 studies using estimated diversification rates, though several recent papers have suggested  
434 that more complex models (such as hidden state SSE models; (Helmstetter *et al.*, 2021)  
435 and a hypothesis driven approach (Morlon *et al.*, 2020) circumvent many of the issues  
436 raised. Therefore, we took an integrative approach to address the role that morphological  
437 traits and environmental conditions played in the evolutionary history of *Saussurea*. The  
438 rates of species diversification are revealed to be negatively correlated with  
439 paleotemperature, and accelerate sharply in the Pliocene toward the present. Similar  
440 patterns of increased diversification with global cooling have been documented in other  
441 flowering plant lineages, e.g. Saxifragales (Folk *et al.*, 2019), rosids (Sun *et al.*, 2020)  
442 and Campanulaceae (Lagomarsino *et al.*, 2016), as well as in mammals (Stadler, 2011)  
443 and birds (Claramunt & Cracraft, 2015). Our trait dependent models detect some  
444 observed phenotypic adaptation associated with diversification changes, and indicate  
445 some unobserved traits also drive diversification, demonstrating a pivotal role of  
446 morphological diversity in this radiating diversification. Accounting for ecological niche  
447 data, we further reveal that acceleration in diversification rates are correlated with climate  
448 lability (PCs of bioclimatic variables), niche breadth and the size of species' range.  
449 Overall, we conclude that tectonic activity of the QTP along with global paleoclimate  
450 cooling provided vast alpine niches for *Saussurea* species with ample adaptive traits,  
451 highlighting the important role of morphological diversity and ecological niche  
452 availability for species radiating to diverse environments.

453 We determined clade ages across *Saussurea* species using whole plastome sequences  
454 and found that the divergence of the main species clades occurred in the Miocene almost

455 simultaneously. Compared to fragment DNA markers, plastomes have been shown to  
456 provide more sufficient phylogenetic signals which are powerful in resolving deep  
457 relationships of plant lineages (Parks *et al.*, 2009; Wicke *et al.*, 2011; Zhang *et al.*, 2020).  
458 Our estimate for the origin of *Saussurea* (ca. 11.8 Ma) is consistent with the result from  
459 single-copy nuclear genes obtained via Hyb-Seq (ca. 12.5 Mya) (Herrando-Moraira *et al.*,  
460 2019) and the result from ITS sequences (12.6-10.3 Mya) (Wang *et al.*, 2009), but was  
461 younger than the result of Xu *et al.* (2019) (ca. 18.5 Mya) using plastome coding regions  
462 and the result of Barres *et al.* (2013) (ca. 20.0 Mya) using chloroplast markers. The study  
463 of Barres *et al.* (2013) included only two species of *Saussurea* and used four chloroplast  
464 markers, *trnL-trnF*, *matK*, *ndhF* and *rbcL*. Different from Xu *et al.* (2019) setting the split  
465 of subtribe Arctiinae and subtribe Saussureinae as a minimum age to 8.0 Mya using the  
466 achene fossil assigned to *Arctium*, our study omitted this calibration because only one  
467 Arctiinae sample (*A. lappa*) was included in both studies and the relationship between  
468 Arctiinae and Saussureinae remains unresolved (Herrando-Moraira *et al.*, 2019; Shen *et*  
469 *al.*, 2020). In addition, we estimated divergence times using whole plastome sequences,  
470 as our prior work showed that including noncoding regions can maximize the resolution  
471 in resolving relationships of *Saussurea* (Zhang *et al.*, 2019a).

472       Recent large-scale studies of species diversification on the QTP have provided  
473 convincing evidence for a Miocene diversification in plant lineages (Ding *et al.*, 2020) as  
474 well as amphibians and reptiles (Xu *et al.*, 2020). A hypothesis for the rich biodiversity  
475 found in mountainous regions like the QTP is uplift-driven diversification—that orogenic  
476 activities create diverse habitats favoring rapid *in situ* speciation of resident lineages  
477 (Xing & Ree, 2017; Chen *et al.*, 2019). Extensive plateau uplift in the Miocene further  
478 intensified the Asian summer monsoon, which increased the precipitation for erosion  
479 through river incision, leading to greater topographic relief (Nie *et al.*, 2018). This would  
480 have promoted the differentiation of microhabitats associated with elevational gradients

481 and slope aspects, increasing the availability of ecological niches for radiating species  
482 (Ding *et al.*, 2020). A previous study indicated that the *Saussurea* radiation was likely  
483 driven by ecological opportunities, similar to those on islands, provided by largely  
484 unoccupied habitats resulting from the extensive QTP uplifts (Wang *et al.*, 2009). Our  
485 work provides compelling evidence of the vital role of ecological opportunities in  
486 *Saussurea* diversification by statistically correlating species niche breadth and  
487 distribution range to the speciation rate. A slight difference is that our result supports a  
488 wide-range radiation rather than an ‘island isolation’, from the positive correlation  
489 between range and speciation rate. We attribute the wide-range radiation of *Saussurea* to  
490 the presence of unique pappus combinations (Shi & Raab-Straube, 2011; Chen, 2015),  
491 which can promote the dispersal power of achenes to occupy more newly created niches.  
492 Therefore, colonizing success benefited by wide-range dispersal helped *Saussurea*  
493 species become one of the most diverse lineages on the QTP.

494 The negative correlation between paleotemperature and diversification rates in  
495 *Saussurea* does not seem surprising given the high species richness of *Saussurea* found at  
496 the high elevations of the QTP. Nonetheless, this insight is progressive for our  
497 understanding of the formation of the QTP flora, as it represents one of the few attempts  
498 to explicitly quantify the relationship between lineage diversification and a  
499 paleoenvironmental variable. Geological evidence suggests that after 15 Mya, global  
500 cooling and the further rise of QTP progressively led to more open, herb-rich vegetation  
501 as the modern high plateau formed with its cool, dry climate (Spicer *et al.*, 2021). Thus,  
502 diversification among *Saussurea* clades could have been driven by increased ecological  
503 niches as suitable cold habitats became available. A sharply accelerated diversification  
504 rate of *Saussurea* was detected in the Pliocene toward the present. The uplift of the  
505 Hengduan Mountains region, at the southeastern margin of the QTP, is generally believed  
506 to have been rapid and recent, occurring mainly between the late Miocene and late

507 Pliocene (Xing & Ree, 2017; Spicer *et al.*, 2020). During the Quaternary glaciation, the  
508 Hengduan Mountains with its deep valleys would have provided numerous micro-refugia  
509 within the altitudinal and aspect heterogeneity (Sun *et al.*, 2017; Spicer *et al.*, 2021). This  
510 can explain why extensive morphological traits occur in parallel and evolved  
511 convergently, a result likely driven by local adaptation to the micro-habitats that were  
512 afforded by the complex and highly dissected landscape of the Hengduan Mountains.

513 Trait dependent analyses demonstrated that species exhibiting cauliferous plant,  
514 glabrous stem, leafy bracts and solitary capitula have higher speciation rates. These traits  
515 are usually observed in the subgenus *Amphilaena* (snow lotus), which is characterized by  
516 attractive leafy bract and is the symbols of snow mountains in the QTP (Shi & Raab-  
517 Straube, 2011; Chen, 2015). Snow lotus has abundant morphological variation and is a  
518 taxonomically complex group, with some new species described recently (e.g. Eckhard  
519 von, 2009; Chen & Yuan, 2015; Zhang, *et al.*, 2019b). Despite having significant  
520 taxonomic characteristics, snow lotus is a non-monophyletic group, demonstrating that  
521 these adaptive traits have multiple origins and arose by convergent evolution. In fact,  
522 specialized leafy bracts, the so-called ‘glasshouse’ morphology, are prevalent among  
523 alpine species, such as in Lamiaceae, Asteraceae, and Polygonaceae (reviewed by Sun *et*  
524 *al.*, 2014). Leafy bracts reportedly protect pollen grains from damage by UV-B radiation  
525 and rain, promote pollen germination by maintaining warmth, enhance pollinator  
526 visitation by providing a vivid visual display during flowering, and facilitate the  
527 development of fertilized ovules during seed development (Tsukaya, 2002; Yang & Sun,  
528 2009; Song *et al.*, 2015). Convergent morphological evolution seems to be common for  
529 plants adapting harsh environments of the QTP, examples include cushion (stemless)  
530 plant, woolly hairs and the leafy bract (Sun *et al.*, 2014; Peng *et al.*, 2015; Yang *et al.*,  
531 2019). Similar to leafy bract, the present of stemless and woolly hairs has been revealed  
532 to occurred multiple times, and is thought to defense cold and arid climate on the plateau

533 (Sun *et al.*, 2014). However, both stemless plants and the presence of woolly hairs appear  
534 to be not associated with an increase in diversification rate of *Saussurea*. A plausible  
535 explanation for this is that species with stemless and woolly hairs are commonly found in  
536 environments of the QTP with extremely high altitude with very low temperature, and  
537 these species usually have long lifespans.

538 Some traits associated with high diversification rates appear to have no evidence for  
539 ecological adaptation, such as solitary capitula and pinnate leaf margin. These may occur  
540 in combination with other important adaptive traits. Some traits were not examined  
541 because they are common across the entire genus, such as two rows of pappus and small  
542 achenes (Shi & Raab-Straube, 2011; Chen, 2015). Although trait dependent analyses  
543 showed several adaptive traits driving the increase of speciation rate, some unobserved  
544 traits were also important for rapid diversification, highlighting the vital roles of  
545 morphological diversity in the evolutionary history of *Saussurea*. Morphological  
546 diversity is an essential but often neglected aspect of biodiversity (Chartier *et al.*, 2021).  
547 Our work provides a valuable guide for conservation efforts in the protection of  
548 morphological diversity of organisms, especially in the context of exacerbated  
549 biodiversity loss due to global warming.

550 Our results provided evidence of a positive relationship between speciation rate and  
551 niche breadth as well as species range. Among the few studies that have tested a niche  
552 breadth–diversification relationship, a clear consensus has not been reached (Sexton *et*  
553 *al.*, 2017). One argument for low niche breadth lineages having greater diversification  
554 rates is that they are more likely to suffer from resource limitations and more susceptible  
555 to range fragmentation, and thus allopatric speciation occurs more frequently (Vrba,  
556 1987). An alternative view is that species with high niche breadth typically have larger  
557 range sizes (Slatyer *et al.*, 2013) and are therefore more likely to have these ranges  
558 fragmented by ecological or geographical barriers over evolutionary time, promoting



559 allopatric speciation (Rolland & Salamin, 2016). We argue that wider ecological niches  
560 can help species diverging in the QTP cope with climatic fluctuation, occupy  
561 microhabitats and promote morphological divergence. Note that anthropogenic activities  
562 have led to landscape modification and habitat fragmentation, alternating the distributions  
563 of a vast array of species (Boivin *et al.*, 2016), even in plateau areas (Chen *et al.*, 2014).  
564 To promote future biodiversity resilience, the conservation of entire unfragmented  
565 landscapes is necessary to preserve niche heterogeneity and enable species migrations at  
566 will. Only this approach will conserve the processes of biodiversity dynamics as well as  
567 the genetic library and the capacity for future adaptation in threatened species (Spicer *et*  
568 *al.*, 2020).

## 569 **Conclusion**

570 Despite substantial processes on the taxonomy, phylogeny and biogeography of plant  
571 lineages on the QTP (reviewed by Wen *et al.*, 2014), our knowledge of the diversification  
572 rates associated with geological activities along with subsequent environmental  
573 fluctuations and biotic interactions is still limited, especially for rapidly radiating species.  
574 Our study integrates *Saussurea* into an marcoevolutionary diversification framework.  
575 Using a genomic data set (plastome sequences) for reconstructing divergence history and  
576 multiple statistical analyses, we quantify the roles of abiotic/environmental and  
577 biotic/species-intrinsic factors in driving diversification of *Saussurea*. Our comprehensive  
578 and large-scale analyses depict a plausible evolutionary scene for *Saussurea*, and provide  
579 insights into the drivers of its radiating diversification. We document a Miocene  
580 diversification pattern in which increased speciation rates are related to global cooling,  
581 and correlate it to clade-specific traits and ecological niches. We hypothesize that the  
582 current mega diversity of *Saussurea* is the result of interactions between geological  
583 activity, global paleoclimate and ecological niche. Our results highlight the vital roles of  
584 morphological diversity and available ecological niches in plants adapting to the

585 changing climate. Given the ongoing global warming and human expansion, causing the  
586 disappearance of numerous undescribed species and extensive occupied habitats, our  
587 present study together with previous macroevolutionary pattern studies (e.g. Condamine  
588 *et al.*, 2018; Folk *et al.*, 2019; Testo *et al.*, 2019; Ding *et al.*, 2020; Sun *et al.*, 2020)  
589 provide valuable theoretical basis for mitigating the threats posed to biodiversity.

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## 607 **Author contributions**

608 HW, HS, TD and XZ developed the idea and designed the experiment; XZ and JBL  
609 performed the statistical analyses; XZ, JBL, TD, HS and HW interpreted the results and

610 wrote the manuscript. XZ, YS, TF, HZ, NL, TB, XH and TD collected the leaf materials;  
611 All authors read, edited and approved the final manuscript.

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858 **Figure legends:**

859 **Fig. 1** Diversification dynamics of *Saussurea* inferred from BAMM analysis. (a)  
860 BAMM identified two shifts in diversification rates (represented by arrows). The time  
861 of three clades beginning to diversify is provided. (b) Rates-through-time plots of all  
862 *Saussurea* species and three main clades separately, with trends in global climate  
863 change over 12 million years (Zachos *et al.* 2008) depicted. (c-d) BAMM tip rates of  
864 three clades and four morphology-based subgenera of *Saussurea*, respectively.

865 **Fig. 2** Paleoenvironment-dependent diversification processes in *Saussurea*. The best-  
866 fit paleoenvironment-dependent model implemented in RPANDA shows negative  
867 dependence between paleotemperatures (a) and speciation rate (b).

868 **Fig. 3** Binary trait dependent diversification of *Saussurea* inferred from HiSSE  
869 analysis. Speciation, extinction and net diversification rates are calculated by the  
870 model-averaged marginal ancestral state reconstruction for four binary traits: (a)  
871 stemless (0) vs. cauliferous (1), (b) stem glabrous (0) vs. densely haired (1), (c) the  
872 absence (0) vs. presence (1) of leafy bracts, and (d) capitula solitary (0) vs. numerous  
873 (1).

874 **Fig. 4** Multistate trait dependent diversification of *Saussurea* estimated from MuSSE  
875 analysis. Marginal distributions of net diversification rates are estimated by the  
876 MCMC run of 5, 000 generations for four multistate traits: (a) leaves margin entire (1)  
877 vs. pinnately lobed (2) vs. both types (3), (b) leaves glabrous (1) vs. sparsely haired  
878 (2) vs. densely haired (3), (c) phyllary in <5 (1) vs. 5 (2) vs. 6 (3) vs. >6 (4) rows, and  
879 (d) phyllary glabrous (1) vs. sparsely haired (2) vs. densely haired (3) vs. appendage  
880 (4).

881 **Fig. 5** Speciation rates of *Saussurea* correlated with ecological factors based on the  
882 QuaSSE best-fitted model and *ES-sim* tests. Both (a) niche breadth and (b) species  
883 range size (log-transformed) show positive sigmoidal curves in QuaSSE analysis with  
884 the midpoints (represented by the red dashed line) of 0.729 and 11.433 on the x-axis  
885 respectively. *EM-sim* tests show significant positive relationships between DR  
886 speciation rates and (c) niche breadth and (d) species range size. Species from three  
887 clades are in different colors.

888

889 **Table 1** Results of RPANDA analyses.

Models	NP	logL	AICc	$\lambda_0$	$\alpha$	$\mu_0$	$\beta$
Constant birth–death (1)	2	-325.7908	655.6424	0.7214	NA	0.3714	NA
$\lambda_{\text{Time}}$ and $\mu_{\text{constant}}$ (2)	3	-325.2570	656.6364	0.6801	-0.0618	0.1562	NA
<b><math>\lambda_{\text{Temp.}}</math> and <math>\mu_{\text{constant}}</math> (3)</b>	<b>3</b>	<b>-324.4240</b>	<b>654.9705</b>	<b>0.7585</b>	<b>-0.0933</b>	<b>0.1610</b>	<b>NA</b>
$\lambda_{\text{constant}}$ and $\mu_{\text{Time}}$ (4)	3	-325.4236	656.9698	0.7020	NA	0.3051	0.0475
$\lambda_{\text{constant}}$ and $\mu_{\text{Temp.}}$ (5)	3	-325.4355	656.9930	0.649	NA	0.1445	0.2067
$\lambda_{\text{Time}}$ and $\mu_{\text{Time}}$ (6)	4	-325.1840	658.5732	0.6840	-0.0460	0.1843	-0.0036
$\lambda_{\text{Temp.}}$ and $\mu_{\text{Temp.}}$ (7)	4	-323.9815	656.168	0.693	-0.0017	0.1910	0.1159

890 Bold columns represent the best model, in which speciation rate is negative dependence ( $\alpha < 0$ ) to  
891 past temperature and extinction rate is constant. Detailed model sets are described in Condamine  
892 *et al.* (2013). Abbreviations: NP, number of parameters; logL, log-likelihood; AICc, corrected  
893 Akaike Information Criterion. Parameter estimates:  $\lambda_0$  and  $\mu_0$ , speciation and extinction rates for a  
894 given environmental variable; and  $\alpha$ ,  $\beta$ , parameter controlling variation of speciation and  
895 extinction with paleo-environment, respectively.  
896

897 **Table 2** Summary of the mean rate values for four binary traits in HiSSE and FiSSE  
 898 analysis.

Trait	HiSSE			FiSSE	
	$\lambda$	$\mu$	$\lambda-\mu$	$\lambda$	<i>p</i> -value
Stemless	0.5947	0.2415	0.3532	0.9011	
<b>Cauliferous</b>	<b>1.0893</b>	<b>0.3821</b>	<b>0.4502</b>	<b>0.9264</b>	0.4416
<b>Glabrous</b>	<b>1.7230</b>	<b>1.2100</b>	<b>0.5128</b>	<b>0.8971</b>	
Densely haired	0.5531	0.2223	0.3308	0.8674	0.4096
Normal	0.8925	0.6849	0.2076	0.8987	
<b>Bracts</b>	<b>1.3951</b>	<b>0.8752</b>	<b>0.5199</b>	<b>0.9412</b>	0.5614
<b>Capitula solitary</b>	<b>1.6920</b>	<b>0.9969</b>	<b>0.6952</b>	<b>1.0825</b>	
Capitula numerous	0.5661	0.5253	0.0408	0.7828	<b>0.0240</b>

899 Traits with higher net diversification rates ( $\lambda-\mu$ ) are in bold. For HiSSE analysis, mean rate values  
 900 are calculated from the model-averaged marginal ancestral state reconstruction, and detailed  
 901 model tests are provided in Supporting Information Table S7. For FiSSE analysis, the significant  
 902 *p*-values for adjusted results are in bold.

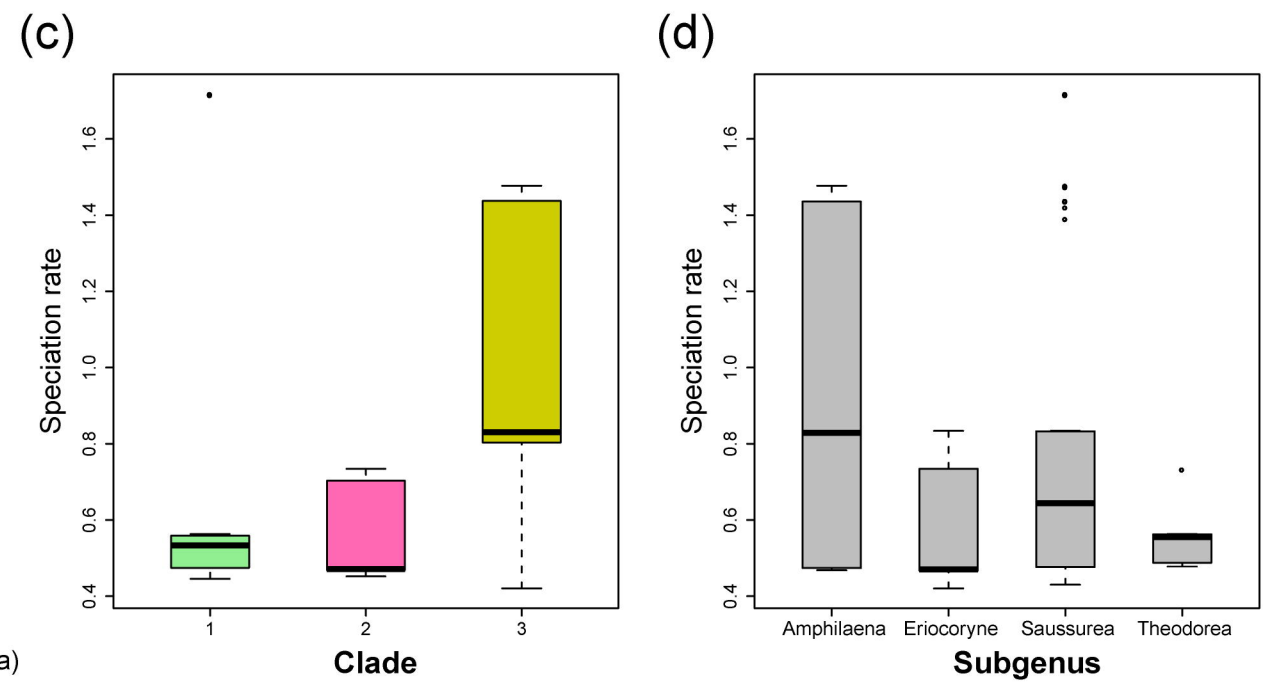
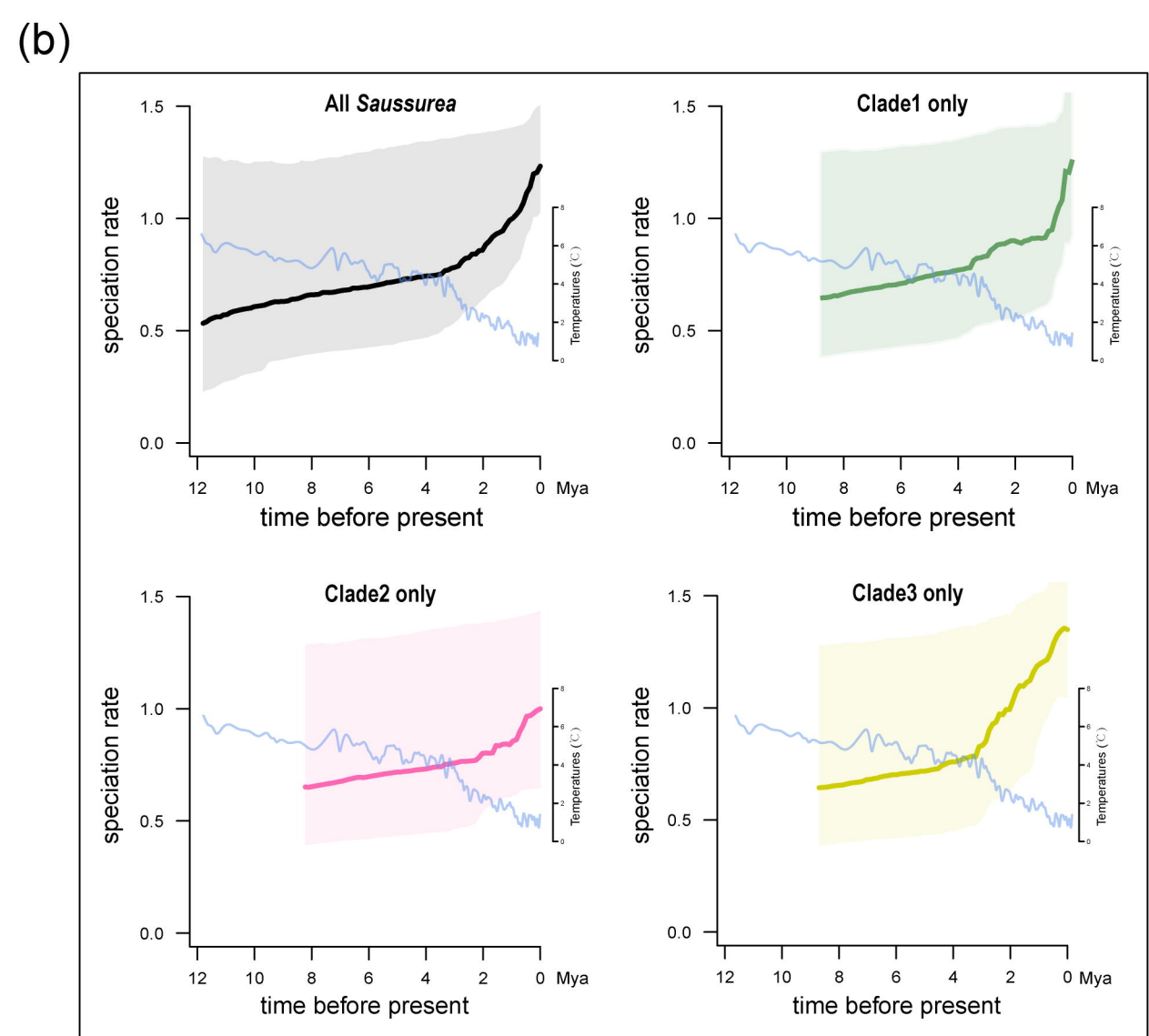
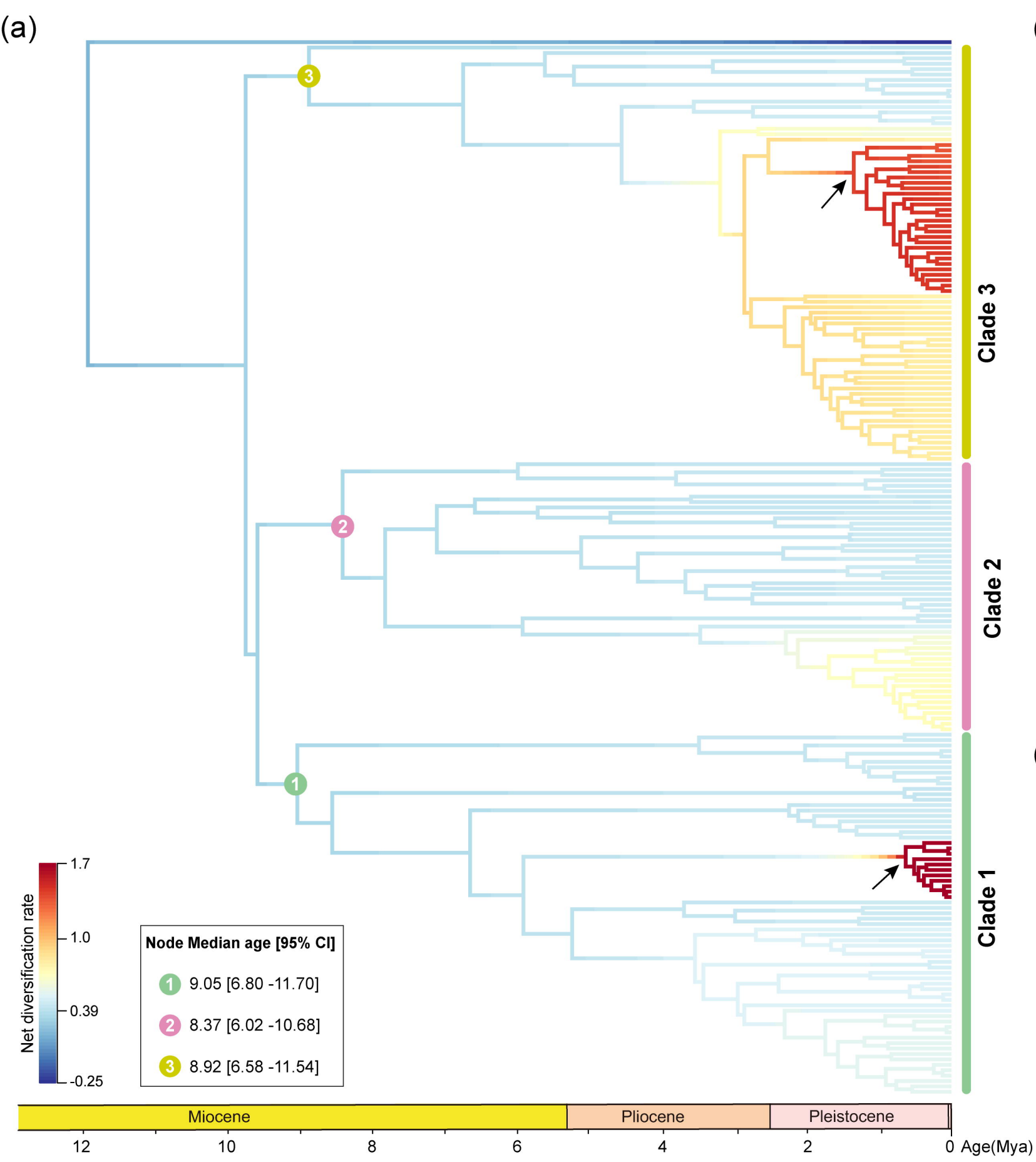
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904 **Table 3** Summary of *ES-sim* tests for correlation between speciation rate and  
 905 continuous ecological factors.

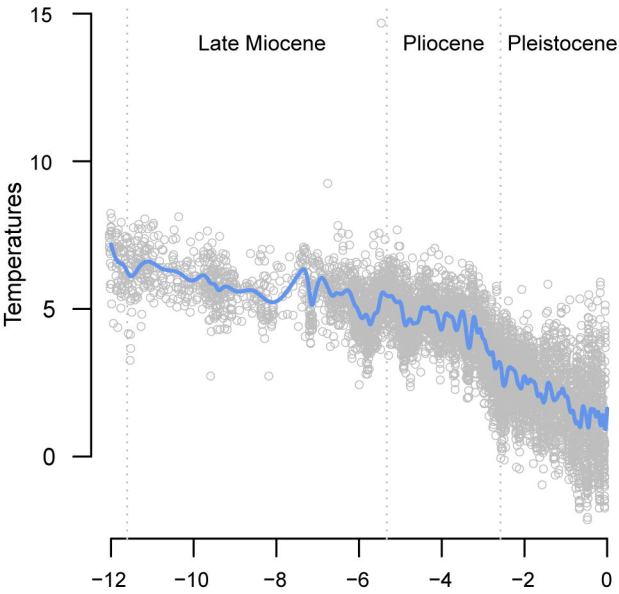
Ecological factors	<i>ES-sim</i> (DR statistic)		<i>ES-sim</i> (Inverse equal splits statistic)	
	$\rho$	<i>p</i> -value	$\rho$	<i>p</i> -value
ClimatePC1	0.170	0.359	0.188	0.335
ClimatePC2	0.098	0.649	0.095	0.635
Niche breadth	0.363	<b>0.027</b>	0.387	<b>0.019</b>
Range size	0.399	<b>0.018</b>	0.411	<b>0.011</b>

906 The significant *p*-values for the correlation are in bold. Rho ( $\rho$ ) is the Pearson's correlation  
 907 coefficient. Both the DR statistic and the default inverse equal splits statistic were used as reliable  
 908 estimators. Detailed test statistic described in Harvey & Rabosky (2018).

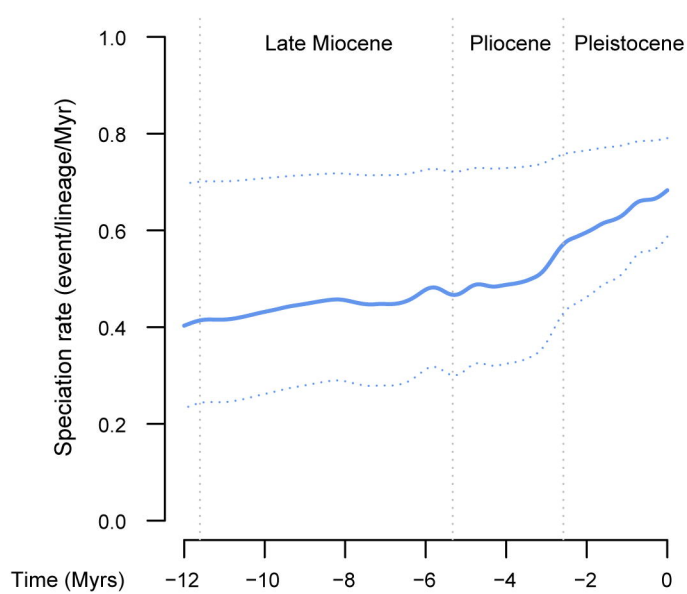
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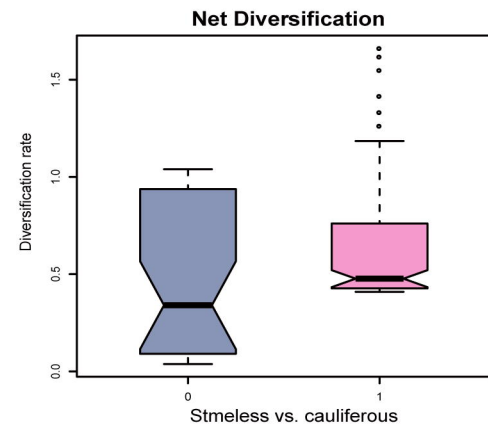
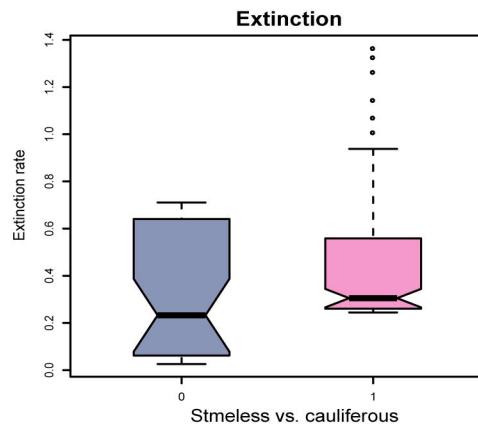
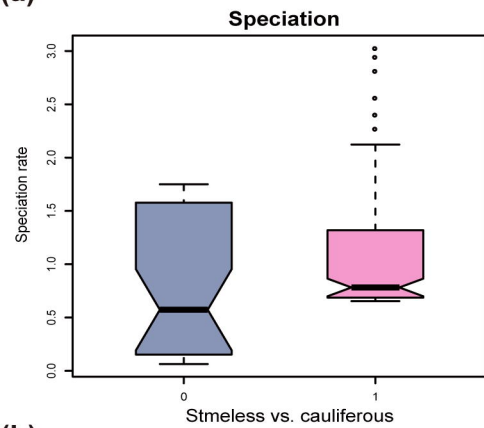
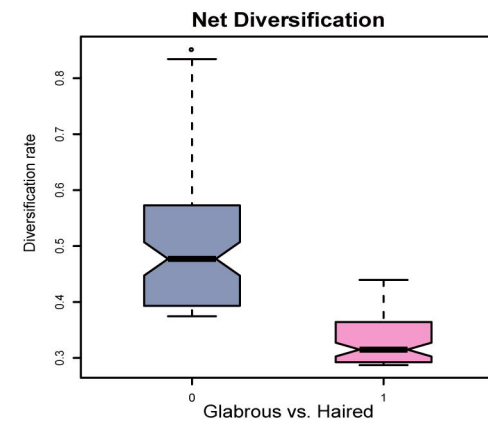
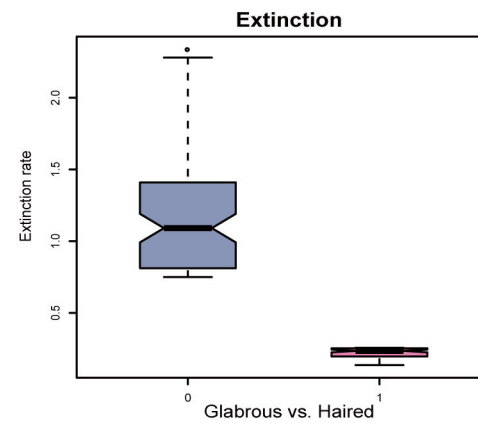
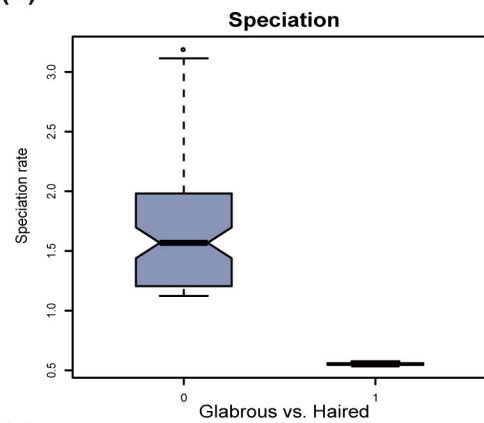
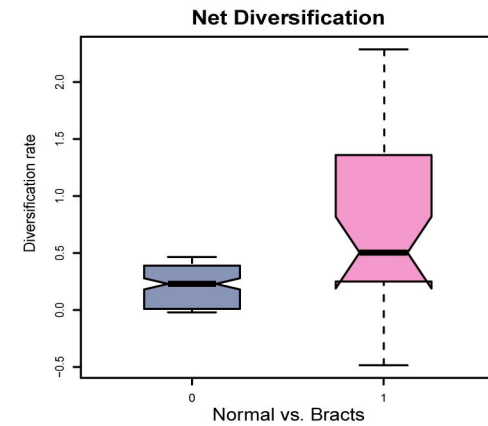
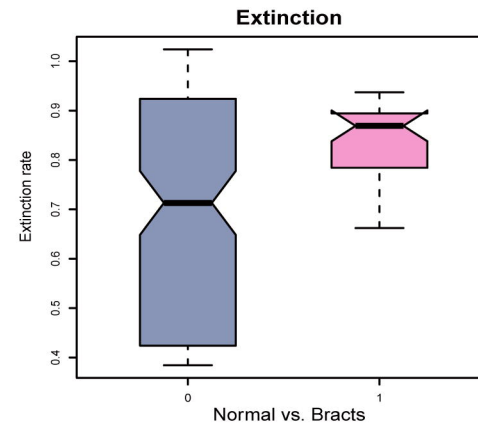
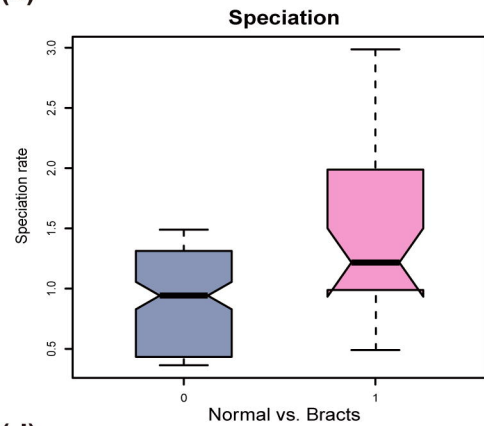
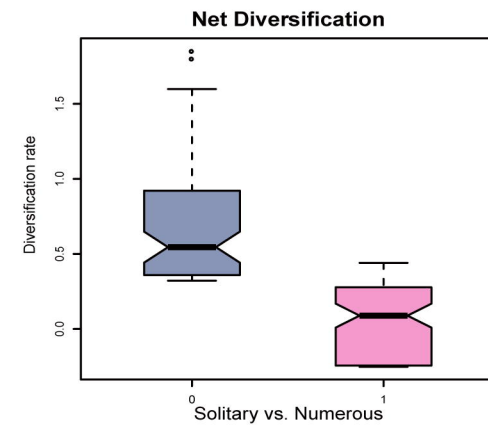
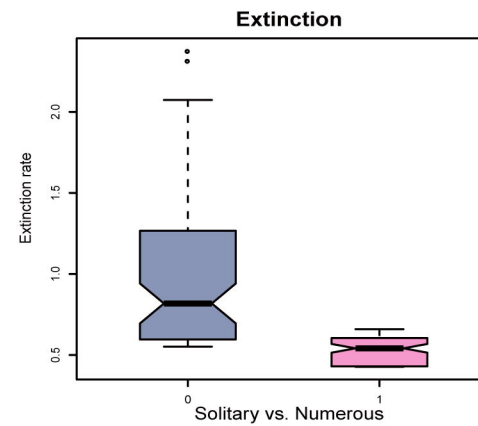
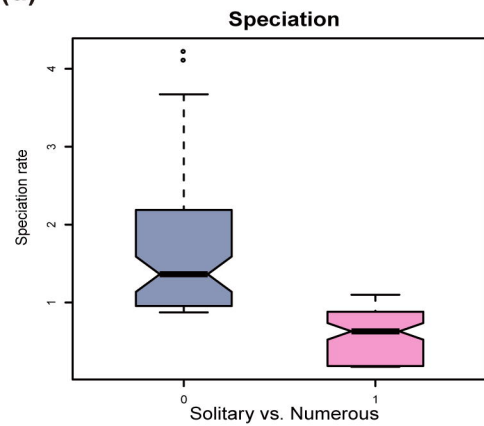


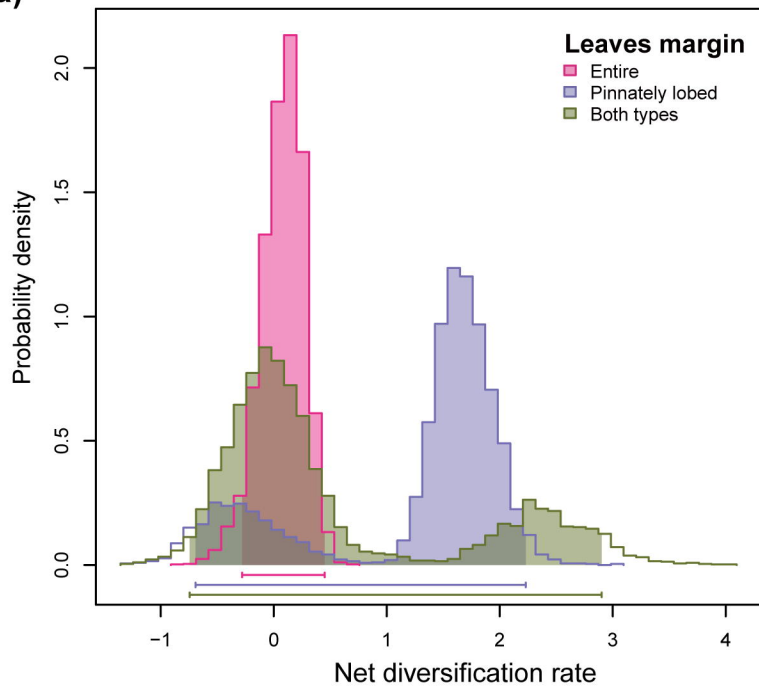
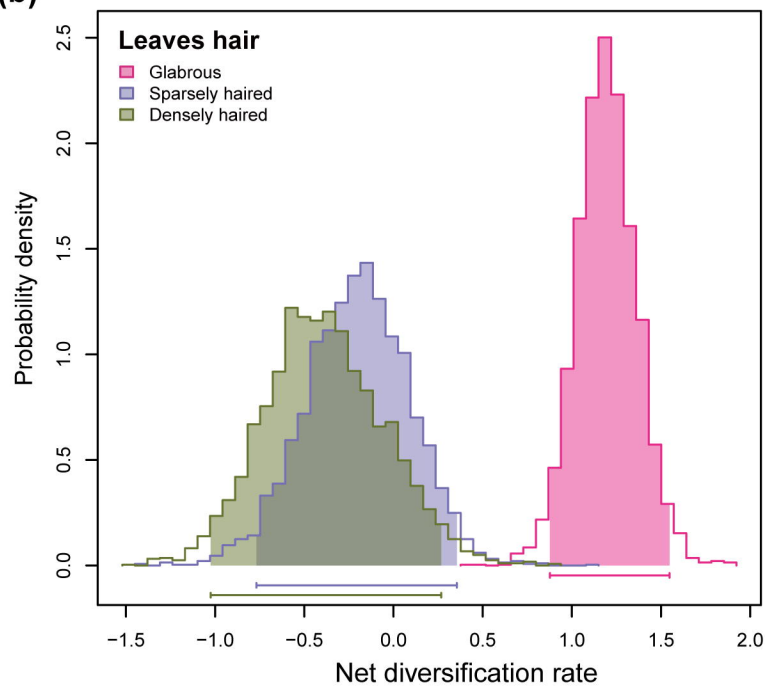
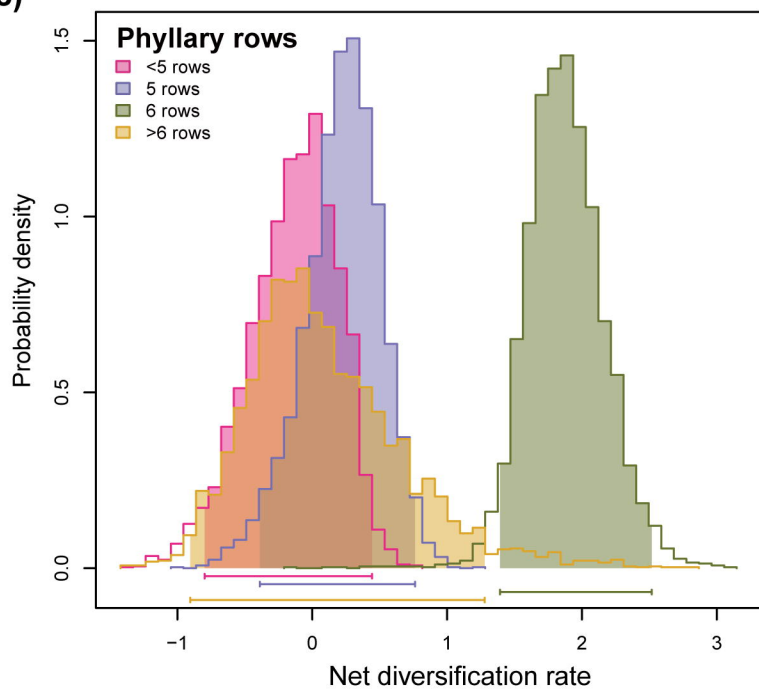
(a) Global paleo-temperatures over the last 12 Myrs



(b) Speciation is negatively correlated to past temperatures



**(a)****(b)****(c)****(d)**

**(a)****(b)****(c)****(d)**