

1 **Title**

2 Adapting to extremes: reconstructing niche evolution in Australian *Acacia* over time and
3 space.

4

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12 **Abstract**

13 Macroevolutionary analysis is increasingly being used to study biodiversity responses to
14 climate change, for example by using phylogenetic node ages to infer periods of
15 diversification, or phylogenetic reconstruction of traits to infer adaptation to particular
16 stresses. Here we apply a new macroevolutionary method to investigate the responses of a
17 diverse plant genus, *Acacia*, to increasing aridity and salinity in Australia from the Miocene
18 to the present. The Niche Evolution Model (NEMo) combines environmental niche
19 modelling with phylogenetic comparative methods in a single statistical framework, to
20 estimate current environmental tolerances, reconstruct the history of niche evolution and infer
21 rates of change in key aspects of environmental tolerance. Using a large database of *Acacia*
22 occurrence records and presence-absence survey sites, we find that both spatial and temporal
23 patterns in niche evolution of *Acacia* are consistent with the aridification history of Australia
24 and suggest high niche lability along both axes, which has allowed *Acacia* to quickly exploit
25 new niches created during the aridification of the continent, and resulting in their current

26 dominance of many habitats across Australia. This study demonstrates that phylogenetic
27 studies of niche evolution can move beyond application of simple trait-based models,
28 allowing the underlying processes of speciation, adaptation and dispersal to be explicitly
29 modelled in a macroecological and macroevolutionary context.

30

31 Keywords: aridity tolerance, salt tolerance, aridification, niche lability, Environmental Niche
32 Modelling, Phylogenetic Comparative Methods.

33

34 **Introduction**

35 The climatic and environmental history of Australia during the Neogene and Quaternary
36 Periods has been a story of increasing aridification. Dry climates may have existed from the
37 Eocene (Carpenter et al 2014), but evidence from charcoal and pollen records reveals an
38 increase in fire frequency and prevalence of fire-adapted, scleromorphic plant taxa, pointing
39 to an expansion of the arid zone from the early Miocene onwards (Byrne et al 2008). As the
40 arid zone expanded, previously widespread mesic forest habitats contracted to the coastal
41 margins, and plant groups responded in different ways (Byrne & Murphy 2020, Crisp et al
42 2004, Weston & Jordan 2017). Some groups (such as *Nothofagus*) remained confined to
43 humid forest conditions and contracted with these habitats to small coastal refuges. Other
44 groups responded to new ecological opportunities provided by the expanding arid zone, and
45 diversified into numerous xeromorphic lineages. Some plant families, especially Proteaceae,
46 Myrtaceae and Fabaceae, contain both mesic and xeromorphic lineages. Even some large
47 genera, such as *Acacia*, *Grevillea* and *Hakea*, have both mesic and xeromorphic
48 representatives. Plant groups such as these, in which close relatives occupy a range of
49 ecoclimatic conditions, provide good case studies for studying the evolution of tolerance to
50 extreme environmental conditions.

51 The expansion of the arid zone in Australia led to increasingly challenging
52 environments characterized by stressful conditions for plant growth, including extreme
53 drought, low soil fertility, frequent fires and high soil salinity. At the same time, areas that
54 are extreme for most plants represent an opportunity for lineages to specialize, escape
55 competition, and diversify. Arid-adapted lineages evolved a range of strategies to cope with
56 water stress, broadly classified into escape, avoidance, and tolerance (Delzon 2015). We see
57 all three strategies in *Acacia*, such as phyllode shedding (escape), scleromorphic structures
58 like sunken stomata or needle-shaped phyllodes (avoidance), and physiological adaptations
59 such as low leaf water potential (tolerance: Moore 2013). The wide range of mechanisms
60 adopted by *Acacia* species to cope with water stress are reflected in their geographic
61 distributions and varied climatic niches.

62 To understand how opportunities provided by the drying environments shaped
63 present-day distribution and diversity, we need to examine the dynamics of lineage
64 adaptation and diversification in response to extreme conditions. Macroevolutionary and
65 macroecological analyses based on phylogenies have provided a variety of ways of
66 investigating adaptation to harsh environments (Bromham et al. 2020). For example, the ages
67 of nodes in molecular phylogenies have been used to infer bursts of diversification associated
68 with periods of drying in *Acacia* (Renner et al. 2020), and the movement of rainforest-
69 adapted lineages into dry environments (Crayn et al. 2006). In addition to relative timing of
70 diversification, phylogenies can provide information on evolution of specific adaptations to
71 extreme conditions. For example, Crisp et al. (2011) inferred the phylogenetic history of
72 traits such as post-fire epicormic resprouting to investigate the timing of adaptation of
73 *Eucalyptus* to increasing fire frequency. Jordan et al. (2008) reconstructed the evolution of
74 stomatal protection structures (which are adaptations to dry climates) in Proteaceae to show
75 that such structures evolved relatively few times in the ancestors of dry-climate clades. As

76 well as physiological and morphological traits, phylogenetic approaches have been applied to
77 reconstructing the evolution of environmental niches. For example, by comparing alternative
78 evolutionary models of climatic niche evolution for clades from different regions, Skeels and
79 Cardillo (2017) showed that within four large genera (*Protea*, *Moraea*, *Banksia*, and *Hakea*),
80 different lineages are evolving towards different climatic optima in different bioclimatic
81 regions. Onstein et al. (2016) used the same approach to show that Proteaceae lineages in
82 open vegetation and closed forest also have different inferred climatic optima.

83 Phylogenetic analyses such as these are useful, but have a number of limitations. To
84 permit the application of standard stochastic models of trait evolution, such as Brownian
85 Motion (BM) or Ornstein-Uhlenbeck (OU), in most macroevolutionary studies, the
86 environmental niche is simplified to a point-estimate for a species, for example by taking the
87 mean value across a species distribution (Evans et al 2009; Kozak & Wiens 2010;
88 Münkemüller et al 2015; Renner et al 2020). But this is problematic for two main reasons.
89 First, it averages away most of the potentially informative variation in environmental
90 conditions across the range of a species. Second, models of trait evolution such as BM may
91 not be appropriate for modelling the evolution of environmental tolerance. In particular,
92 applying trait-evolution models to current environmental data to infer ancestral niches (e.g.,
93 Renner et al 2020, Skeels & Cardillo 2017) assumes that environmental traits such as aridity
94 or temperature evolve randomly and independently along branches for each lineage. This
95 assumption may often be violated because environmental niche evolution is likely to be
96 driven by climatic changes, which most likely apply simultaneously and directionally across
97 all lineages within a given region.

98 More fundamentally, phylogenetic analyses of niche evolution with changing climate
99 compartmentalise the inference of contemporary niche (inferred from occurrence data) and
100 the history of niche evolution (inferred from phylogenetic models). These are treated as two

101 separate problems, and subject to independent analyses with separate statistical frameworks.
102 But contemporary niches are the product of macroevolutionary processes and inferring these
103 processes requires accurate inference of the niche from contemporary distributions, so the
104 two are fundamentally linked. The logical step, then, is to link their analyses too.

105 Here, we infer the history of adaptation to extreme environments by applying a novel
106 method, the Niche Evolution Model (NEMo), that combines Environmental Niche Modelling
107 (ENM) and reconstruction of niche evolution in a single statistical framework. This method
108 uses occurrence records to characterise the distribution of each tip species in a phylogeny,
109 and reconstructs niche evolution by explicitly modelling key driving processes: (1) shift in
110 areas of tolerable conditions, (2) adaptation of lineages to changing conditions, (3) dispersal
111 away from areas that are no longer suitable and into accessible areas of suitable habitat, (4)
112 speciation, and (5) divergence along lineages' environmental tolerances. A strength of this
113 method is that it allows us to infer the placement of these key processes on the phylogeny and
114 to reconstruct ancestral niches at any time point, without making the unrealistic assumption
115 that niches evolve under a simple stochastic trait-based model. Unlike previous approaches,
116 NEMo can infer the occurrence density of these processes of niche evolution on the
117 phylogeny, so it allows us to directly compare the timing of niche shift to periods of climatic
118 change, or compare the timing of niche evolution along different environmental axes, using
119 standard statistical tests, without pre-defining the covariance structure to account for
120 phylogenetic nonindependence. Information on paleoclimate can either be incorporated into
121 the model as prior information, or can be held aside as independent information against
122 which to test the robustness of the inference.

123 We use NEMo to investigate macroevolutionary responses of the Australian plant
124 genus *Acacia* to aridification. *Acacia* is one of the largest and most widely-distributed plant
125 genera in Australia, with over 900 described species occupying nearly all major habitat types,

126 often as dominant taxa. Many *Acacia* species are known to be tolerant of a range of extreme
127 environmental conditions, and several previous studies have used *Acacia* species as case
128 studies for understanding tolerance to extremes, including salinity and other aspects of soil
129 geochemistry (Bui et al. 2014a, 2014b), and water stress (Atkin et al. 2002). There has been
130 rapid progress in understanding the evolutionary history of *Acacia* in recent years (Maslin et
131 al. 2003; Murphy et al. 2010; Mishler et al. 2014). Nearly all *Acacia* species are conspicuous,
132 woody plants, so spatial occurrence records for *Acacia* are abundant and widespread. We
133 combine a curated database of *Acacia* occurrence records with extensive survey data, along
134 with predictors of sampling bias, to characterise the current distribution of species in the
135 phylogeny. We use NEMo to reconstruct the evolution of adaptation to increasing
136 aridification in Australian *Acacia*, focusing on niche evolution in response to increased
137 aridity and changes in the distribution and severity of soil salinity (frequently associated with
138 aridification (Gilkes et al. 2003). Specifically, we ask two questions. First, have periods of
139 rapid niche evolution along the aridity and salinity axes corresponded with major periods of
140 aridification in Australia? Second, on which lineages did adaptation to increased aridity and
141 salinity occur? Answering these two questions may help us understand whether *Acacia*
142 responded to continental aridification by a burst of adaptation, allowing the genus to radiate
143 through the arid zone.

144

145 **Methods**

146 ***1. Phylogenetic and Spatial Data***

147 We based our analysis on a published phylogeny of 508 *Acacia* species, representing half of
148 species currently recognized (Mishler et al. 2014). We timescaled branch lengths under a
149 relaxed clock model using the R package ape (Paradis and Schliep 2018), with the root date

150 constrained between 21 and 23.9 million years ago (the estimated range of crown divergence
151 dates (Miller et al. 2013).

152 To describe the current environmental conditions under which each species is found,
153 we used a published dataset of 132,295 carefully checked presence locations of these 508
154 *Acacia* species, filtered from a total of 750,000 *Acacia* records from the Australian Virtual
155 Herbarium (Misher et al. 2014). We also assembled species presence-absence data from
156 floristic survey databases for New South Wales (BioNet Systematic Flora Survey;
157 environment.nsw.gov.au/research/VISplot.htm), Victoria (Victorian Biodiversity Atlas flora
158 records; environment.vic.gov.au/biodiversity/victorian-biodiversity-atlas), and Western
159 Australia (Flora Surveys of the Yilgarn; naturemap.dbca.wa.gov.au). We only included data
160 from complete floristic surveys conducted later than 2005 (to ensure consistency in
161 taxonomy) under government custodianship (to ensure consistent standards of survey
162 quality). As a result, our presence-absence survey data included 27,850 survey sites across
163 three states of Australia. Survey data are particularly valuable because they represent
164 presence/absence data, not just opportunistic presence records, and are not subject to the
165 same sampling biases as atlas or herbarium occurrence records. These presence-absence data
166 were analysed together with the presence locations. To represent degree of aridity and
167 salinity at each presence location for each species and each survey site, we used information
168 from the geographic layer of maximum monthly aridity index (Williams et al. 2012) and the
169 geographic layer of root zone soil electrical conductivity (Bui et al. 2017).

170 The majority of occurrence records for most species are presence-only records,
171 typically collected opportunistically, so their spatial distributions tend to be biased towards
172 regions that are more easily accessible to researchers, especially along road networks, and
173 areas of particular interest, such as protected areas or hotspots of diversity (Fithian et al.
174 2015; Haque et al. 2017). To correct for this sampling bias, we included two additional

175 factors: distance to the nearest road (using data from www.ga.gov.au/geoscience/national)
176 and whether it is in a protected area, such as a conservation reserve or national park (from
177 www.environment.gov.au/land/nrs/science/capad).

178

179 ***2. Niche evolution model***

180 The Niche Evolution Model (NEMo) uses a Bayesian framework that includes three major
181 components: model of niche evolution, phylogenetic comparative method (PCM), and
182 ecological niche model (ENM). NEMo models species distributions in niche space, not
183 geographic space. The aim is to infer not only species' niches at the contemporary tips of the
184 phylogeny, but also to estimate the amount of change in niches along every branch of the
185 phylogeny. This method has been shown to be effective at reconstructing past periods of
186 niche evolution and better than standard ENMs at predicting aspects of contemporary niches
187 (see Hua et al. submitted for details, available on bioRxiv).

188 The niche evolution component calculates how three aspects of niche (fundamental,
189 available, realized) change under three basic processes (speciation, adaptation, dispersal).
190 “Fundamental niche” represents the range of conditions that each species can tolerate at a
191 given point in time, expressed as a range of values along a relevant environmental axis. We
192 expect species to evolve along this environmental axis over evolutionary time, through the
193 processes of adaptation to new conditions (by selecting for individuals at the extreme values
194 of the distribution) and through speciation along environmental gradients (so that a species
195 splits along its tolerance range leaving one daughter species that is more skewed toward the
196 extreme conditions). “Available niche” describes the range of conditions in areas that are
197 accessible to the species, regardless of whether the species can persist in those conditions.
198 The potential of the species to occupy the available niche is governed by its fundamental
199 niche and dispersal ability. We also include barriers to dispersal as nuisance parameters,

200 which could represent physical or biotic barriers. “Realized niche” consists of the locations
201 where a species is found, which is shaped by the suitability of conditions and the opportunity
202 to disperse to and occupy those areas.

203 The PCM part of the model infers the locations on the phylogeny of adaptation and
204 speciation events, assuming these events are distributed along the branches of the phylogeny
205 according to a Poisson process. Dispersal in niche space is modelled as a continuous process
206 between any two events (adaptation or speciation), or nodes of the phylogeny. The
207 distribution of these events on the phylogeny gives a possible history of niche evolution.
208 Given this history, we use the model of niche evolution to calculate the three aspects of niche
209 of each tip species. The ENM component of the method then assesses the fit of the inferred
210 niches to the presence and/or absence locations of the tip species. The fit of the inferred
211 niches to the data then feeds back to our inferences of the history of niche evolution and the
212 niches of tip species. Details of the method, and description of tests of its performance and
213 reliability, are given in Hua et al. (submitted), and the code with step-by-step instructions for
214 implementing the model can be found at
215 <https://github.com/huaxia1985/NEMo>. Below we briefly describe the parameters of the
216 model applied to this case study.

217

218 ***3. Model Parameters***

219 NEMo has two universal parameters which are assumed to be constant across the phylogeny:
220 adaptation rate and dispersal rate. Additional parameters that are specific to each adaptation,
221 speciation, and dispersal events are: 1) amount of environmental change in an adaptation
222 event, 2) proportion of a tolerance range that is inherited by one descendent species (its sister
223 species inherits the other part) from the ancestral species in a speciation event, 3) where a
224 dispersal barrier locates along the environmental axis during a dispersal process.

225 We apply separate NEMo analyses to the evolution of environmental niches in *Acacia*
226 along aridity and along salinity. For each of these niche axes, the values for the available
227 niche and the fundamental niche at the root of the phylogeny were assumed to follow normal
228 distributions. The starting values for their means and standard deviations were set to their
229 maximum likelihood estimates given the likelihood of each tip species' presence-only data
230 and presence-absence data, assuming that no events of niche evolution occurred on the
231 phylogeny. The starting values for adaptation rate and dispersal rate were arbitrarily set to
232 0.03 and 1, respectively. Dispersal rate had rather flat prior, an exponential distribution with
233 rate of 0.1. The parameters for the niches at the root and the adaptation rate had relatively
234 narrow priors, a lognormal distribution with logarithmic mean of their initial values and
235 logarithmic standard deviation of 0.5. The narrow priors for the root niches were used to
236 ensure that root niches do not exceed observed maximum and minimum values (Williams et
237 al. 2012; Bui et al. 2017) too often during MCMCs. The narrow prior for adaptation rate was
238 used to ensure a finite likelihood value, as parameters of the fundamental niche change
239 proportionally to adaptation rate on a log scale, so there is a narrow range of adaptation rate
240 that gives finite likelihood values. The starting value for the occurrence rate of adaptation
241 events and the occurrence rate of speciation events was arbitrarily set to 0.1. A narrow prior
242 was applied on both occurrence rates, an exponential distribution with rate of 10 to reduce the
243 degree of overestimation on the number of events (see Hua et al. submitted). For both aridity
244 and salinity, we ran 20 independent MCMCs for 1×10^6 generations, with a thinning interval
245 of 1000 and the first 600,000 generations discarded as burnin.

246

247 ***4. Accounting for sampling bias***

248 We used an inhomogeneous Poisson point process (Renner et al. 2015) as the ENM
249 component of NEMo. Because opportunistic sampling bias can influence the niche estimation

250 (Fithian et al. 2015), we modified the likelihood function to account for sampling bias, using
251 the method of Fithian et al. (2015). The log-likelihood of the presence-only data of each tip
252 species is modified as,

$$253 \quad \ell_P = \sum_{i \in I_{PO}} [\log(\lambda_{r_i}) + \delta z_i] - \frac{\mathcal{D}}{N} \sum_{i \in I_{BG}} \lambda_{f_i} \lambda_{a_i} e^{\delta z_i}$$

254 , where z_i are values of the factors that influence sampling effort at the i^{th} location and δ are
255 the coefficients of the two sampling factors we included - distance of the location to the
256 nearest road and whether the location is in a protected area - which are assumed to have the
257 same value for all tip species. The starting values of coefficients were set to their maximum
258 likelihood estimates given the likelihood function, and a rather flat prior was used: an
259 exponential distribution with rate of 0.1. In the second term of the equation, λ_f is the inferred
260 fundamental niche of a tip species, giving the proportion of individuals of the species that can
261 tolerate a certain level of aridity and salinity, and λ_a is the inferred available niche of the tip
262 species, giving the proportion of areas accessible to the species that have certain level of
263 aridity and salinity. If we were not accounting for sampling bias, the second term of the
264 equation would be a direct integral of $\lambda_f \lambda_a$ (Hua et al. submitted), but because each location
265 now has a specific value for sampling bias, we can only approximate the integral by
266 randomly sampling N locations from the surroundings of the species presence locations to
267 construct a set of background locations I_{BG} and averaging the term to integrate over all the
268 background locations. Note that these background locations are only used to numerically
269 approximate the second term of the equation, so they are not pseudo-absence locations
270 (Fithian et al. 2015).

271 For each *Acacia* species, background locations were sampled uniformly from areas
272 within an arbitrary large radius (20 km) of each presence location of the species, so the
273 weight of each background location is $\frac{\mathcal{D}}{N}$, where \mathcal{D} is the total area that falls within the 20 km

274 radius of each species presence location, and N is the total number of background locations,
275 which was ten times the number of the species presence locations, with a minimum of 1000
276 for species with few presence locations. Based on the environmental conditions over all the
277 background locations, we used Sturges' formula (Sturges 1926) to decide the bin size to
278 discretise the aridity index and the electrical conductivity.

279

280 ***5. Estimating rate and amount of niche evolution***

281 The amount of niche evolution per branch is calculated as the sum of the differences in the
282 mean of the realized niche before and after each event (both adaptation and speciation events)
283 inferred along each branch of the phylogeny over the posterior samples, calculating both the
284 net amount (positive or negative) and the absolute amount. The net amount of niche evolution
285 divided by branch length gives the direction of niche evolution, and the absolute amount of
286 niche evolution divided by branch length gives the rate of niche evolution. Last, we
287 calculated the intensity of the inferred environmental change during adaptation events at each
288 time point over the whole phylogeny and compared the distribution of this intensity over time
289 to the aridification history of Australia (see details in Hua et al. submitted).

290

291 **Results**

292 ***1. Spatial Patterns of Niche Evolution***

293 The *Acacia* phylogeny is characterized by a mixture of large, young clades (recent, rapid
294 divergences), and smaller, older clades (longer-persisting lineages: Figure 1). Three recent
295 radiations, each around 1 Ma old, include most present-day species in Northern Australia,
296 Southern Australia, and Eastern Australia. Of these, the Northern Australian clade has the
297 highest average rate of niche evolution on both aridity and salinity axes (Figure 1). The
298 smaller, older clades are mainly in Western Australia, with many extant species originating

299 earlier than the three large radiations. These smaller older clades have, on average, much
300 lower rate of niche evolution on both aridity and salinity axes than the recent radiations
301 (Figure 1). Present-day species that occupy regions that are arid and prone to high salinity
302 (deserts and Mediterranean ecosystems) are found throughout the phylogeny, forming small
303 clusters of related species. In contrast, present-day species that occupy the mesic zone are
304 found mainly in young clades as large clusters of closely-related species.

305

306 The net amount of niche evolution inferred by NEMo along the phylogeny is shown in Figure
307 1. Most of the evolutionary change on both aridity and salinity axes has been pushing species
308 towards tolerance of the extreme conditions. We can see this in the patterns of niche
309 evolution along the aridity axis in different habitat types (Figure 2A). The largest changes in
310 niche are in lineages currently found in the most arid environments, while little or no niche
311 shift is in lineages currently distributed in forests. The same pattern is repeated over the
312 whole phylogeny, and also within each of the recent radiations (Figure 2A). Similarly, on the
313 salinity axis, NEMo inferred the greatest amount of niche evolution towards higher salinity
314 leading to species currently distributed in the Mediterranean ecosystems (Figure 2B). The
315 same pattern is inferred in the three recent radiations, where there is little change along the
316 salinity axis in lineages currently distributed in forests (Figure 2B).

317

318 ***2. Temporal Patterns of Niche Evolution***

319 On the aridity axis, the inferred intensity of environmental change during each adaptation
320 event peaks at 13 Ma and near the present (after 2.5 Ma), corresponding to major drying
321 periods of Australia (Figure 3). On the salinity axis, the inferred intensity of environmental
322 change during each adaptation event peaks near the present (after 2.5 Ma), with two
323 additional lower peaks around 10 Ma and 17 Ma (Figure 3).

324 Across the phylogeny, the net amount of inferred niche evolution due to both
325 adaptation and speciation events along the salinity and aridity axes showed a significant
326 positive correlation (Figure 4; Kendall's rank correlation coefficient=0.25, $p<0.001$).
327 Although significant, the correlation is mostly driven by niche evolution along the terminal
328 branches, and there is a great deal of scatter, with relatively more aridity evolution on tip
329 branches leading to present-day species found in the arid zone (Deserts & Xeric Shrublands),
330 and relatively more salinity evolution on tip branches leading to present-day species found in
331 Mediterranean ecosystems. The greater amounts of aridity and salinity evolution along the tip
332 branches are not due to longer branch length, as NEMo also infers large amount of aridity
333 and salinity evolution along very short tip branches (Figure 4).

334

335 **Discussion**

336 Aridification has been the prevailing environmental trend across most of the Australian
337 continent through the Neogene and Quaternary. In order to understand the history underlying
338 Australia's current plant diversity, we need effective methods for reconstructing the
339 evolutionary responses of plant clades to the environmental changes brought about by
340 aridification. In this study, we use the large, widespread genus *Acacia* as a case study to
341 reconstruct its history of niche evolution along aridity and salinity axes, using the newly
342 developed method NEMo. We reconstruct both spatial and temporal patterns in niche
343 evolution of *Acacia* and show (1) a preponderance of evolutionary change in niches towards
344 more extreme values of aridity and salinity, (2) a concentration of niche evolution in the
345 ancestors of species currently found in arid and Mediterranean regions in both the old clades
346 and the recent radiations, (3) peaks in niche evolution throughout the aridification history of
347 Australia, (4) correlated evolution of aridity and salinity tolerance, particularly along tip
348 branches. As we explain below, these results are consistent with the adaptation of *Acacia*

349 lineages to drier climates, allowing them to exploit new niches opening up through the
350 aridification of the continent. Our results point to an evolutionary flexibility in *Acacia* that is
351 likely to have played a key role in their current near-ubiquitous distribution and dominance of
352 many habitats across Australia.

353 The preponderance of niche evolution towards more extreme values of both aridity
354 and salinity suggests that aridification has been a dominant driver of *Acacia* evolution and
355 diversification across Australia during the Neogene & Quaternary. The concentration of
356 niche evolution on branches leading to species currently found in more arid and salt-affected
357 regions, in both old and recent clades, suggests that the recent radiations of *Acacia* were not
358 triggered by adaptation to aridity and salinity. Instead, extant species occupying arid and salt-
359 affected regions are likely to have evolved from ancestral lineages in mesic areas which do
360 not show high rates of niche evolution along these axes. This interpretation indicates
361 evolutionary flexibility in *Acacia*, which is supported by the high rate of niche evolution
362 along both aridity and salinity axes in the three recent radiations. This enhanced rate of niche
363 evolution was also found by a recent study of diversification in *Acacia* using traditional
364 PCMs, which hypothesized that Pleistocene glacial-interglacial climate cycling (< 2.5Ma)
365 drove rapid climatic adaptation, which promoted diversification and led to large radiations
366 (Renner et al. 2020).

367 Unlike traditional PCMs, NEMo is able to identify key events of niche evolution
368 along the *Acacia* phylogeny. We found that most changes in niche along the aridity axis were
369 in the recent radiations, predominantly after 1Ma and along tip branches. But niche evolution
370 in aridity was also inferred along two branches ancestral to all these radiations (marked with
371 asterisk in Figure 1), between 12 to 15 Ma and around 5Ma (Figure 1). It is likely that the
372 niche evolution events that occurred along the two ancestral branches left descendant
373 lineages of *Acacia* able to tolerate higher aridity than the other lineages in Tropical and

374 Temperate forests and grasslands during the Pleistocene, so that *Acacia* was able to replace
375 many other groups during the drying period of the Pleistocene. These two pulses of niche
376 evolution along the aridity axis are also consistent with the aridification history of Australia.
377 *Acacia* emerged during a period of aridification (28-23Ma), and we found peaks of
378 adaptation to increasing aridity in *Acacia* during the two subsequent drying periods, 14-5Ma
379 and after 5Ma (Byrne et al. 2008).

380 Comparing salt tolerance to aridity tolerance in *Acacia*, we found that the amount of
381 niche evolution along aridity and salinity axes was highly correlated across branches of the
382 *Acacia* phylogeny. This result is consistent with the observation that saline soils are
383 frequently associated with aridity, so many species are adapted to both aridity and salinity
384 (Gong et al. 2017). Correlated evolution was also suggested between salinity and alkalinity
385 tolerance in *Acacia* using phylogenetic independent contrasts (Bui et al. 2014b). The NEMo
386 method offers a way to account for phylogenetic nonindependence in testing correlated
387 evolution, without making *a priori* assumptions about the phylogenetic covariance matrix. In
388 addition, the NEMo method allows us to detect branches that have large amounts of niche
389 evolution along both aridity and salinity axes. These branches are mostly terminal branches
390 scattered across the *Acacia* phylogeny and are mostly ancestral to species currently
391 distributed in areas with both high aridity and high salinity. This result is consistent with high
392 niche lability along both aridity and salinity in Australian *Acacia*, as suggested by the high
393 rate of niche evolution in the three radiations.

394 Inferred peaks of increased salinity are on recent lineages, which is consistent with
395 previous findings of a phylogenetically “tippy” distribution of salt tolerant species in grasses
396 (Bennett et al. 2013; Moray et al. 2015). Consequently, even if salt tolerance has evolved
397 many times through old adaptation events, these events rarely gave rise to persistently salt
398 tolerant lineages of large clades of related salt tolerant taxa, leaving less phylogenetic signal

399 for us to reconstruct adaptation events in the deep part of the phylogeny than adaptation
400 events near the tip of the phylogeny (Bromham 2016). The high niche lability in Australian
401 *Acacia* further agrees with previous hypothesis that the tippy distribution of salt tolerant
402 species is due to high niche lability (Bromham et al. 2016).

403 It is important to note that for the NEMo analysis, as for most macroevolutionary
404 analyses, we have assumed that the phylogeny has accurate topology and provides an
405 approximate scaling of evolutionary events in time. Clearly, phylogenetic estimates of either
406 topology or the timing of evolutionary events are not error-free (Bromham et al. 2018;
407 Guindon 2020; Nie et al. 2019). In particular, uncertainty or bias in branch length estimates
408 arising from imperfect characterisation of molecular rates over time may have a non-trivial
409 impact on macroevolutionary inference (Duchêne et al. 2017). Phylogenies such as this one
410 where the only calibration is a root date may be particularly vulnerable to errors in position of
411 node heights in some parts of the tree (Duchêne et al. 2014). One possible solution is to apply
412 the NEMo analysis on posterior samples of phylogenies, but perhaps a better advance will be
413 to embed the NEMo method into phylogenetic reconstruction, which allows us to directly
414 link niche evolution events to diversification events (Hua et al. submitted). Another issue
415 with our current inference of niche evolution in *Acacia* is incomplete sampling, as the *Acacia*
416 phylogeny only includes half of the known extant species. One obvious solution is to sample
417 more species, but another way to increase the amount of information in the analysis of niche
418 evolution is to incorporate historical data, such as paleoclimate, as priors for the occurrence
419 density of niche evolution events, to supplement current species distribution data (Hua et al.
420 submitted). Here we have used only part of the available information in the inference process
421 in order to demonstrate the utility of the method (so we can compare our inferred
422 evolutionary history to known paleoclimatic history), but the method allows for all available
423 information to contribute to stronger inference.

424

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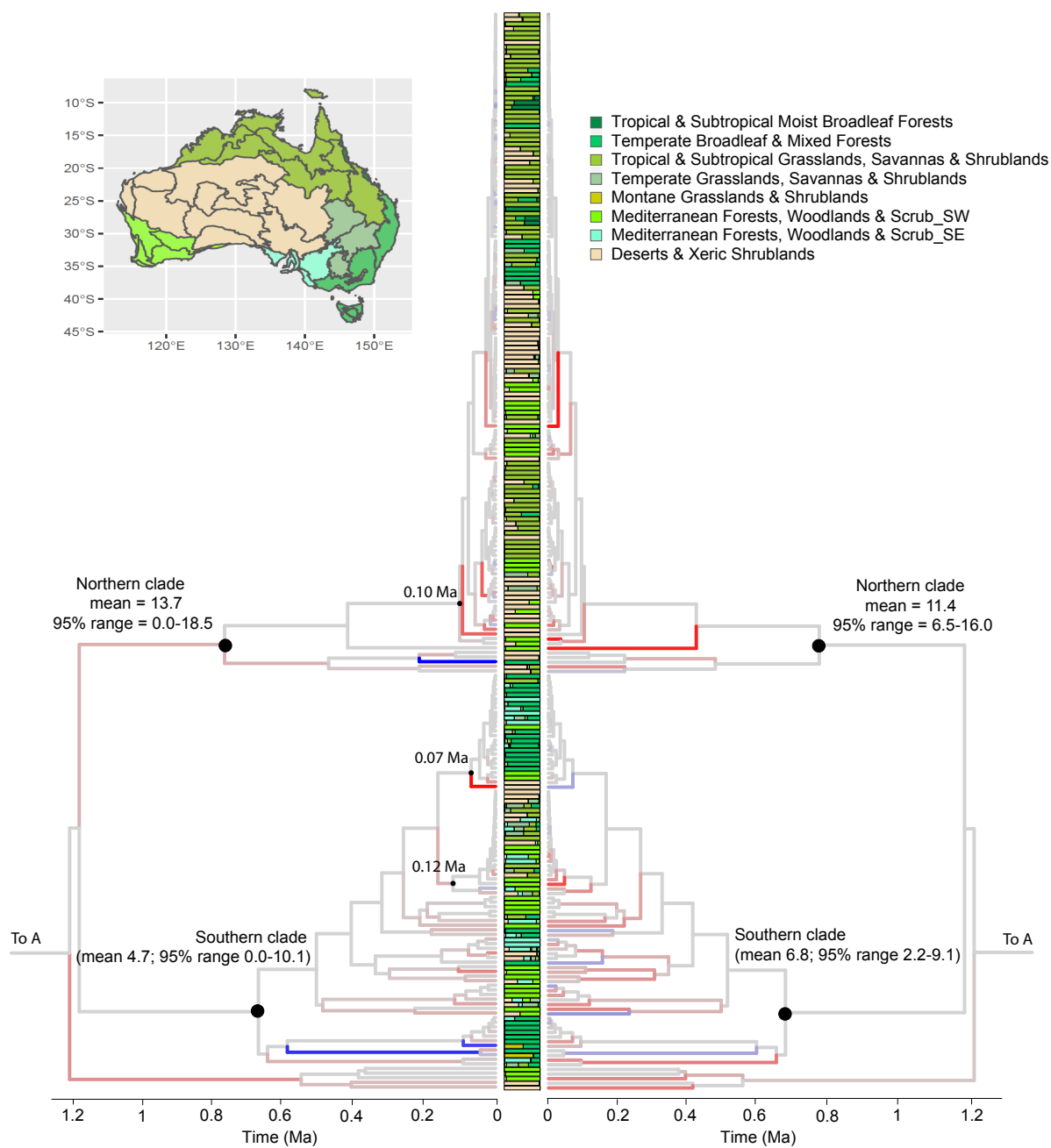
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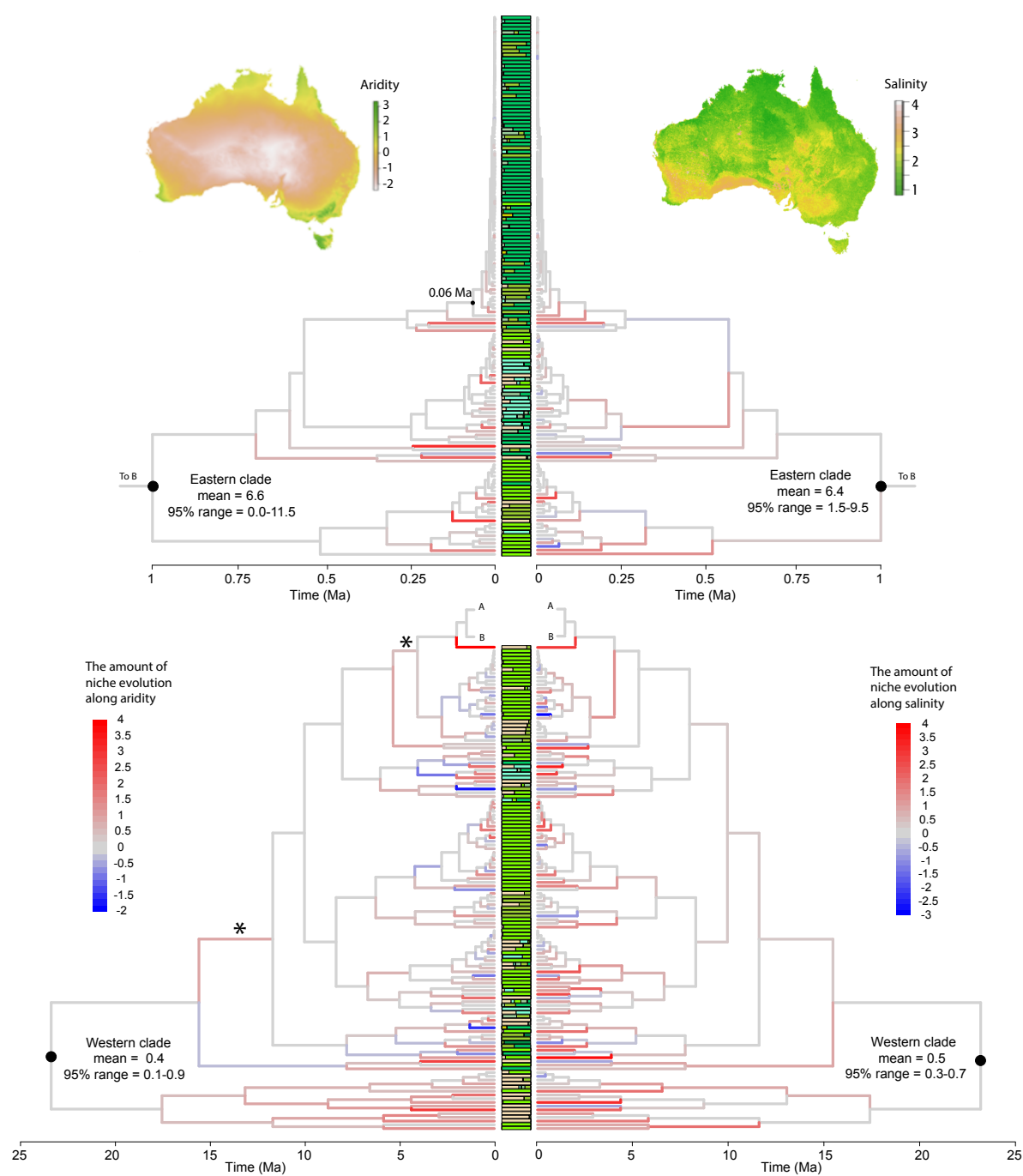
543 Figure 1. Phylogenetic distribution of the inferred amount of niche evolution along axes of
544 aridity (left) and salinity (right). The blue to red color scale gives the sum of the amount of
545 change in the mean of realized niche values before and after each niche evolution event
546 inferred along each branch, averaged over posterior samples. Shifts towards less extreme
547 conditions are in blue and shifts towards more extreme conditions are in red. The ecoregion
548 map shows the current distribution of eight ecoregions in Australia. Each tip species has a
549 stacked bar showing the relative proportion of its current presence locations in each
550 ecoregion, in the same color scheme as the ecoregion map. We also include two additional
551 maps. Left one shows the spatial variation in the aridity index in Australia, with lower values
552 in more arid areas. Right one shows the variation in root zone soil electrical conductivity,
553 with higher values in areas with higher salinity. The mean and the 95% range of estimated
554 rate of niche evolution over all posterior samples are marked for each major clade in the
555 phylogeny.



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557

558 Figure 1 cont.

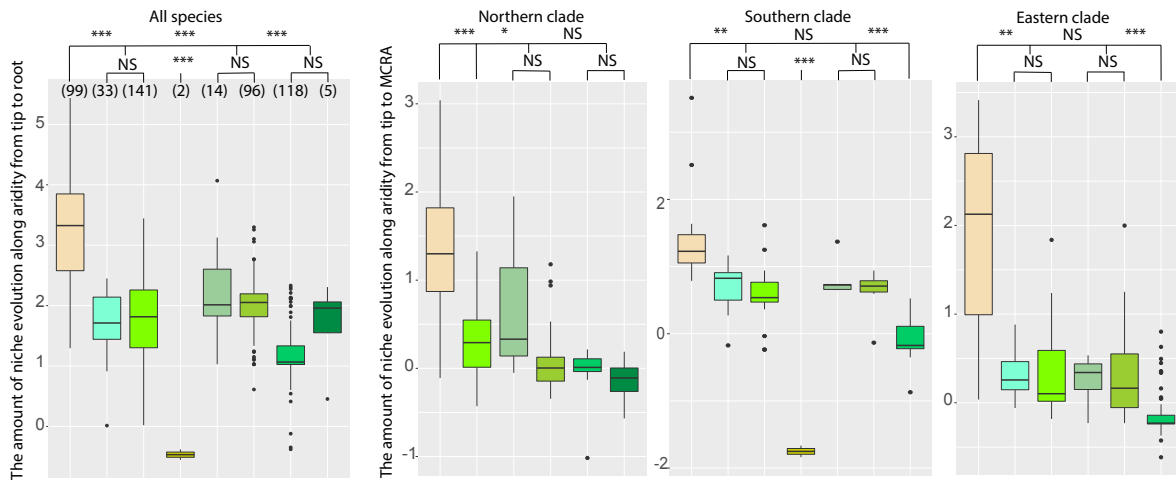


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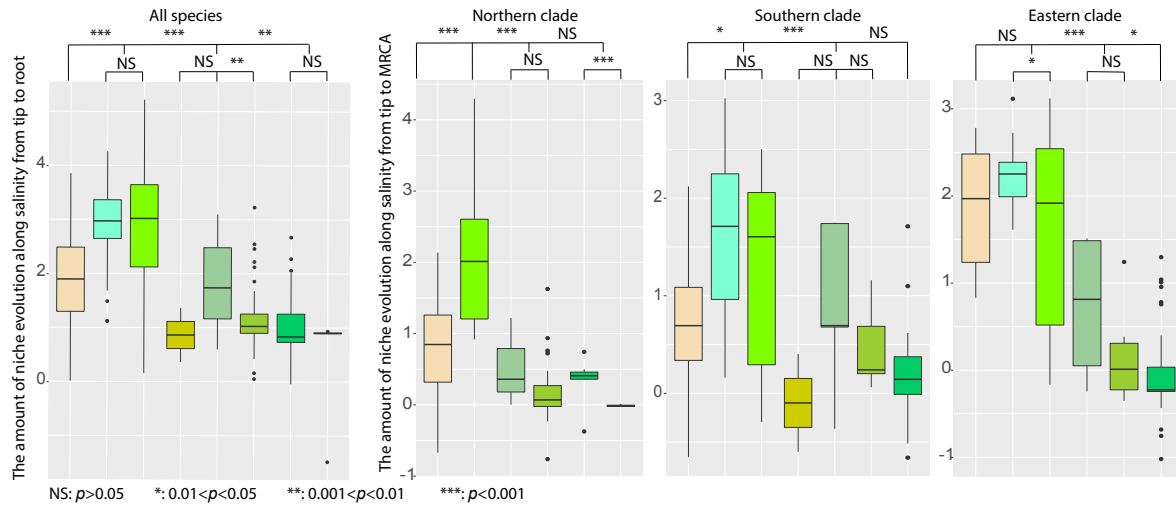
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561 Figure 2. Boxplots of the net amount of niche evolution along aridity axis A) and salinity axis
562 B). “All species” plots the sum of the amount of niche evolution along the branches that
563 connect the root to each tip of the phylogeny. “Northern clade”, “Southern clade”, “Eastern
564 clade” plot the sum of the amount of niche evolution along the branches that connect the
565 MCRA of the three clades of recent radiation to each tip in these clades in Figure 1. Tips are
566 grouped by the ecoregion that the majority of current presence location of the tip species fall
567 in, using the same color scheme as the ecoregion map in Figure 1. T-test is used to first
568 compare within vegetation types: desert, Mediterranean, grasslands, forests. If regions of the
569 same vegetation type do not differ, then these regions are grouped and compared to other
570 vegetation types. The statistical significance of a t-test between two bioregions linked by
571 lines is shown above the corresponding horizontal line.

A) The amount of niche evolution along aridity axis



B) The amount of niche evolution along salinity axis



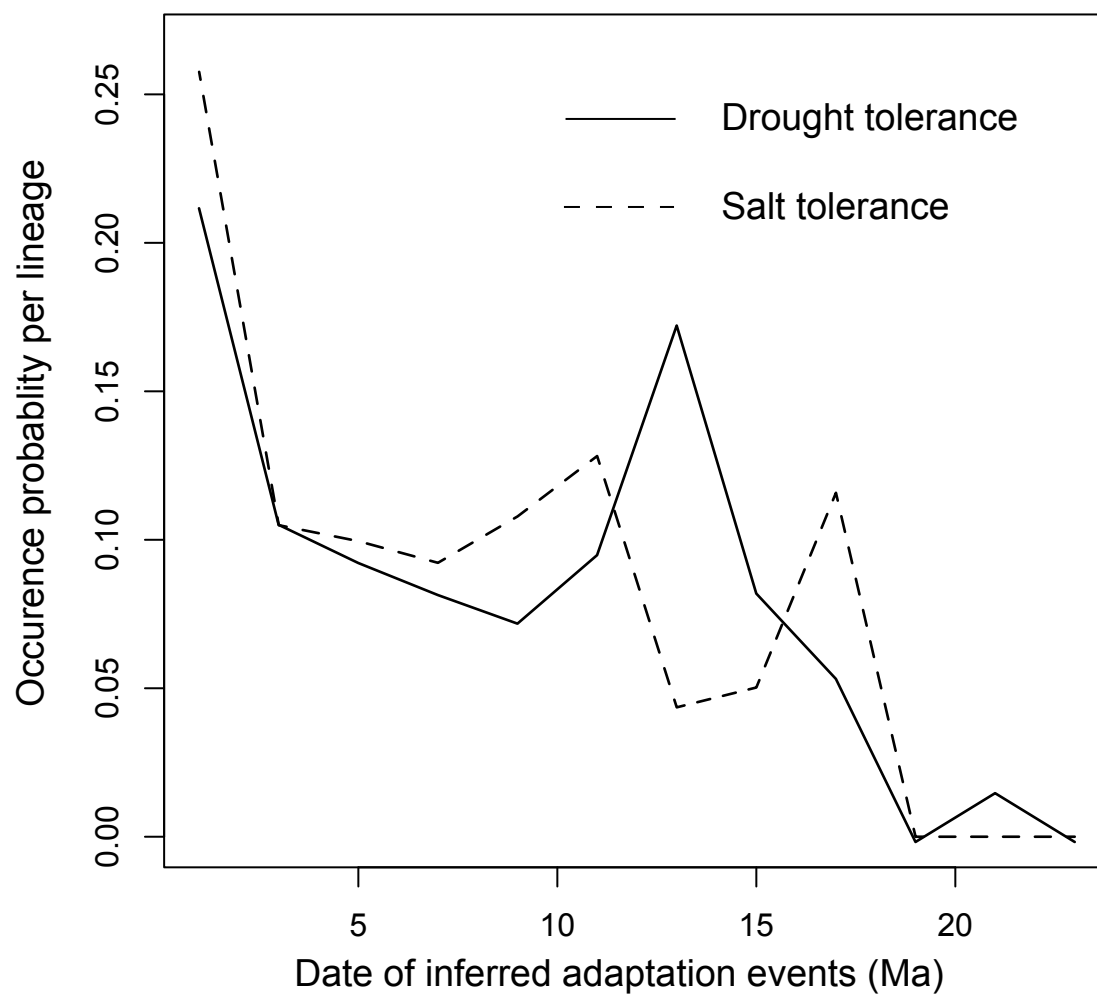
NS: $p > 0.05$ * : $0.01 < p < 0.05$ ** : $0.001 < p < 0.01$ *** : $p < 0.001$

- Deserts & Xeric Shrublands
- Montane Grasslands & Shrublands
- Temperate Grasslands, Savannas & Shrublands
- Tropical & Subtropical Grasslands, Savannas & Shrublands
- Mediterranean Forests, Woodlands & Scrub_SE
- Mediterranean Forests, Woodlands & Scrub_SW
- Temperate Broadleaf & Mixed Forests
- Tropical & Subtropical Moist Broadleaf Forests

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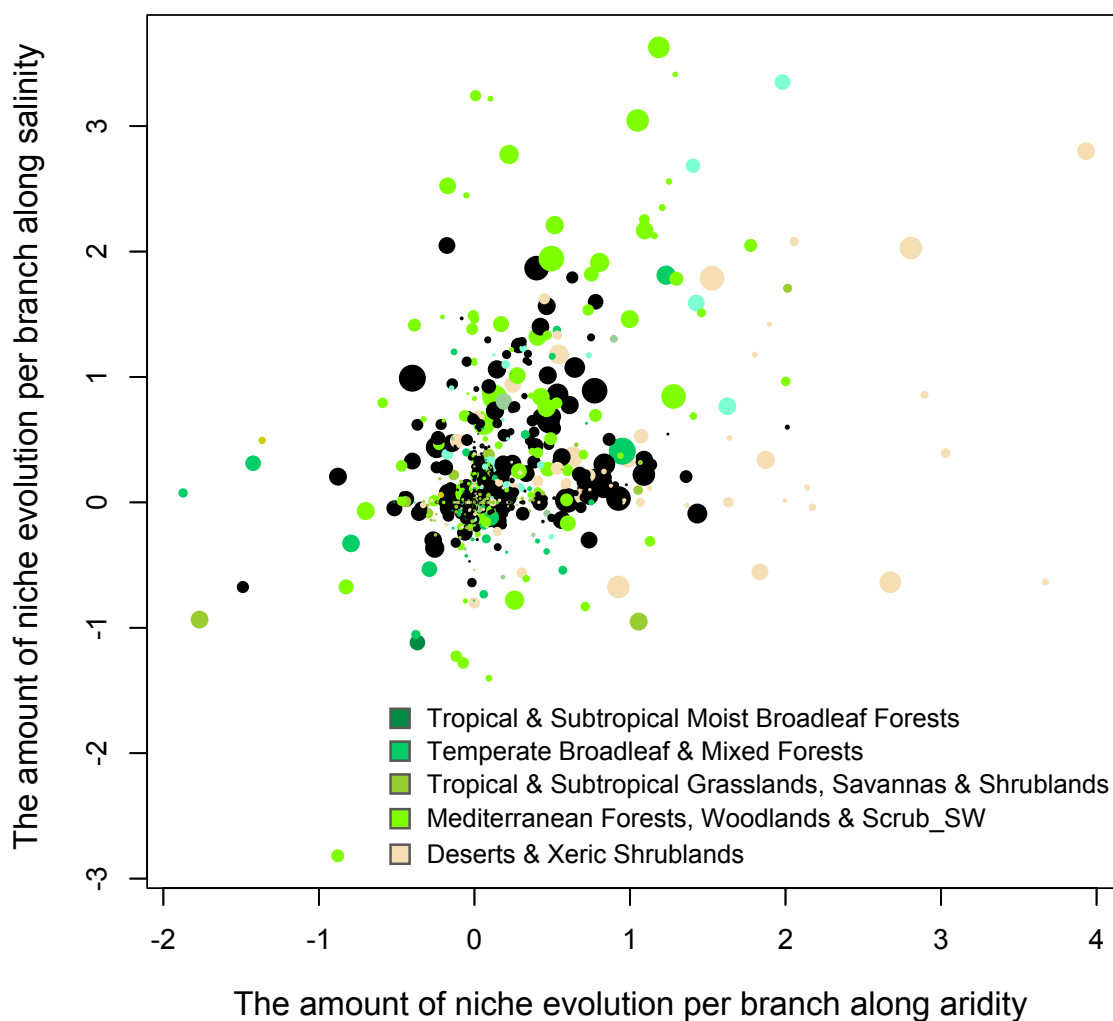
573

574 Figure 3. Histogram of the inferred dates of adaptation events in *Acacia* drought tolerance
575 (solid) and salt tolerance (dashed).



576

577 Figure 4. Correlation in the amount of niche evolution per branch along aridity and salinity.
578 Each datapoint is a branch, with internal branches in black. The size of each datapoint is
579 proportional to the tenth root of the branch length. Terminal branches are colored by the
580 ecoregion that the majority of current presence location of the corresponding tip species fall
581 in, using the same color scheme as the ecoregion map in Figure 1.



582