

Original Article

Rise and fall of a continental mesic radiation in Australia: spine evolution, biogeography, and diversification of *Cryptandra* (Rhamnaceae: Pomaderreae)

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ABSTRACT

The Australian continent has experienced progressive aridification since the Miocene, spurring recent radiations of arid-adapted lineages and the likely decline of mesic biotic groups. While examples of the former have been relatively well-documented, post-Miocene declines of non-arid sclerophyllous floras are less well understood. Here, we present a well-sampled time-calibrated nuclear phylogeny (140 accessions representing 60/65 species) of an Australian plant genus (*Cryptandra* Sm.: Rhamnaceae) and using ancestral range reconstructions and diversification analyses, elucidate its evolutionary history through space and time. We used high-throughput sequencing to recover 30 orthologous nuclear loci and BioGeoBEARS to infer ancestral areas. We show that the present-day distribution of *Cryptandra* can be explained by multiple vicariance events followed by *in situ* diversification with little exchange between regions. All diversification models show a speciation rate decline in *Cryptandra* after its radiation in the Miocene (*c.* 23 Mya). This coincides with aridification episodes across Australia and indicates that diversification of this genus has been negatively affected by the expansion of aridity. We also show that there were no significant differences in diversification rates between spinescent and non-spinescent *Cryptandra* lineages, suggesting that spinescent lineages may be the legacies of selection from extinct megaherbivores.

Keywords: Australian flora; biogeography; *Cryptandra*; high-throughput sequencing; molecular phylogeny; Rhamnaceae; speciation rate decline; spines; vicariance

INTRODUCTION

Several iconic, sclerophyllous, endemic lineages among Gondwanan families (e.g. Proteaceae, Myrtaceae, Goodeniaceae, and Restionaceae) are characteristic features of the Australian flora (Burbidge 1960). Many of these lineages diversified in the Eocene–Miocene, replacing rainforest Gondwanan lineages as the continent progressively cooled and became more seasonal and arid (Crisp *et al.* 2004, Crisp and Cook 2013). Paleorecords have indicated that these temperate Australian sclerophyllous plant groups subsequently declined in dominance and retreated towards the mesic periphery of the continent as the arid zone expanded from the Pliocene to the present (Macphail 2007,

Byrne *et al.* 2011). A signal of diversification decline (i.e. either from lower speciation or higher extinction rates, or both) might therefore be expected in phylogenies of extant sclerophyllous Australian lineages. However, available phylogenetic studies instead tend to show a steady accumulation of mesic temperate species over time, both across the flora generally (Nge *et al.* 2020) and in selected groups (Cardillo and Pratt 2013, Cook *et al.* 2015, Nge *et al.* 2021a, 2022). Broad-scale studies such as that of Nge *et al.* (2020) may not detect recent signals of diversification decline due to low taxonomic sampling (Cusimano and Renner 2010); hence, further studies using densely sampled species-level phylogenies are required.

The Australian plant genus *Cryptandra* Sm. (Rhamnaceae) is a good candidate for addressing this hypothesis. *Cryptandra*, with at least 60 species (Kellermann 2020), is the second-largest genus in Rhamnaceae tribe Pomaderreae (Kellermann *et al.* 2005, Medan and Schirarend 2004; Fig. 1). It is endemic to Australia, and is widely distributed across the continent, with most species in south-western, southern, and eastern mainland Australia and seven taxa in the northern monsoon tropics (Kellermann 2006; Fig. 2). *Cryptandra* species are dwarf to medium-sized shrubs, often with an ericoid habit. Some species have spinescent branches, an uncommon trait among Pomaderreae (only found in two other species of *Trymalium* Fenzl in the tribe) and a trait that is equally unusual for the Australian flora (where leaf spines are more common; Orians and Milewski 2007). These spinescent *Cryptandra* often have a divaricate ‘cage’ architecture growth form similar to many shrubs in New Zealand (Burns 2016, Maurin *et al.* 2022).

Spinescence may protect against medium-sized to large herbivores, and it has been speculated that this may play a role in the evolutionary survival and diversification of spinescent lineages in Australia (Orians and Milewski 2007, Burns 2014). The origins and diversification of spinescent plants closely follow the radiation of large mammals or avian herbivores, as is the case for New Zealand during the Pliocene–Pleistocene (Maurin *et al.* 2022), central Tibet in the Eocene (Zhang *et al.* 2022), and African savannas in the Miocene (Charles-Dominique *et al.* 2016). The diversification of spinescent plants in Australia occurred relatively recently in the Pliocene compared to other continents (Gelin *et al.* 2023). However, whether the evolution of spinescence has resulted in diversification rate increases specifically compared to non-spinescent congeners is not well known. For other defence traits such as extrafloral nectaries, plant families with this trait have 2-fold higher diversification rates than those without (Weber and Agrawal 2014). Similarly, in the large Australian *Solanum* L. radiation, the majority of species are spinescent (suggesting higher diversification rates), although no formal diversification analyses have been conducted to date (Echeverría-Londoño *et al.* 2020). *Cryptandra* would be a good case-study group to investigate further the diversification of spinescence in an Australian context, given the genus is widely distributed across Australia and has both spinescent and non-spinescent lineages.

A small number of *Cryptandra* species have been included in previous phylogenies of Rhamnaceae and Pomaderreae based on ITS and *trnL*-F DNA sequence data (e.g. Richardson *et al.* 2000, one species; Kellermann *et al.* 2005, 15 species; Ladiges *et al.* 2005, 15 species). Later family-wide phylogenies of Rhamnaceae (Onstein *et al.* 2015, Hauenschield *et al.* 2016) also included small numbers of *Cryptandra* species, mostly using previously published DNA sequence data. Comprehensive, multi-gene phylogenies have recently been developed for two Pomaderreae genera: *Pomaderris* Labill. (Nge *et al.* 2021b) and *Spyridium* Fenzl (Clowes *et al.* 2022). Nge *et al.* (2021b) showed that *Pomaderris* was ancestrally widespread and detected a vicariant signal between the western and eastern clades coinciding with the uplift of the calcareous Nullarbor Plain at *c.* 14 Myr (Crisp and Cook 2007).

Here, we present a densely sampled multi-gene phylogeny of *Cryptandra* and use this as the basis to (i) explore continental biogeographic patterns in the genus, (ii) test whether patterns of lineage diversification have been influenced by the post-Miocene progressive aridification of the Australian continent, and (iii) test whether spinescence has affected diversification among *Cryptandra* lineages.

MATERIAL AND METHODS

Sampling and DNA sequencing

We sampled 140 accessions representing 60 species (92% of 65 species in the genus). Multiple samples of two putative species complexes (29 and 18 for *C. tomentosa* Lindl. and *C. amara* Sm. groups, respectively) and five potentially new taxa were included to enable testing of taxonomic boundaries in these groups for ongoing taxonomic research. Sixteen Pomaderreae species and two other Rhamnaceae taxa (nine Pomaderreae genera and two non-Pomaderreae: *Granitites* Rye and *Hovenia* Thunb.) were included as outgroups. Voucher and accession information is in Table S1 in the Supporting Information.

We adopted a high-throughput sequencing (HTS) approach using the OzBaits v.1.0 custom bait set (Waycott *et al.* 2021), which targets 100 single-copy nuclear loci for angiosperms. This bait set has been used successfully for Rhamnaceae (Nge *et al.* 2021b) and other plant groups (Foster *et al.* 2021, Nge *et al.* 2021a, Simpson *et al.* 2022). Approximately 20 mg of silica-dried leaf material sourced from herbarium or field-collected specimens were used for DNA extractions performed by Intertek Group plc (Adelaide, Australia) using sbeadex magnetic bead chemistry. Library preparation steps followed Nge *et al.* (2021b). Briefly, extracted DNA was sheared using a Diagenode Bioruptor Pico sonicator to fragment lengths of *c.* 400–600bp, DNA libraries were constructed using JetSeq Flex DNA Library preparation kit (Bioline), hCLC capture was performed following the manufacturer’s instructions (Arbor Biosciences), and libraries were pooled in equimolar concentrations and sent for Illumina paired-end sequencing (2 × 150 bp) on a lane of a HiSeqX Ten at the Garvan Institute for Medical Research in Sydney.

Bioinformatic data processing

Post-sequencing data processing followed the protocol of Nge *et al.* (2021b). Briefly, raw sequence data were imported into CLC Genomics Workbench (v.7.5.1) for demultiplexing and trimming (remove adapters and barcodes, quality trim *Q* > 20). We used the *de novo* assembly of a single sample (*Pomaderris buchanensis* N.G. Walsh FN 451) to generate a set of Rhamnaceae specific OzBaits references. Following *de novo* assembly in CLC, the resultant contigs were converted into a BLAST database in Geneious v.2022.2.2 (Kearse *et al.* 2012; <https://www.geneious.com>) that was queried using the OzBaits reference sequences for *Arabidopsis thaliana* (L.) Heynh. (maximum expected value 1e-5). The BLAST hits were then extracted and used as a reference to recover per locus read mappings for each of the *Cryptandra* samples in CLC. A majority-rule consensus sequence was extracted for each locus per sample, with ‘Ns’ inserted when coverage was < 5, and multiple sequence alignments were performed using the Muscle (Edgar 2004) plugin

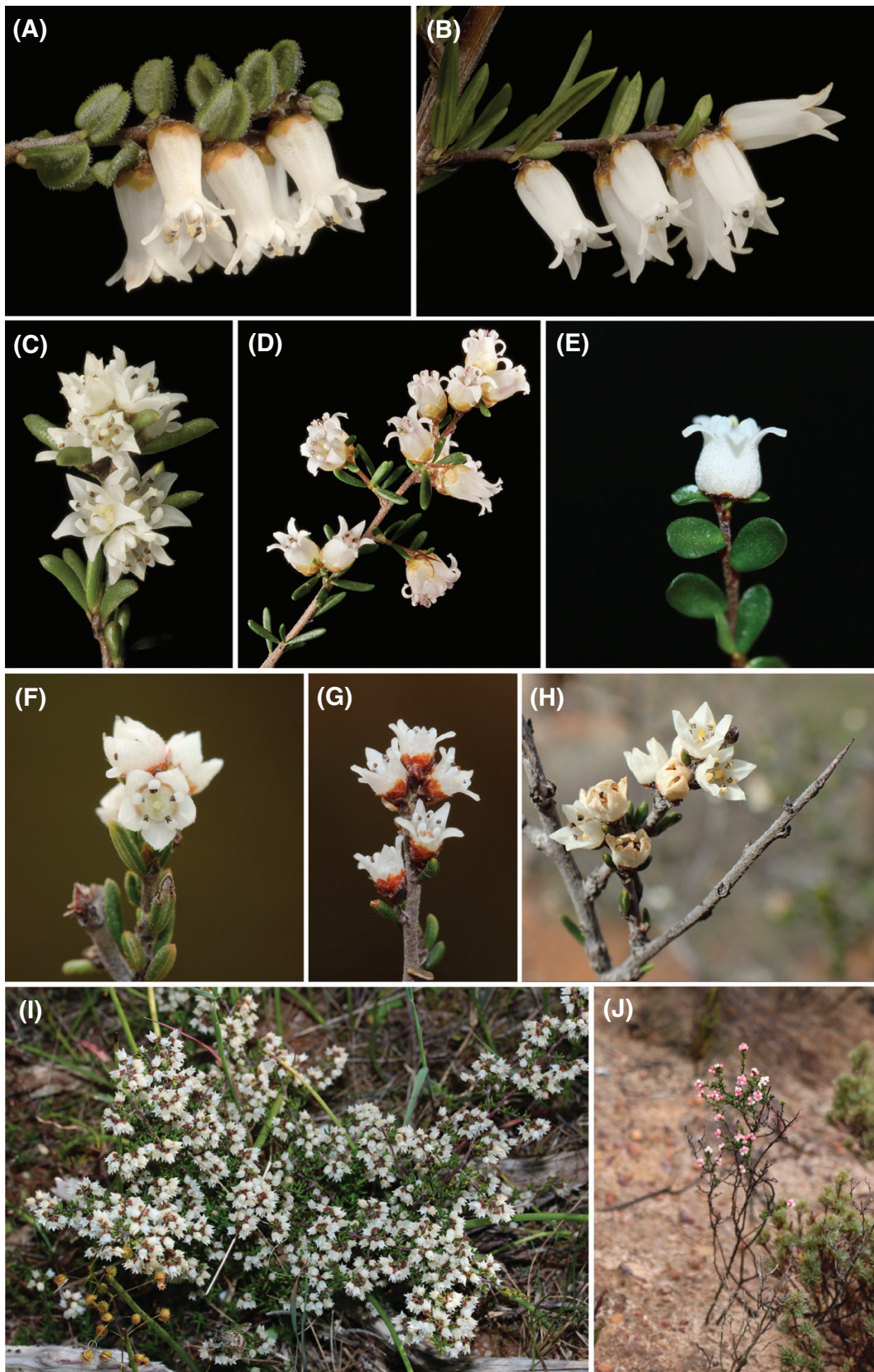


Figure 1. Representative diversity of *Cryptandra*: A, *C. arbutiflora* var. *arbutiflora* (KRT3328); B, *C. arbutiflora* (KRT3234); C, *C. myriantha* Western Australian form (KRT3737); D, *C. connata* (KRT4163); E, *C. amara* (FN814); F, *C. tomentosa*; G, *C. myriantha* South Australian (SA) form; H, *C. setifera* (JK750) showing a spinose branch; I, *C. tomentosa* shrub habit; and J, *C. myriantha* SA habit. Photos: Kevin R. Thiele (A–D), Francis J. Nge (E–G, I, J), Jürgen Kellermann (H).

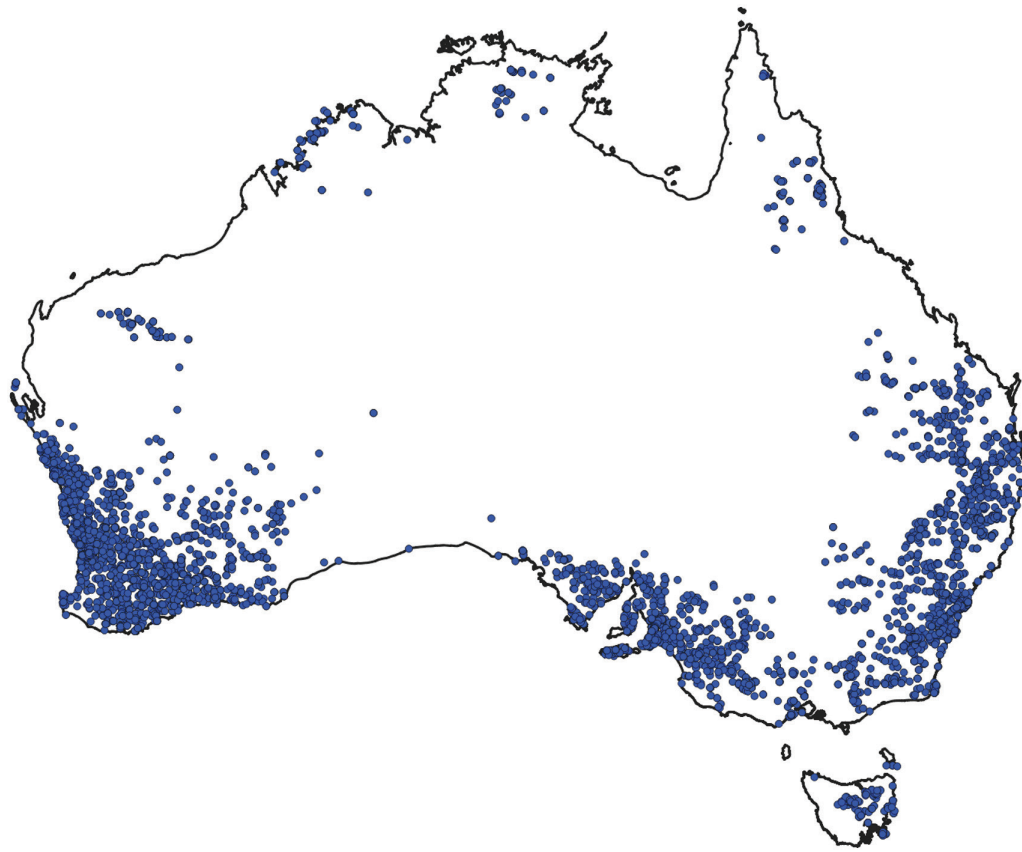


Figure 2. Distribution map of *Cryptandra* based on herbarium vouchered records sourced from the Australasian Virtual Herbarium (avh.ala.org.au).

for Geneious. Paralogs were identified during the BLAST step (before) with the assumption that divergent and overlapping contigs recovered from a single target locus indicated paralogy. We retained 30 putative orthologs from the 100 original targeted nuclear loci comprising the final orthologous dataset.

Phylogenetic analyses and divergence dating

We generated an overall phylogeny comprising 62 taxa of *Cryptandra* (60/65 spp.) and including only one representative per species for divergence time, and biogeographic and diversification analyses (hereafter, 60-ALIGN). For *Cryptandra amara*, we also included two forms of *C. amara* in the phylogeny as they probably represent two distinct entities (*C. sp.* Bundanoon (K.R.Thiele 2511) Kellermann and *C. sp.* Watchimbark (R.L.Barrett *et al.* 9116) Kellermann; see Barker 2005), indicated by ongoing taxonomic work (Kellermann *et al.* unpublished).

Divergence times were estimated using BEAST v.2.4.7 (Bouckaert *et al.* 2014). As there are no reliable fossils of Pomaderreae, we used five Pomaderreae node ages from Nge *et al.* (2021b) as secondary calibrations. These are based on a family-wide molecular clock analysis that used four Rhamnaceae fossil constraints to estimate node ages. Our approach ensures that our results are comparable with previous studies, while enabling the use of our full nuclear dataset instead of a select few loci that overlap with outgroup taxa based on Sanger data. Multiple (five) calibration points were implemented as per recommended best

practice for estimating divergence ages based on secondary calibrations (Sauquet *et al.* 2012). All five calibration points were assigned uniform priors to better account for uncertainties in these analyses (Sauquet pers. comm.): Pomaderreae stem (95% confidence intervals from Nge *et al.* (2021b); 32.4–50 Mya), Pomaderreae crown (32.4–49.69 Mya), *Stenanthemum* Reissek crown (19.7–35 Mya), *Pomaderris* crown (14.7–36.74 Mya), and *Cryptandra* stem (29–45 Mya). The *Cryptandra* crown was unconstrained.

The 60-ALIGN (concatenated 30-gene nuclear) alignment was used for BEAST analyses. The input XML file for BEAST was created in BEAUti v.2.4.7, applying the GTR + I + G substitution model as it is the most parameter-rich model (Abadi *et al.* 2019), relaxed lognormal clock model, and birth-death tree prior. The alignment was unpartitioned to allow for the analyses to converge in adequate time (< 4 weeks) as commonly applied in other HTS studies. Three parallel BEAST runs were performed until convergence (ESS > 200), with up to 800 million Markov Chain Monte Carlo (MCMC) generations and trees sampled every 1000 generations. Convergence of the posterior and other parameters were assessed via Tracer v.1.6 (Rambaut *et al.* 2015) with the first 20% of MCMC discarded as burn-in. After burn-in, all three independent runs were combined using LogCombiner v.2.4.7, summarized in TreeAnnotator v.2.4.7 to a consensus tree, and the tree visualized using FigTree v.1.4.3 (Rambaut 2012). BEAST analyses were conducted on the Institut de Recherche pour le Développement HPC cluster.

Biogeographic analyses

Geographic distributions for all sampled species were determined based on occurrence records obtained from the Australasian Virtual Herbarium online database (AVH; <http://avh.chah.org.au>, accessed June 2021). Records based on cultivated or clearly erroneous localities were excluded. Four broad biogeographic regions were delimited based on previous studies (Cook *et al.* 2015, Nge *et al.* 2021b, 2022), comprising three mesic (W, E, and N) areas and one for the expansive arid centre (A; Table 1). *Cryptandra debilis* A.R.Bean occurs in the wet tropics of Queensland but was coded as being part of N given that the wet tropics region is part of a wider northern Australian region (N) that mostly has a tropical monsoonal climate.

The W and E regions were subdivided into seven and five subregions respectively, based on Interim Biogeographic Regionalisation of Australia (IBRA) regions for W and the phytogeographical regions of Ebach *et al.* (2015) for E. The latter largely correspond to the political state boundaries of eastern Australia, except that 'South Australia' includes parts of western Victoria with a similar Mediterranean climate.

Biogeographic analyses were conducted using BioGeoBEARS v.1.1 (Matzke 2013) in R v.3.5.1 (R Core Team 2016). BioGeoBEARS implements and compares ML versions of dispersal-extinction-cladogenesis (DEC; Ree and Smith 2008), dispersal-vicariance (DIVA; Ronquist 1997), and Bayesian (BAYAREA; Landis *et al.* 2013) biogeographic models. Additional model parameters such as jump dispersal (j), dispersal probability as a function of distance (x), and time stratification, are also available in BioGeoBEARS. For the continental-wide analysis, we implemented 24 (6×4) models across two aridification scenarios in BioGeoBEARS and compared model fits via corrected Akaike Information Criterion scores (AICc;

Akaike 1974), with the lowest score indicating the best fit. A default model used no additional parameters, specifying only the current distributional range of each taxon. In the Scenario 1 models, we included an additional relative distance parameter (x) based on current distributions of taxa. Distances were coded from 1–100 (100 being the furthest relative distance). Scenario 2 models were similar to Scenario 1 with the addition of a time stratification across six 5-Myr time intervals (i.e. throughout the evolutionary history of *Cryptandra*), simulating a gradual retreat of the three mesic regions and expansion of the arid interior as Australia progressively became more arid. Finally Scenario 3 models was similar to Scenario 2 but specified that aridification intensified at 15 Myr and again at 5 Myr, based on current knowledge of the aridification history of Australia (Scenario 3; Fujioka *et al.* 2005, 2009). We tested the model fit for DEC, DIVA, and BAYAREA, with or without a jump dispersal parameter (j), for each of the three scenarios and the default model, resulting in six models per scenario (24 models in total). For subregions within southwest Western Australia (SWA) and eastern Australia (SEA), we only included the default model and not the distance or time stratification parameters, as these regions are based on current vegetation and knowledge of past changes is currently poor to non-existent.

Clade-specific diversification and mass-extinction analyses

Bayesian analyses of macroevolutionary mixtures (BAMM; Rabosky 2014) were used on our dated BEAST phylogeny to estimate and investigate diversification rates (and rate shifts) of *Cryptandra*. A conservative global sampling regime of 80% was specified to account for potential undescribed species in addition to the five species that were not sampled in this study. The BAMM analyses were conducted for 5 million rjMCMC generations and then checked for convergence (ESS value of > 200) via the BAMMtools v.2.1.10 package (Rabosky *et al.* 2014) in R. The first 10% of the BAMM runs were discarded as burn-in. Next, 95% credibility rate shift values were calculated for each branch in the dated tree using BAMMtools with expected core shifts in diversification rate set to one. Subsequently, speciation and extinction curves along with expected number of diversification rate shifts were plotted via BAMMtools in R. Speciation and extinction curves were also estimated in TESS v.2.1.2 (Höhna *et al.* 2016) and compared with those obtained from BAMM, with congruence providing confidence to our results.

We used Compound Poisson Process on Mass-Extinction Times (CoMET; May *et al.* 2016) models implemented in TESS (Höhna *et al.* 2016) in R to test for potential mass-extinction events that may have affected the diversification of *Cryptandra*. CoMET was used instead of other mass-extinction models as it performs better than other models for smaller phylogenies (< 200 tips; Culshaw *et al.* 2019). A conservative sampling fraction of 80% was used for CoMET, similar to our BAMM analyses. We also conducted CoMET analyses with the actual sampling regime (95%), and compared the results. As we are uncertain what the specific extinction fraction would be for a mass-extinction event for *Cryptandra* (due to the paucity of fossils), we tested three different survival probability priors (5% default, 20% and 50%) in CoMET. The autostop function was applied to stop the CoMET runs automatically after convergence (ESS > 200).

Table 1. Geographic regions and subregions of Australia and their associated abbreviations mentioned in text.

Biogeographic regions	Coding abbreviation
Continental Australia	
Southwest Australia (SWA)	W
Eastern Australia (SEA)	E
AMT	N
Arid Eremaean zone	A
SWA subregions	
Geraldton Sandplains	G
Avon wheatbelt	A
Mallee	M
Jarra Forest	J
Swan Coastal Plain	S
Warren	W
Esperance	E
SEA subregions	
Queensland	Q
NSW	N
Victoria	V
Tasmania	T
South Australia	S

CoMET analyses were also conducted for the two subclades of *Cryptandra* including mostly SWA and SEA clades. For these, the conservative sampling fraction was adopted (80%) along with a survival probability of 20%. All other parameters and settings were set to default unless specifically mentioned previously.

To test for the effects of past environmental climate change on speciation and extinction rates of *Cryptandra*, we applied paleoenvironmental-dependent birth-death diversification models (Condamine et al. 2013) implemented in the R package RPANDA v.1.8 (Morlon et al. 2016). Specifically, we compared models of time-dependent as well as two other paleoenvironmental-dependent diversification models: global temperature and sea-level changes through time. These models have been applied successfully for other biotic groups (e.g. Lagomarsino et al. 2016; see Supporting Information for details on the specific models).

Spinose trait dependent diversification

To test whether speciation rates differ significantly between spiny and non-spiny lineages within *Cryptandra*, we conducted a Structured Rate Permutations on Phylogenies (STRAPP; Rabosky and Huang 2015) analysis on our dated tree. STRAPP used the speciation rates derived from our BAMM analyses to test for the significance of a specific trait (in our case, the presence of spinose branches). The spinose trait was coded as a binary character (1–spinose, 0–non-spinose) in an input file for STRAPP.

We estimated the ancestral state (spinose or non-spinose) using empirical Bayesian posterior probabilities via the ‘lik.anc’ function from phytools (Revell 2012) in R, using the ER model (equal rates) as it is the most conservative and parsimonious option, and used the *D* statistic (Fritz and Purvis 2010) in R using the caper package (Orme et al. 2013) to test for a phylogenetic signal of spininess. Lineage accumulation curves were also constructed for spinose and non-spinose lineages, plotting each branching node in the phylogeny to provide further insights into the origin and accumulation of these lineages.

RESULTS

Phylogenetic relationships and divergence times of *Cryptandra*

Our curated nuclear alignment had a total length of 35 560 bp, comprising 30 loci covering 93 sampled taxa including outgroups (Table S3 in the Supporting Information). *Cryptandra* was recovered as monophyletic with strong support, sister to the *Blackallia* C.A.Gardner, *Papistylus* Kellermann, Rye & K.R.Thiele, and *Serichonus* K.R.Thiele clade (Fig. 3, Supporting Information, Fig. S1a). All nodes were resolved with moderate to high support (posterior probability; PP \geq 0.9) in our BEAST Bayesian topology except for two: the *C. minutifolia* Rye subspecies (PP = 0.82) and *C. amara*–*C. spinescens* Sieber ex DC. (PP = 0.75). Clades within the genus largely correspond to geographic partitioning of lineages across Australia, i.e. there are Australian northern monsoonal tropics (AMT), SWA, and SEA-specific clades, with the exception of the arid Eremaean taxa, which comprise phylogenetically distant lineages that are scattered across the phylogeny and share close links with taxa in surrounding mesic regions.

The stem age (origin) of *Cryptandra* was estimated at 30.9 Myr (95% credibility interval, CI: 29–33.3 Myr), based on our BEAST divergence-time analyses (Fig. 3, Supporting Information, Fig. S1). The crown of *Cryptandra* was estimated at 22.4 Myr (95% CI: 19.9–25.9 Myr), which also corresponds with the divergence (stem) of the SWA and SEA + AMT clades. The *C. amara* subclade diverged from its sister northern monsoonal clade (*C. gemmata* A.R.Bean–*C. intratropica* W.Fitzg.) at c. 19.2 Myr (95% CI: 17.0–22.6 Myr), with a crown age of c. 9.9 Myr (95% CI: 6.8–12.9 Myr). The *C. tomentosa* subclade diverged from its sister subclade (*C. recurva*) at c. 17.2 Myr (95% CI: 14.3–21.0 Myr), with the crown of the *C. tomentosa* subclade estimated at c. 11.3 Myr (95% CI: 7.9–14.7 Myr) and the *C. recurva* crown at c. 14.6 Myr (95% CI: 10.7–18.0 Myr). The Tasmanian clade (*C. alpina* Hook.f. and *C. exilis* D.I.Morris) diverged (stem) from mainland lineages (*C. ericoides* Sm.) at 7.3 Myr (95% CI: 4.4–11.0 Myr) and diversified (crown) at c. 3.2 Myr (95% CI: 1.5–4.9 Myr). The two South Australian species (*C. hispidula* Reissek ex F.Muell., *C. campanulata* Schldt.) diverged from other eastern taxa at c. 16.5 Myr (95% CI: 13.8–19.9 Myr) and diverged (crown) from each other at c. 12.3 Myr (95% CI: 7.8–15.8 Myr).

Biogeographic results

The ancestral area for *Cryptandra* was estimated to be widespread (SWA and SEA), based on the best fitting model—the DIVALIKE + *x* model from Scenario 2 with gradual aridification (Fig. 3, Supporting Information, Table S4). For both SWA and SEA + AMT subclades, the DEC + *j* model were the best fit models with the lowest AICc scores (Supporting Information, Table S5). The ancestral areas of these subclades were unresolved (i.e. widespread) and congruent with the ancestral area for the genus being widespread across Australia. For the SWA subclade, the northern Geraldton Sandplains was inferred as the ancestral area for most (20/25 spp.) SWA taxa (Fig. 3). The ancestral area of the SEA subclade was unresolved, however, one of the clades (*C. alpina*–*C. propinqua* A.Cunn. ex Fenzl) was estimated to have originated in the Queensland phytogeographic region. Interestingly, this subregion in SEA is also the one with the highest number of phylogenetically distant lineages (*C. pogonoloba* A.R.Bean, *C. armata* C.T.White & Francis, *C. debilis*–*C. propinqua*) in contrast to other subregions that have monophyletic clades (Tasmania, New South Wales (NSW), South Australia; Fig. 3C).

Major clades within *Cryptandra* largely correspond to geographic regions, however, no regions have a completely monophyletic clade apart from the AMT clade (Fig. 3)—which is nested in a larger SEA clade. For SEA, another smaller clade is present (*C. orbicularis* A.R.Bean–*C. tomentosa*), sister to SWA and arid-zone species. In addition, several other SEA taxa (*C. setifera* Kellermann, *C. magniflora* F.Muell.) do not belong to either of these SEA clades, but share close affinities with other SWA and arid-zone species in a phylogenetically distant clade within the genus. Interestingly, the endemic Pilbara species (*C. monticola* Rye & Trudgen) from northwest Western Australia is sister to two southern temperate lineages (*C. sabulicola* Kellermann from SEA, and *C. recurva* Rye from SWA), which are then sister to a SEA clade (*C. tomentosa*–*C. orbicularis*) instead of other arid-zone lineages. The SWA clade (*C. orbicularis*–*C. crispula* Rye)

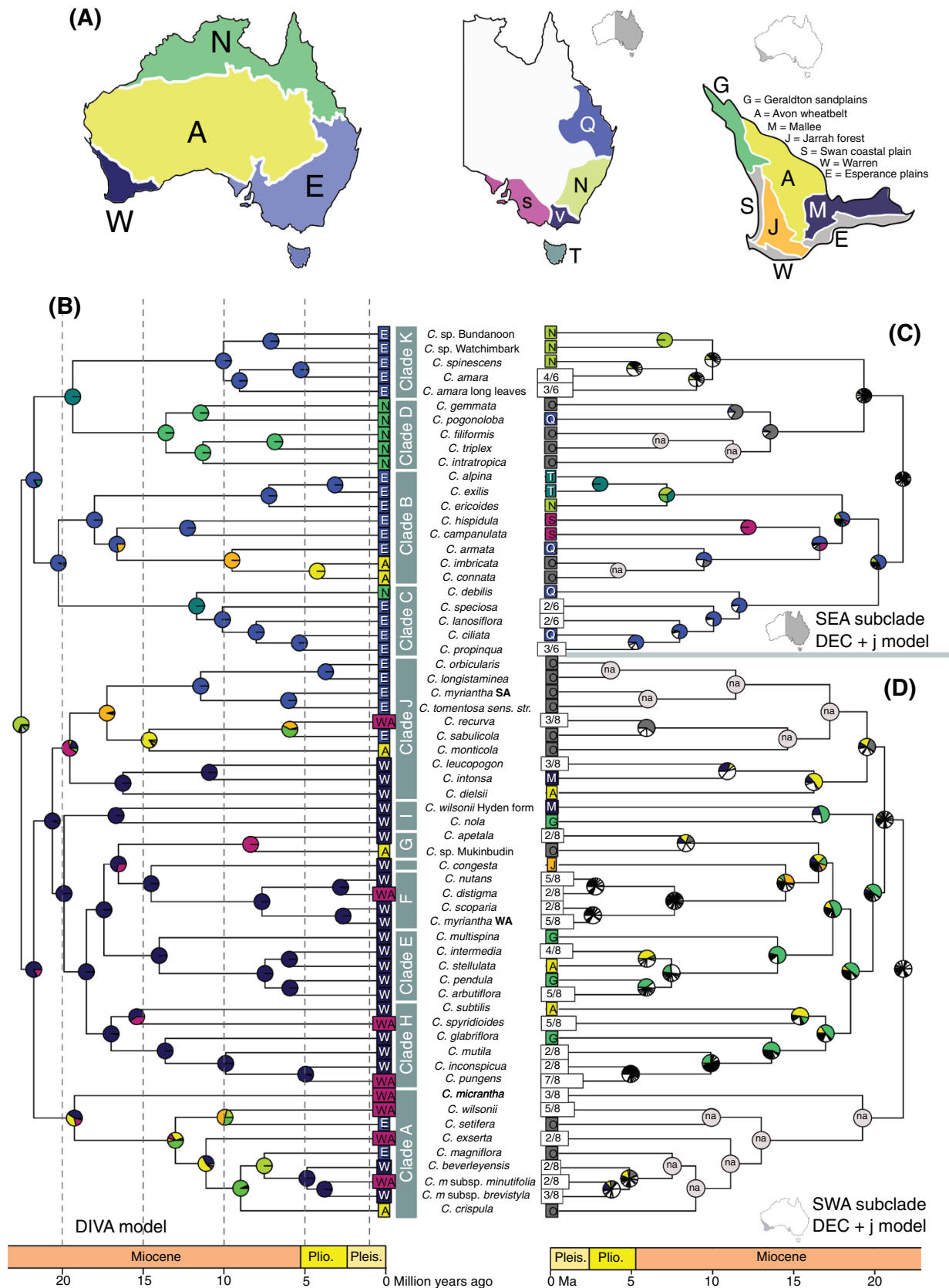


Figure 3. Maximum likelihood ancestral range reconstruction of *Cryptandra* based on the best biogeographic models and dated BEAST tree with 30 nuclear loci. A, Region-delimitation of continental-wide Australia, eastern Australia (SEA), and southwest Western Australia (SWA). Area names for continental Australia are south-western Australia (W), eastern Australia (E), northern Australia (N), the arid zone (A), and (WA) spanning both W and A regions. For the SEA subtree, the subregions are Queensland (Q), New South Wales (N), Victoria (V), South Australia (S), and Tasmania (T), following phylogeographic boundaries defined by [Ebach et al. \(2015\)](#). For the SWA subtree, the subregions are the Geraldton Sandplains (G), Avon wheatbelt (A), Jarrah Forest (J), and Mallee (M), and (O) for other regions not part of the SWA or SEA. Fractions indicate the number of subregions that each taxon occupies. B, Continental-wide biogeographic reconstructions based on the DIVALIKE model. C, D, Biogeographic reconstructions for SWA and SEA subclades based on the DEC + J model.

comprises mostly SWA endemic lineages, but also includes a smaller SEA clade and multiple nested arid-zone taxa.

Up to three separate east-west vicariance events were inferred in *Cryptandra* at different time periods. Both SWA and SEA were important biogeographic links (vicariant) and source areas of dispersal to the arid Eremaean centre. In particular, dispersals or biome transitions into the arid zone occurred since the late Miocene (e.g. *C. imbricata* Rye, *C. connata* C.A.Gardner from SEA, *C. sp. Mukinbudin* (J.A. Wege 2034) Kellermann, *C. distigma* Rye, *C. spyridioides* F.Muell., *C. pungens* Steudel, *C. minutifolia* from SWA; Fig. 3).

Speciation decline in *Cryptandra* towards the present

A substantial negative shift in speciation rates for *Cryptandra* was detected across three diversification models (BAMM, CoMET, RPANDA) in this study (Fig. 4). This slowdown and shift in speciation rate was significant at c. 2.5 Myr based on our CoMET analyses (Bayes Factors > 6), across all tested survival probability regimes and sampling fractions (Fig. 4, Supporting Information, Fig. S2). This decrease in speciation rate was predominantly driven by the SWA clade (see Bayes Factor support in the Supporting Information, Fig. S2d, e). Similarly, the speciation-through-time plot from BAMM also showed a strong decreasing trend towards the present for *Cryptandra* (Fig. 4). No significant diversification rate shift across *Cryptandra* was detected from BAMM (Supporting Information, Fig. S3),

and no mass-extinction events were detected across the evolutionary history of *Cryptandra* from our CoMET analyses (Supporting Information, Fig. S3). Both time-dependent and temperature-dependent diversification models (without extinction, models 3 and 7; Supporting Information, Table S2) were the best models based on the lowest AICc scores from our RPANDA analysis (Supporting Information, Table S6). In both cases, the speciation rate of *Cryptandra* also showed a decline towards the present, similar to our findings from BAMM and CoMET (Fig. 4).

Diversification of spinose lineages in *Cryptandra*

Non-spinose was the inferred ancestral character state for *Cryptandra* (Fig. 5). Lineages exhibiting spinose branchlets arose independently at least eight times in *Cryptandra* (Fig. 5). There were no significant differences between the speciation rates of spinose and non-spinose *Cryptandra* lineages based on STRAPP ($P = 1$; Supporting Information, Table S7) as no significant diversification rate heterogeneity was recovered from our BAMM analyses. Phylogenetic clustering was also noted for spinescent clades as the D statistic tests recovered a significant deviation from no phylogenetic signal (i.e. random structure, Supporting Information, Table S8). The lineage accumulation curves indicate that spinescent species accumulate at a slower constant rate than non-spinescent lineages through time (Fig. 4D).

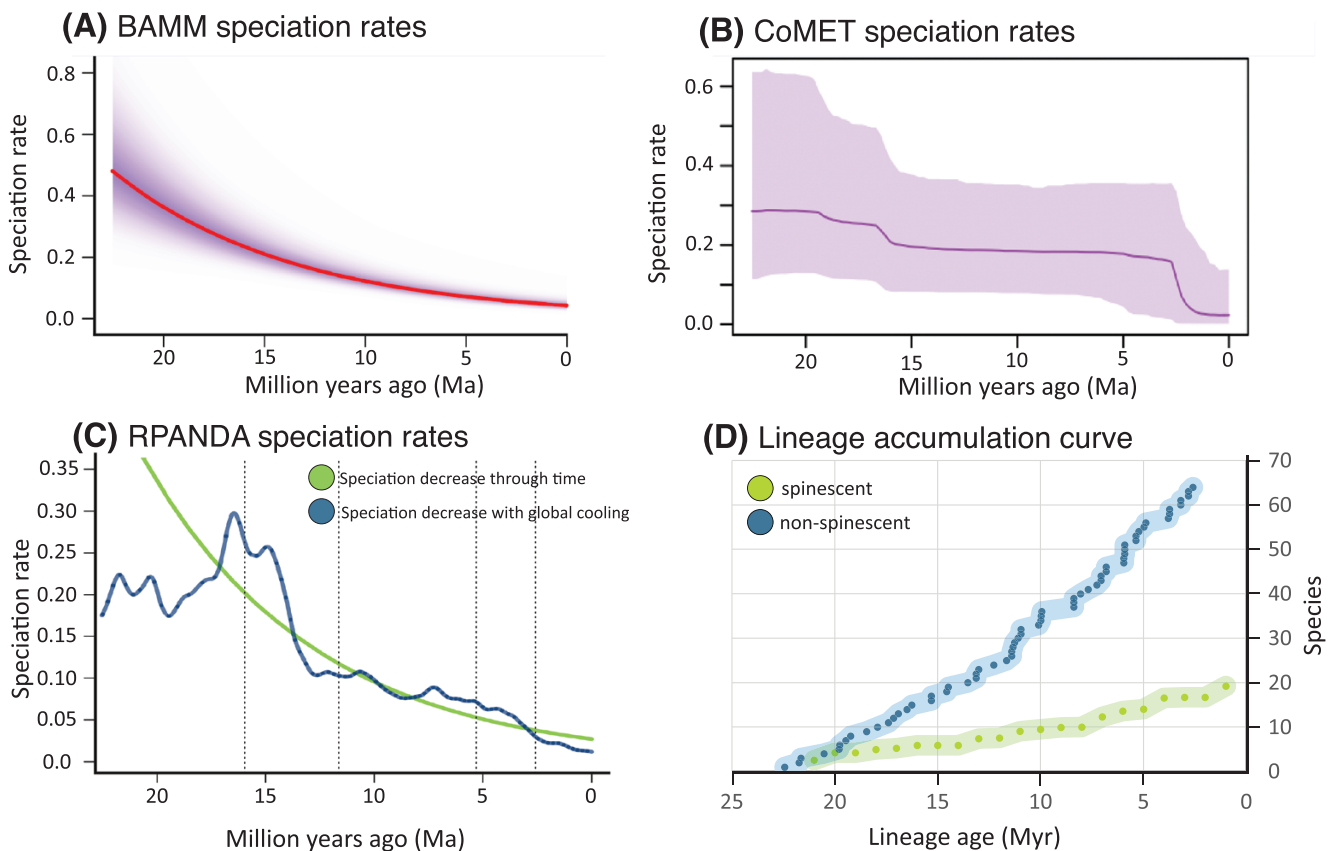


Figure 4. Diversification of *Cryptandra* in Australia through time, with declining speciation rates shown in: A, BAMM; B, CoMET; and C, RPANDA analyses. D, Lineage accumulation curves for spinescent and non-spinescent lineages through time. The CoMET analysis was based on an extinction survival probability of 20%.

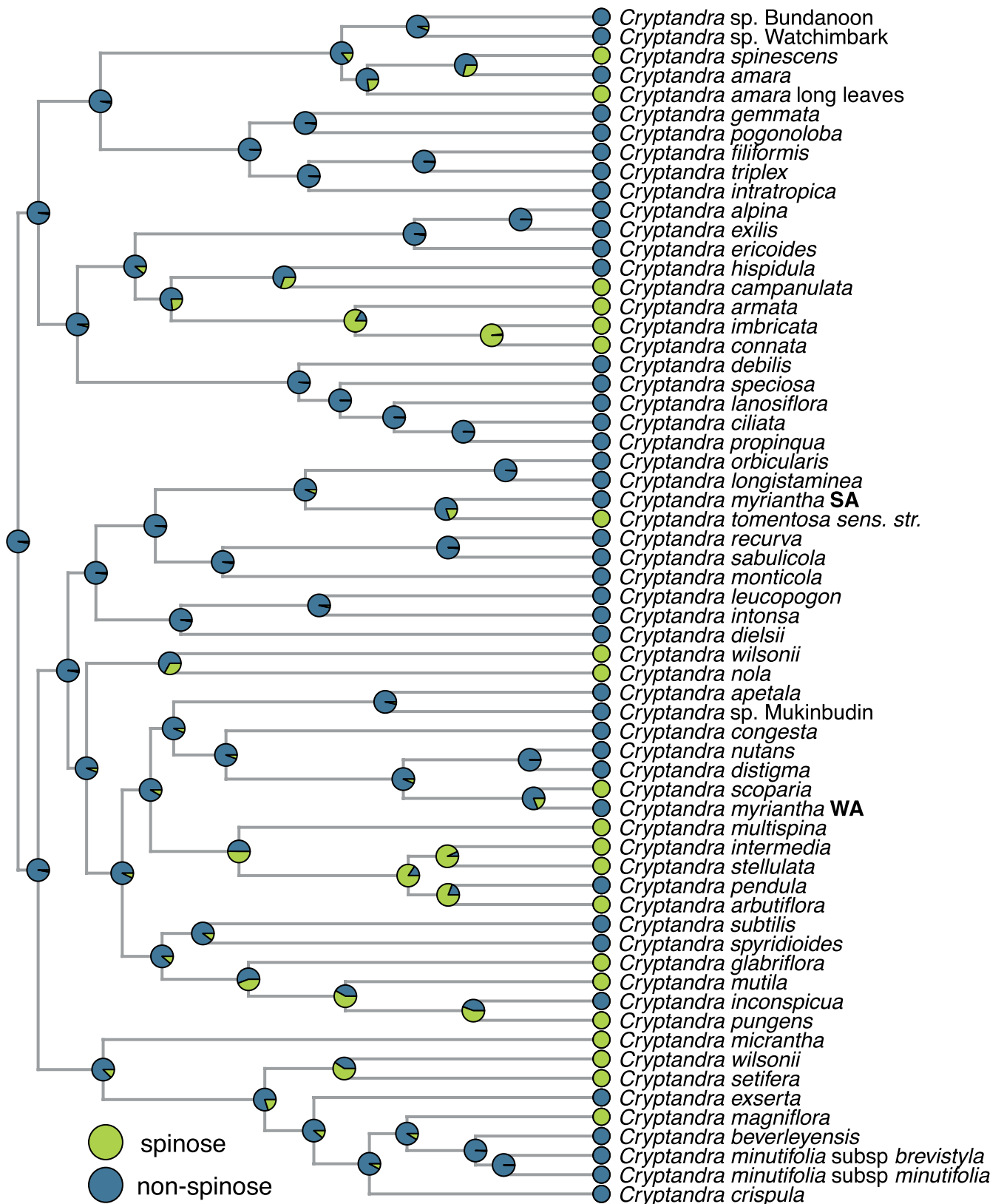


Figure 5. Ancestral state character mapping onto the BEAST topology of *Cryptandra*, for spinescent trait (blue 0–non-spinose, green 1–spinose). Ancestral character estimations were conducted with the equal rates (ER) model using empirical Bayesian posterior probabilities via the 'lik.anc' function from phytools (Revell 2012) in R.

DISCUSSION

Biogeographic history of *Cryptandra*

We present here the most comprehensive dated molecular phylogeny of *Cryptandra* to date. Our divergence dating analyses recovered older age estimates for *Cryptandra* than previous studies (Onstein et al. 2015, Hauenschild et al. 2018), possibly due to a more extensive taxon and genetic sampling, which is known to result in older and probably more accurate age estimates (Linder et al. 2005, Poux et al. 2008). Nevertheless, our divergence-time results overlap with those obtained in previous studies (Onstein et al. 2015, Hauenschild et al. 2016). We estimate that the initial crown radiation of *Cryptandra* occurred in the early Miocene (c. 22.4 Mya) across the Australian continent. *Cryptandra* clades diverged initially into different geographic regions followed by subsequent *in situ* diversification and little floristic exchange apart from dispersing into the arid interior from the late Miocene onwards.

The widespread ancestral area of *Cryptandra* inferred from this study supports the Peripheral Vicariance Hypothesis (Nge et al. 2022), with vicariance of mesic clades towards the more mesic periphery as the arid centre expanded since the Miocene. Vicariance of mesic lineages across Australia has also been shown by biogeographic studies using different methodological approaches on other plant groups such as Pomaderreae (Rhamnaceae), *Calytrix* Labill. (Myrtaceae), and *Eucalyptus* L'Hér. (Myrtaceae) (Ladiges et al. 2005, 2010, Nge et al. 2021b, 2022, Clowes et al. 2022), and is surmised to have also occurred in other plant groups based on their present-day distributions (see Nge et al. 2022). These vicariance events could have been driven by a number of processes including gradual aridification of the continent since the Oligocene, repeated inundation of the southern Eucla Basin in the Eocene and Miocene, and uplift of the Nullarbor Plain c. 13–14 Myr (Dettmann et al. 1992). Crisp and Cook (2007) showed that multiple east-west vicariance events in multiple plant groups clustered around the uplift of the calcareous Nullarbor Plain (c. 13–14 Mya), which presents a barrier for floristic exchange of calcifuge plants. The vicariance event at the crown node of *Cryptandra* predates this event (22.4 Myr, 95% CI: 19.9–25.8 Myr), hence ruling out the Nullarbor as the cause of vicariance for the oldest vicariance event in *Cryptandra* and favours the peripheral vicariance scenario. However, a younger vicariance event of *C. crispula*–*C. setifera* clade within Clade A coincides with the Nullarbor uplift event, and hence it is plausible that this event resulted in the vicariance of this clade as most *Cryptandra* species do not grow on limestone substrates.

The sister relationship between the northern Australian (AMT, Clade D) and SEA clades in *Cryptandra* is not unique, as a similar relationship has been noted in other plant groups (e.g. Cardillo et al. 2017, Jobson et al. 2017, Nargar et al. 2019, Nge et al. 2022). These studies, along with fewer examples of SWA–northern Australian floristic links (Burbidge 1960), reinforce greater connectivity of SEA with a continuous band of mesic vegetation through to northern Australia. Conversely, the SWA flora experienced a greater degree of isolation throughout its evolutionary history (Burbidge 1960, Hopper 1979). The radiation of the northern Australian *Cryptandra* clade in the mid-Miocene (c. 14 Mya) postdates the formation of the monsoonal

climate in that region around the late Eocene (Bowman 2002), as with other plant lineages mentioned previously.

We identified two recent independent dispersals into Tasmania from the Australian mainland, both occurring within the last 5 million years. Only one of these lineages diversified into two species (*C. alpina*–*C. exilis*). *Cryptandra alpina* is the only species in the genus that grows in alpine environments (above 1000 m in elevation). The relatively recent divergence of this species (c. 3.2 Myr) in Tasmania indicates that the regional alpine flora not only comprises ancient paleo-endemics dating back to the Cretaceous (Jordan et al. 2016), but also recent additions to the flora following global cooling in the Pliocene. The diversification and assembly of the Australian alpine flora warrants further investigation.

Similarly, biogeographical links across the subregions of SWA warrant further study. While we found that the Geraldton Sandplains was estimated to be the ancestral area of the SWA subclade in *Cryptandra*, further detailed studies are required incorporating past environmental simulations to rule out methodological artefact, as the Geraldton Sandplains contains the highest number of endemic species within SWA for *Cryptandra*. The Geraldton Sandplains IBRA subregion consists of mainly kwongan heathland vegetation, which is well known for its high plant diversity within SWA: including species richness, endemism (Gioia and Hopper 2017), and threatened species (Gosper et al. 2022). Interestingly, several of these endemic *Cryptandra* species represent old lineages (*C. nola* Rye—16.7 Myr, *C. multispina* Rye—14 Myr, *C. glabrifolia* Rye—13.6 Myr), suggesting the Geraldton subregion could be an important long-term refugium for long-persisting lineages, as shown in a continental-wide angiosperm spatial phylogenetics study by Thornhill et al. (2016). Our definition of long-persisting lineages here refers to isolated, long-branching, species-poor lineages similar to those commonly referred to as ‘relictual’ species. However it is worth noting that extant species representing these lineages are neither necessarily old nor relictual (Grandcolas et al. 2014) hence we use the term ‘long-persisting’ instead. Being situated along the coast, the Geraldton subregion would be relatively more buffered from extreme climatic shifts compared to more inland regions (Nge et al. 2020), indicating higher plant species richness and endemism of near-coastal SWA subregions (e.g. Geraldton Sandplains and Esperance) may be partly explained by a more stable long-term climate (Harrison and Noss 2017, Cai et al. 2023). While *Cryptandra* species also occur in the southern Esperance sandplains subregion of SWA, none of these are strictly endemic to Esperance. By contrast, the Pomaderreae genus *Siegfriedia* C.A.Gardner, and Western Australian species of *Pomaderris* are largely endemic to the Esperance subregion of SWA. Thus competitive exclusion of *Cryptandra* from these incumbents may be a driver in preventing narrow endemics of *Cryptandra* from originating and persisting in that area, as has been shown for other groups across the globe more generally (Betancur-R et al. 2012, Condamine et al. 2019, Pavón-Vázquez et al. 2022).

For the SEA subregions, Victoria is the only subregion with no endemic *Cryptandra* species. This lack of endemics might be linked to a higher extinction rate for that subregion, as documented from the fossil record of a once hyperdiverse sclerophyllous flora in the Stony Creek Basin (Sniderman et al. 2013). A

similar diversity pattern was noted for *Pomaderris* by Nge *et al.* (2021b), with five endemic Victorian species compared to 16 in the adjacent NSW subregion. Similar patterns are also seen for *Spyridium* with Victoria having the fewest endemic species (two spp.) compared to other eastern regions (SA 16 endemic spp., Tasmania four endemic spp., NSW three endemic spp.). Few transitions across SEA subregions were noted for *Cryptandra*, in stark contrast to *Pomaderris* (Nge *et al.* 2021b). The reasons for this are unknown. The confinement of lineages within subregions and failure to radiate after repeated transitions into the arid zone are probably why *Cryptandra* has experienced a speciation rate decline towards the present (i.e. failure to keep up with the changing climate of Australia).

Progressive diversification decline in *Cryptandra*

All diversification models in this study point to a decline in speciation rates towards the present for *Cryptandra*. Congruence across these different models indicates a strong directional trend that is not an artefact of non-identifiability in diversification models (Louca and Pennell 2020, Kopperud *et al.* 2023). Similarly, we are confident that our results are not compromised by insufficient sampling as we sampled almost all extant species within *Cryptandra* and tested different sampling fractions in our models. This decline in speciation rate for *Cryptandra* is in contrast to the recent radiation (*c.* 10 Mya) in eastern Australia of the closely related genus *Pomaderris* (Rhamnaceae; Nge *et al.* 2021b). These different diversification trajectories may be related to their different habitat specializations, with *Pomaderris* predominantly occurring in wet sclerophyll *Eucalyptus* forests whereas *Cryptandra* species are more commonly found in open vegetation such as sclerophyllous shrublands (Walsh and Coates 1997, Walsh and Udovicic 1999, Kellermann and Udovicic 2007, Rye 2007, Kellermann 2020). Polyploid *Pomaderris* have been shown to transition into wetter sclerophyllous habitats in eastern Australia (i.e. novel niche space; Nge *et al.* unpublished). The crown radiation of *Cryptandra* is substantially older than *Pomaderris*, diversifying *c.* 22.4 Myr compared to a recent radiation of *Pomaderris* across SEA at *c.* 12 Myr (Nge *et al.* 2021b). The radiation of *Pomaderris* in the late Miocene coincided with the rise in dominance of wet sclerophyll *Eucalyptus* forests in SEA, shown by the presence of high charcoal levels in the paleorecord indicative of high intensity fire regimes of these forests from the late Miocene onwards (Hill *et al.* 2016, Hill 2017). Recent radiations of extant *Eucalyptus* from 5 Myr as dominant components of the Australian vegetation were also suggested from the dated molecular phylogeny of Thornhill *et al.* (2019). It may be plausible that *Pomaderris* diversified under the rise of *Eucalyptus* in Australia as a result of novel niche opportunities, at the expense of other floristic elements that were once dominant or associated with other vegetation types across the continent (e.g. *Cryptandra* that diversified before the dominance of *Eucalyptus*). This phenomenon is not unheard of, for example ferns have been shown to diversify under the rise of angiosperms (Schneider *et al.* 2004), which pushed for the decline of a once conifer-dominated flora (Condamine *et al.* 2020). Whether habitat specializations are linked to co-diversification/competitive decline across Pomaderreae genera requires further research and explicit analysis.

The decrease in speciation rates for *Cryptandra* appears to be linked to different climatic events throughout its evolutionary history. Our RPANDA results indicate a decline in speciation at *c.* 14 Mya, which coincides with a sharp drop in global temperature and commencement of aridification in Australia following the end of the Mid-Miocene Climatic Optimum. This speciation decline after the Mid-Miocene Climatic Optimum is also evident in our CoMET analyses, albeit to a lesser degree. Following this event, our CoMET and RPANDA temperature-dependant models indicate another speciation decline at *c.* 3–4 Mya, when aridification of Australia further intensified after the Pliocene (Fujioka *et al.* 2005, 2009, Byrne *et al.* 2008). These findings strongly suggest that diversification of *Cryptandra* has been negatively affected by progressive aridification in Australia. The absence of mass-extinction events for *Cryptandra* based on our CoMET analyses suggests that the speciation decline has been gradual, which fits with the known scenario of gradual aridification of the continent. This negative diversification trend for *Cryptandra* can also be seen in our dated phylogeny, where isolated long-persisting species-poor lineages in the arid zone (e.g. *C. monticola*, *C. sp. Mukinbudin*, *C. crispula*) have either failed to diversify or represent lineages that have suffered extinction leaving only one remaining species in their respective lineages. *Cryptandra monticola* is endemic to the Pilbara, and diverged from other *Cryptandra* at 15 Mya. The Pilbara region is a well-known refugium in the arid zone, contains a mosaic of semi-arid to arid vegetation types and is topographically heterogeneous (Pepper *et al.* 2013, Umbrello *et al.* 2020). This pattern of long-persisting lineages in the arid zone is also seen in other Australian plant groups such as *Calytrix* (Nge *et al.* 2022), which also shows speciation decline towards the present (Nge *et al.* unpublished). Our findings based on molecular phylogenies of extant lineages indicate that at least some sclerophyllous Australian plant groups have experienced a decline in diversification towards the present due to widespread aridification across the continent (Byrne *et al.* 2011), reinforcing findings based on the paleorecord (Sniderman *et al.* 2013). The decline in temperate lineages and their associated mesic biomes opened new niches for the diversification of arid-adapted groups (Cabrera *et al.* 2011, Echeverría-Londoño *et al.* 2020, Renner *et al.* 2020, Hammer *et al.* 2021, Čalasan *et al.* 2022). Crayn *et al.* (2006) showed that the former Tremandraceae clade (now in Elaeocarpaceae) radiated across temperate Australia during the late Miocene, coinciding with widespread aridification. However, more sampling is required to test whether that clade also suffered a speciation decline towards the present, as they have only sampled nine out of 50+ species (18%) in that clade. Further studies on densely sampled phylogenies of sclerophyllous lineages are required to test whether recent speciation decline is a general phenomenon of the wider Australian sclerophyllous flora.

The initial crown radiation of *Cryptandra* occurred in both SWA and SEA, with a more extensive radiation in SWA (seen by the number of nodes, Fig. 3). These radiations postdate the Eocene–Oligocene extinction event across the Australian flora (Nge *et al.* 2020), hence drivers for this discrepancy in diversification across regions require another explanation. It has been argued that SWA experienced low levels of relative extinction throughout its history, from the Eocene through to the present (Hopper 2009, Sniderman *et al.* 2013, Nge *et al.* 2020).

Indeed, SWA has a greater number of long-persisting species-poor lineages (nine: *C. congesta* Rye, *C. subtilis* Rye & Hislop, *C. spyridioides*, *C. micrantha* Rye, *C. exserta* Rye, *C. glabriflora*, *C. nola*, *C. wilsonii* Rye, *C. dielsii* Rye) ranging from 11–19 Myr old, compared with SEA that has only two of these lineages that are of younger age (12 Myr old: *C. campanulata* and *C. hispidula*). The generally low diversification rate in SWA for *Cryptandra* adds to the growing body of evidence that the SWA flora is the result of a steady accumulation of species rather than elevated rates of speciation that are commonly seen in other biodiverse regions (e.g. [Givnish *et al.* 2009](#), [Verboom *et al.* 2009](#), [Madriñán *et al.* 2013](#), [Vasconcelos *et al.* 2020](#), [Kandziora *et al.* 2022](#)). For example, at least five speciation events resulting in nine new species of *Cryptandra* occurred in the last 5 Myr in SWA. In contrast, genera in other biodiverse regions such as the Neotropics, Andes, Mediterranean Basin, and Succulent Karoo diversified into hundreds of species each within the same 5 Myr timeframe ([Richardson *et al.* 2001](#), [Klak *et al.* 2004](#), [Valente *et al.* 2010](#), [Lagomarsino *et al.* 2016](#)).

Multiple origins of spinescence in *Cryptandra*

Eight independent origins of branch spinescence in *Cryptandra* suggest that this trait has been regularly selected in the evolution of this genus. Spines as herbivory defence to browsers (e.g. marsupials) has been demonstrated for *Solanum* species in arid Australia ([Symon 1986](#)). However, the origins and diversification of several of these spinescent clades in *Cryptandra* (e.g. *C. multispina*, *C. micrantha*) pre-date the origins of extant marsupial browsers in Australia (post-10 Myr; [Celik *et al.* 2019](#)). Thus, spinescence may have arisen in these lineages in response to non-marsupial (e.g. emu, which diverged at 30 Mya; [Haddrath and Baker 2012](#)) or extinct browsers ([Meredith *et al.* 2009](#)). The extinction of megafauna or other smaller browsers may explain why we did not detect significant differences in diversification rates between present-day spinescent and non-spinescent *Cryptandra* lineages, as the older spinescent clades may be legacies of selection from the past ([Onstein *et al.* 2018](#), [Dantas and Pausas 2022](#)). Indeed, reversals from spinescence to secondary non-spinescence is inferred in several species such as *C. inconspicua* Rye, *C. pendula* Rye, and within *C. amara*, supporting this hypothesis (as we would not expect these lineages to revert if there is ongoing strong selection pressure for spininess). Several species-poor spinescent lineages of *Cryptandra* are currently found in arid to semi-arid regions of Australia and are long-persisting (> 7 Myr: *C. micrantha*, *C. multispina*, *C. wilsonii*–*C. nola*, *C. magniflora*). Having spinescent traits may allow these lineages to persist longer (i.e. have lower extinction rates) than other species in these harsh environments, but nevertheless insufficient to allow for further diversification (i.e. they still have lower speciation rates compared to non-spinescent lineages in more mesic environments, [Fig. 4D](#)). [Onstein *et al.* \(2022\)](#) detected speciation rate slowdowns of spinescent palms following the extinction of megaherbivores during the Cretaceous–Paleogene mass-extinction event. A similar trend was detected in this study for the entire *Cryptandra* genus, although not just limited to spinescent lineages.

[Orians and Milewski \(2007\)](#) suggested that plants with spinescent stems like *Cryptandra* are found primarily on

phosphorus-rich soils where browser densities are greatest. However, spinescent *Cryptandra* lineages occur primarily in SWA and semi-arid areas that are phosphorus-poor ([Rossel and Bui 2016](#)). Our findings are similar to [Cowling and Witkowski \(1994\)](#), indicating that ecological determinants (soil nutrients) and evolutionary selection over longer timescales for spinescence may be uncoupled. [Gelin *et al.* \(2023\)](#) indicated spinescent plant lineages in Australia arose (from 10 Myr) and diversified (from 5 Myr) relatively recently compared to other continents and this recent diversification was correlated strongly with climatic changes (i.e. aridification). Spinescence is uncommon in the Australian flora compared to other continents/regions (at 3.8% compared to the global average of 9%, across eucydots; [Gelin *et al.* 2023](#)), which is surprising as spinescent plants are generally more common in open and arid/seasonally dry environments ([Charles-Dominique *et al.* 2016](#), [Dantas and Pausas 2022](#)). Similarly, the Australian flora is relatively depauperate of succulents compared to other arid to semi-arid regions ([Holtum *et al.* 2016](#)). Further studies incorporating a comparative phylogenetic approach with multiple plant groups from Australia and beyond are required to explain this discrepancy.

Conclusion

Here we present the first comprehensive phylogeny of *Cryptandra*, which allowed us to infer the biogeographic and evolutionary history of this Australian genus. Our study indicates that *Cryptandra* once had a widespread ancestral southern Australian distribution, with subsequent vicariant events separating the mesic clades across the continent. Most species later diversified *in situ* within biomes, with little floristic exchange between regions except into the arid zone. We show that lineages in the arid zone either failed to diversify or diversified and subsequently declined, resulting in long-persisting lineages there. Furthermore, diversification models from this study all show a decline in speciation rate for this genus towards the present, with negative shifts in speciation rates during the Miocene (15 Mya) and Pliocene (5 Mya). These declines coincide with progressive aridification and retreat of mesic biomes across Australia, indicating that diversification of *Cryptandra* has been negatively affected by recent aridification events in evolutionary time. Further studies applying densely sampled molecular phylogenies are needed to investigate whether a recent decline in speciation in response to aridification is a general pattern in the Australian sclerophyllous flora. Spinescent *Cryptandra* lineages did not have higher diversification rates compared with non-spinescent counterparts. Furthermore, they also suffered from the same speciation rate decline, suggesting spinescent traits may have been selected for in the past by extinct megaherbivores but not the present. The densely sampled phylogeny presented in this study also provides a framework for further research on the evolution, ecology, and systematics of *Cryptandra*, and can be combined with other studies to infer common evolutionary patterns in the Australian flora more broadly.

SUPPLEMENTARY DATA

Supplementary data is available at *Botanical Journal of the Linnean Society* online.

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AUTHOR CONTRIBUTIONS

F.J.N. and J.K. designed the study. F.J.N., J.K., and K.R.T. collected the data. F.J.N. compiled and analysed the data. F.J.N. wrote the manuscript with contributions from J.K., E.B., K.R.T. and M.W.

DATA AVAILABILITY

Newly sequenced paired reads from this study have been uploaded to the sequence read archive (SRA) at NCBI (BioProject PRJNA887684, submission number: SUB12122847).

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