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RESEARCH ARTICLE

Phylogeny, evolution, and biogeographic history of *Calandrinia* (Montiaceae)

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PREMISE OF THE STUDY: *Calandrinia* are small, succulent herbs that vary broadly in habitat, morphology, life history, and photosynthetic metabolism. The lineage is placed within the Montiaceae, which in turn is sister to the rest of the Portulacineae (Caryophyllales). *Calandrinia* occupy two distinct biogeographic regions, one in the Americas (~14 species), and one in Australia (~74 species). Past analyses of the Montiaceae present conflicting hypotheses for the phylogenetic placement and monophyly of *Calandrinia*, and to date, there has been no molecular phylogenetic analysis of the Australian species.

METHODS: Using a targeted gene enrichment approach, we sequenced 297 loci from multiple gene families across the Montiaceae, including all named and 16 putative new species of Australian *Calandrinia*, and the enigmatic monotypic genus *Rumicastrum*.

KEY RESULTS: All data sets and analyses reject the monophyly of *Calandrinia*, with Australian and New World *Calandrinia* each comprising distinct and well-supported clades, and *Rumicastrum* nested within Australian *Calandrinia*. We provide the first well-supported phylogeny for Australian *Calandrinia*, which includes all named species and several phrase-named taxa.

CONCLUSIONS: This study brings much needed clarity to relationships within Montiaceae and confirms that New World and Australian *Calandrinia* do not form a clade. Australian *Calandrinia* is a longtime resident of the continent, having diverged from its sister lineage ~30 Ma, concurrent with separation of Australia from Antarctica. Most diversification occurred during the middle Miocene, with lowered speciation and/or higher extinction rates coincident with the establishment of severe aridity by the late Miocene.

KEY WORDS aridity; Australia; Australian Calandrinia; crassulacean acid metabolism (CAM); Montiaceae; Portulacineae; Rumicastrum; targeted bait enrichment.

Over the last ~150 years there has been considerable debate regarding the monophyly, phylogenetic placement, and naming of *Calandrinia* Kunth, an enigmatic group of small, succulent herbs that display broad variation in habitat (Tahir and Ashton, 1989), vegetative morphology (Hershkovitz, 1993; Obbens, 2006, 2011; Ogburn and Edwards, 2015), and photosynthetic metabolism (Winter and Holtum, 2011, 2014; Holtum et al., 2016). *Calandrinia* is a member of the Montiaceae, which in turn is sister to the rest of the Portulacineae (Caryophyllales) (Nyffeler and Eggli, 2010; Ogburn and Edwards, 2015), a clade containing many arid-adapted, succulent plants (Arakaki et al., 2011; Ogburn and Edwards, 2013). *Calandrinia* as currently circumscribed is tri-continental, with ca. 14 species in North and South America and the bulk of species (ca. 74) endemic to Australia. In the New World, species occur in arid and alpine climates along the western cordillera from British Columbia to Chile (Arroyo et al., 1990; Elvebakk et al., 2015; Ogburn and Edwards, 2015), while in Australia, *Calandrinia* is widely distributed across the continent, typically inhabiting arid to semiarid environments, but extending into temperate regions in Tasmania and Victoria and the monsoonal tropics of northern Australia (Obbens, 2006; Tahir and Carolin, 2011; West and Chinnock, 2013).

The monophyly of *Calandrinia* has been in question ever since Carolin's 1987 morphological cladistic analysis theorized that Australian *Calandrinia* were not closely related to New World *Calandrinia* and that the monotypic genus *Rumicastrum* Ulbr, originally described in the family Chenopodiaceae, was closely related to the Australian clade. He recommended that the Australian species be transferred to *Rumicastrum*, but failed to make new

combinations. Hershkovitz (1991, 1993, 1998) supported Carolin's separation of the Australian and New World *Calandrinia* but, believing that *Rumicastrum* was indeed in the Chenopodiaceae, erected the genus *Parakeelya* Hershk., a name taken from an aboriginal vernacular for the Australian species of *Calandrinia*. Although he specified new combinations for all species known at that time into *Parakeelya*, Hershkovitz's genus was not adopted in Australia (e.g., Obbens, 2006, 2011; West and Chinook, 2013; Obbens et al., 2017), as the nonmonophyly of *Calandrinia* was not confidently resolved and the correct placement of *Rumicastrum* (which has nomenclatural priority) was regarded as uncertain (Obbens, 2006).

More recently, molecular phylogenetic analyses of the Montiaceae (Ogburn and Edwards, 2015) generated contrasting hypotheses for the placement and monophyly of *Calandrinia*. In a three-gene (*matK*, *ndhF*, and *phyC*) analysis, *Calandrinia* was supported as monophyletic, albeit with relatively low support (ML bootstrap = 65%, Bayesian posterior probability = 0.90). In a five-gene (*matK*, *ndhF*, *phyC*, ITS, and *ycf3*) analysis, however, *Calandrinia* was paraphyletic, with Australian *Calandrinia* sister to a clade comprising Hectorelleae + Montieae. However, taxon sampling for Australian *Calandrinia* was extremely poor in both analyses, with only one and five species represented in the three-gene and five-gene analyses, respectively.

The present study had multiple goals. First, we aimed to establish whether *Calandrinia* s.l. is monophyletic and to resolve the placement of Australian and New World species within the Montiaceae. Second, we aimed to completely sample all named species of Australian *Calandrinia* as representing a potentially important arid plant radiation that has received scarce attention. Australia is the driest vegetated continent, yet it harbors very few native succulent plant lineages, and only 0.6% of its vascular plants are known to use crassulacean acid metabolism (CAM) (Holtum et al., 2017). This finding is perplexing, as both succulence and CAM are common adaptations to aridity and have evolved numerous times across a diversity of plant lineages that inhabit similar deserts to those found in Australia (Winter and Smith, 1996; Ogburn and Edwards, 2010; Edwards and Ogburn, 2012). *Calandrinia* provides a key opportunity to explore this conundrum, as it is one of the most speciose succulent lineages in Australia and has evolved CAM photosynthesis (Winter and Holtum 2011, 2014). Third, we aimed to explore the biogeographic history and radiation of *Calandrinia* across Australia, assess how and when the lineage likely arrived there, and evaluate how aridification of the continent in the mid-late Miocene may have influenced patterns of lineage diversification. To address these issues, we used a targeted gene enrichment approach (Moore et al., 2018) to sequence hundreds of loci from multiple gene families of interest across *Calandrinia* and the Montiaceae. We provide the first well-supported phylogeny for Australian *Calandrinia*, which includes all named species of Australian *Calandrinia*, several phrase-named taxa, and the enigmatic monotypic genus *Rumicastrum*.

MATERIALS AND METHODS

Taxon sampling

We collected silica-dried leaf, stem, or flower material from across the Montiaceae (Appendix S1, see the Supplemental Data with this article), including multiple species from all recognized genera except *Hectorella* Hook.f., *Lyallia* Hook.f., and *Schreiteria* Carolin, three monotypic, geographically remote genera. Seven of the ~14 described New World *Calandrinia* species (nine individuals total) were included, including multiple accessions of the type species, *Calandrinia ciliata* (Ruiz & Pav.) DC. Material from a specimen of *Rumicastrum chamaecladum* (Diels) Ulbr. was obtained from the National Herbarium of Victoria (MEL). In addition to the Montiaceae, we included outgroup taxa from additional Portulacineae and Molluginaceae, using material and sequence data originally collected for Moore et al. (2018).

As a primary objective of the study was to resolve species relationships within Australian *Calandrinia* (Fig. 1), we collected extensively in this region (Fig. 2). We included 64 of the ~74 species currently recognized in Australia (see Australian Virtual Herbarium: http://avh.ala.org.au/occurrences/search?taxa=Calandrinia#tab_mapView, AVH, 2017): all 48 described species and 16 of 26 undescribed but recognized phrase-named taxa. We sequenced multiple individuals from across the range of species with large geographic distributions, for a total of 77 Australian *Calandrinia* individuals. Of these, 45 were newly collected in the field during six field campaigns between August 2014 and August 2015, while 32 were sequenced from plant material obtained from herbarium specimens at the Western Australian Herbarium (PERTH) and the National Herbarium of Victoria (MEL). In addition, we received field-collected plant material of several taxa from Bob Chinnock (State Herbarium of South Australia) and Attila Kapitany (Melbourne, Australia).

Molecular methods

Genomic DNA was extracted from dried material using a two-step DNA extraction protocol. First, we used the FastDNA Kit (MP Biomedicals, Santa Ana, CA, USA) to extract DNA from 20–40 mg of plant tissue. We followed the manufacturer's protocol, allowing samples to sit on the benchtop for ~2 h following homogenization with both CLS-VF and PPS buffers. After the initial extraction, samples were eluted twice in 75 μL of distilled water, cleaned using a QIAquick PCR Cleanup Kit (Qiagen, Valencia, CA, USA), and eluted twice in 50 μL of EB buffer.

To prepare DNA for library preparation and subsequent bait hybridization, we sonicated ~500 ng of sample DNA using a Covaris S220 focused-ultrasonicator (Covaris, Woburn, MA, USA) at the Brown University Genomics Core Facility. The following parameters were implemented to generate a mean fragment length of 400 bp: peak power 140.0, duty factor 10.0, and cycles/burst 200 for 50 seconds. Library preparation was done using the NEBNext Ultra DNA Library Prep Kit or NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) following the manufacturer's protocol. After library preparation, samples were pooled in groups of 8–9 and combined for hybridization. For bait hybridization, a custom MYbaits kit was used from MYcroarray. Both probe design and bait hybridization were carried out according to the method of Moore et al. (2018). Quantification, combination, and sequencing were performed at the Brown University Genomics Core Facility and the Oklahoma Medical Research Foundation Genomics Facility on Illumina HiSeq 2000 or 2500 to obtain 100-bp paired-end reads.

Sequence assembly

Reads were initially filtered and assigned to individuals using their inline barcodes. Paired reads in which neither barcode matched

FIGURE 1. Species representatives of Australian *Calandrinia*. (A) *Calandrinia* sp. The Pink Hills, (B) *C. tumida* (C), *C. papillata* (D), *C. holtumii* (E), *C. granulifera* (F), *C. pumila* (G), *C. polyandra*, and (H) *C. schistorhiza*.

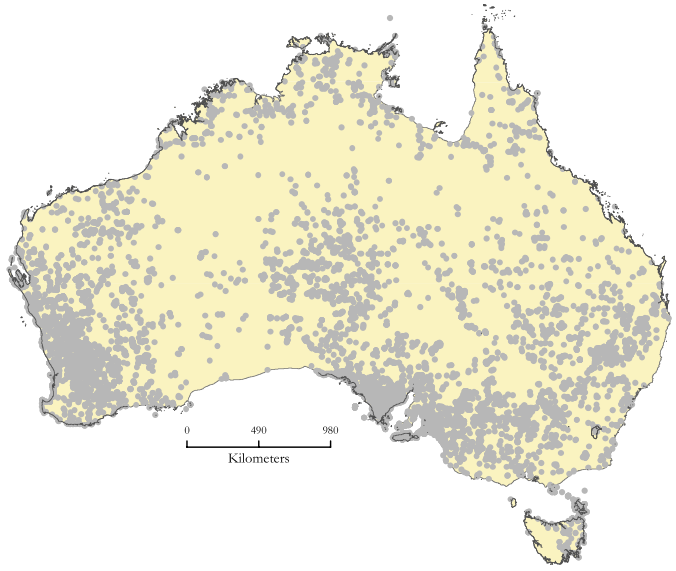


FIGURE 2. Specimen collections of Australian *Calandrinia* downloaded from the Australian Virtual Herbarium. Grey dots represent the 48 described and 26 phrase-named Australian *Calandrinia* taxa.

exactly were discarded. The last five bases and the barcodes were then removed from the remaining reads. Finally, trimmed reads with more than one low-quality base were also discarded. Our sequence processing pipeline (Moore et al., 2018) consisted of three steps: (1) extracting all relevant reads for each gene family and then assembled the reads into contigs, (2) assigning contigs into putative paralogs, and (3) identifying gene duplications within gene families and extracting individual loci. These loci were then used to infer phylogeny with both concatenation and species tree approaches.

Briefly, step 1 classified reads into gene families using them in a BLAST (Altschul et al., 1990) search against a local library of sequences used to design the baits. The "captured" reads were then pooled among individuals belonging to the same family or subgroups within the Montiaceae. SPAdes version 3.1.0 (Bankevich et al., 2012) was used to assemble pooled reads into contigs, and the contigs were then added to the bait sequence database. The trimmed reads were then used in a BLAST search against this combined database (baits reads + preliminary SPAdes assembly), and separate assemblies were made for each individual for each gene family. This process produced a fasta file for each gene family containing all contigs, labeled by individual. Finally, these files were used in a BLAST search against the bait sequence database to delineate exons. Introns were removed and only exons were used in subsequent analyses.

Step 2 classified the assembled contigs from step 1 according to the paralogs within gene families and combined contigs that represent different regions of the same paralog into a single gene sequence. Classification was executed by placing the contigs in a "backbone" phylogeny of each gene family, built from the original bait sequences and additional sequences generated from prior analyses (Moore et al., 2018). The backbone tree was iteratively refined using the add fragments algorithm in MAFFT version 7.017 (Katoh and Standley, 2013; using the localpair option because the fragments to be aligned covered only a portion of the backbone alignment) and the short read classification (-f v) algorithm in RAxML version 8.0.22 (Berger and Stamatakis, 2011; Stamatakis, 2014).

These steps produced gene-family trees containing the original sequences plus clusters of contigs (i.e., putative paralogs). For each of these clusters, the contigs from each individual were combined to form a consensus sequence. These consensus sequences were further analyzed, and if they met certain criteria (i.e., >75 bases long and 75% of the mean length of all consensus sequences from that cluster), they were added to the backbone alignments for each gene family.

Step 3 used a preliminary species tree to (1) identify the location of gene duplications within gene families, (2) extract paralogs arising from these gene duplications as separate loci, and (3) build individual gene trees and concatenated matrices for subsequent analyses. The initial species tree included all individuals in the analysis plus sequences for *Arabidopsis thaliana*, *Glycine max*, *Oryza sativa*, *Populus trichocarpa*, *Solanum tuberosum*, and *Vitis vinifera* (derived from genomes for these model organisms) and was constructed from three plastid loci (*matK*, *ndhF*, and *rbcL*) and the nuclear ITS region. Before this species tree was made, gene trees of these three loci plus ITS were inspected by eye for individuals that were out of place, and these sequences (likely pseudogenes) were removed from the tree. NOTUNG version 2.8.1.6 (Chen et al., 2000; Stolzer et al., 2012) was used to find gene duplications in the gene family trees based on the preliminary species tree, which delimited sets of orthologous sequences, i.e., phylogenetic loci. After a series of quality-control steps (see Moore et al., 2018), alignments were made using the local pair algorithm in MAFFT for the selected loci and sets of individuals. To reduce missing data, we removed all sites with >90% missing data from the alignments. Separate gene trees were then inferred for each locus using RAxML version 8.0.22 (GTRCAT, 1000 rapid bootstrap replicates); these gene trees were then used for coalescent-based species tree analyses.

After the initial run of step 3 of the pipeline (as described above), the plastid/ITS species tree was replaced with the reconstructed ASTRAL species tree (see below), and step 3 was rerun in its entirety. In addition to the updated species tree, we reran step 3 with 116 individuals divided into eight groupings (Appendix S1): Caryophyllales outgroups, Molluginaceae, Portulacineae except for Montiaceae, *Phemeranthus*, *Calandrinia* s.s., Australian *Calandrinia*, Montieae (*Lewisia* + *Montia* + *Claytonia* + *Lewisiopsis*), and the CCM clade (*Cistanthe* + *Calyptridium* + *Montiopsis*).

Species tree reconstruction

We inferred phylogenetic relationships using both concatenation and coalescent-based approaches. Concatenation analyses were performed in RAxML version 8.0.22 (Stamatakis, 2006) using a GTRCAT substitution model, with 1000 rapid bootstrap replicates. Coalescent-based species trees were estimated with ASTRAL II 4.10.2 (Mirarab et al., 2014), a package that analyzes individual gene trees (made with RAxML in step 3 of the pipeline). We used site-only multilocus bootstrapping to generate bootstrap support values. Both ASTRAL and maximum likelihood concatenated RAxML trees were reconstructed using four unique data sets, each with different amounts of missing data. The first two data sets varied in the number of loci present in a given percentage of individuals: all loci present in 85% or more of the individuals (minimum of 98 of 116 individuals, 74 loci; named i98) and all loci present in 35% or more of the individuals (minimum of 40 of 116 individuals, 165 loci; named i40). The other two data sets varied in loci present per major grouping (8 groups, see above): all loci present in two or more groups (297 loci; named g2) and all loci present in seven or more groups (116 loci; named g7). In all data sets, we removed sites (nucleotides) present in <10% of individuals. Finally, to more fully understand the effects of missing data on phylogenetic relationships and branch lengths, we removed all sites present in <50% of the individuals and reran concatenation and coalescent-based phylogenetic analyses using the reduced g2, g7, i40, and i98 data sets. To compare the internal and the terminal branch lengths of the g2, g7, i40, and i98 Australian *Calandrinia* concatenated phylogenies, when sites present in <10% of the individuals were removed and when sites present in <50% of the individuals were removed, we used a two-tailed, paired *t*-test as implemented in R version 3.3.1 (R Core Team, 2017).

Bayesian concordance analysis was performed using BUCKy version 1.4.4 (Larget et al., 2010) based on the posterior distribution of gene trees from analyses in MrBayes 3.2 (Ronquist et al., 2012). BUCKy estimates the genomic support as a concordance factor (CF) for each relationship found across analyses of all individual loci (Ane et al., 2006; Baum, 2007), thereby detecting groups of genes supporting the same topology while accounting for uncertainty in gene tree estimates. We focused BUCKy analyses on the relationships among 12 major Montiaceae lineages: *Phemeranthus*, *Cistanthe* s.s. (sensu Ogburn and Edwards 2015), *Calyptridium*, *Montiopsis*, Montieae, *Calandrinia* s.s., and the six Australian *Calandrinia* subclades (see below). BUCKy does not allow for missing data, and most of our loci were not sampled from across all 116 terminals. However, as we were primarily interested in how these major lineages were related, we could include all loci that were sampled from at least one taxon in each of the lineages. To construct this data set, we renamed taxa to their major lineages, then pruned all but one random exemplar for each lineage from each sample of the posterior distribution of MrBayes trees (for more on this approach, see Moore et al., 2018). BUCKy analyses used four independent runs under default settings, with the option enabled to compute the posterior probability that pairs of loci support the same topology.

Ancestral character state reconstruction

Ancestral character state reconstructions within Australian *Calandrinia* were based on the topology inferred using ASTRAL and the g2 data set (297 loci), with RAxML branch lengths. This choice was guided by the result that coalescent-based species tree estimations were topologically more congruent across all data sets (g2, g7, i40, i98) than trees inferred using concatenated data, which showed considerable topological variation depending on the loci used (Fig. 3; Appendix S2). Moreover, the concordance analyses also supported this topology (Fig. 4). Four morphological traits were characterized for each Australian *Calandrinia* species and mapped onto the tree: stigmatic lobe number, petal number, capsule type (dehiscent vs. indehiscent), and life history (annual vs. perennial). We chose to focus our analyses on these four morphological traits as they were originally used by von Poellnitz (1934), Tahir and Ashton (1989), and Tahir and Carolin (1990) to differentiate Australian *Calandrinia* clades. Ancestral character state probabilities at all internal nodes were inferred based on maximum likelihood, using the ape package version 4.1 of the statistical software R (R Core Team, 2017; Paradis et al., 2004). For each analysis, we modelled these characters as discrete traits, allowed transition rates between states to vary equally, differently, or symmetrically. The model with AIC >2 above the alternative was chosen as the preferred model.

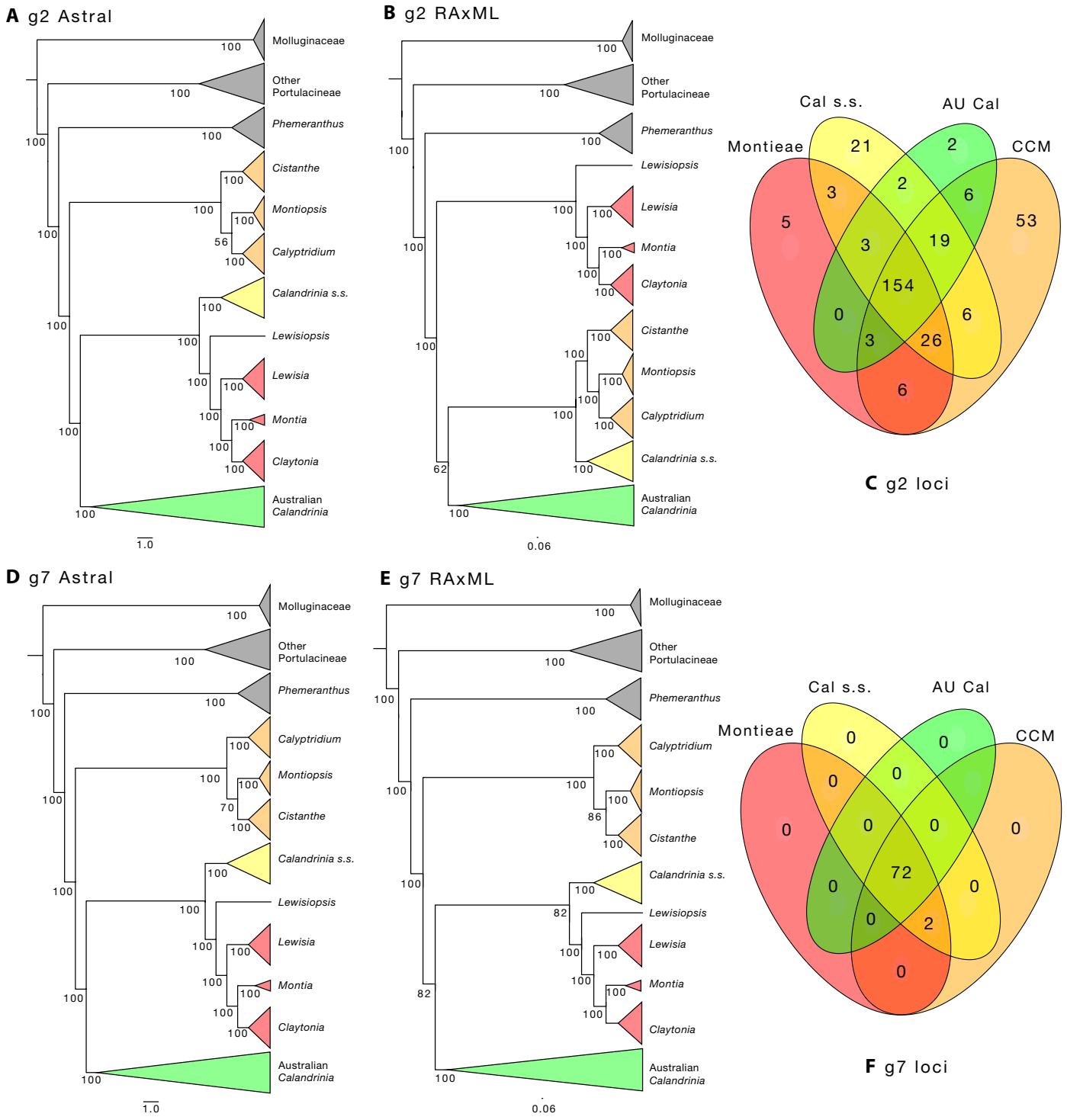


FIGURE 3. Summary trees depicting the major clades in Montiaceae. Bootstrap support percentages are below branches. (A) The g2 data set representing 297 loci ASTRAL consensus topology. (B) The g2 data set representing 297 loci, maximum likelihood RAxML topology. (C) Venn diagram showing the distribution of loci and locus overlap in the g2 data set across groups that show topological conflict in phylogenetic analyses: CCM group (*Cistanthe* + *Calyptridium* +*Montiopsis*), *Calandrinia* s.s., Australian *Calandrinia,* and Montieae (*Lewisia,* + *Claytonia* + *Montia* + *Lewisiopsis*). (D) The g7 data set representing 115 loci, ASTRAL consensus topology. (E) The g7 data set representing 115 loci, maximum likelihood RAxML topology. (F) Venn diagram showing the distribution of loci and locus overlap across groups (CCM, *Calandrinia* s.s., Montieae, Australian *Calandrinia*) that show topological conflict using the g7 data set.

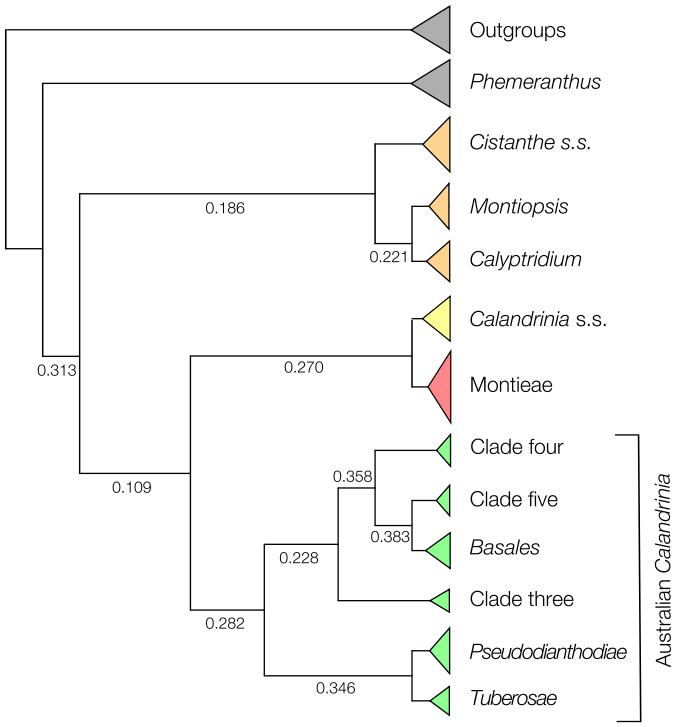


FIGURE 4. Bayesian concordance analysis primary concordance tree for Montiaceae backbone relationships including the six Australian *Calandrinia* clades. The concordance factor, which indicates the percentage of genome support for a given split, is below the node.

Dating, diversification rates, and biogeographic analyses

Divergence times were estimated using a Bayesian approach as implemented in BEAST v2.4.5. (Bouckaert et al., 2014) using the g7 concatenated matrix (116 individuals, 116 loci, 158,436 bp) as it was the shortest. We fixed the tree topology using the g2 ASTRAL topology and estimated branch lengths under a log-normal relaxed clock (Drummond et al., 2006). Since there are no reliable and suitably old fossils of Portulacineae or Molluginaceae, we relied on a secondary calibration to date the divergence of Portulacineae; the stem age of Portulacineae was set to 53 Myr ago (Ma) with a standard deviation of ±2.1 Myr (Arakaki et al., 2011) and parameterized under a normal prior, because it was a secondary calibration point. Of studies that estimate divergence times across Portulacineae lineages (Ocampo and Columbus, 2010; Arakaki et al., 2011; Hernandez-Hernandez et al., 2014), we chose to use the divergence times (and standard deviations) generated by Arakaki et al. (2011) because their dating analyses are informed by a larger number of fossil calibration points. We used the GTR model of substitution with 4 gamma rate categories for the substitution rate. A birth–death speciation process was specified with default priors. The analysis was run twice for 10,000,000 generations, sampled every 1000 generations. Convergence and adequacy of burn-in were inspected visually in Tracer v1.5 (Rambaut and Drummond, 2007), and a maximum clade credibility tree was computed in TreeAnnotator.

We examined both the geographic history of the Montiaceae and Australian *Calandrinia* using a likelihood-based dispersal–extinction–cladogenesis (DEC) ancestral range reconstruction in Lagrange version 20130526 (Ree and Smith, 2008). For the Montiaceae analysis, we coded species as North American, South American, and/or Australian. For the Australian *Calandrinia* analysis, we allowed each tip to occupy one or more of the following five geographic regions: southwestern Australia (SW), western Australia excluding the SW (WA), northern Australia (N), central Australia (C) and east/southeastern Australia (SE). We chose these five regions because they represent floristically distinct and/or geographically separate zones in Australia (Crisp et al., 2004a) and allowed us to reconstruct informative ancestral nodes. We separated the Eremean region of Australia into a western and central zone in efforts to further understand dispersal across the Nullabor Plain, a large limestone karst that is a geographic barrier to dispersal (Crisp and Cook, 2007). We allowed for migration between all adjacent regions using an assigned rate of 1.0. For non-adjacent regions (i.e., N-SW, SE-SW, and WA-SE), where migration between regions required passing through another region, we used a "stepping-stone" model (Fine et al., 2014), assigning a rate of 0.5 to these transitions.

To identify potential shifts in diversification rate within the Australian *Calandrinia* clade, we ran MEDUSA (Alfaro et al., 2009) using a birth–death model, as implemented in the R package GEIGER 2.0.6 (Harmon et al., 2008). We removed all tips except for a species representative for each Australian *Calandrinia* taxa from 500 BEAST posterior trees and ran MEDUSA on the resulting 67-tip species trees. Since taxonomic sampling of Australian *Calandrinia* is essentially complete, with the exception of several undescribed taxa, we did not build a species richness table to account for missing taxa.

We also evaluated diversification in Australian *Calandrinia* using Bayesian analysis of macroevolutionary mixtures (BAMM) (Rabosky, 2014). We set the priors for the analyses using the setBAMMpriors function in BAMMtools (Rabosky et al., 2014). These priors included: expectedNumberofShifts = 1.0; lambaInitPrior = 1.71; lambaShiftPrior = 0.038; muInitPrior = 1.78. We ran BAMM on the maximum clade credibility Australian *Calandrinia* topology (with 67 tips) and allowed possible rate-change events to occur on all branches (minCladeSizeForShift = 1.0). BAMM was run for 10 million generations sampling every 1000. BAMMtools was used to assess convergence and effective sample size and to identify shifts within the 95% credible shift configuration. In addition, we constructed a lineage through time (LTT) plot based on 500 posterior distribution trees from the BEAST analysis to visualize broad shifts in diversification rate through time.

RESULTS

Sequencing results and assembly

After running step 1 and 2 of the pipeline, 1018 loci were available for analysis. The number of loci per gene family (82 gene families in total) ranged between 1 and 39 (mean = 11.4; SD = 8.74). The number of loci recovered per individual ranged between 12 and 364 with the average being 151 loci (Appendix S1). Average locus length was 666.5 (SE ±16.89) nucleotide sites, ranging between 152 and 3468 bp.

Phylogenetic reconstruction

The concatenated matrices of the four data sets, where sites present in <10% of the individuals were removed, had the

following properties: data set i98 = 164,559 nucleotides (bp), 91,811 parsimony-informative sites (is), 61.95% missing data (md); data set i40 = 317,622 bp, 173,194 is, 67.99% md; data set g7 = 158,436 bp, 105,972 is, 49.88% md; data set g2 = 244,257 bp, 152,108 is, 58.05% md. To investigate the effect of missing data on phylogenetic analyses, particularly on the length of terminal branches, we then removed all sites present in <50% of the individuals. The concatenated matrices of the four resulting data sets had the following properties: data set i98 = 65,050 bp, 43,388 is, 25.87% md; data set i40 = 91,466 bp, 62,413 is, 28.96% md; data set g7 = 81,068 bp, 54,897 is, 27.91% md; data set g2 = 91,466 bp, 62,413 is, 28.96% md. Although these data sets had fewer missing data, they also had fewer phylogenetically informative sites, resulting in inconsistent and poorly resolved phylogenetic relationships along the Montiaceae backbone (Appendix S2). No difference in topology or bootstrap support was found across the Australian *Calandrinia* backbone when reconstructing the phylogenies with the reduced matrices (Appendix S2). Furthermore, we found no significant difference in Australian *Calandrinia* branch lengths (both internal and terminal branch lengths) when reconstructing the phylogenies with the reduced matrices versus the original matrices (<10%) except for in the i40 (t = 2.40, df = 66, P = 0.02) and i98 (t = 3.23, df = 66, P = 0.002) data sets, where the terminal branches were significantly shorter with fewer missing data (Appendix S3). Thus, we chose to focus the remainder of our results and discussion on the phylogenetic trees reconstructed with more missing data (i.e., where sites present in <10% of the individuals were removed) and informative sites.

Paraphyly of Calandrinia—There is strong and consistent support for two distinct monophyletic *Calandrinia* clades: New World *Calandrinia* (hereafter referred to as *Calandrinia* s.s.) and Australian *Calandrinia*. These clades are strongly supported and distinct across all data sets and methods of analysis (Fig. 3 and Appendices S4–S10). The type species for *Calandrinia*, *C. ciliata* (Ruiz & Pav.) DC, falls within the New World *Calandrinia* clade. In addition to resolving the monophyly of Australian *Calandrinia,* we confidently place *Rumicastrum chamaecladum* (Diels) Ulbr. within Australian *Calandrinia.* This strongly supported result requires new combinations for all Australian *Calandrinia* species, either into *Rumicastrum* or, if a proposal to conserve the name *Parakeelya* is successful, into *Parakeelya* (Thiele et al., 2018).

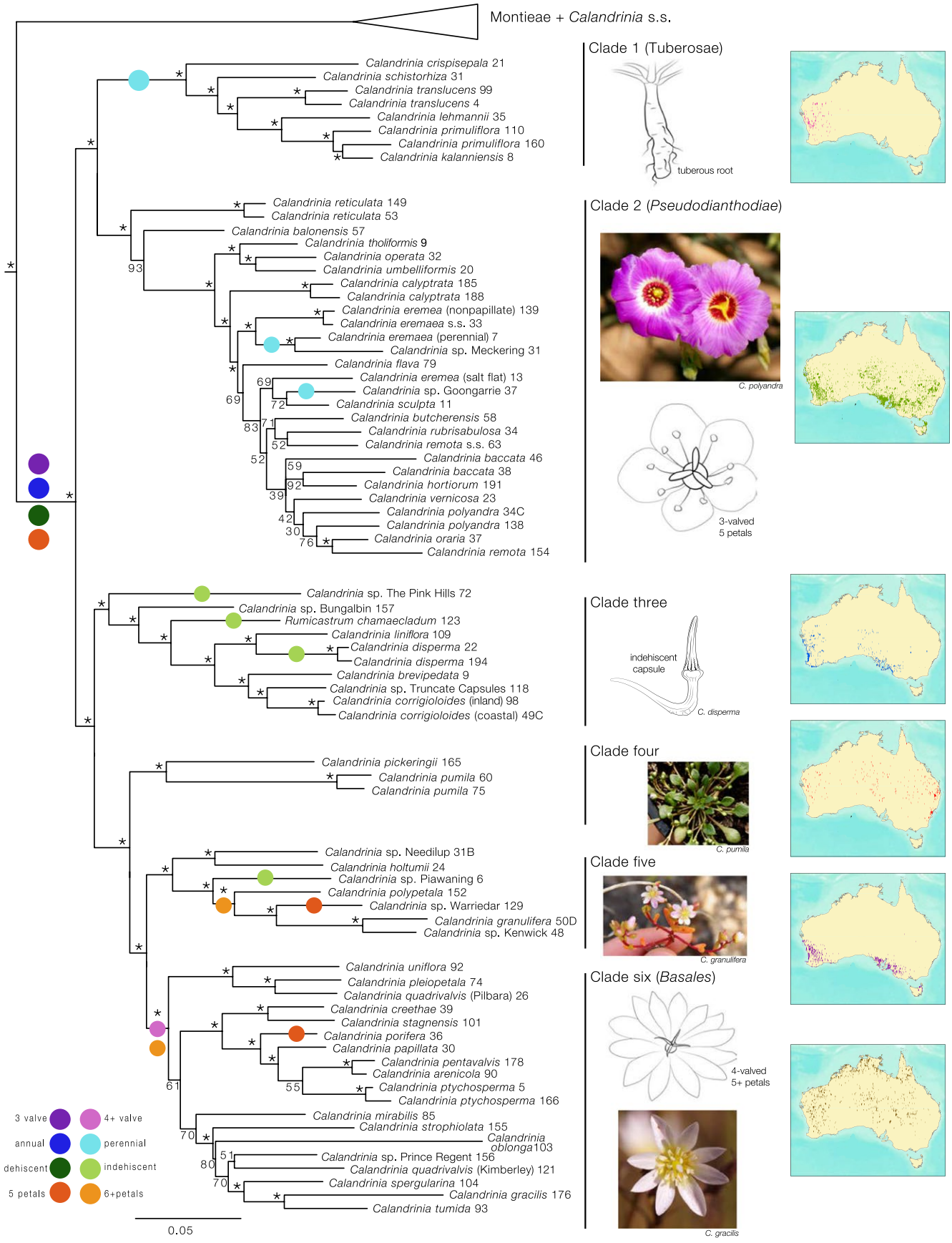
Relations among major Montiaceae lineages—With the exception of *Calandrinia*, all recognized genera within Montiaceae are well supported (100% bootstrap support) in both concatenated and ASTRAL analyses (Fig. 3; Appendices S4–S10). It should be noted, however, that section *Philippiamra* Kuntz within *Cistanthe* was not sampled in this study, and in previous studies it has been shown to be distinct from other *Cistanthe* with strong support (Ogburn and Edwards, 2013, 2015). *Phemeranthus* is resolved as sister to the rest of Montiaceae (100% bootstrap support), and *Lewisiopsis*, *Lewisia*, *Claytonia*, and *Montia* form a well-supported clade (Montieae), with *Lewisiopsis* consistently sister to *Lewisia* + *Montia* + *Claytonia* across analyses and data sets (100% support in all cases). *Cistanthe* s.s., *Calyptridium*, and *Montiopsis* also form a well-supported clade (hereafter called CCM). However, the inferred relationships among these major groups vary notably across analyses and data sets.

Overall, ASTRAL produced consistent, well-supported topologies across data sets, while topologies from concatenation analyses differed (Fig. 3; Appendices S2, S4, S6, S8). In ASTRAL analyses, the CCM clade is recovered as sister (100%) to Montieae + *Calandrinia* s.s., and Australian *Calandrinia.* The only conflict observed in the backbone tree using ASTRAL are relationships within the CCM clade: *Calyptridium* is resolved as sister (100%) to *Cistanthe* s.s. + *Montiopsis* using the g7 and i98 data sets, but *Cistanthe* s.s. is sister (100%) to *Montiopsis* + *Calyptridium* with the other two data sets (g2 and i40). All data sets show low support for *Cistanthe* s.s. + *Montiopsis* (g7, 70%; i98, 62%) and *Montiopsis* + *Calyptridium* (g2, 56%; i40, 60%). Across the concatenated trees estimated with RAxML there is also conflict among these relationships. Specifically, *Calyptridium* is sister to *Cistanthe* s.s. + *Montiopsis* in the i98 and g7 trees, while *Cistanthe* s.s. is sister to *Montiopsis* + *Calyptridium* with the remaining two data sets. The bootstrap support for these relationships is >99% across concatenated trees in all analyses except for g7, where the *Cistanthe* s.s. + *Montiopsis* node received only 86% bootstrap support (Fig. 3). Results from the Bayesian concordance analysis illuminated these differences: while the primary concordance tree recovered *Cistanthe* s.s. as sister to *Montiopsis* + *Calyptridium*, the concordance factor for this relationship (CF = 0.221) was only marginally higher than the one for the competing relationship *Cistanthe* s.s. + *Montiopsis* (CF = 0.211), indicating genome-wide conflict among loci at this node (Fig. 4; Appendix S11).

In contrast to the ASTRAL analyses, topologies based on concatenation and RAxML analysis differed across data sets (Fig. 3 and Appendices S2, S5, S7, S9, S10). Three nodes that exhibit considerable conflict are highlighted here. First, *Calandrinia* s.s. is recovered as sister to the CCM clade (>96% bootstrap support) across all data sets except g7 (Appendix S5), with *Calandrinia* s.s. sister to Montieae, albeit with low support (BS = 82%). Second, the placement of the CCM clade + *Calandrinia* s.s. varied across matrices: in the i40 and i98 trees, the CCM clade + *Calandrinia* s.s. is resolved as sister to Montieae + Australian *Calandrinia* with 100% bootstrap support, while in the g2 tree, CCM + *Calandrinia* s.s. is sister to Australian *Calandrinia* (BS = 66%). Third, the Montieae clade is placed as sister to Australian *Calandrinia* in the i40 and i98 trees (BS = 96%), while species tree reconstructions with the g2 data set place Montieae as sister to CCM + *Calandrinia* s.s. and Australian *Calandrinia.* The concatenation analysis based on data set g7 resolved a very similar topology as recovered with the coalescent based reconstructions, placing Montieae + *Calandrinia* s.s. as sister to Australian *Calandrinia* (Fig. 3).

Bayesian concordance analysis resulted in a primary concordance tree that was congruent with the topology recovered by ASTRAL and g7 Concatenation (Fig. 4). Specifically, it supported *Calandrinia* s.s. as sister to Montieae, with these jointly being sister to Australian *Calandrinia.* Although CF values are typically quite low, indicating that significant portions of the genome support relationships that deviate from the dominant signal (Appendix S11), there are no alternative resolutions that are equally well supported as those in the primary concordance tree. The only exception to this pattern was within the CCM clade, highlighted above.

***Australian* Calandrinia**—Species tree reconstructions using concatenation, coalescence, and Bayesian inference recover six strongly supported clades within Australian *Calandrinia* (Fig. 5). Three of these correspond to the previously described sections *Tuberosae*, *Pseudodianthoidiae*, and *Basales*, while three (clades 3, 4, and 5) have not been previously recognized taxonomically. There is


Montieae + Calandrinia s.s.
Clade 1 (Tuberosae)
tuberous root
Clade 2 (Pseudodianthodiae)
C. polyandra
3-valved
5 petals
Clade three
indehiscent
capsule
C. disperma
Clade four
C. pumila
Clade five
C. granulifera
Clade six (Basales)
4-valved
5+ petals
C. gracilis
Calandrinia crispisepala 21
Calandrinia schistorhiza 31
Calandrinia translucens 99
Calandrinia translucens 4
Calandrinia lehmannii 35
Calandrinia primuliflora 110
Calandrinia primuliflora 160
Calandrinia kalanniensis 8
Calandrinia reticulata 149
Calandrinia reticulata 53
Calandrinia balonensis 57
Calandrinia tholiformis 9
Calandrinia operata 32
Calandrinia umbelliformis 20
Calandrinia calyptrata 185
Calandrinia calyptrata 188
Calandrinia eremea (nonpapillate) 139
Calandrinia eremaea s.s. 33
Calandrinia eremaea (perennial) 7
Calandrinia sp. Meckering 31
Calandrinia flava 79
Calandrinia eremea (salt flat) 13
Calandrinia sp. Goongarrie 37
Calandrinia sculpta 11
Calandrinia butcherensis 58
Calandrinia rubrisabulosa 34
Calandrinia remota s.s. 63
Calandrinia baccata 46
Calandrinia baccata 38
Calandrinia hortiorum 191
Calandrinia vernicosa 23
Calandrinia polyandra 34C
Calandrinia polyandra 138
Calandrinia oraria 37
Calandrinia remota 154
Calandrinia sp. The Pink Hills 72
Calandrinia sp. Bungalbin 157
Rumicastrum chamaecladum 123
Calandrinia liniflora 109
Calandrinia disperma 22
Calandrinia disperma 194
Calandrinia brevipedata 9
Calandrinia sp. Truncate Capsules 118
Calandrinia corrigioloides (inland) 98
Calandrinia corrigioloides (coastal) 49C
Calandrinia pickeringii 165
Calandrinia pumila 60
Calandrinia pumila 75
Calandrinia sp. Needilup 31B
Calandrinia holtumii 24
Calandrinia sp. Piawaning 6
Calandrinia polypetala 152
Calandrinia sp. Warriedar 129
Calandrinia granulifera 50D
Calandrinia sp. Kenwick 48
Calandrinia uniflora 92
Calandrinia pleiopetala 74
Calandrinia quadrivalvis (Pilbara) 26
Calandrinia creethae 39
Calandrinia stagnensis 101
Calandrinia porifera 36
Calandrinia papillata 30
Calandrinia pentavalvis 178
Calandrinia arenicola 90
Calandrinia ptychosperma 5
Calandrinia ptychosperma 166
Calandrinia mirabilis 85
Calandrinia strophiolata 155
Calandrinia oblonga 103
Calandrinia sp. Prince Regent 156
Calandrinia quadrivalvis (Kimberley) 121
Calandrinia spergularina 104
Calandrinia gracilis 176
Calandrinia tumida 93
93
69
72
69
71
83
52
52
59
92
39
42
30
76
61
55
70
51
80
70
3 valve
4+ valve
annual
perennial
dehiscent
indehiscent
5 petals
6+petals
0.05

both strong and uniform support for the monophyly and placement of these six clades across all data sets (Appendices S4–S10). Furthermore, the topologies within *Tuberosae* and clades 3, 4, and 5 are congruent and well supported across all data sets and analyses. Within the *Pseudodianthoidiae* and *Basales* clades, however, there is considerably more uncertainty in species relationships (Fig. 5), regardless of data set and/or method of analysis. Below we provide a brief description for each of the six clades.

Clade 1 aligns well with section *Tuberosae* as circumscribed by von Poellnitz (1934), Tahir and Ashton (1989), and Tahir and Carolin (1990). These species are perennials with a tuberous rootstock, five-petaled, three-carpellate flowers with numerous stamens, and dehiscent capsules. Most species occur in semi-arid to arid regions of Western Australia.

Clade 2 includes most species placed in section *Pseudodianthoideae* by von Poellnitz (1934), Tahir and Ashton (1989), and Tahir and Carolin (1990). These are semi-erect to erect (rarely prostrate to decumbent) annuals (rarely perennials), with five-petaled, three-carpellate flowers that are usually medium to large, mostly with numerous stamens, and have dehiscent, many-seeded capsules. Most occur in temperate to semi-arid parts of southern and central Australia. Though the clade is well supported, many species relationships within it are poorly resolved.

Clade 3 includes *C. disperma*, *C. brevipedata*, *C. corrigioloides*, *C. liniflora*, and *Rumicastrum chamaecladum,* as well as three currently undescribed taxa (*C.* sp. Truncate Capsules, *C.* sp. The Pink Hills, *C.* sp. Bungalbin). The named species, with the exception of *R. chamaecladum*, were placed by von Poellnitz 1934 and Tahir and Carolin, 1990 in section *Pseudodianthoideae*. Most are small, prostrate to decumbent annuals often with many, relatively small, three-carpellate flowers with five or fewer petals and stamens. Most have several-seeded, dehiscent capsules, except *C.* sp. The Pink Hills, *R. chamaecladum*, and *C. disperma,* which have one- or two-seeded, indehiscent capsules. All are endemic to southwestern Australia except for *C. disperma*, which grows through western and central Australia.

Clade 4 comprises *C. pickeringii* and *C. pumila*. Both are prostrate to decumbent, five-petaled, pink-flowered, 3-carpellate annuals that range widely across temperate arid Australia into eastern Queensland and New South Wales. No obvious morphological synapamorphies unite this clade.

Clade 5 is a morphologically diverse group, also with no apparent synapamorphy that differentiates it from the other five clades. Most species are prostrate to decumbent, diminutive annuals with small to very small, three-carpellate, five-petaled flowers. All have several- to many-seeded dehiscent capsules except *C.* sp. Piawaning, which has indehiscent capsules usually containing two seeds. Most species are endemic into southern Western Australia except *C. granulifera*, which is widespread across southern Australia including Tasmania.

Clade 6 aligns well with section *Basales*, as circumscribed by von Poellnitz (1934) and Tahir and Ashton (1989). All are four-carpellate except *C. pentavalvis* and *C. strophiolata*, which are five- and six-carpellate, respectively. All species are decumbent to erect annuals, mostly with medium to large flowers with six or more pink, purple, or white petals and usually numerous stamens. Capsules are generally many-seeded (*C. oblonga* has few, large seeds). Capsules in *C. porifera* open by a terminal pore, while all other species are valvate. Many species in this clade occur in northern, subtropical (monsoonal) Australia, with others occurring in the arid interior.

Ancestral character state reconstructions

Character state reconstructions indicate that ancestral Australian *Calandrinia* was likely an annual plant with three-stigmatic lobes, five petals, and dehiscent capsules (Fig. 5). When ML analyses were conducted for life-history and fruit-type reconstructions, the simplest model with equal transitions rates was favored, though AIC scores between models were typically very similar, and our actual reconstructions inferred shifts in only one direction. Life-history reconstructions under an equal rates model (ER AIC = 33.57; ARD AIC= 35.03) recovered three shifts from annual to perennial; one shift at the base of section *Tuberosae* and two in *Pseudodianthoidieae* (Appendix S12). Evolution of indehiscent fruits, under the favored ER model (ER AIC = 31.71; ARD AIC 33.73), likely occurred four separate times with three of these switches arising in clade 3 and one in clade 4 (Appendix S13). There is considerable intraspecific variation in petal number when a species has six or more petals; for this reason, we chose to bin petal number as five and six plus. Under the favored ER model (ER = 37.56; ARD = 39.51), the derived six plus petal state evolved twice, once in clade 4 and once at the base of *Basales*; there are also several reversals back to five petals from 6 plus petals (Appendix S14). On the basis of AIC values, we chose the more complex, symmetrical transition rates model to reconstruct valve number. We found one shift to a four-valve state from the ancestral three-valved state at the base of section *Basales,* and two shifts to four plus valves within this clade (ER AIC = 45.32; SYM AIC = 43.21; ARD AIC = 52) (Appendix S15).

Divergence time estimation, rate shifts, and biogeographic analyses

The estimated crown age of Australian *Calandrinia* is late Oligocene (27.03 ± 2.95 Myr), with most diversification events occurring during the Miocene (Fig. 6). MEDUSA and BAMM analyses indicate no clade-specific shifts in diversification rate within Australian *Calandrinia*, although BAMM shows a lineage-wide general slowdown or decrease in speciation rate with time (Appendix S17). The inferred slowdown is not surprising given the near absence of any topological divergence events from the Pliocene onward (Fig. 6). The LTT plot (Appendix S16) shows an abrupt and dramatic decline in lineage accumulation at 10 Ma.

Biogeographic reconstruction of the Montiaceae using the time-calibrated phylogeny (Fig. 6; Appendix S17) indicate that Australian *Calandrinia* most likely entered Australia via South America/Antarctica close to the estimated Australia/Antarctica split at ~33

FIGURE 5. Australian *Calandrinia* phylogeny reconstructed with the ASTRAL g2 locus data set (297 loci) with g2 RAxML branch lengths. Bootstrap support values from 1000 bootstrap replicates with greater than 95% support are indicated with a star. Ancestral character states and shifts in character traits are mapped onto the phylogeny, with purple circles representing valve number, blue circles life-history habit, green circles capsule type, and orange circles petal number. Maps correspond to each of the six clades, with colored dots representing species distributions for the given clade. For more ancestral character state reconstruction detail, see Appendices S12–S15.

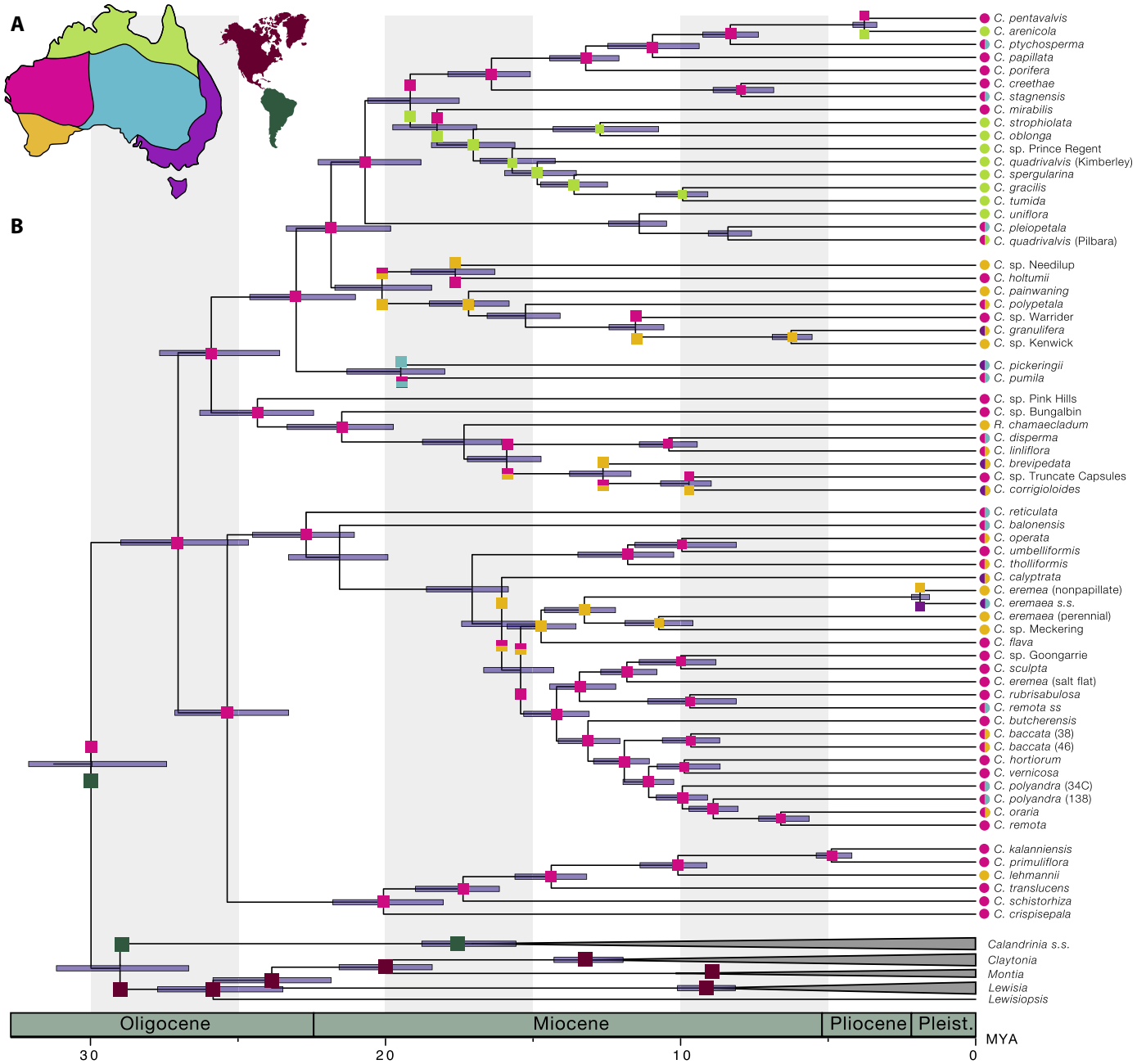


FIGURE 6. (A) Map of Australia colored by geographic region plus North and South America. (B) Time-calibrated phylogeny made with BEAST of Australian *Calandrinia* and its sister lineages. Colored boxes reconstructed at the nodes correspond to the geographic regions as denoted in Fig. 6A. Only the ancestral states with greater than 50% probability are reconstructed on the tree. If there was equal probability of a region at a node, both states were reconstructed.

Ma (Crisp et al. 2004). LaGrange recovered a South America/Australia separation (100% probability) at the node separating Australian *Calandrinia* from Montieae + *Calandrinia* s.s., indicating an ancestral South American distribution. Australian *Calandrinia* likely originated in Western Australia (78% probability) and moved east and south into central and southwestern Australia, respectively, multiple times. There were only two dispersals into northern Australia, and both in section *Basales;* one roughly ~18 Ma, and one within the last few million years (*C. arenicola*).

DISCUSSION

Phylogenetic relationships within the Montiaceae

The most recent phylogenetic evaluation of the Montiaceae (Ogburn and Edwards, 2015), using three-marker (*matK*, *ndhF*, and *phyC*) and five-marker (including ITS and *ycf3*) combined analyses, is congruent with many of the relationships presented here: *Phemeranthus* is sister to the rest of Montiaceae, with *Cistanthe* s.s.,

Calyptridium, and *Montiopsis* forming a well-supported clade (the CCM clade) and *Claytonia*, *Montia*, *Lewisia*, and *Lewisiopsis* (the Montieae) also well supported as monophyletic.

Although the paraphyly of *Calandrinia* s.l. has long been suspected (Carolin, 1987; Hershkovitz and Zimmer, 1997; Hershkovitz, 1998), only here do we finally confirm that *Calandrinia* s.s. and Australian *Calandrinia* are distinct lineages and do not form a clade. Concatenation and coalescent-based methods support alternative scenarios for relationships within the major lineages of Montiaceae; however, concordance analyses, despite only being run on a smaller subset of our loci, show that the top 10 splits (i.e. bifurcations) converge onto the same underlying phylogenetic relationships, those recovered in the g7 RAxML and all ASTRAL analyses (Fig. 3A, B, E; Appendices S2, S6, S8). Discordance in the placement of *Calandrinia* s.s. and the sister lineage to Australian *Calandrinia* does not appear to result from strongly supported conflict at the nodes, as seen in the relationships among *Cistanthe*, *Calyptridium*, and *Montiopsis*, but rather from a low signal-to-noise ratio in the gene trees. It is likely that individual gene trees are resolved with low support because of missing data across many individuals. Similarly, we suspect that the concatenation analyses were only congruent with the ASTRAL backbone topology when analyzing the g7 matrix because the loci represented in this data set (see Fig. 3C compared to Fig. 3F) have the least amount of missing data across the Montiaceae. Concatenation analyses, in general, appeared more sensitive to missing data than ASTRAL analyses. Furthermore, although genomic support for many clades is quite low (typically CF of 0.2–0.3), the entire primary concordance tree (Fig. 4) assembled from the top 10 best-supported splits is perfectly congruent with the ASTRAL topology.

Phylogenetic relationships within Australian *Calandrinia*

Phylogenetic analyses using concatenation, coalescence, and Bayesian inference recover six strongly supported clades within Australian *Calandrinia* (Fig. 5), with *Rumicastrum chamaecladum* nested within clade 3 with strong support. Although the backbone topology of the lineage is consistent across data sets and all three methods of inference, there remains considerable topological uncertainty within certain clades, specifically within *Basales* and *Pseudodianthoideae*. The recovery of low and inconsistent support for many of these relationships may indicate widespread gene flow and introgression during past speciation events. Many of these poorly supported species relationships represent taxa that overlap geographically and share similar, or the same, habitat and niche space.

In addition to hybridization and introgression, low support values and topological uncertainty at the tips may also reflect several complexes of poorly defined species within these two clades, centered on the (mostly) geographically widespread *C. quadrivalvis*, *C. eremaea*, *C. baccata*, *C. polyandra*, and *C. remota*. For example, the *C. eremaea* complex, which is not monophyletic in our analyses, occurs across the southern half of Australia, from Mediterranean climate areas of southwestern Australia and Victoria, to more arid regions of western and central Australia. *Calandrinia eremaea* s.s. is from central southern Queensland and is a small, decumbent to erect annual herb with three stigmas, five petals, eight stamens and reniform to subreniform, colliculate to finely papillate seeds. Most variants deviate notably from the original species description, including individuals with a perennial rather than annual growth form and others with smooth to dull, noncolliculate seeds.

Finally, low support for some relationships may be a product of poor sequence capture with the bait hybridization approach. For example, it is likely that placement of *C. oblonga* is compromised by the fact that we recovered the fewest loci for this taxon (12 loci).

Dating and the biogeographic history of Australian *Calandrinia*

While we are fully aware of the shortcomings (see Schenk, 2016) of utilizing secondary node calibrations to estimate divergence times, no suitable fossils are available for our analyses. We feel that an attempt to date this Australian radiation with secondary calibration is preferable to none at all and recommend interpreting our inferred ages with a reasonable level of skepticism, as we do.

The evolution and radiation of Australian *Calandrinia* has received little attention, as species relationships within the lineage have been largely unresolved. Tahir and Carolin (1990) suggested that Australian *Calandrinia* were Gondwanan in origin (i.e., colonizing Australia before it separated from Antarctica) rather than arriving via long-distance dispersal from South America. They argued that dispersal (e.g., by birds, ocean currents) would have left a "trail" of related species on islands between South America and Australia, including New Zealand, which provide suitable climate and habitat.

Using modern molecular and phylogenetic analyses (Fig. 6; Appendix S18), we provide some support for Tahir and Carolin's biogeographic hypothesis for the radiation of *Calandrinia.* In our BEAST analysis, the node separating the Australian *Calandrinia* clade from its sister lineage (the Montieae clade plus *Calandrinia* s.s) is estimated to be ~30 ± 2.34 Ma, which aligns well with the final separation of Australia from Antarctica at ~33 Mya (Scher and Martin, 2006). The Montieae + *Calandrinia* s.s. clade is primarily distributed in North and South America, with *Calandrinia* s.s. extending into southern Chile, Argentina, and the Falkland Islands (*C. feltonii*) and two *Montia* species (*M. fontana* and *M. australasica*) into Australia. Not included in our phylogenetic analyses are the monotypic *Hectorella* and *Lyallia* lineages. *Hectorella* was recovered as sister to the Montieae by Ogburn and Edwards (2015), and *Lyallia* was placed as sister to *Hectorella*, albeit with low support, by Arakaki et al. (2011). *Lyallia* is endemic to the sub-Antarctic Kerguelen Islands, while *Hectorella* is endemic to the South Island of New Zealand. If these were sister to the Australian *Calandrinia* clade, they could comprise the "trail" of taxa, anticipated by Tahir and Carolin (1990), indicating east to west long-distance dispersal. However, given the dates in our analysis, and their uncertainty, we find it difficult to strongly argue for either hypothesis. The dispersal and radiation of plants between South America and Australia via Antarctica is well known and documented by a rich record of both macro- and microfossils (Eklund et al., 2004; Sanmartín and Ronquist, 2004; Wagstaff and Hennion, 2007; Crisp and Cook, 2013) and also seems to be a reasonable scenario for the Montiaceae. The phylogenetic placement of *Lyallia* and *Hectorella* is critical to these discussions and including these taxa in subsequent work is a high priority.

Regardless of whether Australian *Calandrinia* formed due to vicariance or long-distance dispersal, the lineage is a longtime resident of Australia. Our analyses indicate that the stem lineage of Australian *Calandrinia* originated in mesic, temperate climates of Western Australia during the late Oligocene. The crown lineage then diversified over the next 15 Myr, during the early to mid-Miocene, when the climate in Australia was generally wet,

warm, and stable (Martin, 2006; Byrne et al., 2011). Australian *Calandrinia* radiated across the continent, moving mostly eastward from Western Australia into central Australia and southward to southwestern Australia. The lineage—specifically section *Basales*—reached northern Australia ~18 Ma, during which time the Australian plate was colliding with the Asian plate (Hall 2012). Once in northern Australia, section *Basales* radiated across the tropics (Fig. 6), the only Australian *Calandrinia* clade to do so.

The dramatic paleoclimatic history of Australia provides a framework in which to consider Australian *Calandrinia* diversification. Although Australia became gradually drier as it drifted northward from Antarctica, due both to the establishment of the Antarctic Circumpolar Current (Scher and Martin, 2006; Lyle et al., 2007) and the precipitous decline in global CO_2 levels (DeConto and Pollard, 2003; Edwards and Ogburn, 2012), aridity did not become widely established in Australia until the mid to late Miocene (~13–6 Ma) (Crisp et al., 2004; Martin, 2006; Byrne et al., 2008). During this time Australia experienced sea-level subsidence, the end of regular flows in paleo-drainage systems, the drying-up of seasonal lakes, and general climatic instability (Hill et al., 1999; Martin, 2006; Byrne et al., 2008). The earlier part of this time period (in general, ~20 to 10 Ma) corresponds with diversification of Australian *Calandrinia* in both western and central Australia (Fig. 6). Although MEDUSA and BAMM analyses recover no clade-specific shifts in diversification rate during this time, LTT plots demonstrate a substantial decline in diversification rate from ~10 Ma to present day (Appendix S16). Even though the beginning of the Pliocene saw slightly wetter and warmer temperatures, the mid-Pliocene into the Pleistocene marks the beginning of severe aridity in Australia (Crisp and Cook, 2007). Stony deserts began to form across western and central Australia, and the tropics of northern Australia became drier (Fujioka et al., 2005; Byrne et al., 2008; Fujioka et al., 2009). Major oscillations between glacial and interglacial climates that characterized the Pleistocene enhanced the expansion of aridity in Australia (Byrne et al., 2008; Fujioka et al., 2009). BAMM analyses also detected an overall decrease in speciation with time (Appendix S17), which is not surprising given the virtual absence of divergence events in our phylogeny from 6 Myr onward (Fig. 6).

A decline in diversification rates has also been reported for other Australian plant lineages that had originally diversified and radiated during the mid-Miocene, e.g., *Tetratheca* (Crayn and Rossetto, 2006), Goodeniaceae (Jabaily et al., 2014), Proteaceae (Sauquet et al., 2009; Mast et al., 2012), and Myrtaceae (Thornhill et al., 2015). Many of these lineages likely responded to extreme desertification of the continent during glacial maxima by retreating to refugia. These extreme arid periods seem to either lead to extinction or species maintenance for many lineages (Crisp et al., 2004; Byrne et al. 2008). While these "Gondwanan" lineages were likely constrained to refugia, especially during glacial maxima, arid-adapted migrant lineages—groups that had newly arrived during the mid-late Miocene and Pliocene epochs, e.g., Chenopodiaceae (Shepherd et al., 2004; Kadereit et al., 2010), Poaceae (Gillespie et al., 2009), Triodiinae (Toon et al., 2015), *Lepidium* (Mummenhoff et al., 2001; Crisp et al., 2004a), *Ptilotus* (Hammer et al., 2015), and certain Asteraceae, including Gnaphalieae (Bergh and Linder, 2009) and *Olearia* and *Celmisia* (Wagstaff et al., 2010), radiated across the continent. These newer arrivals, many of which are short-lived, fast-growing, and often C_4 annuals, may have been better adapted to expanding arid conditions. Expansion of these immigrant lineages across Australia may have fundamentally changed community structure, ecosystem properties, and perhaps the competitive ability of established lineages.

CONCLUSIONS

The apparent lack of diversification in Australian *Calandrinia* from the late Miocene onward is paradoxical: why did this succulent, C_3+CAM lineage (Winter and Holtum, 2011, 2014; Holtum et al., 2016), which seems well-adapted to arid landscapes, not diversify as arid environments became more widespread? Did the extreme climate during the Pliocene-Pleistocene simply exceed the climatic range in which the species could thrive? Or was Australian *Calandrinia* outcompeted by more recent arrivals, such as C_4 grasses and chenopods? Many biogeographic studies of arid ecosystems are hampered by a lack of fossil evidence, and the main sources of biogeographic hypotheses for the assembly of the arid Australian flora have been contemporary species distributions. Lacking physical evidence, such as fossils, the continued generation of robust, well-supported, taxon-rich, time-calibrated phylogenies (e.g., Toon et al., 2015; Owen et al., 2017) are imperative to our understanding of the Australian arid zone, as comparisons between multiple, thoroughly sampled model lineages can reveal general patterns in the timing of species radiations and extinctions.

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DATA ACCESSIBILITY

Voucher information is available in Appendix S1. Raw reads are deposited in the NCBI Short Read Archive (PRJNA417446). All trees and final alignments are available in GitHub [https://github.com/lillypine/Calandrinia_phylogeny].

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

LITERATURE CITED

AVH. 2017. Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria. Website http://avh.ala.org.au/occurrences/search?taxa=Calandrinia#tab_mapView [accessed 10 October 2015].

Alfaro, M. E., F. Santini, C. Brock, H. Alamillo, A. Dornburg, D. L. Rabosky, G. Carnevale, and L. J. Harmon. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences, USA* 106: 13410–13414.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.

Ane, C., B. Larget, D. A. Baum, S. D. Smith, and A. Rokas. 2006. Bayesian estimation of concordance among gene trees. *Molecular Biology and Evolution* 24: 412–426.

Arakaki, M., P. A. Christin, R. Nyffeler, A. Lendel, U. Eggli, R. M. Ogburn, E. Spriggs, et al. 2011. Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proceedings of the National Academy of Sciences, USA* 108: 8379–8384.

Arroyo, M. K., E. Medina, and H. Ziegler. 1990. Distribution and $\delta^{13}C$ values of Portulacaceae species of the high Andes in northern Chile. *Botanica Acta* 103: 291–295.

Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Lesin, et al. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19: 455–477.

Baum, D. A. 2007. Concordance trees, concordance factors, and the exploration of reticulate genealogy. *Taxon* 56: 417–426.

Berger, S. A., and A. Stamatakis. 2011. Aligning short reads to reference alignments and trees. *Bioinformatics* 27: 2068–2075.

Bergh, N. G., and H. Peter Linder. 2009. Cape diversification and repeated out-of-southern-Africa dispersal in paper daisies (Asteraceae–Gnaphalieae). *Molecular Phylogenetics and Evolution* 51: 5–18.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, et al. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537–6.

Byrne, M., D. A. Steane, L. Joseph, D. K. Yeates, G. J. Jordan, D. Crayn, K. Aplin, et al. 2011. Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography* 38: 1635–1656.

Byrne, M., D. K. Yeates, L. Joseph, M. Kearney, J. Bowler, M. A. J. Williams, S. Cooper, et al. 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology* 17: 4398–4417.

Carolin, R. 1987. A review of the family Portulacaceae. *Australian Journal of Botany* 35: 383–412.

Chen, K., D. Durand, and M. Farach-Colton. 2000. NOTUNG: a program for dating gene duplications and optimizing gene family trees. *Journal of Computational Biology* 7: 429–447.

Crayn, D. M., M. Rossetto, and D. J. Maynard. 2006. Molecular phylogeny and dating reveals an Oligo-Miocene radiation of dry-adapted shrubs (former Tremandraceae) from rainforest tree progenitors (Elaeocarpaceae) in Australia. *American Journal of Botany* 93: 1328–1342.

Crisp, M., L. Cook, and D. Steane. 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Philosophical Transactions of the Royal Society B* 359: 1551–1571.

Crisp, M. D., and L. G. Cook. 2007. A congruent molecular signature of vicariance across multiple plant lineages. *Molecular Phylogenetics and Evolution* 43: 1106–1117.

Crisp, M. D., and L. G. Cook. 2013. How was the Australian flora assembled over the last 65 million years? A molecular phylogenetic perspective. *Annual Review of Ecology, Evolution, and Systematics* 44: 303–324.

DeConto, R. M., and D. Pollard. 2003. Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric CO_2. *Nature* 421: 245–249.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4: e88–12.

Edwards, E. J., and R. M. Ogburn. 2012. Angiosperm responses to a low-CO_2 world: CAM and C_4 photosynthesis as parallel evolutionary trajectories. *International Journal of Plant Sciences* 173: 724–733.

Eklund, H., and J. E. Cantrill. 2004. Late Cretaceous plant mesofossils from Table Nunatak, Antarctica. *Cretaceous Research* 25: 211–28.

Elvebakk, A., A. Flores, and J. M. Watson. 2015. Revisions in the South American *Calandrinia caespitosa* complex (Montiaceae). *Phytotaxa* 203: 001–023.

Fine, P. V. A., F. Zapata, and D. C. Daly. 2014. Investigating processes of neotropical rain forest tree diversification by examining the evolution and historical biogeography of the Protieae (Burseraceae). *Evolution* 68: 1988–2004.

Fujioka, T., J. Chappell, L. K. Fifield, and E. J. Rhodes. 2009. Australian desert dune fields initiated with Pliocene-Pleistocene global climatic shift. *Geology* 37: 51–54.

Fujioka, T., J. Chappell, M. Honda, I. Yatsevich, K. Fifield, and D. Fabel. 2005. Global cooling initiated stony deserts in central Australia 2–4 Ma, dated by cosmogenic ^{21}Ne-^{10}Be. *Geology* 33: 993–996.

Gillespie, L. J., R. J. Soreng, and S. W. L. Jacobs. 2009. Phylogenetic relationships of Australian *Poa* (Poaceae: Poinae), including molecular evidence for two new genera, *Saxipoaand Sylvipoa*. *Australian Systematic Botany* 22: 413–25.

Hall, R. 2012. Sundaland and Wallacea: geology, plate tectonics and palaeogeography: 32-78. *In* D. Gower, K. Johnson, J. Richardson, B. Rosen, L. Ruber, and S. Williams [eds.], Biotic evolution and environmental change in Southeast Asia. Cambridge University Press, Cambridge, UK.

Hammer, T., R. Davis, and K. Thiele. 2015. A molecular framework phylogeny for *Ptilotus* (Amaranthaceae): evidence for the rapid diversification of an arid Australian genus. *Taxon* 64: 272–285.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* 24: 129–131.

Hernández-Hernández, T., J. W. Brown, B. O. Schlumpberger, L. E. Eguiarte, and S. Magallón. 2014. Beyond aridification: multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytologist* 202: 1382–1397.

Hershkovitz, M. A. 1991. Phylogenetic assessment and revised circumscription of *Cistanthe* Spach (Portulacaceae). *Annals of the Missouri Botanical Garden* 78: 1009–1021.

Hershkovitz, M. A. 1993. Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on phylogeny of the portulacaceous alliance. *Annals of the Missouri Botanical Garden* 80: 333–365.

Hershkovitz, M. A. 1998. *Parakeelya*: a new genus segregated from *Calandrinia* (Portulacaceae). *Phytologia* 84: 98–106.

Hershkovitz, M. A., and E. A. Zimmer. 1997. On the evolutionary origins of the cacti. *Taxon* 46: 217–232.

Hill, R.S., E.M. Trustwell, S. McLoughlin, and M.E. Dettman. 1999. The evolution of the Australian flora: fossil evidence. *In* A.E. Orchard [ed.], Flora of Australia. Melbourne, Australia: CSIRO Publishing: 251–320.

Holtum, J. A., L. P. Hancock, E. J. Edwards, M. D. Crisp, D. M. Crayn, R. Sage, and K. Winter. 2016. Australia lacks stem succulents but is it depauperate in plants with crassulacean acid metabolism (CAM)? *Current Opinion in Plant Biology* 31: 109–117.

Holtum, J. A. M., L. Hancock, E. J. Edwards, and K. Winter. 2017. Facultative CAM photosynthesis (crassulacean acid metabolism) in four species of *Calandrinia,* ephemeral succulents of arid Australia. *Photosynthesis Research* 134: 17–25.

Jabaily, R. S., K. A. Shepherd, A. G. Gardner, M. H. G. Gustafsson, D. G. Howarth, and T. J. Motley. 2014. Historical biogeography of the predominantly Australian plant family Goodeniaceae. *Journal of Biogeography* 41: 2057–2067.

Kadereit, G., E. V. Mavrodiev, E. H. Zacharias, and A. P. Sukhorukov. 2010. Molecular phylogeny of Atripliceae (Chenopodioideae, Chenopodiaceae): implications for systematics, biogeography, flower and fruit evolution, and the origin of C_4 photosynthesis. *American Journal of Botany* 97: 1664–1687.

Katoh, K., and D. M. Standley. 2013. MAFFT Multiple Sequence Alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Larget, B. R., S. K. Kotha, C. N. Dewey, and C. Ané. 2010. BUCKy: gene tree/species tree reconciliation with Bayesian concordance analysis. *Bioinformatics* 26: 2910–2911.

Lyle, M., S. Gibbs, T. C. Moore, and D. K. Rea. 2007. Late Oligocene initiation of the Antarctic Circumpolar Current: evidence from the South Pacific. *Geology* 35: 691–704.

Martin, H. A. 2006. Cenozoic climatic change and the development of the arid vegetation in Australia. *Journal of Arid Environments* 66: 533–563.

Mast, A. R., E. F. Milton, E. H. Jones, R. M. Barker, W. R. Barker, and P. H. Weston. 2012. Time-calibrated phylogeny of the woody Australian genus *Hakea* (Proteaceae) supports multiple origins of insect-pollination among bird-pollinated ancestors. *American Journal of Botany* 99: 472–487.

Mirarab, S., R. Reaz, M. S. Bayzid, T. Zimmermann, M. S. Swenson, and T. Warnow. 2014. ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* 30: 541–548.

Moore, A. J., J. M. de Vos, L. P. Hancock, E. W. Goolsby, and E. J. Edwards. 2018. Targeted enrichment of large gene families for phylogenetic inference: phylogeny and molecular evolution of photosynthesis genes in the portullugo clade (Caryophyllales). *Systematic Biology* 67: 367–383. https://doi.org/10.1093/sysbio/syx078.

Mummenhoff, K., H. Brüggemann, and J. L. Bowman. 2001. Chloroplast DNA phylogeny and biogeography of *lepidium* (Brassicaceae). *American Journal of Botany* 88: 2051–2063.

Nyffeler, R., and U. Eggli. 2010. Disintegrating Portulacaceae: a new familial classification of the suborder Portulacineae (Caryophyllales) based on molecular and morphological data. *Taxon* 59: 227–240.

Obbens, F. 2006. A review of the tuberous *Calandrinia* species (section Tuberosae), including three new species for Western Australia. *Nutsyia* 16: 95–115.

Obbens, F. J. 2011. Five new species of *Calandrinia* (Portulacaceae) from Western Australia with additional information on morphological observations. *Nutsyia* 21: 1–23.

Obbens, F., L. P. Hancock, K. C. Thiele, and E. J. Edwards. 2017. *Calandrinia holtumii* (Portulacaceae), a new and unusual species from arid Western Australia. *Nutsyia* 28: 217–223.

Ocampo, G., and J. T. Columbus. 2010. Molecular phylogenetics of suborder Cactineae (Caryophyllales), including insights into photosynthetic diversification and historical biogeography. *American Journal of Botany* 97: 1827–1847.

Ogburn, M. R., and E. J. Edwards. 2015. Life history lability underlies rapid climate niche evolution in the angiosperm clade Montiaceae. *Molecular Phylogenetics and Evolution* 92: 181–192.

Ogburn, R. M., and E. J. Edwards. 2013. Repeated origin of three-dimensional leaf venation releases constraints on the evolution of succulence in plants. *Current biology* 23: 722–726.

Ogburn, R. M., and E. J. Edwards. 2010. The ecological water-use strategies of succulent plants. *Advances in Botanical Research* 55: 179–255.

Owen, C. L., D. C. Marshall, K. B. R. Hill, and C. Simon. 2017. How the aridification of Australia structured the biogeography and influenced the diversification of a large lineage of Australian cicadas. *Systematic Biology* 66: 569–589.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rabosky, D. L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence onphylogenetic trees. *PLoS ONE* 9: e89543–15.

Rabosky, D. L., M. Grundler, C. Anderson, P. Title, J. J. Shi, J. W. Brown, H. Huang, and J. G. Larson. 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution* 5: 701–707.

Rambaut, A., and A. J. Drummond. 2007. Tracer v1.4. Available from https://beast.bio.ed.ac.uk/Tracer.

Ree, R. H., and S. A. Smith. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57: 4–14.

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, et al. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Sanmartín, I., and F. Ronquist. 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* 53: 216–243.

Sauquet, H., P. H. Weston, C. L. Anderson, N. P. Barker, D. J. Cantrill, A. R. Mast, and V. Savolainen. 2009. Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proceedings of the National Academy of Sciences, USA* 106: 221–225.

Schenk, J. J. 2016. Consequences of secondary calibrations on divergence time estimates. *PLoS ONE* 11: e0148228.

Scher, H. D., and E. E. Martin. 2006. Timing and climatic consequences of the opening of Drake Passage. *Science* 312: 428–430.

Shepherd, K. A., M. Waycott, and A. Calladine. 2004. Radiation of the Australian Salicornioideae (Chenopodiaceae)—based on evidence from nuclear and chloroplast DNA sequences. *American Journal of Botany* 91: 1387–1397.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Stolzer, M., H. Lai, M. Xu, D. Sathaye, B. Vernot, and D. Durand. 2012. Inferring duplications, losses, transfers and incomplete lineage sorting with nonbinary species trees. *Bioinformatics* 28: 409–415.

Tahir, S. S., and P. S. Ashton. 1989. A cladistic analysis of *Calandrinia* (Portulacaceae). *Pakistan Journal of Botany* 21: 158–169.

Tahir, S. S., and R. C. Carolin. 2011. A new species of *Calandrinia* (Portulacaceae) from Northern Territory, Australia. *Proceedings of the Linnean Society of New South Wales* 133: 11–14.

Tahir, S. S., and R. C. Carolin. 1990. Phylogeny and origin of Australian (Portulacaceae). *Pakistan Journal of Botany* 22: 1–4.

Thiele, K., F. Obbens, L. P. Hancock, E. J. Edwards, and J. G. West. 2018. Proposal to conserve the name *Parakeelya* against *Rumicastrum* (Montiaceae). *Taxon* 67: 214–215.

Thornhill, A. H., S. Y. W. Ho, C. Külheim, and M. D. Crisp. 2015. Interpreting the modern distribution of Myrtaceae using a dated molecular phylogeny. *Molecular Phylogenetics and Evolution* 93: 29–43.

Toon, A., M. D. Crisp, H. Gamage, J. Mant, D. C. Morris, S. Schmidt, and L. G. Cook. 2015. Key innovation or adaptive change? A test of leaf traits using Triodiinae in Australia. *Scientific Reports* 5: 12398.

Voon Poelnitz, K. 1934. Die *Calandrinia* Arten Australians. *Feddes Repertorium* 35: 161–173.

Wagstaff, S. J., I. Breitwieser, and M. Ito. 2010. Evolution and biogeography of *Pleurophyllum* (Astereae, Asteraceae), a small genus of megaherbs endemic to the subantarctic islands. *American Journal of Botany* 98: 62–75.

Wagstaff, S. J., and F. Hennion. 2007. Evolution and biogeography of *Lyallia* and *Hectorella* (Portulacaceae), geographically isolated sisters from the Southern Hemisphere. *Antarctic Science* 19: 417–426.

West, J. G., and R. J. Chinnock. 2013. *Calandrinia mirabilis* (Portulacaceae), a spectacular new species from Western Australia with notes on its ecology, seed germination and horticultural potential. *Journal of the Adelaide Botanic Garden* 26: 97–102.

Winter, K., and J. A. C. Smith [eds.]. 1996. Crassulacean acid metabolism: Biochemistry, ecophysiology and evolution. Ecological studies. Springer-Verlag, Berlin, Germany.

Winter, K., and J. A. M. Holtum. 2011. Induction and reversal of crassulacean acid metabolism in *Calandrinia polyandra*: effects of soil moisture and nutrients. *Functional Plant Biology* 38: 576–582.

Winter, K., and J. A. M. Holtum. 2014. Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *Journal of Experimental Botany* 65: 3425–3441.