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Climate niche evolution in C4+CAM *Portulaca* and closely related C3+CAM lineages

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Term: Fall 2020



Abstract

With at least a hundred independent origins among land plants, the CAM and C4 photosynthetic pathways represent one of the most notable examples of global convergent evolution of a complex trait. While biochemically similar, CAM and C4 are generally understood to be two distinct ecological adaptations evolving along separate trajectories. However, the purslanes (*Portulaca*), a globally widespread clade of around 100 species of annual and perennial succulents, are able to operate both CAM and C4 cycles in the same leaf. *Portulaca* likely originated from a facultative CAM ancestor and then evolved a C4 system at least three times while maintaining its CAM capabilities. Here, we use a dataset of 55,000 specimen records curated from the Global Biodiversity Information Facility (GBIF) and the Australian Virtual Herbarium, combined with high-resolution environmental rasters, to investigate the climate niche of *Portulaca*. In comparison to its closest relatives (Anacampserotaceae, Talinaceae, Cactaceae, and *Calandrinia*)—which include facultative and constitutive CAM plants but no C4 plants—*Portulaca* is able to persist in areas experiencing much higher temperatures and precipitation levels. Ancestral character state reconstructions conducted on the best-supported phylogenies for *Portulaca* and Anacampserotaceae allow us to infer the ecological consequences of evolving the C4+CAM pathway as opposed to C3+CAM or strong CAM. Further research into *Portulaca*'s anomalous photosynthetic system will dramatically improve our understanding of which environmental selective pressures have driven land plants to repeatedly evolve CAM and C4 and how this diverse and ecologically important group of plants may respond to future climate change.

Introduction

1. CAM and C4 photosynthesis

During the Oligocene, CO₂ declined rapidly from nearly 1500 ppm to less than 500 ppm over a period of 10 million years, and land plants responded by evolving CAM and C4 photosynthesis dozens of times independently (Edwards and Ogburn, 2012). Rubisco, the enzyme that catalyzes carbon fixation in photosynthetic organisms, becomes inefficient at high temperatures and low atmospheric concentrations of CO₂, binding oxygen molecules instead of CO₂ and initiating an energetically costly process known as photorespiration (Gilman and

Edwards, 2020). In contrast to the more prevalent C3 pathway, C4 photosynthesis and crassulacean acid metabolism (CAM) work by concentrating CO₂ within the leaf such that Rubisco is saturated with CO₂ molecules and never interacts directly with the low-CO₂ external environment. While both forms of photosynthesis use almost identical biochemical pathways—wherein the enzyme PEPC fixes atmospheric CO₂ into a 4-carbon molecule that is then decarboxylated in order to bind with Rubisco—they differ in how they segregate PEPC’s activity from that of Rubisco (Figure 1). C4 photosynthesis employs a spatial separation, where PEPC is active in the mesophyll cells, and the 4-carbon acid must be shuttled to the bundle sheath cells where Rubisco operates, isolated from the stomata and the low-CO₂ environment outside the leaf. Under CAM photosynthesis, Rubisco and PEPC are active in the same cell but are temporally segregated. PEPC fixes carbon while the stomata are open at night and Rubisco is inactive, storing the 4-carbon acid in a vacuole overnight. Then in the daytime, the stomata close, the 4-carbon molecule is released from the vacuole and converted back to CO₂, and Rubisco is activated to begin the Calvin Cycle (Edwards, 2019).

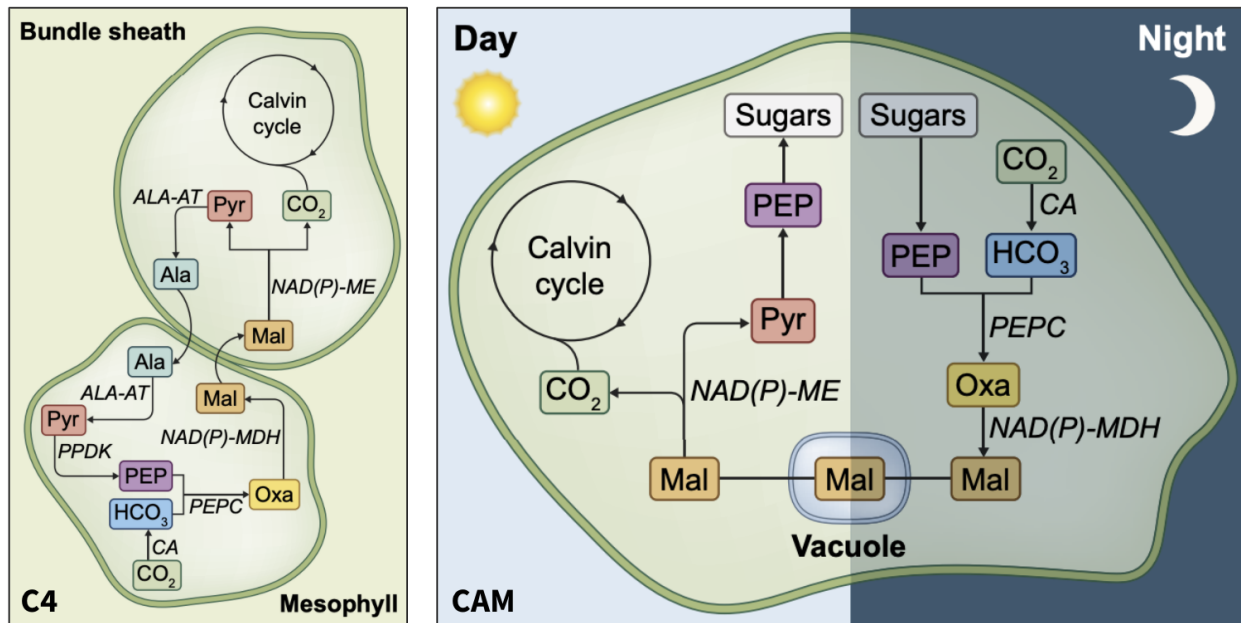


Figure 1. (reproduced from Edwards, 2019) Anatomical comparison between C4 and CAM photosynthesis. C4 (left) spatially segregates PEPC and Rubisco in the mesophyll and bundle sheath cells respectively, while CAM (right) temporally segregates their activity such that PEPC is only active at night and Rubisco is only active during the day.

CAM and C4 also differ in their degree of phenotypic plasticity. For the most part, C4 is relatively static; a plant performs either C4 or C3 photosynthesis its entire life and does not switch between the two. However, the CAM pathway is extremely flexible, such that some lineages like the cacti perform constitutive “strong CAM,” while a surprising number of lineages perform facultative CAM, consisting of a typical C3 cycle supplemented by an inducible CAM cycle that can be upregulated in response to stressors like drought. Some “CAM-cycling” plants are primarily C3, taking in CO₂ during the daytime for direct fixation by Rubisco, but also operate a CAM cycle in the background to re-fix respired CO₂ during the night (Cushman, 2001; Dodd et al, 2002). The identification of low levels of CAM in an astonishing diversity of presumed C3 plants—from tropical tree *Clusia pratensis* to epiphyte *Guzmania monostachia*—has led researchers to hypothesize that the C3+CAM phenotype may be far more widespread than previously believed (Winter, 2019; Winter and Holtum, 2014).

Despite their numerous commonalities, CAM and C4 are generally understood to be two distinct ecological adaptations. The C4 pathway confers high photosynthetic capacity, allowing C4 plants to live in hot temperatures and high light environments (Edwards and Ogburn, 2012). Centers of C4 diversity correspond to arid or semi-arid monsoonal climates with a warm season precipitation regime, and well-known C4 plants include major crops like maize, sugarcane, grasses, and fast-growing weeds in disturbed environments. While only about 3% of land plants are estimated to make use of the C4 pathway, they are responsible for nearly a third of global primary productivity (Sage et al, 2011). The CAM pathway, on the other hand, confers high water use efficiency and drought tolerance, allowing CAM plants to live in water-limited environments (Edwards and Ogburn, 2012). Constitutive CAM plants such as cacti, agave, aloes, and euphorbias are a key component of arid landscapes, but CAM is also found in the aquatic fern *Isoetes* and rainforest epiphytic Bromeliads, which experience considerable water stress despite living in very wet environments (Keeley and Busch, 1984; Kluge et al, 1973).

2. *Portulaca*: the C4-CAM plant

There are a number of biochemical, ecological, and functional reasons that would lead one to expect CAM and C4 to be mutually exclusive; characteristics that facilitate the evolution of one pathway would seemingly disadvantage the other. For example, CAM and C4 require quite different leaf anatomies—differentiated mesophyll and bundle sheath cells for C4 and

enlarged vacuoles for CAM. CAM and C4 utilize the same suite of molecules, albeit regulated through different mechanisms, and therefore the two pathways might place additional strain on the same limited reserves by competing for PEPC or another critical enzyme (Sage, 2002). Because of their different anatomical requirements and adaptation to different environmental conditions, conventional wisdom has held that CAM and C4 evolved along largely independent evolutionary trajectories, perhaps with some lineages being predisposed to develop one or the other photosynthesis pathway, i.e. Kranz anatomy leading to the evolution of C4 or succulence being a necessary prerequisite for the evolution of CAM (Edwards, 2019).

However, the presumed incompatibility between CAM and C4 cannot account for the purslane lineage, *Portulaca*, a genus of about 100 species in the Caryophyllales that for decades has been the only plant lineage documented to be able to operate both CAM and C4 photosynthesis in the same leaf (Ferrari et al, 2019). Last month, a second instance of the C4+CAM phenotype was discovered when CAM activity was identified in the C4 ice plant *Trianthema portulacastrum* (Aizoaceae), raising the possibility that C4+CAM photosynthesis may exist in other lineages as well (Winter et al, 2020). *Portulaca* is globally widespread with centers of diversity in Australia, South America, and Africa, consists mainly of annual and perennial herbaceous plants, and likely evolved from a C3 ancestor with a weak facultative CAM cycle. *Portulaca* appears to have evolved a C4 pathway three separate times while retaining its facultative CAM capabilities, along with some anatomical characteristics like succulence that are frequently associated with CAM (Guralnick et al, 2008; Ocampo et al, 2013; Ocampo and Columbus, 2012). The genus is part of the “Portullugo” clade and its monophyletic relationship with its sister taxon Anacampserotaceae is well supported in Bayesian and Maximum Likelihood inferences, as is its placement in the wider APCT clade which includes *Portulaca* and Anacampserotaceae, as well as Cactaceae and *Talinum*. (Moore et al, 2018) (Figure 2).

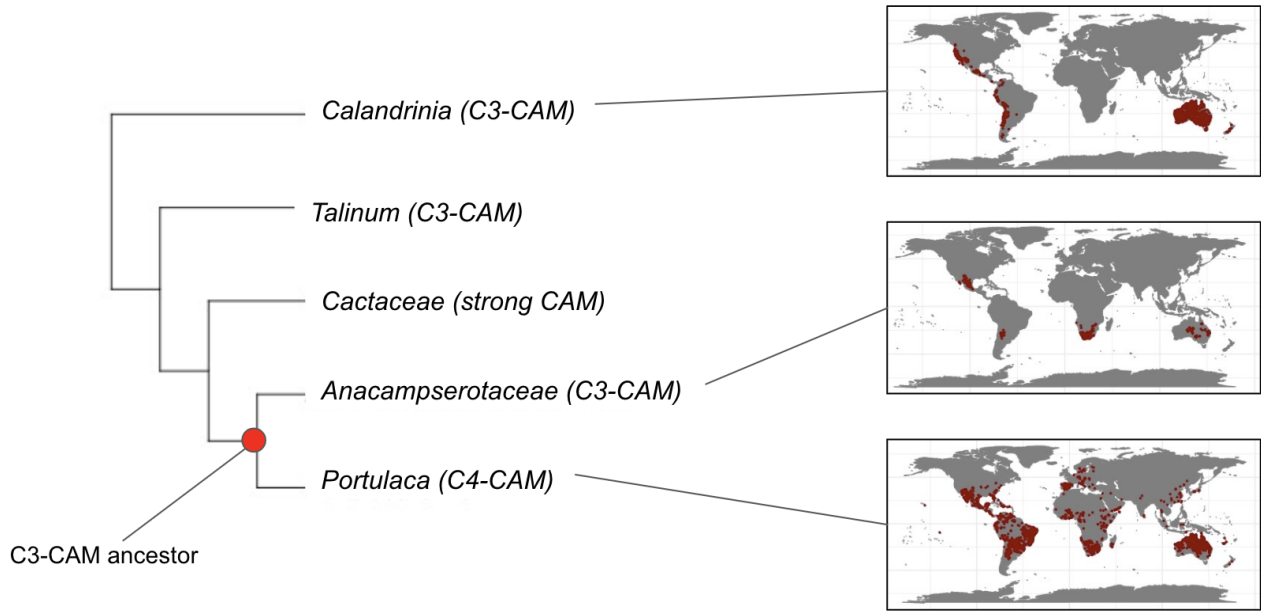


Figure 2. Phylogeny of *Portullugo* groups used in analysis, with *Anacampserotaceae* and *Portulaca*'s shared C3+CAM ancestor marked in red. Global distributions of curated records downloaded from GBIF are shown for *Calandrinia*, *Anacampserotaceae*, and *Portulaca*.

3. Climate niche evolution in *Portulaca* and related C3+CAM lineages

Ecological conditions are a critical selection pressure driving the evolution of C4 and CAM, allowing us to begin to reconstruct the pathways by which such complex traits evolve convergently. The *Portullugo* clade has the potential to significantly enhance our understanding of the ecological implications of evolving C4+CAM versus C3+CAM or constitutive CAM. Here, I investigate how evolving C4+CAM photosynthesis impacted the climate niche evolution of *Portulaca* in comparison to two closely related lineages: *Calandrinia*, a C3+CAM clade in Montiaceae, and *Anacampserotaceae*, *Portulaca*'s sister lineage, which contains facultative and constitutive CAM species but no C4 plants. Despite their different photosynthetic pathways, these plants are similar in many respects, including growth form, degree of succulence, herbaceousness, and annual growth cycle (Figure 3), making them a useful case study for examining the ecological selection pressures that drove the different lineages to evolve CAM, C3, C4, or some combination thereof (Hancock et al, 2018; Moore et al, 2018; Ocampo and Columbus, 2012). The climate niche comparison between *Portulaca* and *Anacampserotaceae* is global, in order to take into account the full ranges of these clades, while the comparison between *Portulaca* and *Calandrinia* is limited to Australia, where highly accurate locality data is

available, in an attempt to balance the inevitable errors that accumulate in biodiversity databases like GBIF. Using environmental layers and species occurrence data, I infer the ecological consequences of evolving C4+CAM, C3+CAM, or full CAM photosynthesis from a shared ancestral C3+CAM phenotype. As outgroups, I also incorporate climate, locality, and phylogenetic data for Cactaceae (strong CAM) and *Talinum* (C3+CAM). I hypothesize that evolving C4 shifted the ecological range of *Portulaca* into warmer and wetter environments, perhaps enabling the lineage to expand into a wider range of climatic conditions than its CAM and C3+CAM relatives.



Figure 3. From left to right: *Portulaca*, *Anacampseros*, and *Calandrinia*. Top row: vegetative growth. Bottom row: flowers. All photos sourced from iNaturalist (see Appendix for DOIs).

Preliminary analyses of the climate niche space of *Portulaca* and *Calandrinia* in Australia have suggested that the two lineages have completely overlapping geographic ranges but only partially overlapping climate niches, with *Portulaca* shifting into wetter and warmer climates. This would indicate that C4+CAM *Portulaca* specializes in the tropical monsoon climate space of its C3+CAM relative, but initial analyses did not find any discernible expansion of *Portulaca* into climate space not occupied by *Calandrinia*, which may be due to Australia's relatively restricted arid climate. The relationship between CAM photosynthesis variants and biogeography in Australian *Portulaca* and *Calandrinia* is particularly interesting in light of Australia's unexplained paucity of CAM plants and complete lack of large native stem succulents

found in other arid regions (Hancock et al, 2018; Holtum et al, 2016). The global Anacampserotaceae and *Portulaca* comparison will provide further evidence as to whether *Portulaca*'s C4+CAM photosynthesis has allowed it to expand into new climate spaces beyond those occupied by closely related lineages.

The hundred or so independent origins of CAM and C4 photosynthesis represent one of the most notable examples of global convergent evolution of highly complex traits. *Portulaca*'s rare C4+CAM photosynthesis contradicts previous hypotheses that ecological and functional selection factors drive the evolution of CAM and C4 along predominantly separate trajectories, as does the presence of both CAM species and C4 species in many major clades (Edwards, 2019; Ferrari et al, 2019). More practically, CAM's drought tolerance and C4's high productivity are both adaptations to environmental stressors, and as climate change places increasing pressure on agriculture, the ability to engineer crops that can grow in less hospitable environments will become increasingly necessary. Efforts are already underway to genetically engineer the C4 pathway in rice and the CAM pathway in poplar (Kajala et al, 2011; Yang et al, 2015). A more thorough understanding of how *Portulaca* operates CAM and C4 together and how that influences its distribution and climatic niche could eventually provide an evolutionary blueprint for a crop that is both highly productive and highly water use efficient, and therefore able to grow and provide food in regions that will be rendered unproductive for current agriculture due to rising temperatures, changing rainfall patterns, and desertification.

Methods

1. Data collection and processing

I downloaded all available specimen collection records for *Portulaca*, Anacampserotaceae, *Calandrinia*, Cactaceae, and *Talinum* from the Global Biodiversity Information Facility (GBIF), supplemented with localities from the Australian Virtual Herbarium (see appendix for download DOIs). This resulted in a raw dataset of approximately 237,000 records for 1,782 species. To combat the inevitable errors that accumulate in widely-sourced biodiversity databases, I thoroughly curated these records using the R package Coordinate Cleaner (Zizka et al, 2019) to remove erroneous localities, such as records with high coordinate uncertainty (greater than 1000 m), very old records (before 1945), records from data sources

other than preserved specimens (i.e. human observation), records with suspiciously high individual counts (i.e. 99999), and coordinates corresponding to points in the ocean, country centroids, and major biodiversity institutions and herbaria, which are likely to have been assigned automatically and are not representative of where an organism naturally occurs. I removed any species with fewer than 10 available localities from the analysis, keeping only one record for each species at a given set of coordinates, and I manually removed localities in ArcGIS Pro that fell far outside of the known distributions of these taxa (i.e. a lone Anacampserotaceae locality in Europe). I also excluded records from *Portulaca oleracea*, *Portulaca pilosa*, *Talinum paniculatum*, and *Talinum fruticosum / triangulare*, as these are globally widespread weeds that would necessarily skew analyses of climatic niche. The final dataset used in the analysis included approximately 55,000 localities for 770 species (Figure 4).

I verified taxonomic names to the fullest extent possible using the iPlant Collaborative Taxonomic Name Resolution Service (Boyle et al, 2013), a detailed *Portulaca* database assembled by Ian Gilman, and the most plausible published phylogeny of each lineage (Hancock et al, 2019; Majure et al, 2019; Moore et al, 2018; Ocampo and Columbus, 2012). I then downloaded environmental data in the form of 30 arcsecond global raster layers from CHELSA, constituting 19 standard bioclimatic variables which are shown in Appendix Table 1. This dataset is more up-to-date than WorldClim's climate data, as it encompasses climatologies from the years 1979-2013, as well as the impact of local topology on climatic variation (Karger et al, 2017). I extracted environmental data for each specimen locality in ArcGIS Pro. All analyses were conducted over a spatial resolution of 1 km² under the geodetic datum WGS84.

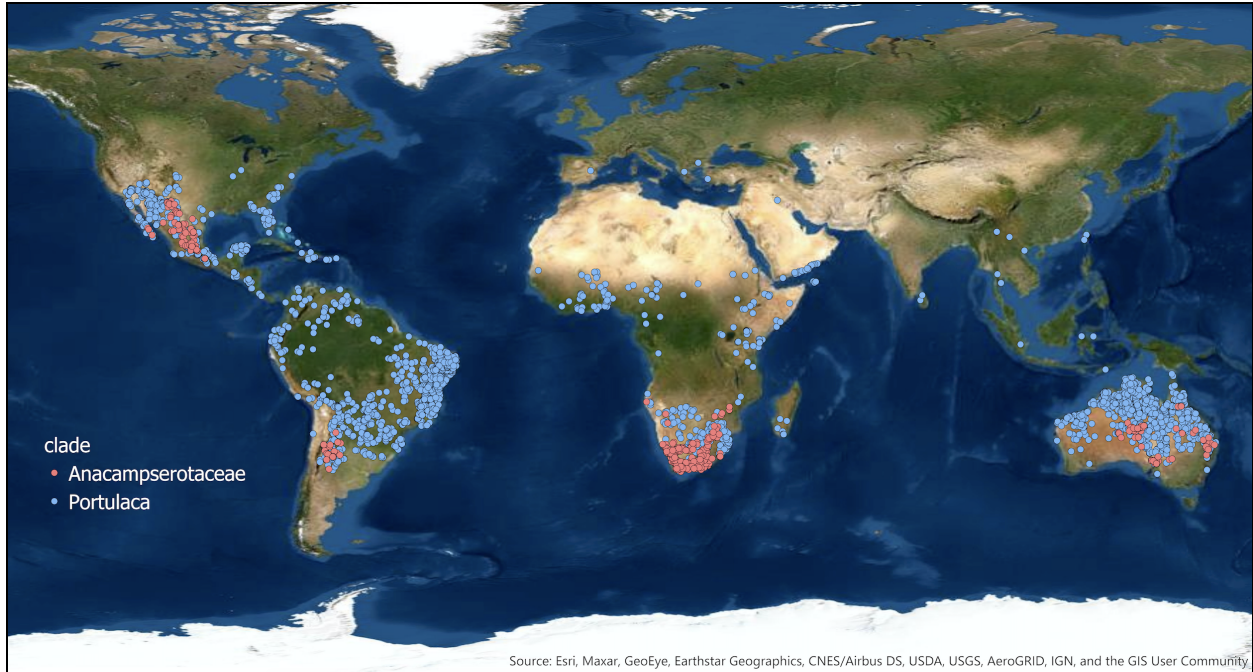


Figure 4a. Global distribution of curated localities for Portulaca (blue) and Anacampserotaceae (red). Note that Anacampserotaceae has a much more restricted geographic distribution than Portulaca.

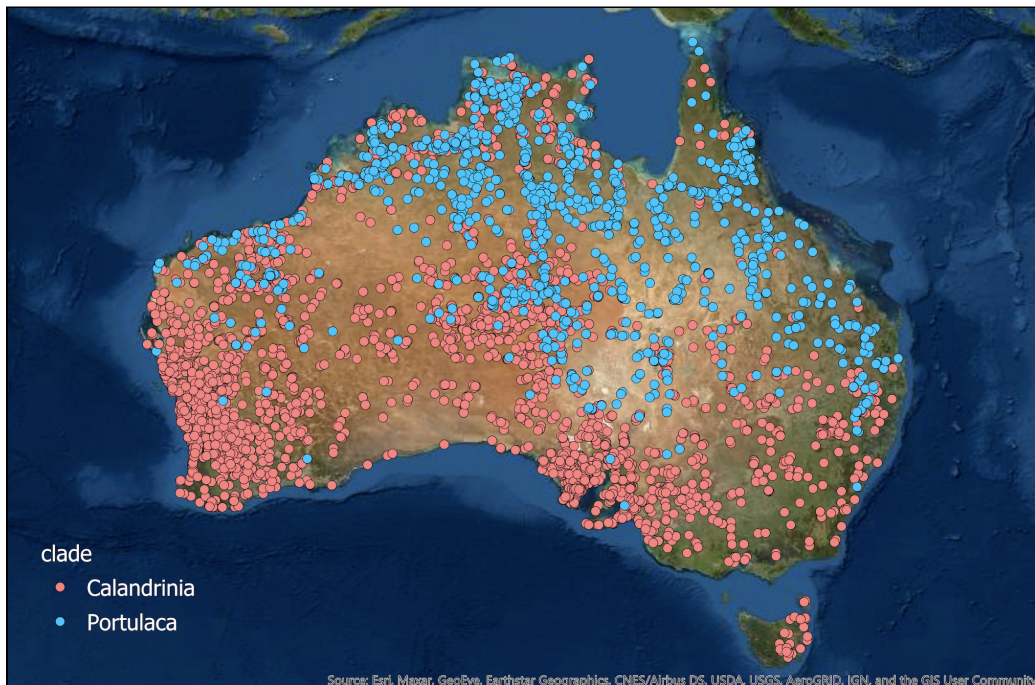


Figure 4b. Australian distribution of curated localities for Portulaca (blue) and Calandrinia (red). While the distributions of the two groups overlap, Portulaca is predominantly confined to the northern half of the continent, the warmer and wetter part of the total climate space of Australia.

2. Climate niche evolution

To attempt to control for the wide differences in geographical range and number of data points between Anacampserotaceae and *Portulaca*, I resampled the *Portulaca* dataset by creating a 500 km² buffer around the four Anacampserotaceae clusters in South Africa, Australia, and North and South America and then only included *Portulaca* localities within 500 km of an Anacampserotaceae locality (Figure 5). I then conducted an unpaired two-samples Wilcoxon test in R on the global Anacampserotaceae-*Portulaca* dataset, the buffered Anacampserotaceae-*Portulaca* dataset, and the Australian *Calandrinia*-*Portulaca* dataset in order to test for statistically significant differences between the two groups across each environmental variable. I used a Bonferroni correction to account for the increase in frequency of Type I errors when performing multiple comparisons simultaneously, which multiplies the p-value by the number of comparisons performed to generate a higher standard for statistical significance. I created species-level boxplots for *Portulaca*, Anacampserotaceae, and *Calandrinia* to confirm that one species inhabiting a particularly extreme climate niche was not unduly influencing the between-group comparisons. Lastly, I compared *Portulaca*'s climate niche to the other taxa in the APCT clade—Anacampserotaceae, Cactaceae, and *Talinum*, using a hypothesis test and exploratory visualizations.

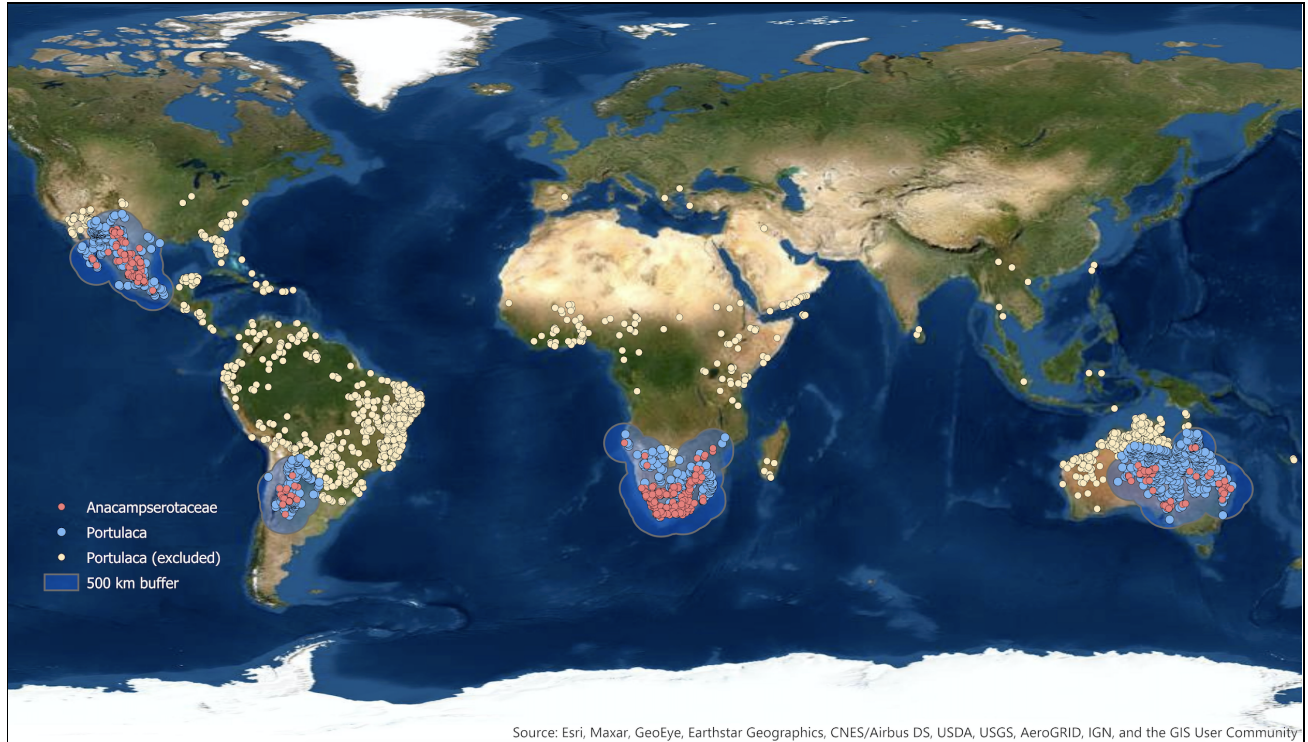


Figure 5. *Portulaca* points included in buffered analysis (blue) represent only *Portulaca* localities within 500 km of an *Anacampserotaceae* locality. *Portulaca* localities located outside of *Anacampserotaceae*'s typical range are depicted in yellow and excluded from this part of the analysis.

3. Phylogenetic analysis

Because the *Anacampserotaceae-Portulaca* comparison is primarily phylogenetic, while the *Calandrinia-Portulaca* comparison is primarily geographic, I conducted phylogenetic analyses only for *Anacampserotaceae* and *Portulaca*. I obtained Newick tree files for the *Portulaca* phylogeny constructed by Ocampo and Columbus (2012) and the *Portullugo* phylogeny constructed by Moore et al. (2018) from members of the Edwards Lab and the Dryad data repository. The Ocampo and Columbus (2012) phylogeny was constructed from 80 samples of *Portulaca*, representing 59 species, 10 subspecies, 3 cultivars, and 3 outgroups from Cactaceae, Talinaceae, and Montiaceae. The gene matrix included nuclear ribosomal DNA loci (ITS, comprising ITS1, the 5.8S gene, and ITS2) and chloroplast DNA loci (protein-coding *ndhF*, *trnT-psbD* intergenic spacer, and *ndhA* intron) (Ocampo and Columbus, 2012). The Moore et al. (2018) phylogeny was constructed from sequences of 60 *Portullugo* individuals and 11 outgroups, using a new set of bait sequences from 19 gene families known to be important for C4

or CAM photosynthesis and 52 other nuclear genes (Moore et al, 2018). In R, I simplified these trees to only include taxa for which I had also collected climate data, resulting in a *Portulaca* phylogeny with 25 tips and an Anacampserotaceae phylogeny with 10 tips, containing representatives of all major lineages within the two groups (Figure 6).

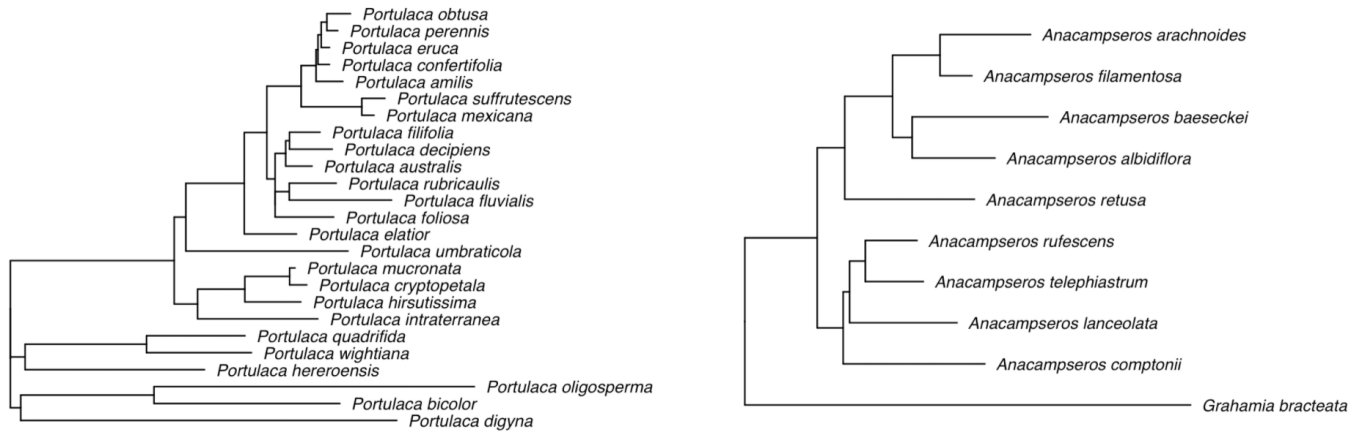


Figure 6. Phylogenies of taxa for which I have both climate and phylogenetic data, sourced from Ocampo & Columbus (2012) and Moore et al. (2018). Because these trees were constructed using different regions of the genome and different methods, they cannot be easily combined into a single phylogeny while maintaining meaningful edge lengths, even though they are well-supported in multiple reconstructions as sister clades (Goolsby et al, 2018; Landis et al, 2020; Moore et al, 2018).

Using the R packages ape, phytools, phylotools, and ggtree (Paradis and Schliep, 2019; Revell, 2012; Yu et al, 2017; Zhang, 2017), I plotted mean annual temperature (MAT) and mean annual precipitation (MAP) averaged across each species onto the tips of the trees to examine the distribution and clustering of these bioclimatic variables within the phylogenies. Then I reconstructed the mean annual temperature and precipitation values at the ancestral node and internal nodes, first under restricted maximum likelihood (REML), then under phylogenetic independent contrasts (PIC). Both methods are used for ancestral reconstruction of continuous quantitative characters under a Brownian motion model of evolution, but REML reconstructs ancestral states in such a way as to maximize their joint likelihood, while PIC reconstructs ancestral states recursively by averaging the character values of their descendants and accounting for branch lengths (Royer-Carenzi and Didier, 2016). I compared the confidence intervals of the values reconstructed for each node under each method and plotted them against each other in order to evaluate the robustness of the reconstruction to the type of method used. I then plotted

the reconstructed states on the phylogeny to compare the climate niche of the ancestral node of *Portulaca* and of Anacampserotaceae and to examine whether their divergence in climate space occurred near the root or closer to the present time.

Results

1. Climate niche evolution: Portulaca and Anacampserotaceae

A Wilcoxon ranked sum hypothesis test found that the difference in climate niche between *Portulaca* and Anacampserotaceae was statistically significant ($p < 2.2 \times 10^{-16}$), and all 19 bioclimatic variables had similarly low p-values when tested individually. Even after a Bonferroni correction was employed to account for the increase in Type I errors from multiple comparisons, all p-values were far below 0.05, indicating that there is considerable difference between the climate niches occupied by *Portulaca* and Anacampserotaceae worldwide. I selected mean annual temperature (MAT) and mean annual precipitation (MAP) as two particularly important species distribution predictors and plotted them against each other in order to visualize the overlap and differentiation between the two clades' climate spaces. *Portulaca* occupies nearly the entire available global climate space, expanding beyond Anacampserotaceae's climate space into warmer and wetter areas, while Anacampserotaceae is primarily clustered in areas experiencing an average temperature between 12°C and 22°C and 0 to 1000 mm of rainfall annually (Figure 7).

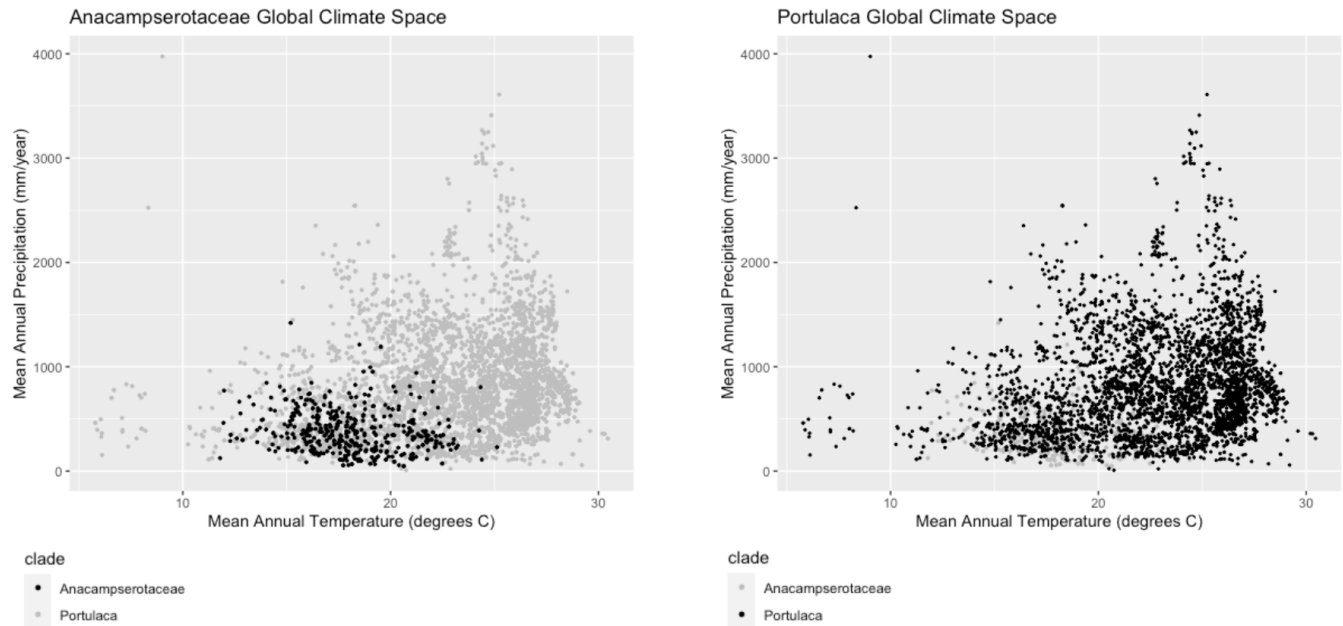


Figure 7. Mean annual temperature (MAT) vs. mean annual precipitation (MAP) for *Anacampserotaceae* (a) and *Portulaca* (b) with respect to each other's climate niche space. *Portulaca* (shown in black on the right) occupies almost the entire available niche space including *Anacampserotaceae*'s niche space, but also extends beyond *Anacampserotaceae* (shown in black on the left) into a warmer and wetter zone.

This difference in climate niche space, while significant, could hypothetically be attributed to the difference in size of the two groups' geographic ranges. Even with *P. oleracea* and *P. pilosa* removed from the analysis, *Portulaca* is widely distributed across Australia, Africa, and North and South America, while *Anacampserotaceae* is confined to four clusters in South Africa, Australia, Argentina, and Mexico. To investigate this possible confounding factor, I repeated the above analysis with only *Portulaca* records located within 500 km² of an *Anacampserotaceae* locality. The statistically significant difference in niche space generated by the Wilcoxon test persisted with $p < 2.2 \times 10^{-16}$, and individual climate variables generated p-values of less than 0.05 with the exception of the precipitation of the driest quarter and the precipitation of the coldest quarter, which were not statistically significant. Precipitation of the driest month and temperature seasonality were not statistically significant after the Bonferroni correction was applied, but all other bioclimatic variables remained significant. When plotting MAT vs. MAP for the buffered dataset, the overlap between the two groups' climate spaces is larger, but *Portulaca* still occupies a region of the total available climate space characterized by higher temperatures and higher precipitation from which *Anacampserotaceae* is entirely absent

(Figure 8). In both the buffered and unbuffered biplots of *Portulaca*'s climate space, the set of points occupying temperatures between 0°C and 10°C correspond to the species *P. perennis*, which has only 11 localities represented in this dataset, all of which are distributed in the mountains of Argentina and likely at very high altitudes.

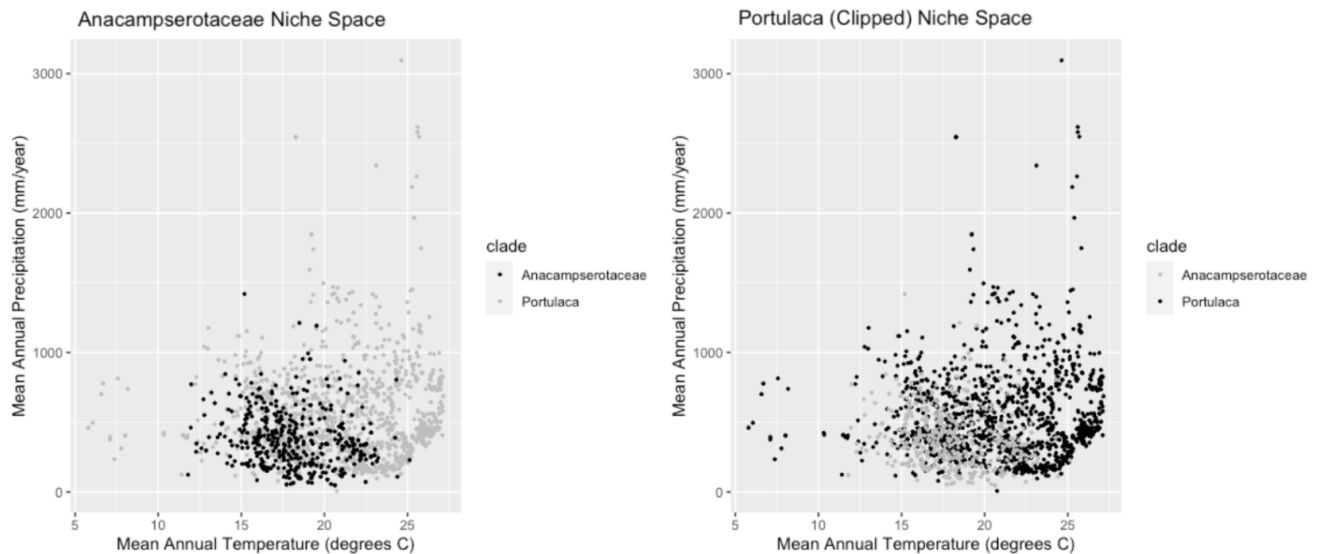


Figure 8. Mean annual temperature (MAT) vs. mean annual precipitation (MAP) for Anacamserotaceae (a) and *Portulaca* (b) with respect to each other's climate niche space. Even when restricting the analysis to *Portulaca* points that roughly co-occur with Anacamserotaceae points, *Portulaca* is clearly moving into a new climate niche characterized by higher temperatures and precipitation.

A few individual bioclimatic variables stand out as particularly notable in this comparison (Figure 9). *Portulaca* experiences a higher mean temperature during the wettest quarter of the year than Anacamserotaceae, indicating that *Portulaca* is shifting into a more tropical, more monsoonal climate space. *Portulaca* also appears to experience more seasonal variation in precipitation than Anacamserotaceae, a climate condition generally presumed to be more stressful for plants. Even within the 500 km² buffer region, *Portulaca* and Anacamserotaceae seem to have differentiated into a warm season precipitation regime and a precipitation regime with very little seasonal variation, such that *Portulaca* experiences the most precipitation under warm temperatures, while Anacamserotaceae experiences roughly the same level of precipitation in the warmest quarter and the coolest quarter. Overall, the climate niche differentiation between *Portulaca* and Anacamserotaceae is robust to a constrained geographic range and does not seem to be unduly influenced by *Portulaca*'s wider global distribution.

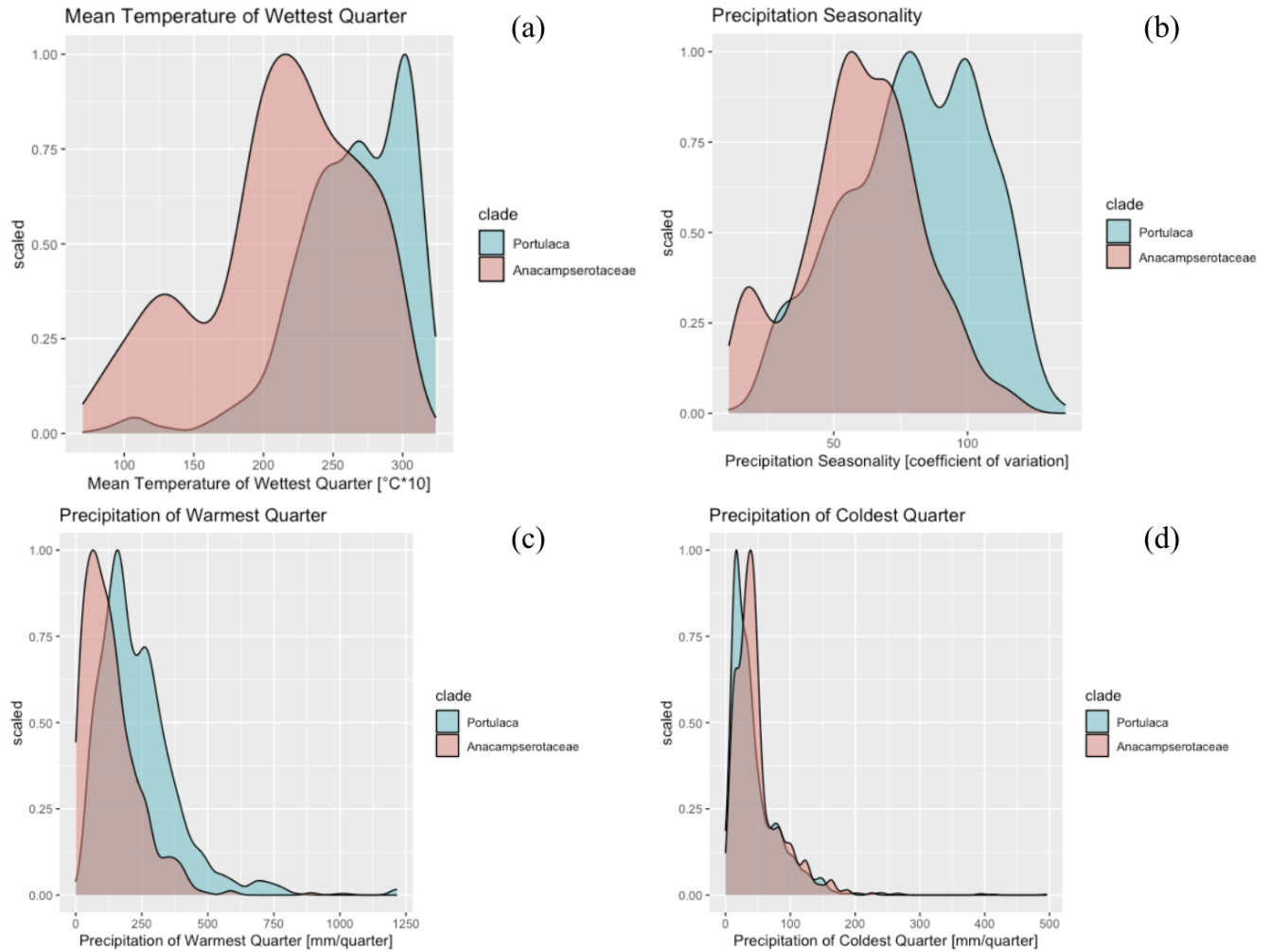


Figure 9. Comparisons between *Anacampserotaceae* and clipped *Portulaca* for a few notable bioclimatic variables. a) *Portulaca* is shifting into a more tropical monsoonal climate space characterized by higher temperatures in the wettest quarter of the year. b) *Portulaca* experiences more seasonal variation in precipitation than *Anacampserotaceae*. c) *Portulaca* appears to live under a warm season precipitation regime, while d) *Anacampserotaceae* has more or less the same level of precipitation in the warmest and coldest quarter.

2. Climate niche evolution: *Portulaca* and *Calandrinia*

As with *Portulaca* and *Anacampserotaceae*, the Wilcoxon ranked sum test found that *Portulaca* and *Calandrinia* occupy significantly different climate niches within the Australian continent ($p < 2.2 \times 10^{-16}$), and all individually tested bioclimatic variables remained significant even after accounting for errors generated by multiple comparisons. Biplots of MAT vs. MAP for *Calandrinia* and *Portulaca* show that *Portulaca*'s climate space entirely overlaps with that of *Calandrinia*, but occupies the high temperature, high precipitation region of the climate space

(Figure 10). Unlike the global Anacampserotaceae-*Portulaca* biplots, where *Portulaca* occupies virtually all of the global climate space suitable for terrestrial vegetation, *Calandrinia*'s MAT vs. MAP plot shows a curiously curved distribution, with moderate precipitation at lower temperatures (10-17°C) and high precipitation at higher temperatures (25-29°C), but with very little precipitation in areas experiencing moderate temperatures. A null climate space of 10,000 randomly plotted points within Australia confirms that this curved shape is representative of the available climate space of the entire continent (Appendix, Figure 1).

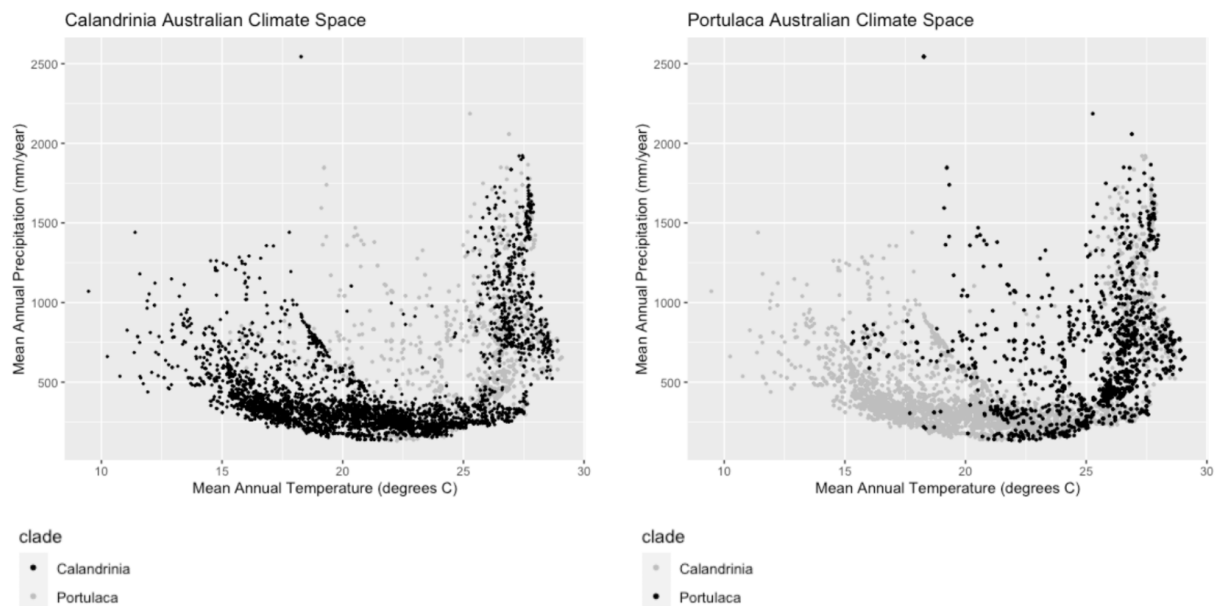


Figure 10. Mean annual temperature (MAT) vs. mean annual precipitation (MAP) for *Calandrinia* (a) and *Portulaca* (b) with respect to each other's climate niche space in Australia. The curved shape of the niche space is representative of the shape of the total available climate space in Australia. *Portulaca*'s niche space entirely overlaps with that of *Calandrinia*, but represents the warmer and wetter part of *Calandrinia*'s total niche.

The individual bioclimatic variables highlighted above for the buffered Anacampserotaceae-*Portulaca* comparison are even more illustrative for *Calandrinia* and *Portulaca* (Figure 11). *Calandrinia*'s climate niche for mean temperature of the wettest quarter is bimodal, with some localities experiencing low temperatures in the wettest quarter, some experiencing high temperatures, but no localities experiencing moderate temperatures. *Portulaca* solely occupies the portion of *Calandrinia*'s climate space experiencing very high mean temperatures during the wettest quarter, suggesting that *Portulaca* is specializing in the tropical, monsoonal climate space of its C3+CAM relative. *Portulaca*, on average, experiences greater

precipitation seasonality than *Calandrinia*, and *Portulaca* clearly inhabits a warm season precipitation zone, while *Calandrinia* inhabits a cold season precipitation zone.

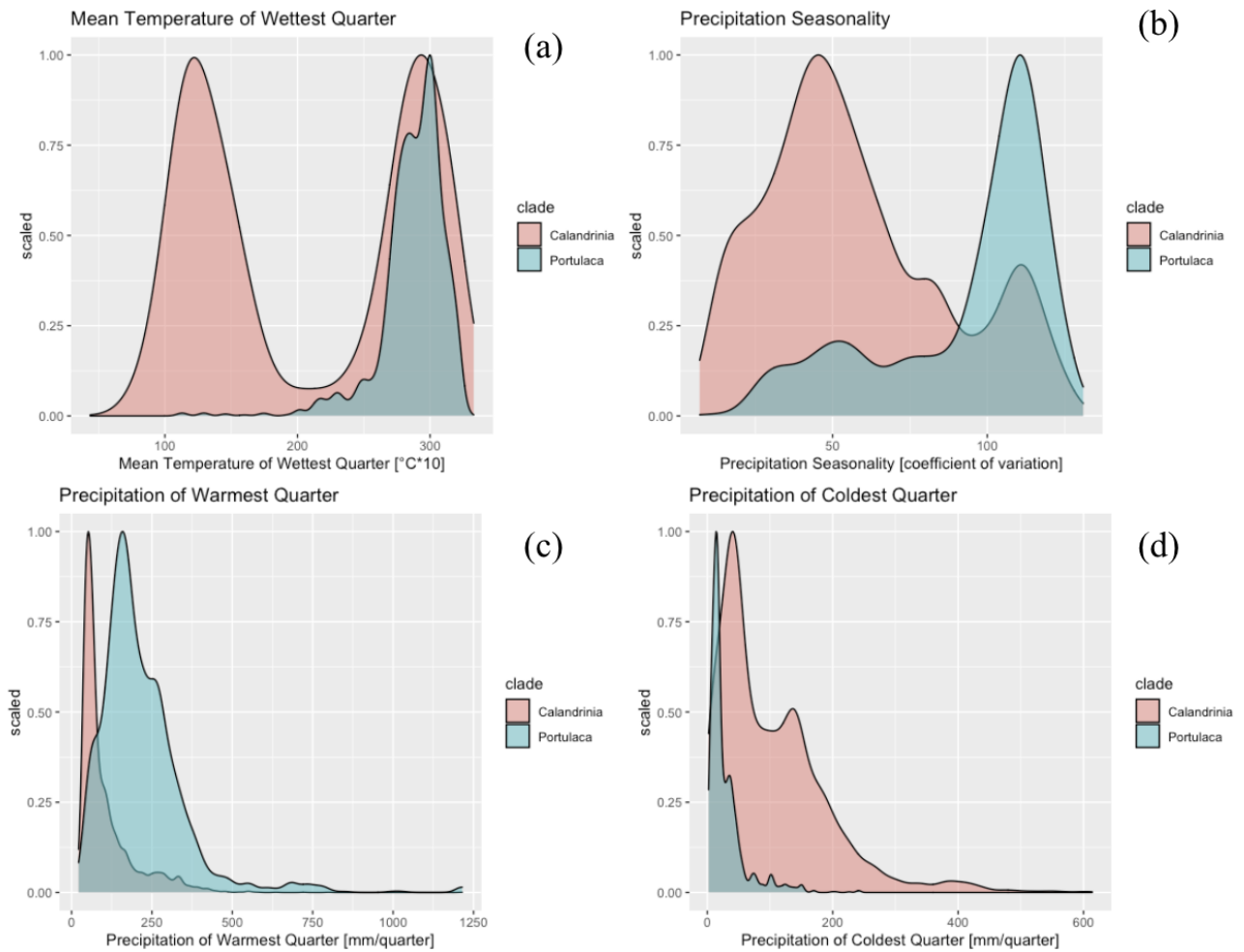


Figure 11. Comparisons between Australian *Calandrinia* and *Portulaca* for a few notable bioclimatic variables. a) *Portulaca* solely occupies the more tropical monsoonal climate space of *Calandrinia* characterized by higher temperatures in the wettest quarter of the year. b) *Portulaca* occupies the subset of *Calandrinia*'s climate space that experiences greater precipitation seasonality. c) *Portulaca* lives under a warm season precipitation regime, while d) *Calandrinia* lives under a cold season precipitation regime.

3. Phylogenetic analysis

To ensure a more accurate phylogenetic comparison, I incorporated global climate and locality data for two outgroups for Anacampserotaceae and *Portulaca*—*Talinum* and Cactaceae which together form the APCT clade in Portullugo. *Talinum*, which includes primarily facultative CAM species, and Cactaceae, which includes some facultative CAM species and a high concentration of constitutive “strong CAM” species, are more similar in their climate space

to Anacampserotaceae than to *Portulaca* (Figure 12). Although the distinction is not quite so clear, *Portulaca* does seem to be moving into a warmer, wetter climate niche space characterized by more seasonal precipitation, in comparison to any other member of the APCT clade.

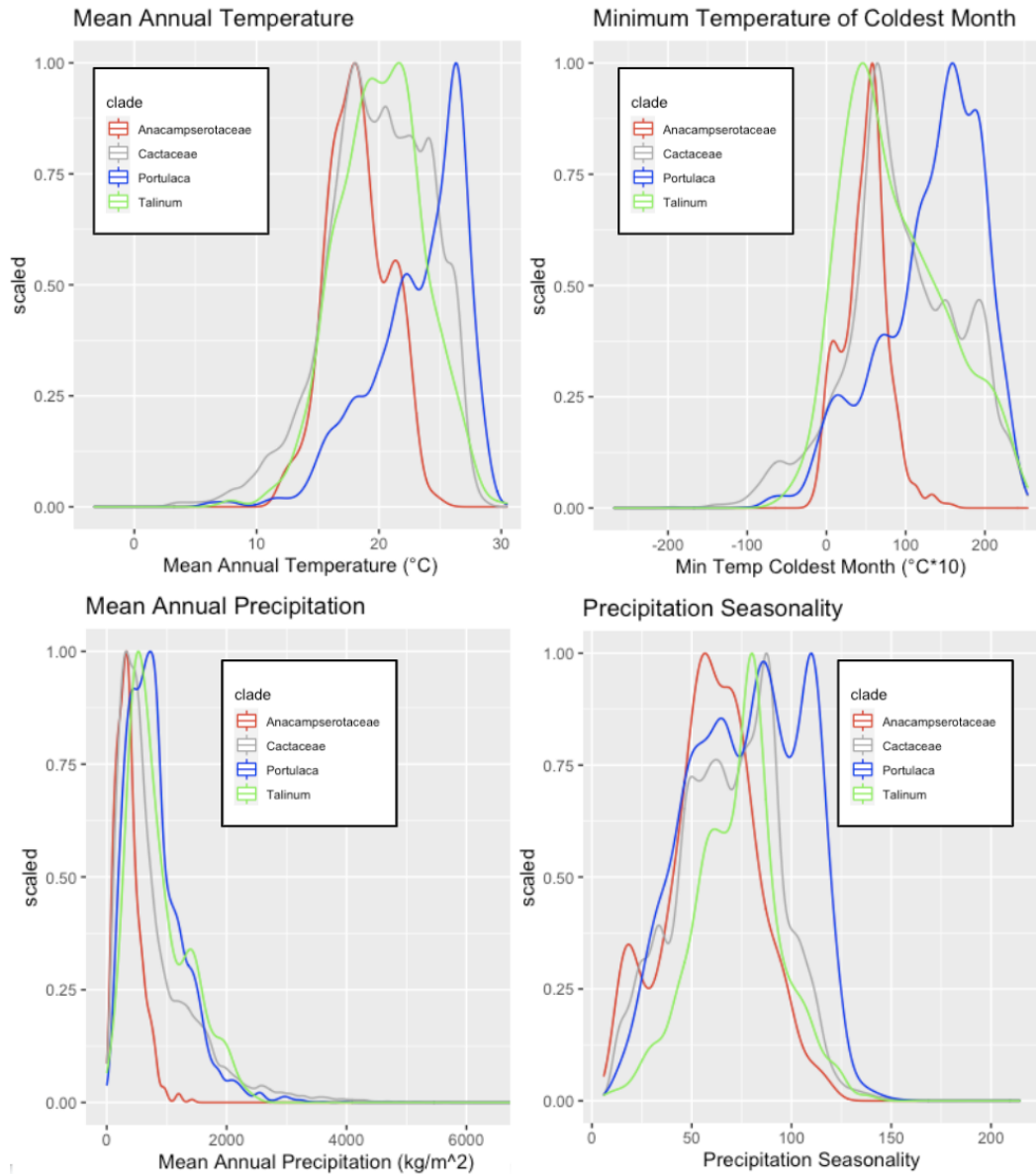


Figure 12. When including *Cactaceae* and *Talinum* as outgroups for *Anacampserotaceae* and *Portulaca*, forming the APCT clade, these were the four bioclimatic variables that showed the most differentiation of *Portulaca* from the rest of the clade. *Portulaca* is moving into a warmer, wetter, and more seasonal climate niche space than that which is occupied by any of its closest relatives.

Restricted maximum likelihood (REML) and phylogenetic independent contrasts (PIC) both reconstructed the ancestral temperature and precipitation niche of *Portulaca* to be higher than the ancestral niche of Anacampserotaceae (Figure 13). Under REML, *Portulaca*'s ancestor is reconstructed to have a mean annual temperature of 22.9°C, while Anacampserotaceae's ancestor is reconstructed to have a mean annual temperature of 17°C. Under PIC, *Portulaca*'s ancestral value is 21.5°C, and Anacampserotaceae's is 17.2°C. Estimates of node character values are well outside the other group's 95% confidence interval, but estimates for PIC vs. REML for the same species are within each other's confidence interval. The reconstructed values for precipitation niche similarly diverge across the two groups but are similar for each reconstruction method. *Portulaca*'s ancestor is estimated to have a mean annual precipitation of 668 mm/year under REML and 626 mm/year under PIC, while Anacampserotaceae's ancestor has a mean annual precipitation of 374 mm/year under both REML and PIC.

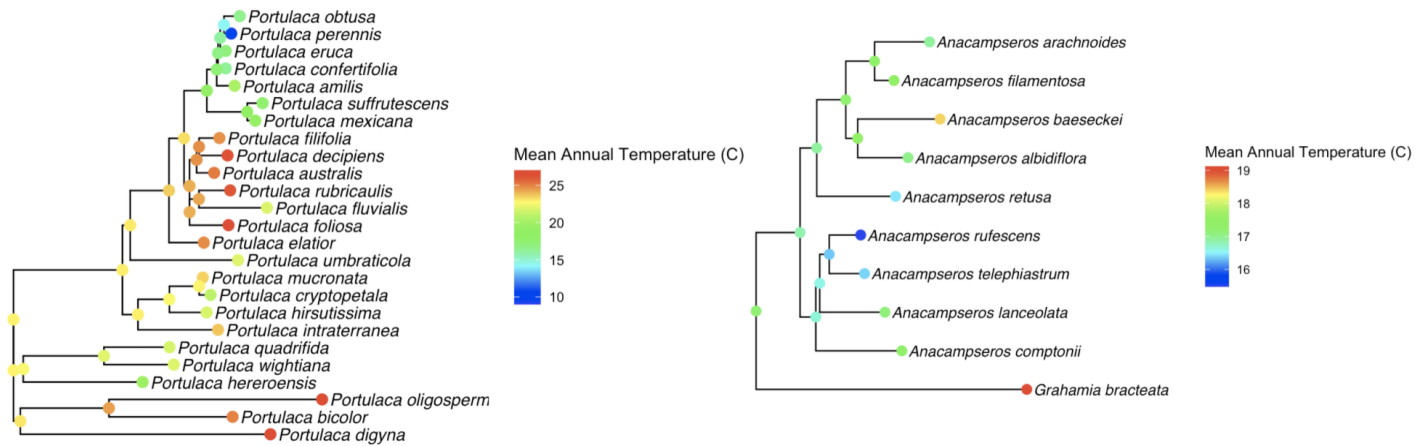


Figure 13a. Restricted maximum likelihood (REML) reconstructions of the ancestral temperature niche of *Portulaca* (left) and Anacampserotaceae (right). Tip nodes represent averages of all localities for each species. Note that the scales are different in order to show a meaningful distribution of colors. *Portulaca*'s ancestral niche is reconstructed to be 22.9°C, 95% CI [1.99-43.8], while Anacampserotaceae's ancestral niche is reconstructed as 17°C, 95% CI [16.6-18.0].

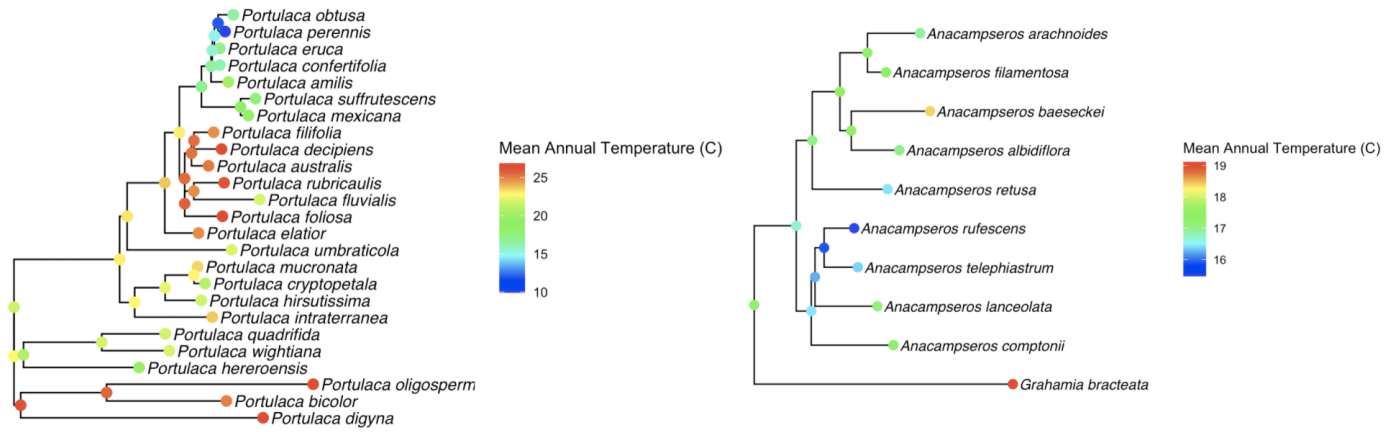


Figure 13b. Phylogenetic independent contrasts (PIC) reconstructions of the ancestral temperature niche of *Portulaca* (left) and Anacampserotaceae (right). Tip nodes represent averages of all localities for each species. Note that the scales are different in order to show a meaningful distribution of colors. *Portulaca*'s ancestral niche is reconstructed to be 21.5°C, 95% CI [20.0-23.1], while Anacampserotaceae's ancestral niche is reconstructed as 17.2°C, 95% CI [16.3-18.3].

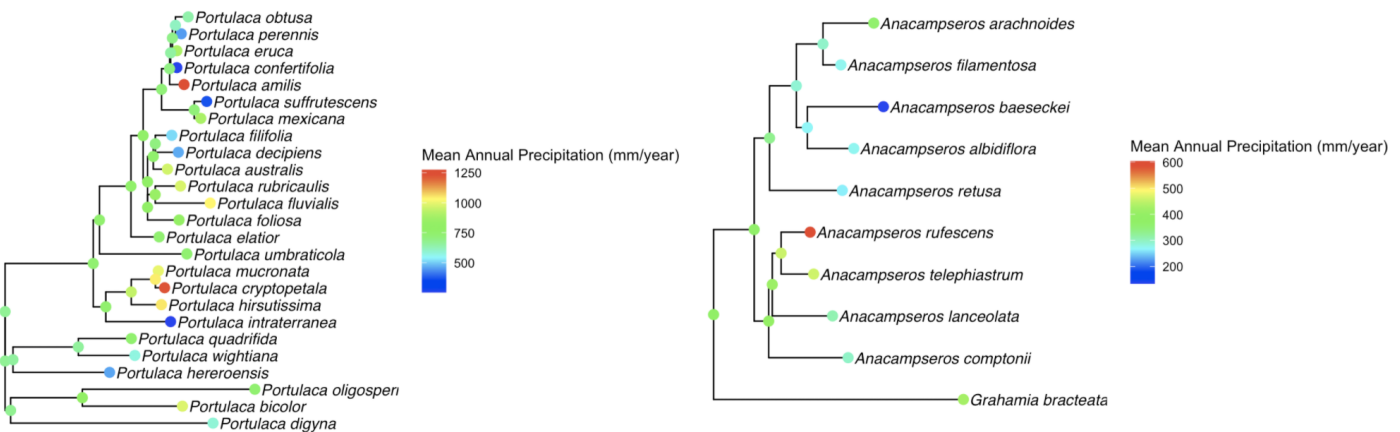


Figure 13c. Restricted maximum likelihood (REML) reconstructions of the ancestral precipitation niche of *Portulaca* (left) and Anacampserotaceae (right). Tip nodes represent averages of all localities for each species. Note that the scales are different in order to show a meaningful distribution of colors. *Portulaca*'s ancestral niche is reconstructed to be 668 mm/year, 95% CI [647-689], while Anacampserotaceae's ancestral niche is reconstructed as 374 mm/year, 95% CI [343-406].

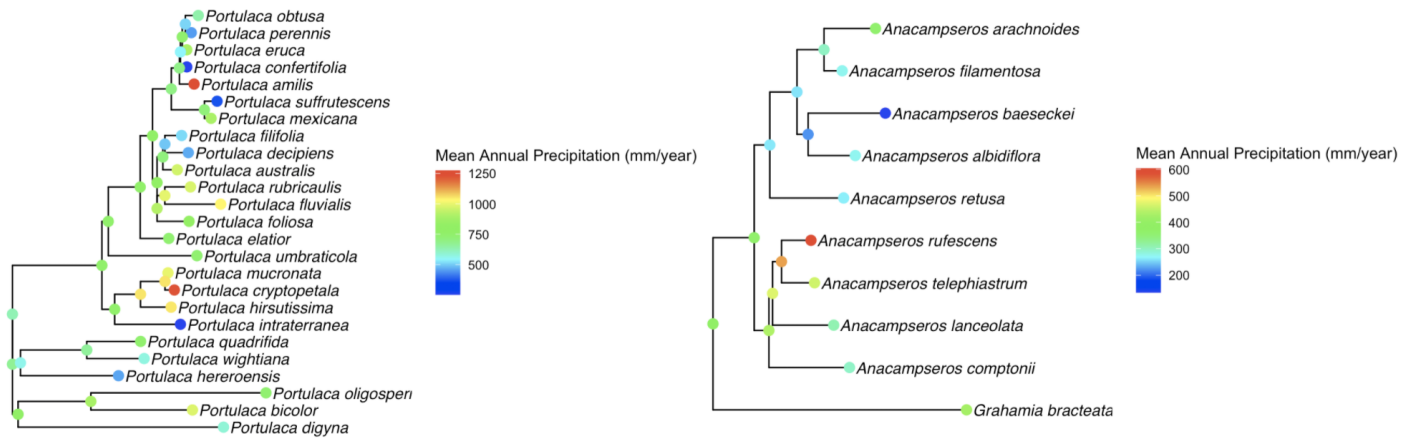


Figure 3d. Phylogenetic independent contrasts (PIC) reconstructions of the ancestral precipitation niche of *Portulaca* (left) and Anacampserotaceae (right). Tip nodes represent averages of all localities for each species. Note that the scales are different in order to show a meaningful distribution of colors. *Portulaca*'s ancestral niche is reconstructed to be 626 mm/year; 95% CI [624-627], while Anacampserotaceae's ancestral niche is reconstructed as 374 mm/year; 95% CI [372-376].

Examining the distribution of character values at the tips of each tree, we see that temperature is more clustered in the phylogeny than precipitation. With the exception of a particularly cold clade that includes the Argentinian outlier *P. perennis*, every tip in *Portulaca* has a higher temperature than any tip in Anacampserotaceae, though there is more overlap between the groups for precipitation niche. The distribution of tips along the tree indicates that there doesn't seem to be any particularly warm or wet *Portulaca* clade that is pulling up the average of the entire group. A comparison of the performance of the REML and PIC ancestral reconstruction methods shows that with a few exceptions, both methodologies reconstruct very similar values for the same node (Figure 14). Most importantly, the ancestral nodes—49 for *Portulaca* and 11 for Anacampserotaceae—are very close to the line and therefore do not differ greatly between REML and PIC reconstructions.

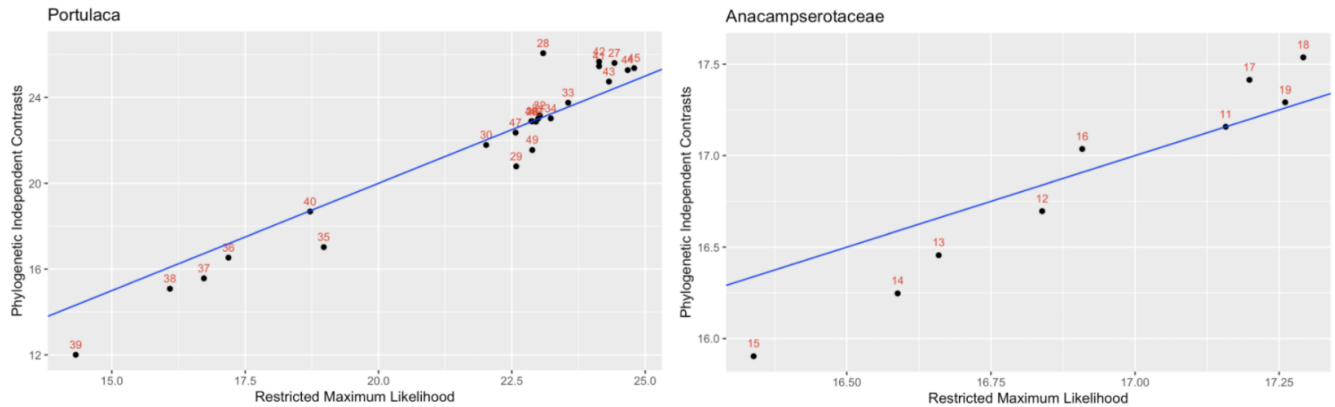


Figure 14. Comparison between the node character values generated by REML and PIC. Points are labelled according to node number on the tree and deviate from the center line in accordance with how different the mean annual temperature values derived by each method are from each other. All points are within acceptable confidence intervals, and the conclusions appear to be robust to the type of ancestral reconstruction method used.

Discussion

This study provides the first comprehensive investigation of climate niche differentiation and evolution between C4+CAM and C3+CAM lineages. As hypothesized, *Portulaca* specializes in the more tropical, monsoonal area of the total climate niche of its C3+CAM relative *Calandrinia*. Additionally, *Portulaca* has expanded its global niche beyond its C3+CAM sister lineage Anacampserotaceae into warmer and wetter regions. This niche expansion is robust even to constraints imposed on the dataset that limited *Portulaca*'s range to roughly the same geographic areas as Anacampserotaceae, strongly suggesting that *Portulaca*'s novel evolution of C4+CAM photosynthesis allowed it to inhabit a wider range of climatic conditions than its C3+CAM relatives, where it experiences higher temperatures, more precipitation, and greater precipitation seasonality, concentrated in the warm season. While *Portulaca*'s Australian niche space and geographic range entirely overlaps with that of *Calandrinia*, *Portulaca* has likely colonized Australia three separate times, while *Calandrinia* has only one origin on the continent (Hancock et al, 2018; Ocampo and Columbus, 2012). This would indicate that *Portulaca* is not found only in the northern half of the continent because it hasn't yet had enough time to spread south, but because of an actual climatic limitation on its distribution.

The ancestral reconstructions of temperature niche and precipitation niche suggest that *Portulaca*'s movement into warmer and wetter spaces occurred near the base of the clade, when

it diverged from Anacampserotaceae and that the clade containing *P. perennis* subsequently diversified into a colder environment than its ancestor. However, ancestral reconstructions necessarily incorporate untested assumptions about edge lengths into their calculations, and therefore one data point for the basal node of *Portulaca* and Anacampserotaceae is not sufficient to draw conclusions here, as it could be impacted by inaccuracies in the rate of evolution along certain branches. To verify that there is a statistically significant difference between the ancestral climatic niche of *Portulaca* and that of Anacampserotaceae, future work should incorporate a sensitivity analysis of the impact of edge length on the ancestral character values. By scaling edge lengths in accordance with a random noise element, a distribution of trees could be generated, sharing the same topology but different edge lengths. From this distribution of ancestral character values, a difference of means hypothesis test could be performed to verify that the climate niche of *Portulaca*'s ancestor was significantly warmer and wetter than that of Anacampserotaceae's ancestor and that this difference is relatively robust to errors in estimated rates of evolution and perhaps even to minor variations in tree topology.

No evidence was found to suggest that one particular clade within *Portulaca* has moved into an extremely warm and wet temperature niche, unduly skewing the average. Noting that *Portulaca*, Anacampserotaceae, and *Calandrinia* share many physiological and ecological similarities despite their different photosynthetic pathways, this points to two possible explanations: 1) *Portulaca*'s unique evolution of C4+CAM within Portullugo enabled it to expand its climate range, persisting in warmer and wetter environments that remained inhospitable to its C3+CAM sister lineages and/or became more competitive in those environments to the point of displacing other plants, or 2) *Portulaca*'s pre-existing range in warmer and wetter climates drove it to evolve the C4+CAM phenotype, while its C3+CAM relatives in more moderate environments did not face similar selection pressures. Given the apparent rarity of the C4+CAM phenotype in the phylogenetic tree, the first explanation seems considerably more likely, as plenty of plant clades inhabiting environments that are similar to or even more warm and wet than that of *Portulaca* have not evolved C4+CAM, even though they've had a far longer time to do so. A reasonable next step to test this hypothesis would be to conduct a wider phylogenetic analysis of climate niche evolution in the APCT clade. This would require testing several methods of grafting the *Portulaca* and Anacampserotaceae phylogenies, which were inferred using different methods and regions of the genome, making them difficult to

combine in a way that produces meaningful edge lengths. Combined phylogenies of this sort represents a major challenge to the development of large-scale phylogenomics and phylodiversity analyses, and possible avenues forward may include: 1) estimating edge lengths for each phylogeny independently, grafting the trees together based on the assumption that they form a clade, time calibrating branches accordingly, and testing time calibrations by adding jitter into the sensitivity analysis; 2) concatenating data for varying genes into a single large matrix with many gaps, and inferring the phylogeny based on this matrix; and 3) choosing the most taxon-restricted dataset with the best character sampling, inferring a constraint tree based on that dataset, and then restricting the Maximum Likelihood search for the overall tree to possibilities that are compatible with the constraint tree (Landis et al. 2020).

Once a joint tree of Anacampserotaceae and *Portulaca* has been successfully inferred, adding Cactaceae and *Talinum* into the analysis should increase our confidence in the proposed conclusion that *Portulaca*'s C4+CAM innovation led to an increase in temperature and precipitation niche evolution at the base of the clade, causing the lineage to diverge from the rest of the APCT clade and diversify into warmer and wetter zones. A number of contradictory Cactaceae phylogenies are in use, but the whole-plastome tree generated by Majure, et al. (2019) is a plausible candidate for an outgroup analysis. Obtaining a phylogeny of intra-clade relationships for *Talinum* may be hampered by undersampling and a handful of widespread weedy taxa, but regardless, the relationships between these two outgroups and *Portulaca* and Anacampserotaceae are well-supported, which is the most important factor for the viability of the climate niche evolution analysis (Moore et al, 2018). Another avenue of further investigation would be to replicate this study in *Trianthema*, the second lineage recently confirmed to be able to perform both C4 and CAM photosynthesis, and a few of its relatives in Aizoaceae that do C3, C3+CAM, CAM, or C4. If *Trianthema* also occupies a higher temperature and higher precipitation niche than its C3+CAM and strong CAM relatives, this would lend further credibility to the association between photosynthesis phenotype and climate niche presented here.

Untangling the complicated relationships between the global convergent evolution of the CAM and C4 pathways, the broad variation of facultative CAM phenotypes, and the present distribution of plant lineages in a rapidly changing global biosphere is a lengthy process, but one that has considerable implications for both our ability to provide sufficient food sources in the

near and long term and our understanding of the fundamental mechanisms of evolution. The rapid niche expansion of *Portulaca* in comparison to Anacampserotaceae provides a counterexample to the theory of phylogenetic niche conservatism—the paradigm under which conserved traits limit the distribution of organisms to particular phylogenetically clustered climate spaces (Ogburn and Edwards, 2015). We know that rapid niche diversification and adaptation to novel climates is possible under certain circumstances, and identifying the particular physiological, genetic, and situational factors that enable one lineage to evolve its niche rapidly while another evolves within a narrow set of climatic conditions is of increasing importance as many organisms face massive environmental upheaval in the form of climate change and land use change. CAM and C4 photosynthesis represent one of the most important adaptations in the evolutionary history of land plants, and *Portulaca* still has much to teach us about evolutionary responses to environmental change and their subsequent ecological consequences.

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Appendix

Code

Scripts and data necessary to reproduce all analyses are available at <https://github.com/noraheaphy/finalproject>.

Data downloads

Portulaca: GBIF.org (14 August 2020) GBIF Occurrence Download <https://doi.org/10.15468/dl.h75e2y>.

Portulaca: Atlas of Living Australia occurrence download at <https://doi.org/10.26197/5f3feac68a017> accessed on Sat Aug 22 01:39:43 AEST 2020.

Anacampserotaceae: GBIF.org (14 August 2020) GBIF Occurrence Download <https://doi.org/10.15468/dl.vw8j2e>

Anacampseros: Atlas of Living Australia occurrence download at <https://doi.org/10.26197/5f3febac807af> accessed on Sat Aug 22 01:43:34 AEST 2020.

Calandrinia: GBIF.org (14 August 2020) GBIF Occurrence Download <https://doi.org/10.15468/dl.zmewj8>

Calandrinia: Atlas of Living Australia occurrence download at <https://doi.org/10.26197/5f3febf79ac91> accessed on Sat Aug 22 01:44:44 AEST 2020.

Cactaceae: GBIF.org (9 October 2020) GBIF Occurrence Download <https://doi.org/10.15468/dl.qg62bc>

Cactaceae: Atlas of Living Australia occurrence download at <https://doi.org/10.26197/5f7fb3d1b627c> accessed on Fri 2020-10-09 11:50 AM AEST.

Talinum: GBIF.org (9 October 2020) GBIF Occurrence Download <https://doi.org/10.15468/dl.zwm94a>

Talinum: Atlas of Living Australia occurrence download at <https://doi.org/10.26197/5f7fb3e192a14> accessed on Fri 2020-10-09 11:50 AM AEST.

CHELSEA bioclimatic variables

Variable name	Bioclimatic variable	Units
Bio1	Annual Mean Temperature	°C*10
Bio2	Mean Diurnal Range	°C*10
Bio3	Isothermality	N/A
Bio4	Temperature Seasonality	standard deviation
Bio5	Max Temperature of Warmest Month	°C*10
Bio6	Min Temperature of Coldest Month	°C*10
Bio7	Temperature Annual Range	°C*10
Bio8	Mean Temperature of Wettest Quarter	°C*10
Bio9	Mean Temperature of Driest Quarter	°C*10
Bio10	Mean Temperature of Warmest Quarter	°C*10
Bio11	Mean Temperature of Coldest Quarter	°C*10
Bio12	Annual Precipitation	mm/year
Bio13	Precipitation of Wettest Month	mm/month
Bio14	Precipitation of Driest Month	mm/month
Bio15	Precipitation Seasonality	coefficient of variation
Bio16	Precipitation of Wettest Quarter	mm/quarter
Bio17	Precipitation of Driest Quarter	mm/quarter
Bio18	Precipitation of Warmest Quarter	mm/quarter
Bio19	Precipitation of Coldest Quarter	mm/quarter

Image sources

Portulaca: Ueda K (2020). iNaturalist Research-grade Observations. iNaturalist.org. Occurrence dataset <https://doi.org/10.15468/ab3s5x> accessed via GBIF.org on 2020-12-08.

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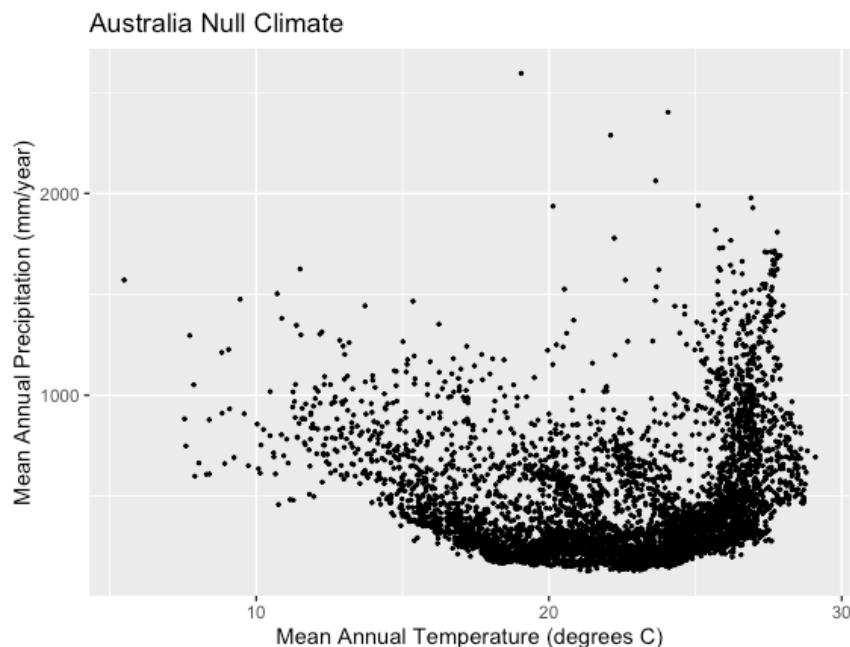
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Supplementary Figure 1. Mean annual temperature (MAT) vs. mean annual precipitation (MAP) for 10,000 randomly sampled points located in Australia. The curved distribution matches the distribution of climate zones across the continent, with a large central desert and savannah surrounded by tropical forest on the northern coast and a more temperate, mediterranean region to the south.