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Disparity, diversity, and duplications in the Caryophyllales

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Summary

- The role whole genome duplication (WGD) plays in plant evolution is actively debated. WGDs have been associated with advantages including superior colonization, various adaptations, and increased effective population size. However, the lack of a comprehensive mapping of WGDs within a major plant clade has led to uncertainty regarding the potential association of WGDs and higher diversification rates.

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- Using seven chloroplast and nuclear ribosomal genes, we constructed a phylogeny of 5036 species of Caryophyllales, representing nearly half of the extant species. We phylogenetically mapped putative WGDs as identified from analyses on transcriptomic and genomic data and analyzed these in conjunction with shifts in climatic occupancy and lineage diversification rate.
- Thirteen putative WGDs and 27 diversification shifts could be mapped onto the phylogeny. Of these, four WGDs were concurrent with diversification shifts, with other diversification shifts occurring at more recent nodes than WGDs. Five WGDs were associated with shifts to colder climatic occupancy.
- While we find that many diversification shifts occur after WGDs it is difficult to consider diversification and duplication to be tightly correlated. Our findings suggest that duplications may often occur along with shifts in either diversification rate, climatic occupancy, or rate of evolution.

Key words: Caryophyllales, climatic occupancy, diversification rates, duplications, phylogenomics.

Introduction

Understanding the causes and correlates of diversification within flowering plants has been a central goal of evolutionary biologists. Genomic and transcriptomic data have reinvigorated hypotheses associating whole genome duplication (WGD) with lineage diversification rate increases (e.g. Levin, 1983, 2002; Barker *et al.*, 2009, 2016; Estep *et al*., 2014; Soltis *et al.*, 2014; Edger *et al.* 2015; Puttick *et al.* 2015; Tank *et al.*, 2015; Huang *et al.*, 2016; McKain *et al.*, 2016; Laurent *et al.*, 2017). It is not self-evident why WGDs would be associated with increases in lineage diversification. One hypothesis suggests that the additional genetic material provides a basis to generate new adaptations (Edger *et al.*, 2015), although this itself assumes a co-occurrence of adaptation and lineage proliferation (Levin, 1983). The apparent lack of precise co-occurrence of adaptation and lineage proliferation has been explained by the potential of a lag model (Schranz *et al.* 2012; Tank *et al.* 2015) where diversification may follow WGD events. In the absence of overwhelming correlative signal, we are often unable to discern true ancient WGD events from aneuploidy without advanced genomic information such as synteny mapping

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(Dohm *et al.*, 2012). Because it is often difficult to distinguish the two, for simplicity we will define WGD broadly to include putative ancient WGD events (paleopolyploidy) and ancient aneuploidy events. WGD events are thought to be a common occurrence and have been associated with an estimated 15% of angiosperm speciation events (Wood *et al.*, 2009). However, whether speciation by WGD is correlated with higher diversification rates remains highly debated (Mayrose *et al.*, 2011; Estep *et al.* 2014; Soltis *et al.*, 2014; Tank *et al.*, 2015; Kellogg *et al.* 2016). Analyses based on recent WGD events have concluded that immediate extinction rates are higher for polyploid plants (Mayrose *et al.*, 2011; Arrigo & Barker, 2012). This may result from small initial population sizes and an increased dependence on selfing. Alternatively, despite the disadvantages of WGD, others have suggested that polyploids may be superior colonizers (Soltis & Soltis, 2000).

Indeed, extreme environments are associated with high levels of WGD, with up to 87% of species restricted to areas that were glaciated during the last ice age consisting of polyploids (Brochmann, 2004). However, in the example from Arctic plants, the high level of WGD has occurred post-glaciation representing a micro-evolutionary period whereas previous studies often focus at much deeper macro-evolutionary time scales (Mayrose *et al.*, 2011; Tank *et al.*, 2015; Soltis *et al.*, 2014). From the perspective of a short timescale, polyploidy has the disadvantages of higher error rates in mitosis (Storchová *et al.*, 2006) and masking of deleterious mutations allowing them to accumulate to higher frequencies in a population (Otto & Whitton, 2000). A suite of advantages however may also arise, including gain of asexuality (Miller *et al*., 2000) and varying effects of heterosis (Comai, 2005). The net role these advantages and disadvantages play on the macroevolutionary scale is difficult to determine from either the purely short-term or purely long-term time scales previously used.

The long-term consequence of WGD is a central question in macroevolution and comparative genomics. However, with a suite of advantages and disadvantages, much debate surrounds the importance and patterns of correlation of WGD (Comai, 2005). While polyploidization events can cause instant speciation, there is no reason to assume that these singular speciation events in themselves would influence large-scale diversification rate shifts when considering lineage survivorship. Instead, there may be other factors, such as the increase in genetic material, perhaps increasing genetic diversity or enabling adaptation, that cause long term shifts in rates of diversification. Adaptations need not be associated with shifts in the tempo

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of diversification and those adaptations and shifts in diversification may not co-occur on the same branch (i.e. there may be a lag time; Donoghue, 2005; Smith *et al*., 2011, Schranz *et al*., 2012; Donoghue & Sanderson, 2015; Tank *et al*., 2015; Dodsworth *et al*., 2016). In the broader context of plant evolution, there are several possible outcomes of WGD in relation to the evolution and diversification of clades: no relationship between WGD and speciation rate or habitat shift/adaptation; WGD coincides with an increase of speciation rate, with or without a lag time; WGD promotes dispersal and habitat shifts, which has mixed relationship with speciation rate; and a mixture (some association, some not), similar to the previous hypothesis but without explicitly promoting dispersal or habitat shift or speciation (e.g. adaptation could be more prominent than dispersal and habitat shift). Here, we contribute to this discussion on diversification and WGDs with an in-depth examination of the intersection of diversification and WGDs happening at a range of scales within the hyperdiverse Caryophyllales.

The Caryophyllales comprise *c*. 12500 species in 39 families (Thulin *et al.*, 2016; APG IV: Chase *et al.*, 2016), representing *c*. 6% of extant angiosperm species diversity. The estimated crown age of Caryophyllales is *c*. 67–121 million yr ago (mega-annum, Ma) (Bell *et al.*, 2010; Moore *et al*., 2010). Species of the Caryophyllales exhibit extreme life-history diversity, ranging from tropical trees to temperate annual herbs, and from desert succulents (e.g. Cactaceae) to a diverse array of carnivorous plants (e.g. the sundews *Drosera* and pitcher plants *Nepenthes*). Such extraordinary diversity makes Caryophyllales a particularly useful system for investigating the relationship between WGD vs diversification and climate occupancy evolution. Our previous analyses using 62 transcriptomes representing 60 species across the Caryophyllales identified 13 well-supported ancient WGD events (Yang *et al.*, 2015). We have since nearly tripled the taxon sampling and assembled a data set comprising high-coverage transcriptomes and genomes from 169 species across the Caryophyllales (Yang *et al.*, 2017), providing even greater power for resolving the number and phylogenetic locations of WGD events. Moreover, the growth in the number of plant taxa on GenBank that are represented by traditional targeted sequences (e.g. *rbc*L, *mat*K, ITS, etc.) and the growth of publicly available collections data (e.g. GBIF, iDigBio) provide excellent opportunities to apply phylogenetic and climate diversification approaches at fine scales in Caryophyllales.

By examining WGDs and diversification within the Caryophyllales, we present an important example. Not only does the dataset examined have a high density of transcriptomic

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sampling, the diversification of the bulk of Caryophyllales occurred during a time frame intermediate to that of most published studies that have probed a link between WGD and macroevolution. This time frame, between 10 and 100 Ma, is important for angiosperms as much of the diversification that has led to the modern flora occurred during this period and most modern angiosperm families appeared by this time. Discussion of speciation rates, climate occupancy shifts, and WGDs would be flawed without accurate mappings of WGD events within this time scale. We compiled a data set with extensive and precise mapping of WGD combined with a species-level phylogeny. The megaphylogeny approach has been used extensively in the past to combine data from many gene regions and across broad taxonomic groups to address evolutionary questions (Smith *et al.*, 2009). Here, we use this approach to help inform analyses from phylogenomic studies, and provide a broad context in which to examine these genomic phenomena. With half of the species sampled, this represents one of the largest and most exhaustive studies of WGDs, diversification rate, and adaptive shifts.

Materials and Methods

Sanger sequencing and assembly

A total of 248 new *matK* sequences were included in this study (Table 1). To generate these sequences, leaf samples were collected in silica in the field or from cultivated material, or were collected from herbarium sheets. DNA was isolated using either the Nucleon Phytopure kit (GE Healthcare Life Sciences, Pittsburgh, PA, USA), using the 0.1 g protocol and following manufacturer's instructions, or using the Doyle & Doyle (1987) protocol, with the addition of 1% PVP-40. An *c*. 950 bp region in the middle of the *matK* gene was amplified and sequenced using custom-designed primers (Table 2). PCRs were performed in 12.5 μl volumes with 0.5 μl of 5 mM primer for both primers, 5–20 ng of DNA template, 0.1 μl of GoTaq (Promega, Madison, WI, USA), 6.25 μl of Failsafe Premix B (Epicentre, Madison, WI, USA), and 4.7 μl of sterile, deionized water. Reactions were run on a Bio-Rad PTC 200 thermocycler (Bio-Rad, Hercules, CA, USA) at Oberlin College. Individual PCRs were cleaned in 16.5 μl reactions containing 10 U of Exonuclease I (Affymetrix, ThermoFisher Scientific, Waltham, MA, USA), 2 U of shrimp alkaline phosphatase (Affymetrix), 8 μl of PCR product, and 8.5 μl of sterile, deionized water. Sanger sequencing of the resulting cleaned PCRs was conducted by Neogenomics (formerly SeqWright; Houston, TX, USA) using an ABI 3730xl automated

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sequencer (Applied Biosystems, ThermoFisher Scientific). The resulting forward and reverse sequences for each reaction were trimmed and *de novo* assembled using default parameters of the Geneious assembler in Geneious versions 5–7 (Biomatters, Auckland, New Zealand).

Molecular data for phylogenetic reconstruction

Nucleotide data from the nuclear ribosomal internal transcribed spacers (ITS) and *phyC* gene, and the plastid loci *matK*, *ndhF*, *rbcL*, *trnH-psbA* spacer, and *trnL-trnF* spacer were used to reconstruct the phylogeny. These data were gathered first using PHLAWD (Smith & Donoghue, 2008; Smith *et al.*, 2009) and then curated and combined with newly sequenced *matK* data for 124 additional species. This yielded the following sampling: ITS 2969 species, *matK* 2270 species, *ndhF* 417 species, *phyC* 172 species, *rbcL* 947 species, *trnH-psbA* 240 species, and *trnL-trnF* 1996 species. We used *mat*K, *rbc*L, and *ndh*F sequences from *Aextoxicon*, *Apium*, *Berberidopsis*, *Campanula*, *Clethra*, *Coffea*, *Echinops*, *Helwingia*, *Ilex*, *Ipomoea*, *Lamium*, *Lonicera*, *Nyssa*, *Polysoma*, *Primula*, *Santalum*, *Valeriana*, and *Viburnum* to represent outgroups.

Phylogenetic reconstruction

We conducted phylogenetic analyses with RAxML v7.2.8 (Stamatakis, 2014) using the full analysis command, -f a, which conducts a rapid bootstrap and then a full maximum likelihood search. The combined bootstrap and maximum likelihood search allows for a more thorough maximum likelihood analysis where the initial rapid bootstrap results prime the maximum likelihood analysis. However, we did not use the rapid bootstrap trees from this analysis and instead, we conducted a full bootstrap, generating the bootstrap dataset using phyx (Brown *et al.*, 2017) and then conducting individual maximum likelihood runs on each constructed bootstrap dataset. This allowed us to conduct SH-like approximate likelihood ratio test (SH-aLRT; Guindon *et al.*, 2010) on the resulting bootstrap set. We conducted bootstraps within gene regions and we retained the individual bootstrap alignments to conduct additional analyses (i.e. bootstrapped alignments contained the same number of gene-specific sites as the empirical alignment). On each of the resulting trees of the bootstrap and the maximum likelihood tree, we conducted SH-aLRTs as implemented in RAxML. These analyses calculate support for each edge while also finding the NNI-optimal topology. RAxML completed the likelihood search for

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each of these bootstrap replicates, however the SH-aLRT analyses often resulted in an improved maximum likelihood topology. The trees that resulted from the SH-aLRT, ML, and bootstrap samples, were used for further analyses. Because several deep relationships within Caryophyllales are hard to resolve without large amounts of molecular data that are unavailable for most of the taxa included in this analysis (Yang *et al.*, 2015), for all phylogenetic analyses we applied the following topological constraint: (Droseraceae, (*Microtea*, (Stegnospermataceae, Limeaceae, (Lophiocarpaceae, (Barbeuiaceae, Aizoaceae))))) as per previous analysis (Brockington *et al.*, 2009; Yang *et al.*, 2015).

Divergence time estimation

Few tractable options for divergence time estimation exist for datasets of the size presented here. We use the penalized likelihood approach (Sanderson, 2003) as implemented in the program treePL (Smith & O'Meara, 2012), which can handle large-scale phylogenies. The early fossil record of the Caryophyllales is sparse with only a few known records (Friis *et al.*, 2011; Arakaki *et al.*, 2011): fossil pollen has been ascribed to Amaranthaceae (*Chenopodipollis*) from the Paleocene of Texas (Nichols & Traverse, 1971); a putative fossil infructescence from within the Phytolaccaceae in the Campanian has also been reported (Cevallos-Ferriz *et al.*, 2008), but this phylogenetic position has been disputed (S. Manchester, pers. comm.) and hence we excluded it; Jordan & Macphail (2003) describe a middle to late Eocene inflorescence from the species *Caryophylloflora paleogenica*, ascribed to Caryophyllaceae; pollen from Argentina within the Nyctaginaceae has been reported from the middle Eocene (Zetter *et al.*, 1999); and fossil pollen and seeds of *Aldrovanda* (Degreef, 1997). The penalized likelihood method performs better when a calibration is used at the root. For this calibration, and because there is no fossil record for the earliest Caryophyllales, we use a secondary calibration from the comprehensive angiosperm divergence time analyses of Bell *et al.* (2010). We attached several other secondary calibrations to major clades where fossils are not available (Ocampo & Columbus, 2010; Arakaki *et al.*, 2011; Schuster *et al.*, 2013; Valente *et al.*, 2013; see Supporting Information Table S1 for detail on placement and calibrations). We conducted a priming analysis to determine the best optimization parameter values. We then performed a cross validation analysis using the random cross validation setting to determine the optimal smoothing parameter value.

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Climate occupancy analyses

We downloaded 6592700 georeferenced occurrences for the Caryophyllales from GBIF (accessed on 6 January 2015 **[Author, please confirm amended text '6 January' is correct]**; http://gbif.org). After removing samples present in living collections, and therefore not necessarily representative of native climates, and removing samples whose localities were over water, 6009552 samples remained. We extracted bioclimatic values for each coordinate using the 2.5 arc-minute resolution data from WorldClim (http://worldclim.org). We only included taxa that had at least three samples in these analyses to reduce potential errors and to have the minimum number of samples required to calculate mean and variance. The resulting overlap of the taxa represented in both the geographic and genetic data was 2843 taxa. We conducted principal component analyses (PCA) on these extracted values. With both the bioclimatic values and the first two axes of the PCA, we conducted ancestral state reconstruction analyses.

We also compared ancestral states and Brownian motion rates of evolution between sister clades (comparing duplicated lineages with their sisters) for mean annual precipitation, mean annual temperature, and principal component axis 1. We calculated ancestral states for continuous characters using a single rate model and compared sister lineages. We also calculated and compared estimates of independent Brownian motion rates for sister lineages.

Diversification analyses

To map diversification rate shifts, we conducted MEDUSA (Alfaro *et al.*, 2009; Pennell *et al.*, 2014) analyses on the maximum likelihood tree and the bootstrap trees. MEDUSA is far more computationally tractable than some other diversification estimation methods. Furthermore, we required the ability to feasibly integrate over the phylogenetic uncertainty within the phylogenetic dataset because of both the nature of the larger phylogenetic dataset and the inherent biological uncertainty within the Caryophyllales. MEDUSA fits a birth-death model of diversification (with parameters r: net diversification (birth - death), and ε: relative extinction (death / birth)) before using stepwise AIC (Burnham & Anderson, 2002) to identify shifts in rates of diversification. These complementary analyses accommodate topological and branch length uncertainty. We employed a birth-death model for 97 chronograms generated from nonparametric bootstrapping of the original matrix, inferring ML trees in RAxML, and estimating divergence times in treePL using the temporal constraints described above. We

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discarded three trees based on poor fossil placement resulting from phylogenetic uncertainty causing fossil placements to conflict.

Whole genome duplication identification

To identify WGDs (procedure described below), we generated a tree based on transcriptomic data. For this tree, we used 178 ingroup data sets (175 transcriptomes, three genomes) representing 169 species in 27 families and 40 outgroup genomes (Tables S1 & S2 in Yang *et al*., 2017). We mapped putative WGD events using multiple strategies: gene tree topology, plotting synonymous distance, and chromosome counts (Yang *et al*., 2015, 2017). For gene tree topology analyses, we performed two alternative strategies for mapping duplication events from gene trees to the species tree: mapping to the most recent common ancestor (MRCA), or mapping to the species tree only when gene tree and species tree topologies are compatible.

To conduct synonymous distance analyses, we performed the following procedure. For all ingroup Caryophyllales transcriptome data sets, we calculated the distribution of paralog synonymous distance following the same procedure as (Yang *et al*., 2015). We reduced highly similar peptide sequences with CD-HIT (-c 0.99 -n 5)(Li & Godzik 2006). We also carried out an all-by-all BLASTP within each taxon using an E value cutoff of 10 and -max_target_seq set to 20. Resulting hits with pident < 20% or niden < 50 amino acids were removed. We removed sequences with ten or more hits to avoid overrepresentation of gene families that experienced multiple recent duplications. We used the remaining paralog pairs and their corresponding CDS to calculate Ks values using the pipeline https://github.com/tanghaibao/bio-pipeline/tree/master/synonymous_calculation (accessed 29 November 2014). The pipeline first carries out pairwise protein alignment using default parameters in ClustalW (Larkin *et al*., 2007), back-translates the alignment to a codon alignment using PAL2NAL (Suyama *et al*., 2006), and calculates the synonymous substitution rate (Ks) using yn00 as part of the PAML package (Yang, 2007), with Nei–Gojobori correction for multiple substitutions (Nei & Gojobori, 1986). We obtained chromosome counts from the Chromosome Counts Database (CCDB; http://ccdb.tau.ac.il accessed 5 October 2015). When multiple counts were reported from different authors or different plants, we erred on the conservative estimate and recorded the lowest number. For species that were not available in the database, we found counts from the

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literature (e.g. Jepson eFlora http://ucjeps.berkeley.edu/eflora/ and Flora of North America http://floranorthamerica.org) or by a consensus from species of the same genera.

Results and Discussion

Phylogenetic results

Phylogenetic analyses showed strong support based on bootstrap and SH-aLRT values for the monophyly of most Caryophyllales families (see Fig. S1). We found strong support for the carnivorous clade including Droseraceae, Ancistrocladaceae, Nepenthaceae, Drosophyllaceae, and Dioncophyllaceae. There was also strong support for this clade as sister to a clade including Frankeniaceae, Tamaricaceae, Plumbaginaceae, and Polygonaceae. However, relationships among the families showed more varied support. There was weak support for the placement of other families relative to other early diverging Caryophyllales (see Fig. S1). There was strong support for Caryophyllaceae sister to Amaranthaceae. There was very weak support for Aizoaceae sister to Phytolaccaceae+Nyctaginaceae. As with previously published analyses, there was no support for the monophyly of Phytolaccaceae in the traditional sense (i.e. including Phytolaccaceae s.s., Petiveriaceae, and *Agdestis*; APG IV) and very weak support for the placement of Sarcobataceae. There was also weak support for the relationships among Limeaceae, Molluginaceae, and the Portulacineae. Many of these relationships have been found to be strongly supported but conflicting in different analyses (Brockington *et al.*, 2009; Soltis *et al.*, 2011; Yang *et al.*, 2015; Smith *et al.*, 2015; Walker *et al.*, 2017). Here, we focused less on the systematic resolution within the Caryophyllales and instead examine the potential relationship of diversification and climate occupancy shifts to WGDs. Therefore, we placed more emphasis on including more taxa over that of more gene regions (i.e. transcriptomes) at the cost of more missing data. Confident resolution of many of the systematic relationships will require genomic and transcriptomic sampling, as well as more thorough taxon sampling (Yang *et al.*, 2017).

Climate occupancy reconstruction results

We performed climate occupancy ancestral reconstruction analyses on the phylogeny of 2843 taxa that included taxa with at least three sampled geographic coordinates (Figs 1–3). We conducted these analyses for visualization and for comparison with diversification and WGD

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results (see below). Results for individual bioclimatic variables and principal components can be found in Figs S2–S4. Bioclimatic variable 1 (mean annual temperature, Fig. 1) showed that there are several strong phylogenetic patterns of clades with preferences for colder or warmer regions. For example, Polygonaceae, Caryophyllaceae, and Montiaceae each are dominated by taxa with preferences for cold environments, although each also contains early-diverging taxa with preferences to warm environments. By contrast, taxa inhabiting warm environments predominate in Cactaceae, Amaranthaceae, Aizoaceae, the carnivorous clade (Droseraceae, Drosophyllaceae, Nepenthaceae, Ancistrocladaceae, Dioncophyllaceae), and the phytolaccoid clade (Nyctaginaceae, Phytolaccaceae, Petiveriaceae, Sarcobataceae, and *Agdestis*). Bioclimatic variable 12 (mean annual precipitation) showed a comparatively consistent pattern of relatively dry to intermediately wet clades throughout the group. Indeed, only a few clades inhabiting wet ecosystems (in this case, the wet tropics) exist in the Caryophyllales, specifically small groups within the carnivorous clade, the phytolaccoids, early-diverging Polygonaceae, and other small groups throughout the Caryophyllales. The principal component loadings are presented in Fig. 2 and Fig. S5. Principal component 1, PCA1, showed significant differentiation throughout the Caryophyllales, as for example, early-diverging Polygonaceae vs the rest of Polygonaceae, early diverging Caryophyllaceae vs the rest of Caryophyllaceae, phytolaccoids vs Aizoaceae, and Portulacineae + relatives vs Cactaceae, to mention a few. These results generally reflect the extensive ecological diversification throughout the group. They also reflect significant diversification in the temperate regions of the world especially within the Caryophyllaceae and Polygonaceae contrasted with extensive diversification in the succulent lineages (especially Aizoaceae and Cactaceae) found in relatively dry and warm environments.

Diversification

Significant shifts in diversification were detected in most major clades (Table 4; Fig. 1). The results from diversification analyses on the maximum likelihood tree and bootstrap tree set were generally congruent with each other. However, there were discrepancies (Fig. 1). The bootstrap set recovered many shifts in Polygonaceae, the carnivorous clade, Caryophyllaceae, some shifts within Cactaceae, phytolaccoids, and Amaranthaceae. Disagreements on the existence and placement of shifts are primarily within Portulacineae, Aizoaceae, and Amaranthaceae. Overall, MEDUSA detected 27 increases in diversification rate using the ML tree and 16 increases using

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the bootstrap trees. Given the relative lack of support of some of the branches in the phylogeny, we find the MEDUSA results on the set of bootstrapped trees to be the most conservative while the ML results are suggestive but not definitive of diversification shifts.

Duplications, diversification, and climate occupancy

WGD analyses showed thirteen putative WGDs that could be mapped to clades (i.e. involve more than one taxon in the dataset; Table 3; Figs 1–3). Many of these were found in early diverging lineages as opposed to nested deep within families, though there are WGDs identified in *Amaranthus* and *Claytonia*. We also found evidence of nested WGDs within the phytolaccoids and Portulacineae. In addition to these deeper WGDs, there were several more recent WGDs that were present in Ks plots but could not be mapped to a clade (Yang *et al.*, 2017). By sampling more extensively, Yang *et al.* (2017) and Walker *et al.* (2017) found additional WGD events within the Caryophyllales. We will surely find additional WGDs events in other lineages as more effort is placed on denser taxon sampling using genomes and transcriptomes. We did not explore WGDs that could only be mapped to terminal branches as we could not verify these phylogenetically. Further discussion of specific results related to the WGDs themselves can be found in Yang *et al*. (2017) and Walker *et al*. (2017).

To better examine whether WGDs coincide with diversification rate shifts, increases and decreases, or notable changes in climate tolerance, we mapped WGDs onto the large phylogenies and summarized the number of species and climate occupancy information for each clade (Tables 3, 4; Figs 1–3). Some WGD events were associated with synchronous diversification events. For example, within Nyctaginaceae, a WGD event occurs on the same branch (leading to Tribe Nyctagineae; Douglas & Spellenberg, 2010) as an increase in diversification rate in both the ML tree and the bootstrapped dataset (Fig. 1, dup:1 div:n). These events were also associated with a shift in life history and occupancy from an ancestral woody habit in the tropics to the largely herbaceous, arid-adapted temperate Nyctagineae. This was also the case for *Amaranthus* (Fig. 1, dup:5 div:x). Other coincident diversification and WGD events in the Droseraceae and Nepenthaceae were only supported by the ML tree. Although these correlated events may, in fact, be accurate, we will reserve more comments for when these are more confidently resolved. Other than these simultaneous shifts and one diversification shift at the base of the MRCA of Nyctaginaceae+Cactaceae, all other shifts in diversification occured more recently than WGD

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events. Several authors have suggested that this lagging pattern may be common at the broader angiosperm scale (Schranz *et al.*, 2015, Tank *et al*., 2015), though the expected distance of the diversification shift from the WGD event was not specified (this is discussed more below). In the results presented here, some diversification events occur shortly after the WGD event, such as within the Amaranthaceae (dup: 6) and Portulacineae (dup: 4). For others, it is difficult to determine whether the diversification events that occur after the WGD events are significantly close to the WGD to warrant suggestion of an association (e.g. dup: 7, dup: 10, dup: 8). More description of a model that would generate a null expectation would be necessary to determine what is 'close enough' (see discussion below).

Many of the other inferred lineage diversification rate shifts were associated with very recent, rapid radiations within genera such as those documented within *Commicarpus* (Nyctaginaceae), *Dianthus* (Caryophyllaceae), *Cerastium* (Caryophyllaceae), *Arenaria* (Caryophyllaceae), and *Salicornia* (Amaranthaceae), to name a few (Table 4). Although polyploids were reported in these clades, we were unable to pinpoint the phylogenetic location of any WGD with our current taxon sampling (e.g. *Dianthus*; Carolin, 1954; Weiss *et al.*, 2002). Increased sampling of transcriptomes and genomes will shed more light in these areas. While we only find a few WGDs that coincide well with diversification rate shifts, it is important to note that the uncertainty in the phylogenies makes it difficult to map anything but the strongest diversification signals. This discrepancy can be seen in the difference between the number of events supported by the ML analyses and those supported by the bootstrap analyses. It is possible that additional sequence data will improve phylogenetic resolution and confidence, and that consequently additional diversification events will emerge.

Equally interesting to the few WGD events associated directly with diversification are the WGD events associated with general shifts in climate tolerance. WGDs in the Polygonaceae, Caryophyllaceae, Montiaceae, and the Tribe Nyctagineae appear to be associated with movement into colder environments (Figs 1, 2, S2, S3). Species arising after the WGD within the Amaranthaceae occupy wetter environments than the sister clade. The WGDs within the carnivorous plants were also associated with shifts in environment as Nepenthaceae are found in very wet environments and the Droseraceae are found in somewhat drier environments, at least comparatively. However, in these cases, perhaps the development of the wide array of

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morphologies associated with carnivory, apart from *Drosophyllum*, is more obviously associated with the WGD (Walker *et al.*, 2017).

While these qualitative assessments suggest potential correlations of shift in the climate occupied and WGDs, more specific and direct comparisons are necessary to quantify the extent of the shifts. For many of the clades experiencing WGD, a direct comparison with a sister clade is difficult because the sister may consist of a single species, another clade with WGD, or another complication. For example, there are WGDs at the base of both Polygonaceae and Plumbaginaceae as well as Nepenthaceae and Droseraceae. However, we made direct comparison of five duplicated lineages (see Fig. 3) in both values (i.e. ancestral states between sister clades) and variances (rate of Brownian motion) of climatic variables. In each case, the duplicated lineage occupied a colder mean annual temperature. This was also the case with the nested WGDs of Portulacineae and the Tribe Nyctagineae.

Of course, we do not suggest that all WGDs are associated with a shift to a colder climate. While such a pattern may exist in some clade (e.g. Caryophyllaceae), we emphasize the we observed a shift in the climate occupied rather than the direction of the shift. This too, may only be the case with the examples shown here. Mean annual precipitation did not exhibit a clear pattern with some clades occupying a higher precipitation and some occupying lower precipitation. Perhaps the best summary of climatic occupancy is the principal components of all the climatic variables. Here, while the shift in units is less easily interpreted, duplicated clades occupied different climatic spaces than sister lineages. This supports the hypothesis that WGD events are associated with adaptations. Here, many of these adaptations are associated with shifts in climatic occupancy. This necessitates further examination within the Caryophyllales as more data is gathered. This also suggests further examination with other angiosperm clades in order to investigate how general these results are.

The rates of climate occupancy evolution show more complicated patterns. While some clades, such as the Portulacineae, showed significant increase in a rate of climate occupancy evolution as compared to the sister clade (e.g. MAT), no clear pattern emerged across all comparisons. There were other shifts in rate such as with MAT and MAP in the Nyctaginaceae and Montiaceae, but these were not as strong as the pattern of climate occupancy itself discussed above.

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We suggest caution with any over interpretation of the results presented here. With each of the patterns presented, it is important to consider them in the context of uncertainty, both inherent in the biological processes that generate the phylogeny and in the analyses associated with large scale datasets. Large phylogenies and datasets allow for broad examinations, but uncertainty makes precise mapping of weaker signals difficult. Furthermore, large datasets often have poor overlap due to lack of data availability, and many of these clades require genomic datasets for accurate resolution (e.g. Arakaki *et al*., 2011; Yang *et al*., 2015; Walker *et al*., 2017). The comparisons of sister clades for climatic occupancy analyses and diversification analyses assume accurate identification of relationships that may differ between datasets. Additionally, large geographic datasets often contain extensive uncertainty and data cleanliness challenges due to the enormous size of the datasets. Furthermore, the biological reality of nested WGDs complicate analyses and interpretations. Focused studies with increased taxon sampling will contribute greatly to our understanding of the patterns presented (as suggested by Edwards *et al*., 2015). Increasing taxon sampling may help, but additional sequence data and specimen data for phylogenetic analyses, WGD mapping analyses, and climate occupancy characterization will improve our precision in these investigations. And so, the results presented here, we hope, will contribute to a growing discussion but will surely not be the last word.

What emerges from these analyses of WGD, diversification, and climate occupancy? It would appear as though, perhaps not unexpectedly, the patterns are complex and mixed. Some WGD are associated directly with diversification events, some WGD are associated with shifts in climate tolerance, some WGD are coincident with shifts in rates of climate occupancy evolution, and still other WGD are associated with known adaptations (carnivory, habit shifts associated with montane habitats, etc.). Some diversification shifts follow WGD events. However, it is unclear whether these events are linked or correlated and, if so, if they are correlated more with diversification than an additional adaptation or other evolutionary pattern or process. As data increase in these groups and as confidence increases in the phylogenetic relationships as well as the placement of both diversification and WGD events, we will be able to better address these questions. However, at least for the Caryophyllales, it does not appear as though diversification is tightly linked with WGD. Instead, for the clades that can be tested, we find shifts in climate occupancy correspond well to WGD.

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Suggestions for moving forward

WGDs are almost certainly one of the dominant processes that contribute to major evolutionary events within plant lineages. This may be in the form of increased diversification, development of novel traits, adaptation to new environments, and many other events (e.g. Schubert & Vu, 2016; Clavijo *et al.*, 2017). However, for several reasons, these events (i.e. WGD and other evolutionary events) may not occur simultaneously. In fact, there may be little to no expectation for the events to occur simultaneously (e.g. Donoghue, 2005; Schranz *et al.*, 2012; Donoghue & Sanderson, 2015; Tank *et al.*, 2015; Dodsworth *et al.*, 2016). In any case, more precise expectations and null models need to be developed to allow for reasonable tests of the correlations among these events. For example, there may be shifts in diversification that follow a WGD, but is it close enough, or frequent enough to infer that the two events are related? Is correlation possible or identifiable if, as is expected, intervening lineages have gone extinct? These questions would benefit from simulation studies where the true correlation pattern is known. Furthermore, more precise connections should be made to the biology of speciation and genome WGDs to better determine why, specifically, WGDs would be expected to correspond with any diversification pattern instead of adaptations, which may or may not correspond with increases or decreases in speciation. While still challenging, investigating the fate of and patterns of selection within individual genes (e.g. subfunctionalization and neofunctionalization) may shed light into the genomic basis of post-WGD and possibly allow for more concrete expectations for diversification. With the availability of genomes and transcriptomes, this is now beginning to become a possibility (e.g. Brockington *et al.*, 2015; Walker *et al.*, 2017). Only when these suggestions are linked to more specific biological hypotheses will we be able to better understand the ultimate impact of WGD in plant evolution.

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Author contributions

S.A.S., J.F.W., Y.Y., M.J.M., and S.F.B. designed research. C.P.D, R.B., N.L., and N.A.D. collected data. S.A.S., J.W.B., and Y.Y. analyzed the data. S.A.S. led the writing. All authors read and contributed to the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 The cladogram with support mapped for the bootstrap replicates described in the Materials and Methods section.

Fig. S2 The chronograms and mapping of temperature variables (bioclimatic variables 2–11) that are not presented in Fig. 1.

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Fig. S3 The chronograms and mapping of precipitation variables (bioclimatic variables 13–19) that are not presented in Fig. 2.

Fig. S4 The chronograms and mapping of PCA axis 2 on the broader Caryophyllales.

Fig. S5 Principal component loadings for bioclimatic variables.

Table S1 Calibrations used for divergence time analysis

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Fig. 1 Chronogram of the Caryophyllales with putative whole genome duplications mapped along with identified diversification shifts. Diversification analyses were performed on the maximum likelihood tree as well as the bootstrap tree set and those shifts that were identified in both groups are shown. The branches are colored based on Bioclim variable 1 (mean annual temperature). The numbers next duplications correspond to the numbers in Table 3 and the letters next to diversification shifts correspond to Table 4.

Fig. 2 The chronograms and mapping of diversification and whole genome duplications are as in Fig. 1 (see key for details **[Author, please confirm amended text 'key' is correct]**). (a) The branches are colored based on Bioclim variable 12 (mean annual precipitation), and (b) based on the principal component analyses (PCA) axis 1.

Fig. 3 Summary of whole genome duplication (WGD) events (with numbers corresponding to those in Table 3), distributions for climatic variables calculated between left and right clades (MAT, mean annual temperature; MAP, mean annual precipitation; PCA1, principal component axis 1), distributions for the rate of climatic variables calculated between left and right clades, and diversification shifts. Numbers along branches denote WGD, with the numbers corresponding to those in Fig. 1 and Table 3. Numbers inside clades denote the number of diversification rate shifts. Estimated species numbers are listed beside clade names. Box plots show the values estimated (ancestral values are listed in the top rows, rates in the bottom rows)

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for both the left and right clades across bootstrap samples. Clades shaded grey denote a WGD; (b–d) have nested WGD.

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Table 1 Voucher information and GenBank accession numbers for newly reported plastid *matK* sequences; families follow APG IV (Angiosperm Phylogeny Group, 2016)

Family	Taxon	Voucher specimen (Herbarium acronym)	Collection locality	NCBI accession number
Achatocarpaceae	*Achatocarpus gracilis* H.Walter	Silvia H. Salas Morales *et al*. 5608 (TEX)	Mexico: Oaxaca	KY952292
Achatocarpaceae	*Phaulothamnus spinescens* A.Gray	Michael J. Moore *et al*. 976 (OC)	USA: Texas	KY952477
Achatocarpaceae	*Phaulothamnus spinescens* A.Gray	William R. Carr 27176 (TEX)	USA: Texas	KY952478
Amaranthaceae	*Allenrolfea occidentalis* (S.Watson) Kuntze	Michael J. Moore 474 (OC)	USA: Texas	KY952314
Amaranthaceae	*Alternanthera caracasana* Kunth	Michael J. Moore 1808 (OC)	USA: Texas	KY952319
Amaranthaceae	*Amaranthus cruentus* L.	Michael J. Moore 356 (OC)	USA: Ohio (cultivated)	KY952320
Amaranthaceae	*Amaranthus* sp.	Michael J. Moore 1801 (OC)	USA: Texas	KY952321
Amaranthaceae	*Amaranthus* sp.	Michael J. Moore 2186 (OC)	USA: Ohio	KY952322
Amaranthaceae	*Amaranthus* sp.	Michael J. Moore 2187 (OC)	USA: Illinois	KY952323
Amaranthaceae	*Atriplex prosopidum* I.M.Johnst.	Hilda Flores Olvera *et al*. 1658 (MEXU)	Mexico: Coahuila	KY952340
Amaranthaceae	*Atriplex* sp.	Michael J. Moore	USA: Texas	KY952338

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		1689 (OC)		
Amaranthaceae	*Atriplex* sp.	Michael J. Moore 1699 (OC)	USA: Texas	KY952339
Amaranthaceae	*Celosia argentea* L. var. *plumosa*	Michael J. Moore 359 (OC)	USA: Ohio (cultivated)	KY952359
Amaranthaceae	*Charpentiera ovata* Gaudich. var. *ovata*	Flora K. Samis 7 (Lyon Arboretum living collection, accession 2011.0034)	USA: Hawaii	KY952360
Amaranthaceae	*Charpentiera tomentosa* Sohmer var. *maakuaensis* Sohmer	Flora K. Samis 6 (Lyon Arboretum living collection, accession 88.0141)	USA: Hawaii	KY952361
Amaranthaceae	*Chenopodium album* L.	Michael J. Moore 344 (OC)	USA: Ohio	KY952362
Amaranthaceae	*Gossypianthus lanuginosus* (Poir.) Moq.	Michael J. Moore 1807 (OC)	USA: Texas	KY952408
Amaranthaceae	*Guilleminea densa* (Humb. & Bonpl. ex Schult.) Moq.	Michael J. Moore *et al*. 2445 (OC)	Mexico: Chihuahua	KY952412
Amaranthaceae	*Kali tragus* (L.) Scop.	Michael J. Moore 453 (OC)	USA: Texas	KY952506
Amaranthaceae	*Nototrichium divaricatum* D.H.Lorence	Flora K. Samis 3 (Lyon Arboretum living collection, accession 96.0036 #3)	USA: Hawaii	KY952468
Amaranthaceae	*Nototrichium humile* Hillebr.	Flora K. Samis 2 (Lyon Arboretum living collection, accession 2001-0254)	USA: Hawaii	KY952469
Amaranthaceae	*Suaeda jacoensis* I.M.Johnst.	Hilda Flores Olvera *et al*. 1662 (MEXU)	Mexico: Coahuila	KY952514

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Amaranthaceae	*Suaeda jacoensis* I.M.Johnst.	Michael J. Moore *et al*. 2617 (OC)	Mexico: Nuevo Leon	KY952515
Amaranthaceae	*Suaeda mexicana* (Standl.) Standl.	Hilda Flores Olvera *et al*. 1654 (MEXU)	Mexico: Coahuila	KY952516
Amaranthaceae	*Tidestromia lanuginosa* (Nutt.) Standl.	Michael J. Moore 1128 (OC)	USA: Texas	KY952521
Amaranthaceae	*Zuckia brandegeei* (A.Gray) S.L.Welsh & Stutz var. *plummeri* (Stutz & S.C.Sand.) Dorn	Joseph L. M. Charboneau 9672 (RM)	USA: Colorado	KY952528
Cactaceae	*Leuenbergeria quisqueyana* (Alain) Lodé	Flora K. Samis 11 (Lyon Arboretum living collection, accession 2000.0281)	USA: Hawaii	KY952473
Caryophyllaceae	*Moehringia macrophylla* (Hook.) Fenzl	Arianna Goodman 1 (OC)	USA: Oregon	KY952464
Caryophyllaceae	*Paronychia lundellorum* Torr. & A.Gray	William R. Carr 17607 (MEXU)	USA: Texas	KY952472
Caryophyllaceae	*Saponaria officinalis* L.	Michael J. Moore *et al*. 1819 (OC)	USA: Indiana	KY952507
Caryophyllaceae	*Schiedea kaalae* Wawra	Flora K. Samis 5 (Lyon Arboretum living collection, accession 92.0513)	USA: Hawaii	KY952509
Caryophyllaceae	*Spergularia salina* J.Presl & C.Presl	Michael J. Moore 1693 (OC)	USA: Texas	KY952512
Didiereaceae	*Alluaudia ascendens* (Drake) Drake	Michael J. Moore 1645	USA (cultivated)	KY952318
Dioncophyllaceae	*Triphyophyllum peltatum* (Hutch. & Dalziel) Airy Shaw	Carel C. H. Jongkind *et al*. 7136 (WAG)	Liberia	KY952524
Droseraceae	*Drosera burmannii* Vahl	Michael J. Moore	USA	KY952400

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	cv. Pilliga Red	1814 (OC)	(cultivated)	
Droseraceae	*Drosera peltata* Thunb.	Michael J. Moore 1817 (OC)	Australia: Tasmania (cultivated)	KY952401
Droseraceae	*Drosera regia* Stephens	Michael J. Moore 1812 (OC)	USA (cultivated)	KY952402
Drosophyllaceae	*Drosophyllum lusitanicum* (L.) Link	Michael J. Moore 1816 (OC)	USA (cultivated)	KY952403
Frankeniaceae	*Frankenia gypsophila* I.M.Johnst.	Michael J. Moore *et al*. 1880 (OC)	Mexico: Nuevo Leon	KY952406
Microteaceae	*Microtea debilis* Sw.	Manuel Rimachi 11128 (TEX)	Peru: Loreto	KY952415
Montiaceae	*Claytonia sibirica* L.	Arianna Goodman 2 (OC)	USA: Oregon	KY952363
Montiaceae	*Phemeranthus parviflorus* (Nutt.) Kiger	Michael J. Moore *et al*. 2214 (OC)	USA: New Mexico	KY952479
Nyctaginaceae	*Abronia angustifolia* Greene	Michael J. Moore *et al*. 2063 (OC)	Mexico: Coahuila	KY952281
Nyctaginaceae	*Abronia angustifolia* Greene	Michael J. Moore *et al*. 896 (OC)	USA: New Mexico	KY952282
Nyctaginaceae	*Abronia bigelovii* Heimerl	Michael J. Moore *et al*. 704 (OC)	USA: New Mexico	KY952283
Nyctaginaceae	*Abronia elliptica* A.Nelson	Norman A. Douglas 2039 (DUKE)	United States: Arizona	KY952284
Nyctaginaceae	*Abronia fragrans* Nutt. ex Hook.	Billie L. Turner 20-22 (SRSC)	USA: Texas	KY952285
Nyctaginaceae	*Abronia fragrans* Nutt. ex Hook.	Glenn Kroh *et al*. 3021 (TEX)	USA: Texas	KY952286
Nyctaginaceae	*Abronia macrocarpa* L.A.Galloway	Steve L. Orzell *et al*. 6492 (TEX)	USA: Texas	KY952287
Nyctaginaceae	*Abronia mellifera* Douglas ex Hook.	N. Elizabeth Saunders BP 19 (SIU)	USA: Wyoming	KY952288

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Nyctaginaceae	*Abronia mellifera* Douglas ex Hook.	N. Elizabeth Saunders BP 20 (SIU)	USA: Wyoming	KY952289
Nyctaginaceae	*Abronia nana* S.Watson var. *nana*	Robert C. Sivinski *et al*. 3108 (NMC)	USA: Arizona	KY952290
Nyctaginaceae	*Abronia umbellata* Lam.	N. Elizabeth Saunders LU 45 (SIU)	USA: California	KY952291
Nyctaginaceae	*Acleisanthes acutifolia* Standl.	James Henrickson *et al*. 22916 (TEX)	Mexico: Coahuila	KY952293
Nyctaginaceae	*Acleisanthes angustifolia* (Torr.) R.A.Levin	Michael J. Moore 460 (OC)	USA: Texas	KY952294
Nyctaginaceae	*Acleisanthes* cf. *purpusiana* (Heimerl) R.A.Levin	James Henrickson 23026 (TEX)	Mexico: Coahuila	KY952309
Nyctaginaceae	*Acleisanthes chenopodioides* (A.Gray) R.A.Levin	Michael J. Moore *et al*. 733 (OC)	USA: Texas	KY952295
Nyctaginaceae	*Acleisanthes crassifolia* A.Gray	Michael J. Moore *et al*. 569 (OC)	USA: Texas	KY952296
Nyctaginaceae	*Acleisanthes diffusa* (A.Gray) R.A.Levin var. *diffusa*	Michael J. Moore *et al*. 624 (OC)	USA: Texas	KY952297
Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A.Levin var. *lanceolata*	Michael J. Moore *et al*. 870 (OC)	USA: New Mexico	KY952298
Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A.Levin var. *lanceolata*	Michael J. Moore *et al*. 903 (OC)	USA: Texas	KY952299
Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A.Levin var. *megaphylla*	Alfred T. Richardson 1666 (TEX)	Mexico: Chihuahua	KY952300

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	(B.A.Fowler & B.L.Turner) Spellenb. & J.Poole			
Nyctaginaceae	*Acleisanthes longiflora* A.Gray	Michael J. Moore 435 (OC)	USA: Texas	KY952301
Nyctaginaceae	*Acleisanthes longiflora* A.Gray	Michael J. Moore *et al*. 571 (OC)	USA: Texas	KY952302
Nyctaginaceae	*Acleisanthes nana* I.M.Johnst.	Jackie Smith *et al*. 798 (TEX)	Mexico: San Luis Potosi	KY952303
Nyctaginaceae	*Acleisanthes obtusa* (Choisy) Standl.	Michael J. Moore *et al*. 984 (OC)	USA: Texas	KY952304
Nyctaginaceae	*Acleisanthes palmeri* (Hemsley) R.A.Levin	George S. Hinton 28620 (TEX)	MexicoNuevo Leon	KY952305
Nyctaginaceae	*Acleisanthes parvifolia* (Torr.) R.A.Levin	Michael J. Moore 452 (OC)	USA: Texas	KY952306
Nyctaginaceae	*Acleisanthes purpusiana* (Heimerl) R.A.Levin	James Henrickson 22709 (TEX)	Mexico: Coahuila	KY952307
Nyctaginaceae	*Acleisanthes purpusiana* (Heimerl) R.A.Levin	Billie L. Turner 6205 (TEX)	Mexico: Coahuila	KY952308
Nyctaginaceae	*Acleisanthes undulata* (B.A.Fowler & B.L.Turner) R.A.Levin	James Henrickson 23195 (TEX)	Mexico: Coahuila	KY952310
Nyctaginaceae	*Acleisanthes wrightii* (A.Gray) Benth. & Hook.	Michael J. Moore *et al*. 620 (OC)	USA: Texas	KY952311
Nyctaginaceae	*Allionia choisyi* Standl.	Norman A. Douglas 2187 (DUKE)	Mexico: Coahuila	KY952315
Nyctaginaceae	*Allionia incarnata* L.	Michael J. Moore *et al*. 1352 (OC)	Mexico: Nuevo Leon	KY952316
Nyctaginaceae	*Allionia* sp.	Michael J. Moore 424 (OC)	USA: Texas	KY952317
Nyctaginaceae	*Andradea floribunda* Allemão	André M. Amorim 2294 (NY)	Brazil	KY952324

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Nyctaginaceae	*Andradea floribunda* Allemão	Jacquelyn Ann Kallunki 701 (NY)	Brazil	KY952325
Nyctaginaceae	*Anulocaulis annulatus* (Coville) Standl.	Richard W. Spellenberg 3162 (NMC)	USA: California	KY952326
Nyctaginaceae	*Anulocaulis eriosolenus* (A.Gray) Standl.	James Henrickson *et al*. 23103 (TEX)	Mexico: Coahuila	KY952327
Nyctaginaceae	*Anulocaulis eriosolenus* (A.Gray) Standl.	Michael J. Moore *et al*. 611 (OC)	USA: Texas	KY952328
Nyctaginaceae	*Anulocaulis hintoniorum* B.L.Turner	Patricia Hernández Ledesma 52 (MEXU)	Mexico: Coahuila	KY952329
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *gypsogenus* (Waterf.) Spellenb. & T.Wootten	Michael J. Moore 402 (OC)	USA: New Mexico	KY952330
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *howardii* Spellenb. & T.Wootten	Thomas Wootten *et al*. s.n. (NMC)	USA: New Mexico	KY952331
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *lasianthus* I.M.Johnston	Michael J. Moore *et al*. 610 (OC)	USA: Texas	KY952332
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *leiosolenus*	Michael J. Moore 493 (OC)	USA: Texas	KY952333
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *leiosolenus*	Michael J. Moore *et al*. 825 (OC)	USA: Nevada	KY952334
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *leiosolenus*	Michael J. Moore *et al*. 853 (OC)	USA: Arizona	KY952335
Nyctaginaceae	*Anulocaulis reflexus* I.M.Johnst.	Michael J. Moore *et al*. 242 (TEX)	Mexico: Chihuahua	KY952336

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Nyctaginaceae	*Anulocaulis reflexus* I.M.Johnst.	Michael J. Moore 483 (OC)	USA: Texas	KY952337
Nyctaginaceae	*Boerhavia anisophylla* Torr.	Norman A. Douglas 2194 (DUKE)	Mexico: Durango	KY952341
Nyctaginaceae	*Boerhavia ciliata* Brandegee	Norman A. Douglas 2145 (DUKE)	USA: Texas	KY952342
Nyctaginaceae	*Boerhavia coccinea* Mill.	Michael J. Moore 366 (OC)	USA: New Mexico	KY952343
Nyctaginaceae	*Boerhavia coulteri* (Hook.f.) S.Watson var. *palmeri* (S.Watson) Spellenb.	Richard W. Spellenberg 13273 (NMC)	USA: Arizona	KY952344
Nyctaginaceae	*Boerhavia dominii* Meikle & Hewson	H. Smyth 42 (NY)	Australia: South Australia	KY952345
Nyctaginaceae	*Boerhavia gracillima* Heimerl	Richard W. Spellenberg 12447 (NMC)	USA: Texas	KY952347
Nyctaginaceae	*Boerhavia intermedia* M.E.Jones	Richard W. Spellenberg 13279 (NMC)	USA: Arizona	KY952348
Nyctaginaceae	*Boerhavia lateriflora* Standl.	Norman A. Douglas 2161 (DUKE)	Mexico: Sonora	KY952349
Nyctaginaceae	*Boerhavia linearifolia* A.Gray	Michael J. Moore *et al*. 581 (OC)	USA: Texas	KY952350
Nyctaginaceae	*Boerhavia purpurascens* A.Gray	Richard W. Spellenberg 13261 (NMC)	USA: Arizona	KY952351
Nyctaginaceae	*Boerhavia repens* L.	J. S. Rose 2	USA: Hawaii	KY952352
Nyctaginaceae	*Boerhavia repens* L.	Richard W. Spellenberg 7183 (NMC)	Yemen: Sana	KY952353
Nyctaginaceae	*Boerhavia* sp.	Erin Tripp *et al*. 4090	Namibia	KY952346

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		(OC)		
Nyctaginaceae	*Boerhavia torreyana* (S.Watson) Standl.	Michael J. Moore *et al*. 633 (OC)	USA: Texas	KY952354
Nyctaginaceae	*Bougainvillea campanulata* Heimerl	Michael Nee 51257 (TEX)	Bolivia: Santa Cruz	KY952355
Nyctaginaceae	*Bougainvillea glabra* Choisy	Michael J. Moore 538 (OC)	USA: Ohio (cultivated)	KY952356
Nyctaginaceae	*Bougainvillea spinosa* (Cav.) Heimerl	J. Saunders *et al*. 3371 (TEX)	Argentina: San Juan	KY952357
Nyctaginaceae	*Bougainvillea stipitata* Griseb.	Michael Nee 50723 (TEX)	Bolivia: Santa Cruz	KY952358
Nyctaginaceae	*Colignonia glomerata* Griseb.	Michael Nee 52523 (NY)	Bolivia	KY952364
Nyctaginaceae	*Colignonia scandens* Benth.	Martin Grantham 63 (SFBG living collection, accession 1996-0202)	Ecuador	KY952365
Nyctaginaceae	*Commicarpus ambiguus* Meikle	Mats Thulin 11015 (UPS)	Somalia: Sanaag	KY952366
Nyctaginaceae	*Commicarpus arabicus* Meikle	Mats Thulin *et al*. 9294 (UPS)	Yemen: Taizz	KY952367
Nyctaginaceae	*Commicarpus arabicus* Meikle	Richard W. Spellenberg 7217 (NMC)	Yemen: Ibb	KY952368
Nyctaginaceae	*Commicarpus arabicus* Meikle	Richard W. Spellenberg 7297 (NMC)	Yemen: Ibb	KY952369
Nyctaginaceae	*Commicarpus australis* (Meikle) Govaerts	Richard W. Spellenberg *et al*. 9469 (NMC)	Australia: Western Australia	KY952370
Nyctaginaceae	*Commicarpus boissieri* (Heimerl) Cufod.	Mats Thulin 11423 (UPS)	Oman: Dhofar	KY952371
Nyctaginaceae	*Commicarpus boissieri*	Carl J. Rothfels *et al*.	Oman: Ash	KY952373

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	(Heimerl) Cufod.	4331	Sharqiyah	
Nyctaginaceae	*Commicarpus brandegeei* Standl.	Patricia Hernández Ledesma 55 (MEXU)	Mexico: Baja California Sur	KY952372
Nyctaginaceae	*Commicarpus coctoris* N.A.Harriman	Richard W. Spellenberg *et al.* 12883 (NMC)	Mexico: Oaxaca	KY952374
Nyctaginaceae	*Commicarpus commersonii* (Baill.) Cavaco	Mats Thulin *et al.* 11836 (UPS)	Madagascar: Toliara	KY952380
Nyctaginaceae	*Commicarpus decipiens* Meikle	Erin Tripp *et al.* 4127 (NMC)	Namibia	KY952375
Nyctaginaceae	*Commicarpus grandiflorus* (A.Rich.) Standl.	Mats Thulin *et al.* 9311 (UPS)	Yemen: Taizz	KY952376
Nyctaginaceae	*Commicarpus greenwayi* Meikle	Mats Thulin 606 (UPS)	Tanzania: Iringa	KY952377
Nyctaginaceae	*Commicarpus helenae* (Roem. & Schult.) Meikle	Richard W. Spellenberg *et al.* 7504 (NMC)	Yemen: Dhamar	KY952378
Nyctaginaceae	*Commicarpus hiranensis* Thulin	Mats Thulin *et al.* 11225 (UPS)	Ethiopia: Harerge	KY952379
Nyctaginaceae	*Commicarpus mistus* Thulin	Mats Thulin *et al.* 9786 (UPS)	Yemen: Mahrah	KY952381
Nyctaginaceae	*Commicarpus parviflorus* Thulin	Mats Thulin 6318 (UPS)	Somalia: Banaadir	KY952382
Nyctaginaceae	*Commicarpus pedunculosus* (A.Rich.) Cufod.	Mats Thulin 1301 (UPS)	Ethiopia: Arussi	KY952383
Nyctaginaceae	*Commicarpus plumbagineus* (Cav.) Standl.	Mats Thulin 10747 (UPS)	Somalia: Togdheer	KY952384
Nyctaginaceae	*Commicarpus plumbagineus* (Cav.)	Mats Thulin *et al.* 11330 (UPS)	Ethiopia: Harerge	KY952385

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	Standl.			
Nyctaginaceae	*Commicarpus plumbagineus* (Cav.) Standl.	Richard W. Spellenberg *et al*. 7374 (NMC)	Yemen: Ta'izz	KY952386
Nyctaginaceae	*Commicarpus praetermissus* N.A.Harriman	Richard W. Spellenberg *et al*. 12905 (NMC)	Mexico: Michoacán	KY952387
Nyctaginaceae	*Commicarpus reniformis* (Chiov.) Cufod.	Mats Thulin 4200 (UPS)	Somalia: Sool	KY952388
Nyctaginaceae	*Commicarpus reniformis* (Chiov.) Cufod.	Mats Thulin *et al*. 8337 (UPS)	Yemen: Hadramaut	KY952389
Nyctaginaceae	*Commicarpus scandens* (L.) Standl.	Michael J. Moore 1127 (OC)	USA: Texas	KY952390
Nyctaginaceae	*Commicarpus scandens* (L.) Standl.	Richard W. Spellenberg *et al*. 12887 (NMC)	Mexico: Puebla	KY952391
Nyctaginaceae	*Commicarpus sinuatus* Meikle	Mats Thulin 10737 (UPS)	Somalia: Woqooyi Galbeed	KY952392
Nyctaginaceae	*Commicarpus sinuatus* Meikle	Richard W. Spellenberg 7144 (NMC)	Yemen: Sana'a	KY952393
Nyctaginaceae	*Commicarpus sinuatus* Meikle	Richard W. Spellenberg 7506 (NMC)	Yemen: Dhamar	KY952394
Nyctaginaceae	*Commicarpus squarrosus* (Heimerl) Standl. var. *squarrosus*	Erin Tripp *et al*. 4049 (NMC)	Namibia	KY952395
Nyctaginaceae	*Commicarpus stenocarpus* (Chiov.) Cufod.	Mats Thulin *et al*. 8062 (UPS)	Yemen: Hadramaut	KY952396
Nyctaginaceae	*Cuscatlania vulcanicola* Standl.	José L. Linares 12938 (MEXU)	El Salvador: Sonsonate	KY952397

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Nyctaginaceae	*Cuscatlania vulcanicola* Standl.	José L. Linares 13440 (MEXU)	El Salvador: Sonsonate	KY952398
Nyctaginaceae	*Cyphomeris gypsophiloides* (M.Martens & Galeotti) Standl.	Michael J. Moore *et al*. 582 (OC)	USA: Texas	KY952399
Nyctaginaceae	*Grajalesia fasciculata* (Standl.) Miranda	José L. Linares 13416 (MEXU)	El Salvador: Sonsonate	KY952409
Nyctaginaceae	*Guapira discolor* (Spreng.) Little	Richard W. Spellenberg 13294 (NMC)	USA: Florida	KY952410
Nyctaginaceae	*Guapira eggersiana* (Heimerl) Lundell	Scott A. Mori 25542/40 (NY)	French Guiana	KY952411
Nyctaginaceae	*Mirabilis albida* (Walter) Heimerl	Norman A. Douglas 2035 (DUKE)	USA: Arizona	KY952416
Nyctaginaceae	*Mirabilis albida* (Walter) Heimerl	William R. Carr 11075 (TEX)	USA: Texas	KY952417
Nyctaginaceae	*Mirabilis alipes* (S.Watson) Pilz	Arnold Tiehm 13461 (TEX)	USA: Nevada	KY952418
Nyctaginaceae	*Mirabilis bigelovii* A.Gray var. *retrorsa* (A. Heller) Munz	James D. Morefield *et al*. 3780 (TEX)	USA: California	KY952419
Nyctaginaceae	*Mirabilis* cf. *glabrifolia* (Ortega) I.M.Johnst.	Michael J. Moore *et al*. 1244 (OC)	Mexico: San Luis Potosi	KY952428
Nyctaginaceae	*Mirabilis* cf. *nesomii* B.L.Turner	George S. Hinton 25567 (TEX)	Mexico: Nuevo Leon	KY952449
Nyctaginaceae	*Mirabilis coccinea* (Torr.) Benth. & Hook.f.	Norman A. Douglas 2133 (DUKE)	USA: Arizona	KY952420
Nyctaginaceae	*Mirabilis coccinea* (Torr.) Benth. & Hook.f.	Steven P. McLaughlin *et al*. 9354 (ARIZ)	USA: Arizona	KY952421
Nyctaginaceae	*Mirabilis comata* (Small) Standl.	Norman A. Douglas 2084 (DUKE)	USA: Arizona	KY952422

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Nyctaginaceae	*Mirabilis decumbens* (Nutt.) Daniels	Richard W. Spellenberg *et al*. 4073 (TEX)	Mexico: Zacatecas	KY952423
Nyctaginaceae	*Mirabilis donahooiana* Le Duc	Alice Le Duc *et al*. 247 (TEX)	Mexico: Michoacán	KY952424
Nyctaginaceae	*Mirabilis exserta* Brandegee	Pedro Tenorio 10586 (MEXU)	Mexico	KY952425
Nyctaginaceae	*Mirabilis gigantea* (Standl.) Shinners	J. Quayle *et al*. 752 (TEX)	USA: Texas	KY952426
Nyctaginaceae	*Mirabilis glabra* (S.Watson) Standl.	Michael J. Moore *et al*. 674 (OC)	USA: New Mexico	KY952446
Nyctaginaceae	*Mirabilis glabrifolia* (Ortega) I.M.Johnst.	Guy Nesom *et al*. 7654 (TEX)	Mexico: Coahuila	KY952427
Nyctaginaceae	*Mirabilis glabrifolia* (Ortega) I.M.Johnst.	Michael J. Moore *et al*. 1325 (OC)	Mexico: Nuevo Leon	KY952429
Nyctaginaceae	*Mirabilis gracilis* (Standl.) LeDuc	Alice Le Duc *et al*. 71 (TEX)	Mexico: Jalisco	KY952430
Nyctaginaceae	*Mirabilis grandiflora* (Standl.) Standl.	EDL 1863 (MEXU)	Mexico	KY952431
Nyctaginaceae	*Mirabilis greenei* S.Watson	George E. Pilz 998 (TEX)	USA: California	KY952432
Nyctaginaceae	*Mirabilis himalaica* (Edgew.) Heimerl var. *chinensis* Heimerl	D. E. Boufford *et al*. 32449 (F)	China: Xizang (Tibet)	KY952433
Nyctaginaceae	*Mirabilis himalaica* (Edgew.) Heimerl var. *chinensis* Heimerl	D. E. Boufford *et al*. 41198 (F)	China: Xizang (Tibet)	KY952434
Nyctaginaceae	*Mirabilis himalaica* (Edgew.) Heimerl var. *chinensis* Heimerl	D. E. Boufford *et al*. 41435 (F)	China: Xizang (Tibet)	KY952435
Nyctaginaceae	*Mirabilis hintoniorum* Le Duc	Patricia Hernández Ledesma 118 (MEXU)	Mexico: Michoacán	KY952436

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Nyctaginaceae	*Mirabilis jalapa* L.	Michael J. Moore s.n.	USA (cultivated)	KY952437
Nyctaginaceae	*Mirabilis laevis* (Benth.) Curran	Andrew C. Sanders *et al*. 29410 (TEX)	USA: California	KY952438
Nyctaginaceae	*Mirabilis latifolia* (A.Gray) Diggs, Lipscomb & O'Kennon	Victor L. Cory 24549 (GH)	USA: Texas	KY952439
Nyctaginaceae	*Mirabilis linearis* (Pursh) Heimerl	Billie L. Turner 21-854 (TEX)	USA: Texas	KY952440
Nyctaginaceae	*Mirabilis linearis* (Pursh) Heimerl var. *decipiens* (Standl.) S.L.Welsh	Michael J. Moore *et al*. 1984 (OC)	Mexico: Coahuila	KY952441
Nyctaginaceae	*Mirabilis longiflora* L.	Michael J. Moore *et al*. 1230 (OC)	Mexico: San Luis Potosi	KY952442
Nyctaginaceae	*Mirabilis longiflora* L. var. *wrightiana* (A.Gray ex Britton & Kearney) Kearney & Peebles	Alice Le Duc 185 (TEX)	USA: New Mexico	KY952443
Nyctaginaceae	*Mirabilis melanotricha* (Standl.) Spellenb.	Michael J. Moore *et al*. 1191 (OC)	Mexico: San Luis Potosi	KY952444
Nyctaginaceae	*Mirabilis melanotricha* (Standl.) Spellenb.	Norman A. Douglas 2067 (DUKE)	USA: New Mexico	KY952445
Nyctaginaceae	*Mirabilis multiflora* (Torr.) A.Gray	Michael J. Moore 1110 (OC)	USA: Texas	KY952447
Nyctaginaceae	*Mirabilis multiflora* (Torr.) A.Gray	Norman A. Douglas 2037 (DUKE)	USA: Arizona	KY952448
Nyctaginaceae	*Mirabilis nesomii* B.L.Turner	Michael J. Moore *et al*. 2179 (NMC)	Mexico: Nuevo Leon	KY952450
Nyctaginaceae	*Mirabilis nesomii* B.L.Turner	Michael J. Moore *et al*. 2643 (NMC)	Mexico: Nuevo Leon	KY952451
Nyctaginaceae	*Mirabilis nyctaginea* (Michx.) MacMill.	William R. Carr 14590 (TEX)	USA: Texas	KY952452

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Nyctaginaceae	*Mirabilis oligantha* (Standl.) Standl.	José L. Panero 2816 (MEXU)	Mexico: Baja California	KY952453
Nyctaginaceae	*Mirabilis oxybaphoides* (A.Gray) A.Gray	George S. Hinton 25572 (TEX)	Mexico: Nuevo Leon	KY952454
Nyctaginaceae	*Mirabilis polonii* Le Duc	Alice Le Duc 259 (MEXU)	Mexico: Nuevo Leon	KY952455
Nyctaginaceae	*Mirabilis pringlei* Weath.	Alice Le Duc *et al*. 63 (TEX)	Mexico: Jalisco	KY952456
Nyctaginaceae	*Mirabilis pudica* Barneby	Arnold Tiehm 10971 (TEX)	USA: Nevada	KY952457
Nyctaginaceae	*Mirabilis texensis* (J.M.Coult.) B.L.Turner	Billie L. Turner 22-417 (TEX)	USA: Texas	KY952458
Nyctaginaceae	*Mirabilis triflora* Benth.	Ramón Cuevas G. *et al*. 3415 (MEXU)	Mexico: Jalisco	KY952459
Nyctaginaceae	*Mirabilis urbani* Heimerl	Mark Fishbein *et al*. 5107 (MEXU)	Mexico: Michoacan	KY952460
Nyctaginaceae	*Mirabilis violacea* (L.) Heimerl	Patricia Hernández Ledesma 63 (MEXU)	Mexico: Distrito Federal	KY952461
Nyctaginaceae	*Mirabilis viscosa* Cav.	Michael J. Moore *et al*. 1824 (NMC)	Mexico: San Luis Potosi	KY952462
Nyctaginaceae	*Mirabilis viscosa* Cav.	Patricia Hernández Ledesma 13 (MEXU)	Mexico	KY952463
Nyctaginaceae	*Neea belizensis* Donn.Sm.	Cyrus L. Lundell 17692 (TEX)	Guatemala: Petén	KY952465
Nyctaginaceae	*Neea cauliflora* Poepp. & Endl.	Schanke S15106 (NY)	Peru	KY952466
Nyctaginaceae	*Neea psychotrioides* Donn.Sm.	Robert L. Wilbur 63654	Costa Rica: Heredia	KY952467
Nyctaginaceae	*Nyctaginia capitata* Choisy	Michael J. Moore *et al*. 617 (OC)	USA: Texas	KY952470
Nyctaginaceae	*Okenia hypogaea* Schltdl. & Cham.	Thomas R. Van Devender *et al*. 92-	Mexico: Sonora	KY952471

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		1069 (NMC)		
Nyctaginaceae	*Pisonia aculeata* L.	C. Martínez 1209 (TEX)	Mexico: Oaxaca	KY952483
Nyctaginaceae	*Pisonia brunoniana* Endl.	J. S. Rose 3	USA: Hawaii	KY952484
Nyctaginaceae	*Pisonia capitata* (S.Watson) Standl.	Ana L. Reina Guerrero *et al*. 2000-193 (NMC)	Mexico: Sonora	KY952485
Nyctaginaceae	*Pisonia capitata* (S.Watson) Standl.	Thomas R. Van Devender *et al*. 2003-17 (TEX)	USA: Arizona	KY952486
Nyctaginaceae	*Pisonia macranthocarpa* (Donn.Sm.) Donn.Sm.	Dennis E. Breedlove *et al*. 30361 (TEX)	Mexico: Chiapas	KY952487
Nyctaginaceae	*Pisonia sandwicensis* Hillebr.	Flora K. Samis 1 (Lyon Arboretum living collection)	USA: Hawaii	KY952488
Nyctaginaceae	*Pisonia sylvatica* Standl.	José L. Linares 13403 (MEXU)	El Salvador: Sonsonate	KY952489
Nyctaginaceae	*Pisonia umbellifera* (J.R.Forst. & G.Forst.) Seem.	Flora K. Samis 12 (Lyon Arboretum living collection, accession 68.0453)	USA: Hawaii	KY952490
Nyctaginaceae	*Pisonia zapallo* Griseb.	Israel G. Vargas *et al*. 2001 (TEX)	Bolivia: Santa Cruz	KY952491
Nyctaginaceae	*Pisoniella arborescens* (Lag. & Rodr.) Standl.	Alice Le Duc *et al*. 231 (NMC)	Mexico: Oaxaca	KY952492
Nyctaginaceae	*Pisoniella arborescens* (Lag. & Rodr.) Standl.	William R. Anderson 13522 (NY)	Mexico: Oaxaca	KY952493
Nyctaginaceae	*Ramisia brasiliensis* Oliv.	Jomar G. Jardim 1507 (NY)	Brazil	KY952495
Nyctaginaceae	*Reichenbachia hirsuta* Spreng.	Michael Nee 47813 (NY)	Bolivia	KY952496
Nyctaginaceae	*Reichenbachia*	Maria Maguidaura	Brazil	KY952497

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	paraguayensis (D.Parodi) Dugand & Daniel	Hatschbach 49218 (NY)		
Nyctaginaceae	*Salpianthus arenarius* Bonpl.	Richard W. Spellenberg 12903 (NMC)	Mexico: Michoacán	KY952503
Nyctaginaceae	*Salpianthus macrodontus* Standl.	Thomas R. Van Devender *et al*. 91-894 (NMC)	Mexico: Sonora	KY952504
Nyctaginaceae	*Salpianthus purpurascens* (Cav. ex Lag.) Hook. & Arn.	Richard W. Spellenberg *et al*. 12885 (NMC)	Mexico: Oaxaca	KY952505
Nyctaginaceae	*Tripterocalyx carneus* (Greene) L.A.Galloway	Norman A. Douglas 2060 (DUKE)	USA: New Mexico	KY952525
Nyctaginaceae	*Tripterocalyx crux-maltae* (Kellogg) Standl.	Arnold Tiehm *et al*. 12213 (TEX)	USA: Nevada	KY952526
Nyctaginaceae	*Tripterocalyx micranthus* (Torr.) Hook.	B. MacLeod *et al*. 751 (TEX)	USA: Colorado	KY952527
Phytolaccaceae	*Agdestis clematidea* Moc. & Sessé ex DC.	George S. Hinton 25023 (TEX)	Mexico: Tamaulipas	KY952313
Phytolaccaceae	*Gallesia integrifolia* (Spreng.) Harms	Michael Nee *et al*. 50072 (TEX)	Bolivia: Santa Cruz	KY952407
Phytolaccaceae	*Hilleria latifolia* (Lam.) H.Walter	Michael Nee 33807 (TEX)	Bolivia: Santa Cruz	KY952413
Phytolaccaceae	*Petiveria alliacea* L.	Lucas C. Majure 4132 (FLAS)	USA: Florida	KY952476
Phytolaccaceae	*Phytolacca americana* L.	Michael J. Moore 342 (OC)	USA: Ohio	KY952480
Phytolaccaceae	*Phytolacca icosandra* L.	Mark H. Mayfield *et al*. 1001 (TEX)	Mexico: Guerrero	KY952481
Phytolaccaceae	*Phytolacca octandra* L.	Juan A. Encina *et al*. 1545 (TEX)	Mexico: Nuevo Leon	KY952482

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Phytolaccaceae	*Rivina humilis* L.	Michael J. Moore 1129 (OC)	USA: Texas	KY952499
Phytolaccaceae	*Seguieria aculeata* Jacq.	Elsa Zardini *et al*. 22101 (TEX)	Paraguay	KY952510
Phytolaccaceae	*Seguieria paraguariensis* Morong	Michael Nee 48735 (TEX)	Bolivia: Santa Cruz	KY952511
Phytolaccaceae	*Trichostigma octandrum* (L.) H.Walter	Michael Nee 47094 (TEX)	Bolivia: Santa Cruz	KY952522
Phytolaccaceae	*Trichostigma peruvianum* (Moq.) H.Walter	Flora K. Samis 10 (Lyon Arboretum living collection, accession 94.0377)	USA: Hawaii	KY952523
Plumbaginaceae	*Aegialitis annulata* R.Br.	Christopher T. Martine 4043 (OC)	Australia: Western Australia	KY952312
Plumbaginaceae	*Limonium limbatum* Small	Michael J. Moore *et al*. 694 (OC)	USA: New Mexico	KY952414
Plumbaginaceae	*Plumbago scandens* L.	Michael J. Moore *et al*. 1828 (OC)	Mexico: San Luis Potosi	KY952494
Polygonaceae	*Eriogonum longifolium* Nutt. var. *longifolium*	Michael J. Moore 1796 (OC)	USA: Texas	KY952404
Polygonaceae	*Eriogonum rotundifolium* Benth.	Michael J. Moore 1769 (OC)	USA: New Mexico	KY952405
Polygonaceae	*Persicaria odorata* LaLlave	Flora K. Samis 9 (Lyon Arboretum living collection, accession 88.0439)	USA: Hawaii	KY952475
Polygonaceae	*Persicaria* sp.	Michael J. Moore 1177	USA: Ohio	KY952474
Polygonaceae	*Reynoutria japonica* (Houtt.) Ronse Decr.	Michael J. Moore 2188 (OC)	USA: Ohio	KY952498
Polygonaceae	*Rumex albescens* Hillebr.	Flora K. Samis 4 (Lyon Arboretum	USA: Hawaii	KY952500

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		living collection, accession 2008-0119)		
Polygonaceae	*Rumex* sp.	Michael J. Moore 1800 (OC)	USA: Texas	KY952501
Polygonaceae	*Rumex* sp.	Michael J. Moore 1805 (OC)	USA: Texas	KY952502
Sarcobataceae	*Sarcobatus vermiculatus* (Hook.) Torr.	Michael J. Moore *et al*. 813 (OC)	USA: Utah	KY952508
Stegnospermataceae	*Stegnosperma cubense* A.Rich.	Silvia H. Salas Morales 2649 (NY)	Mexico: Oaxaca	KY952513
Talinaceae	*Talinum* cf. *aurantiacum* Engelm.	Michael J. Moore *et al*. 1985 (OC)	Mexico: Coahuila	KY952517
Talinaceae	*Talinum fruticosum* (L.) Juss.	Flora K. Samis 8 (Lyon Arboretum living collection, accession 2012.0008)	USA: Hawaii	KY952518
Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	Michael J. Moore 1789 (OC)	USA (cultivated)	KY952520
Talinaceae	*Talinum* sp.	Michael J. Moore *et al*. 1974 (MEXU)	Mexico: Coahuila	KY952519

Table 2 List of primers used to amplify the *matK* sequences newly reported here; within each primer name, the number indicates the approximate position of the primer in nucleotides downstream from the start of *matK*

Primer name	Sequence (5'→3')	Notes
matK.300F.Car	TTG CAG TCA TTG TGG AAA TTC C	Works broadly across most of Caryophyllales, but generally fails in Caryophyllaceae and Frankeniaceae
matK.1350R.Car	GCC AAA GTT CTA GCA CAA GAA	Works broadly across most of

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	AG	Caryophyllales
matK.210F.Car	TTC GGC TAA TGA TTC TCA CCA A	Designed specifically for Caryophyllaceae
matK.1345R.Car	GAG CCA AAG TTC TAG CAC AAG AA	Designed specifically for Caryophyllaceae
matK.1355R.Car	TGT GTT TAC GAG CTA AAG TTC TAG	Designed specifically for Caryophyllaceae
matK.300F.Fra	TCG CTG TCT TTG CTG AAA TTC C	Designed specifically for Frankeniaceae

Table 3 Summary of whole genome duplication (WGD) events at identified clades with distance to diversification shift in the maximum likelihood (ML) and bootstrap (BS) tree sets and climate occupancy information

No.	**Putative WGD**	**Distance to diversification shift in nodes ML(BS)**	**Subtending species (sister)**	**Mean annual temp °C (sister)**	**Mean annual precip mm (sister)**
1	Tribe Nyctagineae within the Nyctaginaceae	0 (0)	123 (40)	17.49 (20.08)	482.9 (997.08)
2	Phytolaccoid clade	6 (6)	182 (407)	19.64 (18.36)	1007.58 (452.47)
3	*Claytonia*	NA	38 (15)	5.28 (7.25)	790.5 (970.36)
4	Portulacineae	1 (1)	1600 (38)	16.19 (19.35)	699.87 (736.42)
5	*Amaranthus*	0 (0)	28 (1)	16.27 (27.09)	797.74 (117.63)
6	Tribe Gomphrenoideae within Amaranthaceae	7 (7)	172 (41)	17.91 (16.65)	871.95 (1289.5)

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7	in Caryophyllaceae (Alsinoideae + Caryophylloideae sensu Greenberg and Donoghue 2011)	9 (9)	793 (13)	11.44 (12.06)	761.43 (720.00)
8	Polygonaceae	13 (13)	670 (70)	16.3. (16.89)	1084.17 (794.28)
9	Plumbaginaceae	na	70 (670)	16.89 (16.3)	794.28 (1084.17)
10	Droseraceae	8 (na)	67 (108)	16.3 (19.08)	1280.57 (1491.72)
11	Nepenthaceae	4 (na)	89 (19)	22.52 (20.05)	2170.5 (1611.63)
12	Ancistrocladaceae	0 (na)	15 (3)	24.17 (25.6)	1899.13 (2882.4)
13	Tamaricaceae	na	19 (3)	14.09 (16.21)	568.32 (469.61)

Numbers correspond to those in Figs 1 and 2. Entries with 'na' suggest no significant shift near node.

Table 4 Summary of diversification shifts with rough correspondence to included taxa

No.	**Family**	**Diversification shift**	**Mean shift (ML)**	**Mean shift (BS)**
a	Cactaceae	*Echinops*	1.7957	2.2008
b	Cactaceae	within *Gymnocalycium*	6.9152	
c	Cactaceae	*Gymnocalycium*	-0.001	0.0555
d	Cactaceae	*Hylocereus*+*Selenicereus*	0.1175	
e	Cactaceae	*Rhipsalis*+*Schlumbergera*+*Echinocereus*+relati	0.051	

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		ves	4	
f	Cactaceae	*Stenocactus*	-0.057	-0.019
g	Anacampserotaceae	*Anacampseros*	0.2624	
h	Portulacaceae	*Portulaca*	0.0427	0.0447
i	Montiaceae	*Montiopsis*	0.9418	
j	Montiaceae	Montiaceae	0.0325	
k	Aizoaceae	*Drosanthemum*+*Delosperma*+*Hereroa*+relatives	0.1469	
l	Nyctaginaceae	*Boerhavia*		0.0747
m	Nyctaginaceae	*Commicarpus*	0.9642	
n	Nyctaginaceae	Tribe Nyctagineae	0.0484	0.0485
o	Nyctaginaceae	*Abronia*+*Tripterocalyx*		-0.084
p	Nyctag.+Aizo+Cact.+relatives	Nyctag.+Aizo+Cact.+relatives	0.0168	0.019
r	Amaranthaceae	*Salicornia*	0.2732	0.1649
s	Amaranthaceae	*Suaeda* clade 1	0.1027	
t	Amaranthaceae	*Suaeda* clade 2	-0.036	-0.028
u	Amaranthaceae	*Atriplex*	0.0384	
v	Amaranthaceae	*Corispermum*	0.1186	
w	Amaranthaceae	*Froelichia*+*Gomphrena*+relatives	0.0217	0.0132
x	Amaranthaceae	*Amaranthus*	0.335	0.204

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				9
y	Caryophyllaceae	*Dianthus*	0.0662	0.0409
z	Caryophyllaceae	*Cerastium*	0.7137	
aa	Caryophyllaceae	within *Arenaria*	0.4606	0.425
bb	Caryophyllaceae	within *Moehringia*	1.0971	0.995
cc	Caryophyllaceae	*Schiedea*	0.2339	0.2767
dd	Polygonaceae	within *Fagopyrum*	-0.04	-0.034
ee	Polygonaceae	*Eriogonum*+relatives	0.0432	0.0364
ff	Nepenthaceae	within *Nepenthes*	0.042	
gg	Ancistrocladaceae	*Ancistrocladus*	0.1426	
hh	Droseraceae	within *Drosera 1*	0.2237	0.2076
ii	Droseraceae	within *Drosera 2*		0.1622

Letters correspond to those in Figs 1 and 2.

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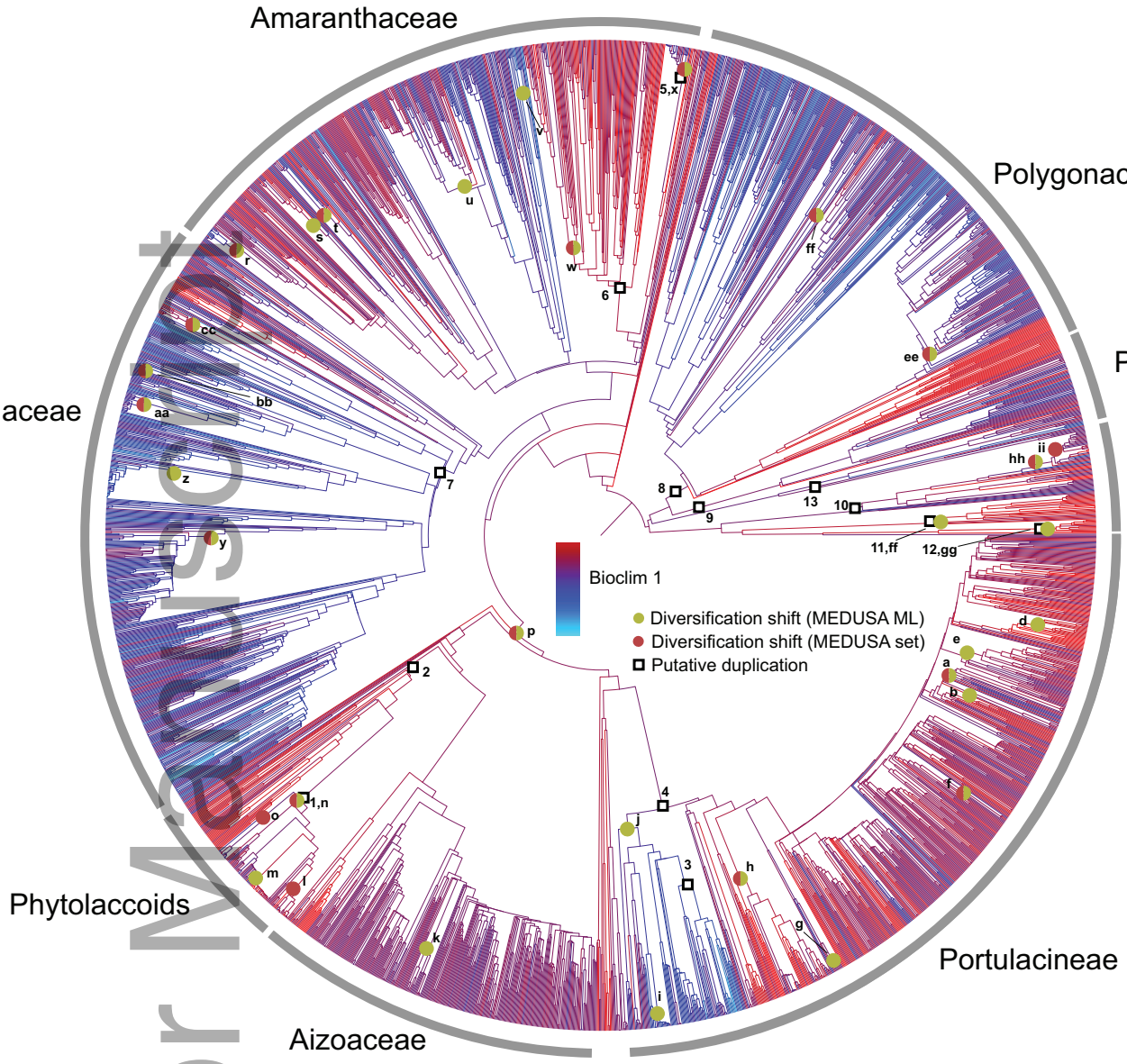

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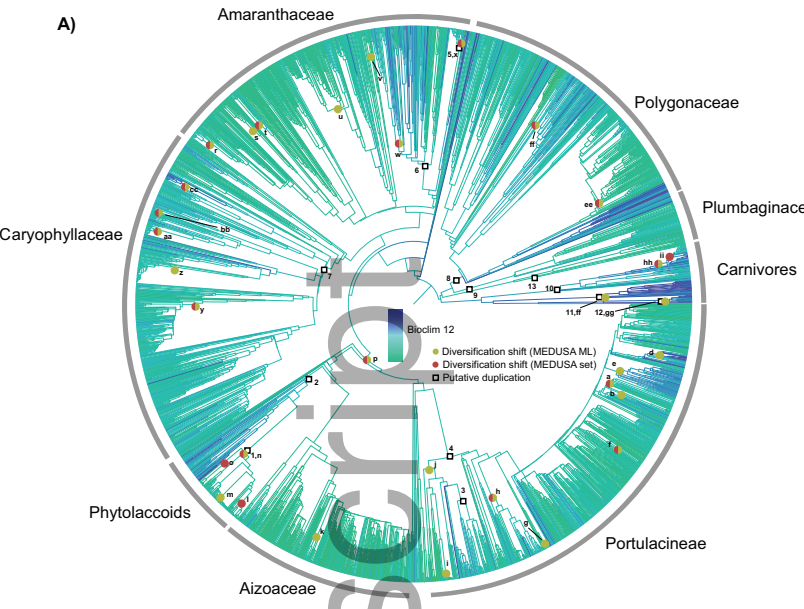

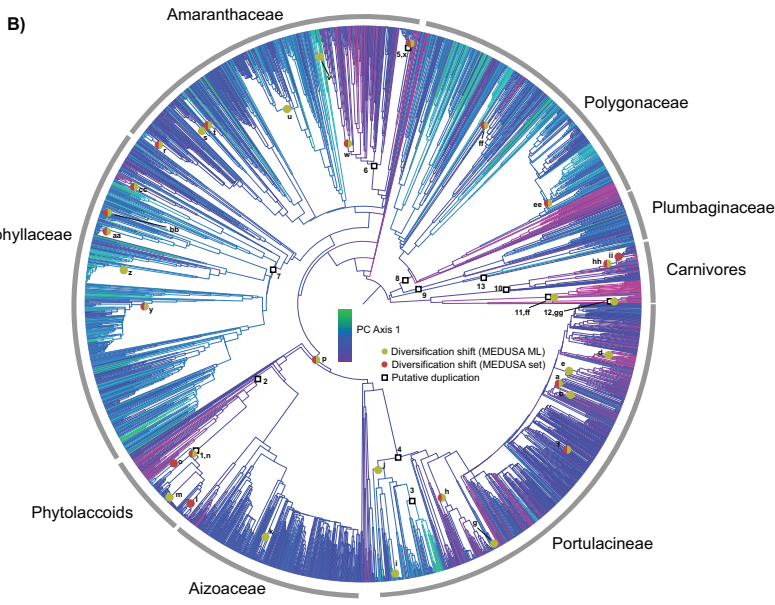

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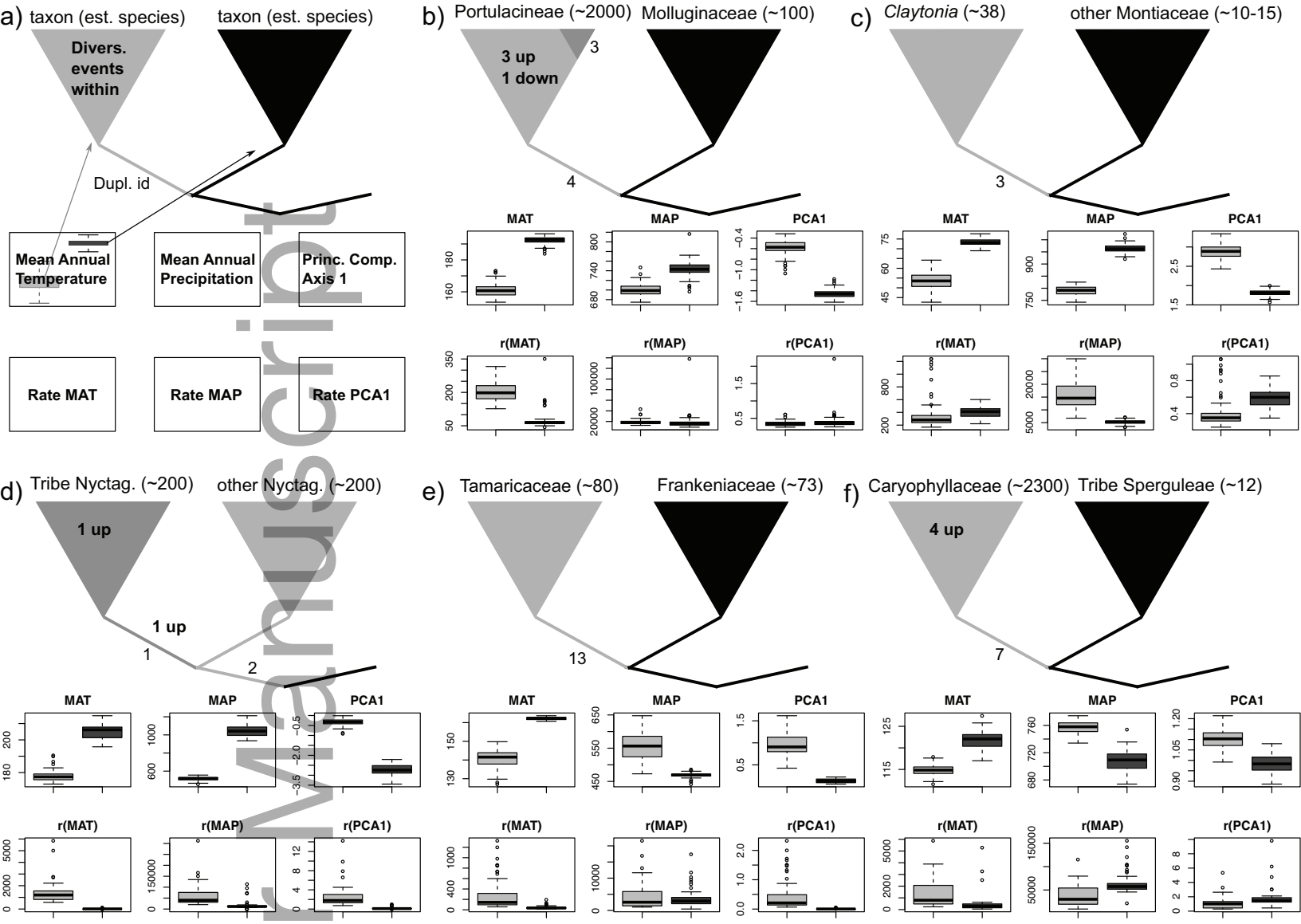

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