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Beyond aridification: multiple explanations for the elevated diversification of cacti in the New World Succulent Biome

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Summary

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- Succulent plants are widely distributed, reaching their highest diversity in arid and semi-arid regions. Their origin and diversification is thought to be associated with a global expansion of aridity. We test this hypothesis by investigating the tempo and pattern of Cactaceae diversification. Our results contribute to the understanding of the evolution of New World Succulent Biomes.
- We use the most taxonomically complete dataset currently available for Cactaceae. We estimate divergence times and utilize Bayesian and maximum likelihood methods that account for nonrandom taxonomic sampling, possible extinction scenarios and phylogenetic uncertainty to analyze diversification rates, and evolution of growth form and pollination syndrome.
- Cactaceae originated shortly after the Eocene–Oligocene global drop in CO₂, and radiation of its richest genera coincided with the expansion of aridity in North America during the late Miocene. A significant correlation between growth form and pollination syndrome was found, as well as a clear state dependence between diversification rate, and pollination and growth-form evolution.
- This study suggests a complex picture underlying the diversification of Cactaceae. It not only responded to the availability of new niches resulting from aridification, but also to the correlated evolution of novel growth forms and reproductive strategies.

Introduction

Arid and semi-arid environments currently comprise the most extensively distributed terrestrial biomes on Earth (McGinnies, 1979; Peel *et al.*, 2007). Under these climatic conditions, drought-stress represents a strong selective pressure on lineages to evolve morphological, physiological and ecological characteristics that allow them to successfully survive and reproduce (Axelrod, 1972; Gibson, 1996). Succulent plants reach their highest diversity under these climatic regimes, as conspicuous elements of a worldwide distributed biome of tropical dry forest, bushland and thicket vegetation poor in grasses and lacking tolerance to fire disturbance – the succulent biome (Schrire *et al.*, 2005). Examples of the succulent biome are the Succulent Karoo in southern Africa, the spiny thicket in Madagascar and the Caatinga in Brazil (Lavin *et al.*, 2004). Although xeric-adapted plants have evolved a wide diversity of strategies to respond to limited water availability, succulent plants most clearly display the relationship between their morphological

traits and climatic conditions. It is perhaps for this reason that species-rich succulent lineages (i.e. lineages within families Cactaceae, Aizoaceae and Agavaceae) have been suggested to originate and radiate in response to a global aridification trend during the late Miocene/Pliocene leading to present-day arid and semi-arid climates (Arakaki *et al.*, 2011; and references therein). Nevertheless, large succulent radiations are not restricted to arid environments. Possibly the richest lineage of succulents is the epiphytic Epidendroideae (Orchidaceae), a group most typical of humid, tropical or temperate conditions (Silvera *et al.*, 2009; Nyffeler & Egli, 2010). In addition, not all succulent lineages are species rich or morphologically diverse, as exemplified by Halophytaceae (1 species), Moringaceae (13 species) and Fouquieriaceae (11 species; Nyffeler & Egli, 2010). Hence, succulent plant diversification may not only respond to aridification.

Different biomes most likely originated in response to aridification trends during the Neogene, for example, the Australian arid zone biota (Byrne *et al.*, 2008), the California floristic

province (Calsbeek *et al.*, 2003) and the Succulent Karoo (Verboom *et al.*, 2009), given that the origin and expansion of arid conditions can constitute a source of novel ecological niches. Yet, few studies have provided detail on the possible ecological mechanisms driving the diversification of species-rich lineages inhabiting these environments, specifically proposing mechanistic explanations for the relation between aridification and an increase in species diversity, or providing tests for alternative explanations (but see Ellis & Weis, 2006; Ellis *et al.*, 2006; Schnitzler *et al.*, 2012). Aridity by itself poorly explains the dramatic increase in species numbers and the morphological and ecological diversity in certain succulent lineages, such as subfamily Ruschioideae (Aizoaceae; Klak *et al.*, 2004), the genus *Euphorbia* (Euphorbiaceae; Bruyns *et al.*, 2011; Horn *et al.*, 2012) or the cactus family (Hernández-Hernández *et al.*, 2011), where additional biotic and abiotic factors may have played important roles. For instance, studies of certain genera within Ruschioideae suggested that diversification occurred primarily via local adaptations to edaphic microhabitats (Ellis & Weis, 2006; Kellner *et al.*, 2011). Additionally, Good-Avila *et al.* (2006) suggested that the evolution of monocarpy and bat pollination drove a second burst of diversification in *Agave* (Agavaceae), leading to its present diversity.

The importance of the evolution of particular pollinating systems in the radiation of lineages with increased diversification rates has been stressed (Johnson, 2010), and it may be also relevant for the diversification of several lineages within Cactaceae (Mandujano *et al.*, 2010; Schlumpberger, 2012). The overlapping geographic distribution of *Agave* and columnar Pachycereae (Cactaceae) may indicate a common pattern of diversification in response to the evolution of similar pollination mechanisms (Valiente-Banuet *et al.*, 1996; Molina-Freaner & Eguiarte, 2003). General floral evolutionary trends in Cactaceae involve shifts from a plesiomorphic bee pollination to other pollination syndromes (Reyes-Agüero *et al.*, 2006; Mandujano *et al.*, 2010; Schlumpberger, 2012; and references therein). In late-diverging members of Cactaceae, the floral pericarpel forms a hypanthium, promoting the appearance of a large diversity of bird-pollinated flowers and allowing adaptation to a wider array of pollinator guilds, such as sphingids and bats (Schlumpberger, 2012, p. 304). Members of subfamily Cactoideae also exhibit the widest range of growth forms, from barrels and small globoses to massive columnars or epiphytic shrubs (Hernández-Hernández *et al.*, 2011). The combination of particular growth forms and pollination guilds might confer an advantage for survival, reproduction and geographical expansion.

Although cacti are present in most biomes throughout the New World, there are geographical hotspots that host numerous species, and some of the most species-rich clades occupy distinct geographical areas. For example, tribe Cacteae is mainly distributed in the southern Chihuahuan Desert (Hernández & Gómez-Hinostrosa, 2005); Hyllocereae in southern Mexico and Central America; Rhipsalideae in southeastern Brazil (Calvente *et al.*, 2011); core Pachycereae in the Sonoran Desert; and Trichocereae in South America (Nyffeler, 2002; Hernández-Hernández *et al.*, 2011). A biogeographic analysis of Cactaceae using a well-sampled phylogeny at a continental level can help to

elucidate whether there is geographic structure in their evolutionary history, indicating if particular biogeographic histories played a substantial role in the diversification of certain lineages.

In this study, we use Cactaceae as a system to investigate the diversification of plant lineages in arid and semi-arid regions of North and South America by testing specific hypotheses about the mechanisms promoting speciation. Insights about the origin and diversification of Cactaceae can also provide clues regarding the origin and expansion of arid biomes in the New World. Our aim is to evaluate if diversification within the family proceeded mostly as synchronous bursts – potentially reflecting a global colonization trend of arid habitats – or if they occurred at different times – for example, if there is a time lag between origination and rapid radiation in different clades, or a lag between aridification and further colonization (Guerrero *et al.*, 2013). Arakaki *et al.* (2011) concluded that major cactus radiations were contemporaneous with radiations in core Ruschioideae in South Africa and agaves in North America, suggesting an association with the global expansion of arid and semi-arid environments. In this study, we investigate the diversification of Cactaceae in greater detail by conducting independent analyses using a phylogeny with a considerably improved taxonomic sampling, and evaluating the potential relevance of growth form, pollination syndrome and biogeographic history in the distribution of species richness within the family. Our results contribute to better understanding the evolutionary history and complex diversification mechanisms of cactus lineages, and provide specific hypotheses for further studies of the possible ecological mechanisms leading to increases in diversification rates in species-rich lineages in New World arid environments.

Materials and Methods

Phylogenetic analysis and divergence time estimation

We used the most taxonomically complete chloroplast dataset currently available for Cactaceae at the genus level, which includes a taxonomic sample of 224 species belonging to 108 genera, representing *c.* 85% of the generic diversity in the family (Hernández-Hernández *et al.*, 2011). It comprises concatenated DNA sequences data from four plastid molecular markers: *rpl16* intronic region, *trnL-trnF* and *trnK-matK* intergenic spacers, and the protein coding *matK*, for a total of 5590 base pairs, which were recently used to elucidate phylogenetic relationships in the family (Hernández-Hernández *et al.*, 2011). Four additional species of Anacampserotaceae, the closest relatives of Cactaceae (Nyffeler, 2007), were included as outgroups. See Supporting Information Table S1 for the list of species, as well as accession and voucher information.

Sequences of each region were aligned automatically using MUSCLE (Edgar, 2004), followed by manual refinement using BioEdit v5.0.6 (Hall, 1999; dataset available in TreeBase, Study ID 11087). To corroborate phylogenetic relationships reported previously (Hernández-Hernández *et al.*, 2011) and evaluate clade support, maximum likelihood (ML) phylogenetic analyses were conducted with RAxML v7.0.4 (Stamatakis, 2006). Each of

the four plastid regions were assigned independent general time reversible (GTR) substitution models with 25 rate categories to model gamma-distributed site-specific rate heterogeneity. Clade support was assessed with 1000 replicates of a nonparametric bootstrap analysis, also conducted with RAxML.

Because fossils that could provide reliable absolute age calibrations near Cactaceae are not available, we implemented a two-step approach to estimate divergence times. First, the age of Cactaceae was estimated based on a representative dataset of eudicots (Eudicotyledoneae) and outgroups. Second, the credibility interval around the estimated age of Cactaceae obtained in the first step was used to calibrate the phylogenetic tree of Cactaceae described earlier. In the first step we used a dataset of 109 species belonging to 34 eudicot orders (including 21 families of Caryophyllales), and representatives of two eudicot outgroups: Ceratophyllales and Monocotyledoneae. We assembled a dataset with nucleotide sequences of the plastid protein-coding genes *atpB*, *rbcl* and *matK*, which were downloaded from GenBank, for a total of 4510 bp (Table S2). Divergence times were estimated with the uncorrelated lognormal (UCLN) relaxed clock available in BEAST v1.6.1 (Drummond & Rambaut, 2007; Methods S1). Twenty three internal nodes were calibrated with critically evaluated fossil-derived ages (Table S3), implemented as lognormal priors in which the mean was equal to fossil age + 10%, and the zero offset was equal to fossil age – 5 million yr (age given in units Million years ago, Ma). Divergence dates within Cactaceae were obtained with the plastid dataset used for phylogenetic estimation (see earlier), using the UCLN relaxed clock in BEAST, with equivalent conditions as described above. The prior age of the root node (stem group Cactaceae) was given a uniform distribution between 22.71 and 42.43 Ma, corresponding to the 95% highest posterior density (HPD) of the age of this node obtained in the eudicot-level analysis (Methods S1).

Diversification rates

The absolute rates of diversification of strongly supported clades within Cactaceae were calculated using methods described in Magallón & Sanderson (2001). These estimators consider the standing species richness and the stem or crown age of a clade in the context of different extinction scenarios, providing absolute estimates of clade net diversification ($r = \text{speciation } (\lambda) - \text{extinction } (\mu)$) conditional on its survival to a given time t (the present). Because absolute extinction rates for clades are unknown, diversification rates were alternatively estimated assuming no extinction ($\varepsilon = 0.0$), and a high relative extinction rate ($\varepsilon = \mu/\lambda = 0.9$).

Assignment of species richness to clades within Cactaceae was not straightforward due to conflicts between classic taxonomic treatments and recent molecular-based phylogenies. We estimated the number of living species in well-supported major clades recovered in our Cactaceae phylogenetic tree and consistently reported in the literature with high support values (i.e. Gibson & Horak, 1978; Gibson, 1982; Wallace, 1995, 2002; Wallace & Cota, 1996; Porter, 1999; Porter *et al.*, 2000; Butterworth *et al.*, 2002; Nyffeler, 2002; Wallace & Dickie, 2002; Arias *et al.*, 2003, 2005; Butterworth & Wallace, 2004,

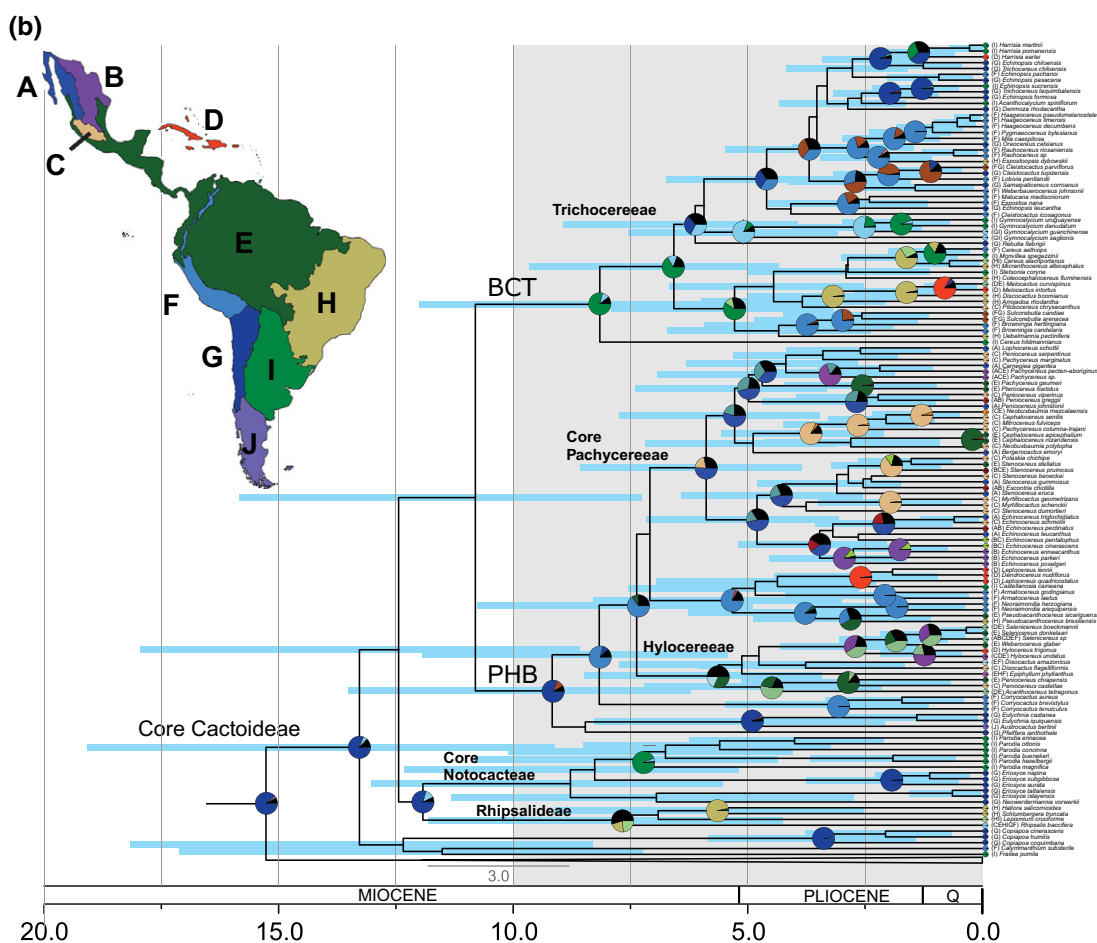
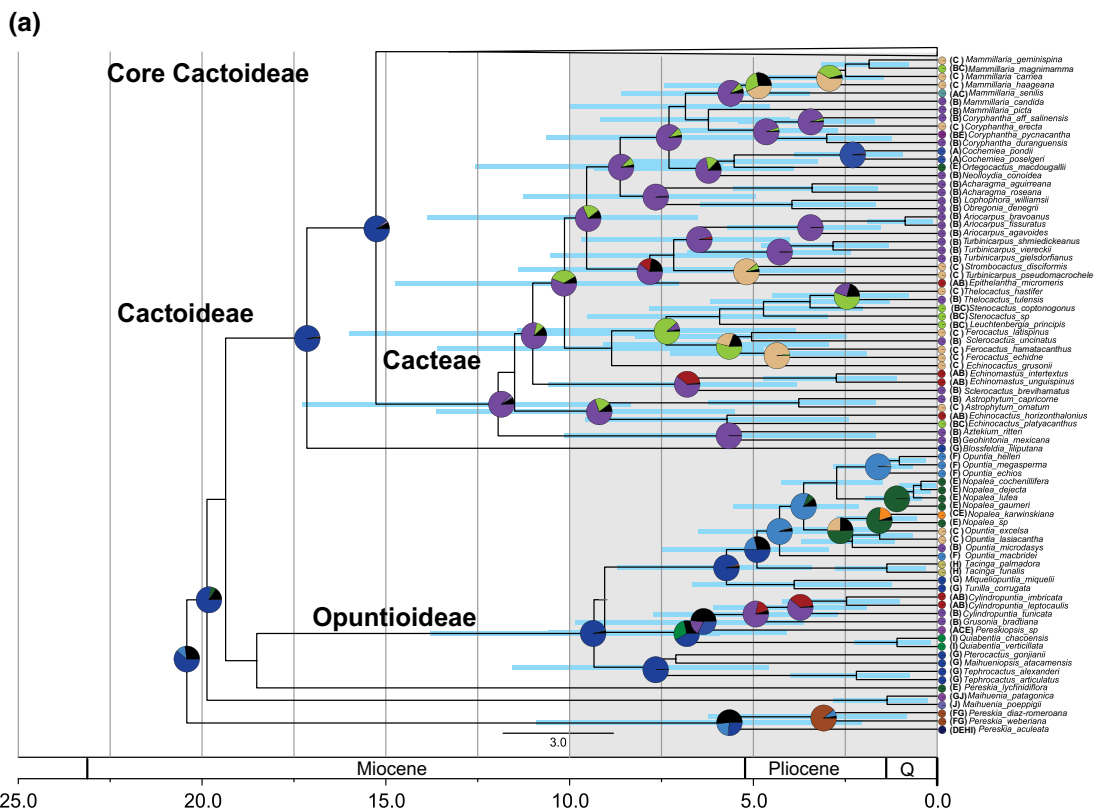
2005; Edwards *et al.*, 2005; Ritz *et al.*, 2007; Griffith & Porter, 2009; Bárcenas *et al.*, 2011; Calvente *et al.*, 2011; Hernández-Hernández *et al.*, 2011; Majure *et al.*, 2012; Schlumpberger & Renner, 2012), based on the species richness of included genera (Hunt *et al.*, 2006; Table S4).

In order to analyze cactus diversification in a phylogenetic and temporal context we implemented MEDUSA (Alfaro *et al.*, 2009), an extension of the birth–death likelihood model (Rabosky, 2006) that allows clade-specific birth–death models, and can detect regions in a time-calibrated phylogeny where diversification rate shifts likely occurred. MEDUSA allows performing diversification analyses in phylogenies lacking a complete taxonomic sampling, as it incorporates a taxonomic likelihood for unresolved terminal clades. We pruned our original 224 taxon phylogeny to a genus-level tree, and assigned diversity to each terminal. To account for phylogenetic uncertainty, we report mean diversification rates and the most frequent rate shifts detected within Cactaceae after running MEDUSA on each of 1000 trees randomly selected from the BEAST posterior distribution.

Biogeographic analyses

In order to maximize the congruence with other studies, we followed the biogeographic scheme of Posadas *et al.* (1997) and Morrone (2001, 2002, 2006) to define operational areas. However, we slightly modified these schemes by observing the geographic ranges of cacti species, and considering regions with high species richness and endemism (Ortega-Baes & Godínez-Alvarez, 2006). We designated the following areas (Fig. 1): (A) Sonoran Desert and Sierra Madre Occidental; (B) Chihuahuan Desert; (C) Central Mexico; (D) the Antilles; (E) Central American and South American Tropical areas; (F) the Andean region of Peru; (G) Andean region of Chile and Argentina; (H) the Caatinga; (I) the Chaco area; and (J) Northern Subantarctic region. For details see Methods S2.

We reconstructed ancestral geographic ranges by utilizing parsimony, ML and Bayesian methods. For the ML framework we used the Dispersal-Extinction-Cladogenesis geography-based method (DEC; Ree, 2005; Ree & Smith, 2008) implemented in LAGRANGE, using the maximum clade credibility (MCC) tree obtained in the BEAST analyses. We used a uniform dispersal matrix to avoid overparameterization. For the parsimony and Bayesian frameworks we (respectively) performed S-DIVA and BBM (Bayesian Binary MCMC) analyses implemented in RASP v2.0b (Yu *et al.*, 2010, 2011; Ali *et al.*, 2012). These methods accommodate phylogenetic uncertainty by averaging the ancestral reconstructions over a sample of user-supplied trees, in this case, the 1000 randomly selected trees obtained with BEAST (see earlier). The ancestral ranges estimated at each node on the MCC chronogram were obtained. The number of maximum unit areas allowed for nodes was set to six. In the BBM analyses we set a null distribution for the ancestral range of the root of the tree, and we ran ten MCMC chains simultaneously for 5×10^4 generations, sampling a state every 100 steps, and discarding the first 100 samples as burnin.



Ancestral states, character coevolution, and diversification correlates

We estimated ancestral states of growth form and pollination syndrome under ML and Bayesian frameworks using BayesMultiState implemented in BayesTraits v1.0 (Pagel & Meade, 2006). For ML analyses, we used the BEAST MCC tree, while for the Bayesian analyses we implemented a series of reversible-jump hyperprior (RJHP) MCMC analyses (Pagel & Meade, 2006) on the 1000 randomly selected BEAST trees. The RJHP approach approximates the posterior distribution of ancestral character states while accounting for phylogenetic uncertainty. We ran three independent chains of 1×10^8 generations with a conservative initial burnin of 25% generations. We used Tracer v1.5 (Rambaut & Drummond, 2007) to check for chain convergence and ESS values.

Growth-form character states analyzed by Hernández-Hernández *et al.* (2011) were here re-scored as binary characters. We assigned (0) to species with a globose solitary, globose caespitose or barrel growth form; and (1) to species with an arborescent, shrubby or columnar growth form. Species within subfamilies Opuntioideae, Maihuenioideae and Pereskioideae lack a growth form comparable to those of members of subfamily Cactoideae; hence, their growth form was scored as nonapplicable. The most widespread, and possibly ancestral, pollination syndrome of Cactaceae is mellitophily or bee pollination (Mandujano *et al.*, 2010; Schlumpberger, 2012), with chiropterophily (bats), ornithophily (birds) and sphingophily (moths) pollination syndromes appearing in derived clades. We thus assigned a pollination guild to species within our phylogeny by reviewing the literature and by examining floral characters in specimens and photographs. We then coded these as binary characters, by assigning (0) to mellitophilic species and (1) to species possessing derived pollination syndromes (chiropterophily, ornithophily and sphingophily).

We conducted a character correlation test for growth form and pollination syndrome using the Discrete module in BayesTraits v1.0 (Pagel, 1994, 1997; Pagel & Meade, 2006), to test for a possible correlation between the appearance of derived pollination syndromes and of a columnar, arborescent or shrubby growth form. Likelihood scores of competing models were obtained with BayesDiscrete (Pagel & Meade, 2006) on the BEAST MCC tree, and their relative fit was compared using a likelihood ratio test (LRT). We also evaluated correlation in a Bayesian context, calculating Bayes factors as the difference between the harmonic mean of marginal log-likelihood ($\log_e L$) scores from MCMC runs for the competing models (Newton & Raftery, 1994). For these, we ran the correlated

and independent models for 1×10^8 generations each over the 1000 BEAST trees, with a conservative initial burnin of 25% generations. These analyses also implemented a RJHP under the same parameters as in the ancestral character reconstruction analyses. A Bayes factor of 5 or greater can be considered as strong evidence for correlated evolution, whereas a Bayes factor smaller than zero supports the independent model (Kass & Raftery, 1995; Pagel & Meade, 2006).

In order to test if diversification rates (as well as speciation and extinction separately) are correlated with character states of growth form and pollination syndrome, we used BiSSE (Binary State Speciation and Extinction) in Diversitree v0.9-1 (Maddison *et al.*, 2007) in a ML framework. This method explicitly incorporates character state change directly into the likelihood assessment of speciation and extinction rates (Maddison *et al.*, 2007). BiSSE includes six state-specific parameters (for states 0 and 1): two speciation rates (λ_0 and λ_1), two extinction rates (μ_0 and μ_1), and two rates of character state change (q_{01} from state 0 to 1, and q_{10} from state 1 to 0). To test hypotheses we compared the AIC scores obtained implementing six different models on the BEAST MCC tree. If diversification rates are correlated with character states, unconstrained models should be favored over the constrained model where parameters λ and μ are set to be equal.

Results

Phylogeny, divergence dates and ancestral areas

The estimated phylogeny of Cactaceae is shown in Fig. S1. The ML analysis resulted in a tree with strong support for major Cactaceae clades, congruent with previous studies (e.g. Nyffeler, 2002; Bárcenas *et al.*, 2011; Hernández-Hernández *et al.*, 2011). Only strongly supported monophyletic lineages were further considered in diversification analyses. The inferred phylogenetic relationships among eudicot orders also conform to relationships found in independent studies (e.g. Wang *et al.*, 2009; Soltis *et al.*, 2011; see Fig. S2).

Cactaceae is estimated to have split from its sister group (i.e. the stem group age) at 32.11 Myr ago (Ma), with a credibility interval (95% highest posterior density (HPD)) spanning between 42.43 and 22.71 Ma. The age of the onset of branching leading to living lineages (i.e. the crown group age) was estimated at 26.88 Ma (37.1–16.67 Ma 95% HPD). The eudicot-level chronogram is shown in Fig. S2 and the Cactaceae chronogram is shown in Fig. 1. The ages of major, well-supported Cactaceae clades are shown in Table 1.

Fig. 1 Cactaceae chronogram resulting for the dating analyses in BEAST and results of the biogeographic analyses with BMM in RASP. Pie charts show the probability values of the ancestral areas reconstructed at each node. (a) Chronogram edited to show the *Pereskia*, *Maihuenia*, Opuntioideae and Cactaceae clades. (b) Chronogram edited to show the Core Cactoideae clade and sub-clades. The map at the top-left shows the classification of Cactaceae for biogeographic analyses: (A) Sonoran Desert and Sierra Madre Occidental; (B) Chihuahuan Desert; (C) Central Mexico; (D) the Antilles; (E) Central American and South American Tropical areas; (F) Northern Andes; (G) Andean region of Chile and Argentina; (H) the Caatinga, including the Cerrado; (I) the Chaco area, including the Chaco, Pampa and Monte; and (J) Northern Subantarctic region.

Table 1 Estimated ages and diversification rates of clades supported by high bootstrap values

	% bs	# spp.	Stem group age (Ma)	Crown group age (Ma)	Eqn 6, stem Epsilon = 0	Eqn 6 stem Epsilon = 0.9	Eqn 7 crown Epsilon = 0	Eqn 7 crown Epsilon = 0.9
Angiosperms		269 323	350 (350–350)	241.71 (241.46–241.95)	0.036	0.029	0.049	0.042
Caryophyllales ¹	52	11 155	111.93 (107.55–116.35)	102.68 (96.90–108.73)	0.083	0.063	0.084	0.068
Caryophyllales ²	99	11 152	102.68 (96.90–108.73)	100.76 (94.71–106.93)	0.091	0.068	0.086	0.069
Cactaceae	95	1405	32.11 (22.71–42.43)	26.88 (16.67–37.10)	0.226	0.154	0.244	0.182
<i>Pereskia</i> ³	100	9	20.41 (16.68–28.46)	5.67 (2.05–10.89)	0.108	0.029	0.265	0.094
<i>Maihuenia</i>	100	2	19.86 (15.34–28.25)	1.36 (0.26–2.83)	0.035	0.005	0.00	0.00
Opuntioideae	100	186	18.51 (13.33–26.54)	9.34 (5.93–13.8)	0.282	0.160	0.485	0.313
Cylindropuntieae ⁴	100	56	9.05 (NA)	6.81 (4.09–10.58)	0.445	0.207	0.489	0.267
MTAC Clade ⁵	76	35	9.33 (5.93–13.8)	7.66 (4.58–11.55)	0.381	0.159	0.374	0.187
Opuntieae ⁶	100	95	9.04 (NA)	5.73 (3.42–8.7)	0.504	0.259	0.674	0.4
<i>Opuntia</i> + <i>Nopalea</i>	87	75 + 4	5.73 (3.42–8.7)	4.9 (2.95–7.48)	0.763	0.38	0.750	0.433
Cactoideae ⁷	99	1298	19.36 (14.75–27.54)	17.15 (12.67–24.46)	0.37	0.252	0.378	0.281
Cactoideae ⁸	100	1297	17.15 (12.67–24.46)	15.27 (10.94–21.85)	0.418	0.284	0.424	0.316
Cacteeae	100	356	15.27 (10.94–21.85)	11.94 (8.33–17.27)	0.385	0.236	0.434	0.297
<i>Aztekium</i> clade	89	3	11.94 (8.33–17.27)	5.67 (1.67–10.16)	0.208	0.062	0.316	0.121
<i>Echinocactus</i> and <i>Astrophytum</i>	97	12	11.5 (NA)	9.22 (5.50–13.64)	0.096	0.016	0.044	0.013
<i>Sclerocactus</i> clade ⁹	100	20	11 (7.74–16)	6.8 (3.84–10.6)	0.272	0.097	0.339	0.149
<i>Ferocactus</i> clade	45	51	10.14 (7.03–14.73)	8.86 (4.72–13.62)	0.388	0.177	0.366	0.196
<i>Ariocarpus</i> clade	92	33	9.54 (6.53–13.89)	7.82 (4.86–11.4)	0.367	0.150	0.358	0.177
Mammilloid clade	97	237	9.54 (6.53–13.89)	8.62 (5.83–12.56)	0.573	0.336	0.554	0.366
Core Mammilloid ¹⁰	100	210	8.62 (5.83–12.56)	7.3 (4.86–10.63)	0.620	0.358	0.638	0.416
Core Cactoideae	100	942	15.27 (10.94–21.85)	13.28 (9.12–19.08)	0.448	0.298	0.463	0.339
<i>Copiapoa</i>	100	21	12.34 (8.3–18.15)	3.38 (1.40–5.84)	0.247	0.089	0.696	0.309
<i>Eulychnia</i> + <i>Austrocactus</i>	100	7	9.17 (6.21–13.51)	4.90 (2.09–8.27)	0.212	0.051	0.256	0.085
<i>Corryocactus</i>	99	12	8.16 (5.42–11.93)	3.06 (1.13–5.48)	0.305	0.091	0.586	0.225
PHB clade	50	230	8.16 (5.42–11.93)	7.37 (4.89–10.76)	0.666	0.389	0.644	0.424
Core Pachycereae ¹¹	99	138	7.09 (NA)	5.89 (3.85–8.57)	0.695	0.379	0.719	0.448
Pachycereinae	95	39	5.89 (3.85–8.57)	5.28 (3.47–7.74)	0.622	0.266	0.563	0.287
Stenocereinae	100	99	5.89 (3.85–8.57)	4.8 (3.05–7.16)	0.780	0.404	0.813	0.485
<i>Echinocereus</i>	100	67	4.8 (3.05–7.16)	3.47 (2.04–5.2)	0.876	0.423	1.012	0.570
Hylocereae (s.s.)	31	57	5.62 (3.42–8.48)	5.12 (2.96–7.3)	0.719	0.336	0.654	0.358
Hylocereae ¹²	94	67	7.37 (4.89–10.76)	5.62 (3.42–8.48)	0.571	0.275	0.625	0.352
Rhipsalideae ¹³	100	53	11.92 (NA)	7.67 (4.26–11.82)	0.333	0.153	0.427	0.231
Core Notocacteeae	99	92	11.92 (NA)	8.78 (5.54–13.03)	0.379	0.194	0.436	0.258
BCT clade	95	177	6.57 (4.34–9.66)	5.28 (3.16–7.9)	0.788	0.445	0.849	0.544
Trichocereae	33	230	6.57 (4.34–9.66)	6.12 (3.93–8.93)	0.828	0.483	0.775	0.510
<i>Gymnocalycium</i>	51	49	6.12 (3.93–8.93)	5.08 (3.09–7.55)	0.636	0.287	0.630	0.336

Numbers in bold indicate the highest estimated diversification rates.

¹Caryophyllales Order including *Rhabdodendron*.

²Caryophyllales members after *Rhabdodendron* split.

³only Andean and southern South American *Pereskia*.

⁴cylindrical-stemmed opuntias (*Quiabentia*, *Pereskiaopsis*, *Grusonia*, *Cylindropuntia* clade).

⁵spherical-stemmed opuntias (*Maihueniopsis*, *Tephrocactus*, *Pterocactus*).

⁶flattened stemmed opuntias (*Opuntia*, *Nopalea*, *Tacinga*, including *Miqueliopuntia*+*Tunilla*).

⁷including *Blossfeldia*.

⁸after *Blossfeldia* split.

⁹includes *Echinomastus*.

¹⁰includes *Coryphantha*, *Mammillaria*, *Cochemia*, *Ortegocactus* and *Neolloydia*.

¹¹traditional Pachycereae excluding *Acanthocereus*, *Peniocereus* subgen. *Pseudoacanthocereus*, *Corryocactus*, and *Pseudoacanthocereus*.

¹²Hylocereae (s.s.) plus *Peniocereus* subgenus *Pseudoacanthocereus* and *Acanthocereus*.

¹³excluding *Pfeiffera*.

Ancestral areas estimated with among parsimony, ML and Bayesian methods were congruent (Table S5), and we selected the results from the Bayesian analyses (Fig. 1) for further discussion. The Andean region of Chile and Argentina, including the southern Andean region of Bolivia, was found to be the

most probable area of Cactaceae origin, as well as of Opuntioideae and Cactoideae (Fig. 1b, Table S5). According to our results, expansion towards unoccupied regions occurred gradually within each lineage. The ancestor of Cactaceae was inferred to occupy the Chihuahuan Desert, after a presumed long

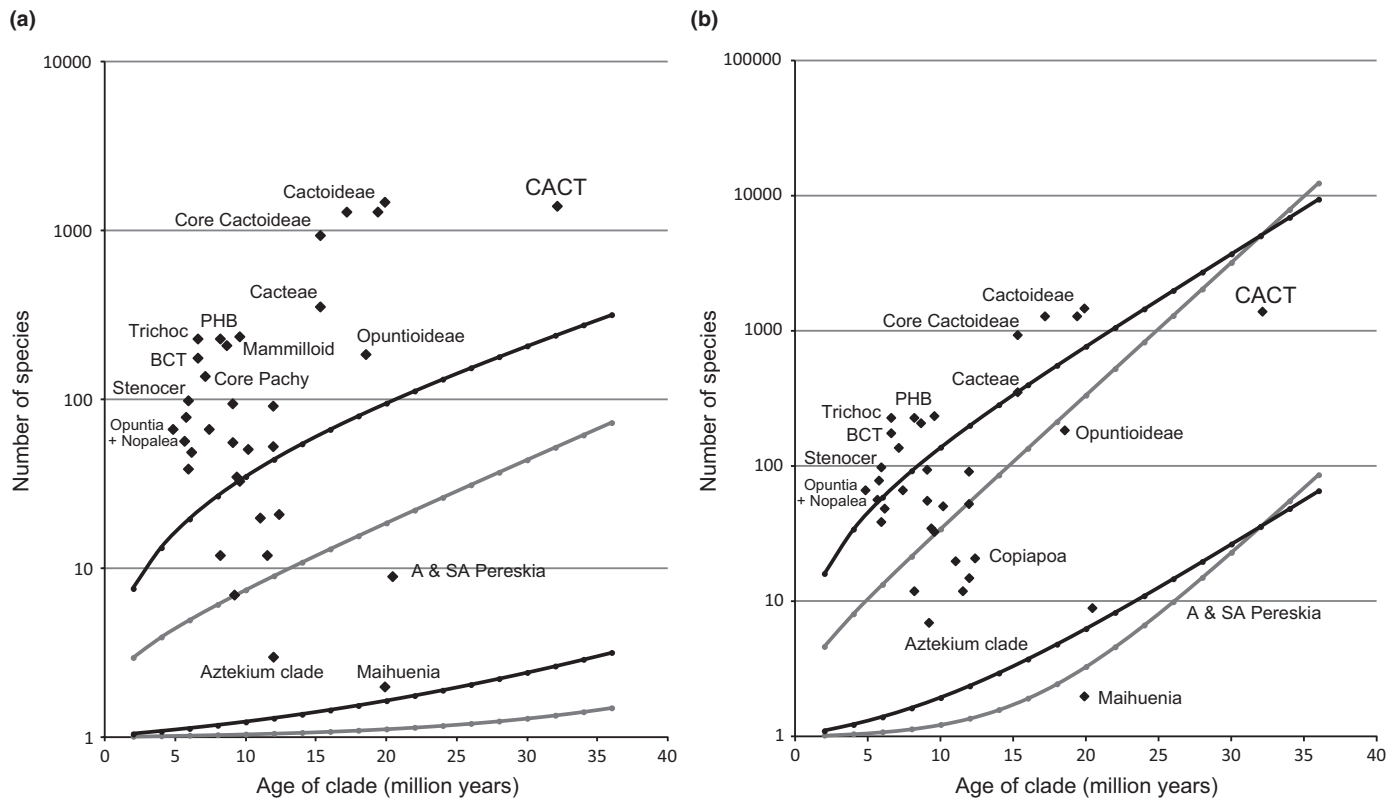


Fig. 2 Absolute diversification rates for main clades in Cactaceae and confidence intervals for expected species diversity. Rates were estimated using Eqns 6 and 7 in Magallón & Sanderson (2001). (a) The 95% confidence interval of expected species diversity through time of a clade that diversifies with a rate equal to that of Caryophyllales as a whole. (b) The 95% confidence interval of expected species diversity through time of a clade that diversifies with a rate equal to that of Cactaceae as a whole. Gray lines indicate expected species richness in the absence of extinction ($\epsilon = 0.0$), and black lines indicate expected species richness under a high relative extinction ($\epsilon = 0.9$). Cactaceae clades were plotted according to crown group age and standing species diversity. Clades that fall above the upper limit of the highest confidence interval are considered extremely species rich. Clades that fall below the lower limit of lowest confidence interval are considered extremely species poor.

dispersal from the Andean region of Chile, Argentina and Bolivia.

Diversification rates

The absolute diversification rates (r) estimated for major, well-supported Cactaceae clades, considering no extinction ($\epsilon = 0.0$) and a high relative extinction rate ($\epsilon = 0.9$), are shown in Table 1. The average diversification rates estimated for crown Cactaceae ($r_{0,0} = 0.244$ speciation events per million years (sp Myr⁻¹) for $\epsilon = 0$, and $r_{0,9} = 0.182$ sp Myr⁻¹ for $\epsilon = 0.9$) are considerably higher than those estimated for Caryophyllales ($r_{0,0} = 0.068$ and $r_{0,9} = 0.084$ sp Myr⁻¹), and for angiosperms as a whole ($r_{0,0} = 0.042$ and $r_{0,9} = 0.048$ sp Myr⁻¹; Magallón & Castillo, 2009). Lineages with the highest diversification rates are Opuntieae (particularly the *Opuntia* + *Nopalea* clade), the Mammilloid clade (particularly the core Mammilloid clade), the PHB clade (particularly the core Pachycereae, Pachycereinae, Stenocereinae and Hylocereae clades, and the genus *Echinocereus*), and the BCT clade (particularly the Trichocereae clade and the genus *Gymnocalycium*; see Table 1). These clades, together with several other Cactaceae major groups, exceed the upper 95% confidence interval of

the species diversity through time expected for a clade that diversifies with a rate equal of that of Caryophyllales or Cactaceae as a whole, and under a scenario of high extinction rate (Fig. 2).

MEDUSA detected several shifts in diversification relative to background levels (Fig. 3). The most frequently detected increases in diversification rates occur in the *Opuntia* + *Nopalea* clade, at the base of the Cactoideae subfamily, in the Mammilloid clade with high rates at the *Mammillaria* + *Coryphanta* clade, and in the BCT clades. Shifts occurring at lower frequency (at least 50% of the random trees) include the terete-stemmed *Grusonia* + *Cylindropuntia* clade and the PHB clade.

Character evolution and diversification correlates

Ancestral character state reconstructions of pollination syndrome and growth form in major Cactaceae clades are shown in Table S6, and results from the ML analyses are illustrated in Fig. 4. Those ancestral character states reconstructed with the highest probability values were consistent in both the ML and the Bayesian analyses. A mellitophilic pollination syndrome was found to be ancestral in Cactaceae ($P = 0.73/0.99$ for ML/Bayesian analyses, respectively), with shifts towards other pollination syndromes

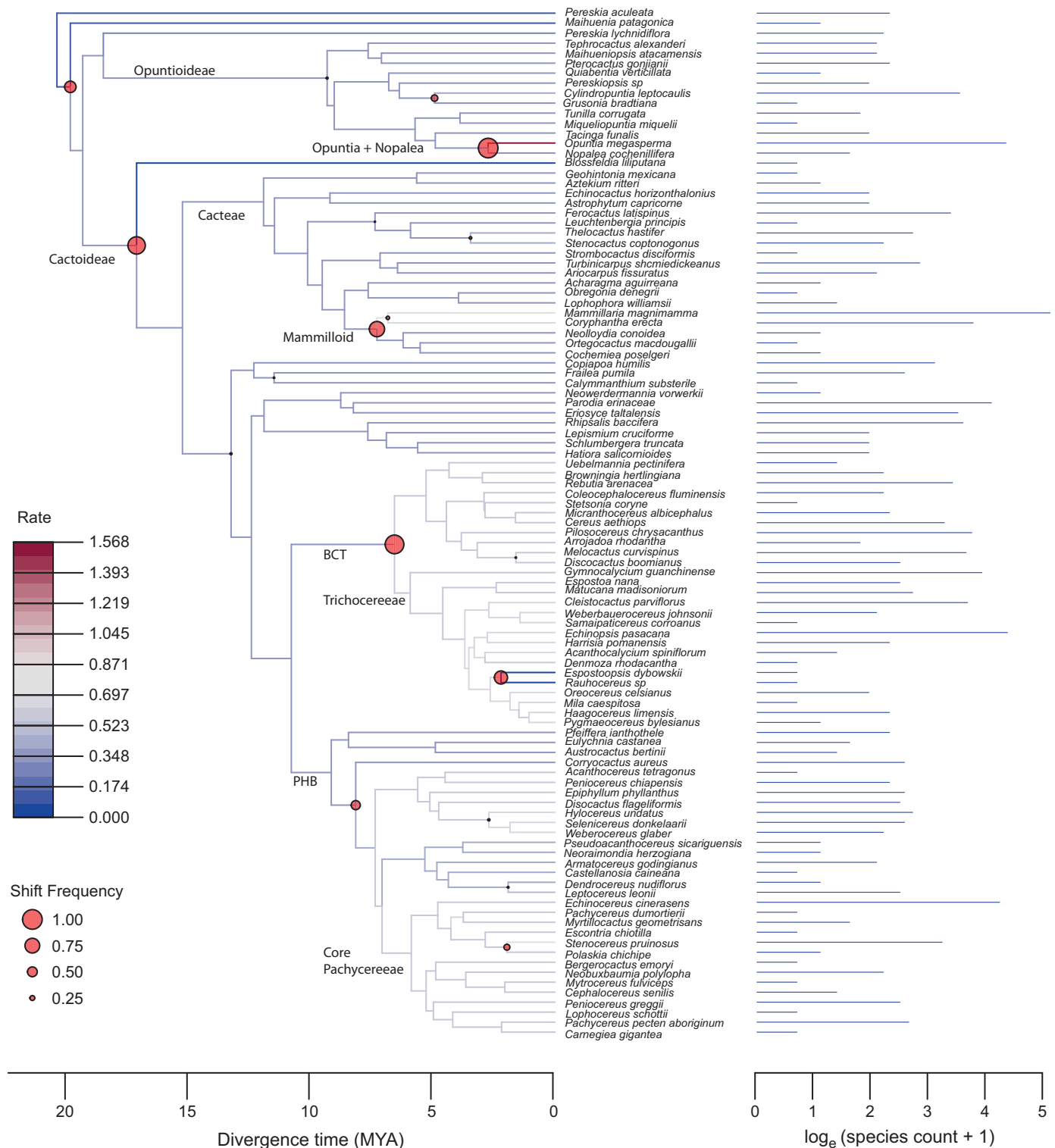


Fig. 3 Results of the MEDUSA diversification rates analyses over the 1000 randomly selected trees. Rate shift frequencies are indicated with red circles, and branches are colored according to median net diversification rates across the 1000 trees. Ma, million years ago.

in the opuntoid flattened-stemmed clade (including *Tacinga*, *Opuntia* and *Nopalea*) ($P=0.99/0.99$), and in the core Cactoi-deae only with Bayesian analyses ($P=0.96$, $P=0.46$ in ML). However, these two clades include mellitophilic members. The ancestral growth form of the family could not be reconstructed

because representatives of subfamilies Opuntioideae and Pereski-oidae lack comparable growth forms with members of Cactoi-deae. In Cactoi-deae, an arborescent, shrubby or columnar growth form was reconstructed as ancestral ($P=0.9/1.0$), with shifts towards a globose or barrel form early in *Blossfeldia*, in the North

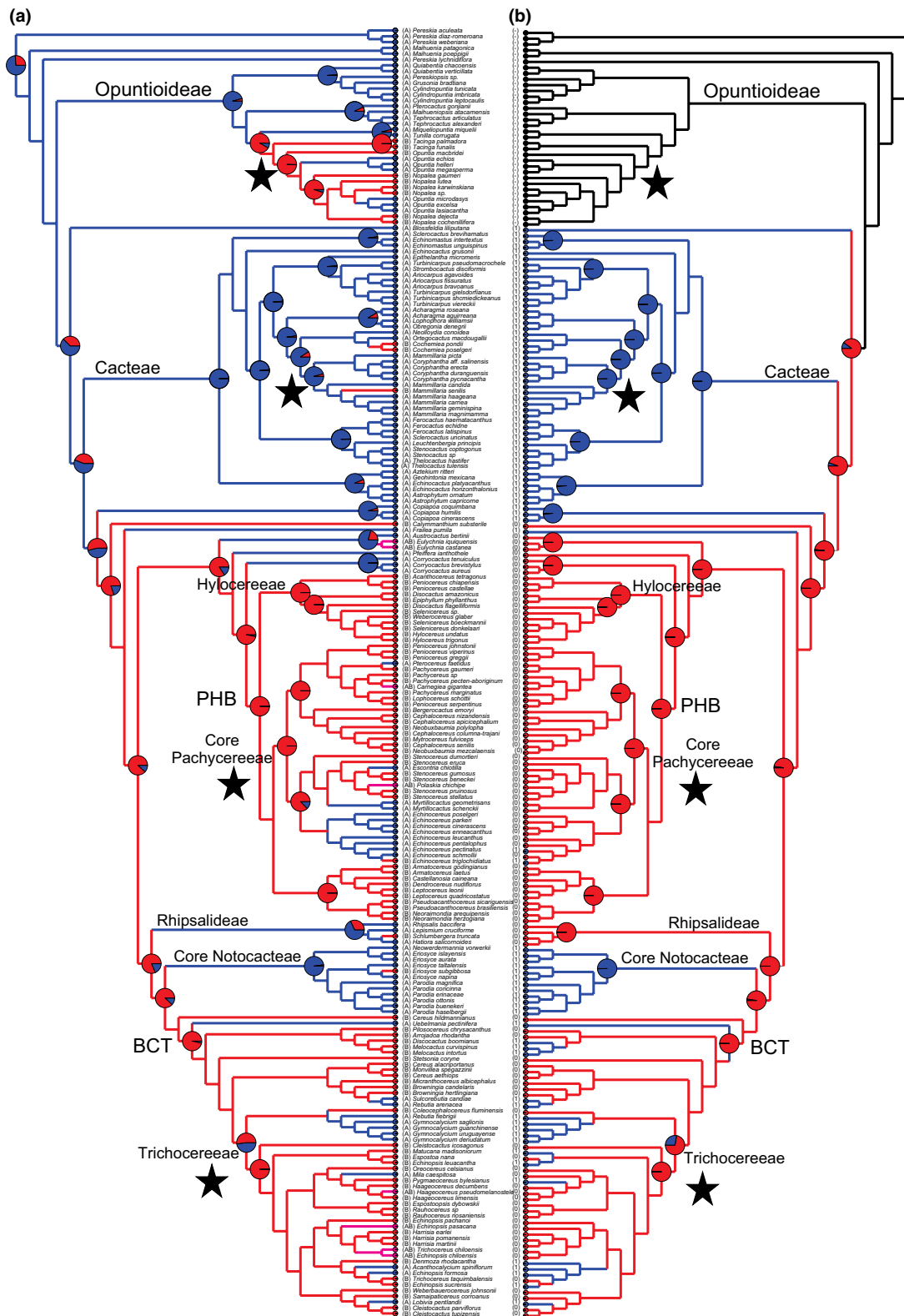


Fig. 4 Character states mapped on the maximum likelihood (ML) phylogenetic tree and ancestral character reconstruction. (a) Ancestral reconstructions of pollination syndromes: mellitophilic species (blue) and species with other pollination syndromes (red; i.e. sphingophily, chiropterophily or ornithophily). (b) Ancestral reconstructions of life form: species with a globose or barrel life form (red) and species with other life forms (blue; i.e. arborescent, shrubby or columnar). Species within subfamilies Opuntioideae, Maihuenioideae and Pereskioideae lack a growth form comparable to those of members of subfamily Cactoideae; hence, their growth form was scored as nonapplicable. Pie charts show the probability states reconstructed with ML at each node, obtained with BayesTraits. Stars located next to nodes indicate the positions of diversification rate shift increases inferred in MEDUSA.

American Cactaceae clade ($P=0.99/0.99$) and the South American core Notocactaceae clade ($P=0.99/0.99$), with independent shifts scattered throughout the BCT clade (Fig. 4). To statistically evaluate a possible concordance in pollination guild and growth-form shifts along the Cactaceae phylogeny we conducted a correlation test with BayesTraits. With the ML approach, we conducted LRTs for each of the 1000 randomly selected trees. Probability values obtained were very low (lower than 5×10^{-4} ; see Fig. S3), suggesting a significant improvement with the correlated model, and thus a possible correlation between the evolution of growth form and pollination syndrome. Similar results were obtained with the Bayesian MCMC approach. The correlated model yielded an harmonic mean of $c. -136.38$, while that of the independent model was $c. -213.59$, leading to a Bayes factor of 154.4, supporting the correlated evolution between growth form and pollination syndrome.

In order to investigate a correlation between character change and diversification in Cactaceae, we implemented variants of the BiSSE model. Results on parameter estimates and the comparison of AIC scores obtained for growth form and pollination syndrome models are shown in Tables S7 and S8, respectively. Results show a clear state-dependence in diversification rates, as speciation rates were on average two times higher in lineages with an arborescent, shrubby or columnar growth form, and with a derived pollination syndrome such as chiropterophily, ornithophily and sphingophily. In both traits there is a clear difference in transition rates, which are on average six times higher in favor of a transition rate from a globose to an arborescent or columnar form, and from a mellitophilic towards a derived pollination syndrome.

Discussion

Cactaceae diversification is congruent with a Miocene origin of the American Succulent Biome

The Succulent Biome comprises highly fragmented, globally distributed patches of vegetation on a climate characterized by erratic, unpredictable rains, with succulents as one of the predominant growth forms together with sclerophyllous shrubs that do not resist fire (Lavin *et al.*, 2004; and references therein). Plant groups in this biome show a strong geographic phylogenetic structure, and a considerable number of endemic and geographically-restricted species (Lavin *et al.*, 2004; Schrire *et al.*, 2005; Thiv *et al.*, 2011). This biome is characterized by low immigration rates and the presence of local specialists; many genera shared among patches, but few shared species (Thiv *et al.*, 2011). To understand the origin and expansion of the succulent biome in the New World, we investigate the geographic phylogenetic structure and biogeographic history of Cactaceae, and evaluate if clades with distinct geographic distributions originated contemporaneously, possibly as a response to a global aridification trend.

The cactus family has been previously used as a model to understand the origin of arid biomes. Arakaki *et al.* (2011) provided an estimation of divergence dates and diversification rates of Cactaceae by using a similar approach to the one presented

here. They also used a two-step approach for the estimation of dates. First, they used an assembled order-level matrix to estimate Cactaceae crown dates with a similar number of taxa, but including 83 chloroplast genes and 13 calibration fossil dates. In spite of that effort to increase the number of genomic regions, general relationships within eudicots and outgroups obtained by Arakaki *et al.* are similar to the ones presented here, and to relationships reported in previous studies (e.g. Wang *et al.*, 2009; Soltis *et al.*, 2011). Consequently, we focused our efforts on the calibration strategy by increasing the number of temporal calibrations to include 23 divergence events obtained from the fossil record. Additionally, we used a lognormal distribution for each calibration to accommodate uncertainty in the paleontological information in the priors rather than relying upon single data points. Our smaller gene dataset allowed utilization of an uncorrelated lognormal clock method that has the advantage of considering differences in the rates of substitution among lineages without assuming rate autocorrelation (Drummond & Rambaut, 2007). Furthermore, we included more than twice the number of Cactaceae representatives with respect to the previous study, which allowed a more detailed investigation of diversification dynamics within the family.

Although our estimated dates for crown and stem Cactaceae are similar to those obtained by Arakaki *et al.* (2011), our estimated dates for clades within Cactaceae are generally younger (Table 1), and consequently our inferred diversification rate estimates are higher. According to our estimates, Cactaceae became differentiated $c. 32.11$ (42.43–22.71) Ma, slightly after the Eocene–Oligocene boundary, and the onset of the diversification of the family into extant lineages is estimated in the late Oligocene, $c. 26.88$ (36.85–22.71) Ma. These dates are younger than the large global drop in atmospheric CO₂ concentrations occurring in the Eocene (Zachos *et al.*, 2008), indicating a possible scenario for the origin of the ancestors of modern succulent lineages with crassulacean acid metabolism. Moreover, the estimated times of origin of other succulent groups are generally similar. For example, the origin of *Euphorbia* (Euphorbiaceae) was recently estimated at 36.59 (47.24–28.99) Ma (Bruyns *et al.*, 2011); Aizoaceae and Didiereaceae (Caryophyllales) at $c. 32.3$ and 28.25 Ma, respectively (Hernández-Hernández, 2010), and Agavaceae (Asparagales) at $c. 35$ –30 Ma (Good-Avila *et al.*, 2006). However, extreme succulence and other specialized adaptations to dry habitats are derived conditions within each of these groups (Applequist & Wallace, 2000; Klak *et al.*, 2003; Bruyns *et al.*, 2011; Hernández-Hernández *et al.*, 2011; Horn *et al.*, 2012), suggesting that these attributes evolved from nonsucculent ancestors that were presumably preadapted to xerophytic habitats in the Oligocene, under warmer, more stable climates (Graham, 2011).

Although evidence for increasing aridity since the Cretaceous is available (Ziegler *et al.*, 2003), floras of arid environments apparently are substantially younger, mostly being no older than the late Miocene or early Pliocene (Moore & Jansen, 2006; and references therein), especially in the New World. Modern vegetation of the Sonoran Desert has been estimated to date from the Pleistocene (2.59 Ma; Axelrod, 1979); the Pliocene–Pleistocene

(5.33–2.59 Ma; Phillips & Comus, 2000); or the middle Miocene (13.82 Ma; Van Devender, 2000). Arid conditions leading to what is now the Chihuahuan Desert are thought to have appeared in the middle Miocene (Morafka, 1977), and fossil grasses from Mojave Desert suggest that Mediterranean chaparral-type grasslands were established there also by the Miocene (Tidwell & Nambudiri, 1989). By the end of the early Miocene, New World ecosystems included early versions of the desert, shrubland, savanna and grassland biomes, derived from drier elements present in older habitats (Graham, 2011, and references therein).

Our dating analysis indicates a time lag between the origin and diversification of Cactaceae, with the latter taking place mainly during the last 15–10 Myr (Table 1, Fig. 1), in agreement with a proposed Miocene expansion of New World arid and semi-arid vegetation. The estimated time of diversification of other (non-succulent) North American xerophytic plant genera also agree with increasing aridity during the Miocene. For instance, *Prosopis* (Fabaceae) – the mesquite – originated during the late Miocene, but diversified during the Pliocene (Catalano *et al.*, 2008); the strongest diversification phase of *Bursera* (Burseraceae) took place during the Miocene (De-Nova *et al.*, 2012); *Triquila* (Boraginaceae) diversified in the early to late Miocene (Moore & Jansen, 2006); and *Nolana* (Solanaceae; Dillon *et al.*, 2009), *Agave* (Agavaceae; Good-Avila *et al.*, 2006) and *Ephedra* (Gnetophyta; Loera *et al.*, 2012) diversified in the middle to late Miocene. The apparent contemporaneous origin of the largest clades, as well as species-rich lineages in Aizoaceae and Agavaceae, has been invoked as an indicator of a global aridification trend (Arakaki *et al.*, 2011). Nonetheless, it is difficult to reject an independent origin of each cactus lineage given the large 95% HPD intervals that were obtained for estimated dates (see Fig. S4). Unfortunately, given the scarcity of fossil evidence in arid biomes, it is difficult to obtain more reliable calibration dates to improve analyses and date estimates by reducing HPD intervals. An adequate test for the hypothesis of synchronous origin and diversification would require further statistical analyses with the inclusion of other xeric-adapted lineages radiating independently in different geographical regions.

Major cacti genera radiated during their colonization of North America

Our biogeographic analyses allowed us to evaluate whether radiating lineages with the highest diversification rates are geographically concentrated sharing particular areas of origin, or are dispersed through the family's distribution. In agreement with previous studies (i.e. Buxbaum, 1969; Leuenberger, 1986; Wallace & Gibson, 2002; Ocampo & Columbus, 2010), our results support a South American origin for Cactaceae, in the central Andean region of northern Chile, north-west Argentina, Bolivia and Peru (Fig. 1; Table S1). Many species of Cactaceae are endemic to this region, which has long been regarded as the source of numerous angiosperm lineages (Raven & Axelrod, 1974). Aridity in the central Andes can be traced back to the late Jurassic, and sedimentological records of the Atacama Desert

reveal climate stability for the region even during the Pleistocene climatic fluctuations (Hartley *et al.*, 2005). The Andean uplift did not commence until 30 Ma (Hartley *et al.*, 2005 and references therein), reinforcing the arid conditions by excluding moisture from the Amazon Basin (Placzek *et al.*, 2009). Additionally, the rise of the Andes could provide novel niches favoring cactus diversification (Hoorn *et al.*, 2010). We estimated several independent expansions within all major Cactaceae clades from this area into different geographic regions (Fig. 1). However, the lineages that expanded into North America contain some of the most species-rich genera, which also exhibit the highest rates of diversification (Table 1).

Two opuntoid clades were associated with a diversification rate increase in the MEDUSA analysis: the flat-stemmed *Opuntia* + *Nopalea* clade and the terete-stemmed *Cylindropuntia* and *Grusonia*. Most species belonging to these clades are distributed in North America, especially in the Chihuahuan Desert (Gómez-Hinostroza & Hernández, 2000; Hernández *et al.*, 2001; Powell & Weedín, 2004; Griffith & Porter, 2009; Majure *et al.*, 2012). Other increases in diversification rates were detected in clades possessing the most elevated absolute diversification rates within the family: *Echinocereus* and the Mammilloid clade. *Echinocereus* includes *c.* 67 species of short, cylindrical-stemmed cacti (Hunt *et al.*, 2006), and has the highest diversification rate in the family ($r_{0,0} = 1.01$ and $r_{0,9} = 0.57$ sp Myr⁻¹). These species are distributed in deserts and semideserts of central and north-western Mexico, and southwestern USA. *Mammillaria*, which belongs to the Mammilloid clade, is the largest genus in the family, with *c.* 163 species (Butterworth & Wallace, 2004; Hunt *et al.*, 2006). It also reaches its maximum species richness and morphological diversity in arid regions of Mexico, with numerous microendemic species in the Chihuahuan Desert. All of these clades represent independent expansions into North American arid biomes (Table S5, Fig. 1).

Different hypotheses have been suggested to explain the large number of species in the Opuntoid, Mammilloid and *Echinocereus* clades (i.e. apomixis: see Pinkava, 2002; polyploidy: see Cota, 1993; Cota & Philbrick, 1994; Cota & Wallace, 1995; Pinkava, 2002; the presence of latex and resin canals in *Mammillaria*: see Farrell *et al.*, 1991); however, detailed studies on the ecological mechanisms driving speciation in these lineages are needed to confirm them.

Novel pollination syndromes and growth forms occur in lineages with high diversification rates

Although aridification might provide environmental conditions fostering the origin of xerophytic plant lineages, other mechanisms promoted elevated diversification rates in some of them. Our taxonomic sample allowed us to provide more detail on clades and lineages associated with increased diversification rates within Cactoideae, the most diverse Cactaceae subfamily, than previously reported (Arakaki *et al.*, 2011). We detected the BCT and PHB clades (and major lineages within them) as having significantly high species richness (Table 1, Fig. 2), as well as being associated with significant increasing shifts in diversification rate

(Fig. 3). The BCT clade includes South American columnar, arborescent and shrubby species from tribes Trichocereae and Cereae. The PHB clade includes species with similar growth forms in the North American tribes Pachycereae and Hylocereae, which also includes epiphytes (Hernández-Hernández *et al.*, 2011).

The derived conditions of bird, bat and moth pollination tend to occur in members of the PHB and BCT clades (Grant & Grant, 1979; Gibson & Nobel, 1986; Barthlott & Hunt, 1993; Cota, 1993; Nobel, 2002; Fleming *et al.*, 2009). Derived pollination syndromes can provide effective barriers to gene flow, contributing to the origin of new lineages (Xu *et al.*, 2012), with an impact on diversification rates (Smith *et al.*, 2008; van der Niet & Johnson, 2012 and references therein). The evolution towards bat or bird pollination might provide important benefits to plants, because these animals deposit a large amount and variety of pollen genotypes on stigmas and, compared with pollinators such as ants or bees, are long-distance dispersers (Fleming *et al.*, 2009).

Although derived Cactoideae clades most conspicuously include shrubs (Fig. 4 and see Hernández-Hernández *et al.*, 2011), members of the North American PHB and the South American BCT clades convergently evolved towards arborescent and columnar growth forms, with a shift towards epiphytic habit in Hylocereae (Hernández-Hernández *et al.*, 2011). In addition to particular floral pollinating systems, columnar or arborescent growth forms in the context of the relatively short vegetation in dry forests and semi-arid regions might facilitate pollination by moths, bats or birds (Fleming *et al.*, 2009). A correlation between derived pollination syndromes and an arborescent, shrubby or columnar growth form in BCT and PHB members has already been suggested (Schlumpberger, 2012), and here we tested this hypothesis of correlation under ML and Bayesian frameworks.

We found strong evidence suggesting that bat, bird or moth pollination syndromes are associated with a columnar, shrubby or arborescent growth form in the Cactaceae phylogeny. We used BiSSE to test if shifts towards novel pollination syndromes and a shrubby or arborescent growth form in the Cactoideae are coincident with shifts in diversification rates. Our results strongly indicate a correlation between character state and increase in speciation rate (Tables S7, S8). Thus, we suggest that the outstanding diversification of core Pachycereae, Hylocereae and Trichocereae is related to the evolution of derived pollination syndromes, with a possible trend towards a specialization for bats in North America and moths in South America, both facilitated by an arborescent, shrubby or columnar growth form. In the context of an arid environment where plant populations have low densities, the evolution of a pollination mechanism that increases pollen-transfer efficiency can be helpful to overcome mate-finding Allee effects and to continue to reproduce successfully (Ghazoul, 2005; Gascoigne *et al.*, 2009; Livshultz *et al.*, 2011), particularly in long-lived and slow-growing species such as many cacti.

In conclusion, our estimated dates of origin and diversification of Cactaceae and major clades within the family are

congruent with a Miocene expansion of arid biomes in the American continent. However, disparity among diversification rate estimates for clades originating at similar times suggests different underlying diversification drivers. The *Opuntia*+*Nopalea*, the *Mammillaria*+*Coryphantha*, the PHB (including the core Pachycereae and Hylocereae), and the BCT clades (including Trichocereae) were identified as having higher-than-expected species richness and elevated diversification rates, and to be associated with rate increases in the phylogeny. The large morphological and ecological diversity encompassed by each of these clades, as well as by other succulent lineages, and the fact that other related xeric-adapted lineages without these diversity and species-richness originated at similar times indicates that the diversification of major succulent plant radiations might be better explained by a complex set of attributes contingent to each clade evolving in each particular arid habitat.

According to our results, the high diversification rates that characterize speciose genera such as *Opuntia*, *Mammillaria* and *Echinocereus* might be associated with their geographic expansion during the recent aridification of North America (particularly the expansion of the Chihuahuan Desert) during the Miocene. In the case of the core Pachycereae, Hylocereae and Trichocereae, which are relatively younger lineages, their high species richness may have resulted from the origin of novel pollination syndromes associated with changes in growth forms in several clade members. We hypothesize that the presence of these characters fostered an increase in the diversification rates of lineages within the BCT and PHB clades, which include the tallest members in dry forests and semi-arid regions of South and North America. Nevertheless, the ecological mechanisms that led to increases in speciation rates in these clades require more investigation.

It has been pointed out that the origin of extant biodiversity in the Neotropical region, the most species-rich region on Earth (e.g. Prance, 1977; Gentry, 1982), cannot be attributed to the action of one or a few events during key time intervals, but rather has resulted from complex ecological and evolutionary processes including both abiotic and biotic factors (Antonelli & Sanmartín, 2011; Rull, 2011). Similarly, hypotheses for the origin of biodiversity in arid regions must take into account the complexity and diversity of possible drivers of diversification in water-limited environments. It has been shown that aridification can shape the evolution not only of functional but also of reproductive traits (Livshultz *et al.*, 2011). However, further field studies providing ecological explanations for the mechanistic processes determining diversification in xerophytic lineages are still pending.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Cactaceae phylogeny resulting from the RAxML analyses.

Fig. S2 Consensus chronogram resulting of the eudicot dating analyses in BEAST.

Fig. S3 Probability values obtained for LRTs conducted for each of the 1000 randomly selected trees to test for correlation among growth form and pollination syndrome.

Fig. S4 Age of major Cactaceae clades vs number of species, showing 95% highest posterior density (HPD) intervals for estimated dates.

Methods S1 Details on the estimation of divergence times.

Methods S2 Detailed description of biogeographical regions designated for this study.

Table S1 Taxa, voucher information and GenBank accessions for the Cactaceae plastid dataset used in the present study

Table S2 GenBank accessions for the eudicot dataset used in the present study

Table S3 Calibration and minimal age constraints used in the eudicot-wide tree to date the Cactaceae crown group

Table S4 Number of species included in each genera within major Cactaceae clades

Table S5 Results on the ancestral area reconstruction analyses

Table S6 Probability values of the ML and Bayesian ancestral state reconstruction analyses for pollination syndrome and growth form

Table S7 Results of the BiSSE analyses for growth form

Table S8 Results of the BiSSE analyses for pollination syndrome

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