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DISENTANGLING THE EFFECTS OF KEY INNOVATIONS ON THE DIVERSIFICATION OF BROMELIOIDEAE (BROMELIACEAE)

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The evolution of key innovations, novel traits that promote diversification, is often seen as major driver for the unequal distribution of species richness within the tree of life. In this study, we aim to determine the factors underlying the extraordinary radiation of the subfamily Bromelioideae, one of the most diverse clades among the neotropical plant family Bromeliaceae. Based on an extended molecular phylogenetic data set, we examine the effect of two putative key innovations, that is, the Crassulacean acid metabolism (CAM) and the water-impounding tank, on speciation and extinction rates. To this aim, we develop a novel Bayesian implementation of the phylogenetic comparative method, binary state speciation and extinction, which enables hypotheses testing by Bayes factors and accommodates the uncertainty on model selection by Bayesian model averaging. Both CAM and tank habit were found to correlate with increased net diversification, thus fulfilling the criteria for key innovations. Our analyses further revealed that CAM photosynthesis is correlated with a twofold increase in speciation rate, whereas the evolution of the tank had primarily an effect on extinction rates that were found five times lower in tank-forming lineages compared to tank-less clades. These differences are discussed in the light of biogeography, ecology, and past climate change.

KEY WORDS: BiSSE-BMA, key innovation, macroevolution, speciation extinction rates.

Species richness is unevenly distributed within the tree of life, and understanding the evolutionary processes that generated and shaped this diversity is a great challenge. Different factors have been identified as drivers of the underlying processes that give rise to these patterns such as biogeographic history, responses to climate changes, and the evolution of key innovations, that is, morphological, physiological, and ecological traits promoting diversification (Sanderson and Donoghue 1994; Heard and Hauser 1995; Hughes and Eastwood 2006; Mayhew 2007; Hoorn et al. 2010; Antonelli and Sanmartín 2011). The importance of key innovations for the evolutionary success of particular taxonomic groups in the tree of life has often been hypothesized and is still vividly debated (Hodges and Arnold 1995; Sargent 2004; Ree 2005; Johnson et al. 2011; Vamosi and Vamosi 2011; Drummond et al. 2012).

The neotropical plant family Bromeliaceae (Poales, 3352 species; Luther 2012) has undergone an extraordinary radiation, especially in the epiphytic niche of tropical forest canopies, which resulted in the highest number of flowering plant epiphytes in the world after Orchidaceae (Gentry and Dodson 1987; Benzing 2000). Bromeliaceae display a striking ecological versatility, occupying a wide range of habitats from hyperarid deserts to rainforests and high-altitude grasslands at more than 5000 m a.s.l. (Smith and Till 1998), and several of its lineages underwent extensive diversification in the biodiversity hotspots of Latin America, for example Tillandsioideae and Puyoideae in the Andes, and Bromelioideae in the Brazilian Cerrado and Atlantic rain forest (Myers et al. 2000; Simon et al. 2009; Givnish et al. 2011). The success of Bromeliaceae is often attributed to the evolution of certain key innovations, in particular of (1) the unique leaf trichomes which allow for water and nutrient uptake via the leaf surface, (2) the tank habit, which serves as an external water and nutrient reservoir and facilitates independence from the substratum; and (3) Crassulacean acid metabolism (CAM), a physiological pathway that reduces water loss during photosynthesis (Benzing 2000; Crayn et al. 2004; Quezada and Gianoli 2011). Although the leaf trichomes represent a synapomorphy for the family, the tank habit and CAM pathway evolved several times independently within the family, and have been seen as responsible traits for a higher diversification in lineages that evolved them (Crayn et al. 2004; Schulte et al. 2009; Givnish et al. 2011). Nevertheless, little is known about to what extent the acquisition of these new traits led to changes in speciation and extinction rates. For the tank habit, it has been argued that the evolution of the external water and nutrient storage structure, coupled with a mechanism to take up water and nutrients over the leaf surface via the leaf trichomes, tank bromeliads became largely liberated from the constraints of the substratum. This in turn opened up new and manifold ecological niches, such as the canopy of the different tropical forests (Pittendrigh 1948; Benzing 2000; Schulte et al. 2009), and might have had a positive effect on speciation rates. Similarly, the evolution of the tank habit might have had an effect on extinction rates by increasing the survival of tank bromeliad lineages over time by aiding them to endure harsher climatic conditions or to survive in more favorable habitats than tank-less bromeliads. For the CAM photosynthetic pathway, it has been postulated that the main advantage of this trait lies in the higher ecological amplitude of CAM plants in comparison to C_3 plants, and thus the capacity to inhabit a greater variety of ecological niches (Lüttge 2010). Similarly to the case in tank bromeliads, the opening of new niches might have had a positive effect on speciation rates in CAM lineages. The CAM pathway might also have led to a decrease in the extinction rates through providing the plants with a higher physiological plasticity, which might have enabled them to adapt more quickly to changing environmental conditions (Lüttge 2010).

Thus, the evolution of the tank habit and the CAM pathway might have had different effects on speciation and extinction rates across bromeliad lineages. However, the impact of these putative key innovations on the diversification of Bromelioideae has not been thoroughly investigated in a statistical framework yet.

Several phylogeny-based approaches have been developed to estimate rates of diversification and test hypotheses of key innovations based on the symmetry of the trees (Chan and Moore 2002; Heard and Mooers 2002; Paradis 2011) or on the patterns of branching times (Rabosky 2006; Alfaro et al. 2009; Rabosky and Glor 2010; Morlon et al. 2011; Silvestro et al. 2011; Stadler 2011; Etienne et al. 2012). Although these approaches can estimate changes of speciation and extinction rates through time and between clades, they do not explicitly link such changes to the evolution of a trait, for example a key innovation. Correlating the character evolution with changes of diversification rates can be accomplished by using stochastic reconstructions of the trait along a phylogeny to estimate the diversification rates under different character states (Ree 2005). However, it has been demonstrated that such approaches can lead to biases in cases where the evolution of a trait and changes in speciation and extinction rates are correlated (Maddison 2006). Thus, a likelihood framework that allows for the joint estimation of evolutionary rates of a binary character and the speciation and extinction rates associated to its states was developed, the binary state speciation and extinction approach (BiSSE; Maddison et al. 2007). In addition to the original maximum likelihood (ML) BiSSE approach, a Bayesian implementation was developed to incorporate parameter and phylogenetic uncertainties (FitzJohn et al. 2009; Johnson et al. 2011).

Model testing in trait-correlated diversification is usually based on ML test statistics, such as the likelihood ratio tests, where models with speciation and extinction rates correlated to the evolution of a character are compared against models with rates constrained to be equal (Maddison et al. 2007). Although these test statistics yield reliable measures of model fit in ML analyses, for rigorous Bayesian model selection, it is desirable to account for the parameter uncertainty and for the priors that determine to which degree each parameter is penalized (Xie et al. 2011). To this purpose, a first application of Bayesian model selection for trait-correlated diversification has been used by Goldberg and Igić (2012), with model marginal likelihoods approximated by a nonparametric method.

Although Bayesian approaches to estimate trait-dependent speciation and extinction rates can already incorporate different sources of uncertainty, the parameter estimation is still often based on a single best-fitting model that might lead to an overstated degree of precision (Burnham and Anderson 2002) and has the disadvantage of relying on debatable threshold values. This problem is particularly pronounced when several models obtain comparably good fit (Beier et al. 2004).

Here, we develop a new Bayesian implementation of BiSSE, named BiSSE–BMA, that calculates the fit of different models of diversification using thermodynamic integration to estimate the respective marginal likelihoods (Lartillot and Philippe 2006) while incorporating parameter uncertainties and priors. Furthermore, we implement Bayesian model averaging (BMA; Hoeting et al. 1999; Wasserman 2000) to overcome the difficulty of choosing between competing models with similar fit and generate parameter estimates that incorporate the uncertainty on model selection. This study aims to examine diversification rates in one of the major lineages of bromeliads, the Bromelioideae (33 genera, 936 species; Luther 2012), and to test hypotheses of key innovations. A new molecular data set for Bromelioideae based on five plastid and one nuclear markers is generated to reconstruct a dated phylogeny of the subfamily. The BiSSE–BMA approach is then used to examine the effect of two putative key innovations, the tank habit and the CAM physiology, on the diversification of the group considering spatial and temporal settings of the clade.

Materials and Methods

A molecular data set of 140 bromeliad species was assembled to reconstruct the phylogenetic relationships in Bromelioideae, with representatives from the subfamilies Brocchinioideae, Hechtioideae, Pitcairnioideae *s.s.*, Puyoideae, and Tillandsioideae used as outgroups based on previous molecular studies in the family (Crayn et al. 2004; Barfuss et al. 2005; Schulte and Zizka 2008; Givnish et al. 2011). Details on taxon sampling, voucher deposition, and GenBank accession numbers are provided in Table S1. Using genomic DNA isolated by CTAB procedure (Weising et al. 2005), and the plastid markers *atp*B-*rbc*L spacer, *trn*L-*trn*F spacer, *trn*L intron, and *mat*K with part of the adjacent 3’ *trn*K intron were amplified as detailed by Schulte et al. (2009). The nuclear phytochrome C gene (*phyC*) was amplified using the primers *phyc974f* and *phyc1145r* (M. H. Barfuss, University of Vienna, pers. comm.). The PCR reaction mix included 22 μL ABgene AB-0619/LD (Thermo Scientific), 1 μL of each primer (10 mM), and 2 μL DNA solution. Thermal cycling conditions were as follows: 1 cycle of 2 min at 95°C, followed by 35 cycles with 95°C for 30 sec, 59°C for 30 sec, 70°C for 2 min, the latter prolonged by 10 sec/cycle after 15 cycles. Contiguous sequences were assembled and edited in the software Geneious (Drummond et al. 2011) and alignments assembled using MAFFT (Katoh et al. 2009).

PHYLOGENETIC ANALYSES

For phylogenetic reconstructions, the plastid and the nuclear regions were first analyzed separately using ML, and then visually inspected to assess the presence of conflicts between data sets. The total data set was analyzed with independent partitions for plastid and nuclear regions after detecting the absence of incongruences. GTR+G+I substitution models were selected using jModelTest (Posada 2008) independently for the plastid and the nuclear data partitions based on the AIC scores (Akaike 1973). Phylogenetic relationships and divergence times were jointly estimated applying the Bayesian relaxed molecular clock with uncorrelated lognormal rates using BEAST (version 1.6.1; Drummond and Rambaut 2007). The Yule prior on node ages was selected against the birth–death prior after model testing by Bayes factors as implemented in Tracer. Because of the absence of reliable fossils of Bromeliaceae, we applied secondary calibrations based on Givnish et al. (2011). Thus, a normal distribution with mean 30.97 Ma (central 95% range 17.25–44.69 Ma) was assigned to the root of the bromeliad phylogeny based on the crown age of the family Bromeliaceae. On the same basis, the split between subfamily Bromelioideae and its sister group Puyoideae was calibrated with a normal prior with mean 11.92 Ma (central 95% range). The analyses were run for 50 million Markov Chain Monte Carlo (MCMC) generations, sampling every 2500 generations, and the resulting phylogenies were summarized in a consensus tree (Fig. S1) after excluding a burnin proportion of five million generations. Phylogenetic analyses were also run under parsimony, ML, and Bayesian inference using PAUP* (Swofford 2002), raxmlGUI (Stamatakis 2006; Silvestro and Michalak 2012), and MrBayes (Ronquist et al. 2012; Supplementary Information; Molecular alignments and BEAST trees are available at Dryad doi:10.5061/dryad.0dm16).

TRAIT-CORRELATED DIVERSIFICATION

A new Bayesian implementation of the BiSSE method, termed BiSSE–BMA, was developed for this study to account for phylogenetic uncertainty, estimate the model marginal likelihoods, and incorporate the uncertainty of the model selection by Bayesian model averaging. Posterior sampling was obtained via MCMC using uniform sliding window proposals with reflection at the boundary (Ronquist et al. 2007) to randomly update the rates of state transition (q_{01}, q_{10}) and the net diversification and relative extinction from which speciation (λ_1, λ_1) and extinction (μ_0, μ_1) rates are derived (Silvestro et al. 2011). Uniform bounded priors were assigned to the net diversification (range 0–3), relative extinction (range 0–1), and state transition rate (range 0–1). The estimation of the model marginal likelihood was obtained via thermodynamic integration (TI) using the “quasi-static” algorithm (Lartillot and Philippe 2006) to sample a progression of distributions ranging from the posterior to the prior at the two extremes.

This progression was obtained by raising the likelihood ratio to the power of a parameter β that ranged from a value of 1 (i.e., MCMC samples from the posterior distribution) to 0 (i.e., MCMC samples from the prior only). A path of 20 β values was drawn from evenly spaced quantiles of a β distribution with shape B(0.3, 1) as in Xie et al. (2011) to place more values close to 0, where the acceptance probability changes more rapidly. The marginal likelihood L_{TI} of each model was obtained by integrating the likelihood along the path of power values β (Lartillot and Philippe 2006). Log Bayes factors (BF) were calculated as twice the difference between the log marginal likelihoods and interpreted according to Kass and Raftery (1995). Relative probabilities of the models were obtained from the respective marginal likelihoods as

$$P(m_i|D) = \frac{\exp(\Delta L_{TI}(m_i))}{\sum_{j=1}^{M} \exp(\Delta L_{TI}(m_j))}, \quad (1)$$

where $\Delta L_{TI}(m_i)$ is the difference between the log marginal likelihoods of model i and the best-fitting model m_{best} and M is the set of models tested on each trait. Bayesian model averaging was performed by randomly resampling the posterior parameters from different models proportionally to the respective relative probability and pooling them in a single posterior distribution (cf. Beier et al. 2004). Thus, given posterior samples of size S, the number of MCMC states randomly retained from each model m_i is

$$R(m_i) = \frac{\mathrm{S}}{P(m_{best})} P(m_i). \quad (2)$$

The BiSSE–BMA was implemented based on the R library Diversitree (R Development Core Team 2011; Fitzjohn 2012) and incorporated within the open source program BayesRate version 1.5, available at http://sourceforge.net/projects/bayesrate/.

Two traits were tested as putative key innovations for a total of 114 ingroup species representing 31 of the 33 currently recognized genera (Luther 2012). Photosynthetic pathway coded as C_3 (11 species: representing $\rho_{C3} = 14\%$ of all C_3 species) or CAM (86 species: $\rho_{CAM} = 11\%$) and external water storage strategy coded as presence (76 species: $\rho_{TANK} = 12\%$) or absence (38 species: $\rho_{TANK\text{-}LESS} = 17\%$) of the tank habit. Measures of the carbon isotope ratio $\delta^{13}C$ were used to define the photosynthetic pathway following Crayn et al. (2004). For each trait, 40 models were tested with differently linked or unlinked parameters (e.g., $\lambda_0 \sim \lambda_1$; $\mu_0 \sim \mu_1$; $q_{01} \sim q_{10}$; Tables S2, S3). Among these models we included pure-birth processes by setting μ_0 and/or $\mu_1 = 0$, and irreversible trait evolution by setting either $q_{01} = 0$ or $q_{10} = 0$. In all cases, the "skeletal tree" approach described by FitzJohn et al. (2009) was applied to correct for incomplete taxon sampling, assuming random sampling associated to each character state, using the sampling fractions indicated above. Based on initial test runs, we set the first 5000 generations as burnin fraction, and the sampling frequency to 100 in the analyses. The marginal likelihood of the different models was calculated on the BEAST consensus tree by running 50,000 MCMC iterations under each of the 20 β values. The best-ranking models reaching a cumulative probability of 0.95 were then applied in a second analysis for parameter estimation, that is, sampling only from the posterior (β = 1). The relative probabilities obtained by TI in a Bayesian framework were compared to the models' fit in ML analyses. The latter scores were calculated as Akaike weights after performing ML optimizations (as implemented in Diversitree) under each of the 40 models. Although all models can be used for BMA, setting a cumulative probability threshold allowed a drastic reduction of the computational burden of the analyses while accounting for 95% of the model uncertainty. To incorporate phylogenetic uncertainty in the parameter estimates, we ran the MCMC sequentially on a random sample of 100 trees from the BEAST analysis with 20,000 iterations on each tree, after a burnin fraction applied on each tree (Silvestro et al. 2011). The joint posterior sample obtained by BMA was used to calculate mean and maximum a posteriori (MAP) of each parameter along with the respective 95% credibility intervals. For comparison, an MCMC analysis with the same settings was also carried out under the unconstrained six-rate model.

ANCESTRAL STATE RECONSTRUCTION

The presence of a correlation between the evolution of a trait and the rates of speciation and extinction has the effect of biasing ancestral state reconstructions based on Markov models that do not account for such dependency (Maddison 2006). Thus, to infer the evolution of the photosynthetic pathway and tank habit, we applied the BiSSE likelihood function as implemented in Diversitree to obtain marginal estimates of the ancestral states at the nodes. The reconstructions were performed on the BEAST consensus tree, based on a sample of 1000 sets of rate parameters (λ_0, λ_1; μ_0, μ_1; q_{01}, q_{10}) randomly drawn from the BiSSE–BMA posterior distribution. The mean relative probabilities of each ancestral state for all internal nodes were summarized over the 1000 replicates with the respective mean and standard deviation. The analyses were repeated on a sample of 100 BEAST trees to assess the robustness of the results against phylogenetic uncertainty.

Results

DIVERSIFICATION OF BROMELIOIDEAE

The four different phylogenetic analyses (maximum parsimony, ML, and Bayesian inference with MrBayes and under relaxed molecular clock with BEAST) yielded largely congruent results albeit with different levels of resolution (Figs. 1, S1–S4). In the following, results from the BEAST analysis with the respective posterior probabilities (PP) are presented. The

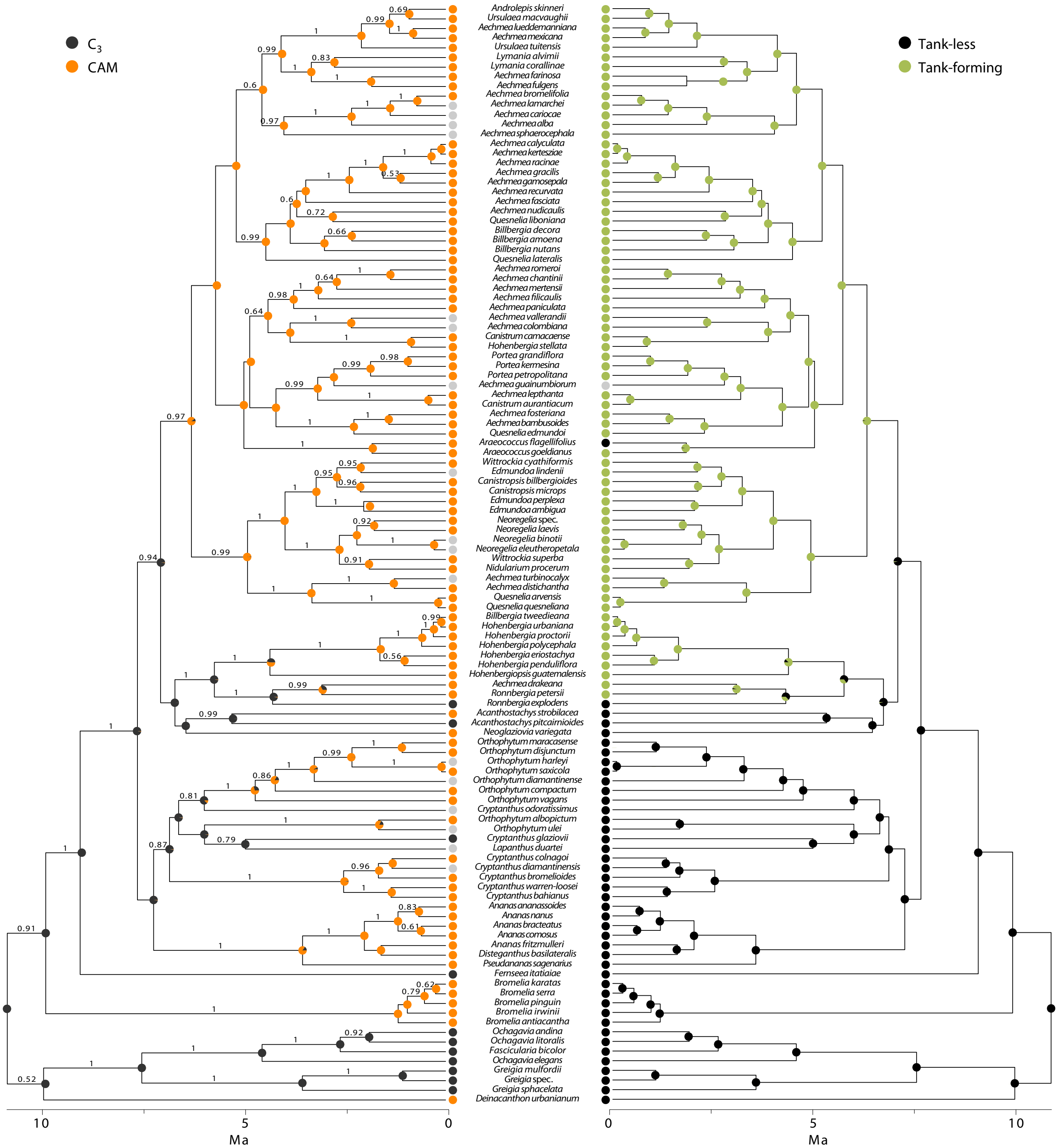

Figure 1. Evolution of tank habit and photosynthetic pathway. Evolution of the photosynthetic pathway (left) and water impounding tank (right) reconstructed on the dated consensus phylogeny of Bromelioideae based on the BiSSE–BMA results. The pie charts at the internal nodes show the relative probabilities assigned to each ancestral state. Light gray circles at the tips represent missing data. Branch lengths display divergence time in million of years (Ma) and the values above branches are the posterior probabilities estimated by BEAST.

monogeneric subfamily Puyoideae forms a highly supported monophyletic clade (PP 1) and is found in sister group position to a monophyletic Bromelioideae. The first diverging lineages of the latter are (1) a highly supported clade comprising the mesophytic genera *Greigia*, *Ochagavia*, and *Fascicularia* (PP 1); (2) the monospecific genus *Deinacanthon*; (3) *Bromelia* (PP 1), the latter depicted in sister group position to a clade with *Fernseea* as sister group to the highly supported Eu-bromelioids sensu Schulte et al. (2009) (PP 1). Within the latter, the next diverging groups are a highly supported clade harboring the three genera *Ananas, Pseudananas*, and *Disteganthus* (PP 1) and a weakly supported clade (PP 0.87) comprising the genera *Cryptanthus*, *Orthophytum*, and *Lapanthus*. These are found in sister group position to a moderately supported clade (PP 0.94) containing the remainder of the subfamily, which represent the core bromelioids sensu Schulte et al. (2009) plus the genera *Neoglaziovia* and *Acanthostachys*, which are shown as early diverging lineages within this clade. Within the core bromelioids, several highly supported clades are found, for example a Nidularioid clade comprising the genera *Nidularium*, *Neoregelia*, *Wittrockia*, *Canistropsis*, and *Edmundoa* (PP 1), a clade unifying the representatives of *Aechmea* subgen. *Ortgiesia* (*A. calyculata*, *A. racinae*, *A. kertesziae*, *A. gamosepala*, *A. gracilis*, and the type of the subgenus *A. recurvata*). The phylogenetic reconstruction demonstrates the substantial polyphyly of the largest bromelioid genus *Aechmea* and indicates the nonmonophyly of several other bromelioid genera (i.e., *Billbergia*, *Canistrum, Hohenbergia*, and *Quesnelia*).

Relaxed molecular clock analyses (Figs. 1, S1) placed the stem age of Bromelioideae in the mid-Miocene, 11.87 Ma (95% credibility interval [CI] = 8.25–15.55; Fig. S4), and the crown age of the extant lineages was inferred to 10.89 Ma (CI = 7.60–14.55). The origin of the core group was dated to the late Miocene, 7.08 Ma (CI = 4.71–9.67).

EVOLUTION OF TWO PUTATIVE KEY INNOVATIONS AND DIVERSIFICATION

The joint analysis of character evolution and diversification showed that both the photosynthetic pathway and the tank habit are significantly correlated to changes in the rates of speciation and extinction of Bromelioideae. The effect of these two traits can however be differentiated.

Based on the fit of the 40 models of diversification correlated with the photosynthetic pathway, 11 of the models had to be retained for BMA to reach a cumulative probability of 0.95 (Tables 1, S2). Evolutionary shifts between C_3 and CAM physiologies were found to correlate with changes in speciation rates in nine of the retained models (with cumulative probability = 0.89), whereas variations of extinction rates were assumed in eight models (with cumulative probability = 0.69). The posterior parameter estimates obtained by BMA show that the evolution of CAM photosynthesis is correlated to a two times higher speciation rate (λ_1 = 1.25; CI = 0.59–2.12) compared to C_3 lineages (λ_0 = 0.52; CI = 0.23–0.98; Table 2; Fig. 1). Crassulacean acid metabolism physiology also appears to be correlated with higher extinction rates (μ_0 = 0.12, CI = 0–0.64; μ_1 = 0.48, CI = 0–1.40). This difference, however, drastically reduces when based on the MAP estimators rather than the arithmetic mean (cf. Silvestro et al.

Table 1. Model fit of trait-dependent diversification: photosynthetic pathway. Eleven models with different constraint settings and degrees of freedom (df) were selected to cumulate a relative probability of 0.95. Subscript numbers 0 and 1 refer to C_3 and CAM, respectively. The best model has only a slightly better fit than the following, and overall greatest relative probabilities are given to models with unconstrained speciation and irreversible transitions from C_3 to CAM.

BiSSE (constrained) models							Physiology (C_3/CAM)		
λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	df	L_M	BF	Rel. prob.
		0			0	4	−259.51	0	0.41
		0	0		0	3	−260.5	1.97	0.15
					0	5	−260.54	2.06	0.14
		$\mu_0 = \mu_1$			0	4	−260.98	2.93	0.09
			0		0	4	−261.85	4.67	0.04
$\lambda_0 = \lambda_1$			0		0	3	−262.02	5.02	0.03
$\lambda_0 = \lambda_1$					0	4	−262.57	6.11	0.02
		0		$q_{01} = q_{10}$		4	−262.4	5.77	0.02
		$\mu_0 = \mu_1$		$q_{01} = q_{10}$		4	−262.58	6.13	0.02
				$q_{01} = q_{10}$		5	−262.77	6.52	0.02
$\lambda_0 = \lambda_1$				$q_{01} = q_{10}$		4	−263.2	7.37	0.01
Cumulative probability									0.95

Table 2. Model fit of trait-dependent diversification: tank habit. Five models with different constraint settings and degrees of freedom (df) were chosen for BMA cumulating a relative probability of 0.95. Subscript numbers 0 and 1 refer to absence/presence of tank, respectively. The best model, positively supported by Bayes factor test (BF), assumes equal speciation rates, null extinction for tank-forming species, and equal transition rates.

BiSSE (constrained) models							Tank (present/absent)		
λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	df	L_M	BF	Rel. prob.
$\lambda_0 = \lambda_1$			0	$q_{01} = q_{10}$		3	−260.43	0	0.58
$\lambda_0 = \lambda_1$				$q_{01} = q_{10}$		4	−261.54	2.22	0.19
			0	$q_{01} = q_{10}$		4	−262.48	4.12	0.07
		$\mu_0 = \mu_1$		$q_{01} = q_{10}$		4	−262.62	4.4	0.06
				$q_{01} = q_{10}$		5	−263.16	5.47	0.04
Cumulative probability									0.95

Table 3. Trait-correlated diversification. Posterior estimates of the rates of speciation, extinction, and state transition obtained by Bayesian model averaging (BMA) are given as mean and maximum a posteriori (MAP) with 95% credibility interval (CI).

		Posterior rates (BMA)					
		λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}
Physiology(C_3/CAM)	Mean	0.52	1.249	0.115	0.482	0.121	0.002
	MAP	0.41	1.014	0	0.002	0.100	0
	95% CI lower	0.233	0.590	0	0	0.008	0
	95% CI upper	0.981	2.123	0.639	1.397	0.247	0.016
Tank(present/absent)	Mean	0.902	0.941	0.54	0.105	0.012	0.012
	MAP	0.761	0.763	0.458	0	0.010	0.010
	95% CI lower	0.499	0.569	0.083	0	0.002	0.002
	95% CI upper	1.39	1.409	1.03	0.585	0.023	0.023

2011), in which case extinction is almost negligible under both character states ($\mu_0 = 0$, $\mu_1 = 0.002$). In seven of the retained models, the trait is set to be irreversible, that is, loss rate $q_{10} = 0$ (cumulative probability = 0.89). Thus, the rates of evolutionary shifts between photosynthetic pathways are highly asymmetric, with a comparatively high rate of shifts from C_3 to CAM, but low rates of reversal transition (Table 3).

Of the 40 trait-correlated diversification models associated with the tank habit, five models obtained a cumulative probability of 0.95. The best-fitting model (relative probability = 0.58) sets equal speciation rates for tank-less and tank-forming clades but different extinction rates, which are assumed to be zero in the tank-forming lineages (i.e., pure-birth model). In total four of the five models retained for BMA assume different extinction rates for tank forming and tank-less bromelioids (cumulative probability = 0.886). The evolution of the tank habit was found to have very low and symmetric rates of gain and loss in all retained models (Tables 2, S3). After BMA, the speciation rates under the two character states are still found to be almost identical, varying by less than 5% (Table 3). However, the evolution of the tank habit is correlated with a fivefold decrease in extinction rates ($\mu_0 = 0.54$, CI = 0.08–1.03; $\mu_1 = 0.11$, CI = 0–0.59; Table 3; Fig. 2) and a sixfold decrease in the extinction fraction ($a = \mu/\lambda$), that is, $a_0 = 0.60$ in the tank-less lineages and $a_1 = 0.11$ in the tank-forming clades. Considering the MAP values, the difference in extinction is even more pronounced (Table 3) because the extinction rate for tank-forming species is reduced to 0.

The posterior estimates of the rate parameters obtained under the six-rates unconstrained model showed for both photosynthetic pathway and tank habit similar patterns to those inferred by BiSSE–BMA (Fig. S5). However, the estimated rates were generally found to be greater than those obtained after BMA (particularly for extinction and transition rates), with credibility intervals on average 7 and 37% larger for photosynthetic pathway and tank habit, respectively (Table 3; Fig. S5).

ANCESTRAL STATE RECONSTRUCTIONS

The inferred physiology of the subfamily's common ancestor is C_3. The evolutionary history of the trait estimated from a highly asymmetric model of trait evolution (Table 3) shows that C_3 photosynthetic pathway was retained relatively long during the early splits followed by multiple independent changes to CAM physiology (Fig. 1A). In our reconstruction, we find independent shifts to CAM in the genera *Deinacanthon* and *Bromelia*, in the *Ananas* clade, and repeatedly within the *Orthophytum*/*Lapanthus* clade and within the core Bromelioideae.

The reconstructed evolution of the water-impounding tank based on the model estimated by BMA reveals that the trait is highly conserved with only few shifts reconstructed throughout the diversification of the whole subfamily. The common ancestor to Bromelioideae is inferred to have a tank-less habit and this condition is shared by a large number of early diverging lineages (Fig. 1B). The tank habit appears only within the core bromelioids where it is present in the large majority of its lineages.

Discussion

The evolution of the CAM physiology and of the tank habit was reconstructed based on an expanded phylogeny of Bromelioideae to test their respective effect on the speciation and extinction rates within the subfamily. We found that both traits fulfill the criteria for key innovations, that is, they are new and beneficial traits that led to an increased net diversification in the clades that evolved them (e.g., Sanderson and Donoghue 1994; Hodges and Arnold 1995). Our results corroborate previous key innovation hypotheses (Benzing 2000; Crayn et al. 2004; Schulte et al. 2009; Quezada and Gianoli 2011), demonstrated their validity in a robust probabilistic framework, and revealed unsuspected asymmetries among the rates of speciation and extinction that can be attributed

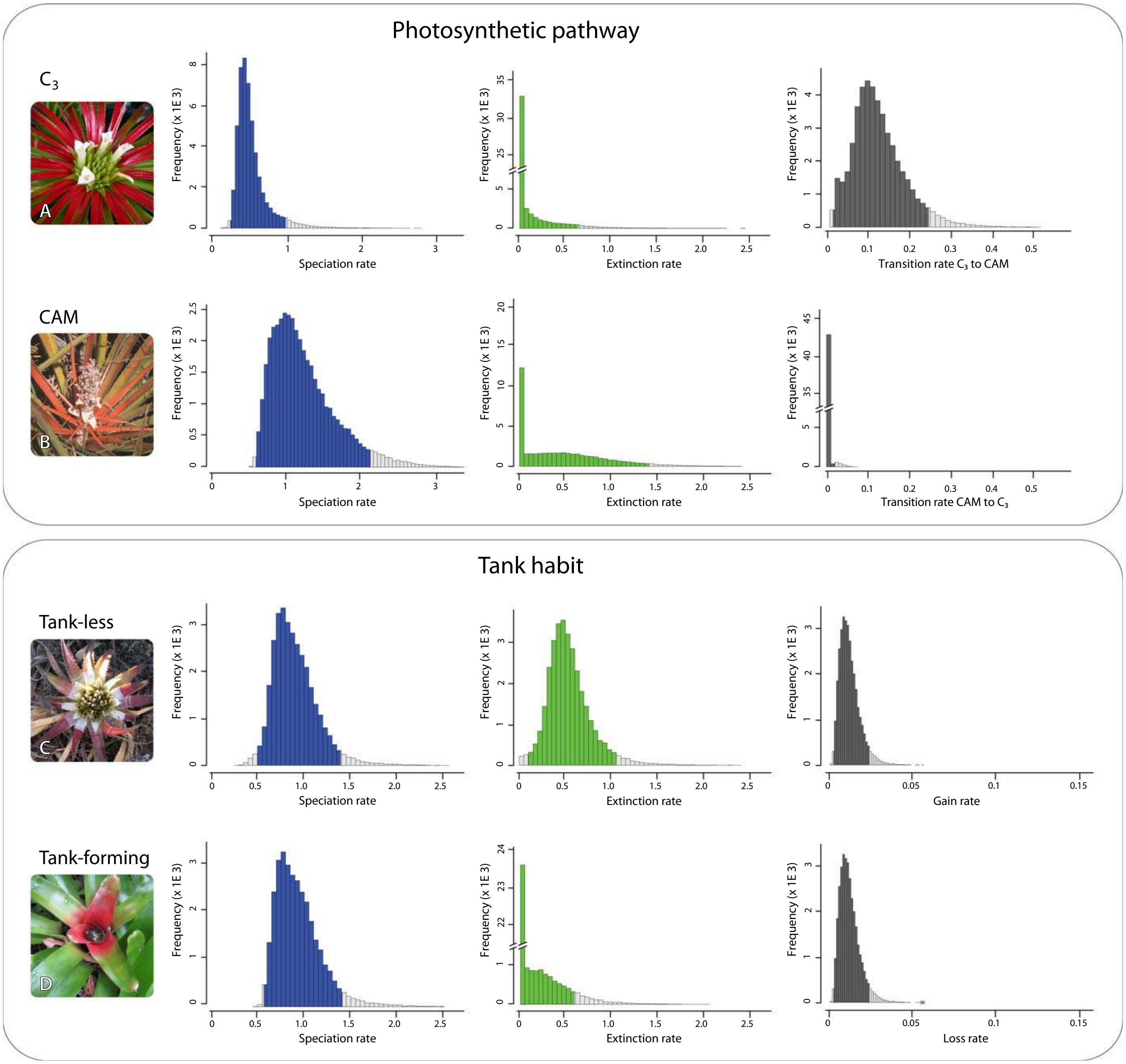

Figure 2. Trait-dependent rates. Posterior estimates (BMA) of speciation rates (blue), extinction rates (green), and state-transition rates (gray) associated with the evolutionary changes of the photosynthetic pathway (C$_3$/CAM) and with the presence/absence of the tank habit. Parameter values and respective credibility intervals are reported in Table 3. Photos: (A) C$_3$ species *Orthophytum navioides* (by R. Louzada); (B) CAM species *Bromelia pinguin*; (C) tank-less *Orthophytum hatschbachii*; (D) tank-forming *Neoregelia mucugense* Chapada Diamantina, Brazil.

to the evolution of the two traits. Indeed, the CAM physiology was found to mainly correlate with higher speciation, whereas the tank habit was associated with lower extinction. Previous studies that implied that these two traits can be seen as key innovations were mainly based on empirical assessments based on the distribution of character states and clade sizes. The only statistical tests to examine this hypothesis further were conducted by Quezada and Gianoli (2011) who found increased diversification associated to CAM photosynthesis, but were based on a much lower taxon sampling and did not attempt a joint reconstruction of the trait evolution and clade diversification.

Our ability to understand the causes and mechanisms of speciation and extinction and their correlation to trait evolution is partly linked to power of the analytical tools available. In this study, we developed a new Bayesian implementation of the BiSSE approach and used it to evaluate the fit of 40 models of diversification for each trait using marginal likelihoods. Using BMA, here applied for the first time in diversification analysis, we obtained posterior rates that account for different sources of uncertainty, including the uncertainty among models. The tested models represent all possible combinations of constrained equal speciation, extinction, and transition rates, ranging between a two-parameter pure-birth process with symmetric speciation and transition rates, and the full six-parameter BiSSE model. Although other model constraints can potentially be tested, in this study the analyses were focused on rate (a)symmetries to detect key innovation effects and on whether the set of free parameters could be reduced without significantly penalizing the likelihood of the data. This is particularly important as a recent simulation study by Davies et al. (2013) showed that the accuracy of the rate estimates using BiSSE might be low when the trees are smaller than a few hundred taxa, but that the performance improves significantly by reducing the number of parameters in the model, for example by assuming symmetric rates of speciation, extinction, or transition. These observations highlight the importance of comparing a wide range of models to verify whether some of the parameters can be subtracted from the model. Noticeably, the 16 models utilized for rate estimation after BMA had four parameters on average, and for neither of the traits was the full birth–death model with six parameters retained. In fact, the analyses using the unconstrained six-rate model yielded wider credibility intervals around the parameter estimates than the retained less complex models. This can be explained as result of an unjustified complexity of the model, because indeed for both traits the unconstrained model obtained relative probabilities smaller than 0.01. Finally, because the BiSSE approach might be prone to limited power in discerning among models of trait-dependent diversification (Davis et al. 2013), the use of BMA to incorporate model uncertainty represents a solution to generate more robust rate estimates. The BiSSE–BMA implementation can be extended to analogous models that have been recently developed to analyze diversification correlated with multistate and quantitative traits (Fitzjohn 2010), geographic ranges (Goldberg et al. 2011), or sets of binary traits (Fitzjohn 2012).

A parallelized implementation of BiSSE–BMA may render the estimation of model marginal likelihoods on a distribution of trees rather than just on the consensus tree more feasible in the future, as well as the incorporation of phylogenetic uncertainty in the model selection. Alternatively, because the model fit based on ML and AIC yielded similar rankings (albeit not identical; Tables S2, S3) to those obtained by TI, Akaike weights can be considered as a reasonable approximation for model averaging in cases of limited computational capacity or very large data sets. In addition, sampling algorithms that can move across different models and jointly estimate the parameters, might provide an efficient alternative to BMA, as shown in other comparative phylogenetic methods (e.g., Eastman et al. 2011) and potentially expand the set of models explored.

EVOLUTION OF PHOTOSYNTHETIC PATHWAY

The evolution of the CAM photosynthetic pathway was found to be correlated to an over twofold increase of the speciation rate compared to C_3 lineages. The existence of a correlation between CAM physiology and extinction remained unclear due to large uncertainties around the estimated rates and to the fact that the hypothesis of zero extinction could not be ruled out for neither of the two photosynthetic pathways. The BiSSE–BMA results indicate a trend to acquire CAM in the evolution of Bromelioideae with few reversals to C_3 physiology, as highlighted by the strong asymmetry of the transition rates. The tendency to evolve and maintain the CAM physiology across clades of Bromelioideae reflects the evolutionary advantage of this photosynthetic pathway and its key innovation effect (Crayn et al. 2004; Givnish et al. 2011; Quezada and Gianoli 2011). The main evolutionary advantage of the CAM photosynthetic pathway is seen in the higher physiological plasticity of CAM plants compared to their C_3 counterparts, which facilitates the former to react more efficiently to the highly dynamic spatio-temporal environmental conditions of many tropical habitats, such as in tropical rain forests (Lüttge 2010).

Despite the fact that the majority of Bromelioideae species perform CAM, about 90% based on Crayn et al. (2004), the reconstructed evolution of the photosynthetic pathway using BiSSE–BMA revealed that the ancestral state in the clade is C_3 and that shifts to CAM repeatedly occurred throughout the evolution of the subfamily (Fig. 1; Table S4). The reconstruction of the ancestral states performed using a standard Markov model of evolution, i.e., without accounting for correlated changes in speciation and extinction rates, reconstructs the C_3 physiology as a “derived” state evolving from a CAM ancestor at the origin of the subfamily. This highlights the importance of incorporating the interdependence between the diversification process and trait evolution in ancestral state estimation (Maddison 2006).

The CAM physiology evolved independently multiple times throughout the diversification of bromeliads (Crayn et al. 2004), appearing in five of the eight recognized subfamilies. The majority of the C_3 species among Bromelioideae represent the descendants of early diverging lineages (the “basal bromelioids”; Schulte et al. 2009) and are distributed today in mesic environments in the North/Central Andes (Givnish et al. 2011). The hypothesis of a C_3 origin of the subfamily is corroborated by the similarity to the sister group Puyoideae, about 200 species with Andean distribution, prevalently performing C_3 photosynthesis (Crayn et al. 2004; Jabaily and Sytsma 2013). The evolution of the water-saving CAM physiology might have allowed early bromelioids to successfully expand and diversify in semi-arid biomes such as the Brazilian Cerrado and Caatinga, and clearly contributed to their success as epiphytes. Thus, the correlated increase of the speciation rate can be attributed to the improved tolerance to drought, elevated evaporation, and highly variable environments that opened new ecological opportunities for speciation in areas with scarce precipitations (e.g., the Cerrado), irregular water availability (e.g., rocky outcrops), or subject occasional periods of drought (e.g., Atlantic forest; Crayn et al. 2004; Lüttge 2010; Quezada and Gianoli 2011).

TANK HABIT

A strong key innovation effect was detected in correlation to the evolution of the water-impounding tank. Tank-less Bromelioideae represent a group of early diverging lineages that today include over 200 species, whereas the tank habit is gained and shared by a group that today comprises over 600 species (Fig. 1; Luther 2012). The small and symmetric transition rates estimated by BiSSE–BMA indicate that this trait changed very rarely throughout the diversification of the subfamily. The reconstructed evolution of the tank showed that the ancestral Bromelioideae were tank-less plants like their sister group Puyoideae and that the evolution of the tank habit appeared concurrently with the emergence and diversification of the core bromelioid clade, thus corroborating earlier findings (Schulte et al. 2009; Givnish et al. 2011).

Our analyses revealed that the increased net diversification of tank-forming Bromelioideae derived from a drastic decrease of the extinction rate in comparison to the tank-less lineages. Key innovation effects deriving from decreased extinction have been postulated as the result of either improved dispersal capability and wider geographic distribution, or more efficient defense against predation (Vamosi and Vamosi 2011 and citations therein). In Bromelioideae, the lower extinction rates in tank-less lineages can be partly ascribed to the first hypothesis, which considers the effects of a key innovation in the light of the species biogeography. The majority of tank-less species inhabit semiarid to arid open habitats and are especially diverse in the Brazilian Cerrado (Smith and Downs 1974; Schulte et al. 2009). As terrestrial or lithophytic, slow growing xerophytes they are weak competitors and only successful at sites where most other plants fail or struggle to survive (such as on rock cliffs, open sandy soils). The tank-less bromelioids are able to endure harsh environmental conditions but field and greenhouse observations suggest that their realized niche lies at the edge of their physiological tolerance. The tank-forming Bromelioideae, in contrast, are able to grow as epiphytes, because they possess a structure, the tank, in which the leaves can collect and absorb the water, allowing them to collect and uptake water directly from the precipitations, thus freeing the plants from their dependence on the roots for water uptake (Benzing 1990, 2000). These features grant them access to the environmentally more favorable canopy of the tropical rainforests, in particular the Brazilian Atlantic forest, where they contribute substantially to its extraordinary biodiversity (Kress 1989; Benzing 1990).

Over the past 10 million years vegetation changes have deeply affected the extension and continuity of the Atlantic forest and the Cerrado domain, by strong climatic oscillations during the Pleistocene, which lead to repeated expansions and contractions of both rainforests and Cerrado (Pennington et al. 2004; Antonelli et al. 2009; Carnaval et al. 2009; Hoorn et al. 2010; Antonelli and Sanmartín 2011). Phases of higher aridification and potentially increased frequency and intensity of fire might have had a deeper impact on species occurring in the already dry biomes thus causing a higher proportion of extinction among the tank-less bromelioids (Simon et al. 2009). Thus, the lower extinction rates in tank-forming Bromelioideae might be the result of their ability to find shelter in overall more favorable and mesic environments, such as the Atlantic rainforest.

Our findings showed that the processes of speciation and extinction might be responding differently to different factors (Vamosi and Vamosi 2011) and that phylogeny-based analyses of the trait-correlated diversification can be used to compare the effects of putative key innovations. In this study, we showed that two intrinsic traits contributed significantly to shaping the diversity of Bromelioideae. Although both the CAM photosynthetic pathway and the water-impounding tank were found to correlate with increased net diversification within the bromeliad subfamily, the two traits had different impacts on the speciation and extinction rates. The evolution of the water saving CAM physiology, which may be linked to the evolutionary success of Bromelioideae in tropical environments (Benzing 2000; Givnish et al. 2011), was found correlated with a twofold increase of the speciation rate. However, the tank habit allowed the development of the epiphytic life form by providing an alternative system of water storage and absorption and freeing the plants from the dependence on the roots for water uptake (Benzing 1990; Gravendeel et al. 2004). Thus, tank-forming bromelioids gained the ability to successfully compete in the canopy of the tropical rain forests where they diversified under a low extinction rate. On the contrary, the terrestrial, tank-less lineages underwent a five times higher extinction rate, being exposed to harsher environmental conditions in semi-arid biomes such as the Cerrado and Caatinga.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Dated phylogenetic reconstruction of Bromelioideae obtained with a Bayesian relaxed molecular clock.
Figure S2. Phylogenetic reconstruction of Bromelioideae obtained with a maximum parsimony analysis.
Figure S3. Phylogenetic reconstruction of Bromelioideae obtained with a maximum likelihood analysis.
Figure S4. Phylogenetic reconstruction of Bromelioideae obtained with a Bayesian analysis.
Figure S5. Posterior estimates of speciation rates (blue), extinction rates (green), and state-transition rates (gray) associated with the evolutionary changes of the photosynthetic pathway (C_3/CAM) and with the presence/absence of the tank habit.
Table S1. Studied material.
Table S2. List of the 40 models with different constraint settings tested for the C_3/CAM binary trait, sorted by their marginal likelihood.
Table S3. List of the 40 models with different constraint settings tested for the presence absence of tank habit binary trait, sorted by their marginal likelihood.
Table S4. The mean relative likelihoods of the ancestral states (C_3/CAM; tank present/absent) were calculated over 1000 maximum likelihood reconstructions based on the BiSSE–BMA posterior rate estimates along with the respective standard deviations.