

Accelerated diversification and functional trait evolution in Velloziaceae reveal new insights into the origins of the *campos rupestres*' exceptional floristic richness

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- **Background and Aims** The greater diversity of plant clades in the Neotropics compared to their relatives in Africa is a pervasive pattern in biogeography. To better understand the causes of this imbalance, we studied the diversification dynamics of the monocot family Velloziaceae. In addition to being conspicuously richer in the Neotropics compared to the Palaeotropics, many species of Velloziaceae exhibit extreme desiccation tolerance (i.e. 'resurrection' behaviour), and other ecological specializations to life on rocky outcrops, poor sandy soils, open vegetation and seasonally dry climates. Velloziaceae is also ecologically dominant in the *campos rupestres*, a habitat having exceptionally high plant diversity and endemism in Brazil.
- **Methods** We reconstructed a densely sampled time-calibrated molecular phylogeny and used state-dependent and state-independent models to estimate rates of lineage diversification in relation to continent-scale geographical occurrence and functional traits associated with desiccation tolerance and water storage capacity.
- **Key Results** Independent shifts to faster diversification occurred within two Neotropical lineages, *Vellozia* and *Barbacenia*. The *Vellozia* radiation was associated with the presence of conspicuous aerial stems, and was followed by decreasing diversification rates during the Oligocene, a time of rising global temperatures and expanding open areas around the world. The *Barbacenia* radiation was faster and more recent, occurring during the cooling conditions of the Miocene, and associated with the acquisition of aquiferous parenchyma on the leaves.
- **Conclusions** High species richness of Velloziaceae in South America has been driven by faster diversification in lineages predominantly occurring in the *campos rupestres*, putatively by the evolution of adaptive strategies in response to independent climatic events. The radiation of *Vellozia* in particular might have played a key role in the assembly of the *campos rupestres* vegetation.

Key words: Adaptive radiation, *Barbacenia*, ecological strategies, Pandanales, mountain diversity, Neotropics, *Vellozia*.

INTRODUCTION

The unevenness of biodiversity across geographical regions is famously exemplified by the latitudinal gradient of species diversity (Wiens and Donoghue, 2004; Mittelbach *et al.*, 2007), but other disparities are similarly compelling. Within the tropics, a conspicuous biogeographical pattern is the depauperate species richness of Palaeotropical lineages compared to their Neotropical relatives, as seen in palms, bromeliads, Chrysobalanaceae, Malpighiaceae and Vochysiaceae (Hughes *et al.*, 2013; Linder, 2014; Couvreur, 2015). Most of these groups are found in tropical rainforests and the origins of extant species are concentrated in the Quaternary (Hughes *et al.*, 2013; Couvreur, 2015). By contrast, alternative habitat-specific diversification patterns have also been documented in seasonally dry and open habitats (Pennington *et al.*, 2006; Simon *et al.*, 2009; Hughes *et al.*, 2013; Koenen *et al.*, 2013; Pennington and Lavin, 2016). In addition, open upland and montane habitats

in both the Palaeo- and Neotropics display astonishing plant diversity and endemism, as exemplified by the Páramos in the Andes (Madrñan *et al.*, 2013) and the Eastern Afromontane biodiversity hotspot in Africa (Mittermeier *et al.*, 2004).

The distinctive open vegetation of *campos rupestres* (literally, 'rocky fields') that occur in the highlands along the Espinhaço Range in eastern Brazil is another example of exceptional plant diversity in mountains (Giulietti and Pirani, 1988; Fiaschi and Pirani, 2009). *Campos rupestres* cover less than 1 % of Brazil's area (Sano and Almeida, 1998; Supplementary Data Fig. S1), but its flora has 1951 endemic species, 40 % of its total; it is one of the most species-rich vegetation types recognized by the Brazil Flora Group (BFG, 2015). It extends from the transition zone between the Cerrado *sensu lato* and the Atlantic rainforest vegetation types in the south, to the xeric *caatinga* in the north, and westward to the Chapada dos Veadeiros as disjunct patches within the Central Brazilian Cerrado *sensu stricto* (Giulietti and Pirani, 1988; Fiaschi and Pirani, 2009). At broad scales, *campos*

rupestris habitats have often been included within the Cerrado domain rather than in the Atlantic Forest complex based on their herbaceous and shrubby physiognomy (WWF, 2012). However, more precisely it is a distinctive vegetation type within the Cerrado domain (BFG, 2015; Pirani *et al.*, 2015; Rando *et al.*, 2016). This unique flora has been intensively studied from a taxonomic perspective (i.e. BFG, 2015; Pirani *et al.*, 2015), but our understanding of the assembly and evolutionary history of the *campos rupestris* remains fragmentary (Conceição *et al.*, 2016).

The *campos rupestris* habitat occurs on high plateaus and isolated mountain peaks separated by lowland vegetation (Giulietti and Pirani, 1988; Giulietti *et al.*, 2005), similar to other montane sky island systems around the world (Hughes and Eastwood, 2006; Mairal *et al.*, 2017). This leads to the expectation of plant radiations frequently associated with mountains, driven by orogeny and isolation of habitat patches (Hughes and Atchison, 2015; Xing and Ree, 2017). Several studies have suggested this kind of vicariant divergence in *campos rupestris* lineages (e.g. Trovó *et al.*, 2013; Rando *et al.*, 2016), but there is a lack of evidence of a causal link between these geographical patterns and orogeny (see Conceição *et al.*, 2016). The few studies that have inferred time-calibrated phylogenies for *campos rupestris* lineages found species diversification concentrated in the Miocene (Rapini *et al.*, 2007; Antonelli *et al.*, 2010) or Pleistocene (de Souza *et al.*, 2013; Ribeiro *et al.*, 2014; Rando *et al.*, 2016). These studies focused on specific lineages of Asclepiadoideae, Fabaceae and Orchidaceae that differ in their relative diversity in this habitat. However, many of the most conspicuous and diverse plant lineages in *campos rupestris*, e.g. Eriocaulaceae, Velloziaceae, Xyridaceae, and some genera within Asteraceae, Bromeliaceae and Melastomataceae (Giulietti and Pirani, 1988), have received little empirical attention (Fiaschi and Pirani, 2009). Specifically, absolute or relative diversification rate estimates for these lineages are still lacking (but see Simon *et al.*, 2009; Trovó *et al.*, 2013). Moreover, because Eriocaulaceae, Velloziaceae and Xyridaceae are notably more diverse in the Neotropics than in Africa (Giulietti and Hensold, 1990; Mello-Silva, 1995), the characterization of species diversification in these clades may thus aid our understanding of the origins and maintenance of differences between Palaeo- and Neotropical plant diversity, particularly for seasonally dry open shrubby or grassy habitats that have received less attention than tropical forests (Fiaschi and Pirani, 2009; Hughes *et al.*, 2013).

In addition to the archipelago-like distribution of the *campos rupestris*, similarities in habit, life form, leaf shape and pubescence among diverse *campos rupestris* taxa have generated hypotheses of convergent evolution and replicated adaptive radiations in this habitat (Coile and Jones, 1981; Harley, 1988). The freely drained sandy or rocky soils, together with high daily temperatures and seasonal rain regimes favour the establishment of resurrection and drought-resistant plants (Alcantara *et al.*, 2015; Oliveira *et al.*, 2016). These abiotic conditions have been considered the main drivers of adaptive divergence in *campos rupestris* (Harley, 1988). According to the traditional adaptive radiation hypothesis, episodes of explosive species accumulation in a lineage are associated with rapid ecological niche divergence followed by decreasing diversification rates through time (Linder, 2008; Gavrillets and Losos, 2009; Yoder *et al.*, 2010). To date, only one study has studied the role of ecological divergence in the diversification of a *campos rupestris*

lineage, *Minaria* (Asclepiadoideae), but did not find evidence for it (Ribeiro *et al.*, 2014). Support for the hypothesis of adaptive radiation in the *campos rupestris* thus remains elusive.

In this study, we focus on Velloziaceae (Pandanales), the most abundant and species-rich vascular plant family in the *campos rupestris* flora (Porembski and Barthlott, 2000; Conceição *et al.*, 2007a, b; Alcantara *et al.*, 2015; BFG, 2015). Its species generally occur on rocky and shallow soils, especially on rocky inselbergs in both Africa and South America. Most are heliophytes, and range from small trees and shrubs to small rosettes, large stem rosettes and graminoid forms (Ayensu, 1973; Mello-Silva, 1995) (Fig. 1). The family includes mat-forming species that can colonize exposed rock and facilitate the establishment of other plants (Porembski and Barthlott, 2000), tree-like species whose stems support specific epiphytes (Werneck and Espírito-Santo, 2002), and species with densely ramified underground stem-systems that facilitate the capture and concentration of nutrients in sandy soils (Oliveira *et al.*, 2015, 2016). It also displays specializations for survival in seasonally dry areas. For example, all the African species are desiccation tolerant, i.e. *resurrection* plants (Gaff, 1977; Porembski and Barthlott, 2000), and the South American species show seasonally variable drought resistance or desiccation tolerance (Aidar *et al.*, 2010). Several species from the *campos rupestris* are not desiccation tolerant, but are extremely drought resistant (Alcantara *et al.*, 2015). The diversity of water-related ecological strategies has been suggested as key traits underlying the dominance and distribution of Velloziaceae species in the *campos rupestris* (e.g. Alcantara *et al.*, 2015). It is also associated with the adaptive divergence and diversification of *campos rupestris* plants more generally (Harley, 1988; Giulietti and Pirani, 1988).

Phylogenetic investigation of traits, geography and diversification rate can be used to assess whether the imbalance between South American and African lineages of Velloziaceae is caused by higher diversification rates in the Neotropics, and to test the specific hypothesis of adaptive radiation in Neotropical clades. Velloziaceae includes approx. 265 species, of which about 80 % are restricted to the *campos rupestris* (Mello-Silva, 1995), all members of *Barbacenia* and *Vellozia*. These two genera each have approx. 120 species, few of which occur outside the *campos rupestris* (Mello-Silva, 2015). Other members of Velloziaceae include the small genus *Barbaceniopsis*, with 4 species restricted to Andean outcrops (Ibisch *et al.*, 2001), and the genus *Xerophyta* (approx. 34 species) which occurs on African inselbergs (Mello-Silva *et al.*, 2011). The only East Asian species, *Acanthochlamys bracteata*, has been consistently recovered as the sister group of the rest of the family (Behnke *et al.*, 2000; Mello-Silva, 2005; Chase *et al.*, 2006; Mello-Silva *et al.*, 2011).

Our first objective in this study is to reconstruct an improved and dated molecular phylogeny of Velloziaceae in order to infer lineage diversification rates through time and across clades. We want to understand whether the species imbalance between African and South American clades can be explained by different rates of speciation and/or extinction, and if changes in rate are temporally associated with changes in climate. We also wish to test for associations between diversification rates and putatively adaptive functional traits, namely the presence of conspicuous aerial stems and the presence of aquiferous parenchyma between vascular bundles in the leaves. These morphological traits are associated with important ecophysiological

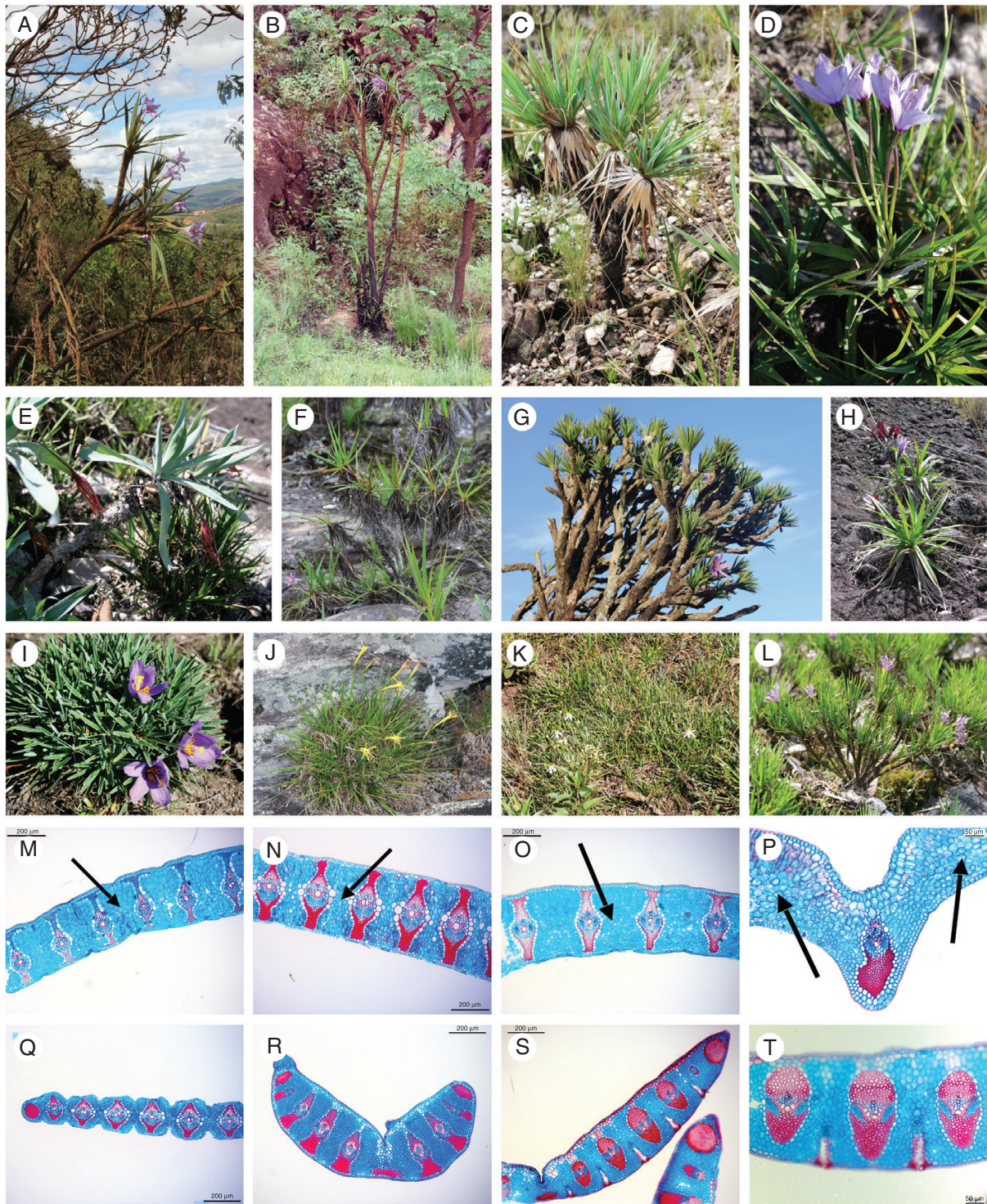


FIG. 1. Examples of the species and the functional traits analysed. (A–L) Velloziaceae species displaying absence (D, H, I, J and K) or presence (A, B, C, E, F, G and L) of conspicuous aerial stems. (M–T) Anatomical sections of the leaves, showing presence (M, N, O, P) or absence (Q, R, S, T) of the aquiferous parenchyma between the bundles on the leaves (indicated by arrows). Scale bars: 200 µm in M–O and Q–S; 50 µm in P and T. Blue colour indicates tissues with primary cell walls (parenchyma and phloem), red/magenta color indicates tissues with secondary cell walls (fiber and xylem). (A) *Vellozia aloifolia* Mart. (B) *Vellozia glabra* J.C.Mikan. (C) *Vellozia variabilis* Mart. ex Schult. & Schult.f. (D) *Vellozia caruncularis* Mart. ex Seub. (E) *Barbacenia spiralis* L.B.Sm. & Ayensu. (F) *Vellozia fruticosa* L.B.Sm. (G) *Vellozia compacta* Mart. ex Schult. & Schult.f. (H, M) *Barbacenia celiae* Maguire. (I) *Vellozia cryptantha* Seub. (J, Q) *Barbacenia riedeliana* Goethart & Henrard. (K, S, T) *Vellozia gramineae* Pohl. (L, R) *Vellozia semirii*. (N) *Barbacenia involucrata* L.B.Sm. (O, P) *Barbacenia glabra* Goethart & Henrard. Credits: A–D, F, G, J–L: S. Alcantara; E, I: R. Mello-Silva; H: J. G. Rando; M–T: K. Drequeceler.

strategies in this group. The presence of aquiferous parenchyma between vascular bundles in the leaves allows water storage and survival during longer seasonal dry periods (Warming, 1893; Diogo, 1926), while the development of conspicuous aerial stems is a surrogate of desiccation tolerance and plant productivity/competitive ability (Alcantara et al., 2015; Material S2). We hypothesize that these traits played a role in driving the diversification in Velloziaceae. We also evaluate if bursts of species diversification were followed by decreasing rates indicative of density-dependent diversification, i.e. an early burst and rate slowdown trajectory of diversification typical of an evolutionary radiation. In this sense, our second main objective is to test for specific signals of adaptive radiation, based on (1) an association between functional traits and diversification rate shifts and (2) investigation of the diversification rates dynamic itself. Since Velloziaceae is the most conspicuous floristic element in the *campos rupestres*, we also discuss the implications of our findings in the context of the assembly of the *campos rupestres* flora more generally.

MATERIAL AND METHODS

Taxon sampling and phylogeny reconstruction

We sampled a total of 150 species of Velloziaceae, represented by 158 accessions, and eight outgroup species, representing six families. We follow the phylogeny-based delimitations of the five genera in the family (Mello-Silva et al., 2011). Sampling included 48 of the 104 species accepted in *Barbacenia*, 77 of the 122 species accepted in *Vellozia*, complete sampling of *Acanthochlamys* and *Barbaceniopsis* (i.e. one and four species, respectively) and 20 of 34 species in *Xerophyta*. Estimates of species diversity for *Barbacenia* and *Vellozia* are based on the most recent accounts available on the Flora do Brasil website (Mello-Silva, 2015), with inclusion of the only Andean species that does not occur in Brazil (*Vellozia andina* Ibisch, R. Vásquez & Nowicki) and nine additional species (four of *Barbacenia* and five of *Vellozia*) recently described (Mello-Silva and Menezes, 2014; Mello-Silva and Sasaki, 2016). For the three other genera, we used data from The Plant List website (<http://www.theplantlist.org>, accessed 20 June 2015). In this sense, our sampling of species within genera can be considered phylogenetically random, since we did not focus on specific groups and/or characteristics.

In addition to published sequences of 46 Velloziaceae species and four outgroups from Mello-Silva et al. (2011), we sequenced reproductive specimens collected during extensive fieldwork carried out over approx. 3 years in Velloziaceae-rich areas of Brazil, in addition to cultivated individuals (i.e. most samples of *Xerophyta*, kindly provided by the Universität Bonn, Germany). This resulted in new molecular sequences for 112 Velloziaceae specimens, representing 104 species, and four additional outgroup taxa; sequences were deposited in GenBank and vouchers were deposited at SPF (Material S3). Fresh leaf tissues were dried in silica gel (Chase and Hills, 1991) and sequenced in the Pritzker Laboratory of Molecular Systematics at The Field Museum, Chicago, IL, USA. We performed total DNA extraction followed by amplification and sequencing of the following molecular markers: (1) *trnL*-intron – *trnL-F* spacer (Taberlet

et al., 1991); (2) *trnH-psbA* spacer (Shaw et al., 2005); (3) *atpB-rbcL* spacer (Chiang et al., 1998); and (4) internal transcribed spacer (ITS) nrDNA (Sun et al., 1994). Laboratory procedures and primers used for amplification are described in Material S3. The phylogenetic utility of these markers was previously demonstrated by Mello-Silva et al. (2011). The raw chromatograms were assembled into contigs in Sequencher 2.0 (Gene Codes Corporation, Ann Arbor, MI, USA), and then were checked by eye and, when necessary, edited using the same software. We created an alignment for each marker using MUSCLE 3.8 (Edgar, 2004) with subsequent manual adjustments, and concatenated them into a single matrix.

We performed Bayesian phylogenetic reconstruction using MrBayes 3.2 (Ronquist et al., 2011), partitioning the combined matrix by gene, setting *Acorus gramineus* Aiton (Acoraceae) as the outgroup (Chase et al., 2006) and coding alignment indels as missing data. Substitution model parameters were unlinked across partitions. To account for model uncertainty, we used reversible-jump Markov chain Monte Carlo (RJMCMC) in MrBayes to integrate over all sub-models of the GTR class, allowing both among-site rate heterogeneity (Γ) and invariant sites (I), in proportion to their marginal posterior probabilities (Huelsenbeck et al., 2004). Four simultaneous chains were run starting from random trees for 10 million generations, sampling every 500 generations. We used the average standard deviation of split frequencies, Potential Scale Reduction Factor of each parameter (PSFR), average effective sample sizes (ESS) and visual examination of the likelihood trace in the software as provided by Tracer 1.6 (Rambaut et al., 2013) to evaluate stationarity and convergence of the runs, discarding the first 25 % of samples as burn-in. Posterior probabilities of clades were summarized on the majority-rule consensus tree.

Estimation of divergence times

We estimated a time-calibrated phylogeny of Velloziaceae by Bayesian inference using relaxed molecular clock models in BEAST 2.2 (Bouckaert et al., 2014). We based topological priors (i.e. the monophyly of Velloziaceae and of Pandanales) on the results from the non-clock MrBayes analysis. We excluded the two most distant outgroups (*Acorus gramineus* and *Encholirium scutor*), as including them in exploratory runs led to very large confidence intervals for the root age of the tree, and much longer times until MCMC convergence.

There are no known fossils of Velloziaceae, so for calibration purposes we used the oldest fossil known for the order Pandanales, represented in our matrix by members of Velloziaceae and three outgroups: *Cyclanthus bipartitus* Poit. ex A. Rich. and *Thoracocarpus bissectus* (Vell.) Harling (Cyclanthaceae), and *Pandanus pygmaeus* Thouars (Pandanales). We used standard procedures (Magallón, 2004; Iles et al., 2015) to assign the oldest fossil of Pandanales to the stem node of the order. To identify this fossil, we carried out an extensive review of the literature on monocot fossils, cross-referenced with a recent review of monocot fossils suitable for molecular dating (Iles et al., 2015). We obtained two candidates: inflorescences of *Mabelia* and *Nuhliantha* (Triuridaceae) dating from the Turonian period (93.5–89.3 Mya; Gandolfo et al., 2002), and an infructescence of Pandanales, *Gruenbachia pandanoides* (Herman and Kvacek,

2010), dating from 83.6–72 Mya. The position of *Gruenbachia pandanoides* within Pandanaceae is still uncertain (Iles et al., 2015), and the Turonian Triuridaceae fossils are older, so we used the latter to establish the minimum age of Pandanales. We specified a lognormal prior with mean $M = 15$ and standard deviation $S = 0.75$, resulting in a median of 101 Mya.

As with the MrBayes analysis, we partitioned the data by gene and used RJMCMC to average over substitution models using the BEAST add-on package RBS (Bouckaert et al., 2013). Models and clock rates were unlinked across the four data partitions. We used a birth–death tree prior and a relaxed, uncorrelated lognormal (UCLN) molecular clock (Drummond et al., 2006). All specifications for the analysis are included in the input file (available upon request). We performed four independent runs, each for 50 million generations and a sampling frequency of 5000. To check for stationary and convergence, we examined parameter ESS values and distributions in Tracer 1.6 (Rambaut et al., 2013). Results were combined using LogCombiner (distributed with BEAST 2.2), discarding 20 % as burn-in. We used TreeAnnotator (distributed with BEAST 2.2) and FigTree 1.4.1 (Rambaut, 2009) to produce the maximum clade credibility (MCC) tree, which we used for downstream analyses. We compared node ages recovered by this analysis to those of other studies based on different calibrations (Janssen and Bremer, 2004; Mennes et al., 2013; Hertweck et al., 2015; Magallón et al., 2015).

Diversification analyses

We analysed diversification rates on the MCC tree pruned of all outgroups and seven redundant terminals of six species (two terminals representing different morphological varieties of *Barbacenia flava* were retained). The final tree included 151 terminals (150 species).

We used this final tree as input to BAMM 2.5, which uses Bayesian RJMCMC to infer heterogeneity in rates of speciation, extinction and trait evolution on phylogenetic trees (Rabosky, 2014). To account for incomplete taxon sampling, we included sampling fractions of each genus of Velloziaceae. We ran BAMM for 10 million generations, sampling every 10 000 generations, and analysed the results using BAMMtools 2.1.6 (Rabosky et al., 2014). We discarded the first 10 % of samples as burn-in and verified chain stationarity by inspecting plots of the likelihood trace and calculating ESS values of parameters.

We used BAMMtools to summarize differences in diversification across the phylogeny of Velloziaceae, and calculated marginal odds ratios for rate shifts on individual branches and Bayes factors (BFs) for the number of distinct rate regimes (Shi and Rabosky, 2015). We recorded the distinct shift configurations in the 95 % credible set and identified the core shifts with BFs exceeding 5. We summarized the number of shifts in the 20 shift configurations that together account for > 50 % of the posterior distribution. We considered a marginal odds ratio > 50 as strong evidence favouring a shift in net diversification rate on a given branch. We performed macroevolutionary cohort analysis (Rabosky et al., 2014), identifying sets of taxa likely to share the same diversification regime. We calculated mean rates of diversification across the Velloziaceae phylogeny and generated rates-through-time plots for specific clades.

To cross-check these results we used MEDUSA, a maximum-likelihood approach of estimating diversification rate shifts (Alfaro et al., 2009), using the R (R Core Team, 2015) package geiger v. 2.0.6 (Harmon et al., 2015). We accounted for incomplete phylogenetic sampling by adding unsampled species to their respective genera (i.e. *Barbacenia*, *Vellozia* and *Xerophyta*).

We also tested directly whether speciation rates differ between African and South American clades of Velloziaceae by the Hidden-State Speciation and Extinction model (HiSSE) (Beaulieu and O'Meara, 2016). HiSSE extends previous state-dependent diversification models (e.g. BiSSE; Maddison et al., 2007) by allowing an unobserved binary character to influence diversification dynamics in addition to the observed character, mitigating the risk of Type I error (Rabosky and Goldberg, 2015; Beaulieu and O'Meara, 2016). We coded geography as a binary trait (occurrence in Africa vs. South America), pruning the sole Asian species, *Acanthochlamys bracteata*, from the tree. We used the R package hisse 1.1 (available at <https://cran.r-project.org/web/packages/hisse/index.html>) to evaluate the fit of alternative models by their average Akaike weight (w_i) (Burnham and Anderson, 2002) and estimate confidence intervals for parameters of the best-fit model. We evaluated two models: BiSSE, having no hidden states; and HiSSE-partial, in which only one observed state (i.e. 'South America occurrence', which accounts for most species and genera of Velloziaceae) can transition between 'hidden' states. Model fit was assessed by w_i (Burnham and Anderson, 2002).

Correlates of diversification: climate, morphology and ecology

We placed the rate shifts inferred by BAMM into geological periods and their associated climatic conditions (Jaramillo et al., 2006; Zachos et al., 2008). We also tested if the presence of aquiferous parenchyma between vascular bundles in the leaves and the development of conspicuous aerial stems were linked to diversification rate using the HiSSE model (Beaulieu and O'Meara, 2016) as described in the previous section. We evaluated three models: BiSSE, having no hidden states; HiSSE-partial, in which only one observed state can transition between 'hidden' states; and HiSSE-full, with unrestricted transitions. We also performed stochastic character mapping (Huelsenbeck et al., 2003) implemented in phytools (Revell, 2012) to estimate and visualize the phylogenetic history of these functional traits. For SSE analyses, in addition to considering the whole phylogeny, we conducted lineage-specific analyses to account for rate heterogeneity within Velloziaceae. We specifically focused on the two South American lineages of the family: (1) the genus *Barbacenia* and (2) the genera *Barbaceniopsis* + *Vellozia*, which emerge as sister clades and constitute the most diverse lineage of Velloziaceae.

RESULTS

Phylogeny and divergence time estimation

In total, the combined matrix used for phylogeny estimation had 166 terminals, representing 158 ingroups and eight outgroups, and 4037 characters, of which 2611 had informative variation.

The majority-rule consensus tree from the MrBayes analysis (Fig. S4) strongly supports the monophyly of Velloziaceae genera, as found by Mello-Silva *et al.* (2011). In general, infra-generic relationships are poorly supported in the two largest genera of Velloziaceae (*Barbacenia* and *Vellozia*) and are more strongly supported in the smaller genera *Barbaceniopsis* and *Xerophyta*.

The substitution models with highest posterior support for each data partition in the MrBayes and BEAST analyses are shown in Material S5. Heterogeneity in substitution rates estimated by BEAST indicates that the data do not fit a strict molecular clock model (Fig. S6). The BEAST MCC tree (Fig. S8) supports the same major clades as MrBayes (Fig. S4). Comparison with previous divergence time estimation studies (Table 1) shows congruence with the divergence dates obtained here (approx. 101 Mya) for the crown group age of Pandanales (i.e. the node used here for calibration). We estimated an age of 74.3 Mya (97–52 Mya) for the crown node of Velloziaceae (Fig. 2).

Diversification rates in Velloziaceae

Both BAMB and MEDUSA indicated heterogeneity in diversification rates across the phylogeny (Fig. 2 and Material S7). There is strong support for two rate shifts (Table 2 and Material S7). Although a three-shift model had the highest posterior probability with BAMB, its BF is only 3.7) over the two-shift model. Examination of the most credible rate shift configurations from BAMB (see Fig. 3A) indicates some uncertainty about the precise number and locations of rate shifts (Material S9), but it is clear that two occur *within* the two largest Neotropical genera, *Barbacenia* and *Vellozia* (Fig. 3B), a result also obtained by MEDUSA (Material S9). We present the mean (model-averaged) diversification rates estimated along every branch of the Velloziaceae tree by

BAMB (Fig. 2). In *Vellozia*, the shift is to a regime with higher initial net diversification that declines through time, while for *Barbacenia*, the shift is of greater magnitude to a higher constant rate (Fig. 2). We refer to the clades descending from these shifts as *core-Vellozia* and *core-Barbacenia*, respectively. Apart from these two clades, rates across the rest of the family are more or less homogeneous, except for weak signals of slowing diversification rates in the monotypic *Acanthoclamys* (node 1) (Figs 2 and 3; Material S9). This third rate shift has low support (BF = 1.83).

MEDUSA shows strongest support for two independent shifts in macroevolutionary regime (we report the results of the analysis including sampling fractions, because there was no difference from the analysis that omitted incomplete sampling). They are inferred to be more recent than those inferred by BAMB (Fig. 2), nesting within the *core-Barbacenia* and the *core-Vellozia* clades (Material S7).

Estimates of net diversification by BAMB are higher and more variable (uncertain) in the South American clades of Velloziaceae [λ : 0.18, quantiles (25–75 %): 0.15–0.25; net diversification: 0.17, quantiles: 0.125–0.195] than in the African clade (λ : 0.1, quantiles: 0.07–0.14; net diversification: 0.08, quantiles: 0.075–0.081). This is reflected in a relatively complex estimate of macroevolutionary cohorts in the family (Fig. S10), and in comparisons across genera and infra-generic clades of Velloziaceae (Fig. 4), as well as the variable rates of speciation and extinction estimated from BAMB simulations (Fig. S11). The geography-dependent diversification analysis corroborates more variable rates in South America, with the highest support for a model in which the rate of diversification there, in conjunction with a ‘hidden’ binary character (HiSSE-partial model, see Table 3), is about four times higher than the background diversification rates within Velloziaceae (i.e. the rate observed in both the African lineage and the South American representatives that are not associated with the ‘hidden’ state).

TABLE 1. Crown group (c.g.) and stem lineage (s.l.) ages (Mya) estimated from the fossil calibration carried out here and from previous literature available, with confidence interval indicated

Source	Pandanales s.l.	Pandanales c.g.	Velloziaceae s.l.	Velloziaceae c.g.	N	Study details
This study (UCLN)	131.1 (93–175)	101.24 (91–118)	101.24 (91–118)	74.3 (52–97)	150	Pandanales tree-dating, focus on divergence times of Velloziaceae, use of 1 fossil calibration point
Janssen and Bremer (2004) (NPRS)	124	114	108	14	2	Secondary calibration of the monocots tree-root, based on a previous calibration using 8 fossil points
Mennes <i>et al.</i> (2013) (UCLN)	110 (96–123)	92 (69–110)	NA	NA	2	Monocots tree-dating, focus on positioning of Triuridaceae and divergence dates of Pandanales, use of 4 fossil calibration points
Hertweck <i>et al.</i> (2015) (PL)	116 (110–122)	103 (95–111)	PL: ~90	NA	1	Monocots tree-dating based on 11 fossil calibration points
Magallón <i>et al.</i> (2015) (UCLN & PL)	UCLN: 110.5 (96.5–123) PL: 118.31 (114–121)	UCLN: 72.35 (53–100) PL: 103.44 (99–109)	UCLN: 72.35 (53–100) PL: 103.44 (99–109)	NA	4	Angiosperms tree-dating, focusing on the ages of angiosperms families and based on 137 fossil calibration points

N: number of species of Velloziaceae sampled in each study. UCLN: estimations based on BEAST analyses using a UCLN model. PL: point age estimations on a maximum likelihood (ML) tree with penalized likelihood method. NPRS: estimations based on non-parametric rate smoothing. NA: not applicable.

TABLE 2. Pairwise Bayes factor matrix computed for models with different rate shift configurations simulated over the Velloziaceae dataset (k = number of rate shifts)

k	0	1	2	3	4	5	6	7	p.p.
0	1.00	0.12	0.001	0.0004	0.0003	0.0002	0.0003	0.0003	0.001
1	8	1.00	0.01	0.003	0.002	0.002	0.003	0.003	0.004
2	696	87	1.00	0.27	0.20	0.21	0.26	0.24	0.190
3*	2576	322	3.7	1.00	0.74	0.76	0.98	0.87	0.360*
4	3504	438	5.03	1.36	1.00	1.03	1.33	1.19	0.240
5	3392	424	4.87	1.32	0.97	1.00	1.29	1.15	0.120
6	2624	328	3.77	1.02	0.75	0.77	1.00	0.89	0.046
7	2944	368	4.23	1.14	0.84	0.87	1.12	1.00	0.026
8	2816	352	4.05	1.09	0.80	0.83	1.07	0.96	0.012

Values above the diagonal represent the prior probability of the corresponding model with k shifts. Values below the diagonal represent the Bayes factor (BF) evidence in favour of the model with k shifts (indicated by the list number) relative to the simpler model with k shifts (indicated in the column number). Last column indicates the relative posterior distribution (p.p.) of the models with k shifts. Usually, the overall best model from a BMM analysis is the model with the highest BF relative to the null model, M_0 ($k = 0$). However, model probabilities for rarely sampled models are probably inaccurate (<http://bamm-project.org/postprocess.html#bayesfactors>), which seems the case of the null model for the Velloziaceae dataset, and the posterior probabilities indicate that the model with three shifts (marked by *) is the most frequent among the 95 % credible set of rate shift configurations sampled with BMM (see text).

BF > 20: evidence for one model over another; BF > 50: very strong evidence in favour of the numerator model; BF < 5: weak evidence in favour of the numerator model.

TABLE 3. The fit of alternative models of geography-dependent diversification for the family Velloziaceae (trait states: Africa and South America occurrence, see text)

Model	Model specification	logL	AICc	Δ AIC	w_i
bisse.null	hidden.states=FALSE, rates=c(1,1,0,0), $N=4$	-476.75	961.77	30.55	2.1E-07
bisse	hidden.states=FALSE, rates=(1,2,0,0), $N=6$	-471.12	954.84	19.04	6.7E-05
null.hisse	hidden.states=TRUE, rates=c(1,1,2,2), $N=5$	-462.70	935.8	4.58	0.0919
hisse.partial	hidden.states=TRUE, rates=c(1,2,0,3), $N=10$	-455.82	931.22	0	0.9079

The best model fitted, based on Δ AIC and Akaike weights (w_i), is denoted in bold. AICc: Akaike information criterion corrected for sample size. N = number of parameters of each model; logL = log-likelihood of the model.

Correlates of diversification: climate, morphology and ecology

The inferred shift to higher diversification rates was more recent in *Barbacenia* (13.4 Mya; middle/late Miocene: 15–8 Mya) than in *Vellozia* (23.1 Mya; Oligocene/Miocene transition, 33–13 Mya; Fig. 2). These periods correspond to distinct climate scenarios, with the late Oligocene/early Miocene marked by increasing temperature and humidity culminating in the Middle Miocene Climatic Optimum (approx. 15 Mya), followed by drying/cooling climatic conditions in the late Miocene (Fig. 2).

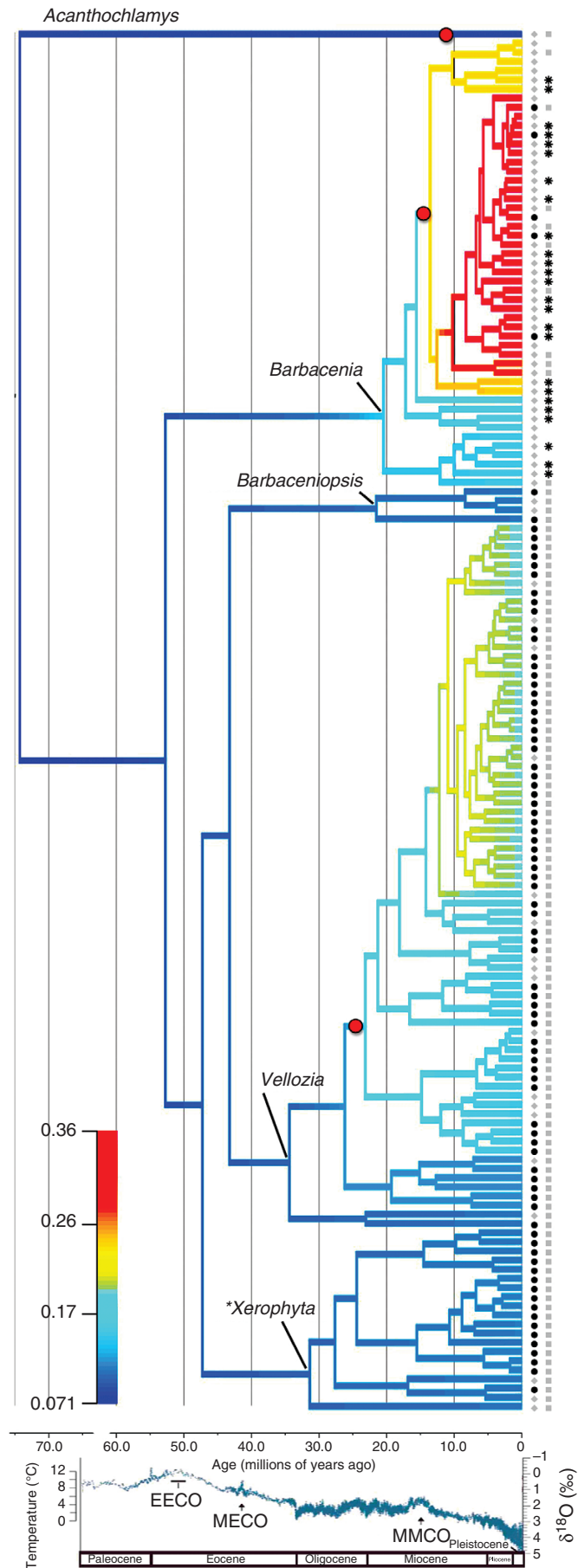
Ancestral state reconstructions (Figs S12 and S13) indicate that aerial stems and aquiferous parenchyma had already evolved in *Vellozia* and *Barbacenia*, respectively, by the time shifts to higher diversification occurred in each clade. The state-dependent diversification analyses give highest support to a model in which the acquisition of aquiferous parenchyma between bundles, in conjunction with a ‘hidden’ binary character, is associated with higher diversification rates in Velloziaceae as a whole (HiSSE-partial model, see Table 4). Our data indicated no variation of this trait in the most diverse lineage (*Vellozia* + *Barbaceniopsis* clade), with none of the species sampled in this clade showing the presence of aquiferous parenchyma between bundles.

HiSSE analyses do not support the hypothesis that the development of aerial stems is linked to higher diversification; instead, the best-fit model is one in which variation in diversification is

associated with a hidden binary trait, but not associated with aerial stems (HiSSE-null model; Table 4). Similarly, restricting the scope of the analysis to just *Barbacenia* yields no support for any state-dependent diversification model (BiSSE-null; Table 4). However, for the *Vellozia* + *Barbaceniopsis* clade, the best-fit model is HiSSE-partial, followed by HiSSE-full, and average net diversification of lineages with aerial stems is more than twice the rate of those without aerial stems.

DISCUSSION

Our primary results indicate that the disparity of species richness between Africa and South America in Velloziaceae is the result of accelerated species diversification within the two most diverse genera of the family (Fig. 2), which are restricted to the Neotropics. These rate shifts are significantly correlated with transitions in the states of functional traits (Fig. 1; Table 4; Figs S12 and S13), suggesting an adaptive component in these radiations. Diversification rate heterogeneity in the core-*Vellozia* clade is consistent with the density dependence expected in mature radiations and by traditional hypothesis of adaptive radiation. The timing of the two rate shifts in Velloziaceae, coupled with the signals of adaptive radiation in *Vellozia*, add new insights into the assembly of the *campos rupestres* habitat itself, as discussed below.



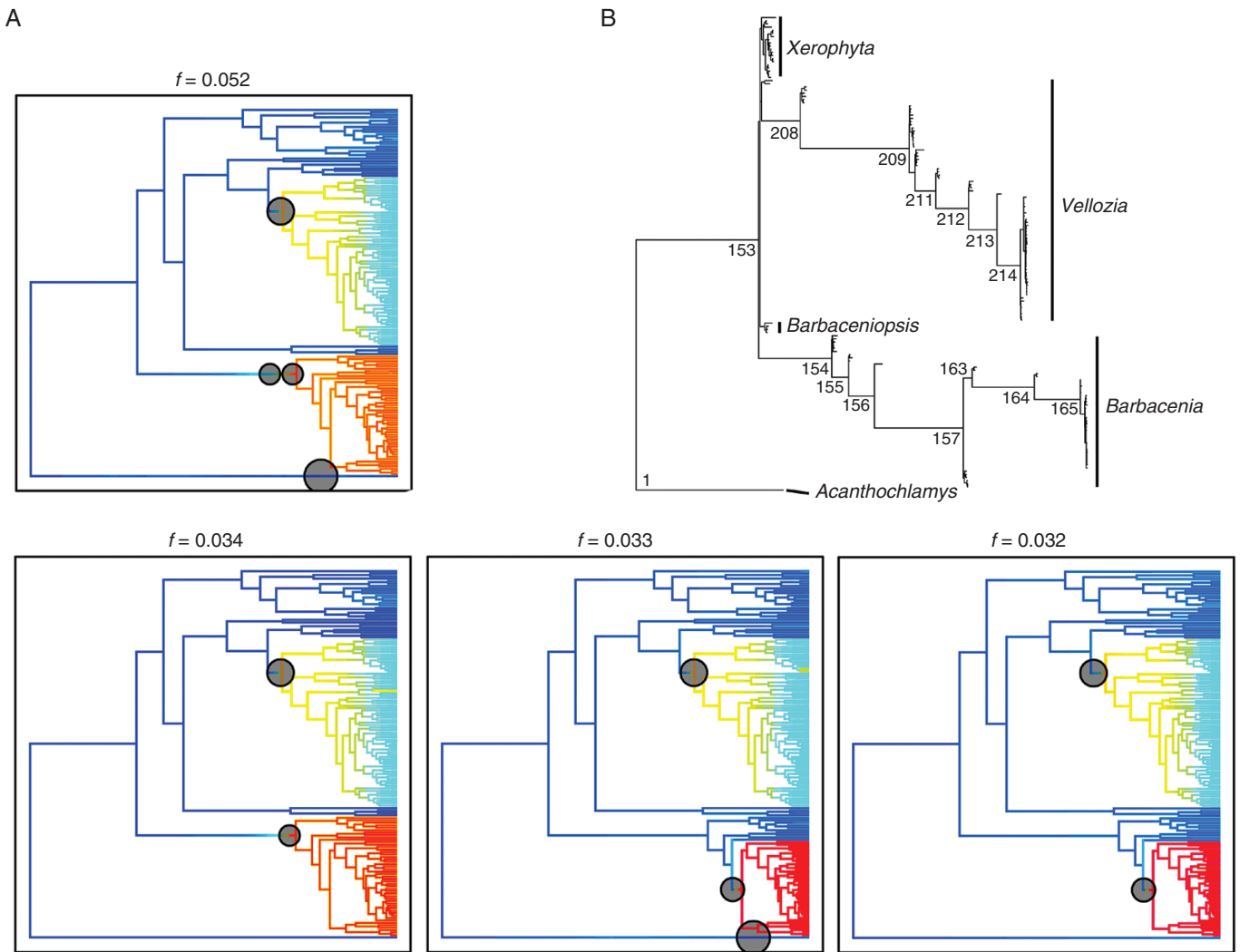


FIG. 3. BAMB results of rate shifts in Velloziaceae. (A) Top four most credible shift sets recovered by BAMB with associated probabilities (f). Grey circles indicate the location of estimated shifts. Rate values represent new lineages per million years, with rates increasing from blue to red (online version). (B) Phylogeny of Velloziaceae with branch lengths transformed to Bayes-factor support for rate shifts.

African versus South American diversification of Velloziaceae

The diversification analyses clearly support the hypothesis that the imbalance between Palaeo- and Neotropical floristic diversity results from higher speciation rates in South America (e.g. Gentry, 1982; Linder, 2014; Couvreur, 2015). However, occurrence in Africa versus South America per se does not explain much of the variation in diversification of Velloziaceae. Instead, rate heterogeneity is nested within the South American clades, with independent shifts in *Vellozia* around 23 Mya and in *Barbacenia* around 13 Mya. Similar patterns of higher speciation in Neotropical lineages have been shown in other plant groups centred in Andean and Amazon

regions (e.g. Bell and Donoghue, 2005; Hughes and Eastwood, 2006; Bardon et al., 2013; Koenen et al., 2015). The main biogeographical factors proposed to explain higher speciation in the Neotropics are area, time for speciation and climatic stability (reviewed by Couvreur, 2015). However, climatic instability during the Quaternary resulting in population isolation in refugia has also been invoked to explain recent diversification in the region (Haffer, 1969; Prance, 1974). Here, neither the shifts nor average differences in the diversification rates seem to be related to overall clade ages, since the South American *Vellozia*–*Barbaceniopsis* clade and the African *Xerophyta* are sister clades (i.e. exhibit the same stem lineage ages) but

FIG. 2. Chronogram of Velloziaceae, highlighting net diversification rate (r) simulated by BAMB, with rates increasing from blue to red. Rate values represent new lineages per million years. The African clade is shown by an asterisk (*Xerophyta*) and states of the traits evaluated for association with diversification rates are displayed in the tips. Trait A: development of conspicuous aerial stems; states: black circles: presence; grey diamonds: absence. Trait B: aquiferous parenchyma between vascular bundles in the leaves; states: black asterisks: presence; grey squares: absence. Bottom graphic shows global mean temperature according to Zachos et al. (2008). EECO: Early Eocene Climatic Optimum; MECO: Mid-Eocene Climatic Optimum; MMCO: Mid-Miocene Climatic Optimum. Age (in million years) and geological epoch are indicated at the bottom of the phylogeny and temperature graphic, respectively.

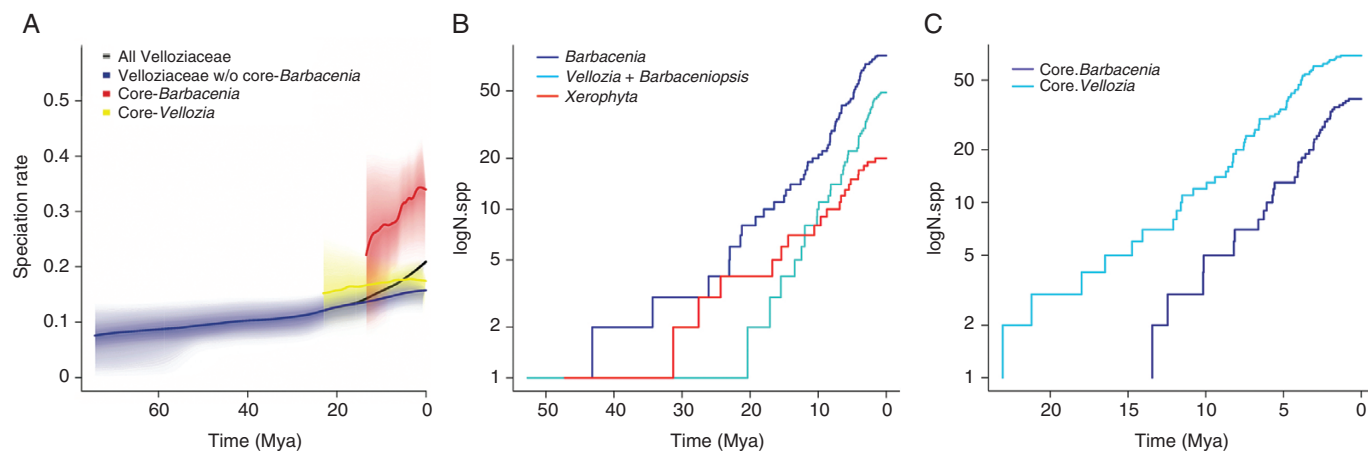


FIG. 4. (A) Estimated speciation rates in Velloziaceae lineages and (B, C) estimated logarithm of the number of species ($\log N.spp$) accumulation through time (in millions of years) for selected lineages of Velloziaceae. All Velloziaceae: includes all five genera of the family; core-*Barbacenia* + core-*Vellozia*: the infra-generic clades within these genera exhibiting the highest probability for having suffered shifts in their diversification rates relative to the background rates simulated by BAMM analyses (delimited by nodes 157 and 209, respectively).

exhibit different diversification dynamics (Fig. 2). In addition, the South American genus *Barbacenia*, which split from the [*Vellozia*–*Barbaceniopsis*]–*Xerophyta* clade approx. 10 Myr earlier (i.e. showing the older stem age between the three major clades of Velloziaceae), is the clade showing the most recent crown age and the highest diversification rate in the family (Fig. 2). The total geographical range of *Xerophyta* in Africa is larger than that of the South American genera of Velloziaceae, and range sizes of individual species also tend to be larger in Africa compared to the *campos rupestres* endemics in South America (Mello-Silva et al., 2011); however, it is difficult to speculate whether small-scale geographical range has influenced diversification rates.

Most diversification in Velloziaceae is concentrated within the Neogene (mainly in the Miocene), although the origin estimated for the stem lineages of the genera occurring in both South America and Africa dates from the early Eocene (approx. 55–40 Mya). This pattern is consistent with expectations of ancient origins and persistence of old lineages under a ‘museum’ hypothesis for the Neotropics. Even considering the alternative scenarios with more recent rate shifts (as reconstructed by MEDUSA), diversification in Neotropical Velloziaceae is still older than that predicted by higher diversification rates caused by Pliocene/Quaternary climatic events. The relatively homogeneous background rates of diversification in Velloziaceae suggest that catastrophic events did not cause higher extinction rates in Africa than in other tropical areas, as proposed by Raven and Axelrod (1974). Indeed, evidence for catastrophic climatic events in Africa is largely lacking (Couvreur, 2015) and many studies have found bursts of diversification since the early Oligocene to the Miocene in African clades (Schnitzler et al., 2011; Linder, 2014), similar to our findings in Velloziaceae. Despite being relatively old, such shifts in diversification linked to environmental changes contradict the ‘museum’ model of tropical biodiversity, which assumes steady diversification under climatic stability (see Koenen et al., 2015).

Current evidence, including our results, suggests that independent bursts of diversification across time, in different

lineages, have generated spatial imbalances in tropical diversity, instead of a single factor (Linder, 2008; Hughes et al., 2013; Couvreur, 2015). Likewise, multiple nested rate shifts accounting for the high diversity of angiosperm lineages (Magallón and Sanderson, 2001) appear to be a general pattern, instead of single rate shifts explaining exceptionally species-rich plant groups (Smith et al., 2011; Koenen et al., 2013). This study is one of the first to show that faster diversification underlies higher Neotropical diversity in lineages from open areas, as most studies to date have focused on rain forests or dry forests (Hughes et al., 2013). A notable exception is Leguminosae, in which rate shifts have been documented in Neotropical lineages found in grasslands and savannas (Simon et al., 2009; Koenen et al., 2013), as well as in the *campos rupestres* and Andean *páramos* (Drummond et al., 2012; Koenen et al., 2013; de Souza et al., 2013; Rando et al., 2016). Likewise, contrasts in diversity in these legume clades are closely associated with particular distinctive ecologies, and potentially with ecological (biome) shifts, rather than with the strict geographical history of clades (Lavin et al., 2004; Koenen et al., 2013). Our results also imply that continental vicariance did not directly influence Velloziaceae diversification, as the pattern of rate shifts is more complex, independently nested within the two South American lineages, and requiring hidden states unrelated to continental areas of occurrence per se. Vicariance may have been important, however, to the diversification of the Andean representatives of the family (Mello-Silva et al., 2011): the genus *Barbaceniopsis* and the species *Vellozia andina* split from their sister lineages around 23–21 Mya, concomitant with the initial South/Central Andean uplift (Hoorn et al., 2010).

Vellozia: adaptive radiation in the campos rupestres?

The core-*Vellozia* clade shows a trajectory of diversification in line with a ‘mature’ evolutionary radiation (*sensu* Linder, 2008), i.e. an initial burst followed by declining rates. This could reflect niches filling toward an equilibrium (Benton and Emerson, 2007; Rabosky, 2014), and/or greater extinction as

TABLE 4. The fit of alternative models of state-dependent diversification for the family Velloziaceae (traits: aquiferous parenchyma and development of aerial stems) and for the two most diverse subclades of the family (trait development of aerial stems only, see text)

Lineage - trait	Model specification	logL	AICc	ΔAIC	w_i
Model					
Velloziaceae - aquiferous parenchyma					
bisse.null	hidden.states=FALSE, rates=c(1,1,0,0), N=4	-516.38	1041.03	11.776	0.0027
bisse	hidden.states=FALSE, rates=(1,2,0,0), N=6	-513.21	1039.001	9.747	0.00756
null.hisse	hidden.states=TRUE, rates=c(1,1,2,2), N=5	-527.92	1066.258	37.004	9.1E-09
hisse.partial	hidden.states=TRUE, rates=c(1,2,0,3), N=10	-503.83	1029.254	0	0.9895*
hisse.full	hidden.states=TRUE, rates=c(1,2,3,4), N=12	-509.85	1045.98	16.726	0.0002
Velloziaceae - development of aerial stem					
bisse.null	hidden.states=FALSE, rates=c(1,1,0,0), N=4	-543.53	1095.34	20.57	3.4E-05
bisse	hidden.states=FALSE, rates=(1,2,0,0), N=6	-542.78	1098.16	23.39	8.3E-06
null.hisse	hidden.states=TRUE, rates=c(1,1,2,2), N=5	-532.17	1074.77	0	0.9999*
hisse.partial	hidden.states=TRUE, rates=c(1,2,0,3), N=10	-538.5	1098.59	23.82	6.7E-06
hisse.full	hidden.states=TRUE, rates=c(1,2,3,4), N=12	-537.44	1101.16	26.39	1.9E-06
<i>Barbacenia</i> - development of aerial stem					
bisse.null	hidden.states=FALSE, rates=c(1,1,0,0), N=4	-149.18	307.28	0	0.8165*
bisse	hidden.states=FALSE, rates=(1,2,0,0), N=6	-149.2	312.40	5.12	0.0632
null.hisse	hidden.states=TRUE, rates=c(1,1,2,2), N=5	-149.86	311.12	3.83	0.0002
hisse.partial	hidden.states=TRUE, rates=c(1,2,0,3), N=10	-149.18	324.16	16.88	0.1202
hisse.full	hidden.states=TRUE, rates=c(1,2,3,4), N=12	-149.19	331.04	23.76	5.7E-06
<i>Vellozia</i> + <i>Barbaceniopsis</i> - development of aerial stem					
bisse.null	hidden.states=FALSE, rates=c(1,1,0,0), N=4	-292.75	594.03	10.04	0.0045
bisse	hidden.states=FALSE, rates=(1,2,0,0), N=6	-287.46	588.09	4.09	0.0875
null.hisse	hidden.states=TRUE, rates=c(1,1,2,2), N=5	-287.79	586.38	2.38	0.2052
hisse.partial	hidden.states=TRUE, rates=c(1,2,0,3), N=10	-280.93	583.99	0	0.6759*
hisse.full	hidden.states=TRUE, rates=c(1,2,3,4), N=12	-280.93	590.44	6.44	0.0269

The best model fitted, based on ΔAIC and Akaike weights (w_i), is denoted by *. AICc: Akaike information criterion corrected for sample size. N = number of parameters of each model; logL = log-likelihood of the model.

niches become more finely divided among proliferating species (Jansson and Dynesius, 2002; Linder, 2008). A previous study found that diversification of water-related adaptations could be important to niche divergence in *Vellozia* (Alcantara et al., 2015). The development of conspicuous aerial stems (as a surrogate trait for drought resistance and loss of desiccation tolerance in these species) associated with higher diversification rates in representatives of the *Vellozia* + *Barbaceniopsis* clade corroborates this (Fig. 2).

The Oligocene/Miocene transition time inferred for the diversification increase in *Vellozia* is coincident with a global increase in temperature, fossil angiosperm pollen diversity, particularly of Poaceae and Asteraceae, and areas of C_3 grassland dominance (Jaramillo et al., 2006). We hypothesize that in this environment, the development of conspicuous aerial stems (associated with drought resistance) allowed greater plant productivity and size (Alcantara et al., 2015), and enabled *Vellozia* to escape restricted occurrence in exposed outcrops and expand to open grasslands. It would have allowed the colonization of warmer microhabitats with a deeper soil layer, surrounding the exposed rock outcrop habitats characteristic of *campos rupestres*. In these soils, seasonal water availability is less restricted, and consequently the competition with other grassland-related species more intense (Alpert, 2006). Thus, this ecological release can be seen as a key opportunity for *Vellozia* radiation. A better comprehension of the adaptive radiation of *Vellozia* in the *campos rupestres* will require a deeper understanding of the origins of alternative drought resistance strategies in the family. It will also be important to consider

how other ecological features, such as responses to nutrient soil availability (Ayensu, 1973; Oliveira et al., 2016) and altitude (Mello-Silva, 1995), root specializations (Oliveira et al., 2015), fire resistance (Conceição and Orr, 2012), and interactions with pollinators and herbivores (Sazima and Sazima, 1990; Landau et al., 1999; Jacobi and Sarto, 2007), might have contributed to diversification in *Vellozia*.

The ecological diversity and endemism of *Vellozia* and other plant clades of the *campos rupestres* have been long cited as examples of adaptive radiation (Coile and Jones, 1981; Harley, 1988). Givnish (2015) argues that adaptive radiation does not require explosive diversification in its initial phase; instead, its evolutionary signature is simply closely related to species occupying different ecological niches in sympatry, or to a convergence of adaptive life forms in distantly related species of different lineages occupying similar niches in different locations. The only previous study to test for adaptive diversification in a *campos rupestres* lineage found no signal of ecological divergence, despite the fact that *Minaria* (Apocynaceae) radiated only around 1.5 Mya (Ribeiro et al., 2014). Other recent studies have inferred dated molecular phylogenies of *campos rupestres* lineages and found patterns that suggest evolutionary radiations, but did not explicitly test adaptive hypotheses (Koenen et al., 2013; de Souza et al., 2013; Rando et al., 2016). Our understanding of the evolution and distribution of ecological strategies in this habitat is still in its infancy, preventing a detailed assessment of how ecological divergence has contributed to lineage diversification. Thus, further studies focusing on the evolution and characterization of life forms

(e.g. Mello-Silva, 1995; Alves and Kolbek, 2010) and ecological strategies in *Vellozia* (Oliveira et al., 2005, 2015, 2016; Teodoro, 2014; Alcantara et al., 2015), within this background of diversification rate slowdown, are promising routes toward a better understanding of adaptive radiation in the *campos rupestres*.

Diversification in Barbacenia: habitat specialization and ongoing high speciation

The rate shift inferred within *Barbacenia* between 15 and 8 Mya (approx. 13.4 Mya) is consistent with a ‘pull-of-the-present’ effect observed in constant birth–death models (Stadler, 2013) and a high turnover rate characteristic of young radiations (*sensu* Linder, 2008). It does not exhibit the density-dependent signature of declining rates with the filling of ecological niche space (Morlon et al., 2011). Similar patterns have been observed in South African Aizoaceae (Klak et al., 2004) and South American *Lupinus* (Hughes and Eastwood, 2006), although the age of the shift in *Barbacenia* is notable for being relatively old compared to these and similar cases (see Linder, 2008). It coincides with the period following the Middle Miocene Climatic Optimum (approx. 15 Mya), marked by a drying/cooling climate (Zachos et al., 2008). In *Hoffmannseggella* (Orchidaceae), diversification was associated with the expansion of *campos rupestres* outcrops during the Miocene cooling, as surrounding forested areas retracted (Antonelli et al., 2010). In that case, populations restricted to the outcrops expanded their geographical range and reached secondary contact before the completion of reproductive isolation in allopatry, leading to speciation by hybridization (Antonelli et al., 2010). We have as yet no evidence that hybridization has contributed to diversification in *Barbacenia*. Alternatively, based on the restrictive conditions of life in exposed outcrops (e.g. Alpert, 2006; Porembski and Barthlott, 2000), we tested whether a morphofunctional trait associated with such stressing conditions is associated with higher diversification rates.

The acquisition of aquiferous parenchyma between bundles in the leaves, a trait that within Velloziaceae is mostly present in species of *Barbacenia*, could have improved survival in drier and colder conditions by increasing water accumulation within the plant (Warming, 1893; Menezes, 1973). The ensuing expansion of *Barbacenia* populations might have led to accelerated diversification by increased habitat specialization and allopatric isolation. Many species of *Barbacenia* exhibit very small distribution ranges (Mello-Silva, 2009), and several of those are restricted to shadow and colder rocky crevices/caves found near waterfalls, in contrast to the majority of Velloziaceae, which are heliophytes. This habitat specialization strategy might have provided special advantages in the relatively small competition pool in the outcrops (Alpert, 2006).

In summary, contrasting ecological strategies seem to have driven the radiations of *Barbacenia* and *Vellozia*. While a preference for rock outcrops generally characterizes Velloziaceae as a whole, *Vellozia* apparently escaped this ecological restriction by evolving drought resistance (as indicated by aerial stems). *Barbacenia*, by contrast, evolved the existent desiccation tolerance mixing it with water retention in the leaves (i.e. by the aquiferous parenchyma between bundles) and thus

became even more specialized to the outcrops during the colder and drier late Miocene. A better characterization of the habitat and fine-scale distribution of *Barbacenia* and *Vellozia* under a phylogenetic perspective is currently underway (S. Alcantara et al., in preparation) and will investigate these hypotheses in more detail.

Velloziaceae and the origin of campos rupestres

The spectacular floristic diversity of the *campos rupestres* raises compelling questions about its evolutionary origins and ecological dynamics. In this regard, understanding the evolutionary history of the Velloziaceae is tantamount to revealing the time and mode of this assembly. The association between the *campos rupestres* phytophysognomy and representatives of Velloziaceae is so emblematic that arborescent and showy flowered species of *Vellozia* are popularly known as ‘queens of the *campos rupestres*’. The macroevolutionary evidence for an adaptive *Vellozia* radiation helps us understand the diversification dynamics that underlie the extant huge species diversity in the *campos rupestres*.

The Oligocene/Miocene diversification burst of the core-*Vellozia* clade is coincident with the global increase of Poaceae and Asteraceae (Jaramillo et al., 2006), families that also have conspicuous representation in the *campos rupestres*. A general pattern of temporal congruence in the origin and diversification of other *campos rupestres* clades with *Vellozia* would reinforce the importance of the Oligocene increase in global temperature and C₃ grasslands to the origin of the *campos rupestres* in Eastern/Central Brazil. In addition, the *Barbacenia* radiation during the Miocene cooling corroborates the importance of the expansion of rocky outcrops for the diversification of this flora (Antonelli et al., 2010). Importantly, these results push back the origin of the *campos rupestres*, which has generally been inferred around the Miocene, based on inferred clade ages in Asclepiadoideae (Rapini et al., 2007). According to Conceição et al. (2016), these Miocene-age lineages would exhibit increasing diversification into the Quaternary, exemplified by recent Pleistocene diversification in *Calliandra* (Leguminosae) (de Souza et al., 2013), *Minaria* (Apocynaceae) (Ribeiro et al., 2014) and *Chamaecrista* (Leguminosae) (Rando et al., 2016). Quaternary diversification is in agreement with the ‘evolutionary pump’ model (Morton, 1972), proposed for West African tropical mountain vegetation and also invoked to explain the high plant diversity in the *campos rupestres*, as resulting from successive habitat expansion/retraction events in response to Pleistocene climatic oscillations (Harley, 1988, 1995; Alves and Kolbek, 1994; Giulietti et al., 1997; Rapini et al., 2008). It posits that mountaintops were interglacial refugia, accelerating divergence among vicariant populations during warmer and moister interglacial periods. During cooler and drier glacial periods, the *campos rupestres* would have expanded to lower altitudes, while forests would have retracted. Our results highlight the importance of global climatic oscillations in the diversification of both *Barbacenia* and *Vellozia*, although this diversification is much older than the Quaternary glacial cycles predicted by the ‘evolutionary pump’ model.

Habitats with similar vegetation physiognomies (i.e. outcrops inhabited by desiccation-tolerant plants, and surrounded

by patches of shallow soil dominated by grasses and xerophytic shrubs) and ecological disturbance (i.e. fire and seasonal rain regimes) around the world have been described as typically old, climatically buffered, infertile landscapes (OCBILs; Hopper, 2009). These habitats, such as the Pantepuis of the Guayana Shield, south-west Australia and the southern African savannas, are highly diverse and endemic-rich landscapes that may share ecological and evolutionary patterns with the *campos rupestres* of the Espinhaço Range (Conceição et al., 2016; Oliveira et al., 2016; Mucina, 2018). The origin of the Espinhaço Range landscapes culminated with crustal flexures and overlap caused by east–west pressure at the end of Neoproterozoic, around 900 Mya (Almeida-Abreu & Pflug, 1994), characterizing the long geological stability predicted for OCBILs (Hopper, 2009). Mucina and Wardell-Johnson (2011) refined this concept by proposing old stable landscapes (OSLs), where soil infertility is a function of landscape age. They also added fire regime predictability as a new dimension of the theory, because it affects the composition and function of biotas and shapes several biomes (Mucina and Wardell-Johnson, 2011). Thus, the OSL highlands would not be cooler and dryer refuges during Pleistocene interglacial periods, but rather refuges for fire-sensitive lineages since the Tertiary (Mucina, 2018). According to this hypothesis, OSL lineages are usually highly specialized, and display low dispersal capability and high phylogenetic conservatism (Mucina and Wardell-Johnson, 2011; Mucina, 2018). The climatic stability of OSLs allows the persistence of these lineages for long periods, characterizing these landscapes as refuges, cradles and/or ‘museums’ of biodiversity that shelter high concentrations of endemic diversity. The Espinhaço Range houses more than 10 % of plant species reported to Brazil and, on average, 40 % of them are endemic (BFG, 2015). In addition, the long-term persistence of South American Velloziaceae lineages (Fig. 2), their high phylogenetic conservatism and habitat specialization, especially in *Barbacenia*, is consistent with expectations for OSLs.

Based on the ecological similarities between OSL areas in the *campos rupestres* and in Africa, and the distribution patterns of the monocot families Eriocaulaceae, Xyridaceae and Velloziaceae, these groups have been previously interpreted as Gondwanan in origin (see Mello-Silva et al., 2011). However, the vicariance of South America and Africa (i.e. prior to 110 Mya) pre-dates our inferred crown age of Velloziaceae (i.e. approx. 75 Mya). The divergence of *Acanthochlamys* has been dated to the Cretaceous, too young to be attributed to the breakup of Gondwana. In addition, the stem ages of the African and South American Velloziaceae genera are concentrated between 55 and 40 Mya (Fig. 2), during the Palaeocene–Eocene Thermal Maximum (PETM; Zachos et al., 2008). All previous studies reporting molecular clock analyses that included Velloziaceae representatives also contradict a Gondwanan origin (see Table 1 and references therein).

Because of the fire-prone nature and open vegetation landscape, the *campos rupestres* have been considered part of the Cerrado (Simon et al., 2009; Trovó et al., 2013). The flora of the Cerrado was assembled from independent plant lineages from different biomes, mostly over the past 4–5 Myr (Simon et al., 2009), in line with recent estimates for a few *campos rupestres* lineages, especially Leguminosae genera (de Souza et al., 2013; Rando et al., 2016). However, *campos rupestres*

groups show higher levels of endemism and have higher species turnover than Cerrado flora, suggesting distinct biogeographical processes (see Rando et al., 2016). Although physiognomically similar, the *campos rupestres* are historically, ecologically and floristically distinct from the Cerrado, with great diversity derived from *in situ* radiations resulting in microendemic species in *campos rupestres* (Giulietti and Pirani, 1988; Pirani et al., 2015; Conceição et al., 2016). Our results corroborate the first studies focusing on *campos rupestres* lineages (Rapini et al., 2007; Antonelli et al., 2010) that suggested older ages than those of most Cerrado lineages (Simon et al., 2009; Hughes et al., 2013).

Despite the differences between Cerrado and *campos rupestres*, overall biodiversity estimates usually inflate the number of plant species in the Cerrado hotspot by the inclusion of *campos rupestres* (Alves et al., 2007; Strassburg et al., 2017). It is crucial, however, to recognize the distinct ecological conditions (in particular the edaphic ones) and historical processes that have moulded Cerrado and *campos rupestres*, especially given current threats to the conservation of both. The *campos rupestres* might be considered a hotspot within the Cerrado hotspot, similar to what has been proposed for the Páramos within the Tropical Andes hotspot (Madrriñan et al., 2013). However, unlike the Páramos, the exceptionally high species diversity of the *campos rupestres* seems to be derived from generally older evolutionary events. The highest diversification rates in Velloziaceae reaches 0.36 species/Myr since the late Miocene within the core-*Barbacenia* clade and approx. 0.25 species/Myr within the core-*Vellozia* clade, while in other groups (e.g. *Calliandra*, Koenen et al., 2013) highest diversification rates have been observed within the last 5 Mya. Although we still lack average rate estimates for most *campos rupestres* lineages, the different time of species origins of most clades studied so far indicates a more complex macroevolutionary scenario than that reported for the Páramos and other relatively ‘recent’ species-rich biomes reported by Madrriñan et al. (2013) (e.g. the Mediterranean Basin, the Succulent Karoo, the Cerrado, the Cape Floristic Region, the California Floristic Province and south-west Australia).

In this study, we corroborate the hypothesis that adaptive radiation has been crucial to the origin of a dominant lineage in the *campos rupestres*. Despite the ecological restrictions of Velloziaceae to rock/shallow soils and open vegetation habitats in both Africa and South America, the evolution of diversified water-related strategies in the two genera that occur in the *campos rupestres* seems to have driven their diversification. We also corroborate the hypothesis of increased diversification in South American lineages associated with transitions in ecological strategies as the main factor that has driven species imbalance between New and Old World Velloziaceae, echoing several other Pantropical plant families and the idiosyncratic origins of different Neotropical biomes.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Figure S1: map displaying the distribution of *campos rupestres* vegetation. Material S2: description and codification of the functional traits

analysed. Material S3: A, vouchers of the specimens analysed; and B, laboratory procedures and primers used for molecular analyses. Figure S4: majority-rule consensus tree from the Bayesian phylogenetic analysis. Material S5: substitution models prevalent for each data partition from the analyses performed in MrBayes and BEAST. Figure S6: A, coefficient of variation; and B, standard deviation of the UCLD rates estimated from the MCMC runs in BEAST analyses. Material S7: results of the MEDUSA analysis. Figure S8: major clade credibility tree, time-calibrated through the UCLN rate model. Material S9: results of the BAMM simulations. Figure S10: macroevolutionary cohorts in Velloziaceae. Figure S11: A, speciation; B, extinction; and C, net diversification rates estimated by BAMM for African and South American lineages of Velloziaceae. Figure S12: Bayesian reconstruction of ancestral states of the functional trait “development of conspicuous aerial stems”. Figure S13: Bayesian reconstruction of ancestral states of the functional trait “aquiferous parenchyma between the bundles on the leaves”.

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