Research

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Fast diversification through a mosaic of evolutionary histories characterizes the endemic flora of ancient Neotropical mountains

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Mountains are among the most biodiverse areas on the globe. In young mountain ranges, exceptional plant species richness is often associated with recent and rapid radiations linked to the mountain uplift itself. In ancient mountains, however, orogeny vastly precedes the evolution of vascular plants, so species richness has been explained by species accumulation during long periods of low extinction rates. Here we evaluate these assumptions by analysing plant diversification dynamics in the *campo rupestre*, an ecosystem associated with pre-Cambrian mountaintops and highlands of eastern South America, areas where plant species richness and endemism are among the highest in the world. Analyses of 15 angiosperm clades show that radiations of endemics exhibit fastest rates of diversification during the last 5 Myr, a climatically unstable period. However, results from ancestral range estimations using different models disagree on the age of the earliest *in situ* speciation events and point to a complex floristic assembly. There is a general trend for higher diversification rates associated with these areas, but endemism may also increase or reduce extinction rates, depending on the group. Montane habitats, regardless of their geological age, may lead to boosts in speciation rates by accelerating population isolation in archipelago-like systems, circumstances that can also result in higher extinction rates and fast species turnover, misleading the age estimates of endemic lineages.

1. Background

Species richness is not evenly distributed on the globe's surface. Unequal allocation of biodiversity among different areas occurs both within and between latitudinal zones [1–3], and similar environments are often associated with similar evolutionary patterns. Mountains are a good example of this trend [3–5]. Mountain chains worldwide, but especially those in the tropics, have called attention to their impressive number of plant species, many of which are found nowhere else on the planet (e.g. [6,7]).

There are several explanations for the high species richness and endemism in the flora of tropical mountains. In the absence of fossil records, molecular phylogenetic trees have been used as a proxy to estimate the age of assemblage and the

rates of *in situ* speciation in these areas (e.g. [4]). Studies of this kind have become increasingly frequent after the popularization of sequencing techniques and advances in methods for reconstructing and extracting further information from phylogenetic data [6–10]. Lessons learned from these studies are many (see also [11,12]), but can be summarized in three main points: (i) montane floras are hyper-diverse because the altitudinal gradient creates multiple distinct niches (e.g. micro-climates), allowing more plant species to coexist in a relatively smaller area; (ii) mountaintops create continental archipelago-like systems that impose barriers to gene flow, leading to fast speciation rates in endemic lineages; and (iii) the origin of endemic montane radiations can be linked in time to the age of mountain uplift, when both the archipelago-like system and the altitudinal gradients are formed. These concepts and hypotheses, although well established for floras in mountains of recent orogeny such as the Andes [6,8], the Kinabalu mountains [7] and the Hengduan mountains [5,9], are still poorly understood for floras found in the mountains of ancient geological ages.

A montane flora that has received relatively little attention from diversification studies in spite of its immense species richness is the endemic flora of the *campo rupestre*. The *campo rupestre* is an ecosystem associated with the areas of higher altitude in eastern South America, mainly distributed over the pre-Cambrian Espinhaço Range [13–15]. Due to constant erosion through roughly the last 500 Myr, altitudes in the *campo rupestre* are today mostly between 1000 and 2000 metres high. Despite its lower elevation when compared to mountain chains of recent orogeny, this is still a prominent slope in contrast with the surrounding areas, which are mostly approximately 500 metres lower in elevation [16]. The *campo rupestre* presents an exceptionally rich and highly endemic flora, including *ca* 15% of all species of angiosperms found in Brazil (approx. 5000, almost half of them endemics), in only 1% of the country's area (approx. 70 000 km^2) [17,18]. This exceptional diversity and endemism have classified the *campo rupestre* as a UNESCO heritage site [19] and led to its recognition as a floristic unity among South American phytochoria [20] and recently as two biogeographic provinces in their own right (i.e. the Southern Espinhaço Province and the Chapada Diamantina Province [21]), emphasizing the floristic singularity of these areas in contrast with surrounding ecosystems (i.e. Caatinga, Cerrado and Brazilian Atlantic Forest). Through particular growth forms, *campo rupestre* plant radiations form a unique landscape, rich in small-leaved and sclerophyllous shrubs and giant rosettes [22].

Given its distinct ancient geological history and the relative stability of its landscape along the Cenozoic, the evolution of the remarkable diversity of the *campo rupestre* flora has been explained by different mechanisms to those invoked for other mountain systems. The pre-Cambrian orogeny of the Espinhaço range, preceding the early evolution of vascular plants [23], means that it is unrealistic to link the first events of *in situ* speciation to mountain uplift, a common approach in studies with young mountain ranges (see references above). By contrast, studies focusing on plant ecology have hypothesized that the geological and climatic stability of these old mountains have created refugia for plant diversity. It is then assumed that exceptional species richness and endemism in this area is, first, a consequence of slow diversification of old endemic lineages and, second, the survival of species and lineages that go extinct in less stable areas [17,24]. However,

the few studies that actually present age estimates for *campo rupestre* plant radiations show contrasting results, estimating the age of these from as old as the Early Miocene (e.g. 23–15 Ma (million years before present) for Velloziaceae [25]) to as recent as the Pleistocene (e.g. 2 Ma for *Chamaecrista* [26]).

Even though comparable in species richness and endemism to other well-studied montane systems (e.g. the Andean Páramos [6]), no study has yet combined multiple datasets to investigate the age and diversification patterns of the *campo rupestre* flora. Tracing the diversification history of such a species-rich flora is complex, but general patterns can be inferred if data from multiple plant lineages is examined using a comparative approach. In this study, we present a comprehensive description of diversification dynamics associated with the assembly of the *campo rupestre* flora. We aim to test two premises that have been frequently suggested in the literature: (i) that the endemic flora in the *campo rupestre* is as ancient as its landscape, i.e. by estimating ages of first diversification events, and (ii) that the exceptional species richness in the *campo rupestre* results from species accumulation through long periods of time (i.e. by expecting lower rates of extinction in endemic lineages when contrasted to non-endemic lineages).

2. Material and methods

(a) Selected taxa and tree calibration

The main source of evidence for this study is 15 species-level phylogenetic trees of angiosperm lineages with high species diversity in *campo rupestre* areas. Species sampling and molecular coverage vary among groups, but the 15 empirical replicates confer robustness to the results presented here. The following criteria were used to select these lineages: (i) they represent both woody and herbaceous species, and both monocots and eudicots; (ii) they represent groups with high species diversity in the *campo rupestre* [18], including endemic species and their closest non-endemic relatives (from surrounding areas or not); and (iii) their phylogenetic trees have relatively high species-level coverage considering the total estimated species diversity in their groups (ideally above 50% of the species are sampled, again considering both endemics and non-endemic closest relatives). For a detailed reasoning behind species sampling see electronic supplementary material, short literature review.

The 15 time-calibrated phylogenetic trees represent nine families and 2099 species. This dataset includes 753 species endemic to our focal area, which we estimate to represent a third of the endemic flora of this region [18]. Molecular sequences for generating the phylogenetic trees were mostly retrieved from published studies. Calibration methods are a sensitive matter and we took great care to avoid bias in dating analyses of each tree. A detailed description of how calibration points were chosen for each tree (including nodes calibrated in each tree and offset, mean and standard deviation values for calibration points) is available in electronic supplementary material, Trees. To attest that estimates found for each group were realistic, we further compared them to ages found in an alternative analysis (i.e. [27]) and found high similarity between age estimates. A summary of these comparisons can be also found in electronic supplementary material, Trees.

(b) Area assignation

Tips in each phylogenetic tree were assigned a geographical range using distribution points obtained from the GBIF public repository [28] after a round of standard data cleaning (i.e. deleting duplicates, points in the sea, outliers and centroids of countries). We generated a shape file of our focal area by extracting areas of altitude above

1000 m from four topographic regions (retrieved from a map of topographic areas by IBGE [16]): 'Serras do Espinhaço/Tabatinga/Quadrilátero Ferrífero', 'Planaltos e Serras da Diamantina', 'Planalto Central Brasileiro' and 'Planalto da Canastra'. The final shape file is highly similar to the definition of *campo rupestre* (in its broad sense) by several studies (see review in [17]), but may also include a few patches of Cerrado that are in higher altitudes. Points were plotted over this shape file (available on the Dryad folder linked to this paper) for visual inspection and range scoring. All tips were scored in a matrix of two presence/absence columns (*campo rupestre* and non-*campo rupestre*). When possible, these distribution matrices were further contrasted to the taxonomic literature for each group (see electronic supplementary material, Literature review) to confirm that scoring is in agreement with specialized literature. Note that we considered *campo rupestre* in its broadest sense, including most areas of higher altitude (above 1000 m) in the eastern side of tropical South America. Further details on range assignation can be also found in electronic supplementary material, Biogeography.

(c) Biogeographical and diversification analyses

Unless otherwise stated, all analyses were performed in R [29]. All files resulting from these analyses as well as R code used can be found on the Dryad folder linked to this paper.

To estimate ages for *in situ* speciation events and describe diversification dynamics in the *campo rupestre*, we contrasted results from several analytical frameworks. First, we used functions of the R package BioGeoBEARS [30] to reconstruct the historical distribution of each group and to extract the ages of nodes with highest probability of *in situ* diversification in the *campo rupestre*. BioGeoBEARS test diversification-independent biogeographical models and estimate ancestral range based on maximum likelihood. The best fit for DEC or DEC + J models were selected comparing AIC values between models before each run and ancestral range estimations were performed based on the model that best-fitted range distributions in each tree. To account for phylogenetic uncertainty and broad age confidence intervals, we also cross-validated these results using 100 simulations of biogeographic stochastic mapping [31] on a sample of 100 trees from the posterior distribution of trees in each group. These results, alongside best models for each group and probabilities for each node in each phylogenetic tree can be found in electronic supplementary material, Biogeography.

Second, we relied on the GeoHiSSE set of models (from [32]) to estimate the parameters of speciation, extinction and net-diversification (speciation minus extinction) in each range. This framework enables fitting a series of models to characterize the relative effects of state-dependent diversification and/or hidden (unobserved) states on diversification. For each tree, we fitted two sets of 18 geographic-state speciation and extinction models (36 models in total) using functions of the R package hisse [33]. For all aspects concerning using these models to estimate speciation, extinction and net-diversification rates, we followed recommendations outlined in Caetano et al. [32]. The models derive from the 18 models proposed in Caetano et al. [32] and are distinct in the sense that jump dispersal between areas are forbidden in the first set but allowed in the second set. For each tree, we also incorporated a unique, clade-specific sampling fraction to deal with unbalanced sampling of species in different ranges (see electronic supplementary material, Diversification). The set of three best models (AIC values at electronic supplementary material, Diversification) were averaged according to their Akaike weights and used to estimate diversification rate parameters based on maximum likelihood. Tip-rates were then extracted and contrasted among the different range categories using Conover's test for pairwise comparisons.

SSE models have been also shown to outperform conventional trait models (i.e. that do not account for diversification) in ancestral character reconstructions [34] and have been also recently advocated for biogeographic range reconstructions [35]. In this context, the GeoSSE full model (with all parameters free to vary) was also used to estimate ancestral ranges in a scenario where range reconstruction is diversification dependent (in contrast with the diversification-independent models DEC and DEC + J). In this case, however, we decided to use the original GeoSSE model (without hidden states) to infer probabilities of ancestral ranges at the nodes of the tree, because the probabilities of hidden areas at the nodes were difficult to interpret. In this and in the BAMM analyses, trees had to be further pruned to exclude outgroup taxa that had poor species-level coverage before analyses (see details in electronic supplementary material, Diversification). Time-stratified analyses were not considered necessary because, in theory, both analysed areas (*campo rupestre* and non-*campo rupestre*) were constantly adjacent to each other and available for colonization throughout all time slices for all groups.

To estimate speciation rates through time in the *campo rupestre* radiations of each group, we used BAMM v. 2.5.0., which uses rjMCMC (reversible-jump Markov chain Monte Carlo) to test different combinations of shifts in diversification rates and speciation and extinction parameters that are allowed to vary through time. BAMM indicates the best diversification regime without requiring established models to be selected *a priori*. Priors for *lambda* values and expected number of shifts were defined empirically using the tree and an estimation of the total number of species in each group in the R package BAMM tools [36]. To account for unbalanced species sampling among clades in each phylogenetic tree, we also incorporated sampling fractions per clade in most analyses. When species sampling was almost complete or only slightly unbalanced throughout the tree, we used a global sampling fraction instead. Analyses were run twice for each group using different seed numbers in each run and 10 million MCMC generations in all cases. A minimum ESS of 200 was confirmed, and speciation rates through time were measured by selecting the stem node of the most recent common ancestor for each *campo rupestre* radiation. Mean lambda values were estimated from the crown node of each radiation using the functions of the package BAMMtools.

The 15 species-level phylogenetic trees are natural replicates that confer robustness to our results. However, to analyse them independently could also raise some concerns: (i) taxonomic sampling bias in each phylogeny could mean that most of the species assembled are representing just one scenario (given an observed preference by taxonomists for studying endemic radiations); (ii) there is some concern that ages estimated independently for several phylogenies they may not represent a single evolutionary history (given that all species are related, phylogenetic trees should ideally be calibrated in a holistic framework). Taking these concerns in consideration, we also estimated ages for *campo rupestre in situ* speciation events using the complete seed plant phylogenetic tree of Smith & Brown [27]. We extracted a list of all species names indicated as 'native to' and '*campo rupestre*' (highland rocky field) from the Flora do Brasil 2020 [18] dataset. We crossed the names in this list with the list of tips in this tree and found 1,122 matches (i.e. *campo rupestre* species that were sampled in the tree, corresponding to 290 endemic and 832 native but non-endemic). These were again scored in a 'presence/absence' matrix of geographical range distribution for each tip. We performed a similar maximum-likelihood optimization in BioGeoBEARS as described above to recover node probabilities for each range.

3. Results

(a) Estimated age of first diversification events

Results for age estimates of *in situ* diversification events vary depending on which models are used to perform the analyses.

Diversification-independent ancestral range estimation (i.e. models DEC and DEC + J; figure 1, 'DEC/DEC + J') recover the first events of *in situ* speciation (i.e. oldest nodes with highest probability of endemism in the *campo rupestre*) mainly from the Late Miocene to Pliocene (i.e. from 15 to 2.5 Ma) time slices (figure 2a). Analyses using the same models in the angiosperm-wide phylogenetic tree return similar results ('eudicots' and 'monocots' in figure 2a). In this analysis, the great majority of the *in situ* speciation events in the *campo rupestre* are from the Pleistocene (711 events, 73% of the total). Therefore, our results indicate that the floristic assembly of the current *campo rupestre* flora dates back from the Late Miocene, with highest diversification during the Pleistocene. Biogeographic stochastic mapping on samples of 100 trees for each clade are also largely comparable, although first events of endemism can be older due to phylogenetic uncertainty (see electronic supplementary material, Biogeography).

By contrast, the results of ancestral range estimation retrieved from diversification-dependent models (i.e. GeoSSE model) often recover an older scenario for the *campo rupestre* floristic assembly (figure 1, 'GeoSSE'). In this scenario, it is frequently observed that the oldest nodes and branches in each phylogenetic tree are either *campo rupestre* endemics or widespread, implying that some of these lineages may have established in the *campo rupestre* a relatively long time before the rise of recent radiations of endemic species took place. In some lineages, the oldest nodes with highest probability of *campo rupestre* endemism are dated from the Oligocene to the Early Miocene (i.e. from 33 to 15 Ma; figure 2b), contrasting estimates using diversification-independent models. Nevertheless, most of the *in situ* speciation events are still in the Pleistocene (534 events, 63% of the total).

(b) Comparisons between speciation and extinction rates in endemics and non-endemics lineages

The set of three best GeoHiSSE models were different for each clade (see electronic supplementary material, Diversification), highlighting the singularity of diversification dynamics in each group. Parameter estimates extracted from tip-rates also show distinct diversification dynamics between monocot and eudicot lineages (figure 3).

Combined analyses of all clades (figure 3a) show that endemic lineages have a two-fold increase in speciation rates (medians 0.26 for widespread, 0.49 for *campo rupestre* endemics, and 0.25 for non-*campo rupestre* lineages; henceforward all medians are given in this order; all values are in the scale of lineage per million years) and that lowest extinction rates are observed in the lineages of widespread distribution (0.004, 0.11 and 0.15), resulting in higher net-diversification rates in both endemics and widespread lineages (0.47, 0.44 and 0.17). However, when these clades are divided according to their major groups, it is observed that eudicots (figure 3b) and monocots (figure 3c) present diversification dynamics that are distinct from one another and thus contribute differently to the general pattern. Endemic eudicots present higher rates of both speciation (0.22, 0.49, 0.24) and extinction (0.003, 0.18, 0.14), still resulting in faster net-diversification (0.43, 0.49, 0.17). On the other hand, endemism does not drive faster speciation rates (0.54, 0.34, 0.34) or net-diversification (0.91, 0.26, 0.31) in monocots, where widespread and endemic lineages group have lower extinction rates than non-*campo rupestre* lineages (0.01, 0.08, 0.21). In this group, fastest

rates of both speciation and net-diversification are observed in widespread lineages. These results highlight that switching ranges affect diversification in these lineages, but that responses are variable and lead to distinct range-dependent diversification patterns depending on the group.

(c) Speciation rates across time in endemic radiations

Species endemic to the *campo rupestre* are often strongly phylogenetically clustered in only a few clades within each group. We identified clades that were observed to radiate *in situ* in both diversity-dependent and diversity-independent analyses, with at least 15 descendent tips and 75% of endemism among tips. Although some of them managed to disperse out of the *campo rupestre*, these clades were here interpreted as '*campo rupestre* radiations' (marked with a black line and numbers in figure 1) based on these arbitrary thresholds. Five groups (*Minaria*, *Habenaria*, *Dyckia*, *Myrcia* and Melastomateae) did not pass the threshold and were not considered here to have *campo rupestre* radiations, despite great species richness in these areas. By contrast, three of the 15 groups presented two radiations each (*Chamaecrista*, *Paepalanthus* and Velloziaceae), resulting in a total of 13 *campo rupestre* radiations. Rates through time were extracted from these 13 radiations based on BAMM analyses, with results showing that increases in speciation rates are particularly significant during the last 5 Myr, with several lineages experiencing the burst of diversification during this period (figure 2c).

4. Discussion

(a) Fast speciation through rapid and recent radiations

Most of the current endemic flora in the *campo rupestre* results from speciation events that took place rapidly in the last 5 Myr. This period is characterized by strong climatic instability and rapid glaciation cycles starting around 5 Ma (Pliocene), and becoming more intense between 3 and 2 Ma (Pleistocene), when most of the *in situ* speciation events were observed in all lineages. Similar results were recovered in both the analyses with the 15 lineages and in the whole seed plant phylogenetic tree (figure 3a), highlighting congruence between datasets.

Strong decline in temperatures interposed with the periods of mild and warm climates (i.e. Milankovich cycles, [37]), as well as changes in precipitation regimes, may have caused several alterations in niche suitability through a short period of time across the steep altitudinal gradient of the *campo rupestre* (e.g. [38]). Multiple events of range expansions and contractions could have then promoted speciation by vicariance among adjacent mountains (during range contraction) and occasional hybridization events when still closely related species are found in sympatry again (during range expansion). Climatic instability can also promote pulses of high speciation in areas of uneven topography by specializations to different micro-climates in species of endemic lineages [39]. This creates a species-pump effect that is prominent in montane landscapes and shown to be an important driver of plant diversification in naturally fragmented habitats ([40,41]; also called 'flickering connectivity', [42]). Empirical evidences also support the link between the rapid, recent diversification in the *campo rupestre* and the species-pump scenario (already hypothesized by [43]; and [44]). Among them, the frequent records of natural hybrids
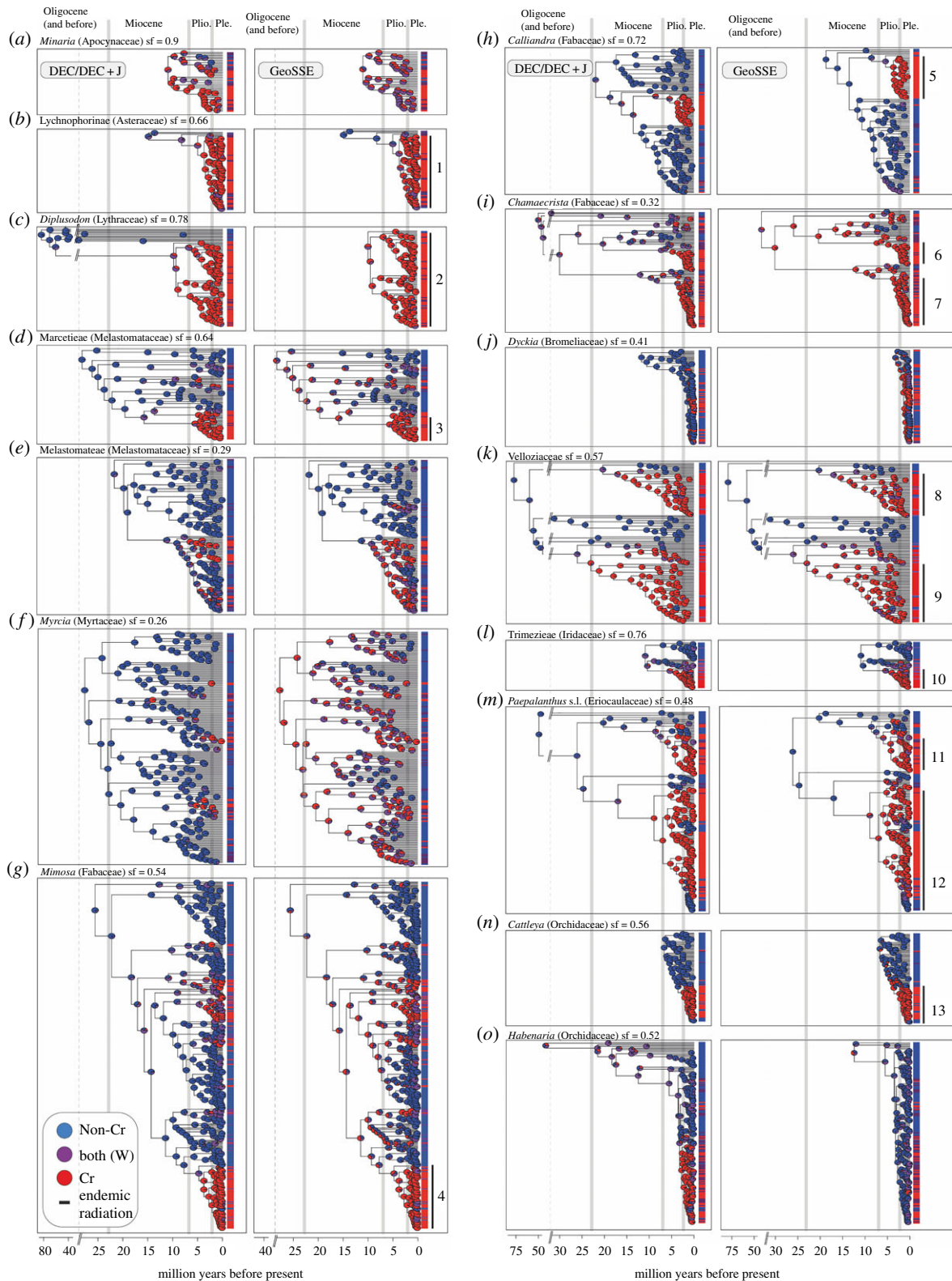
Figure 1. Ancestral range estimations in chronograms of 15 clades with high species diversity in the *campo rupestre*. Pie charts at nodes represent marginal probabilities of each range estimated using DEC/DEC + J models (on the left. DEC was used in *Habenaria*, Lychnophorinae, Marcetieae and Trimezieae; remaining reconstructions used DEC + J models) and GeoSSE model (on the right). Clades marked with black lines are interpreted as 'endemic radiations' and numbered (1 to 13) to facilitate interpretation of rates through time in figure 2c. Note that outgroups were excluded in analyses with the GeoSSE model, so trees are slightly different. Sampling fractions (sf) are given for each clade (sampling fractions in *Habenaria* and Melastomateae correspond to their Neotropical lineages). (Online version in colour.)

(e.g. [45,46]), the poor phylogenetic resolution among species in several *campo rupestre* endemic radiations (e.g. [26,40]) and the projections of range expansions during glacial periods inferred for species that today are restricted to mountaintops (e.g. [38]).

(b) A complex assembly and a mosaic of evolutionary histories

These results emphasize that recent radiations are common across the tree of life (see also [47]) and may be completely
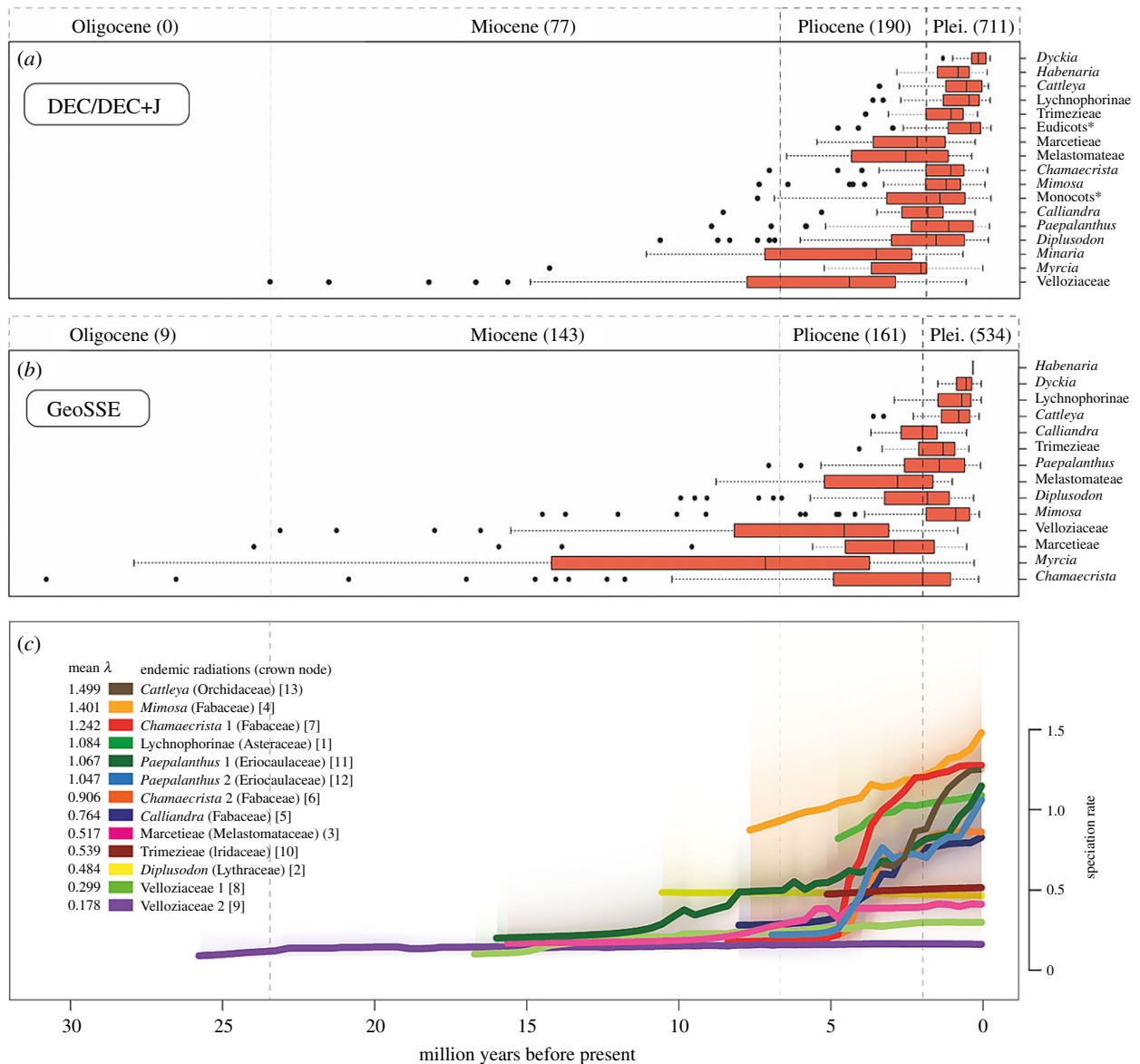
Figure 2. Boxplots summarize ages for nodes with the highest probability of *in situ* speciation (i.e. endemic to the *campo rupestre*) in reconstructions using (*a*) DEC/DEC + J models and (*b*) GeoSSE model. Numbers in brackets indicate how many *in situ* speciation events are recovered in each period. Asterisks highlight ages inferred using the whole seed plant phylogenetic tree. (*c*) Speciation rates through time and mean speciation rates (in units of species formed per million years per lineage) inferred from BAMM for 13 *campo rupestre* radiations. Numbers refer to radiations marked in figure 1. Colour shading represents uncertainty on rate reconstructions at any point in time. (Online version in colour.)

unrelated to the age of mountain uplift, in the case of montane biotas. Recent and rapid radiations have been also observed in other ancient landscapes (e.g. the Cape [48] and the Tepuis [49]), many of which might have been also perturbed by recent climatic instability. This shows that the link between orogeny, floristic assembly and diversification may be more complex than frequently stated and that different geological histories can result in overall similar macroevolutionary patterns.

Despite the strong evidence for rapid and recent radiations, results from ancestral range estimations that incorporate the effect of diversification rates show that some of these floristic elements may have been present in the area much earlier. The small number of old *in situ* speciation events in the *campo rupestre* may be a consequence of constant extinction rates through time (see also [50]), or even of dramatic extinction events in the past where only a few lineages survived. The constant fragmentation of populations through the same processes that lead to increased speciation (i.e. climatic instability and species pump, as described above) can also result in high occurrence of species

that are restricted to few mountaintops. Given their small population size and restricted distribution, these can be easily and stochastically eliminated, increasing the turnover of species across time and leading to the observable pattern of recent radiations in phylogenetic trees (e.g. as in [51]). High rates of speciation and extinction are not mutually exclusive, especially in areas with a large number of narrow endemic species where high rates of extinction are potentially an inescapable consequence of high speciation rates (e.g. [52]).

Although our results do not favour this scenario for monocots, eudicot lineages indeed present higher rates of both extinction and speciation associated with endemic lineages, partially corroborating this hypothesis. In this scenario, species-poor, endemic, and phylogenetically isolated lineages, including monotypic genera (e.g. *Rupestrea* (14 Ma) and *Eriocnema* (8 Ma) in Melastomataceae [53]; *Accara* in Myrtaceae (25 Ma) [54]) may represent relics from previously diverse clades that have undergone severe extinction, but for which few species survived to the present. Furthermore, the extant
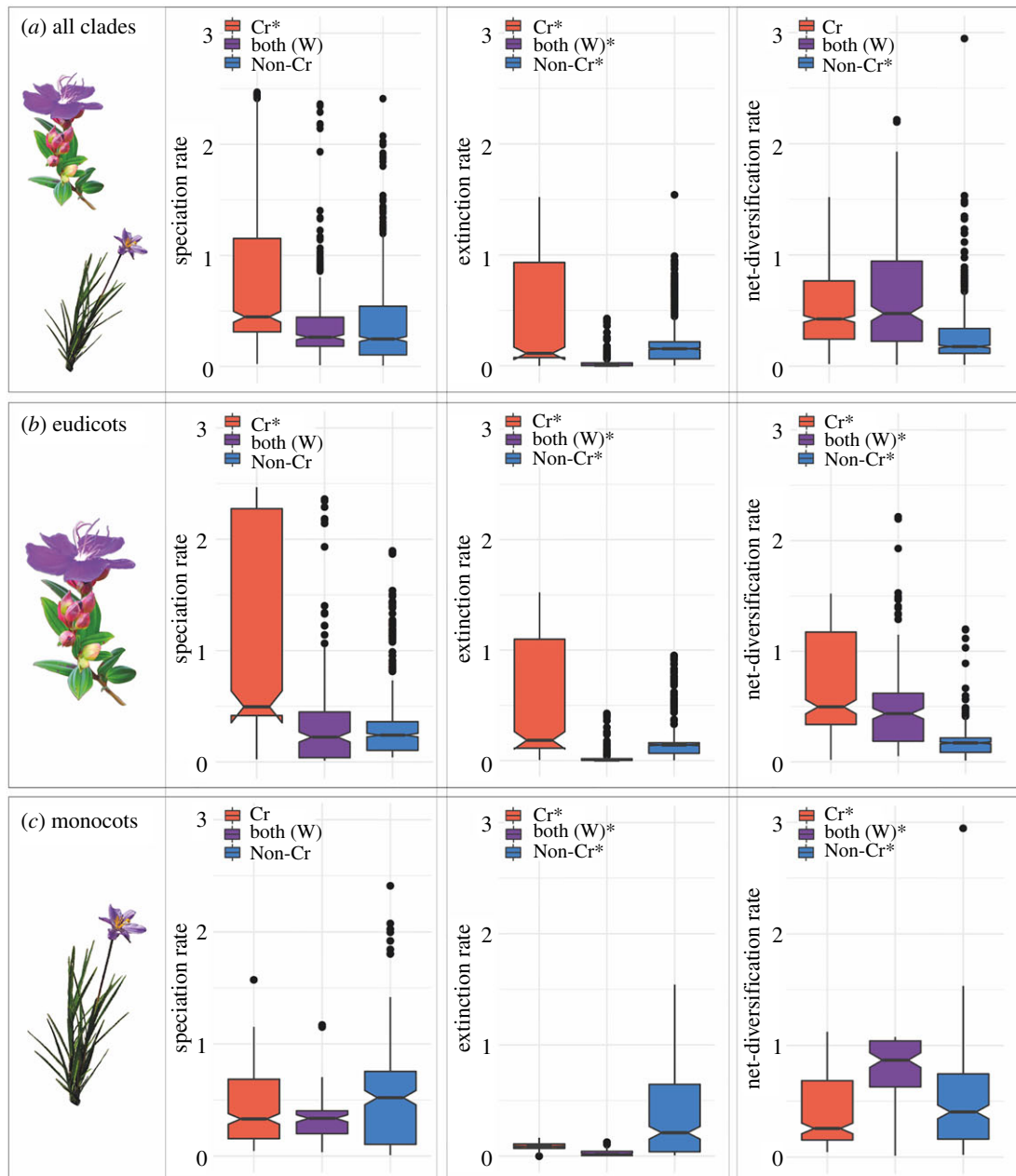
Figure 3. Boxplots representing diversification rate distribution (from left to right: speciation rates, extinction rates and net-diversification rates) obtained from GeoHiSSE analyses in 15 clades (nine clades of eudicots and six of monocots) with conspicuous species diversity in the *campo rupestre*. Rates were estimated using the three best models from a set of 36 models for each clade (see electronic supplementary material, Diversification). 'Both (W)' indicates widespread species, 'Cr' indicates species endemic to the *campo rupestre* and 'Non-Cr' indicates species that do not occur in the *campo rupestre*. Asterisks indicate rates that are significantly different from the others ($p < 0.01$) according to pairwise comparisons using Conover's test for multiple comparisons of independent samples. Plant stickers show a *Vellozia* sp. (Velloziaceae—monocots) and a *Pleroma* sp. (Melastomataceae—eudicots). (Online version in colour.)

endemic flora of the *campo rupestre* is recognized for having an above average proportion of narrow endemic and threatened species [55]. Monocots, on the other hand, present a pattern of diversification that is closer to what has been suggested in the *campo rupestre* literature, where range evolution does not significantly change speciation rates, but reduce extinction rates and may lead to a more constant species accumulation through time [17].

In this sense, it seems reasonable to infer that phylogenetic data corroborates current speculation and evidence that the *campo rupestre* is both a museum of lineages and a cradle of species (e.g. [56]). Also, it is important to note that the age of the flora is not necessarily linked to the age of the vegetation, as other now extinct lineages depicting similar functional ecologies could have existed before the extant

radiation of *campo rupestre* endemics. However, the lack of fossil record for the region [57] still limits our ability to extrapolate with certainty what was the floristic composition of the *campo rupestre* flora in the past.

(c) Uneven diversifications dynamics and the role of traits

The fact that we did not recover the same pattern for range-dependent speciation and extinction rates throughout all clades demonstrates that the biology of each lineage certainly affects how the environment drives their diversification. Plant life in the *campo rupestre* requires particular attributes to survive in generally harsh conditions, such as nutrient-poor soils, cold weather, high solar radiation, climatic seasonality

and occasional fire events [58]. Traits related to survival in these habitats, such as resistance to desiccation and fire, are crucial (e.g. [25,26]) and probably affect survival chances differently in each lineage. The distinct diversification dynamics in eudicots and monocots could be also related to their predominant life forms (predominantly woody and herbaceous/geophytic, respectively). Other inherited mechanisms that help isolated populations to hastily adapt to new micro-climates or particular edaphic conditions in these areas may also contribute to accelerating speciation during range contractions and expansions (e.g. as in [59]).

Particular reproductive mechanisms (i.e. dispersal and pollination strategies) may also help promoting or demoting barriers to gene flow between isolated populations and consequently the increase or decrease chances of speciation or extinction (e.g. [59,60]). However, there seems to be no predominant pollination systems among the *campo rupestre* radiations investigated here. For example, some *Barbacenia* (Velloziaceae) are pollinated by vertebrates [61], while many Melastomataceae are buzz-pollinated [62] and Eriocaulaceae are generalists [63]. In the context of seed dispersion, however, none of the prolific radiations have fruits dispersed mainly by animals and most are wind or self-dispersed [64]. Further investigation into the putative link between traits (both associated to survival and reproduction) and high diversification rates will be crucial to better understand macroevolutionary dynamics in the *campo rupestre*.

(d) Caveats

This study is intended as a first step in unlocking general patterns related to the macroevolution of the endemic flora in these exceptionally rich but relatively neglected Neotropical mountains. As presented here, understanding the evolution of floristic assemblies is complex and there are several biases that can affect this and similar studies. Even though strategies to minimize these caveats have been applied (e.g. usage of several empirical replicates), some biases are typical of macroevolutionary research and must be further addressed in future studies. For instance, including more species and molecular markers to infer better phylogenetic trees, as well as refining calibration analyses, will improve conclusions drawn from our analyses (e.g. see [65]). Also, better definitions of *campo rupestre* (e.g. based on climatic and soil layers) will likely enhance discretization of this area for future biogeographical studies. Finally, patterns observed in the reconstructions using diversification-dependent models may also be a consequence of the methods not being able to distinguish high transition rates from speciation/extinction dynamics. We thus suggest that all of these results should be re-evaluated in close scrutiny when better data and methods are available.

5. Conclusion and prospects

These results emphasize how age estimates and diversification dynamics for major biotic assemblages are complex to unravel. Several routes can lead to high species richness in one area through a mosaic of different evolutionary processes, possibly depending on the intrinsic particularities of each group. Perhaps due to high species turnover, unstable environments can mislead age estimates for biotas by generating patterns of recent radiations even when the floristic assembly, in terms of lineages, may be older. As a result, the mountains of both recent and ancient uplift may present radiations of similar age and diversification rates. This also shows that the age of a landscape may represent a confounding factor in estimating the age of a biota and that recent and rapid radiations may be common in many areas perturbed by recent climatic instability. We also highlight important gaps in the overall understanding of montane plant evolution that have to be filled in both the *campo rupestre* and other mountain systems. For instance, weighting the relative contributions of traits and biogeographical history in the diversification dynamics of montane radiations is required. Biogeographical studies that aim to understand historical connections between these and other adjacent or non-adjacent floras are desirable and will contribute to untangling the roles of adaptive and exaptive traits in the diversification of the *campo rupestre* flora. Additional evidence for the species-pump speciation model and comparative studies revealing how various environmental gradients affect the strength of this pattern across mountain chains is also highly desirable.

References

1. Mittelbach GG *et al.* 2007 Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol. Lett.* **10**, 315–331. (doi:10.1111/j.1461-0248.2007.01020.x)

2. Cowling RM, Bradshaw PL, Colville JF, Forest F. 2017 Levyns' Law: explaining the evolution of a remarkable longitudinal gradient in Cape plant diversity. *Trans. R. Soc. S. Afr.* **72**, 184–201. (doi:10.1080/0035919X.2016.1274277)

3. Antonelli A *et al.* 2018 Geological and climatic influences on mountain biodiversity. *Nat. Geosci.* **11**, 718–725. (doi:10.1038/s41561-018-0236-z)

4. Hughes CE, Eastwood R. 2006 Island radiation on a continental scale: exceptional rates of plant

diversification after uplift of the Andes. *Proc. Natl Acad. Sci. USA* **103**, 10 334–10 339. (doi:10.1073/pnas. 0601928103)

5. Hughes CE, Atchison GW. 2015 The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. *New Phytol.* **207**, 275–282. (doi:10.1111/nph.13230)

6. Madriñán S, Cortés AJ, Richardson JE. 2013 Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Front. Genet.* **4**, 192. (doi:10. 3389/fgene.2013.00192)

7. Merckx VS *et al.* 2015 Evolution of endemism on a young tropical mountain. *Nature* **524**, 347–350. (doi:10.1038/nature14949)

8. Lagomarsino LP, Condamine FL, Antonelli A, Mulch A, Davis CC. 2016 The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytol.* **210**, 1430–1442. (doi:10.1111/nph.13920)

9. Xing Y, Ree RH. 2017 Uplift-driven diversification in the Hengduan Mountains, a temperate biodiversity hotspot. *Proc. Natl Acad. Sci. USA.* **114**, E3444–E3451. (doi:10.1073/pnas.1616063114)

10. Pouchon C, Fernández A, Nassar JM, Boyer F, Aubert S, Lavergne S, Mavárez J. 2018 Phylogenomic analysis of the explosive adaptive radiation of the *Espeletia* complex (Asteraceae) in the tropical Andes. *Syst. Biol.* **67**, 1041–1060. (doi:10.1093/sysbio/ syy022)

11. Hoorn C, Perrigo A, Antonelli A. 2018 *Mountains, climate and biodiversity*. Hoboken, NJ: John Wiley & Sons.

12. Perrigo A, Hoorn C, Antonelli A. 2020 Why mountains matter for biodiversity. *J. Biogeog.* **47**, 315–325.

13. Saadi A. 1995 A geomorfologia da Serra do Espinhaço em Minas Gerais e de suas margens. *Geonomos* **3**, 41–63. (doi:10.18285/geonomos.v3i1. 215)

14. Hasui Y, Carneiro CDR, de Almeida FFM, Bartorelli A. 2012 *Geologia do brasil*. São Paulo, Brazil: Beca.

15. Schaefer CE *et al.* 2016 The physical environment of rupestrian grasslands (Campos Rupestres) in Brazil: geological, geomorphological and pedological characteristics, and interplays. In *Ecology and conservation of mountaintop grasslands in Brazil* (ed. GW Fernandes), pp. 15–53. Cham, Switzerland: Springer.

16. IBGE. 2018 Instituto Brasileiro de Geografia e Estatística. See www.ibge.gov.br (accessed 15 November 2018).

17. Silveira FA *et al.* 2016 Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant Soil* **403**, 129–152. (doi:10.1007/s11104-015-2637-8)

18. BFG. 2018 The Brazil Flora Group. Brazilian Flora. 2020 Project –Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. See http://floradobrasil. jbrj.gov.br (accessed 30 July 2018).

19. UNESCO - United Nations Educational, Scientific and Cultural Organization. 2005 See www.unesco. org/new/en/natural-sciences/environment/

ecological-sciences/biosphere-reserves (accessed 20 July 2018).

20. Prance GT. 1994 A comparison of the efficacy of higher taxa and species numbers in the assessment of biodiversity in the neotropics. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **345**, 89–99. (doi:10. 1098/rstb.1994.0090)

21. Colli-Silva M, Vasconcelos TNC, Pirani JR. 2019 Outstanding plant endemism levels support the creation of *campos rupestres* provinces in the Neotropical region. *J. Biogeogr.* **6**, 1723–1733. (doi:10.1111/jbi.13585)

22. Rapini AL, Ribeiro PL, Lambert SA, Pirani JR. 2008 A flora dos *campos rupestres* da Cadeia do Espinhaço. *Megadiversidade* **4**, 16–24.

23. Morris JL *et al.* 2018 The timescale of early land plant evolution. *Proc. Natl Acad. Sci. USA* **115**, E2274–E2283. (doi:10.1073/pnas.1719588115)

24. Morellato LPC, Silveira FA. 2018 Plant life in campo rupestre: new lessons from an ancient biodiversity hotspot. *Flora* **238**, 1–10. (doi:10.1016/j.flora.2017. 12.001)

25. Alcantara S, Ree RH, Mello-Silva R. 2018 Accelerated diversification and functional trait evolution in Velloziaceae reveal new insights into the origins of the *campos rupestres* exceptional floristic richness. *Ann. Bot.* **122**, 165–180. (doi:10.1093/aob/mcy063)

26. Rando JG, Zuntini AR, Conceição AS, van den Berg C, Pirani JR, de Queiroz LP. 2016 Phylogeny of *Chamaecrista* ser. *Coriaceae* (Leguminosae) unveils a lineage recently diversified in Brazilian *campo rupestre* vegetation. *Int. J. Plant Sci.* **177**, 3–17. (doi:10.1086/683846)

27. Smith SA, Brown JW. 2018 Constructing a broadly inclusive seed plant phylogeny. *Am. J. Bot.* **105**, 302–314. (doi:10.1002/ajb2.1019)

28. GBIF. 2018 Global Biodiversity Information Facility. See www.gbif.org (accessed 1 December 2018).

29. R Development Core Team. 2019 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

30. Matzke NJ. 2013 Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Front. Biogeogr.* **5**, 242–248. (doi:10.21425/F55419694)

31. Dupin J, Matzke NJ, Särkinen T, Knapp S, Olmstead RG, Bohs L, Smith SD. 2016 Bayesian estimation of the global biogeographic history of the Solanaceae. *J. Biogeogr.* **44**, 887–899. (doi:10.1111/jbi.12898)

32. Caetano DS, O'Meara BC, Beaulieu JM. 2018 Hidden state models improve state-dependent diversification approaches, including biogeographical models. *Evolution* **72**, 2308–2324.

33. Beaulieu JM, O'Meara BC. 2016 Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Syst. Biol.* **65**, 583–601. (doi:10.1093/sysbio/syw022)

34. Goldberg EE, Igić B. 2008 On phylogenetic tests of irreversible evolution. *Evolution* **62**, 2727–2741.

35. Ree RH, Sanmartín I. 2018 Conceptual and statistical problems with the DEC+ J model of founder-event

speciation and its comparison with DEC via model selection. *J. Biogeog.* **45**, 741–749.

36. Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huang H, Larson JG. 2014 BAMM tools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods Ecol. Evol.* **5**, 701–707. (doi:10.1111/2041-210X.12199)

37. Lomolino MV, Riddle BR, Whittaker RJ. 2017 *Biogeography*. Oxford, UK: Oxford University Press.

38. Barres L, Batalha-Filho H, Schnadelbach AS, Roque N. 2019 Pleistocene climatic changes drove dispersal and isolation of *Richterago discoidea* (Asteraceae), an endemic plant of *campos rupestres* in the central and eastern Brazilian sky islands. *Bot. J. Linn. Soc.* **189**, 132–152. (doi:10.1093/botlinnean/boy080)

39. Stebbins GL. 1974 *Flowering plants: evolution above the species level*. Cambridge, MA: Belknap Press.

40. Antonelli A, Verola CF, Parisod C, Gustafsson ALS. 2010 Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biol. J. Linn. Soc.* **100**, 597–607. (doi:10.1111/j.1095-8312.2010. 01438.x)

41. Nevado B, Contreras-Ortiz N, Hughes C, Filatov DA. 2018 Pleistocene glacial cycles drive isolation, gene flow and speciation in the high-elevation Andes. *New Phytol.* **219**, 779–793. (doi:10.1111/nph. 15243)

42. Flantua SG, O'dea A, Onstein RE, Giraldo C, Hooghiemstra H. 2019 The flickering connectivity system of the north Andean Páramos. *J. Biogeog.* **46**, 1808–1825. (doi:10.1111/jbi.13607)

43. Harley RM. 1988 Evolution and distribution of Eriope (Labiatae), and its relatives, in Brazil. In *Proceedings of a workshop on neotropical distribution patterns*. (eds P Vanzolini, WR Heyer). Rio de Janeiro, Brazil: Academia Brasileira de Ciências.

44. Alves RJV, Kolbek J. 1994 Plant species endemism in savanna vegetation on table mountains (Campo Rupestre) in Brazil. *Vegetatio* **113**, 125–139.

45. Conceição ADS, De Queiroz LP, Borba EL. 2008 Natural hybrids in *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae): genetic and morphological evidence. *Plant Syst. Evol.* **271**, 19–27. (doi:10.1007/s00606-007-0621-3)

46. Ribeiro PL, Pereira ACS, Borba EL, Giulietti AM. 2018 Genetic and morphological diversity and evidence of hybridization in the 'sempre-vivas' (*Comanthera*, Eriocaulaceae) endemic to the Chapada Diamantina, Bahia, Brazil. *Flora* **238**, 60–71. (doi:10.1016/j.flora. 2017.09.013)

47. Diaz LFH, Harmon LJ, Sugawara MTC, Miller ET, Pennell MW. 2019 Macroevolutionary diversification rates show time dependency. *Proc. Natl Acad. Sci. USA* **116**, 7403–7408. (doi:10.1073/pnas. 1818058116)

48. Pirie MD, Oliver EGH, Mugrabi De Kuppler A, Gehrke B, Le Maitre NC, Kandziora M, Bellstedt DU. 2016 The biodiversity hotspot as evolutionary hot-bed: spectacular radiation of Erica in the Cape Floristic Region. *BMC Evol. Biol.* **16**, 190. (doi:10.1186/ s12862-016-0764-3)

49. Salerno PE, Ron SR, Señaris JC, Rojas-Runjaic FJM, Noonan BP, Cannatella DC. 2012 Ancient tepui summits harbor young rather than old lineages of endemic frogs. *Evolution* **66**, 3000–3013. (doi:10.1111/j.1558-5646.2012.01666.x)

50. Verboom GA, Boucher FC, Ackerly DD, Wootton LM, Freyman WA. In press. Species selection regime and phylogenetic tree shape. *Syst. Biol.*

51. Koenen EJ, Clarkson JJ, Pennington TD, Chatrou LW. 2015 Recently evolved diversity and convergent radiations of rainforest mahoganies (Meliaceae) shed new light on the origins of rainforest hyperdiversity. *New Phytol.* **207**, 327–339. (doi:10.1111/nph.13490)

52. Buerki S, Jose S, Yadav SR, Goldblatt P, Manning JC, Forest F. 2012 Contrasting biogeographic and diversification patterns in two Mediterranean-type ecosystems. *PLoS ONE* **7**, e39377. (doi:10.1371/journal.pone.0039377)

53. Goldenberg R, Almeda F, Sosa K, Ribeiro RC, Michelangeli FA. 2015 *Rupestrea*: a new Brazilian genus of Melastomataceae, with anomalous seeds and dry indehiscent fruits. *Syst. Bot.* **40**, 561–571. (doi:10.1600/036364415X688862)

54. Vasconcelos TNC *et al.* 2017 Myrteae phylogeny, calibration, biogeography and diversification patterns: increased understanding in the most species rich tribe of Myrtaceae. *Mol. Phylogenet. Evol.* **109**, 113–137. (doi:10.1016/j.ympev.2017.01.002)

55. Silva JMC, Rapini A, Barbosa LC, Torres RR. 2019 Extinction risk of narrowly distributed species of seed plants in Brazil due to habitat loss and climate change. *PeerJ* **7**, e7333. (doi:10.7717/peerj.7333)

56. Fiorini CF, Miranda MD, Silva-Pereira V, Barbosa AR, Oliveira UD, Kamino LHY, Mota NFDO, Viana PL, Borba EL. 2019 The phylogeography of Vellozia auriculata (Velloziaceae) supports low zygotic gene flow and local population persistence in the campo rupestre, a Neotropical OCBIL. *Bot. J. Linn. Soc* **191**, 381–398. (doi:10.1093/botlinnean/boz051)

57. PBDB. 2018 The Paleobiology Database. See https://paleobiodb.org (accessed 16 September 2018).

58. Oliveira RS, Abrahão A, Pereira C, Teodoro GS, Brum M, Alcantara S, Lambers H. 2016 Ecophysiology of campos rupestres plants. In *Ecology and conservation of mountaintop grasslands in Brazil* (ed. GW Fernandes), pp. 227–272. Cham, Switzerland: Springer.

59. Lousada JM, Lovato MB, Borba EL. 2013 High genetic divergence and low genetic variability in disjunct populations of the endemic *Vellozia compacta* (Velloziaceae) occurring in two edaphic environments of Brazilian campos rupestres. *Braz. J. Bot.* **36**, 45–53. (doi:10.1007/s40415-013-0001-x)

60. Franceschinelli EV, Jacobi C, Drummond M, Resende M. 2006 The genetic diversity of two Brazilian *Vellozia* (Velloziaceae) with different patterns of spatial distribution and pollination biology. *Ann. Bot.* **97**, 585–592. (doi:10.1093/aob/mcl007)

61. Sazima M. 1977 Hummingbird pollination of *Barbacenia flava* (Velloziaceae) in the Serra do Cipó, Minas Gerais, Brazil. *Flora* **166**, 239–247. (doi:10.1016/S0367-2530(17)32141-2)

62. Renner SS. 1989 A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. *Ann. Missouri Bot. Gard.* **76**, 496–518. (doi:10.2307/2399497)

63. Ramos CO, Borba EL, Funch LS. 2005 Pollination in Brazilian *Syngonanthus* (Eriocaulaceae) species: evidence for entomophily instead of anemophily. *Ann Bot.* **96**, 387–397. (doi:10.1093/aob/mci191)

64. Conceição AA, Rapini A, do Carmo FF, Brito JC, Silva GA, Neves SP, Jacobi CM. 2016 Rupestrian grassland vegetation, diversity, and origin. In *Ecology and conservation of mountaintop grasslands in Brazil* (ed. GW Fernandes), pp. 105–127. Cham, Switzerland: Springer.

65. Condamine FL, Nagalingum NS, Marshall CR, Morlon H. 2015 Origin and diversification of living cycads: a cautionary tale on the impact of the branching process prior in Bayesian molecular dating. *BMC Evol. Biol.* **15**, 65. (doi:10.1186/s12862-015-0347-8)

66. Vasconcelos TNC, Alcantara S, Andrino CO, Forest F, Reginato M, Simon MF, Pirani JR. 2020 Data from: Fast diversification through a mosaic of evolutionary histories characterizes the endemic flora of ancient Neotropical mountains. Dryad Digital Repository. (doi:10.5061/dryad.x69p8czf6)

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