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Phylogeny strongly drives seed dormancy and quality in a climatically buffered hotspot for plant endemism

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- **Background and Aims** Models of costs and benefits of dormancy (D) predict that the evolutionarily stable strategy in long-term stable environments is for non-dormancy (ND), but this prediction remains to be tested empirically. We reviewed seed traits of species in the climatically buffered, geologically stable and nutrient-impoverished *campo rupestre* grasslands in Brazil to test the hypothesis that ND is favoured over D. We examined the relative importance of life-history traits and phylogeny in driving the evolution of D and assessed seed viability at the community level.
- **Methods** Germination and viability data were retrieved from 67 publications and ND/D was determined for 168 species in 25 angiosperm families. We also obtained the percentage of embryoless, viable and dormant seeds for 74 species. Frequencies of species with dormant and non-dormant seeds were compared with global databases of dormancy distribution.
- **Key Results** The majority of *campo rupestre* taxa (62.5 %) had non-dormant seeds, and the ND/D ratio was the highest for any vegetation type on Earth. Dormancy was unrelated to other species life-history traits, suggesting that contemporary factors are poor predictors of D. We found a significant phylogenetic structure in the dormancy categorical trait. Dormancy diversity was highly skewed towards the root of the phylogenetic tree and there was a strong phylogenetic signal in the data, suggesting a major role of phylogeny in determining the evolution of D versus ND and seed viability. Quantitative analysis of the data revealed that at least half of the seeds produced by 46 % of the surveyed populations were embryoless and/or otherwise non-viable.
- **Conclusions** Our results support the view that long-term climatic and geological stability favour ND. Seed viability data show that *campo rupestre* species have a markedly low investment in regeneration from seeds, highlighting the need for specific *in situ* and *ex situ* conservation strategies to avoid loss of biodiversity.

Key words: *Campo rupestre*, *Cerrado*, community, ecophylogenetics, embryoless seeds, evolutionarily stable strategy, OCBIL, P-deficient soils, refugia, regeneration ecology, rupestrian grassland, seed viability.

INTRODUCTION

Long-term climatic fluctuations can affect large-scale patterns of biodiversity, and centres of diversity and endemism are commonly associated with climatic and geological stability (Jansson, 2003; Carnaval and Moritz, 2008; Médail and Diadema, 2009). Therefore, identifying and protecting refugia have become a top conservation priority under projected climate change scenarios because of their ability to facilitate the survival of biota under adverse conditions (Keppel *et al.*, 2012). However, the question of how long-term climatic and geological stability influence plant life-history traits that determine species niches has rarely been explored.

Remarkable ecological and evolutionary singularities have been shown for old, climatically buffered, infertile landscapes (OCBILs) in contrast to young, often disturbed and fertile landscapes (YODFELs; Hopper, 2009). These OCBILs occur to a greater or lesser extent in at least 12 biodiversity hotspots, and

they function as biodiversity refugia containing extremely high levels of plant endemism, which can sum to 79 % (Hopper *et al.*, 2016). In OCBILs, there is a clear predominance of slow-growing plant species that largely rely on clonal reproduction for persistence, lack specialized means for seed dispersal, have specialized nutritional mechanisms and are resilient to fire and fragmentation but not to soil removal (Hopper, 2009; Oliveira *et al.*, 2015; Silveira *et al.*, 2016). As a result of long-term stability, plant populations from OCBILs seem to make little investment in migration mechanisms (Hopper, 2009), but no study has yet attempted to link biome-wide regeneration strategies with both life-history traits and historical stability.

Seed germination is a key component of the regeneration niche (Grubb, 1977), and it is one of the earliest traits expressed by plants and thus is under strong natural selection (Donohue *et al.*, 2010). Seed dormancy (D) is a state of inhibited germination (Simpson, 1990) that may confer adaptive value by helping to restrict the timing of germination to periods when

environmental conditions are favourable for seedling establishment. Global datasets indicate that D tends to increase with increasing seasonality, but it is a dominant trait across all vegetation zones on Earth (Jurado and Flores, 2005; Baskin and Baskin, 2014). Since D is a condition that helps regulate the timing of seed germination, it enables seeds to avoid germination during briefly favourable periods for seedling establishment (Linkies *et al.*, 2010; Baskin and Baskin, 2014). Delaying germination is also a bet-hedging strategy that spreads the risk of reproductive failure in unpredictably variable (stochastic) environments (Cohen, 1966; Venable, 2007; Poisot *et al.*, 2011; Moreira and Pausas, 2012).

On the other hand, D comes at the cost of increased seed mortality before germination occurs, e.g. due to physiological ageing, predation by animals and microbial decay (Long *et al.*, 2015). Thus, the benefits of D should outweigh its costs with increase in environmental uncertainty, whereas non-dormant seeds are expected to be more advantageous under a scenario of long-term climatic stability. In fact, models predict that the evolutionarily stable strategy in long-term constant environments is for non-dormant seeds regardless of adult longevity, the timing of reproduction and population age/stage structure (Rees, 1994). This prediction, however, remains to be tested empirically. Moreover, seed dormancy is not evenly distributed among plant functional groups such as different growth forms, dispersal modes and seed dispersal seasons (Wang *et al.*, 2009; Baskin and Baskin, 2014; Rubio de Casas *et al.*, 2015). This suggests that investigating life-history traits that drive D is a promising way of gaining insight into the evolution of D.

Lack of germination under favourable conditions cannot be attributed directly to seed dormancy without detailed examination of seed viability (Baskin *et al.*, 2006; Silveira, 2013). Therefore, assessing seed viability is especially crucial in severely phosphorus (P)-impoverished landscapes, where P limitation leads to drastic constraints in plant sexual reproduction (Fujita *et al.*, 2014). However, embryo presence and viability are often overlooked in germination studies, and disentangling the contributions of the multiple factors that result in lack of germination is crucial not only for distinguishing between dormant and non-germinable seeds but also to understanding the potential of regeneration from seeds at the community level.

Here we report the occurrence of primary dormancy (dormancy at the time of seed maturity) and seed viability for the *campo rupestre*, megadiverse heterogeneous grasslands (Silveira *et al.*, 2016). The *campo rupestre* is an OCBIL that occurs in eastern Brazil and is traditionally included as part of the *Cerrado* biome, i.e. Neotropical savanna (Oliveira-Filho and Ratter, 2002), a biodiversity hotspot (Myers *et al.*, 2000). Dated phylogenies suggested that unrelated endemic lineages of *Cerrado* started to diversify 9.8 million years ago (Mya) (Simon *et al.*, 2009). Evidence also indicates that diversification of some *campo rupestre* lineages pre-date diversification of *Cerrado* lineages by several million years, suggesting that the *campo rupestre* was the first open habitat in eastern South America (Hughes *et al.*, 2013). On a short-term time-scale, the climatic regime in the *campo rupestre* is seasonal, with markedly dry winters and wet summers and increasing aridity and temperatures towards the north of its range (Silveira *et al.*, 2016). In the long term, however, the *campo rupestre* has been buffered from past climatic extremes. Although there have been

shifts in temperature and aridity, *campo rupestre* did not undergo any significant expansion during the middle Holocene or during the Last Glacial Maximum, probably due to its strong edaphic isolation (Alves and Kolbek, 1994; Barbosa and Fernandes, 2016). The resulting vegetation stability may have favoured the existence of several areas of endemism and refugia (Collevatti *et al.*, 2009; Bonatelli *et al.*, 2014; Ribeiro *et al.*, 2014; Barbosa *et al.*, 2015). In fact, the *campo rupestre* is a major centre of endemism, with the highest percentage of endemic species (1951 endemic out of 4928 species, 39.6 %) among all other vegetation types in Brazil (BFG, 2015). Furthermore, *campo rupestre* occurs in severely P-impoverished, shallow, acidic and excessively drained quartzite-derived or ironstone soils, mostly above 900 m and up to 2033 m above sea level (Giulietti *et al.*, 1997; Jacobi *et al.*, 2007; Alves and Kolbek, 2010; Oliveira *et al.*, 2015). Here, plants often experience strong winds, high irradiance, frequent fires, high daily fluctuations in temperature and water shortage during the dry season (Silveira *et al.*, 2016).

In this study, we review the ecology and evolution of seed dormancy of *campo rupestre* plant species. Specifically, we tested the following hypotheses. (1) Long-term geological and climatic stability favour seeds that are non-dormant. (2) Life-history traits play a more important role than phylogeny in determining seed dormancy occurrence. (3) Plants growing in P-impoverished soils make low investments in sexual reproduction, with unusually high levels of embryoless and non-viable seeds. Further, we discuss the implications of our findings for predicting the impact of future rapid climate change in the regeneration niche of species in the *campo rupestre* flora.

MATERIALS AND METHODS

Literature review on campo rupestre seed germination

We conducted a comprehensive literature search using three online search services to include experimental germination studies on seeds collected at *campo rupestre* sites. To find relevant papers, we used a topic search in Web of Science, an all-indexes search in Scielo (a National Open-Access Scientific Library; www.scielo.br) and a broad search at Google Scholar (search terms in Supplementary Data Appendix S1), which yielded a total of 50, 9 and 1160 studies, respectively (until 5 September 2015). In addition, we included unpublished data of the present authors. We combined the results from all searches and removed duplicates.

Studies were included in the database if they met the following criteria: (1) peer-reviewed indexed literature and Masters and PhD theses written in English or Portuguese; review papers were not included; (2) germination experiments were performed with fresh seeds, given that dormancy cannot be safely determined in stored seeds (Baskin *et al.*, 2006); (3) seeds were collected in *campo rupestre sensu stricto* (Silveira *et al.*, 2016); (4) control treatments were performed whenever a dormancy-breaking treatment was conducted; and (5) species-level identification was provided by the authors.

In total, 67 studies met our criteria, providing data for 184 species in 17 families. We also report empirical data for 26 species belonging to 16 families (ten additional families) to increase representation of non-studied taxa (original data from

germination and viability experiments in [Supplementary Data Appendix S2](#)). In total, we gathered information for 210 species (plus 25 subspecies, varieties or populations) belonging to 27 families. This corresponds to 20 % of the families and 4.2 % of the species in the *campo rupestre*. To investigate the potential for regeneration from *campo rupestre* seeds, a quantitative analysis was performed in which we included species only when viability tests were performed or when scarification provided reliable information on maximum seed viability. Since among-population variation in seed dormancy and germination is commonplace ([Anderssen and Milberg, 1998](#)), we considered different populations as different evolutionary units, using numbers to distinguish among them. Given that we found information on *Lychnophora pinaster* seeds collected in two different years, we averaged the values of viability and germinability of this species.

For each species/population examined, we recorded study site (region, location and coordinates), collection date or dispersal period, species dispersal mode (biotic or abiotic), growth form (tree, succulent, shrub or herb), microhabitat (xeric and/or mesic), experiment length (number of days), incubation temperature, germinability (germination %), germination time or rate (e.g. mean germination time, mean velocity, germination rate index), embryoless seeds (%), viable seeds (%) and pre-germination treatments when available (e.g. scarification, gibberellic acid). Species names were updated according to the Brazil Plant Species List available at Reflora (<http://reflora.jbrj.gov.br>) in September 2015.

Information on species geographic distribution and growth form was obtained at the specieslink network (<http://www.splink.org.br>) and from the Reflora database. Information on dispersal mode was inferred from fruit morphology (e.g. [Barroso et al., 1999](#)). Species microhabitat (xeric, mesic or xeric and mesic) was determined from information available in the papers or by consulting the authors and taxonomy experts. Date of seed collection or fruit dispersal phenology was used to determine the time of seed dispersal, which was further divided into four seasons according to rainfall data for the region ([Madeira and Fernandes, 1999](#)). The early rainy season is from October to December, the late rainy season from January to March, the early dry season from April to June and the late dry season from July to September. The dry season was considered unfavourable for seedling establishment due to the occurrence of frequent fires, high daily temperature fluctuations and drought ([Giulietti et al., 1997](#); [Madeira and Fernandes, 1999](#); [Silveira et al., 2012](#)).

Determination of primary dormancy category

There are many definitions of D. In this study, we define seed dormancy as the failure of viable seeds to germinate when environmental conditions, including water, temperature, light and gases, are favourable for germination of non-dormant seeds ([Vleeshouwers et al., 1995](#); [Bewley, 1997](#); [Hilhorst, 2011](#)). Due to limited information on most *campo rupestre* species, we did not attempt to assign dormancy classes *sensu* [Baskin and Baskin \(2004\)](#). Our primary aim was to determine whether seeds are primarily dormant or non-dormant.

Seeds were considered to be non-dormant if ≥ 70 % of viable seeds germinated in about 4 weeks. They were considered to be dormant if less than 30 % of viable seeds germinated in about 4 weeks, and pre-germination treatments improved germination. We also classified seeds as dormant for intermediate cases in which germination occurred at higher percentages only at a very limited temperature range (assumed to be conditionally dormant *sensu* [Baskin and Baskin, 2014](#)), and < 70 % of viable seeds germinated in about 4 weeks. The D/non-dormancy (ND) classification was considered non-conclusive when: (1) seed viability was less than 10 %; (2) less than 70 % of seeds germinated in about 4 weeks and there was no reference value for seed viability and no increase in germination percentage following treatment for dormancy break. Populations classified as non-conclusive were excluded from categorical data analyses, but populations with a low percentage of viable seeds were used in quantitative analyses.

Database of seed dormancy in other vegetation zones

[Baskin and Baskin \(2014\)](#) collected a massive amount of information on seed dormancy and germination from all main vegetation zones worldwide. Comparison of *campo rupestre* data on seed dormancy with the [Baskin and Baskin \(2014\)](#) database allows us to situate *campo rupestre* within its encompassing biome and other vegetation zones. Therefore, we used the [Baskin and Baskin \(2014\)](#) database to extract information on occurrence of primary D in savannas (i.e. tropical dry woodlands, natural savannas and grasslands) and in three other vegetation types (tropical evergreen forest, tropical semi-deciduous forest and tropical deciduous forest) occurring in southeast Brazil that have differences and similarities of climatic patterns compared with those in *campo rupestre*. We then compared data for populations growing in *campo rupestre* with data for species growing in these four vegetation zones.

Statistical and phylogenetic analyses

The χ^2 test was used to assess differences in the frequencies of D and ND between the five vegetation zones and life-history traits – i.e. dispersal mode (abiotic or biotic), dispersal period (early rainy, late rainy, early dry or late dry seasons), geographic distribution (endemic or non-endemic), growth form (herb or shrub) and microhabitat (xeric or mesic). Levels within variables with a small sample size, namely succulent and tree (within growth form category) and ‘xeric and mesic’ (within microhabitat category), were excluded from analysis to prevent loss of test reliability ([Zar, 2012](#)). Bonferroni correction (α/n) was applied in χ^2 tests whenever multiple comparisons were made.

To explore the main pattern of association between all categorical variables, we computed a global distance matrix based on the above-mentioned variables and applied a principal coordinates analysis (PCoA) to compute the two main axes of variation encompassed in the global distance matrix. Finally, to visualize the groups based on the categorical variables, we plotted a factorial map, using the functions in [Pavoine et al. \(2009\)](#). These analyses were performed with the package ‘ade4’ ([Dray](#)

and Dufour, 2007) in the R environment (R Development Core Team, 2014).

To assess the role of phylogeny in explaining seed dormancy in *campo rupestre*, populations were arrayed on a phylogenetic tree. Briefly, species relationships were obtained using ‘PhyloMatic’ (<http://phylodiversity.net/phyloMatic/>). We then manually corrected and improved the ultrametric tree resolution in ‘Mesquite’ (<http://mesquiteproject.org/>) based on a number of recent studies of molecular phylogeny. To estimate branch lengths (i.e. time since divergence), we dated 55 nodes according to several studies (Supplementary Data Appendix S3) and positioned undated nodes evenly in the tree with the ‘bladj’ algorithm of Phylocom software (Webb et al., 2008).

To assess the existence of phylogenetic signal in the dormancy categorical trait, we used two distinct approaches: (1) the Maddison and Slatkin (1991) method, which compares the minimum number of trait-state changes across a phylogenetic tree with a null model (100,000 randomizations), in which the trait states were randomized in the tips of the tree; and (2) decomposition of dormancy trait diversity among the nodes of the phylogenetic tree to test whether dormancy diversity is clustered near the root of the phylogenetic tree [tips/root skewness test based on the distance of nodes to the root of the tree, with 10 000 randomizations, according to Pavoine et al. (2010)]. To obtain the distance matrix based on the categorical dormancy trait, we used the generalization of Gower’s distance according to Pavoine et al. (2009). For quantitative traits [dormancy index $D \times (D + ND + 1)^{-1}$ and $\log(x + 1)$ -transformed percentages of embryoless and non-viable seeds], we examined the phylogenetic signal with Blomberg’s K test with 100 000 randomizations (Blomberg et al., 2003; Münkemüller et al., 2012). All analyses were performed in the R environment (R Development Core Team, 2014), using the ‘picante’ package for Blomberg’s K test (Kembel et al., 2010), the ‘phylo.signal.disc’ function (developed by Enrico L. Rezende, University of Roehampton, UK) for the Maddison and Slatkin (1991) method and the ‘decdiv’ function for decomposition of trait diversity among the nodes of the phylogenetic tree (Pavoine et al., 2010).

RESULTS

Ecology and evolution of seed dormancy

We were able to classify the D/ND status in 168 populations (155 species) from *campo rupestre* (Supplementary Data Appendices S4 and S5). The majority of populations (105; 62.5%) had non-dormant seeds, and 63 populations (37.5 %) had dormant seeds. We could not assign dormancy status in 55 species.

Frequencies of D and ND in *campo rupestre* were significantly different from those in all the other tropical vegetation zones. Non-dormancy was statistically more frequent in *campo rupestre* than in any of the other four vegetation zones. Evergreen tropical rainforest had the second highest frequency of ND, followed by tropical semi-deciduous forests and savannas and tropical deciduous forests, which had the lowest frequencies of ND (Fig. 1) (Supplementary Data Appendix S6).

The χ^2 tests did not show a correlation between seed dormancy category and any life-history traits: dispersal mode,

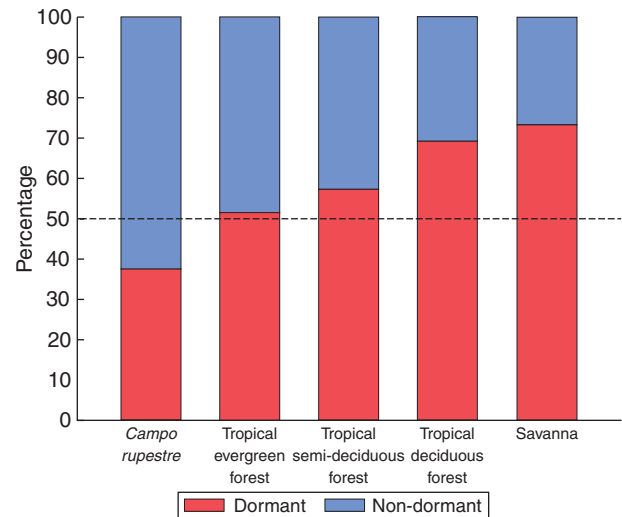


FIG. 1. Biome-wide percentage of seed dormancy categories among *campo rupestre* (this study) and tropical evergreen forests, tropical semi-deciduous forests, savannas and tropical deciduous forests (Baskin and Baskin, 2014). D and ND frequencies were significantly different among all vegetation zones, except for savannas and tropical deciduous forests, according to the χ^2 test (Appendix S5).

TABLE 1. Lack of association between dormancy category and life-history traits: dispersal mode (abiotic or biotic), dispersal period (early rainy, late rainy, early dry or late dry season), geographic distribution (endemic or non-endemic), growth form (herb or shrub), microhabitat (xeric or mesic). Bonferroni correction was applied in χ^2 tests due to the multiple comparisons, using the significance level of $P < 0.01$

Variables	χ^2 test	
	χ^2	P
Dispersal mode	0.83	0.362
Dispersal time	8.077	0.044
Geographic distribution	2.442	0.118
Growth form	1.6834	0.194
Microhabitat	4.9452	0.026

dispersal period, geographic distribution, growth form or microhabitat (Table 1). The PCoA applied in the global distance computed with dormancy and life-history traits highlighted the lack of association between these variables. The two main axes of the PCoA explained 39 % of the total variance. The first axis separated dormant and non-dormant populations, whereas the second axis separated endemic from non-endemic populations. The other life-history traits were not associated with the first two axes, with either D or ND classification (Fig. 2).

We found a strong phylogenetic signal in the dormancy categorical trait using the Maddison and Slatkin (1991) method ($P < 0.00001$), and the partition of dormancy diversity was highly skewed towards the root of the tree ($P < 0.001$; Fig. 3). The node with the highest seed dormancy trait diversity (10.2 % of total diversity) was the one that diverges between monocots and eudicots (Fig. 3). Overall, dormancy was concentrated in a few extant families, and it was especially rare in the

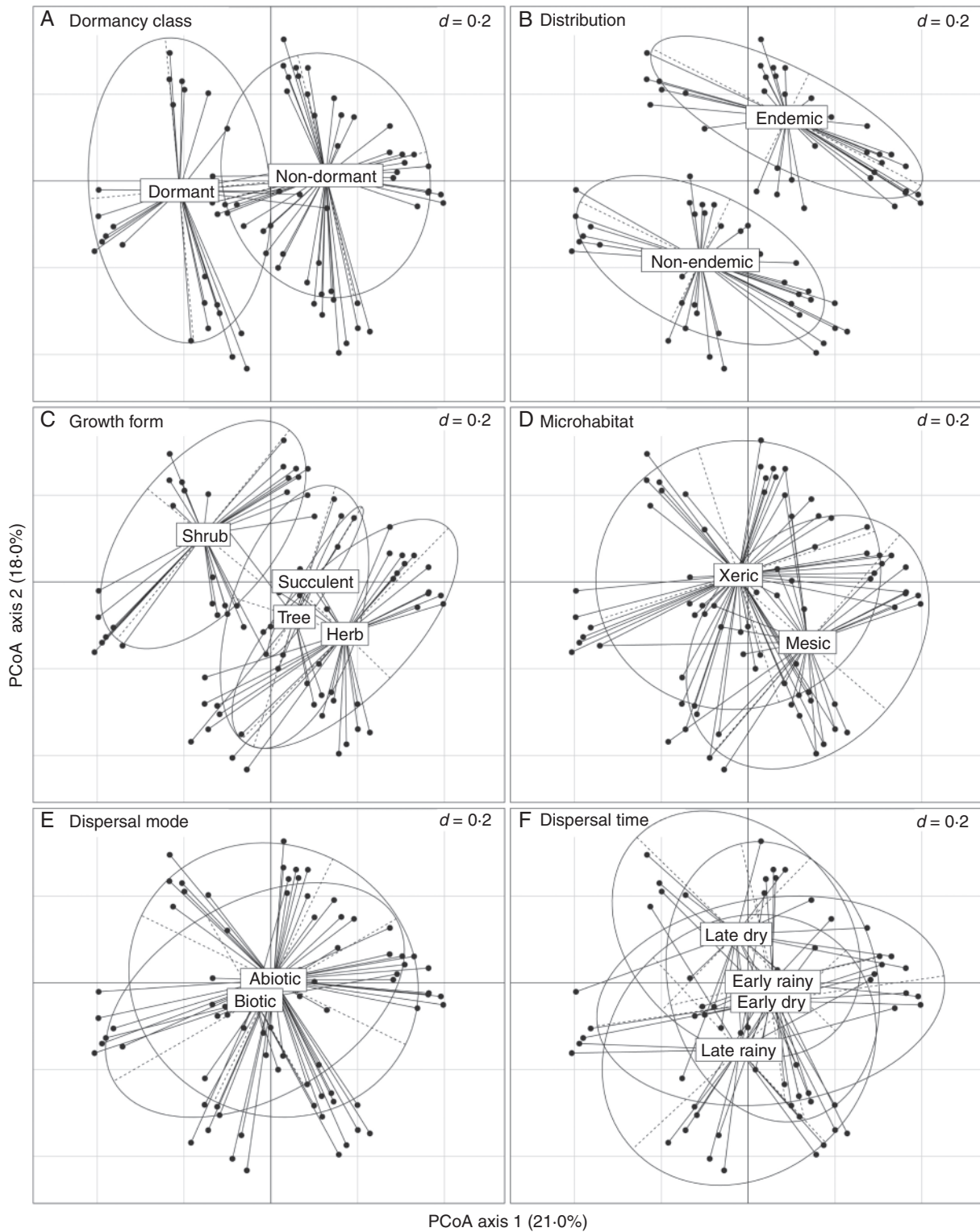


FIG. 2. Principal coordinates analysis (PCoA) applied to the global distance computed with the dormancy categorical trait and five life-history traits, using the generalization of Gower distance according to Pavoine *et al.* (2009). Each panel shows a factorial plot representing the levels of each categorical variable at the centroid of species assigned to each level. The percentage of variation explained by each axis is shown in parentheses. Vertical and horizontal grid lines are separated by 0.2 units (d) at each axis scale.

monocots, with Poaceae and Cyperaceae having the highest dormancy percentages. Seed dormancy was more common in the eudicots, with Fabaceae and Verbenaceae showing highest percentages of species with dormant seeds. In the Melastomataceae, D appears to have evolved multiple times (Fig. 3).

embryoless and/or non-viable seeds seems to be phylogenetically determined (Supplementary Data Appendix S7). Overall, we found a wide variety of patterns for seeds from *campo rupestre* in which families had contrasting levels of dormancy, viability and embryo presence (Fig. 4).

The potential for regeneration from seeds

Reliable information for embryo presence, viability and dormancy was available for 83 populations of 74 species in 21 families. Blomberg’s *K* test shows the phylogenetic signal in the quantitative assessment of dormancy ($K=0.646$; $P=0.00001$), confirming the results obtained using the Maddison and Slatkin (1991) method and diversity partitioning analysis in the dormancy categorical trait. The phylogenetic signal was also significant for the percentage of embryoless ($K=1.469$; $P=0.00001$) and non-viable ($K=0.2264$; $P=0.03456$) seeds. Therefore, the high prevalence of populations that produce

DISCUSSION

Despite the widely recognized association between centres of diversity and endemism with climatic and geological stability, very little is known about how long-term stability influences species traits that determine regeneration niches. We compiled a database with information on seed dormancy and viability in *campo rupestre* to test hypotheses on the life-history traits that drive the adaptive value of germination behaviour in long-term stable environments. To our knowledge, the D/ND ratio found for *campo rupestre* is the lowest on Earth (Baskin and Baskin, 2014). In *campo rupestre*, the D/ND ratio was markedly lower than that in the surrounding types of vegetation, which have

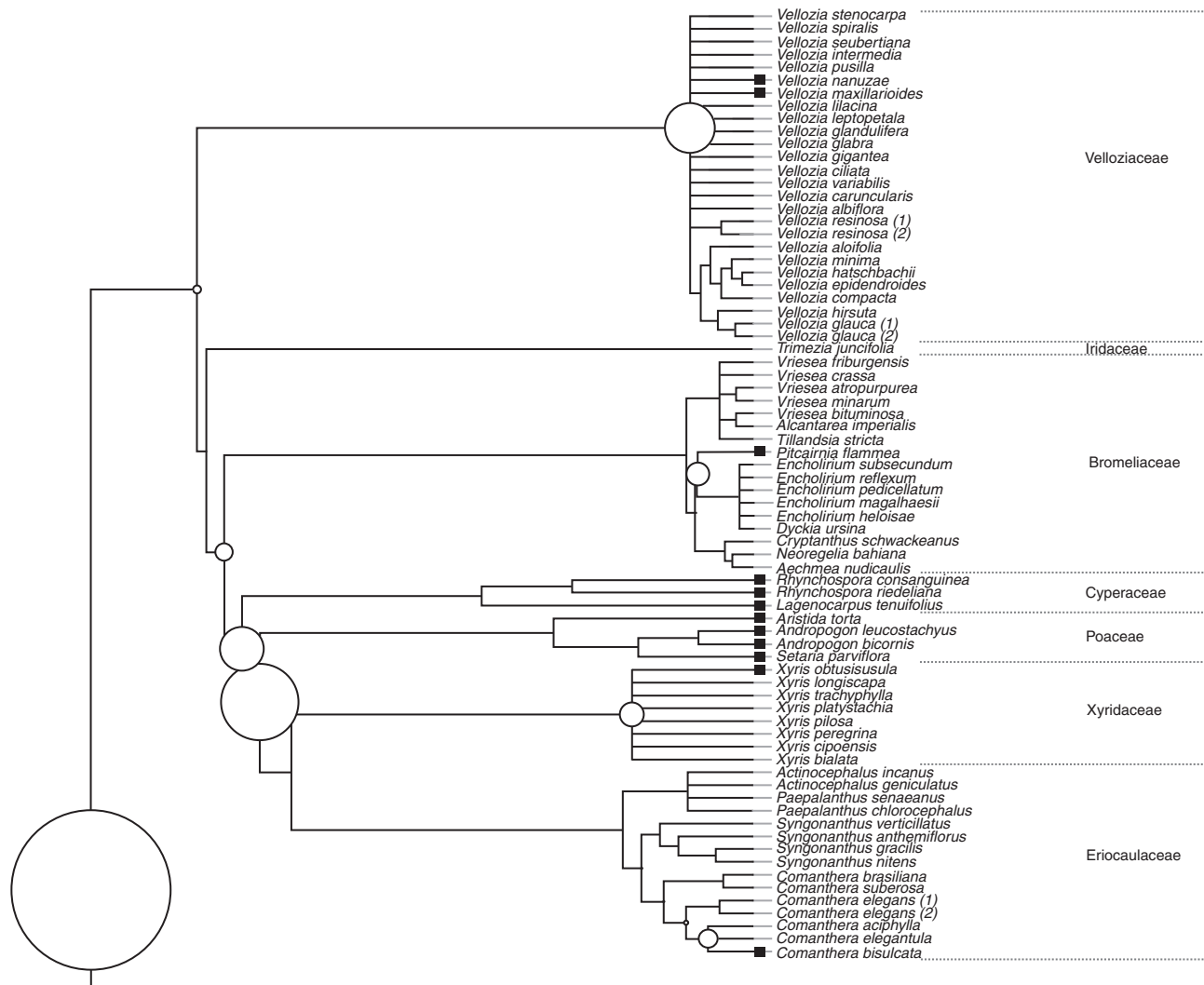


FIG. 3. Continued.

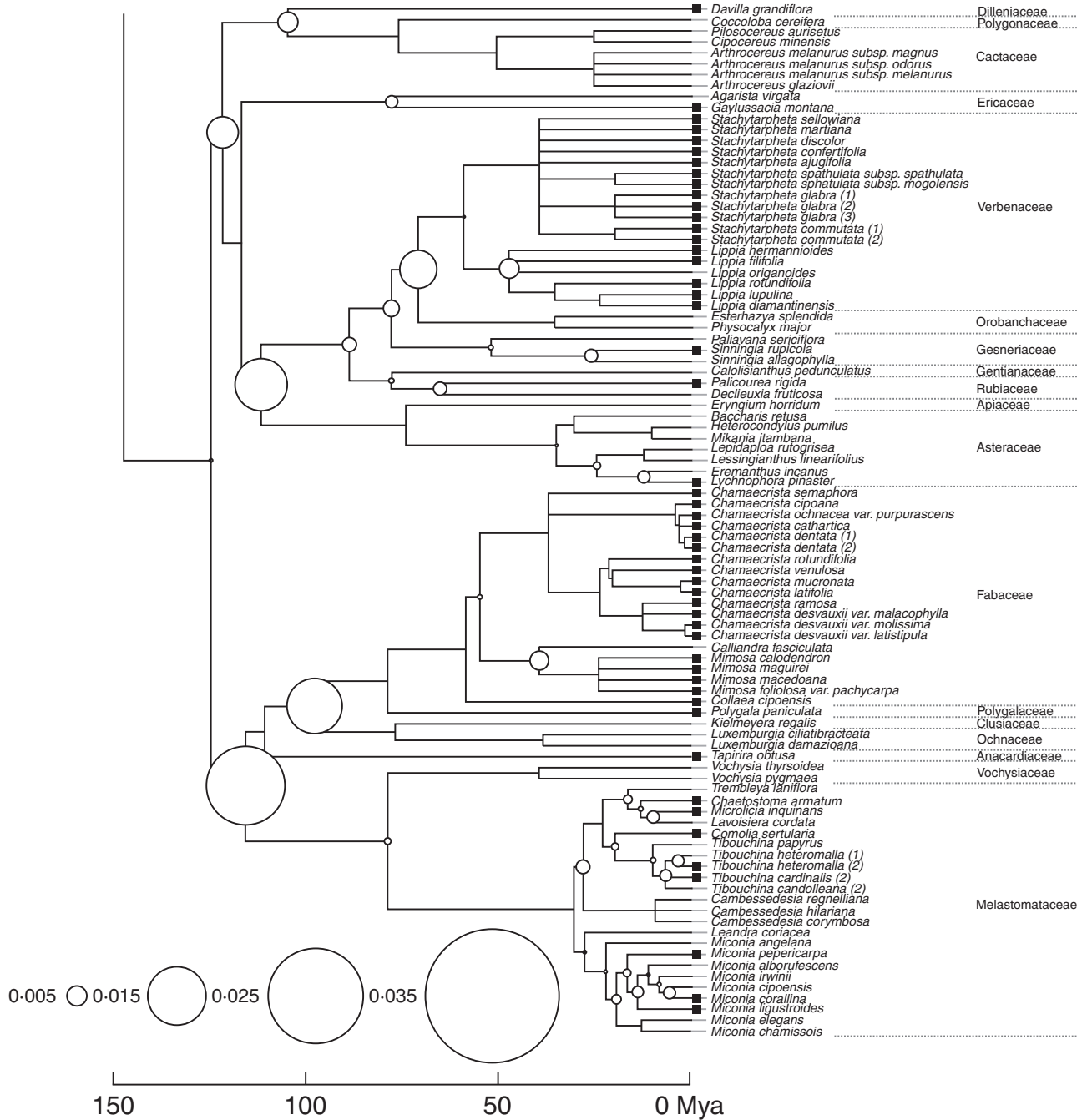


FIG. 3. Decomposition of dormancy trait diversity along the nodes of the phylogenetic tree assembled for the *campo rupestre* species. Black squares at the tips of the tree denote populations classified as primarily dormant. A time scale (Mya) is shown below the tree. The total quadratic entropy (TQE) for the dormancy categorical trait diversity is 0.994, and the circles at the nodes indicate the contribution of the node to total dormancy diversity. Scale is given at the bottom of the tree.

gone through major and extensive area changes during periods of climatic fluctuation, especially during the Quaternary (Carnaval and Moritz, 2008; Carnaval *et al.*, 2009; Werneck *et al.*, 2011, 2012), thus supporting our first hypothesis that long-term geological stability and climatic buffering favours non-dormant seeds. We found no significant correlations between seed dormancy and life-history traits, thereby providing no support for our second hypothesis. Phylogeny, rather than ecology, was better correlated with seed dormancy categories,

indicating that geological-climatic history played a more important role than contemporary factors in driving seed dormancy. We also found an unprecedentedly wide variation in the percentage of embryoless and non-viable seeds within a single vegetation type, which showed a strong phylogenetic signal, suggesting strong biome-wide limitations in sexual reproduction across different taxa. Notably, *campo rupestre* had major differences with regard to the known patterns of germination behaviour, which seems to agree with evidence of low

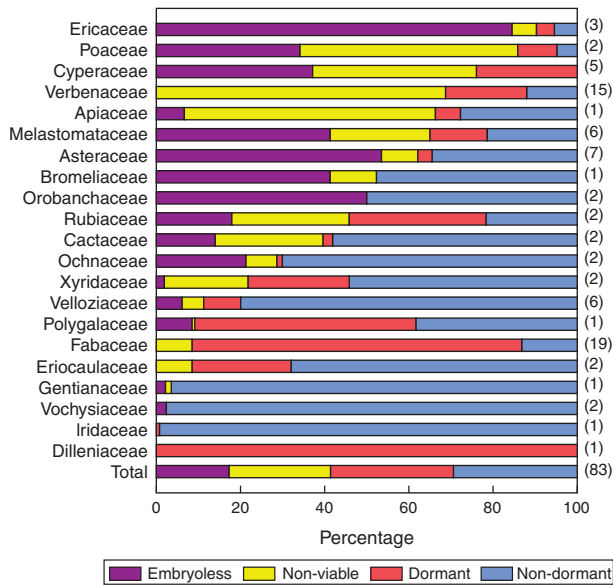


Fig. 4. Average percentages of embryoless, non-viable, non-dormant and dormant seeds of *campo rupestre* species groups per family. Families are ranked by total percentage of non-germinable seeds (embryoless plus non-viable). Total percentage of viable seeds is the sum of the percentages of dormant and non-dormant seeds. Numbers in parentheses on the right side of the bars indicate numbers of sampled populations (n).

investment in dispersal mechanisms in plant communities from OCBILs (Hopper, 2009).

Costs and benefits of seed dormancy in *campo rupestre*

Without accounting for the historical geological–climatic component, we would expect a seasonal environment, such as the *campo rupestre*, to have proportions of dormant seeds similar to that of savannas and tropical deciduous forests, and a greater proportion than that of aseasonal environments, such as the tropical evergreen rainforest and tropical semi-deciduous forests (Jurado and Flores, 2005; Baskin and Baskin, 2014). At least for Melastomataceae from *campo rupestre*, dormancy evolved in species dispersing seeds during the late rainy season, shortly before conditions become unfavourable for seedling establishment (Silveira *et al.*, 2012), thereby confirming the predictions of a significant association between dispersal time and seed dormancy in seasonal environments (Rubio de Casas *et al.*, 2015). However, our results differ from those of other studies (Bu *et al.*, 2008; Wang *et al.*, 2009) in that we found no correlation of D with dispersal period, suggesting that seasonality is not a strong selective force driving D in *campo rupestre* at the community level.

It is not yet clear how non-dormant species manage to germinate in the field at a time that is suitable for seedling establishment. Unlike other seasonal environments, life-stage transitions (e.g. germination, flowering, seed dispersal) of *campo rupestre* plants show tremendous diversity in phenological patterns (Madeira and Fernandes, 1999; Le Stradic, 2012; Belo *et al.*, 2013), suggesting no obvious outcome of the complex interaction between life-history traits and environmental filters. For

instance, seed dispersal and germination of two *campo rupestre* endemic species of *Leiothrix* (Eriocaulaceae) occur under unfavourable conditions for seedling establishment, resulting in enormous mortality rates of seedlings during the subsequent dry period, as predicted (Coelho *et al.*, 2008). It seems that these *Leiothrix* species and many other *campo rupestre* species rely on long-term survival of adult plants for persistence, since long-lived perennials, resprouters and clonal plants are recurrent in this environment (Alves, 1994; Silveira *et al.*, 2016). This strategy of persistence through long-lived individuals is markedly different from that of annual plants, which depend on seedling survival and/or formation of a seed bank for persistence and therefore delay germination, which reduces risk (Venable, 2007).

Seeds must remain viable for dormancy to be adaptive, otherwise they may die before germination occurs (Donohue *et al.*, 2010). Our results indicate that many species produce seeds with low viability at the time of dispersal. Although some typical *campo rupestre* species maintain their viability for more than 1 year (Munné-Bosch *et al.*, 2011; Cheib and Garcia, 2012; Garcia *et al.*, 2012, 2014), some agents of mortality, such as predators, can effectively reduce seed set. Future studies should focus on quantifying seed mortality and identifying its various sources to allow more general conclusions on seed persistence in the soil (Long *et al.*, 2015).

Phylogeny correlates with dormancy categories

All analyses consistently indicated a strong phylogenetic signal in seed dormancy, indicating that closely related species are more similar regarding D category than expected by chance. Additionally, skewness of D diversity towards the root in the phylogenetic tree shows that the rate of dormancy evolution was high in the past, during the major species divergences in angiosperms, and suggests a stable evolution of dormancy in recent lineages. This trait conservatism may be at least partly explained by *campo rupestre*'s history of recent speciation (Bitencourt and Rapini, 2013) and by its geographically structured speciation (Alves and Kolbek, 1994). Furthermore, in particular, transitions from ND to other D states have been considered to be uncommon throughout the evolutionary history of flowering plants, suggesting that ND is a derived state unlikely to be changed, although some such changes have occurred (Willis *et al.*, 2014).

Since seed dormancy occurrence was not correlated to any of the life-history traits analysed here, phylogeny alone was its best predictor. In Melastomataceae, however, seed dormancy is a very labile trait, known to be influenced by life-history factors (Silveira *et al.*, 2012). Therefore, although phylogenetic constraints probably prevent multiple evolution of seed dormancy in the vast majority of clades, some families, such as Melastomataceae, may evolve under more relaxed constraints.

Finally, we acknowledge that our database of *campo rupestre* seeds may be biased towards certain clades, thus limiting the extent of our conclusions on the evolution of seed dormancy. Nevertheless, biased samples are the case in all biodiversity studies (Hortal *et al.*, 2015), including those that consider seed biology (Baskin and Baskin 2014). In addition, our database comprises several species in eight of the ten most species-rich

families of *campo rupestre* and in eight of its nine most species-rich genera (Silveira et al., 2016). Poaceae and Cyperaceae are especially abundant in *campo rupestre*, and therefore they may be underrepresented in our database. However, these two families exhibited extremely low seed viability, indicating that most of their species probably do not rely on seeds as a means of reproductive assurance (Le Stradic et al., 2015). Thus, despite the limited size of the database, we are confident that it is representative of the *campo rupestre* vegetation.

The potential for regeneration from seeds

Seeds of taxa from *campo rupestre* showed diverse percentages of embryo presence and viability. At least half of the seeds produced by 46 % of the populations were embryoless and/or non-viable, thus indicating a very low potential for regeneration from seeds. Given that the soil of *campo rupestre* is one of the most P-deficient soils in the world (Oliveira et al., 2015), this result is in line with the hypothesis that P limitation is associated with low investment in sexual reproduction (Fujita et al., 2014). Other possible causes of the low levels of seed viability are inbreeding depression (Lamont and Wiens, 2003; Montoro and Santos, 2007; Holmes et al., 2008; Vos et al., 2012), since many *campo rupestre* species are locally rare (Silveira et al., 2016). In addition, the percentage of embryoless and non-viable seeds had a strong phylogenetic signal, indicating that geological-climatic historical factors also play a role in determining seed quality. Further studies are necessary to determine whether sexual versus asexual reproductive systems provide evidence for trade-offs that influence recruitment success in nutrient-impooverished environments (Vico et al., 2016).

The highly diverse patterns of seed viability across taxa also point to a great challenge in relying on seeds to preserve biodiversity of *campo rupestre*. The overall mean of 41 % of embryoless and non-viable seeds indicates that it will be challenging to obtain the amount of seeds necessary for the effective use of native species in landscape-scale restoration (Merritt and Dixon, 2011). To date, many restoration techniques have been inefficient in restoring plant communities of *campo rupestre* (Matias et al., 2009; Le Stradic et al., 2014a, b), and low seed viability is the best explanation for these failures (Le Stradic et al., 2014a). In this regard, our data show that phylogeny plays an important role in determining seed viability percentages, providing a useful tool for species selection and devising new approaches for seed-based restoration techniques.

Future scenario

There is a clear predominance of stress-tolerant strategies in *campo rupestre* plant communities (Negreiros et al., 2014), which may confer unusually high resistance of the species to climate change (Harrison et al., 2015). Nevertheless, we still cannot entirely appreciate the effects of climate change on this vegetation type, since there is a huge knowledge gap regarding plant regeneration. Since phylogeny seems to be the main driver of regeneration strategy, our results suggest that phylogenetic inertia determines the ability of seeds of most species to respond physiologically to specific environmental cues. The

phylogenetic approach may therefore be useful to improve our ability to predict germination patterns across community and neglected clades (Ribeiro et al., 2016). This is important since the speed of climate change and the rate of habitat destruction are much higher than our ability to generate scientific data on the seed biology of native species, and effective conservation strategies are therefore necessary. However, investigations of germination timing in the field and seedling growth and survival under both present and future conditions also are necessary to better understand plant recruitment from seeds in the *campo rupestre*.

Altogether, our study provides support for the view that long-term climatic buffering and geological stability, along with soil infertility, are associated with a markedly low investment in regeneration from seeds (Hopper et al., 2016; Silveira et al., 2016). This view, however, raises the question of how plants adapted to long-term stability will respond to a fast-changing environment. Models of future climate scenarios have predicted large losses of environmentally suitable areas for *campo rupestre* until the 2080s, regardless of other existing anthropic impacts (Bitencourt et al., 2016). The high percentage of non-germinable seeds reported here, combined with the lack of specialized seed dispersal mechanisms in *campo rupestre* (Silveira et al., 2016), suggests that these species are not likely to migrate to suitable sites in the face of climate change. Therefore, persistence of the plant communities in current areas and *ex situ* conservation strategies are of utmost importance in avoiding loss of biodiversity and associated ecosystem services.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Appendix S1: key words used for search in three academic search engines. Appendix S2: germination experiments and viability assessments. Table S1: list of species and information concerning the collection date, locations of the studied species, number of sampled individuals and replicates used in germination and viability trials. Appendix S3: details of the resolution of polytomies and determination of node ages of the phylogenetic tree with 168 species from *campos rupestres*, southeastern Brazil. Table S2: estimated age of nodes numbered in Figure S1. Figure S1: phylogenetic tree with the 168 species from *campos rupestres*, southeastern Brazil. Appendix S4: Table S3: mean percentage of embryoless, non-viable, non-dormant and dormant seeds for 26 species of *campo rupestre*. Appendix S5: Table S4: dormancy or non-dormancy in seeds in 176 populations from *campo rupestre*. Appendix S6: Table S5: comparison of dormancy and non-dormancy absolute frequencies between different vegetation zones. Appendix S7: Figure S2: phylogenetic tree assembled for the *campo rupestre* species with quantitative data.

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