UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

CECILIA FONSECA FIORINI

EVOLUTIONARY PROCESSES AND THE ORIGIN OF PLANT BIODIVERSITY IN OLD NEOTROPICAL SKY-ISLANDS:
A GENOMIC AND MODEL-BASED APPROACH

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutora em Biologia Vegetal.

Área de Concentração: Morfologia, sistemática e diversidade vegetal

BELO HORIZONTE – MG

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"Quando despersonalizamos o rio, a montanha, quando tiramos deles os seus sentidos, considerando que isso é um atributo exclusivo dos humanos, nós liberamos esses lugares para que se tornem resíduos da atividade industrial e extrativista. Do nosso divórcio das integrações e interações com a nossa mãe, a Terra, resulta que ela está nos deixando órfãos, não só aos que em diferente graduação são chamados de índios, indígenas ou povos indígenas, mas a todos."

Ailton Krenk, *Ideias para adiar o fim do mundo,* 2019.

RESUMO

Os Neotrópicos concentram uma grande parte da biodiversidade vegetal mundial e foi demonstrado que montanhas desempenham um papel importante neste padrão. A cadeia de montanhas compreendendo Espinhaço e a Chapada-Diamantina abrigam os campos rupestres, um mosaico de vegetação herbácea-arbustiva altamente diversa, em *sky-islands* (acima de 900m) de solos rochosos ou arenosos pobres em nutrientes. Neste estudo, usamos dois grupos que ocorrem nos campos rupestres e apresentam características ecológicas contrastantes (*Bulbophyllum* sect. *Didactyle*, Orchidaceae, e *Vellozia auriculata*, Velloziaceae) para compreender o processo evolutivo que leva às elevadas diversidades de espécies e de espécies endêmicas observadas nessa vegetação. Observamos a ocorrência de estruturação geográfica, sendo mais forte para *V. auriculata*, espécie que apresenta menor vagilidade. Para *B. involutum*, o fluxo gênico entre as localidades de Espinhaço foi elevado, corroborando a noção de que as sementes pequenas e leves de orquídeas são capazes de dispersão a longa distância. No entanto, nossos dados também sustentam que características ambientais ou eventos demográficos passados podem ser fatores importantes para a diferenciação populacional. De um modo geral, a distribuição da variabilidade genética de *B.* sect. *Didactyle* reflete a geografia das populações, contudo algumas espécies da seção não foram recuperadas como monofiléticas. Além da importância das disjunções e da variabilidade ambiental para a diferenciação entre populações, demonstramos também que a hibridação pode ser um mecanismo importante para a origem e manutenção da biodiversidade de campos rupestres.

Palavras chave: Campos rupestres, *Bulbophyllum*, complexo de espécie, *Vellozia auriculata*, filogeografia

ABSTRACT

A large proportion of the world's plant biodiversity is concentrated on the Neotropics and mountains have been shown to play an important role on this pattern. The Espinhaço/Chapada-Diamantina chain harbors the highly diverse campos rupestres, a sky-island herbaceous-shrubby vegetation mosaic, that occurs above 900 m on nutrient poor, rocky and sandy soils. In this study we used two groups occurring in the campos rupestres and presenting contrasting ecological characteristics (*Bulbophyllum* sect. *Didactyle*, Orchidaceae, and *Vellozia auriculata*, Velloziaceae) to understand the evolutionary process leading to the high species and endemic diversity observed on this vegetation. We observed geographical structure, and this structure was stronger for the less vagile *V. auriculata*. For *Bulbophyllum involutum* the gene flow between Espinhaço localities was high, corroborating the notion that the small and light seeds of orchids are able of long-range dispersion. However, our data also support that environment or past demographic events might be important factors driving population differentiation. Overall, the distribution of the genetic variability of *B.* sect. *Didactyle* reflects the population geography, but some species have not been recovered as monophyletic. Beyond the importance of disjunction and environmental variability for population differentiation, here we demonstrate that hybridization might be and critical engine for the origin and maintenance of the campos rupestres biodiversity.

Keywords: Campos rupestres, *Bulbophyllum*, species complex, *Vellozia auriculata*, phylogeography.

SUMÁRIO

INTRODUCTION

The Neotropics concentrates a high proportion of the plant biodiversity of the planet (28%; Antonelli et al., 2015), and mountains have been shown to play an important role on the diversification and maintenance of its biodiversity (Flantua, O'Dea, Onstein, Giraldo, & Hooghiemstra, 2019; Rahbek et al., 2019). Besides to the well-known Andes, South America harbors another cordillera: the Espinhaço/Chapada-Diamantina chain, with altitudes up to 2,072 m and > 1,000 km of latitudinal extension in eastern Brazil. However, differently from the recent Andean cordillera, the Espinhaço/Chapada-Diamantina chain arose from Proterozoic orogenic events (Uhlein, Paim, Tassinari, & Pedreira, 2015) and was partially modified since the Pliocene by compressional tectonic event (Saadi, 1995).

The Espinhaço/Chapada-Diamantina chain and other nearby highland outcrops harbor the campos rupestres vegetation, a sky-island herbaceous-shrubby vegetation mosaic, that occurs above 900 m on nutrient poor, rocky and sandy soils. It is located in Cerrado, Caatinga and Atlantic Rain Forest provinces and is highly diverse. They maintain 15% of Brazilian plant diversity on an area representing less than 1% of the country's land area (Silveira et al., 2016). The campos rupestres are an example of old, infertile and climate-buffered landscapes (OCBILs; Silveira et al., 2016). It has been suggested that in this kind of environment the association of a buffered climate with an infertile soil is the pillar for the origin and maintenance high diversity and endemism, an idea that is supported by new evidence on montane biodiversity (Rahbek et al., 2019). An important prevision of the Ocbil theory is that high specialization and limited seed dispersal would lead to prolongedly isolated population systems, resulting in independent evolution by drift or selection (Hopper, 2009). Indeed, outcrops can maintain stable microhabitats sustaining in situ microrefugia during climatic changes (Schut et al., 2014).

The campos rupestres are especially rich in endemics, as 40% of its plants are restricted to small patches of the environment's distribution. It has been recurrently suggested that due to the disjunct aspect of the campos rupestres geographic isolation might drive independent evolution of populations, differentiation and speciation, a processes called topography-driven-isolation (Steinbauer et al., 2016). However, as happens to other Neotropical environments (Leal, Palma-da-Silva, & Pinheiro, 2016), populational studies using genetic markers do not show a consensual pattern for the campos rupestres, reflecting the intricate and complex processes of Neotropical diversification (Rull, 2013). Generally, plants presenting low dispersion capability show high spatial genetic structure (Barbosa, Fiorini, Silva-Pereira, Mello-Silva, & Borba, 2012; Collevatti, de Castro, Lima, & Telles, 2012; Fiorini et al., 2019), while vagile species present a more homogeneously distributed genetic diversity (Barres, Batalha-Filho, Schnadelbach, & Roque, 2019).

Along with topography-driven-isolation, hybridization is another process driving population differentiation and speciation. It was considered an evolutionary dead end in the past, but its potential in creating and maintaining biodiversity is now clear (Abbott et al., 2013; Mallet, 2007; Seehausen, 2013). It is estimated that around 25% of plant and 10% of animals participate of hybridization processes (Mallet, 2007) and it is hypothesized that plant engage in hybridization more often due to their more open and plastic patterns of morphogenesis (Gottlieb, 1984). However, while hybridization is frequent in some plants, in other groups it is rare or virtually absent (Whitney, Ahern, Campbell, Albert, & King, 2010). Also, the fact that ancestral polymorphism, mutations and disruptive selection can interfere with the hybrid phenotype hampers the identification of hybrids (Rieseberg, 1995).

In this study we used two groups occurring in the campos rupestres presenting contrasting ecological characteristics in relation to the extension of their geographical distribution and mode of dispersion to understand the evolutionary process leading to the high species and endemic diversity observed on the campos rupestres. They are *Bulbophyllum* sect. *Didactyle* (Lindl.) Cogn (Orchidaceae) and *Vellozia auriculata* Mello-Silva & N.L.Menezes (Velloziaceae).

Bulbophyllum sect. *Didactyle* is composed of seven species: *Bulbophyllum perii* Schltr., *B. popayanense* F. Lehm. & Kraenzl., *B. tripetalum* Lindl. and *B. weddellii* (Lindl.) Rchb. f., and the *B. exaltatum* species complex sensu Ribeiro et al. (2008), that includes *B. exaltatum* Lindl., *B. involutum* Borba, Semir & F. Barros, *B. meridense* Rchb.f. (Smidt, 2007). The group includes also two natural hybrids: *B. ×cipoense* Borba & Semir and *B. ×guartelae* Mancinelli & E.C.Smidt (Smidt, 2007). The *B. exaltatum* species complex is distributed over the entire campos rupestres and included several other taxa previously described and currently synonymized (Smidt, 2007). Brazil is the center of diversity of the section *Didactyle*, which has wide distribution in Espinhaço and occurs less frequently also in outcrops in the states Goiás, Roraima, and other tropical countries in South America. Only one of its species occurs exclusively outside Brazil, *B. popayanense*.

Like other *Bulbophyllum* species, *B.* sect. *Didactyle* species are pollinated by flies (myiophily), their small and light seeds are anemochoric, and individuals are able to propagate vegetatively, due to its reptant rhizomes. The populations of the *B. exaltatum* complex have high habitat specificity. Although they present anemochoric dispersion, it is very frequent that only a small portion of adjacent outcrops with virtually identical habitats are colonized. It is also important to note that those outcrops that are colonized often have a high demographic density. These facts indicate the existence of some subtle characteristic that determines the occurrence of the group.

Vellozia auriculata is part of the Velloziaceae family, whose centers of diversity and endemism are the campos rupestres in the Espinhaço Range. *V. auriculata* is one of the tallest species in the dracenoid group of *Vellozia*, reaching up to 4 m in height. It is a micro-endemic species with disjunct distribution in the Diamantina Plateau (Southern Espinhaço range) and is the only species in the dracenoid group that is flexible in terms of habitat specificity as it can be epilitic in both quartzite and canga, or psamophilic. Floral characteristics suggest that *V. auriculata* is pollinated by bees and fruit and seed characteristics suggest barocoric or slightly anemocoric dispersion, preventing the seeds from migrating over long distances. As the species of *B.* sect. *Didactyle*, *V. auriculata* is capable of vegetative propagation.

With the studies of populations of *V. auriculata* and of *B.* sect. *Didactyle* species, we intend to answer questions about the environmental regionalization (e.g.; does lineages composition mirrors the geographical structure of eastern Neotropical rock sky-islands?, Chapter 1), and the role of disjunction (e.g., does geographic disjunction among sky-island lead to genetic disruptions among orchid populations?, Chapters 3 and 4) and hybridization (e.g., are there hybrids on *B.* sect. *Didactyle*?) in promoting the diversification on the campos rupestres of the Espinhaço/Chapada-Diamantina chain and other highland outcrops on eastern Brazil. For this purpose, we scanned *B.* sect. *Didactyle* using the ddRAD methodology; additionally, to access *V. auriculata* variability we sequenced fragments from the plastid DNA and phenotyped genomic Inter Simple Sequence Repeats. The data was analyzed under a model-based approach, using coalescent-based and simulation methods, along with other multivariate techniques.

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CHAPTER 4 – THE PHYLOGEOGRAPHY OF *VELLOZIA AURICULATA* (VELLOZIACEAE) SUPPORTS LOW ZYGOTIC GENE FLOW AND LOCAL POPULATION PERSISTENCE IN THE CAMPO RUPESTRE, A NEOTROPICAL OCBIL

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The phylogeography of *Vellozia auriculata* (Velloziaceae) supports low zygotic gene flow and local population persistence in the campo rupestre, a Neotropical OCBIL

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The campo rupestre is a Neotropical azonal vegetation. Its disjoint distribution and the fact that it is an old climatic buffered infertile landscape (OCBIL) have been associated with the high diversity and endemism observed in this environment. Here, we tested whether a micro-endemic species from campo rupestre shows: (1) limited zygotic gene flow; (2) lower gametic than zygotic gene flow structure; (3) substrate-driven genetic structure and (4) no evidence of Pleistocene local extinction or recolonization. By sequencing intergenic plastid regions, phenotyping inter simple sequence repeats (ISSR) and modelling present and past species suitability distributions for *Vellozia auriculata* we conclude that (1) zygotic gene flow is limited; (2) gametic gene flow is recurrent, but limited by elevation and distance; (3) there is no support for genetic structure driven by substrate and (4) Pleistocene climatic changes did not restrict the species to refugia, with local persistence. As long-term gene flow restrictions may lead to differentiation and speciation, our data helps to corroborate that the campo rupestre is both a cradle (due to low zygotic gene flow, prolonged isolation and consequent differentiation) and a lineage museum (due to local survival during climate oscillations). We highlight two distinct evolutionarily significant units (ESU), providing information for better conservation practice.

KEYWORDS: endemism – gene flow – Pleistocene – population genetics – SDM.

INTRODUCTION

The Neotropics occupy 14% of the land surface of the earth, but are home to 28% of plant diversity of the planet (Antonelli *et al.*, 2015). Localized in the Neotropical region, Brazil is the country with the highest number of plant species and the campo rupestre stands out among its several phytophysiognomies, presenting the highest density of endemic species (BFG, 2015). The Brazilian campo rupestre is a mosaic of azonal vegetations, located in the Cerrado, Caatinga and Atlantic Rain Forest provinces, characterized by disjunct areas associated with quartzite outcrops, generally above 900 m a.s.l. (Giulietti & Pirani, 1988). This phytophysiognomy occurs mainly on the Espinhaço Range, a rocky chain of > 1000 km of latitudinal extension, home to *c*. 15% of Brazilian plant species, 40% of which are endemics,

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in only 0.78% of the area of the country (Rapini *et al.*, 2008; BFG, 2015; Silveira *et al.*, 2016).

The campo rupestre is a naturally fragmented environment and has been identified as an ancient, infertile, climatically buffered landscape (OCBIL; Silveira *et al.*, 2016). OCBIL theory proposes that buffered climate and soil infertility are important factors for maintenance of the observed diversity and endemism in those environments, and that multifactorial selective regimes would lead to specific patterns, including reduced dispersibility of seeds (Hopper, 2009). Indeed, campo rupestre species generally present seed dispersal syndromes that favour short-distance dispersion (Conceição *et al.*, 2016), and population genetic studies of campo rupestre species point to high differentiation between demes (e.g. Borba *et al.*, 2001, 2007; Jesus *et al.*, 2001; Ribeiro *et al.*, 2008; Barbosa *et al.*, 2012; Lousada, Lovato & Borba, 2013).

OCBIL theory also proposes that the combination of high specialization, limited seed dispersal and buffered climate would lead to population systems in which isolation for long periods of time and consequent independent evolution, by drift or selection, is a common process (Hopper, 2009). Due to their physical characteristics, outcrops are able to maintain stable microhabitats and would have had *in situ* microrefugia during climatic changes, as supported by theoretical studies (Main, 1997; Schut *et al.*, 2014). These factors suggest that OCBILs, as the campo rupestre, could be both lineage cradles, where new lineages are constantly generated, and lineage museums, where older lineages persist through evolutionary time (Bitencourt & Rapini, 2013).

Some groups show particularly high levels of richness, endemism and micro-endemism in the campo rupestre. Velloziaceae, which comprises *c.* 240 species and is predominantly Neotropical, exhibit its highest diversity and endemism on mountains in eastern Brazil, especially in outcrop areas of Espinhaço Range, in the Brazilian states of Minas Gerais and Bahia (Menezes, Mello-Silva & Mayo, 1993; Alcantara, Ree & Mello-Silva, 2018). Population genetic studies of species of *Vellozia* Vand. from campo rupestre support the occurrence of differentiation between demes localized in distinct rock islands, but the occurrence of cytoplasmic gene flow across short scales (distances of 10 km or less) is still an open question (Franceschinelli *et al.*, 2006; Barbosa, 2011; Lousada *et al.*, 2011, 2013; Barbosa *et al.*, 2012).

Vellozia auriculata Mello-Silva & N.L.Menezes (Velloziaceae) is one of the micro-endemic *Vellozia* spp. occurring disjunctly in outcrop landscapes along the Planalto Diamantina, between latitudes −17° and −18° of the Espinhaço Range. The distance between *V. auriculata* localities range from 2.3 km to 70.0 km (27.0 km, on average), in a total area of 1000 km^2 (Mello-Silva & Menezes, 1999). It is one of the tallest *Vellozia* spp., reaching *c.* 4 m in height, and occurs epilithically or as a psammophile, which has been shown to correlate with the pattern of genetic structure in other *Vellozia* spp. (Lousada *et al.*, 2013). There are no floral biology studies for this species, but the large bright purplish and yellow flowers of *V. auriculata* are visited, and probably pollinated, by large bees (pers. obs.), despite some species of the genus with distinct floral morphology being also pollinated by birds (Franceschinelli *et al.*, 2006). Morphological characteristics suggest that *V. auriculata* seed dispersal is barochoric, but eventually the small seeds could be secondarily dispersed by rainwater drainage or gales (pers. obs.). The individuals are capable of vegetative propagation by rooting of fallen branches.

Vellozia auriculata is classified as 'Endangered' on the Red Lists of Threatened Species of fauna and flora of the Minas Gerais state (Drummond *et al.*, 2008), and all populations are immediately threatened by recurrent artificial fires. In addition, one of its populations occurs in a sand mining area (population b1, Fig. 1).

Due to its characteristics, *V. auriculata* is a good model for studying phylogeographic patterns of campo rupestre micro-endemic plant species. In this work, using plastid DNA regions and inter simple sequence repeat (ISSR) phenotypes, we test whether there is genetic structure across the distribution of *V. auriculata*, a pattern that would be expected under low gene flow between populations, or differential substrate-driven selection. As cytoplasmic DNA is generally maternally transmitted in monocots (Greiner, Sobanski & Bock, 2015), we expected that patterns of plastid genetic diversity will reflect the effective seed flow (seed flow followed by establishment, growth and reproduction, on a scale of many generations). Complementarily, the ISSR markers capture variation from nuclear and organelle genomes (but due to genome size disparity, it is more prone to show information about the nuclear genome), also providing information about the effective pollen flow (pollen flow followed by fertilization, seed formation, establishment, growth and reproduction, on a scale of many generations). Here, we also test whether there is evidence that during Pleistocene glacial cycles the populations survived *in situ*, showing no signs of centralized refugia or contraction followed by expressive demographic or geographical expansions. The occurrence of Pleistocene refugia has being observed in plants from other Neotropical phytophysiognomies rich in endemic species (e.g. Ramos, Lemos-Filho & Lovato, 2009; Pinheiro *et al.*, 2013; Buzatti *et al.*, 2017; Camps *et al.*, 2018; Melo *et al.*, 2018). However, the proposed climate buffering

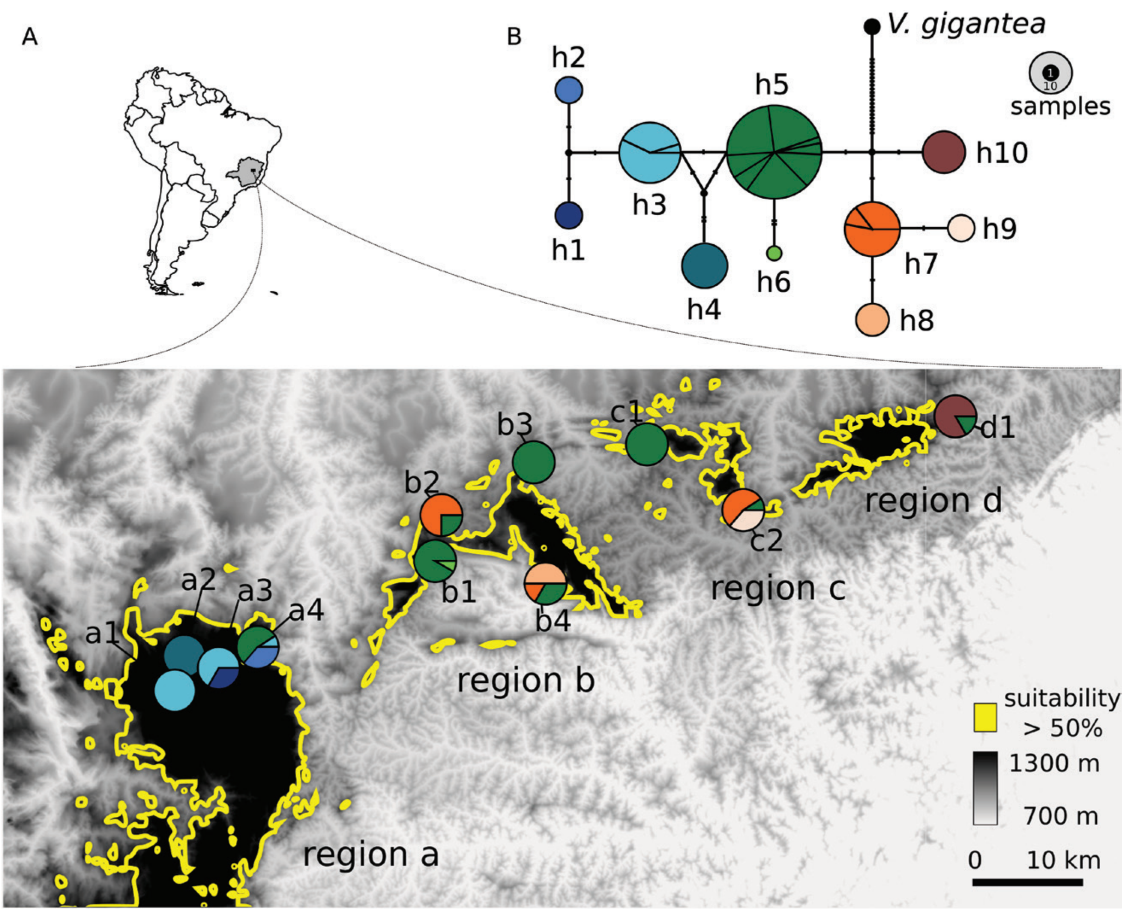


Figure 1. A, Map of the geographical distribution and B, haplotype network of the 11 populations of *Vellozia auriculata* sampled in this work. In the map, the colours indicate the occurrence of the plastid DNA haplotypes h1–h10, according to the colours presented in the network. Elevational variation is represented in greyscale. Limits of occurrence suitability > 50% are presented in yellow lines. For population names, see Table 1. In the network, the diameter of the circles is proportional to the sampling. When present in more than one population, the haplotypes circles on the network are subdivided and the angle of each slice is proportional to the frequency of the haplotypes on populations.

of the campo rupestre and the biological characteristics of its plant species (Silveira *et al.*, 2016) challenge the applicability of the refugium hypothesis to this environment.

Here, we seek to answer the following questions. (1) Are zygotic and gametic gene flow limited in a micro-endemic species from campo rupestre, despite the short distance between populations? (2) Are plastid makers more structured through space than nuclear markers, suggesting that in campo rupestre gametic gene flow may be more frequent than zygotic gene flow? (3) Is there substrate-driven genetic structure in a campo rupestre micro-endemic species? (4) Are there signs of Pleistocene refugia for a micro-endemic species from campo rupestre? We evaluated the magnitude and geographical distribution of genetic diversity, estimated the dates of divergence among lineages, investigated the patterns of cytoplasmic and nuclear genetic diversity and structure between groups of populations, and explored the occurrence of changes in demography and geographical distribution in *V. auriculata* with molecular markers and species distribution models (SDM).

MATERIAL AND METHODS

Genetic data collection

We collected and dried young leaves of individuals from 11 populations, covering the entire geographical range and edaphic occurrence of *V. auriculata* (Table 1). These localities occur in four main 'islands' of high suitability, which are isolated by elevations below 800 m (Miranda, 2012). In this work, we referred to such islands as geographical regions 'a', 'b', 'c' and 'd' (Fig. 1). We collected samples at a minimum distance

Table 1. Populations of *Vellozia auriculata* sampled in this study. The groups used in AMOVA, diversity indexes and geographical groups of Geneland are shown. Pop: population, X: longitude, Y: latitude, AMOVA schemes: (1) geographical with four groups, (2) geographical with three groups and (3) edaphic with two groups. *N*, number of samples; *h*, haplotype diversity, π, nucleotide diversity; σ, standard deviation; Gl, group inferred by Geneland; P, percentage of polymorphic loci; I, Shannon index and H_e, mean expected heterozygosity

Pop	Locality	X	Y	Altitude (m)	AMOVA schemes			plastid DNA					ISSR			
					1	2	3	*N*	Haplotype	*h (σ)*	π (σ)	GI	*N*	*P*	I(σ)	H_e(σ)
a1	Nascente do Córrego das Éguas	-43.35	-18.22	1537	a	a	rock	12	h3 (12)	0 (0)	0 (0)	1	18	0.73	0.36 (0.02)	0.235 (0.016)
a2	Morro do Alecrim	-43.35	-18.19	1336	a	a	rock	12	h4 (12)	0 (0)	0 (0)	2	17	0.72	0.33 (0.02)	0.219 (0.016)
a3	Pico Dois Irmãos	-43.32	-18.21	1561	a	a	rock	12	h1 (4), h3 (8)	0.4848 (0.1059)	0.000735 (0.000604)	1	16	0.76	0.35 (0.02)	0.225 (0.015)
a4	Mata do Izidoro	-43.28	-18.19	1212	a	a	rock	11	h2 (4), h3 (1), h5 (6)	0.6182 (0.1038)	0.001185 (0.000861)	3	15	0.76	0.38 (0.02)	0.251 (0.016)
b1	Serra de Pedra Menina	-43.14	-18.12	1437	b	b+c	rock	12	h5 (11), h6 (1)	0.1667 (0.1343)	0.000253 (0.000304)	4	16	0.78	0.36 (0.02)	0.235 (0.015)
b2	Serra Dois Irmãos	-43.13	-18.08	1246	b	b+c	rock	12	h5 (3), h7 (9)	0.4091 (0.1333)	0.000620 (0.000536)	5	13	0.76	0.37 (0.02)	0.244 (0.016)
b3	Penha de França	-43.06	-18.03	1013	b	b+c	sand	12	h5 (12)	0 (0)	0 (0)	4	0	-	-	-
b4	Serra do Ambrósio	-43.05	-18.13	880	b	b+c	sand	12	h5 (4), h7 (2), h8 (6)	0.6667 (0.0910)	0.001148 (0.000834)	6	17	0.73	0.35 (0.02)	0.234 (0.016)
c1	Ribeirão de Areia	-42.96	-18.02	1097	c	b+c	sand	11	h5 (11)	0 (0)	0 (0)	4	0	-	-	-
c2	Oeste da Serra Negra	-42.88	-18.07	1362	c	b+c	sand	11	h5 (1), h7 (6), h9 (4)	0.6182 (0.1038)	0.000661 (0.000565)	5	0	-	-	-
d1	Parque Estadual da Serra Negra	-42.70	-17.99	1069	d	d	sand	12	h5 (2), h10 (10)	0.3030 (0.1475)	0.000459 (0.000440)	7	15	0.73	0.35 (0.02)	0.231 (0.015)
Total								129		0.7923 (0.0255)	0.001552 (0.000971)		127	0.99	0.43 (0.02)	0.280 (0.014)

of 10 m apart, as the plants are capable of vegetative propagation. Vouchers are deposited in the herbarium BHCB of the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (*BHCB 106375*, *117217*, *123137*, *105142*).

For the phylogeographic study, we obtained sequences of *rpl32-trnL* and *psbD-trnT* plastid DNA intergenic regions using protocols of CTAB DNA extraction (Doyle & Doyle, 1987), PCR amplification and Sanger sequencing described in Barbosa *et al.* (2012). We built consensuses of forward and reverse sequences using Staden Package v.1.7.0 (Staden, 1996) and the alignment with Muscle implemented in MEGA 7 (Kumar, Stecher & Tamura, 2016). We visually inspected the alignment matrix to detect spurious polymorphic sites and encode indels according to the modified complex indel coding scheme (MCIC, Simmons & Ochoterena, 2000) with SeqState 1.4.1 (Müller, 2005). Sequences were deposited in GenBank (accession codes, MG953391–MG953412).

To test population geographical and edaphic structure in the genome as a whole, we used ISSR. We phenotyped with replicates a subset of individuals from three localities using 22 primers. For the seven primers that gave well-defined bands (Supplementary Table S1), we calculated the error rate as the ratio between the total number of mismatched phenotypes (band presence versus band absence) and the product of the number of replicated phenotypes and the number of replicates (Pompanon *et al.*, 2005). Including only bands ranging from 300 to 1500 bp (as the amplification of fragments out of this interval is less reliable), we observed an error rate of 5.05%, similar to that observed in another ISSR study (Casazza *et al.*, 2013). Using these seven primers, we phenotyped 127 individuals from the eight previously known populations, as three of 11 populations sampled for the plastid DNA study were discovered after we carried out the ISSR phenotyping. The addition of individuals from localities discovered after the data acquisition are not expected to change the observed patterns (as discussed below, populations from regions b and d form a group and there is evidence of isolation-by-distance, based on ISSR data; due to this, it is likely that the variability of populations not sampled was already represented). Amplification reactions were conducted in a total volume of 19 µL containing one unit Taq polymerase, 1× reaction buffer [75 mM Tris - HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2SO_4$)], 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.32 µM primer and genomic DNA. The program consisted of pre-dissociation at 94 °C for 4 min, 37 amplification cycles with dissociation at 94 °C for 1 min, annealing at 47.6 °C or 50 °C for 1 min (Supplementary Table S1), extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. We included negative controls in all PCRs. We separated PCR products at 60 V for 4 h, using 1.5% agarose gel electrophoresis with 0.5× TAE buffer, stained the gel with ethidium bromide and photographed it under UV light. To construct an array of ISSR phenotypes based on presence (1) or absence (0) of bands, we used a 100 bp DNA standard (Ludwig), assuming that fragments with similar electrophoretic mobility are homologous for the same primer. To ensure that the observed patterns are not derived from association of samples from a given population or region on a single gel, we split samples from a same locality across multiple gels.

PLASTID DNA ANALYSES

We calculated haplotype (h) and nucleotide (π) diversities with Arlequin v.3.5.1.3 (Excoffier & Lischer, 2010) and inferred the plastid haplotype network with the integer neighbor-joining networks algorithm with 'old-style = 1', implemented in POPART 1.6 beta (Leigh & Bryant, 2015).

We performed analyses of molecular variance (AMOVA), φ_{PT} and φ_{RT} calculations using GenAlEx 6.5 software (Peakall & Smouse, 2006), for three distinct hierarchical schemes: (1) geographical with four groups; (2) geographical with three groups and (3) edaphic with two groups (epilithic or psammophile) (Table 1). Scheme 1 (regions a–d) follow geographical islands with > 50% suitability for the species (Miranda, 2012). As geographical regions b and c are connected by an area of higher elevation than the one which separates these regions from a and d, we set also scheme 2 (regions a, b+c and d). φ_{PT} and φ_{RT} significance values were tested by 9999 permutations.

We detected population structure using Geneland Bayesian inference of spatial clustering (Guillot, Mortier & Estoup, 2005). We tested the existence of one to 11 geographical groups in ten independent runs, composed of 100 000 iterations, with initial burnin of 20%, using the spatial model with correlated allelic frequencies. To test the occurrence of isolation-by-distance, we performed Mantel test (Mantel, 1967) from matrices of mean numbers of site-to-site genetic substitutions and geographical distances between populations, with GenAlEx 6.5 (Peakall & Smouse, 2006).

For populations (Table 1) and the species, we verified adherence of mismatch distributions to demographic and geographical expansion models with the sum of square deviations (SSD), and calculate raggedness statistic in Arlequin 3.5.1.3 (Excoffier & Lischer, 2010). The neutrality tests *D* (Tajima, 1989) and *Fs* (Fu, 1997) were also evaluated with Arlequin 3.5.1.3. We calculated the R^2 index (Ramos-Onsins & Rozas, 2002) and tested its significance by simulating 1000 replicates with DnaSP 5.10 (Librado & Rozas, 2009).

We specified the Bayesian phylogenetic inference with BEAUti 1.8.1 and implemented it in BEAST 1.8.1 (Drummond *et al.*, 2012). Based on the Bayesian information criterion (BIC), implemented in MEGA 7 (Darriba *et al.*, 2012; Kumar *et al.*, 2016), we selected the HKY model for partitions encoding point mutations. We included indel binary encoding as an independent data partition, using Dollo's stochastic model, based on the premise that characters lost in a deletion will not be re-acquired. We empirically calculated nucleotide frequencies, fixing it to observed proportions in the data set and we inferred trees under a constant size model. For temporal calibration, we set the divergence between *V. auriculata* and *V. gigantea* N.L.Menezes & Mello-Silva to 5.4371 Mya (σ = 1.5), following results of temporal calibration methodology presented on supplementary material. We performed 10 000 000 iterations (with initial burnin of 20%), verified convergence of results and effective sample sizes (ESS) with Tracer v.1.6 (http://tree.bio.ed.ac.uk/software/tracer/, last accessed November 2017), summarized trees with TreeAnnotator v.1.8.1 and visualized the result using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, last accessed November 2017).

We compared diversification hypotheses using approximate Bayesian computation (ABC; Beaumont, 2010), implemented in DIY-ABC 2.1.0 (Cornuet *et al.*, 2014). We evaluated five evolutionary scenarios: one scenario of fragmentation of previously continuous distribution (Fig. 2A); and four scenarios of colonization through the founder effect, one from each of the four geographical regions of high suitability for *V. auriculata* (Fig. 2B--E). Bottlenecks/founder effects were modelled by an abrupt reduction of population sizes from *Ne* to *Nb* (Fig. 2, Supplementary Table S2). We simulated 2 000 000 sequences in each of the scenarios and computed the summary statistics of simulated and observed data. We selected the most explanatory model from a logistic regression, which indicated the probability of each scenario in relation to the deviations between observed and simulated data (Fagundes *et al.*, 2007; Beaumont, 2008).

To estimate effective immigration rates (*M*) and effective population sizes (Θ) we used Migrate-N v.3.6.11 (Fig. 3; Beerli, 2006). Also, with Migrate-N, we compared four past demographic models, varying the number of geographical clusters and the level of connectivity between these regions. Models are represented in Fig. 3 and can be described as (1) four geographical regions (a–d) connected as stepping stones (Fig. 3B); (2) four geographical regions (a–d) interconnected (Fig. 3C); (3) three geographical regions (a, b+c and d) connected as stepping stones (Fig. 3C) and (4) three geographical regions (a, b+c and d) interconnected (Fig. 3E). Under each model, we used one long Bayesian heated chain of 2 000 000 steps (sampled every 100 generations), with a burnin of 400 000 steps, with a uniform prior ranging from 0 to 10 000 for the estimates of *M* and a uniform prior ranging from 0 to 0.1 for estimates of Θ. The estimated number of immigrants per generation *Nm* was calculated as $Nm = M_{i\text{-}j} \times (\Theta_{i} + \Theta_{j})$, as plastid markers are haploid and *V. auriculata* is monoecious. Modes of estimated *M* and Θ were used in the calculation.

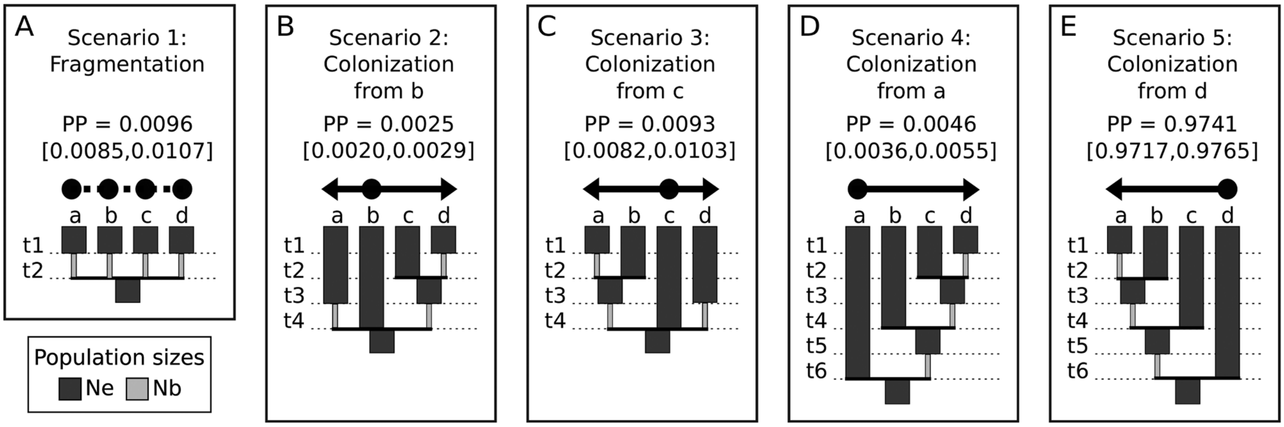


Figure 2. Scenarios tested using approximate Bayesian computation (ABC) for *Vellozia auriculata*, based on plastid DNA haplotypes. A–E, Scenario 1: fragmentation of previously connected distribution (A); scenario 2: colonization from region b (B); scenario 3: colonization from region c (C); scenario 4: colonization from west to east (D) and scenario 5: colonization from east to west (E). The positions of regions a–d are shown in Fig. 1. PP: posterior probability; t1–t6: divergence times (time is not in scale); Ne: population size, Nb: population size during a bottleneck (scenario 1) or colonization population size (founding effect, scenarios 2–5). Parameter priors for all scenarios and estimates for scenario 5 are presented on Supplementary Fig. S2.

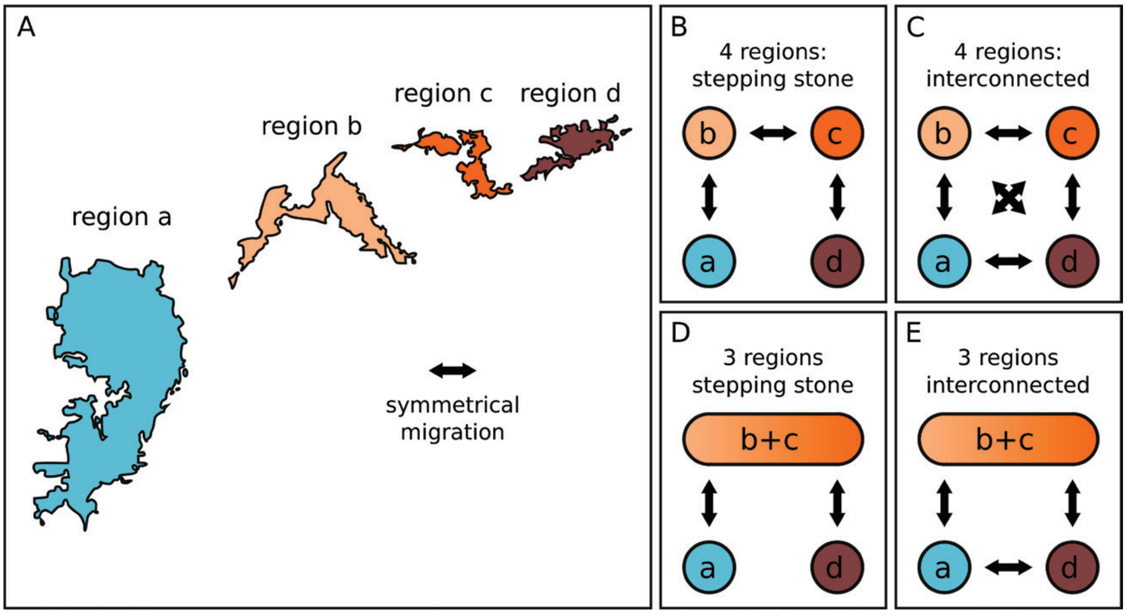


Figure 3. Scenarios tested in Migrate-N model selection for *Vellozia auriculata*, based on plastid DNA haplotypes. A–E, Scenario 1: four geographical regions (a–d) connected as stepping stones (B); scenario 2: four geographical regions (a–d) interconnected (C); scenario 3: three geographical regions (a, b+c and d) connected as stepping stones (D) and scenario 4: three geographical regions (a, b+c and d) interconnected (E). The limits of the four islands of habitat suitability are shown in A and the positions of these regions are shown in Fig. 1.

ISSR ANALYSES

We calculated diversity parameters (P, proportion of polymorphic loci; I, Shannon index of information; H_e, mean expected heterozygosity), Nei's unbiased genetic distance (Nei, 1978), AMOVA, φ_{PT} and φ_{RT} using GenAlEx v.6.5 (Peakall & Smouse, 2006). For AMOVA, we organized the group in a hierarchy according to schemes 2 and 3 (Table 1). The significance values of φ_{PT} and φ_{RT} were tested by 9999 permutations.

After conversion of the presence/absence matrix to compatible format, we constructed an unrooted neighbor-joining dendrogram from Nei's unbiased genetic distance in MEGA 7 (Kumar *et al.*, 2016) and obtained bootstrap values for this dendrogram with AFLP-SURV, using 1000 replicates (Felsenstein, 1989; Vekemans *et al.*, 2002). To test the occurrence of isolation-by-distance, we performed a Mantel test (Mantel, 1967), from matrices of Nei's unbiased genetic distance and geographical distance between populations, with GenAlEx 6.5 (Peakall & Smouse, 2006).

To test the existence of distinct genetic groups and assign individuals to populations, we carried out a Bayesian analysis using STRUCTURE 2.3.4 (Falush, Stephens & Pritchard, 2007). We varied the number of presumed gene pools (*K*) from one to ten, performing ten independent runs composed of 500 000 MCMC iterations for each *K*, with an initial burnin of 20% and models of correlated allele frequencies and admixture. To infer the number of genetic groups (populations), we calculated the mean of each probability value of *K* during all runs, as suggested by Pritchard, Stephens & Donnelly (2000), and the Delta *K* statistic according to Evanno, Regnaut & Goudet (2005) using STRUCTURE HARVESTER (Earl & Holdt, 2012).

SPECIES DISTRIBUTION MODELS

We used SDM to estimate the occurrence suitability for *V. auriculata*, testing 16 algorithms of SDM: Bioclim; boosted regression trees (BRT); classification or regression tree (CART); domain, generalized additive models (GAM); generalized boosted models (GBM); generalized linear models (GLM); maximum likelihood analysis of species occurrence probability from presence-only data for modelling species distributions (maxlike); Mahalanobis distance; Maxent; mixture discriminant analysis (MDA); multivariate adaptive regression spline (MARS); random forest (RF); recursive partitioning and regression trees (RPART); regularization paths for generalized linear models via coordinate descent (GLMnet) and support vector machine (SVM, Franklin & Miller, 2009). To test the best prediction model, we use original records obtained by field collections carried out in the distribution area of *V. auriculata* in 2011 by Miranda (2012) and, when necessary, 1000 pseudoabsences in training models. Presence points were obtained using GPS of high precision (*c.* 1 m) across *V. auriculata* populations (Miranda, 2012). Pseudoabsences were selected with the upper limit of 800 m in the Espinhaço Range. The same limit was used for model predictions, because *V. auriculata* is restricted to campo rupestre. All models were run in R software package SDM (Naimi & Araujo, 2016), except Maxent, which was run in Maxent software (Steven, Miroslav & Robert, 2018). To validate models, we used presence and absences data collected in expeditions independently of training data (Miranda, 2012). We use bioclimatic variables from WorldClim and elevation (SRTM) as predictors (Hasenack *et al.*, 2010). As the elevation variable has *c.* 90 m of resolution, bioclimatic variables were resized accordingly. To reduce variable correlation, which may affect some algorithms, we tested correlation between them with a threshold of 0.7. As some variables were highly correlated, we used the five least correlated variables (elevation, annual mean temperature, mean diurnal range, isothermality and precipitation of driest month), each one representing a set of highly correlated variables. To evaluate models, we used the area under the curve (AUC), based on presences and absences independently collected (see Miranda, 2012). To build the suitability projection of past scenarios of species distribution (21 ky and 120–140 ky), we used the algorithm with the highest AUC and the climatic models CCSM and MIROC from WorldClim (http://www.worldclim.org/, last accessed February 2018). We ran new models with all points of occurrence and subtracted current prediction by each scenario prediction to show the change of suitability between past and current scenarios.

V. AURICULATA CONSERVATION

Associating results from plastid DNA and ISSR data, we described evolutionarily significant units (ESU) and independent management units (MU), according to Moritz (1994).

RESULTS

PHYLOGEOGRAPHIC ANALYSES OF PLASTID DNA

Amplifications of plastid DNA intergenic regions *rpl32-trnL* and *psbD-trnT* provided *c.* 800 and 1000 base pairs, respectively. After sequencing and trimming, the alignment of 129 individuals of *V. auriculata* and one individual of *V. gigantea* comprised 533 sites for *rpl32-trnL* and 797 sites for *psbD-trnT*. These regions were concatenated for analyses. In *V. auriculata*, we observed nine polymorphic sites (one transition and eight transversions) and one imperfect poly T microsatellite of variable length. We chose to use the poly T in the analyses because it was consistent on forward and reverse sequencing. Of the ten polymorphisms we found, eight were informative and, in combination, revealed ten haplotypes (Table 2). With addition of *V. gigantea*, 18 point mutations were included in the matrix.

Haplotype diversity for *V. auriculata* was 0.7923 (σ = 0.0255) and total nucleotide diversity was 0.001552 (σ = 0.000971). In the populations, nucleotide diversity ranged from 0 to 0.001185 (σ = 0.000861) and haplotype diversity from 0 to 0.6667 (σ = 0.0910, Table 1). The

Table 2. Description of the ten plastid DNA haplotypes of *Vellozia auriculata*, derived from the concatenation of the alignments of the *rpl32-trnL* and *psbD-trnT* plastid DNA intergenic regions. Numbers indicate positions in alignment; dots indicate that the character state is the same as that of haplotype h1 SeqState Indel codification is presented after the poly T sequence.

	rpl32-trnL															*psbD-trnT*			
	162	224	323	486	[497	498	499	500	501	502	503	504	505]		513	19	358	616	617
h1	T	G	T	C	-	-	-	-	-	-	-	-	-	1	A	C	G	T	A
h2	.	.	.	.	-	-	-	-	-	-	-	-	-	1	C	.	.	.	C
h3	.	.	G	.	-	-	-	-	-	-	-	-	-	1	C	.	.	.	.
h4	.	.	G	.	T	T	-	-	-	-	-	-	-	3	C	.	T	G	.
h5	.	.	G	.	T	-	-	-	-	-	-	-	-	2	C	.	.	.	.
h6	.	T	G	.	T	-	-	-	-	-	-	-	-	2	C	A	.	.	.
h7	.	.	G	T	T	-	-	-	-	-	-	-	-	2	.	.	.	.	.
h8	A	.	G	T	T	-	-	-	-	-	-	-	-	2	.	.	.	.	.
h9	.	.	G	T	T	-	-	-	-	-	-	-	-	2	.	.	.	.	C
h10	.	.	G	T	T	T	T	T	T	T	T	T	C	0	C	.	.	.	.

number of haplotypes per population ranged from one to three, with an average of 1.9, and the populations with the greatest diversity were c2, b4 and a4 (Table 1). The seven haplotypes that occur in terminal positions in the haplotype network were restricted to single populations (Fig. 1). Haplotype h5 was the most frequent (39%), occurring in the four geographical regions of high suitability for *V. auriculata*; it was present in the majority of the populations, except the three located at the extreme west of the distribution (Fig. 1).

The geographical AMOVA with four groups showed most of the variation (57%) among populations within geographical regions, with 16% of the variation between groups. In the geographical AMOVA with three groups, although most of variation was also concentrated within geographical regions (48%), we observed 28% of variation between them. In the AMOVA between populations from distinct edaphic conditions, we found only 6% of the variation between groups, with 67% of the variation being among populations within them. In all three cases, φ_{PT} values were significant ($P < 0.001$) and > 0.73; φ_{RT} values were also significant ($P < 0.001$), ranging from 0.059 to 0.277 (Table 3).

Geneland genetic-geographical analysis indicated occurrence of seven groups in all independent parallel runs (Table 1, Supplementary Fig. S1). Such groups are compatible with those defined in AMOVA with three groups, since populations of region b are grouped with populations of region c in groups 4 and 5. The Mantel test was not significant ($R = 0.279$, $P = 0.097$), suggesting no isolation-by-distance.

With Bayesian phylogenetic inference, ESS > 1300 was reached, but few branches had posterior probabilities (PP) > 95%; only the clades of haplotypes h1, h2 and h3, and h7, h8 and h9 were recovered with high PP (Fig. 4). The analysis suggests that all current haplotypes of *V. auriculata* have a common ancestor at 1.0391 Myr (95% HPD: 0.0003–2.0324 Myr) and clades formed by h1–h3 haplotypes and h7–h9 originated at 0.2727 Myr (95% HPD: 0.0.0001–0.6769 Myr) and 0.2055 Myr (95% HPD: 0.0001–0.5151 Mya), respectively.

Table 3. AMOVA of *Vellozia auriculata* populations based on plastid DNA. The populations were grouped according to Table 1 and all φ values were significant ($P < 0.001$). AMOVA schemes: (1) geographical with four groups; (2) geographical with three groups and (3) edaphic with two groups. Sum of squares and degrees of freedom are presented between parenthesis (s.s./d.f.).

Source of variation	AMOVA schemes for plastid DNA			AMOVA schemes for ISSR	
	1	2	3	2	3
Among groups	16% (40.0/3)	28% (38.9/2)	6% (13.7/1)	5% (195.8/2)	4% (120.7/1)
Among populations within groups	57% (55.6/7)	48% (56.7/8)	67% (82.3/9)	7% (255.3/5)	9% (330.5/6)
Within populations	27% (35.5/118)	25% (35.5/118)	27% (35.6/118)	87% (2569.6/119)	87% (2569.6/119)
φ_{RT}	0.161	0.277	0.059	0.052	0.042
φ_{PT}	0.735	0.753	0.731	0.127	0.127

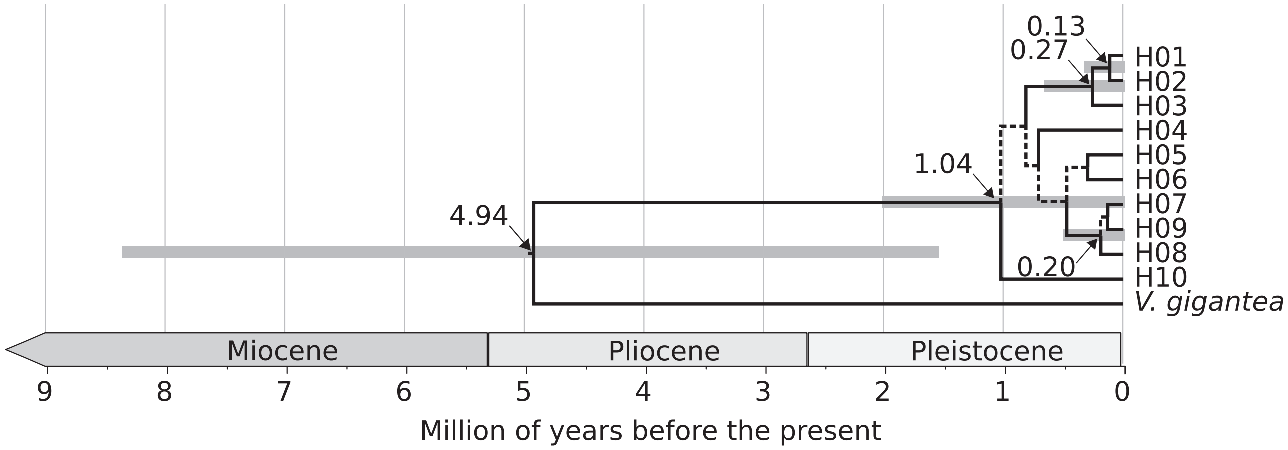


Figure 4. Bayesian phylogenetic inference of *Vellozia auriculata* plastid DNA haplotypes. Branches with posterior probability (PP) > 0.99 are shown in solid lines and branches with PP < 0.50 are shown in dashed lines. The ages of the clades with PP > 0.95 are presented by arrows and the bars indicate the 95% HPD intervals for the divergence times.

Table 4. Neutrality tests based on plastid DNA for *Vellozia auriculata*. *: $P < 0.001$

Population	Tajima's D	FS	R2	Raggedness index	SSD (demographic)	SSD (spatial)
a3	1.3564	2.3279	0.2424	0.7355	0.1828	0.1283
a4	1.8024	1.5454	0.2606	0.3984	0.1246	0.0871
b1	–1.4514	0.4318	0.2764	0.7500	0.0370	0.0152
b2	0.6879	1.9611	0.2045	0.6839	*0.3347	0.0914
b4	1.7228	1.5987	0.2525	0.2176	0.0598	0.0463
c2	–0.5063	0.3227	0.2096	0.1167	0.0092	0.0091
d1	–0.2481	1.3844	0.1515	0.6694	0.2787	0.0502
V. auriculata (total)	0.2793	–0.3545	0.0826	0.0628	0.0191	0.0132

The neutrality tests were not significant ($P > 0.05$). The SSDs of mismatch distributions and the raggedness statistics from populations or species (Table 4) were not significant, except the adjustment to the demographic expansion model of population b2, that had $P < 0.001$. Of the five models tested in ABC, scenario 5 presented PP significantly higher than others (0.9741, 95% HPD = 0.9717–0.9765), indicating colonization from east to west (Fig. 2E, Supplementary Fig. 2). For scenario 5, the mode of estimated population size for each island size was 8500 (95% HPD = 4690–9860) and the mode of bottleneck population size (colonization population size) was one (95% HPD = 1–10, Supplementary Table S2).

Migrate-N model selection and parameter estimate indicated that migration between regions is best explained by a model with three groups, when all these groups are interconnected (PP = 0.793, Fig. 3E, Table 5). For this scenario, estimates of M and Θ are presented in Table 6; the average number of immigrants per generation between regions a and b+c was $Nm_{a\text{-}bc} = 0.8941$, between regions a and d $Nm_{a\text{-}d} = 0.5459$ and between regions b+c and d $Nm_{bc\text{-}d} = 1.0215$.

Table 5. Migrate-N model selection. Maximum likelihood estimate (lnMLE) and model posterior probability (PP)

Model	lnMLE	PP
Four regions, stepping stone	–1853.56	0.003
Four regions, interconnected	–1849.44	0.163
Three regions, stepping stone	–1850.83	0.041
Three regions, interconnected	–1847.86	0.793

ISSR

The seven primers selected resulted in 147 loci ranging from 300 to 1500 bp, 84% of which were polymorphic (Supplementary Table S1). Eighteen percent of the individuals had missing data, ranging from 10 to 20%. Populations had mean expected heterozygosity (H_e) and Shannon index (I) averages of 0.234 and 0.356, respectively. AMOVA indicated that 87% of variation was found within populations (all schemes $\varphi_{PT} = 0.127$, $P < 0.001$; scheme 2: $\varphi_{RT} = 0.052$; scheme 3: $\varphi_{RT} = 0.042$). In the AMOVA hierarchized according to three geographical groups, we observed only 5% of variation between geographic regions and 7% between populations within regions; in the AMOVA hierarchized according to substrate types, we found only 4% of variation between edaphic groups and 9% among populations within these groups (Table 3).

The similarity dendrogram reflected the geographical distribution of populations (Figs 1, 5). The separation between region a and the others had high support (95% bootstrap), but similarity relations between populations of regions b and d had low support. The Mantel test indicated that there is a significant positive correlation between geographical and genetic distances ($R = 0.845$, $P = 0.01$). The genetic distances used in Mantel tests are presented in Supplementary Table S3.

STRUCTURE indicated the occurrence of two genetic groups, mainly represented by western (populations a1, a2, a3 and a4) and the central-eastern (populations b1, b2, b4 and d1) regions (Fig. 6). Populations b1 and b2, which occupy an intermediate position in the geographical distribution, included several individuals with mixed composition. Also, four individuals from populations a1, a2 and d1 showed mixed genetic composition.

SPECIES DISTRIBUTION MODELS

Maxent gave the highest AUC in the validation (0.95; details in Supplementary Fig. S5 and S6) and thus was chosen as the best model. This model predicted high suitability zones in regions where populations of *V. auriculata* were sampled for the current scenario (0 ky, Fig. 7A). Past model projections indicated decrease in suitability in all scenarios and climatic models (Fig. 7B, D, F), but showed persistence of the current pattern of suitability discontinuity (Fig. 7C, E, G). No scenario presented evidences for connection of populations in the past and the change in suitability in all scenarios

Table 6. Migrate-N distribution of the estimates of parameters Θ and M, for the best model (scenario 4: three interconnected regions, Fig. 3 E). Q, quantile

Parameter	Mean	Mode	Q2.5	Q25	Q50	Q75	Q97.5
Θ_a	0.0011	0.0010	0.0000	0.0003	0.0014	0.0017	0.0029
Θ_{bc}	0.0008	0.0008	0.0000	0.0001	0.0012	0.0013	0.0025
Θ_d	0.0015	0.0008	0.0000	0.0001	0.0012	0.0013	0.0031
$M_{a\text{-}bc}$	1072.2	496.7	0.0	180.0	876.7	1000.0	2720.0
$M_{a\text{-}d}$	859.4	303.3	0.0	46.7	656.7	706.7	2366.7
$M_{bc\text{-}d}$	1413.0	663.3	0.0	266.7	1103.3	1273.3	3686.7

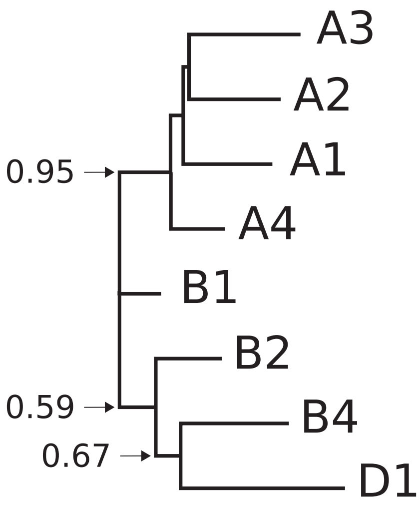


Figure 5. Unrooted neighbor-joining dendrogram of *Vellozia auriculata* populations, using Nei's unbiased genetic distance, based on 147 ISSR loci. Bootstrap values > 50% are presented. For population names see Table 1.

presented similar results (correlation above 0.95). Even if all scenarios presented highly suitability reduction in current areas of occurrence of *V. auriculata* (Fig. 7C, E, G), they showed persistence of suitability, even when subtle, in all regions corresponding to current suitability islands (regions a–d), except region d during the last interglacial (Fig. 7F).

DISCUSSION

Vellozia auriculata, a micro-endemic species from campo rupestre, shows limited zygotic migration among populations and low genetic structure of gametic flow markers (ISSR) when compared to plastid DNA markers; there is no sign of substrate-driven genetic structure and no evidence of expansion from Pleistocene refugia or habitat fragmentation due to Pleistocene climate oscillations. These results indicate that (1) seed flow between populations is low and even virtually absent between major areas of the species occurrence, (2) gene flow between populations occurs mainly via pollen and (3) populations persisted locally during Pleistocene (i.e. presented prolonged local persistence).

The pattern of restricted zygotic gene flow among *V. auriculata* populations is supported by the strong structure highlighted by Geneland and the plastid DNA AMOVA and by the low average number of immigrants per generation between regions estimated by Migrate-N, in accordance with the absence of obvious mechanism for seed dispersion in this species. The isolation driving this pattern is not expected to be recent, as SDM supports discontinuity of habitat suitability during Pleistocene, in both glacial and interglacial periods. Also, haplotypes that originated *c.* 270 000 kya are not shared between *V. auriculata* populations that are separated by distances < 4 km, suggesting that even short distances of low suitability could be gene flow barriers for this and other micro-endemic species from the campo rupestre. Other plants occurring in the campo rupestre and in outcrops across the globe show similar patterns, and this has been associated with low effective seed vagility in these environments (e.g. Byrne & Hopper, 2008; Palma-Silva *et al.*, 2011; Barbosa *et al.*, 2012; Collevatti *et al.*, 2012; Bonatelli *et al.*, 2014; Pinheiro *et al.*, 2014).

Genetic drift has been an important factor on the evolution of *V. auriculata*, for colonization of new environments (founder effect) and for the isolation-driven differentiation between populations (Steinbauer *et al.*, 2016). The model selected in ABC suggests that the current distribution of *V. auriculata* was attained by occasional dispersion events of few individuals, as the mode of posterior distribution for colonization population size is one. This is also supported by the absence of correlation between genetic and geographical distances for plastid DNA data and the low intrapopulation haplotype diversity, again suggesting low capacity of effective seed dispersion of this micro-endemic species from the campo rupestre. The scheme of three geographical groups tested in the plastid DNA AMOVA (scheme 2) retained most of the species interregional structuring and was compatible

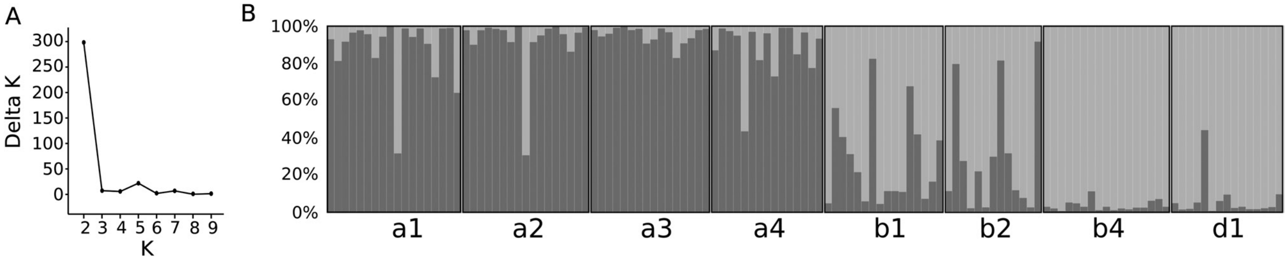


Figure 6. Bayesian clustering analysis of eight populations of *Vellozia auriculata*, based on 147 ISSR loci. The delta K graphic (A) and the summary plot of estimates of ancestry vector (B) are presented. On B, the shades of grey represent different genetic clusters and populations are separated by vertical bars. For population names see Table 1.

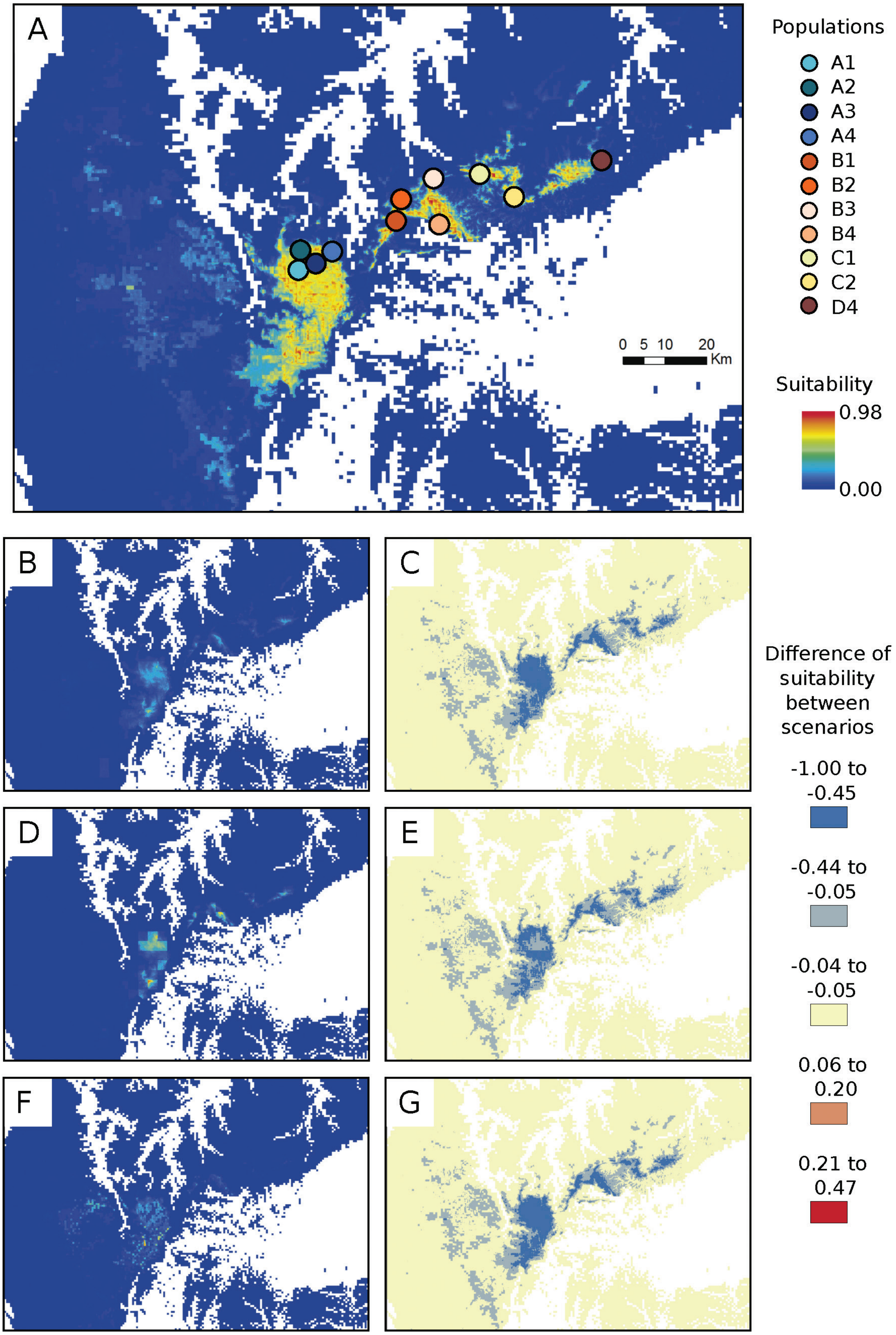
with the geographical division proposed by Geneland and Migrate-N model selection, suggesting that for *V. auriculata* elevations of < 700 m are more restrictive to occasional events of gene flow than those between 700 and 800 m, as corroborated by the SDM estimates of environmental unsuitability on lower lands.

The plastid data and the ISSR phenotypes suggest that substrate has little relevance for the genetic structure, contrasting with the pattern observed in *V. compacta* Mart. (Lousada *et al.*, 2013). For *V. auriculata*, the small portion of variation observed between edaphic groups may even include geographical structuring, since psammophile populations are distributed to the east and epilithic populations to the west of the species distribution. Survival in different substrate types is expected to require specific adaptations (Poot, Hopper & van Diggelen, 2012), and it is possible that the genome of isolated populations of plants occupying different substrates will be at least partially differentiated (Wu, 2001). However, according to the ABC scenario with the highest PP, *V. auriculata* emerged as a psammophile species, subsequently reaching rocky environments. Next-generation sequencing studies could be performed to examine the existence of nuclear genes related to adaptation or edaphic plasticity in *V. auriculata* and to obtain better parameter estimates in ABC for this and other species from the campo rupestre.

The population differentiation in *V. auriculata* is the lowest reported among *Vellozia* spp. phenotyped with ISSR markers (Barbosa, 2011; Lousada *et al.*, 2011, 2013), probably due to its smaller range of occurrence and smaller distance between populations. However, STRUCTURE analysis and the similarity dendrogram indicate the existence of regional groups a and b+c+d for *V. auriculata*, suggesting that gametic gene flow is partially limited, despite the small distance between populations. The existence of groups due to limited gametic gene flow should, however, be interpreted with caution, as the occurrence of isolating by distance indicated by the Mantel test could bias the clustering (Perez *et al.*, 2018). Also, despite the large number of ISSR loci analysed and the geographical and biological coherence of the results presented here, it is important to be aware of ISSR reproducibility, homology and dominance limitations (Roux & Wieczorek, 2009). Future studies with more sophisticated techniques are desirable to deeper explore the intensity of gametic gene flow and existence of genetic clusters across the distributions of campo rupestre endemic species, such as *V. auriculata.*

Our study corroborates the hypothesis that gametic gene flow in the campo rupestre may be more frequent than zygotic gene flow, as plastid DNA haplotypes are more structured through space than ISSR phenotypes. As the mutation rate giving origin to ISSR polymorphisms is expected to be higher than the mutation rate of plastid DNA (Roux & Wieczorek, 2009), this result suggests prolonged low zygotic gene flow compared to gametic gene flow and can be interpreted with confidence. If, instead, ISSR was expected to have lower mutation rates than plastid DNA, the observed similarity between populations could represent incomplete lineage sorting and not a sign of recurrent gene flow. Other plants occurring in OCBILs exhibit the same pattern of greater structuring of plastid markers, indicating that this could be a common motive in this type of vegetation (Barbosa, 2011; Palma-Silva *et al.*, 2011; Pinheiro *et al.*, 2014; Tapper *et al.*, 2014a, b; Hmeljevski *et al.*, 2017). Even when pollen gene flow is recurrent, isolation by low seed gene flow may lead to conflicts between nuclear and plastid genes and consequent reproductive isolation and speciation (Greiner *et al.*, 2011; Greiner & Bock, 2013; Barnard-Kubow, So & Galloway, 2016). Speciation by gene conflict (Crespi & Nosil, 2013) may be common in OCBILs, and studies exploring this issue should be performed.

Geographical retractions and expansions caused by Pleistocene climatic changes have been invoked to explain the differentiation of campo rupestre populations (e.g. Barbosa *et al.*, 2012; Collevatti *et al.*, 2012; Barres *et al.*, 2019). However, in agreement with the hypothesis of prolonged persistence of OCBIL


A
Populations
A1
A2
A3
A4
B1
B2
B3
B4
C1
C2
D4
0 5 10 20
Km
Suitability
0.98
0.00
B
C
D
E
F
G
Difference of suitability between scenarios
-1.00 to -0.45
-0.44 to -0.05
-0.04 to -0.05
0.06 to 0.20
0.21 to 0.47

lineages and the high environmental specificity of campo rupestre plants (Hopper, 2009; Silveira *et al.*, 2016), the phylogeographical patterns of *V. auriculata* do not corroborate that Pleistocene glacial and interglacial periods were triggers for its population differentiation. The SDM indicates lasting suitability discontinuity between regions and shows that, despite the reduction of suitability in past scenarios, SDM indicates that current areas presenting highest suitability match the suitable areas of glacial and interglacial periods. Thus, our analyses support the prolonged persistence in isolation of *V. auriculata* populations during Pleistocene climate oscillations across its current distribution. There is evidence that local persistence during Pleistocene climatic oscillations also occurred in *Minaria* T.U.P.Konno & Rapini (Ribeiro *et al.*, 2014), *Encholirium horridum* L.B.Sm. (Hmeljevski *et al.*, 2017) and in plant species occurring in Australian OCBILs (Byrne & Hopper, 2008; Tapper *et al.*, 2014a, b; Nistelberger *et al.*, 2015). It is expected that other plants from the campo rupestre had prolonged persistence on isolation. The spread of this pattern in contrast to the pattern of recolonization from refugia frequently observed in currently more continuous Neotropical environments, such as savannas and forests (Leal, Palma-Silva & Pinheiro, 2016), should be addressed in future work. Plants with widespread distribution in the campo rupestre could offer interesting opportunities to test this hypothesis. It is important to highlight that Neotropical biodiversity evolution was a complex process (Rull, 2013; Rull, 2015; Antonelli *et al.*, 2018), and thus it is not expected that campo rupestre flora presents strictly concordant responses to past climatic changes. Organisms with distinct niche and life strategies are expected to experience different demographic processes that will lead to idiosyncratic genetic patterns (Massatti & Knowles, 2014).

The low suitability of the eastern region (d) during Pleistocene glacial periods showed by SDM seems to contrast with ABC result, which suggests that *V. auriculata* originated in the eastern region of its distribution. However, the origin of *V. auriculata* pre-dates Pleistocene climatic oscillations, and thus these results are not contradictory. Instead, combined with the occurrence and abundance of the endemic haplotype h10 in population d1, these results suggest that *V. auriculata* persisted locally in eastern populations, even when suitable habitats were limited in this region, again supporting the occurrence of prolonged persistence with isolation of this micro-endemic species from the campo rupestre. Also, niche evolution could be a possible explanation for the estimated low suitability using current data, as Miranda (2012) observed a niche gradient across *V. auriculata* distribution.

The results we presented here support that the western region of occurrence of *V. auriculata* (a) comprise an ESU (Moritz, 1994) distinct from the centre-east region (b+c and d). In addition, the occurrence of exclusive plastid DNA haplotypes in populations a2, a3, a4, b1, b4, c2 and d1 makes each of them an independent MU (Moritz, 1994). It is fundamental that *in situ* and *ex situ* management and conservation plans take into account the high interregional and interpopulation differentiation of *V. auriculata*, including comprehensive protection actions for effective conservation of genetic variability of this species. The extinction of populations in the centre would be especially critical, since it could restrict gametic gene flow between the species distribution edges, potentially decreasing genetic diversity and compromising the future viability of populations. Currently, these populations are not included in conservation units and are subject to anthropogenic impacts that must be urgently interrupted.

We conclude that for *V. auriculata* (1) zygotic gene flow is limited even on short scales, (2) gametic gene flow is recurrent, but limited by distance and environmental unsuitability and (3) climatic changes of Pleistocene did not restrict the species to refugia, with local persistence of populations. Our data contribute to the understanding of the high diversity observed in the campo rupestre by providing more arguments in support of the hypothesis that campo rupestre is both a cradle (due to low zygotic gene flow between populations, which favours population isolation and differentiation) and a lineage museum (since it is able to maintain *in situ* survival of populations during climate changes, Silveira *et al.*, 2016). As genetic studies of non-model organisms are now more accessible than ever, we hope to see new

Figure 7. SDM of *Vellozia auriculata* using Maxent, which presented the best performance between the algorithms tested, for current (A) and past (B, D, F) modelled distributions. B, 21k (maximum glacial) model CCSM; D, 21k (maximum glacial) model MIROC and F, 120–140k. C, E and G show changes in suitability in relation to current conditions, where negative values indicate reduction and positive values indicate increase of suitability. C, 21k (maximum glacial) model CCSM; E, 21k (maximum glacial) model MIROC and G, 120–140k.

advancements on phylogeographical understanding of campo rupestre flora in the coming years.

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SUPPORTING INFORMATION

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

Supplementary Figure S1. Geneland Bayesian inference of spatial clustering. A, Number of clusters along the chain after burnin. B, Spatial distribution of genetic-geographic groups.
Supplementary Figure S2. Model selection and model checking for scenario 2.
Supplementary Figure S3. Parameter priors and subsequent estimates based on the most likely scenario, colonization from east to west (scenario 5).
Supplementary Figure S4. SDM of *V. auriculata* for the study area, training of Maxent algorithm (best performance between the algorithms tested) for current and past scenarios.
Supplementary Figure S5. Validation between algorithms training for *V. auriculata* for the study area.
Supplementary Figure S6. Detail of result of validation of SDM between algorithms, for the occurrence area of *V. auriculata*.
Supplementary Table S1. Primers used in the ISSR amplifications of *V. auriculata* and their respective polymorphism rates.
Supplementary Table S2. Parameters priors for all scenarios and estimates for Scenario 5 (scenario with highest posterior probability on the ABC analysis), based on cpDNA of *Vellozia auriculata*. Ne: population size after colonization, Nb: population size during a bottleneck (scenario 1) or colonization population size (founding effect, scenarios 2–5), t1–t6, divergence times; Dist, prior distribution; uni, uniform; Min, minimum prior value; Max, maximum prior value; Con, constraints and Q, quantile.
Supplementary Table S3. Genetic distance between *V. auriculata* populations. Number of mean substitutions for cpDNA (below diagonal) and unbiased distance of Nei (1978) from ISSR phenotypes (above diagonal).

FINAL CONSIDERATIONS

The association between the high diversity of the campos rupestres, an old Neotropical sky-islands environment on eastern South America, and its disjunct aspect have long been stated. Here we confirm, refine and renew this idea using two groups presenting contrasting biologic characteristic. *Bulbophyllum* sect. *Didactyle* (Orchidaceae) evolved trough a complex pattern of isolation, gene flow and hybridization, and some of its previously recognized species are not recovered as monophyletic. The distribution of the genetic diversity of *Bulbophyllum* sect. *Didactyle* mirrors the geographic distributions of the populations and the main geographical breaks of the Espinhaço/Chapada-Diamantina chain, while a new link among Chapada dos Veadeiros, Serra do Cabral, and Serra da Canastra (regions R04, R06 and R09, respectively, on Chapter 1) was observed.

On a species level, both *B. involutum* and *Vellozia auriculata* (Velloziaceae) presented geographical structure, despite this structure was stronger for the less vagile *V. auriculata*. For both species the overall gene flow estimates between populations was low, despite for *B. involutum* they were high across the Espinhaço range. The high gene flow between Espinhaço range *B. involutum* populations corroborates the notion that the small and light seeds of orchids are able of long-range dispersion, while the fact that eastern and western *B. involutum* populations in the Chapada Diamantina are highly differentiated supports that the environment or the past demography of this species might be important factors driving population differentiation. Since many of campo rupestres species present limited vagility, as occurs to *V. auriculata*, it is expected that gene flow between their populations are generally limited, in accordance with the topography-driven-isolation theory, but it is likely that environmental factor also play a role in this differentiation, as probably occurs in *B. exaltatum*.

Beyond the importance of disjunction and environmental variability for population differentiation, this study shows that hybridization might be and critical engine for the origin and maintenance of the campos rupestres biodiversity. Hybridization is a widespread phenomenon on *Bulbophyllum* sect. *Didactyle*, as at least five from its seven currently circumscribed species are presently involved on interspecific crosses. As the phylogenetic propensity of hybridization is high, it is likely that hybridization is also an important event on other sections of *Bulbophyllum*, influencing the high diversity of this highly speciose genus. Further phylogeographical, niche modeling and landscape genetics work might help to improve the disentanglement of the complex patterns related to the evolution of biodiversity on the campos rupestres. Also, the genetic of hybridization might be explored in deep, using functional gene annotation and trait-based studies, to shed light on the role adaptative introgression and connections between admixture with reproductive barriers generating the exuberant diversity of orchids and the campos rupestres.