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Phylogeography and ecological niche modelling in *Eugenia uniflora* (Myrtaceae) suggest distinct vegetational responses to climate change between the southern and the northern Atlantic Forest

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In this study, we evaluate phylogeographic patterns and predictions of ecological niche modelling (ENM) for *Eugenia uniflora* (Myrtaceae), a widely distributed taxon in the Atlantic forest domain, to understand the effect of past climatic oscillations on the demographic history of this species. An analysis of phylogeographic population structure and demography was conducted on *E. uniflora* from 46 localities in natural environments across the distribution range of the species based on three plastid markers. ENM was also performed to predict suitable environments and areas of dramatic decrease in future suitability for the species under distinct representative concentration pathways (RCPs). *Eugenia uniflora* exhibited higher haplotype and nucleotide diversity in the southern part of its distribution than in the northern part. Two divergent lineages were revealed in the phylogenetic analysis of haplotypes, with an estimated divergence at *c*. 4.9 Mya. The populations in the northern and central regions of the range probably experienced population growth, whereas populations in the southern region are marked by historical demographic stability. ENM results indicate that the distribution of *E. uniflora* was fragmented in cool periods and was broader and more connected during warm periods during Pleistocene. The results suggest distinct evolutionary histories in southern to northern populations, indicating region-specific responses to changes. *Botanical Journal of the Linnean Society*, 2016

ADDITIONAL KEYWORDS: chloroplast DNA – geographical variation – pitanga – population structure – South America.

INTRODUCTION

Investigating the evolutionary history of species can lead to an increased understanding of the interactions between past climatic events and the evolutionary processes that contributed to current patterns of diversity (Hewitt, 2000; Duminil *et al.*, 2010). In this context, molecular phylogeographic approaches facilitate an increased understanding of the role that historical events play in the geographical patterns of genetic variability within and among species (Knowles & Maddison, 2002; Avise, 2009). Studies combining ecological and phylogenetic/phylogeographic analysis have provided information regarding the origin and evolutionary history of species (Wiens & Donoghue, 2004; Ricklefs, 2010) and improved our understanding of the processes structuring genetic variation across landscapes (Knowles, 2009; Chan, Brown & Yoder, 2011; Collevatti *et al.*, 2013; Alvarado-Serrano & Knowles, 2014; Diniz-filho *et al.*, 2014; Thode *et al.*, 2014).

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In South America, recent studies have combined ecological niche modelling (ENM) and phylogeographic approaches, highlighting various patterns of diversification and demographic histories in different taxa (Carnaval & Moritz, 2008; Carnaval *et al.*, 2009, 2014; Martins, 2011; Collevatti *et al.*, 2012; Valdez & D'Elía, 2013). However, the impact of past climatic changes on many Neotropical species has yet to be explored. Further investigations of historical phylogeography on each specific biome and ecoregion in South America are fundamental in the assessment of how species have responded to past climatic and environmental changes and to predict how they might cope with current and future changes.

The Atlantic forest is the second largest tropical forest in South America, covering an area of $> 1\ 000\ 000\ km^2$ along the Brazilian coast and extending to eastern Paraguay and northeastern Argentina (Joly *et al.*, 1999; Oliveira-Filho & Fontes, 2000; Ribeiro *et al.*, 2009). Complex factors including strong seasonality, sharp environmental gradients and orographically driven rainfall (resulting from easterly tropical Atlantic winds) result in a diverse landscape in this ecoregion (Joly *et al.*, 1999; Martins, 2011). This specific complex is referred to as the Atlantic Forest Domain (AFD) (Joly *et al.*, 1999) and includes open, mixed and closed evergreen forest and semi-deciduous and deciduous forests. The evergreen forest runs along the coastline, covering mountain slopes at low to middle elevations (≤ 1000 m), with semi-deciduous forest extending across a plateau (usually > 600 m) in the centre and southeastern interior of Brazil (Morellato & Haddad, 2000; Oliveira-Filho & Fontes, 2000). Surveys of terrestrial plant occurrences in the AFD have demonstrated that species can occupy more than one phytogeographical niche in this domain (Stehmann *et al.*, 2009; Barros & Morim, 2014). Previous studies have suggested that the AFD was historically fragmented with open areas during the Pleistocene (Behling, 1997, 1998, 2002; Lichte & Behling, 1999), presenting widely isolated patches of forest (Ledru, 1998; Behling & Negrelle, 2001). Comparative phylogeography has aided in the identification of putative refugia and zones of secondary contact for vertebrates from the Brazilian Atlantic forest (Martins, 2011; Porto *et al.*, 2012; Silva *et al.*, 2012); however, this type of information remains scarce for plant species (Turchetto-Zolet *et al.*, 2013). Moreover, phylogeographic studies of species in the AFD occupying more than one phytogeographical region in this domain may reveal useful information regarding how such species coped with past climatic changes, and how these events may have driven the evolutionary history of populations.

Eugenia uniflora L., 'pitanga' or Brazilian cherry, belongs to Myrtaceae, one of the most species-rich angiosperm families in the Neotropics (Govaerts *et al.*, 2011). *Eugenia uniflora* grows in a variety of phytogeographical regions in the AFD, including the Atlantic forest (rainforest), semi-deciduous forest (Oliveira-Filho & Fontes, 2000), steppe grassland (Roesch *et al.*, 2009) and the adjacent restinga ecosystem (Scarano, 2002) from the northeastern to southern regions of Brazil, northern Argentina and Uruguay (Fig. 1A). Moreover, this species exhibits several habits. For instance, it is a shrub or small tree in the sandy coastal-plain vegetation near the ocean in southeastern and northeastern Brazil (in adjacent restinga) or a tree in the southern part of the AFD (dense ombrophilous forest, steppe grassland, pioneer formation and riparian forests) (Oliveira-Filho & Fontes, 2000; Almeida, Faria & Da Silva, 2012; Lucas & Bünger, 2015). In the south, this species extends from the coast up to 400 km inland (in riparian forests). Previous studies have revealed the various biological properties of this species (Da Silva *et al.*, 2005; Oliveira *et al.*, 2008; Malaman *et al.*, 2011; Santos *et al.*, 2012; De Oliveira Jucoski *et al.*, 2013; Rodrigues *et al.*, 2013), making this species a target for commercial exploitation. Recent studies of this species using molecular dating were performed to reveal its genetic diversity and differentiation (Margis *et al.*, 2002; Salgueiro *et al.*, 2004; Ferreira-Ramos *et al.*, 2007, 2014) and the regulation of gene expression and metabolic pathways (Guzman *et al.*, 2014). Nonetheless, phylogeographic studies have not yet been performed in this species or other Myrtaceae, which are often underrepresented in phylogeographic studies in South America (Turchetto-Zolet *et al.*, 2013).

The aim of this study was to investigate the phylogeographic and demographic history of *E. uniflora* throughout its distribution to understand how past climatic oscillations have effected its current genetic variation. Particularly, this study aims to answer the following questions: (1) How is the genetic diversity of *E. uniflora* geographically distributed? (2) What was the distribution range of *E. uniflora* during the Quaternary glacial and interglacial periods? (3) Is the genetic diversity of this species a result of past habitat fragmentation or recent range expansion? (4) Is there a common or distinct pattern of genetic variation and demography along its entire distribution?

MATERIAL AND METHODS

Sampling and DNA extraction

Samples of *E. uniflora* were collected from 46 localities (hereafter referred to as populations) across its

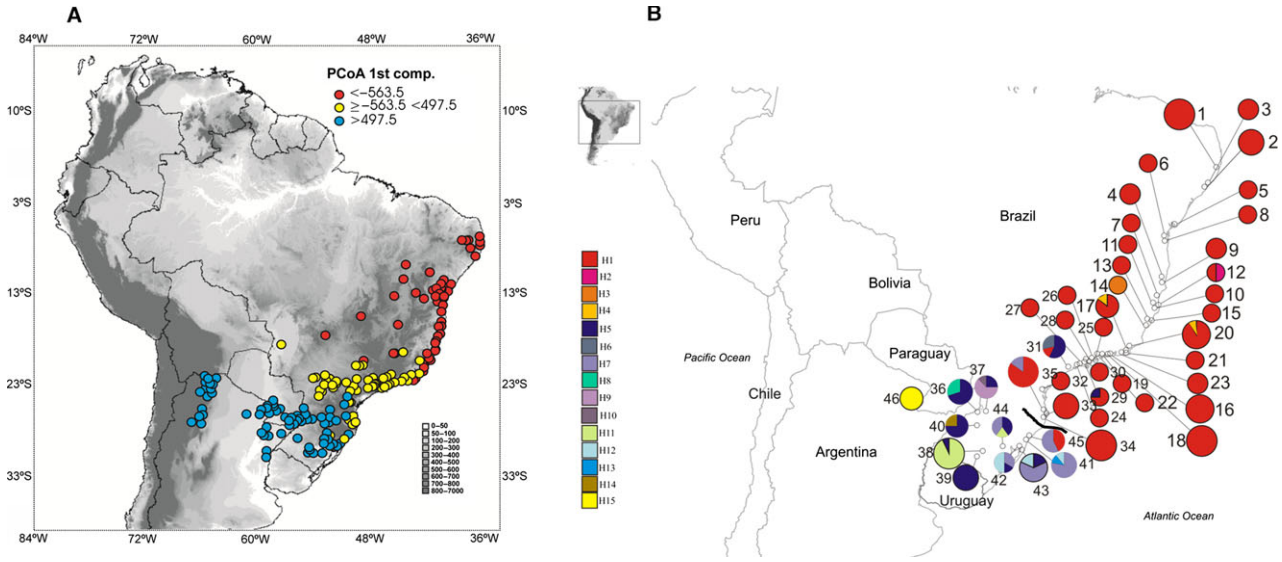

Figure 1. Maps relating to the geographical distribution and the study area of *Eugenia uniflora* in the Atlantic forest domain. (A), Current occurrence records of *E. uniflora* used to generate and validate the ecologic niche models based on field observations and databases. The colours represent the first component of PCoA (70% of variance) for which precipitation variables and altitude were the main contributors. B, Distribution of plastid haplotypes (coloured circles) in *E. uniflora* populations (white circles). Different colours were assigned for each haplotype according to the legend shown on the left side of the figure. Circle size represents the sample size and circle sections represent the haplotype frequency in each sampled population. For details on the population codes and localities, see Table 1. The dotted line on the map indicates the northern and southern division of populations.

distribution. Care was taken to collect only individuals from natural environments. Three hundred and five individuals were collected, ranging from three to 14 individuals per population) (Table 1, Fig. 1B). Representative voucher specimens were collected for two individuals of *E. uniflora* and deposited in the Instituto de Ciências Naturais (ICN) Herbarium of Department of Botany at the Federal University of Rio Grande do Sul (UFRGS) under the numbers ICN167404 and ICN167405.

The distance among populations ranged from 20 to 3300 km. Leaf samples were collected from each individual and dried in silica gel. One individual each from *E. brasiliensis*, *Myrcianthes cisplatensis* (Cambess.) O.Berg, and *Myrcianthes gigantea* (D.Legrand) D. Legrand (Myrtaceae), previously analyzed by Cruz *et al.* (2013), were included in the analysis as outgroup. Total genomic DNA was isolated from powdered leaves with liquid N_2 using the Cetyl trimethylammonium bromide method (Doyle & Doyle, 1990). The quantity and quality of DNA were assessed by electrophoresis and visualization on 1.0% agarose gels.

DNA sequencing

The first step involved screening several markers to locate polymorphisms. Plastid *psbA–trnH, trnS–trnG, trnC–ycf6* and *trnL–trnF* intergenic spacers and *rps16* and *rbcL* genes (Kress *et al.*, 2005; Kress & Erickson, 2007; Shaw *et al.*, 2007) and nuclear ITS (Schultz & Wolf, 2009) were tested in representative samples of 50 individuals. The plastid *psbA–trnH, trnS–trnG* and *trnC–ycf6* intergenic spacers were then selected to amplify in all 305 individuals. Then, the molecular analysis was based on plastid DNA markers that were chosen due to their ability to reconstruct historical demographic events. Some advantages of these markers are their coalescent time and lack of recombination (Duminil *et al.*, 2010). These markers may provide important insight into which processes have influenced modern genetic diversity and how this occurred. The polymerase chain reaction (PCR) was performed based on two programs set according to the melting temperatures of the primers as follow: *psbA–trnH, trnS–trnG, trnC–ycf6* and *trnL–trnF* (94 °C for 5 min, followed by 35 cycles of 94 °C for 50 s, 50 °C for 50 s, and 72 °C for 50 s); *rps16*, *rbcL* and ITS (94 °C for 5 min, followed by 35 cycles of 94 °C for 50 s, 54 °C for 50 s, and 72 °C for 50 s). Each 20 μL PCR reaction included 10 ng genomic DNA, 2.5 mM $MgCl_2$, 0.25 mM dNTP mix, 1× PCR buffer, 0.05 U Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 0.25 μM each primer. Nuclear and plastid PCR products were sequenced from both ends

Table 1. Sample information. Genetic diversity indices and neutrality tests for plastid DNA regions of the 47 populations of *Eugenia uniflora*

No. in Fig. 1B	Geographical origin (abbreviated sample site)	Latitude-Longitude (S-W)	Phytogeographical Region (group)	Sample size	Haplotype	Haplotype diversity/	Nucleotide diversity	Tajima's D	Fu's FS
1	Itamaracá, PE, Brazil (PERN)	07°45′20″–34°49′31″	Adjacent Restinga ecosystem (N)	13	H1	0.0000	0.000000	0.0000	0.0000
2	Barra de Santo Antônio, AL, Brazil (SANT)	9°24′58′–35°30′32.76″	Adjacent Restinga ecosystem (N)	9	H1	0.0000	0.000000	0.0000	0.0000
3	São Miguel dos Milagres, AL, Brazil (SMMI)	9°15′54′–35°22′18′68″	Adjacent Restinga ecosystem (N)	5	H1	0.0000	0.000000	0.0000	0.0000
4	Arraial D'Ajuda, BA, Brazil (ARRA)	16°27′42″–39°03′43″	Adjacent Restinga ecosystem (N)	5	H1	0.0000	0.000000	0.0000	0.0000
5	Imbassaí, BA, Brazil (IMBA)	12°26′28″–38°00′0.5″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
6	Morro de São Paulo, BA, Brazil (MSPO)	13°23′1″–38°54′32″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
7	Mucuri, BA, Brazil (MUCR)	18°04′02″–39°32′11″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
8	Tassimirim, BA, Brazil (TASS)	13°35′12″–38°55′53″	Adjacent Restinga ecosystem (N)	3	H1	0.0000	0.000000	0.0000	0.0000
9	Trancoso, BA, Brazil (TRAN)	16°35′22″–39°05′47″	Adjacent Restinga ecosystem (N)	6	H1	0.0000	0.000000	0.0000	0.0000
10	Praia de Barra Nova, ES, Brazil (BNOV)	18°56′51″–39°44′26″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
11	Sítio do Caju, ES, Brazil (CAJU)	18°51′09″–39°45′21″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
12	Conceição da Barra, ES, Brazil (CBAR)	18°36′57″–39°44′02″	Adjacent Restinga ecosystem (N)	4	H1. H2	0.6667	0.000359	1.6330	0.5400
13	Barra do Sahy, ES, Brazil (SAHY)	19°53′21″–40°05′23″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
14	Reserva Indígena Guarani, ES, Brazil (GUAR)	19°57′05″–40°10′01″	Adjacent Restinga ecosystem (N)	3	H3	0.0000	0.000000	0.0000	0.0000
15	Praia de Itaparica, ES, Brazil (PITA)	20°23′23″–40°18′52″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
16	Barra da Tijuca, RJ, Brazil (BART)	23°01′00″–43°26′00″	Adjacent Restinga ecosystem (N)	12	H1	0.0000	0.000000	0.0000	0.0000
17	Buzios. RJ, Brazil (BUZI)	22°45′20″–41°56′47″	Adjacent Restinga ecosystem (N)	7	H1. H4	0.2857	0.000154	−1.0062	−0.0947
18	Grumarí, RJ, Brazil (GRUP)	23°02′48″–43°31′11″	Adjacent Restinga ecosystem (N)	14	H1	0.0000	0.000000	0.0000	0.0000
19	Ilha Grande, RJ, Brazil (IGRA)	23°11′00″–44°19′00″	Adjacent Restinga ecosystem (N)	3	H1	0.0000	0.000000	0.0000	0.0000

Table 1. *Continued*

No. in Fig. 1B	Geographical origin (abbreviated sample site)	Latitude-Longitude (S-W)	Phytogeographical Region (group)	Sample size	Haplotype	Haplotype diversity/	Nucleotide diversity	Tajima's D	Fu's FS
20	Macaé, RJ, Brazil (MACA)	22°17′35″–41°40′28″	Adjacent Restinga ecosystem (N)	12	H1. H4	0.1667	0.000090	−1.1405	−0.4757
21	Arraial do Cabo, RJ, Brazil (ARRC)	22°57′53″–42°00′53″	Adjacent Restinga ecosystem (N)	3	H1	0.0000	0.000000	0.0000	0.0000
22	Trindade, RJ, Brazil (TRIN)	23°20′43″–44°43′02″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
23	Praia Seca, RJ, Brazil (SECA)	22°56′11″–42°18′39″	Adjacent Restinga ecosystem (N)	5	H1	0.0000	0.000000	0.0000	0.0000
24	Camburi, SP, Brazil (CAMB)	23°46′39″–45°39′15″	Adjacent Restinga ecosystem (N)	5	H1	0.0000	0.000000	0.0000	0.0000
25	Picinguaba, SP, Brazil (PICI)	23°21′24″–44°51′47″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
26	Prumirim, SP, Brazil (PRUM)	23°22′45″–44°57′41″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
27	Santiago, SP, Brazil (SANG)	23°48′46″–45°32′17″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
28	Praia Vermelha, SP, Brazil (PVER)	23°30′33″–45°10′23″	Adjacent Restinga ecosystem (N)	4	H1	0.0000	0.000000	0.0000	0.0000
29	Praia Mococa, SP, Brazil (MOCO)	23°34′19″–45°17′58″	Adjacent Restinga ecosystem (N)	4	H1. H5	0.5000	0.000809	−0.7545	1.7161
30	Praia de Fortaleza, SP, Brazil (FORT)	23°31′37″–45°10′01″	Adjacent Restinga ecosystem (N)	3	H1	0.0000	0.000000	0.0000	0.0000
31	Jardim Botânico, SP, Brazil (JBOT)	23°38′44″–46°37′32″	Dense Ombrophylous Forest (N)	7	H1. H5. H6	0.6667	0.000718	−1.3584	0.5409
32	Antonina, PR, Brazil (ANTO)	25°25′45″–48°42′42″	Adjacent Restinga ecosystem (N)	3	H1	0.0000	0.000000	0.0000	0.0000
33	Florianópolis, SC, Brazil (SFLP)	27°26′33″–48°22′11″	Adjacent Restinga ecosystem (N)	14	H1	0.0000	0.000000	0.0000	0.0000
34	Bombinhas, SC, Brazil (BOMB)	27°12′08″–48°29′11″	Adjacent Restinga ecosystem (N)	13	H1. H7	0.2821	0.000608	−0.4237	2.7797
35	Lagoa Conceição, SC, Brazil (LAFL)	27°35′36″–48°26′08″	Adjacent Restinga ecosystem (N)	9	H1	0.0000	0.000000	0.0000	0.0000
36	Derrubadas, RS, Brazil (DERR)	27°12′16″–53°51′18″	Dense Ombrophylous Forest (S)	10	H5. H8	0.4667	0.000252	0.8198	0.8180

Table 1. *Continued*

No. in Fig. 1B	Geographical origin (abbreviated sample site)	Latitude-Longitude (S-W)	Phytogeographical Region (group)	Sample size	Haplotype	Haplotype diversity/	Nucleotide diversity	Tajima's D	Fu's FS
37	Irai, RS, Brazil (IRAI)	27°11′24″–53°14′55″	Dense Ombrophylous Forest (S)	8	H5. H9. H10	0.6071	0.000424	0.0694	−0.2236
38	Caçapava do Sul, RS, Brazil (CAPS)	30°30′22″–53°28′22″	Steppe grassland (S)	14	H5. H11	0.1429	0.000077	−1.1552	−0.5948
39	Caçapava do Sul, RS, Brazil (CAPP)	30°46′16″–56°54′36″	Steppe grassland (S)	9	H5	0.0000	0.000000	0.0000	0.0000
40	Ijui, RS, Brazil (IJUI)	28°18′37″–53°55′39″	Steppe grassland (S)	8	H5. H14	0.4286	0.000924	−0.5833	0.3512
41	Lagoa de Itapeva, RS, Brazil (ITAP)	29°24′18″–49°51′50″	Pioneer formation (Coastal) (S)	9	H7. H12. H13	0.4167	0.000539	1.3926	0.7581
42	Palmares do Sul, RS, Brazil (PALM)	30°14′44″–50°28′17″	Pioneer formation (Coastal) (S)	6	H5. H7. H12	0.7333	0.000934	0.6876	0.8952
43	Capivari, RS, Brazil (CAPI)	30°08′40″–50°30′42″	Pioneer formation (Coastal) (S)	11	H5. H7. H12	0.5818	0.000627	0.2431	−0.4754
44	Gravataí, RS, Brazil (GRAV)	29°55′00″–51°53′00″	Pioneer formation (Coastal) (S)	5	H5. H7. H11	0.8000	0.000539	1.9122	3.6435
45	Torres, RS, Brazil (TORR)	29°21′16″–49°43′56″	Pioneer formation (Coastal) (S)	7	H1. H7	0.5714	0.001232	0.4852	3.1494
46	Corrientes, Argentina (ARGE)	26°28′20″–58°52′08″	Steppe grassland (S)	8	H15	0.0000	0.000000	0.0000	0.0000
	Total			305		0.5508	0.001023	−0.5615	−2.5916

AL, Alagoas; BA, Bahia; ES, Espirito Santo; PR, Paraná; PE, Pernambuco; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo. Northern group (N), Southern group (N).

by dideoxy chain-termination using a BigDye Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions, and run on an ABI-3100 automatic sequencer (Applied Biosystems). All fragments were sequenced in the forward and reverse directions and detected polymorphisms were validated by visually checking the original chromatograms. Sequences were deposited in the GenBank database (accession numbers KP719023–KP719085) (Table S1).

Multiple sequence alignments were obtained using MUSCLE (Edgar, 2004) implemented in MEGA 6 (Tamura *et al.*, 2013). All analyses were performed using the nucleotide sequences of concatenated plastid DNA regions (*psbA–trnH*, *trnC–ycf6* and *trnG–trnS*).

Genetic diversity, population structure and phylogeographic pattern

Standard diversity indices including nucleotide (pi) and haplotype (h) diversities for individual and overall populations and analysis of molecular variance (AMOVA, Excoffier, Smouse & Quattro, 1992) were performed in ARLEQUIN 3.5.1.2 software (Excoffier & Lischer, 2010). The AMOVA was conducted using 1000 permutations among collection sites and Φ_{ST} (pairwise differences). The occurrence of phylogeographic structures was inferred by testing for differences between G_{ST} and N_{ST} using PERMUT 2.0 (Pons & Petit, 1996) with 1000 permutations. G_{ST} coefficients were dependent on haplotype frequencies, whereas N_{ST} considers sequence differences between the haplotypes and the genetic distance between them. Thus, an N_{ST} value that is higher than the G_{ST} value indicates that closely related haplotypes are observed more frequently in a given geographical area than would be expected by chance (Pons & Petit, 1996).

Bayesian analysis of population structure using BAPS v6 (Corander, Sirén & Arjas, 2008; Cheng *et al.*, 2013) was employed to analyse the population genetic structure by clustering sampled individuals into groups. This method is based on the Markov chain Monte Carlo simulation approach to group population samples into variable user-defined numbers (K) of clusters. The BAPS analysis was used to partition the populations in a number of K groups using spatial information to detect the most likely genetic structure among the 46 populations (Cheng *et al.*, 2013). This method was conducted using two to 46 groups (K), with ten replicates for each K value. The optimal K cluster population partition was determined by the highest marginal log-likelihood.

Genealogical relationships among haplotypes were estimated with the median-joining method (Bandelt, Forster & Röhl, 1999) implemented in NETWORK 4.2.0.1 (available at www.fluxus-engineering.com). Phylogenetic analysis and divergence time estimates of haplotypes were performed using a Bayesian approach, as implemented in BEAST 1.8.2 (Drummond *et al.*, 2012). The priors used included the birth–death model, the GTR+I+G model with four gamma categories and the relaxed molecular clock. The substitution model was selected by Akaike information criterion (AIC) implemented in jModelTest 0.1.1 (Posada, 2008). The substitution rate used was 3.6×10^{-10} ($\pm 5.4 \times 10^{-11}$) substitutions per site per year, as previously estimated for plastid regions of Myrtaceae from South America (Thornhill & Macphail, 2012). Two independent analyses of 10^8 generations were run. The convergence of the Markov chains was checked and effective sample sizes (ESS > 200) confirmed in TRACER 1.6 (Rambaut *et al.*, 2014). TreeAnnotator, part of the BEAST software package, was used to select the maximum clade credibility tree. Statistical support of branches was measured in Bayesian posterior probabilities (PP).

Demographic history

The demographic patterns of *E. uniflora* populations were assessed using different approaches for the plastid DNA sequence data sets. Two groups of neutrality tests were computed: (1) Tajima's *D* (1989) and Fu & Li's (1993) *F** and *D**, which considers the frequency of mutation (segregating sites); and (2) Fu's (1997) *FS*, which is based on haplotype distribution. In addition, mismatch distributions were simulated under the sudden-demographic expansion and spatial-demographic expansion models. All tests were performed using ARLEQUIN and DnaSP (Librado & Rozas, 2009). These analyses were performed in two ways: first all 46 populations were considered separately; and secondly, populations were grouped into northern and southern groups (according to results, see Fig. 1B). In addition, changes in population size over time for the species as a whole and for northern and southern groups were estimated using Bayesian skyline plot analysis (BSP, Drummond *et al.*, 2005) performed in BEAST. The priors for this analysis were the same as those used in the phylogenetic analysis of haplotypes, as previously described. The computation of BSP and convergence checking were performed in TRACER.

LAMARC 2.1.8 (Kuhner, 2006) was used to estimate the demographic parameters theta (Θ), growth rate (g) and migration rate (M). The estimates of theta were calculated as $\Theta = 2\ \mu N_e$, where N_e is the effective population size and μ represents the mutation rate per nucleotide and per generation. Exponential growth rate (g) was calculated as

$\Theta_t = \Theta_{now}\exp(-gt\mu)$, where t is time in mutational units. Migration rate was calculated as $2N_em/\Theta$, where m is the per generation migration rate. Bayesian estimation was used with ten initial chains of 100 000 steps (burn-in of 10 000) and two final chains of 1 000 000 steps (burn-in of 100 000). The priors were kept at the default setting. The analysis was run twice and checked for convergence and effective sample size values (ESS ≥ 200) using TRACER. The most probable estimates (MPE) were obtained as the credibility interval around the estimate for each parameter.

Ecological niche modelling

ENM was performed using MAXENT 3.3.3k (Phillips, Dudík & Schapire, 2004; Phillips, Anderson & Schapire, 2006), which consists of a machine-learning algorithm (Phillips *et al.*, 2006). This algorithm was preferred because it represents the most widely used method of maximum entropy for ENM and has been useful in present plant distributions and reconstructions of potential distributions at the Pleistocene Last Glacial Maximum (LGM) (Waltari *et al.*, 2007; Carnaval & Moritz, 2008; Werneck *et al.*, 2012). Geographical coordinates for this analysis were obtained from fieldwork and the following online databases: Global Biodiversity Information Facility (http://www.gbif.org; January 2015) and Species Link (http://splink.cria.org.br; January 2015). To ensure the utilization of native distribution coordinates only, online data was filtered based on personal observations and the help of a taxonomist (Dr. Marcos Sobral, Universidade Federal de São João Del-Rei). For modelling settings, the function 'Auto features' was selected since different sample sizes of the populations in the feature types and regularization constants permitted the use of all feature types by default. Distributions were modelled through the 'cross-validate' parameter, applying a maximum number of iterations at 1000, with ten replicates. All other additional parameters were set to the default settings in the software. For validation of models, the area under receiver operating characteristic curve (AUC-ROC or AUC) and the true skill statistic (TSS), which selects the 'max SSS' for the calculation of the TSS (Liu *et al.*, 2013), were performed. The identical threshold was also assumed for mapping the predictions. Climatic variables represented the average climates from 1950 to 2000 and consisted of the 19 bioclimatic variables, with 2.5 arcminutes of resolution (*c.* 4.5 km). Projections for past climate conditions were also developed for three periods: LGM *c.* 21 ka; LIG *c.* 120–140 ka; and mid-Holocene *c.* 6 ka. The layers representing all present and past climatic periods were obtained from WORDCLIM (http://www.worldclim.org/bioclim). To represent the LGM climate, climatic simulations were derived from coupled atmosphere-ocean general circulation models (AOGCM), CCSM3 (Collins *et al.*, 2006) and MIROC-ESM (Braconnot *et al.*, 2006) at identical resolutions. ENM data were converted into binary predictions, which developed a consensus projection in the LGM through areas predicted in the CCSM and MIROC-ESM. The resulting layers were converted into binary predictions based on the same 'max SSS' threshold. A principal coordinate analysis (PCoA) was performed using PAST 2.16 (Hammer *et al.*, 2001), which searches for distinct niche properties in an attempt to identify environmentally-based separation of groups among different areas.

For future projections, AOGCMs were used for 2070 (average between 2061 and 2080) under two representative concentration pathways (RCPs) for CCSM4 and MIROC-ESM models. RCPs are climate projections used in the Fifth IPCC Assessment, downloaded from WorldClim 1.4 (available at http://www.worldclim.org/CMIP5), which are already downscaled and calibrated (bias corrected). A consensus was developed for 4.5 and for 8.5 RCPs through multiplying the predictions based on CCSM4 and MIROC-ESM climate data. For that, the continuous predictions obtained were transformed for all models into binary count layers, assuming the 'max SSS' threshold (Liu *et al.*, 2013).

RESULTS

Genetic diversity, structure and phylogeographic analysis

The sequencing of the plastid *trnC–ycf, psbA–trnH* and *trnS–trnG* intergenic spacers generated fragments of 755–758, 472–480 and 638–669 base pairs (bp) in length, respectively. The total combined aligned matrix comprised 1857 sites, of which 15 were variable and three contained gaps. Fifteen haplotypes were observed by combining plastid DNA data for the 305 sequenced individuals. The haplotype diversity (h) ranged from 0 to 0.8 and nucleotide diversity (Pi) ranged from 0 to 0.0012 in the 46 populations studied. Total haplotype and nucleotide diversities were 0.550 and 0.0010, respectively (Table 1). The highest haplotype diversity was observed in two populations from the southern region, GRAV (0.800) and PALM (0.733), and two from the northern region, Jardim Botânico, SP, Brazil (JBOT) (0.666) and CBAR (0.666). Most populations from northern forest physiognomies presented no polymorphisms, whereas southern populations showed a high level of polymorphism (Table 1). Haplotype H1 was the most frequent, occurring in 35 of the 46 populations.

Considering the northern and southern groups presented in Figure 1B, haplotypes H1, H2, H3 and H4 were present only in northern populations, except for H1, which was a widely distributed haplotype present in populations from northern and southern groups. Haplotypes H5–H15 were present only in southern populations, except H5, which was found in two northern populations (JBOT and MOCO) (Fig. 1B). Whereas most haplotypes in the network were separated by a single mutational step, haplotypes H14 and H15, from southern populations, were separated by two and four mutational steps, respectively. Another important pattern was evident in haplotype sharing between northern and southern haplogroups, occurring primarily with central and more frequent haplotypes (H1 and H5, respectively). This pattern was congruent with areas where the ecological conditions were intermediate between the niches at the most extreme latitudes (Fig. 1A), although the terminal haplotypes in the phylogenetic tree are not shared between populations in northern and southern regions of the AFD.

Hierarchical AMOVA indicated significant differentiation between northern and southern groups ($F_{CT} = 0.585$, $P < 0.001$), although the differentiation due to variation among populations in each region was higher ($F_{ST} = 0.878$, $P < 0.001$) (Table 2). G_{ST} value (0.635) was smaller than the N_{ST} value (0.753), suggesting the presence of phylogeographic structure in *E. uniflora* populations.

The network analysis revealed the presence of two main haplogroups, which were geographically structured: one corresponded to the four haplotypes (H1, H2, H3 and H4) from northern populations and the other corresponded to the nine haplotypes (H5, H6, H7, H8, H9, H10, H11, H12 and H13) from southern populations (Figs 1B and 2A, Table 1). Generally, the phylogenetic relationships of haplotypes presented the same two major geographically structured clades identified in the haplotype network analysis (Fig. 2B), which was in accordance to the geographical distribution of lineages (Figs 1 and 2A, B). Given the low sequence divergence in this species, support values for most nodes were moderate to low, especially within the main clades. The haplotypes from northern and southern regions were grouped separately in two well-supported clades, although haplotype H1 is a widely distributed haplotype and also occurs in southern populations (Fig. 2B). The estimated divergence time between *E. uniflora* plastid haplotypes and the outgroup was *c*. 16 Myr . The divergence time between the northern and southern haplogroup of *E. uniflora* was *c*. 4.9 Myr.

The optimal solution identified by BAPS using spatial analysis was K = 6 (Table 3, Fig. S1). The results analysis indicated a pattern of structuring concordant with the different phytogeographical regions in the AFD (Table 3). Cluster 1 and cluster 2 grouped populations from the adjacent restinga ecosystem and coastal pioneer formation along the southern coast [BOMB and Torres, RS, Brazil (TORR) populations]. Cluster 3 grouped populations from dense ombrophilous forest edges and steppe grassland and cluster 5 grouped only populations from southern coastal pioneer formation. Populations Caçapava do Sul, RS, Brazil (CAPS) and Corrientes, Argentina (ARGE) from steppe grassland grouped in clusters 4 and 6, respectively.

Demographic history of *E. uniflora* populations

No significant effect of population retraction followed by expansion was observed when all populations where analysed together, either with Tajima's D (−0.561, $P = 0.318$) or Fu's FS (−2.591, $P = 0.218$) neutrality tests, although negative values were observed (Table 4). Negative values of these statistics indicate an excess of rare alleles or new mutations in the genealogy resulting from either population expansion or genetic hitchhiking (Fay & Wu, 2000). When the northern and southern populations were analysed separately, Fu's test was significant ($FS = -4.407$, $P < 0.005$) for northern populations, indicating a departure from neutrality. The BSP (Fig. 3) mostly supported these conclusions, suggesting that *E. uniflora* from southern regions underwent a constant

Table 2. Analysis of molecular variance (AMOVA) based on plastid DNA (*psbA–trnH*, *trnG–trnS* and *trnC–ycf6*) sequences

Source of variation	d.f.	% of variation	Fixation index
Among populations	46.00	80.37	FST: 0.80369
Within populations	266.00	19.63	
Between groups of populations	1	58.55	FCT: 0.58551
Among populations within groups	45	29.25	FSC: 0.70574
Within populations	266.00	12.20	FST: 0.87803

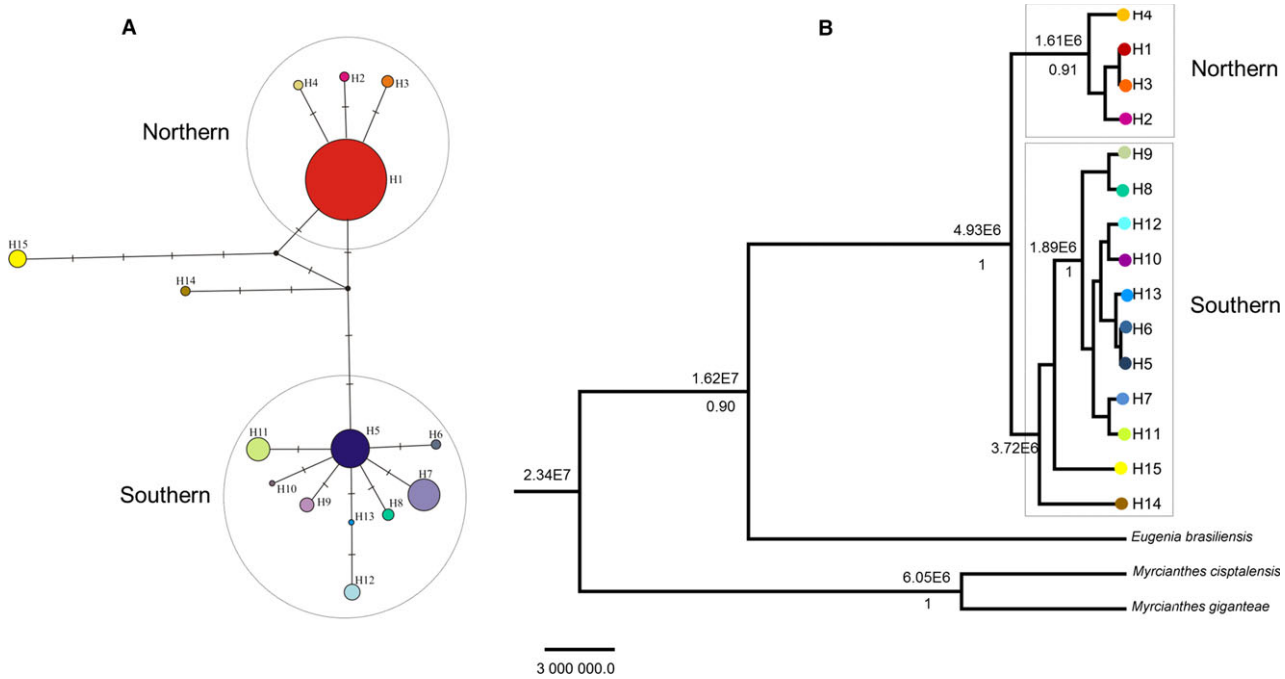

Figure 2. Relationships among haplotypes and estimated divergence times of *Eugenia uniflora* lineages. (A), Median-joining network based on plastid DNA sequences of *E. uniflora*. The circumference size is proportional to the haplotype frequency. The number of mutations is shown on the branches and the small black circles represent median vectors. B, Relationships and divergence time estimates of *E. uniflora* lineages performed in BEAST. The tip colours correspond to the haplotypes described in Figure 1B; the numbers below branches represent the supports to the nodes (posterior probability); the numbers above the branches represent nodes dating the time to the most recent common ancestor. The time scale is in millions of years (Myr). Samples from one individual each of *Eugenia brasiliensis*, *Myrcianthes cisplatensis* and *M. giganteae* were included as outgroups.

Table 3. Bayesian analysis of population structure (BAPS) results. Clusters correspond to the best partition of the data (log maximum likelihood ¼ –1794.6602;P¼0.999)

Cluster	Localities	Region within AFD/colour in Fig. S1
Cluster 1	PERN, SANT, SMMI, ARRA, IMBA, MSPO, MUCR, TASS, TRAN, BNOV, CAJU, CBAR, SAHY, PITA, BART, BUZI, GRUP, IGRA, MACA, ARRC, TRIN, SECA, CAMB, PICI, PRUM, SANG, PVER, FORT, ANTO, SFLP, LAFL	Adjacent Restinga ecosystem/Red
Cluster 2	GUAR, MOCO, BOMB, TORR	Pioneer formation (Coastal) and Adjacent Restinga ecosystem/Green
Cluster 3	JBOT, DERR, IRAI, CAPP, IJUI	Dense Ombrophylous Forest edges and Steppe grassland/Blue
Cluster 4	CAPS	Steppe grassland/Yellow
Cluster 5	ITAP, PALM, CAPI, GRAV	Pioneer formation (Coastal)/Pink
Cluster 6	ARGE	Steppe grassland/Light blue

effective population size and stability, whereas populations from northern regions showed moderated population growth (Fig. 3A, B). The coalescent analyses also indicated more population growth in northern than in southern groups (Table 5) with negligible gene flow among southern and northern populations (< 0.15 migrants per generation).

Ecological niche modelling

Two hundred and 63 geographical coordinates were obtained and refined, representing reliable points for the complete range of the natural population of *E. uniflora*. After filtering, 235 geographical coordinates were used. The first component of the PCoA encompassed 70% of the variance (eigenvalue 2.7)

Table 4. Summary of neutrality test analyses for *Eugenia uniflora* populations and group of populations

Parameter	All populations	Population from North	Population from South
Tajima's D	−0.5615	−1.2169	0.6521
Fu and Li's (1993) F'	0.3653	0.6525	0.5182
Fu and Li's (1993) D'	1.0226	0.0749	0.6724
Fu's (1997) Fs	−2.5916	−4.4072	0.02761
Mismatch distribution	0.10076	0.00006	0.02924

and resulted in at least three major groups (Fig. 1A). Statistical validation for the ENM indicated that the models were successful in the following predictions: for the present and 6 ka TSS = 0.68, standard deviation SD = 0.14; for LGM = 0.71, SD 0.23; for 120–140 ka = 0.66, SD 0.15; for the present and 6 ka AUC = 0.95, SD 0.01; for LGM AUC = 0.89, SD 0.02; and for 120–140 ka AUC = 0.95, SD 0.01. The most relevant areas predicted in ENM for the present are indicated for at least five South American countries, with most of those areas in Brazil and Argentina, although with considerable prediction also for Paraguay and the Bolivia-Peru border (Fig. 4A). Small suitable areas in the north of the continent were also identified, which were disconnected from the primary distribution regions, and mainly located in the littoral regions of Venezuela. Although we excluded specimens that had been cultivated, suitable areas were predicted surrounding the Bolivia–Peru border and in Venezuela that probably represent cultivated areas, since this species is widely cultivated and easily adapts to different environments. The ENM indicates that most suitable areas of distribution for this species were in the AFD region, adjacent restinga and seasonally dry tropical forests. However, occurrences were also indicated for areas in other ecosystems, such as the rainforests of Brazil and Argentina. At the LGM, the complete distribution ranges had not changed considerably in comparison to present distribution and stable areas were practically the same as those from the projection for the LGM (Fig. 4A). Oher patterns that emerged from the analysis included: (1) higher connectivity of predicted distribution between two large biogeographic areas was lost (the AFD region and a part predicted for sub-Andean Forests); and (2) potential expansions

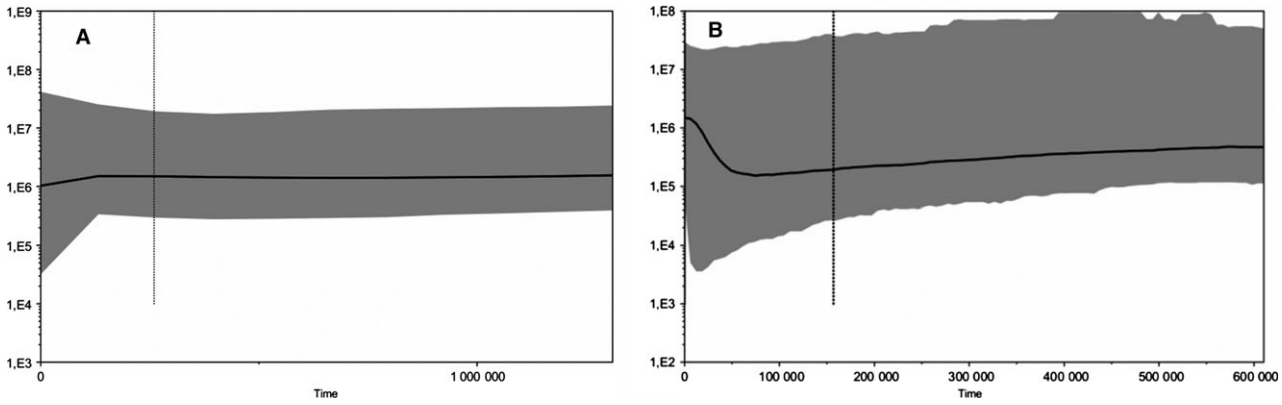

Figure 3. Estimates of population sizes using Bayesian skyline plot for *Eugenia uniflora* plastid DNA, considering (A) southern populations and (B) northern populations, and showing the effective fluctuation in population size over time. The thick solid line represents the median estimates and the shaded area represents the 95% confidence interval.

Table 5. Estimate of demographic parameters theta (Θ), grow rate (g) and migration rate (M) for *E. uniflora* populations

	North	South
LAMARC analysis		
Theta (±)	0.000425 (0.000120–0.000997)	0.001238 (0.000519–0.002379)
Growth (±)	920.4565 (−451.6490–973.0166)	707.8861 (−444.1758–970.7216)
M1 (±)	0.041 (0.014–0.042)	–
M2 (±)	0.119 (0.02–0.123)	–

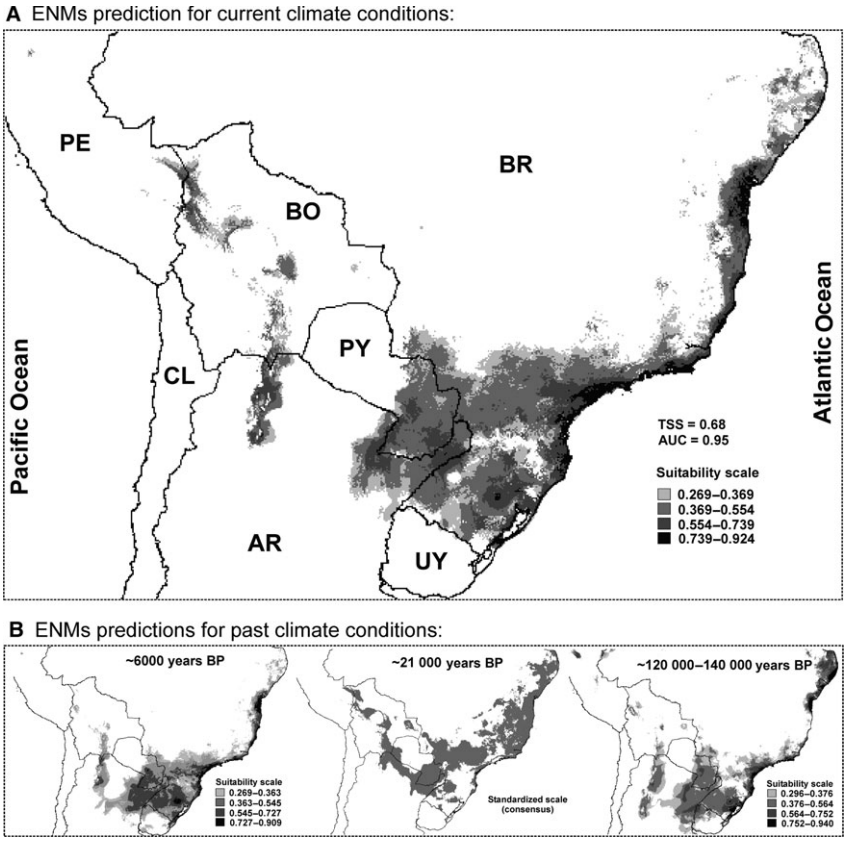

Figure 4. Ecological niche modelling (ENM) predictions for *Eugenia uniflora.* (A), Prediction for the present climate conditions. B, Distribution of past climatic conditions (~6000 BP; Last Glacial Maximum LGM ~21 000 BP; and ~120 000–140 000 BP).

for areas of higher altitudes were observed, corresponding to the highlands of southern Brazil (Fig. 4A, B). Palaeodistribution modelling indicates suitable environments for the occurrence of *E. uniflora* since the LGM (around 21 ka) in most areas in which it presently occurs. However, this potential distribution appeared fragmented during the cooler periods and was broader and more connected during warmer periods and in present-day climate conditions (Fig. 4). Fragmentation was more pronounced in southern populations than in northern populations, which is congruent with the genetic results (FCT: 0.58551). Moreover, a southern–northern disjunction was observed during the cooler periods (LGM), with connections established under warmer periods (LIG, mid-Holocene and present-day climatic conditions).

Projections based on both future scenarios, for 4.5 and 8.5 RCPs (Fig. 5A, B), showed a possible reduction in suitable areas for *E. uniflora*. The most pessimistic prediction (for the 8.5 RCPs) for global warming under the 2070 greenhouse gas concentration showed the most dramatic reduction in predicted distribution of the species (Fig. 5). Areas would be lost principally in the southern-central portions of the AFD, namely in Argentina and Paraguay, roughly corresponding to most of the AFD areas in Misiones province and its surrounding areas. Meanwhile, there are indications of expansions over the Brazilian plateaus, which are currently occupied by the *Araucaria* forest and subtropical highland grasslands.

DISCUSSION

The connection between phylogeographic and palaeodistribution modelling studies has played a key role in understanding how past climatic changes have influenced the demographic history of natural populations of many taxa (Carstens & Knowles, 2007). It is also fundamental to understand the evolutionary dynamics of a domain, such as the AFD, with complex landscapes where distinct evolutionary mechanisms are also likely to interact at various spatial scales. Although recent advances have improved understanding of the demographic history of many AFD taxa, knowledge of how past climate

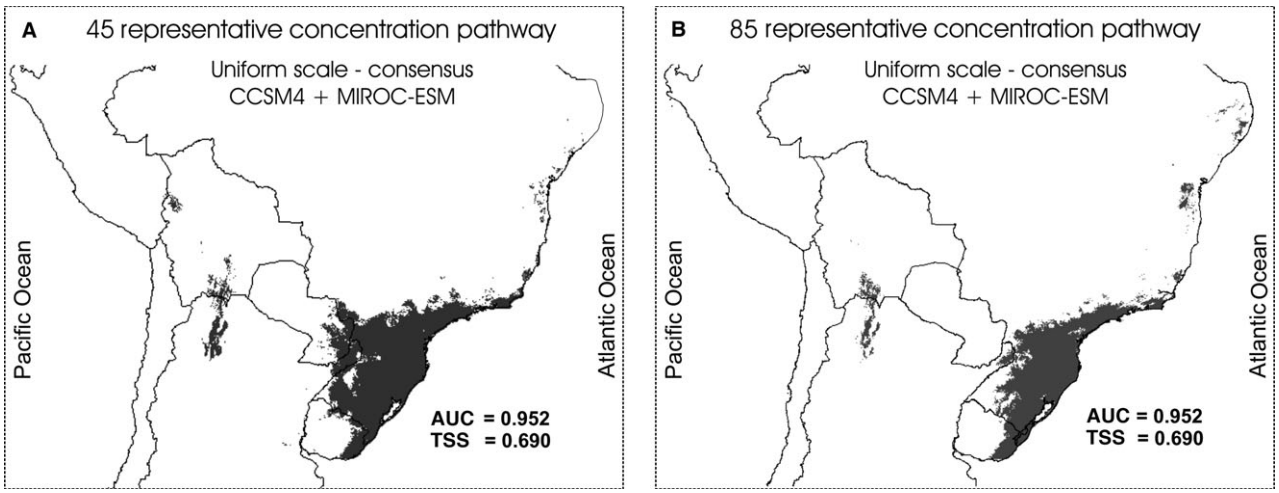

Figure 5. Future predictions of *E. uniflora* distribution: (A) consensus for 4.5 RCPs (B) consensus for the most pessimistic 8.5 RCPs.

changes, especially during glacial and interglacial periods, have affected these taxa remains incomplete. As previously demonstrated in another phylogeographic study (Turchetto-Zolet *et al.*, 2013), past climatic changes may distinctly affect different taxa belonging to a complex landscape such as the AFD. In this context, it is important to understand how widely distributed species in the AFD, such as *E. uniflora* (Fig. 1), responded to the past climatic changes throughout its distribution.

Genetic diversity, structure and phylogeographic analysis

Genetic diversity analysis for *E. uniflora* populations revealed a higher haplotype and nucleotide diversity in the southern than in the northern part of the distribution (see Fig. 1B and Table 1 for the north-south division for populations of this species). The overall genetic diversity indices for *E. uniflora* (h = 0.5508 and Pi = 0.001023) were smaller than reported for other plants such as *Plathymenia reticulata* Benth. (h = 0.900 and Pi = 0.0025) (Novaes *et al.*, 2010), *Schizolobium parahyba* (Vell.) S.F.Blake from the Atlantic forest (h = 0.907 and Pi = 0.00239) (Turchetto-Zolet *et al.*, 2012), *Calibrachoa heterophylla* (Sendtn.) Wijsman from the southern Atlantic coastal plain (h = 0.879 and Pi = 0.41) (Mäder *et al.*, 2013) and *Petunia axillaris* (Lam.) Britton, Sterns & Poggenb. from pampa grasslands (h = 0.70 and Pi = 0.22) (Turchetto *et al.*, 2014). Conversely, genetic diversity indices of *E. uniflora* were higher than found in *Recordia reitzii* (Moldenke) Thode & O'Leary (h = 0.060 and Pi = 0.0015), a tree species endemic to restricted area of Atlantic rainforest (Thode *et al.*, 2014). A similar pattern of higher genetic diversity in southern areas was also seen in *Epidendrum fulgens* Brongn., an orchid species occurring on coastal sand dunes and granitic outcrops in the Brazilian Atlantic forest (Pinheiro *et al.*, 2011).

The results of this study suggest two main evolutionary lineages for *E. uniflora,* which are from distinct geographical regions, namely the southern and northern part of the AFD. These lineages showed low haplotype sharing and long-term divergence (Fig. 2B). This divergence is not surprising when the biology of this species, its distribution and the molecular markers used are taken into consideration. Plastids are inherited maternally and the seed dispersal syndrome is predominantly zoochory (Gressler, Pizo & Morelatto, 2006). Low migration rates were observed between the northern and southern genetic units, suggesting the presence of seed dispersion barriers between northern and southern populations for this species. This barrier could be related to the occurrence of bird dispersion and also associated with different phytogeographical regions where *E. uniflora* occurs. Dispersion ability is crucial to plants in the establishment of new populations on unoccupied sites and it represents a critical component of biological diversity and gene flow maintenance. Some studies of birds from the AFD have demonstrated divergence between northern and southern groups (Raposo do Amaral *et al.*, 2013; Batalha-Filho *et al.*, 2014). The strongest differentiation between northern and southern populations of *E. uniflora* is located south of Torres (IPAP, see Table 1 and Fig. 1A). This region, located approximately between 29° and 30°S, is recognized historically as an important phytogeographical boundary, called the 'Portal de Torres' (Rambo, 1950). This

pattern of differentiation was also reported for *E. fulgens* (Pinheiro *et al.*, 2011). The northern populations of *E. uniflora* were found growing close to the coast, whereas southern populations were located in the inland region. Indeed, nearly all northern populations are associated with restinga vegetation, which is a more connected landscape compared to southern populations, occurring in the pampas domain and associated with riparian forests (disjunct patches of forests). The restinga environment is characterized by sandy and dry soil and the riparian forest is predominantly nutrient-rich, with high levels of organic material and water accumulation. Plants from restingas are morphologically different from plants in other localities (e.g. inland populations) when considering the habit of the tree. However, no characteristics have been described as different between them. The fact that low gene flow and a long period of divergence between the two groups suggests past isolation resulting in genetic differentiation. Perhaps these two lineages have already reproductive isolation, although experiments are necessary to support this hypothesis.

Phylogenetic analysis of *E. uniflora* lineages indicated the formation of two clades that diverged *c*. 4.9 Mya. Thornhill & Macphail (2012) used a chronogram based on ITS and plastid sequences calibrated using macrofossils and pollen fossils data to understand diversification in Myrtaceae. These authors discovered that *E. uniflora* is the sister group of *Hexachlamys edulis* (O.Berg) Kausel & D.Legrand and proposed a *c*. 20 Ma divergence in *Eugenia*, plus the realocation of *Hexachlamys* into tribe Myrteae. The data in the current study is based only on plastid markers and obtained *c*. 16 Mya in divergence between *Eugenia* and *Myrcianthes* O.Berg. As *Myrcianthes* and *Hexachlamys* are genera distributed preferentially inside the South American continent, and the time of divergence in *Eugenia* is earlier than Pleistocene events that influenced the coastal landscape, we suggest that *E. uniflora* most probably originated as a tree in the southern part of its distribution in dense ombrophilous forest, steppe grassland, pioneer formation in secondary forest or riparian forests. Genetic diversity results obtained for the northern and southern groups observed in the present study support the notion of a probable southern origin. The appearance of northern group and its morphology probably is a result of adaptation to salt and coastal environments.

A substructure with high genetic differentiation among populations (Haplotype network, AMOVA results) was found in the southern distribution of *E. uniflora*. This substructure may be a consequence of historical fragmentation in this region, generating organismal responses dependent on life history traits and ecological tolerance. Similar results were also observed for species of *Petunia* Juss. (Lorenz-Lemke *et al.*, 2006, 2010; Turchetto *et al.*, 2014), *Calibrachoa heterophylla* (Mäder *et al.*, 2013), *Schizolobium parahyba* (Turchetto-Zolet *et al.*, 2012) and *Vriesea gigantea* Gaudich. (Palma-Silva *et al.*, 2009) using the plastid molecular markers.

Demographic history and ecological niche modelling

Populations from northern regions experienced moderate changes in their effective population sizes, exhibiting signatures of recent demographic expansion and a lower genetic structure; whereas populations from southern regions experienced smaller or no changes in effective sizes, thus presenting demographic stability, high diversity and genetic structuring. The regional differences in the AFD, with moderate demographic fluctuations in the northern and stability in the southern regions, suggest that species-specific traits may be causing differential tolerance to past climatic changes. Different demographic histories across regions were previously demonstrated for other species in the AFD (d'Horta *et al.*, 2011; Turchetto-Zolet *et al.*, 2012). This latitudinal diversity gradient may reflect the distinct influence of Pleistocene glacial-interglacial cycles in geographical space (Hewitt, 2000, 2004). A recent study of the *Rhinella crucifer* species complex (a group of endemic toads with a widespread distribution in the Brazilian Atlantic forest; Thomé *et al.*, 2014) revealed regional differences for this species, with moderate population growth in the north and central regions and stability in the southern AFD.

The ENM used in the present study predicted fragmentation in the southern regions of *E. uniflora* distribution (Fig. 4), suggesting an effect of historical fragmentation in southern populations with lineage persistence. This fragmentation appears to affect the geographical distribution of *E. uniflora*, causing population differentiation and lineage divergence (Fig. 2B). Many populations in the southern region (see Fig. 1B) possess unique haplotypes, which may suggest that the population of *E. uniflora* persisted in multiple localities during glacial cycles where the ecological conditions were appropriate. During the Quaternary, glacial cycles resulted in cold, dry conditions interrupted by warmer, wet periods, which consequently led to the expansion and retraction of northern tropical forests. Previous studies have demonstrated that in the dry and cold climate of glacial periods, species of the grasslands formation in southern South America could advance 700 km northward and that during this period rainforest persisted in sites near rivers and valleys (Behling &

Lichte, 1997; Behling & Pillar, 2007). The location, size and existence of forest refugia during the glacial maximum were dependent on the ecological and environmental tolerances of each species (Moritz *et al.*, 2000). *Eugenia uniflora* appears to have been able to maintain stable populations throughout the late Pleistocene in its southern distribution, even during peaks of glaciations when forests became fragmented and the climate was temperate (Behling, 2002). In contrast, populations from the northern AFD appear to have been demographically affected by late Pleistocene glaciations. The current southern distribution limit of *E. uniflora* reaches temperate regions with discontinuous forest, namely the riparian forest from humid Chaco and portions over the pampas grasslands (steppes grassland) in eastern Argentina and Uruguay. The persistence of temperate southern populations during glacial periods has also been reported for some other plant species (Azpilicueta, Marchelli & Gallo, 2009; Jakob, Martinez-Meyer & Blattner, 2009; Tremetsberger *et al.*, 2009; Cosacov *et al.*, 2010; Mathiasen & Premoli, 2010; Premoli, Mathiasen & Kitzberger, 2010; Vidal-Russell, Souto & Premoli, 2011).

Taken together, the results of this study indicate an important effect of Pleistocene climatic change on the demographic history of *E. uniflora*, strongly supporting a scenario of two distinct evolutionary and demographic patterns for this species from its southern to northern distribution in the AFD. Two distinct clades of lineages were observed in these regions and low seed dispersion was detected between populations from southern to northern regions. The scenario depicted by the analytical approach in the present study suggests that northern *E. uniflora* populations were isolated in one refugium, subsequently followed by a population expansion. In contrast, the southern populations were fragmented in multiple refugia. Private alleles found across most of the populations in the southern suggest the long-term persistence of these populations, rather than recent migrations. The results of this study highlight the contribution of species growing in different phytogeographic regions in the AFD, helping in understanding of past vegetation and climate dynamics in this Neotropical region. Understanding the distribution of the phylogenetically distinct lineages of *E. uniflora* highlights the importance of including spatially refined measures of phylogenetic diversity coupled with a genomic approach. The results of this study also provide insight into the conservation efforts for this species, since predictions have suggested a possible reduction in the presence of suitable areas for *E. uniflora* in the future, particularly at the south-central portions of the AFD, principally in Argentina and Paraguay, where we identified a rare lineage. Understanding the processes that generate and maintain genetic diversity within species over time may have strong implications for the conservation management of the AFD, such as the protection of centres of genetic diversity and rare lineages. This species has been the subject of breeding and commercial exploitation and the results presented here may help in guiding sustainable commercial harvest and preserving the genetic diversity and rare lineages in this species. *Eugenia uniflora* occurs in some geographical areas that harbour great genetic diversity and includes unique lineages that should be protected.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bayesian analysis of population structure (BAPS) results. Clusters correspond to the best partition of the data (log maximum likelihood ¼ –1794.6602; P¼ 0.999) and are indicated by differently coloured Voronoi's polygons. Collection sites are indicated by circles.

Table S1. GenBank accession numbers, geographical origin, and collector of plastid DNA (*trnG–trnS, psbA–trnH* and *trnC–ycf6*) regions in *Eugenia uniflora* samples.