



## Holocene southward expansion in seasonally dry tropical forests in South America: phylogeography of *Ficus bonijesulapensis* (Moraceae)

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We conducted a phylogeographical and niche modelling study of the tree *Ficus bonijesulapensis*, endemic to Brazilian seasonally dry tropical forests (SDTFs), in order to evaluate the effects of Quaternary climatic fluctuations on population dynamics. The *trnQ-5'rps16* region of plastid DNA was sequenced from 15 populations. Three phylogeographical groups were identified by the median-joining algorithm network and spatial analysis of molecular variance (SAMOVA) ( $F_{CT} = 0.591$ ): a central-west, a central-east and a scattered group. The central groups had higher total haplotype and nucleotide diversities than the scattered group. Ecological niche modelling suggested that, since the Last Interglacial (130 kyr BP), the central and north regions have been relatively stable, whereas the southern region of the species distribution has been less stable. The phylogeographical groups showed concordance with the floristic units described for SDTFs. The low genetic diversity, unimodal mismatch distribution and unfavourable climatic conditions in the southern region suggest a recent southward expansion of the range of the species during the Holocene, supporting the hypothesis of the southward expansion of SDTFs during this period. The central and northern regions of the current distribution of *F. bonijesulapensis*, which are consistent with arboreal caatinga and rock outcrop floristic units, were potential refugia during Quaternary climatic fluctuations. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 177, 189–201.

**ADDITIONAL KEYWORDS:** caatinga – eastern Tropical South America – fig tree – habitat stability – limestone outcrops – Quaternary history.

### INTRODUCTION

Seasonally dry tropical forest (SDTF) occurs in seasonally dry regions with annual rainfall of < 1600 mm and at least 5–6 months with < 100 mm of precipitation (Graham & Dilcher, 1998). Recently, SDTFs have been proposed as one of the major biomes

(Pennington, Lavin & Oliveira-Filho, 2009), with a large number of deciduous tree species (Pennington *et al.*, 2009; Santos *et al.*, 2012). Although there is a wide distribution of SDTFs in South America, most occur as isolated patches or nuclei. One of the largest areas of South American SDTFs is the caatinga domain: a thorny and xeric woodland with several months of severe drought and a complex mosaic of soil types, usually with high fertility (Queiroz, 2006;

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Werneck, 2011). The caatinga is amongst the least understood and most degraded forest types in Brazil (Santos *et al.*, 2011), and this ecosystem has been severely affected by an extensive loss of trees as a result of livestock grazing and fire (Leal *et al.*, 2005; Santos *et al.*, 2011). The current caatinga biome covers an area of *c.* 800 000 km<sup>2</sup> in north-eastern Brazil, corresponding to *c.* 10% of the total territory of the country. It has been hypothesized that, during dry periods in the Last Glacial Maximum (LGM), the caatinga biome in Brazil was probably part of a continuous SDTF range, linked to Misiones and Piedmont nuclei (Prado, 2000), known as the Pleistocenic Arc (Prado & Gibbs, 1993). Caatinga also occurs as remnants and enclaves in the cerrado biome, mainly in limestone outcrop regions. The boundaries, nomenclature and classification of caatinga vegetation have been thoroughly examined elsewhere (Queiroz, 2006; Rodal, Barbosa & Thomas, 2008) and it can be divided into eight floristic units (Santos *et al.*, 2012). One such unit is the 'rock outcrop caatinga', of which limestone karst outcrops are the most common.

The phytogeography and ecology of rock outcrop vegetation in Brazil are still poorly understood (Scarano, 2007). Rock outcrops are considered to be islands (inselbergs) as a result of their irregular distribution and isolation, being surrounded by other forest types or agricultural land. In addition to limestone karst outcrops, various other types of isolated rock outcrops occur throughout Brazil in diverse biomes (< 10% of territory), including granite (e.g. Barbará *et al.*, 2007), ironstone (e.g. Jacobi *et al.*, 2007), crystalline (e.g. Gomes & Alves, 2010) and quartzite (e.g. Lousada, Lovato & Borba, 2013) outcrops. The limestone outcrops in SDTFs occur in only a small number of Brazilian biomes; the trees that occur in these forests are subject to climatic and edaphic stresses, and are capable of growing on rocky and seasonally water-deficient soils (Felfili *et al.*, 2007; see also Supporting Information Fig. S1). The rocky microhabitats have fostered the evolution of highly adapted plant species with unique abilities to use scarce resources and survive in conditions of edaphic water stress (Trejo-Torres & Ackerman, 2002; Felfili *et al.*, 2007). Because these formations are so different from other formations of the SDTF, understanding the typical physiognomy and floristic composition of these outcrops is extremely important, particularly considering their rarity and endemism (Pedralli, 1997; Santos *et al.*, 2007).

The sustainable use of the limestone in the karst outcrops is a critical issue for these environments as they are unique regions with vast biological richness. However, outcrops are often exploited commercially by the mineral industry and the calcareous cement that comes from these environments is a worldwide

commodity subject to over-exploitation in the short term. Furthermore, the threat of diminishing limestone reserves worldwide has been noted (Geological Survey, 2011). As discussed above, limestone karst outcrops in SDTFs are typically dispersed in 'islands' throughout the landscape that are physically isolated from each other. This spatial configuration, as seen in other limestone outcrop environments, probably limits gene flow and reproductive strategies (Barbará *et al.*, 2007, 2009), and promotes high levels of diversity and species endemism (Porembski, 2007).

*Ficus bonijesulapensis* R.M.Castro (Moraceae) is a fig species with a discontinuous geographical distribution that is endemic to outcrops of carbonate rock in dry forests (Castro & Rapini, 2006). It grows on the rock surface and in the fissures of the rocks (Fig. S1). *Ficus calyptroceras* (Miq.) Miq is the most closely related species, but it is easily distinguished by the lack of glabrescence in the leaves; *F. bonijesulapensis* commonly has a dense indumentum (Castro & Rapini, 2006). Although *Ficus* L. is a keystone genus in the Tropics, as it provides resources to many frugivore vertebrates, its population genetic structure has rarely been investigated (Dev *et al.*, 2011; Yu & Nason, 2013; Honorio Coronado *et al.*, 2014). Although little is known about the mating system of *Ficus* spp., the trees have a vital symbiotic relationship with pollinating wasp species (Weiblen, 2002), and figs are classic examples of generalized seed-dispersal mutualisms (Shanahan, Compton & Corlett, 2001). Pollen and diaspores can be dispersed over long distances, between 5.8 and 40 km (Nason, Herre & Hamrick, 1996; Zavodna *et al.*, 2005).

Phylogeographical studies on SDTFs in Brazil are rare, especially those involving tree species. To our knowledge, the only two published studies to date are from Caetano *et al.* (2008) and Collevatti *et al.* (2012), whose results were consistent with the Pleistocene Arc hypothesis. This hypothesis states that, during the LGM in the Pleistocene, the range of SDTFs had reached their maximum expansion and formed a continuous and extensive vegetation (Prado & Gibbs, 1993; Pennington, Prado & Pendry, 2000). A recent study based on palaeodistribution modelling and palynological evidence has proposed that, during the LGM, the distribution of SDTFs was more fragmented than at the present day and that, during the Holocene, a southern expansion of this vegetation type occurred (Werneck *et al.*, 2011); this hypothesis is a clear contradiction of the Pleistocene Arc hypothesis noted above. Studies on tree species of the cerrado and Atlantic Forest of eastern Tropical South America (ETSA) are more common, and have revealed patterns of recent range expansion and high levels of genetic differentiation among populations. These patterns are frequently hypothesized to have been highly

influenced by Quaternary climatic oscillations (Collevatti, Grattapaglia & Hay, 2003; Ramos, Lemos-Filho & Lovato, 2009; Novaes *et al.*, 2010, 2013; Lima *et al.*, 2014).

In this context, we conducted a phylogeographical and niche modelling study of *F. bonijesulapensis*, endemic to Brazilian SDTFs. We compared the evolutionary history of the species with the two hypotheses about the historical biogeography of SDTFs in the region, i.e. retraction (Prado & Gibbs, 1993; Pennington *et al.*, 2000) or expansion (Werneck *et al.*, 2011) during the Holocene. In addition to the generated occurrence datasets and sequencing of a non-coding plastid DNA region, we used environmental niche predictive modelling to determine species occurrences (Pearson & Dawson, 2003), and we applied the maximum entropy machine-learning algorithm (Phillips & Dudík, 2008) to evaluate the effects of Quaternary climatic fluctuations on population dynamics.

## MATERIAL AND METHODS

### SAMPLED POPULATIONS AND AREAS

Fifteen populations of *F. bonijesulapensis* were sampled across most of the distribution range of the species (Table 1; Fig. 1). Geographical distances among sampled limestone karst outcrops ranged from 58 to 1178 km. The outcrops vary significantly in size, ranging from *c.* 0.5 to 2000 ha. Many outcrop habitats have disappeared as a result of exploitation for limestone; for example, in the vicinity of the MAT (19°34'14.52"S/44°01'00.58"W) and VIP (15°28'57.87"S/48°52'19.12"W) populations, as well as the SRN, BJL and LAS populations, the exploitation of limestone was recorded. The current landscape is dominated by planted pastures for livestock grazing, except in designated biological reserves at the Morro do Chapéu, Bahia State Park (MDC) and Vale do Peruaçu National Park (JAN). The height of the outcrops varies and can reach up to 100 m above ground level, as recorded in the JUV population (see Fig. S1). *Ficus bonijesulapensis* was selected as the most frequent tree species during previous analyses of limestone outcrops.

One hundred and twenty-six individuals were analysed, and sample size varied according to population size. In the field, trees of up to 98 cm in diameter at breast height (dbh; mean = 26.05 cm, SE = 2.99 cm,  $n = 67$ ) and 19 m in height (mean = 7.81 m, SE = 0.53 m,  $n = 67$ ) were observed. The absolute density of the species is *c.* 6.1 individuals ha<sup>-1</sup> (ind ha<sup>-1</sup>) (SE = 2.7 ind ha<sup>-1</sup>) in the nine measured outcrops. The phytosociological structure was not analysed for all sampled outcrops because of difficulties in access

or the presence of bees; to collect samples from these individuals, leaves were obtained with the use of a slingshot. Leaf samples were cut and immediately dried in silica gel. A specimen of the species was deposited at the Missouri Botanical Garden – MOBOT (collection number 1780). The floristic unit of the vicinity surrounding the outcrop in which *F. bonijesulapensis* occurred was classified into three types based on the definitions provided by Santos *et al.* (2012): rock outcrop caatinga, arboreal caatinga and cerrado.

### DNA EXTRACTION AND ANALYSIS

Total DNA was extracted from leaves based on the protocol described by Vieira *et al.* (2010). The quantity and quality of DNA were assessed by electrophoresis and visualization on 0.8% agarose gels. Initially, we searched for variability in plastid DNA markers by sequencing ten individuals from distant populations. From this, we selected the six regions with the most potentially informative characters, as described by Shaw *et al.* (2007): *trnQ-5'rps16*; *ndhF-rpl32*; *trnD-trnT*; *psbJ-petA*; *atpI-atpH*; and *trnS-trnG*. Amplifications were carried out following Vieira *et al.* (2010). Among them, we selected the region *trnQ-5'rps16*, which presented considerable length (960 bp) and the highest number of polymorphic sites. A detailed protocol for sequencing reactions is available on request. Sequencing products were run on a MegaBACE sequencer, according to the manufacturer's instructions. The sequences were checked and assembled using PHRED (Ewing & Green, 1998; Ewing *et al.*, 1998), PHRAP (<http://www.phrap.org/>) and CONSED (Gordon, Abajian & Green, 1998). Sequences were aligned using CLUSTAL-W (Thompson, Higgins & Gibson, 1994) implemented in the software MEGA 4.0.1 (Tamura *et al.*, 2007), with default gap penalties and manual verification. The GenBank accession numbers of the *trnQ-5'rps16* sequences are JQ436933–JQ436946.

### DIVERSITY INDICES AND PHYLOGEOGRAPHICAL STRUCTURE

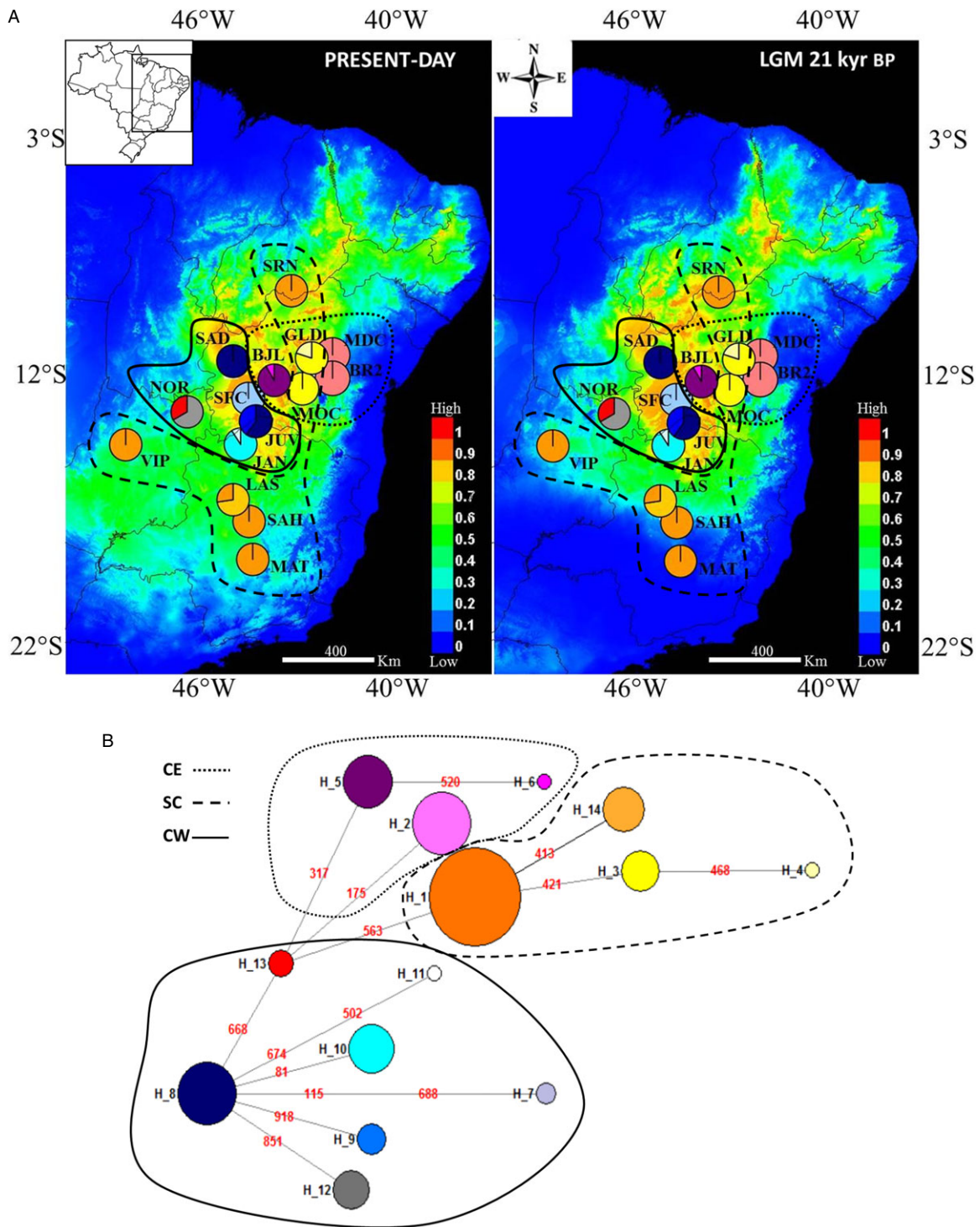
Molecular diversity indices ( $N_h$ , number of haplotypes;  $\pi$ , nucleotide diversity) were calculated using DNASP 4.10.8 (Rozas *et al.*, 2003). The allelic (haplotype) diversity ( $H_D$ ) was calculated using the rarefaction method (Petit, El Mousadik & Pons 1998). Average within-population haplotype diversity ( $H_s$ ), total diversity ( $H_T$ ) and genetic differentiation based on haplotype frequencies ( $G_{ST}$ ) were obtained with the program PERMUT (Pons & Petit, 1996), and  $N_{ST}$  was obtained by taking into account similarities between haplotypes (ordered alleles). When  $N_{ST} > G_{ST}$ , the

**Table 1.** Sampled *Ficus bonijesulapensis* populations with their identification code (ID), geographical location of limestone karst outcrops in eastern Tropical South America and genetic diversity indices

Populations, state	ID	Latitude/longitude	Altitude (m)	Floristic unit	N	Haplotype ( $N_a$ )	Group	$H_D$	$\pi \times 10^{-2}$
São Raimundo Nonato, Piauí	SRN	08°55'25"S/42°36'10"W	354	Rock outcrops caatinga	11	H1 (11)	SC	0.000	0.000
Morro do Chapéu, Bahia	MDC	11°33'06"S/41°14'14"W	1083	Rock outcrops caatinga	11	H2 (11)	CE	0.000	0.000
Lapa Doce, Bahia	GLD	12°19'53"S/41°36'23"W	703	Rock outcrops caatinga	5	H3 (4) H4 (1)	SC	0.400	0.042
BR-242, Bahia	BR2	12°27'38"S/40°59'52"W	597	Rock outcrops caatinga	5	H2 (5)	CE	0.000	0.000
Mocambo, Bahia	MOC	12°47'22"S/42°29'37"W	499	Rock outcrops caatinga	3	H3 (3)	SC	0.000	0.000
Bom Jesus da Lapa, Bahia	BjL	13°02'35"S/43°16'35"W	445	Rock outcrops caatinga	12	H5 (11) H6 (1)	CE	0.167	0.017
São Félix do Coribe, Bahia	SFC	13°26'48"S/44°09'41"W	623	Arboreal caatinga	2	H7 (2)	CW	0.000	0.000
São Desidério, Bahia	SAD	12°22'52"S/44°56'58"W	584	Arboreal caatinga	10	H8 (10)	CW	0.000	0.000
Juvenília, Minas Gerais	JUV	14°22'49"S/44°16'51"W	575	Arboreal caatinga	10	H8 (6) H9 (4)	CW	0.533	0.056
Januária, Minas Gerais	JAN	15°10'24"S/44°13'30"W	519	Arboreal caatinga	11	H10 (10) H11 (1)	CW	0.182	0.057
Nova Roma, Goiás	NOR	13°42'24"S/46°51'08"W	822	Cerrado	9	H12 (6) H13 (3)	CW	0.500	0.105
Vila Propício, Goiás	VIP	15°27'59"S/48°53'42"W	727	Cerrado	7	H1 (7)	SC	0.000	0.000
Lassance, Minas Gerais	LAS	17°54'00"S/44°34'51"W	550	Cerrado	11	H1 (3) H14 (8)	SC	0.446	0.046
Santo Hipólito, Minas Gerais	SAH	18°17'22"S/44°11'16"W	561	Cerrado	8	H1 (8)	SC	0.000	0.000
Matosinhos, Minas Gerais	MAT	19°33'09"S/44°04'14"W	776	Cerrado	11	H1 (11)	SC	0.000	0.000

CE, central-east group; CW, central-west group; SC, scattered group;  $H_D$ , haplotype diversity;  $N$ , sampled individuals;  $N_a$ , number of distinct haplotypes found in individuals sampled ( $N$ );  $\pi$ , nucleotide diversity.





**Figure 1.** A, Geographical distribution of haplotypes sampled and phylogeographical groups of *Ficus bonijesulapensis* in south-eastern to north-eastern Brazil. B, Median-joining (MJ) network-estimated relationships among the haplotypes. Circles are proportional to haplotype frequency in the MJ network. Lines drawn between haplotypes in (B) represent mutation events and red numbers represent the position of the mutations in the sequenced region. The haplotypes and group distribution in (A) are shown in comparison with ecological niche modelling (ENM) for the species at the present day and at the predicted climatic conditions of the Last Glacial Maximum (LGM), 26 kyr BP. In the maps, red corresponds to regions with the highest probability of *F. bonijesulapensis* occurrence and blue corresponds to the least probable regions. Larger polygons correspond to the phylogeographical groups: CE, central-east group; CW, central-west group; SC scattered group.

existence of a phylogeographical structure is indicated (Pons & Petit, 1996). Neutrality tests (Fu, 1997) were also conducted. The expected and observed mismatch distribution analysis (MDA) was estimated using DNASP 4.10.8 (Rozas *et al.*, 2003) to test for population expansion. The MDA of the sequence data was constructed to compare it with the Poisson expectation for constant and varying population sizes (Slatkin & Hudson, 1991).

The spatial analysis of molecular variance (SAMOVA) was assessed using SAMOVA version 1.0 (Dupanloup, Schneider & Excoffier, 2002). We ran a series of independent analyses with a range of  $k$  values (2–7). The phylogenetic relationships among the haplotypes were estimated using the median-joining algorithm implemented in NETWORK 4.5 (Bandelt, Forster & Rohl, 1999). This method allows for the simple reconstruction of phylogenetic trees based on the minimum spanning networks generated from the plastid DNA sequence; it has been shown to be a powerful tool to define groups (Ramos *et al.*, 2009; Ribeiro *et al.*, 2011). Molecular diversity indices were also calculated for separate groups identified by SAMOVA.

#### DISTRIBUTION MODELLING

We modelled the range of the ecological niche of *F. bonijesulapensis* in SDTF vegetation, using environmental variables from BIOCLIM (<http://www.worldclim.org/bioclim>; Hijmans *et al.*, 2005) and the machine-learning maximum entropy model MAXENT version 3.3.3e (Phillips & Dudík, 2008). The distribution model predicts the availability of suitable environments for the species. To date, MAXENT has been successfully used to generate distribution map predictions for several species (e.g. Elith *et al.*, 2006; Hans, Niels & Michael, 2011; Lozier & Mills, 2011; Thode *et al.*, 2014). Current climatic distribution was projected at 30 arc s (1 km<sup>2</sup>) spatial resolution (Hijmans *et al.*, 2005). The area under the receiver operating characteristic curve (AUC) is often used to measure consistency in model predictions of species distributions (Pearson *et al.*, 2006). Usually, AUC values > 0.9 are suggestive of high-accuracy models (Swets, 1988) and an efficient indicator of model performance (Manel, Williams & Ormerod, 2001). We modelled the ecological niche from the present day to the LGM (21 kyr BP). We used multiple runs of cross-validation (20 replicates) in which the occurrence data are randomly split into a number of equal-sized groups (Phillips & Dudík, 2008). The model uses all the data for validation, resetting random testing percentage to zero, thus making better use of small datasets. In the optimization for the species, we used

2000 maximum iterations and a convergence threshold equal to 0.001.

## RESULTS

### GENETIC DIVERSITY

Fourteen haplotypes were detected in the analysis of the *trnQ–5' rps16* non-coding region for *F. bonijesulapensis* (Supporting Information Table S1). The 960 base pairs of the consensus sequence had 945 conserved positions and 15 variable sites, five of which were transitions, four were transversions and six were insertion–deletions. The sequence exhibited a high AT content (76.5%), with several mononucleotide repeats [up to ten T<sub>(n10)</sub> simple-sequence repeats, plastid SSRs]. The phylogenetic relationships among the 14 haplotypes revealed three major groups of haplotypes, with haplotype H13 being intermediate (Fig. 1). The haplotype network had a star-shaped topology with the majority of haplotypes spreading out from H8 or H13.

The haplotype diversity ( $H_D$ ) for each population was in the range 0.000–0.533 and the nucleotide diversity ( $\pi$ ) was in the range (0.000–0.105)  $\times 10^{-2}$  (Table 1). No population had more than two haplotypes and 60% of the haplotypes were unique (Table 1). The populations JUV, NOR, LAS and GLD showed the highest levels of genetic diversity among all populations, presenting high levels of both haplotype diversity and nucleotide diversity (Table 1).

### PHYLOGEOGRAPHICAL STRUCTURE

A clear phylogeographical structure was observed for *F. bonijesulapensis*, with most of the phylogenetically related haplotypes occurring in populations that were geographically close to each other. The level of differentiation among the analysed populations was high ( $G_{ST} = 0.837$ , SE = 0.056;  $N_{ST} = 0.911$ , SE = 0.033) and the significant difference among these two indices supports the observed structure (Table 2). The SAMOVA result for two groups ( $k = 2$ ,  $F_{CT} = 0.471$ ) was as follows: one group contained almost all central populations and another contained the populations from the south plus a few populations scattered throughout the north and central region of the species distribution. However, the most appropriate grouping of populations was three ( $k = 3$ ,  $F_{CT} = 0.591$ ). In the scenario of three groups,  $F_{CT}$  was higher than  $k = 2$ , whereas, for  $k \geq 4$ , the group structure was no longer apparent, i.e. at least one group was represented by a single population (Supporting Information, Table S2). The three-group configuration divides the central group identified in the two-group scenario into two. The three groups identified using SAMOVA are consistent with the three groupings in the haplotype

**Table 2.** Diversity indices for the three phylogeographical groups of *Ficus bonijesulapensis* in eastern Tropical South America

Parameters	Total	Scattered group	Central-west group	Central-east group
$N_h$	14	4	7	3
$H_D$	0.848	0.461	0.779	0.537
$\pi \times 10^2$	0.148	0.026	0.052	0.061
$H_S$ (SE)	0.148 (0.054)	0.119 (0.077)	0.243 (0.116)	0.056 (0.055)
$H_T$ (SE)	0.910 (0.048)	0.624 (0.151)	0.940 (0.069)	0.667 (0.256)
$G_{ST}$ (SE)	0.837 (0.056)	0.809 (0.085)	0.741 (0.127)	0.917 (NC)
$N_{ST}$ (SE)	0.911 (0.033)	0.870 (0.106)	0.769 (0.121)	0.960 (NC)
$N_{ST}-G_{ST}$	0.074	0.061	0.028	0.043

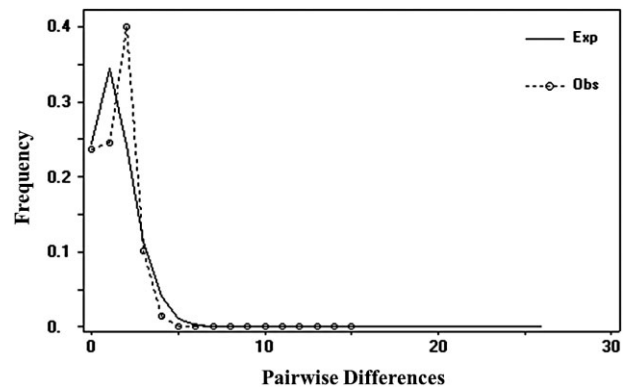
$G_{ST}$ , genetic differentiation among all populations;  $H_D$ , haplotype diversity;  $H_S$ , within-population gene diversity;  $H_T$ , total haplotype diversity;  $N_h$ , number of haplotypes;  $N_{ST}$ , differentiation for ordered alleles; SE, standard error;  $\pi$ , nucleotide diversity.

network (Fig. 1). Each group included exclusive haplotypes, most of which were phylogenetically related, and consisted of geographically proximal populations. We named the groups according to their geographical distribution: central-west group (SFC, SAD, JUV, JAN and NOR populations), central-east group (MDC, BR2 and BJJL populations) and scattered group, which included the northernmost population (SRN), two central populations (GLD and MOC) and all of the southernmost populations (VIP, LAS, SAH and MAT) (Fig. 1; Table 1). The central-west group occurs in arboreal regions of the caatinga biome, whereas the central-east group occurs in the rock outcrops caatinga physiognomy. The scattered group includes populations of cerrado dry forest enclaves in the southern region and of populations occurring in the rock outcrops caatinga biome in the north and central regions (Table 1).

The southern populations of the scattered group showed much lower levels of genetic diversity than the central-west and central-east groups (Fig. 1; Table 1). The four southern populations in the scattered group had only two haplotypes, one of which was also found in the northernmost population. The central-west and central-east groups had high levels of genetic diversity with many endemic and derived haplotypes, with higher haplotype diversity ( $H_D$ ), total haplotype diversity ( $H_T$ ) and nucleotide diversity ( $\pi$ ) (Table 2). Thus, the central-west and central-east groups could be considered sources of the genetic diversity in *F. bonijesulapensis*. Altogether, these features are consistent with a scenario of increased genetic stability in the central groups and relatively recent occupation of the southern region by northern propagules.

POPULATION DYNAMICS

The neutrality tests showed no significant values for any of the three groups (central-west, central-east



**Figure 2.** Mismatch distribution histogram for plastid DNA haplotypes, indicating expected numbers of pairwise differences under a population expansion scenario (Exp) and the observed values in *Ficus bonijesulapensis* samples (Obs) in south-eastern to north-eastern Brazil.

and scattered) or for the entire population. However, the mismatch distributions of the variable sites were unimodal for all populations together (Fig. 2) and for the three groups, i.e. left skewed, single-peak histograms (available on request). Unimodal mismatch distributions are expected for populations that have undergone past expansion, whereas, for populations that have maintained a relative stability over time, mismatch distributions are expected to be multimodal (Rogers & Harpending, 1992).

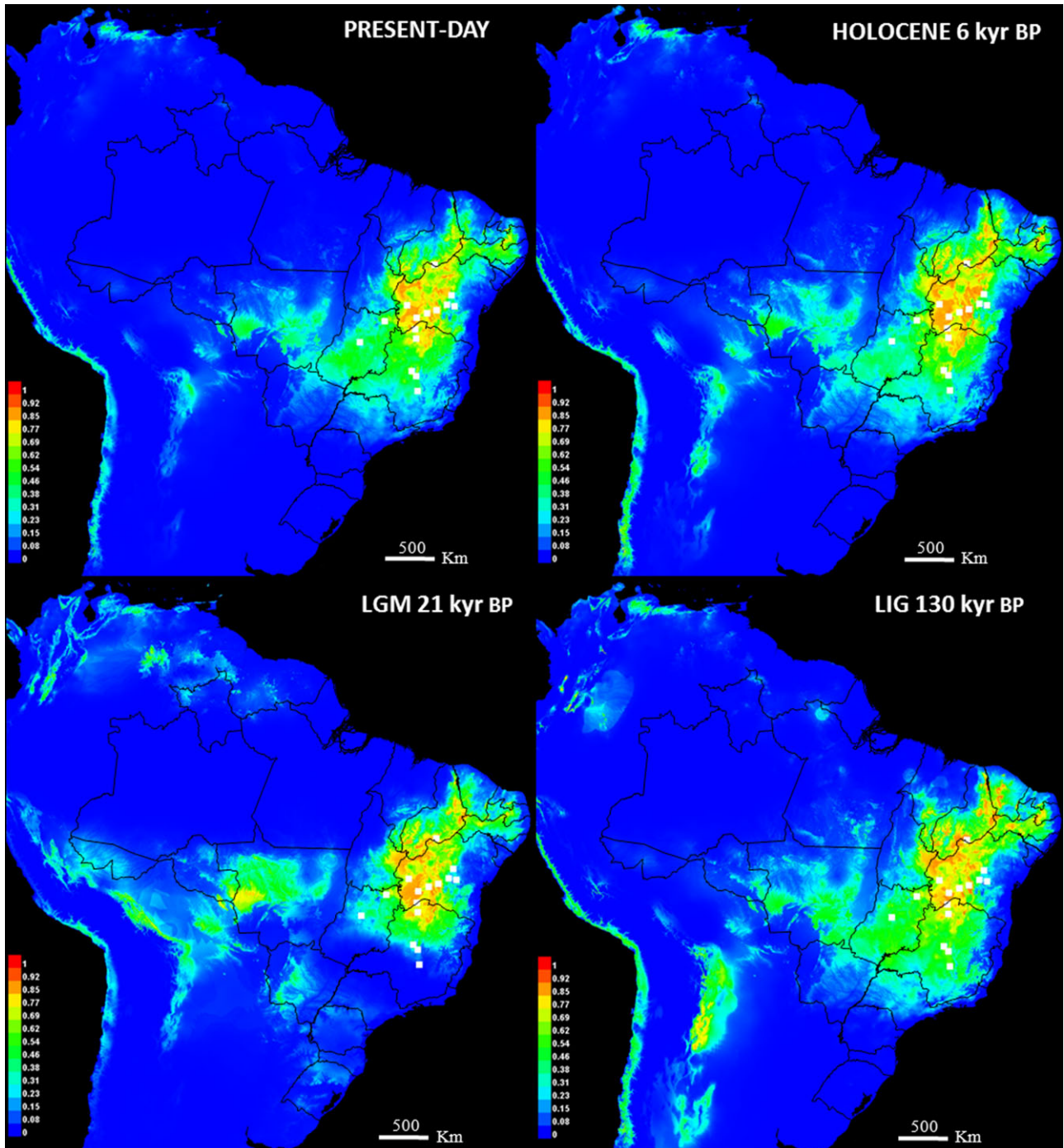
As noted above, the northernmost population, SRN, shares a haplotype with the southernmost populations SAH and MAT, all within the scattered group. This suggests a phylogeographical link and some gene flow between populations in the north and south, although the hypothesis of an ancestral polymorphism shared among populations cannot be ruled out.



## ECOLOGICAL NICHE MODELLING (ENM)

The ENM performed satisfactorily, as shown by the high value of the AUC of 0.991. The average AUC test for replicate runs was 0.930, and the standard deviation

was 0.046. The predicted distribution of *F. bonijesulapensis* was successfully estimated for four periods of time: present day, Holocene (6 kyr BP), LGM (21 kyr BP) and Last Interglacial (LIG) (130 kyr BP) (Fig. 3). The distribution was similar among present



**Figure 3.** Predictive ecological niche modelling for *Ficus bonijesulapensis* under four climatic scenarios. Red indicates regions with a higher probability of occurrence and blue indicates lower probability regions. White dots show the species occurrence locations used for training. LGM, Last Glacial Maximum; LIG, Last Interglacial.



**Table 3.** Contribution of environmental variables used in ecological niche modelling of *Ficus bonijesulapensis* in eastern Tropical South America

Variable	Description	Environmental contribution (%)	Permutation importance
Bio14	Precipitation of driest month	41.5	55.7
Alt	Altitude	40.7	33.8
Bio4	Temperature seasonality	8.1	8.1
Bio16	Precipitation of wettest quarter	8.1	2.3
Bio3	Isothermality	1.5	0.0
Bio17	Precipitation of driest quarter	0.1	0.1

Except for altitude, all variables were taken from WorldClim and ordered according to the heuristic estimates of their relative contributions to the MAXENT model (Phillips & Dudík, 2008).

day, Holocene and LIG conditions, i.e. similar range expansion, which were considerably different from LGM conditions. The main difference was that, in the LGM, the species distribution was significantly reduced in comparison with the present day and the southern region of its current range was unfavourable for the species. During all periods, the most favourable region for the occurrence of *F. bonijesulapensis* remained the same, in north-eastern Brazil, particularly in the western part of Bahia State. Populations in the southern region of the current species distribution were characterized by sharing exclusive haplotypes, whereas the central and north-eastern part of the current distribution (potential refugium) in general has unique and possibly higher within-population gene diversity and total haplotype diversity (Table 2). The environmental variables that most influenced the MAXENT model were the levels of precipitation during the driest month and the altitude (Table 3).

## DISCUSSION

### PHYLOGEOGRAPHICAL STRUCTURE AND BIOGEOGRAPHICAL CONCORDANCE

To our knowledge, this study represents the first phylogeographical analysis of a plant species endemic to the limestone outcrops of north-eastern Brazil and is only the second to focus on a typical SDTF tree species. *Ficus bonijesulapensis* populations are phylogeographically structured and this structure is congruent with the biogeographical subdivisions of SDTFs in north-eastern Brazil (Santos *et al.*, 2012). The patterns of genetic diversity distribution of the species allowed us to define three different phylogeographical groups: central-west, central-east and a scattered group (Fig. 1; Table 1). The first two groups correspond to two different SDTF floristic units in which *F. bonijesulapensis* occurs, namely arboreal caatinga and rock outcrops caatinga, respectively. The southern part of the scattered group corresponds to

enclaves within the cerrado SDTF and remaining populations of rock outcrops caatinga. Recently, Santos *et al.* (2012) have shown that these three floristic units (arboreal caatinga, rock outcrops caatinga and cerrado) are distinct as a result of different species composition and environmental variables. This agreement between phylogeographical and biogeographical patterns has been demonstrated recently for a cerrado tree species (Novaes *et al.*, 2013), and our study may represent the first instance for a tree endemic to the SDTF of ETSA. Persistent divergent ecological pressures, ecological barriers to gene flow or historical factors could have played a role in creating these consistencies (Avice, 2000; Weiss & Ferrand, 2007; Médail & Diadema, 2009); however, further research is necessary to better understand the convergence of these processes.

The endemic haplotypes (e.g. H5 and H6 from the B JL population, and H2 from the MDC and BR2 populations) are separated from the H1 haplotype by two or three mutational steps. Despite this genetic distance, these four haplotypes occur in populations located in close proximity to one another, in the Chapada Diamantina mountain range. It is likely that the geography of the region presents a barrier to gene flow and that the haplotypes are endemic to the area (Werneck, 2011).

### POPULATION DYNAMICS DURING THE QUATERNARY CLIMATIC FLUCTUATIONS

The two different sources of data used in this study suggest that *F. bonijesulapensis* underwent a recent expansion into the southernmost extent of its range; this corresponds to enclaves of SDTFs in the cerrado. According to the genetic data assessed here, the southern part of the scattered group is significantly less diverse than the central-west and central-east groups. Moreover, the mismatch distribution of sequence variation analysis was typically unimodal for all groups (Fig. 2), indicating that its populations

probably experienced a recent demographic or spatial expansion (Rogers & Harpending, 1992). Overall, the phylogeographical analysis confirmed the predictions of higher genetic diversity in the stable central region than in adjacent unstable regions (southern region) and genetic signatures of recent range expansion (Werneck *et al.*, 2011). The ENM data showed that, in the southern region, the environmental conditions during the LGM were clearly unfavourable for the species and differed from the present day conditions. However, conditions in central and northern regions were favourable for the species during all four periods studied: LIG, LGM, Holocene and present day. These results indicate that the southern populations are probably younger than the central groups, as SDTFs are more likely to have occurred in the caatinga in north-eastern Brazil during the LGM climate scenario (based on our results and those of Werneck *et al.*, 2011). Our evidence of a progressive southward expansion for *F. bonijesulapensis* since the LGM is consistent with the hypothesized dispersal scenarios for SDTFs (Mayle, 2004; Werneck *et al.*, 2011). Thus, the recent southward expansion of *F. bonijesulapensis* probably occurred in the Holocene, supporting the hypothesis of SDTF expansion during the Holocene (Werneck *et al.*, 2011), not retraction (Prado & Gibbs, 1993; Pennington *et al.*, 2000).

Evidence of recent southward range expansion has been repeatedly observed in phylogeographical studies conducted in ETSA on tree species (Collevatti *et al.*, 2003; Ramos *et al.*, 2009; Novaes *et al.*, 2010, 2013; Ribeiro *et al.*, 2011) and some animal species (Carnaval *et al.*, 2009). This pattern observed for distinct species with widely varying characteristics and habitats suggests the influence of large-scale environmental variables on the dynamics of the ETSA biota. Currently, the variable most often assumed to explain these patterns in ETSA is the climatic fluctuations of the Quaternary, which have been shown to be a significant factor in shaping the genetic structure of species in other parts of the globe (e.g. Weiss & Ferrand, 2007). It is hypothesized that, during the LGM period, drier and cooler conditions brought about species extinction in some regions, as, for example, supposedly in the southern part of the cerrado and Atlantic Forest in ETSA, causing a retraction of the range of the species northwards (Novaes *et al.*, 2010). Subsequently, with the end of the LGM, more favourable climatic conditions allowed the species to expand its range again, recolonizing the regions at its southern limits (Collevatti *et al.*, 2003; Ramos *et al.*, 2009; Novaes *et al.*, 2010, 2013; Ribeiro *et al.*, 2011).

A currently debated controversy for this scenario is when these events occurred. Traditionally, because of drier and cooler conditions throughout most of South

America (Werneck *et al.*, 2011), the LGM period is believed to be the most probable period of SDTF species expansion in southern ETSA, whereas cerrado and Atlantic Forest species would have had their ranges reduced during this period (Prado, 2000; Pennington *et al.*, 2004). Recently, in their analysis of palaeodistribution modelling and palaeopalynological data, Werneck *et al.* (2011) argued that, during the LGM, SDTFs in ETSA may have had their ranges reduced, together with cerrado and Atlantic Forest biotas, as SDTF species may have been unable to survive in drier and cooler conditions. Our ENM simulations undoubtedly corroborate this hypothesis, as the occurrence of *F. bonijesulapensis* in the southern limits of the species range during the LGM is highly unlikely (Fig. 1). During the Holocene, the climatic variables returned to conditions similar to the present day which were favourable for *F. bonijesulapensis* re-expansion to the south. According to our ENM results, the unfavourable conditions for *F. bonijesulapensis* at its southern limits during the LGM are mainly a result of the levels of precipitation during the driest month and altitude.

The source of the populations that promoted the recent range expansion could be northern. Northern and southern populations (scattered group) have haplotypes from the same phylogenetic group (Fig. 1) and share a common haplotype, H1. This unexpected link between the northern and southern populations has also been shown for *Astronium urundeuva* Engl., an insect-pollinated, wind-dispersed tree that occurs in dry tropical climates (Caetano *et al.*, 2008). In their study, Caetano *et al.* (2008) demonstrated that *A. urundeuva* populations in the northern (e.g. Piauí State) and southern (e.g. Minas Gerais State) regions share haplotypes which are different from three central-western Brazilian populations. This link suggests more biogeographical affinity among northern and southern populations than with central populations, whereas ecological and historical factors are probably responsible for the links between populations (Pennington *et al.*, 2004).

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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:

**Figure S1.** Limestone karst outcrops in the sampled populations.

**Table S1.** Distribution and frequency of plastid DNA haplotypes in each population of *Ficus bonijesulapensis*.

**Table S2.** Spatial analysis of molecular variance of *Ficus bonijesulapensis* in eastern Tropical South America.