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Large-scale phylogeography of the disjunct Neotropical tree species *Schizolobium parahyba* (Fabaceae-Caesalpinioideae)

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ABSTRACT

Neotropical rainforests exhibit high levels of endemism and diversity. Although the evolutionary genetics of plant diversification has garnered increased interest, phylogeographic studies of widely distributed species remain scarce. Here we describe chloroplast and nuclear variation patterns in *Schizolobium parahyba* (Fabaceae), a widespread tree in Neotropical rainforests that harbor two varieties with a disjunct distribution. Chloroplast and nuclear sequence analyses yielded 21 and 4 haplotypes, respectively. Two genetic diversity centers that correlate with the two known varieties were identified: the Southeastern Atlantic forest and the Amazonian basin. In contrast, the populations from southern and northeastern Atlantic forests and Andean-Central American forests exhibited low levels of genetic diversity and divergent haplotypes, likely related to historical processes that impact the flora and fauna in these regions, such as a founder's effect after dispersion and demographic expansion. Phylogeographic and demographic patterns suggest that episodes of genetic isolation and dispersal events have shaped the evolutionary history for this species, and different patterns have guided the evolution of *S. parahyba*. Moreover, the results of this study suggest that the dry corridor formed by Cerrado and Caatinga ecoregions and the Andean uplift acted as barriers to this species' gene flow, a picture that may be generalized to most of the plant biodiversity tropical woodlands and forests. These results also reinforce the importance of evaluating multiple genetic markers for a more comprehensive understanding of population structure and history. Our results provide insight into the conservation efforts and ongoing work on the genetics of population divergence and speciation in these Neotropical rainforests.

1. Introduction

Neotropical rainforests, such as Amazonian, Atlantic, Andean and the Central American forests, exhibit high levels of plant diversity (Burnham and Graham, 1999; Fiaschi and Pirani, 2009), where some species are present in more than one rainforest area. These rainforest areas are currently not continuous. The Atlantic forest is isolated from the other two largest South American forest blocks, the Amazonian and the Andean forests, by a drier climate zone that is comprised of predominantly dry forests and savannas. The Caatinga and Cerrado regions, which are dominated by open vegetation, separate the Atlantic Forest from Amazonia, whereas the Chaco, an area of savanna-like vegetation in the southern depressions of South America, divides the Atlantic Forest from the Andean Forest (Rizzini, 1997; Silva and Cateletti, 2003). The Cerrado, a fire-prone savanna in central Brazil, and the Caatinga, a thorn scrub vegetation area in the semi-arid Northeast of Brazil, comprise 34% of the country area. These seasonally dry ecoregions currently present a major barrier to gene flow between the Amazonian and Atlantic rainforest taxa (Prado and Gibbs, 1993). However, palynological studies have indicated previous floristic connections between the Amazon and Atlantic forest in northeast Brazil (Behling et al., 2000). In addition, the gallery forest network along the streams crossing the savannas in central Brazil have been suggested as another potential link between these areas (Oliveira and Fontes, 2000). In northern South America, the Amazonian basin is separated from the rain forests of the Chocó biogeographic province by the Andean uplift that occurred in the late Tertiary (Gregory-Wodzicki, 2000), which created a barrier between the

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Pacific and Amazonian lowlands of South America. More recently, the closing of the Panama Isthmus in the Late Pliocene (~3.5 Ma) has enabled migration between Central and South America (Coates and Obando, 1996).

Some plant species are not evenly distributed within a neotropical region, but are disjunctly scattered among different areas. Therefore, studies involving widespread species with a disjunct distribution will contribute to an understanding of past and present species distributions and biodiversity and may provide key insights into the evolutionary history of the neotropics. Moreover, phylogeographic studies of disjunctly distributed species garner interest because they provide information concerning the past and present relationships between different geographical regions and the genetic structure within species, helping to identify barriers to gene flow.

Schizolobium parahyba (Vell.) Blake (Fabaceae) is a widespread species in four of the five types of Neotropical rainforest environments (Atlantic forest, Amazonian, Andean and Central America forests). *Schizolobium parahyba* includes two varieties: *Schizolobium parahyba* var. *parahyba* (pedicels not jointed; anthers 2.3–3.2 mm) and *S. parahyba* var. *amazonicum* (Ducke) Barneby (pedicels jointed 2–6.5 mm above base; anthers (1.2) 1.3–2.3 mm). Both varieties are disjunctly distributed: *S. parahyba* var. *parahyba* thrives in the Brazilian Atlantic forest (from Bahia 13°S to Santa Catarina 25°S) and in Paraguay and Argentina, whereas *S. parahyba* var. *amazonicum* is distributed from the Amazon basin in Brazil and Bolivia to southeastern Mexico (Fig. 1a) (Barneby, 1996). *S. parahyba* does not grow in the savanna and in the typical xeromorphic Caatinga or in elevations higher than 700 m above sea level. It is hermaphroditic but self-incompatible, pollinated by bees and harbors wind-dispersed seeds (Pietrobom and Oliveira, 2004). This species is recognized as an ecologically and economically important forest tree. Because it is one of the fastest growing tree species, it is extensively used in reforestation projects and has been widely introduced in tropical regions as an ornamental and shade tree.

This study is one of the first for comprehensive population genetic analysis of a widespread Neotropical tree species that is distributed among four different wet forest areas. We analyzed sequence variation of both chloroplast (cpDNA) and nuclear (nrDNA) markers, which exhibit different modes of inheritance, to elucidate the evolutionary history of this species. The purpose of this work included (i) the analysis of the genetic diversity and its distribution among populations and the identification of hotspots of diversity to define more accurate conservation criteria for this taxon; (ii) the assessment of whether the *S. parahyba* genetic structure suggests the existence of recently colonized areas throughout its distribution in Neotropical rainforests and (iii) the evaluation of the possible role of the dry corridor formed by Cerrado–Caatinga–Chaco and the Andean uplift as a barrier to this species' gene flow.

2. Materials and methods

2.1. Sampling strategy

Twenty populations and 198 individuals spanning most of the range of *S. parahyba* were sampled. The distance between populations ranged 100–6000 km. The 13 populations and 118 individuals sampled from the Atlantic Forest were classified as *S. parahyba* var. *parahyba*. The 80 individuals sampled from Amazonian (three population samples), Andean (two populations), and Central American (two populations) forests were classified as *S. parahyba* var. *amazonicum* (Table 1 and Fig. 1b; Barneby, 1996). Fertile individuals, or those thicker than 50 cm in diameter at breast height, were sampled at random. Three to 26 individuals located from 50 m to 20 km apart were sampled in each population as cambium and leaf materials (silica gel dried). The cambium was collected according to the protocols described for tropical tree species by Colpaert et al. (2005).

2.2. DNA isolation, amplification and sequencing

Total genomic DNA was isolated using the CTAB method (Doyle and Doyle, 1987). Ten chloroplast genome regions were analyzed by amplification and sequencing: *psbA–trnH*, *rpoC1*, *rpoB*, *accD*, *rbcLa*, *ndhJ*, *ycf5* (Kress and Erickson, 2007), *matK* (Wojciechowski et al., 2004), *trnL–trnF* (Kress et al., 2005) and *trnL* intron (Taberlet et al., 2007). Three regions were chosen for analysis based on sequence quality and polymorphism extent: *psbA–trnH*, *trnL–trnF* and *matK*. In addition, the 5.8S nrDNA and flanking ITS1 and ITS2 regions were PCR-amplified using forward ITS1 and reverse ITS2 primers (White et al., 1990). Our PCR protocol involved initial

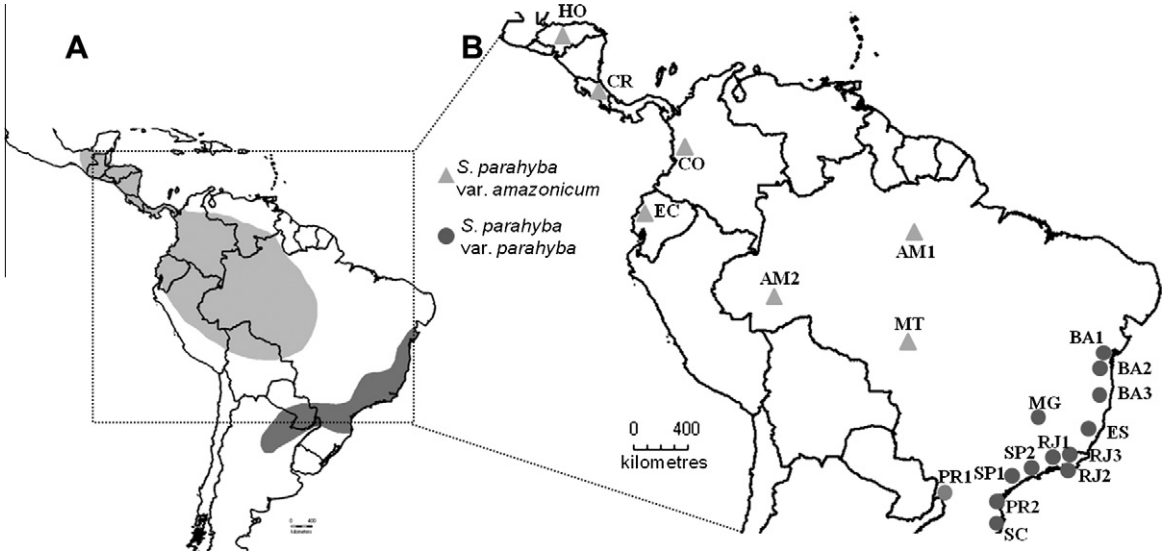

Fig. 1. Natural distribution range (A) and sampled populations of *S. parahyba* (B). *S. parahyba* var. *parahyba* and *S. parahyba* var. *amazonicum* are represented by circles and triangles, respectively. The sampled populations are: Santa Catarina (SC), Paraná (PR), São Paulo 1 (SP1) São Paulo 2 (SP2), Rio de Janeiro 1 (RJ1), Rio de Janeiro 2 (RJ2), Bahia 1 (BA1), Bahia 2 (BA2), Mato Grosso (MT), Amazonas (AM), Ecuador (EC), Colombia (CO), Costa Rica (CR) and Honduras (HO).

Table 1
Location of the sampled *S. parahyba* (var. *parahyba* and var. *amazonicum*) populations and the estimated diversity indexes.

Geographic origin (label)	Collected variety	Rain forest area	Sample size	Latitude	Longitude	Number haplotypes cpDNA (ITS)	Haplotype diversity cpDNA (ITS)	Nucleotide diversity cpDNA (ITS)
Santa Catarina State, Brazil (SC)	*parahyba*	Atlantic forest	13	27°2′N	48°35′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Paraná State, Brazil (PR1)	*parahyba*	Atlantic forest	3	25°34′N	54°29′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Paraná State, Brazil (PR2)	*parahyba*	Atlantic forest	4	25°22′N	48°51′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
São Paulo State, Brazil (SP1)	*parahyba*	Atlantic forest	7	24°44′N	48°9′W	2 (1)	0.571 (0.000)	0.00025 (0.00000)
São Paulo State, Brazil (SP2)	*parahyba*	Atlantic forest	20	23°29′N	45°4′W	4 (2)	0.542 (0.327)	0.00047 (0.00052)
Minas Gerais State, Brazil (Mglinets)	*parahyba*	Atlantic forest	12	20°8′N	44°6′W	2 (*)	0.167 (*)	0.00007 (*)
Rio de Janeiro State, Brazil (RJ1)	*parahyba*	Atlantic forest	10	23°4′N	44°42′W	2 (2)	0.182 (0.600)	0.00039 (0.00096)
Rio de Janeiro State, Brazil (RJ2)	*parahyba*	Atlantic forest	18	22°29′N	43°27′W	2 (2)	0.366 (0.538)	0.00016 (0.00086)
Rio de Janeiro State, Brazil (RJ3)	*parahyba*	Atlantic forest	5	22°52′N	42°29′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Espírito Santo State, Brazil (ES)	*parahyba*	Atlantic forest	11	19°53′N	40°5′W	4 (1)	0.378 (0.000)	0.00057 (0.00000)
Bahia State, Brazil (BA1)	*parahyba*	Atlantic forest	3	14°45′N	39°14′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Bahia State, Brazil (BA2)	*parahyba*	Atlantic forest	9	15°5′N	39°17′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Bahia State, Brazil (BA3)	*parahyba*	Atlantic forest	3	18°4′N	39°32′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Mato grosso State, Brazil (MT)	*amazonicum*	Amazonian forest	26	10°41′N	55°29′W	3 (1)	0.369 (0.000)	0.00016 (0.00000)
Amazonas State, Brazil (AM1)	*amazonicum*	Amazonian forest	4	3°8′N	59°57′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Amazonas State, Brazil (AM2)	*amazonicum*	Amazonian forest	26	8°43′N	69°0′W	3 (1)	0.483 (0.000)	0.00088 (0.00000)
Ecuador (EC)	*amazonicum*	Andean forest	5	1°2′N	44°6′W	1 (2)	0.000 (0.333)	0.00000 (0.00053)
Colombia (CO)	*amazonicum*	Andean forest	5	3°20′N	72°42′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Costa Rica (CR)	*amazonicum*	Central American forests	9	9°51′N	83°33′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
Honduras (HO)	*amazonicum*	Central American forests	5	14°36′N	86°16′W	1 (1)	0.000 (0.000)	0.00000 (0.00000)
POPs var. *parahyba*	–	–	118	–	–	13 (2)	0.851 (0.416)	0.00168 (0.00067)
POPs var. *amazonicum*	–	–	80	–	–	8 (2)	0.752 (0.476)	0.00215 (0.00076)
All populations	–	–	198	–	–	21 (4)	0.907 (0.694)	0.00239 (0.00826)

denaturation at 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 . Each of our 20 μL PCR reactions included 10 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.25 mM dNTP mix, 1× PCR buffer, 0.05 U of Platinum Taq DNA polymerase (Invitrogen) and 0.25 μM of each primer. Nuclear and plastid PCR products were sequenced from both ends by dideoxy chain-termination using a BigDye Kit (Applied Biosystems), according to the manufacturer's instructions, and run on an ABI-3100 automatic sequencer (Applied Biosystems). Approximately 1500 bp of the *matK* gene was sequenced with four additional internal primers (Wojciechowski et al., 2004). All the detected polymorphisms were validated by visually checking the original electropherograms. Low quality sequences were repeated by performing new sequencing reactions. Sequence identities were certified using the BLASTn algorithm against plant DNA sequences deposited at NCBI (http://www.ncbi.nlm.nih.gov). *Schizolobium parahyba* sequences generated in this study were deposited in GenBank under the accession numbers FJ668603–FJ668631 and GQ167768–GQ167774.

2.3. Data analysis

Individual consensus sequences were aligned using CLUSTALW (Thompson et al., 1994) implemented in MEGA4 (Molecular Evolutionary Genetics Analysis) version 4.0 (Tamura et al., 2007). Equal weights were assigned to all variations, and each indel was considered a single mutational event and codified as a 5th state character. All the analyses were performed with two datasets, i.e. with three concatenated cpDNA regions (*matK*, *psbA–trnH* and *trnL–trnF*) and with nrDNA (ITS) sequences.

Genealogical relationships among haplotypes were estimated with two network approaches, the median-joining method (Bandelt et al., 1999), implemented in Network 4.2.0.1 (Fluxus Technology Ltd. at www.Fluxus-engineering.com), and the statistical parsimony (Templeton, 1992), implemented in TCS (Clement et al., 2000). Molecular diversity estimates were calculated using Arlequin version 3.1 (Excoffier et al., 2005) and DNAsp 5.0 (Librado and Rozas, 2009). Population pairwise F_{ST} comparisons were calculated using Arlequin software ($P < 0.001$), and the occurrence of phylogeographic structures was inferred by testing for significant differences between G_{ST} and N_{ST} using PERMUT 2.0 (Pons and Petit, 1996) with 1000 permutations. N_{ST} considers sequence differences between the haplotypes. Thus, an N_{ST} value higher than the G_{ST} value indicates that closely related haplotypes are observed more often in a given geographical area than would be expected by chance (Pons and Petit, 1996). A spatial analysis of molecular variance (SAMOVA) was employed using the SAMOVA 1.0 software (http://web.unife.it/progetti/genetica/ Isabelle/samova/html) (Dupanloup et al., 2002) to identify spatial boundaries among the 20 sample localities. One hundred simulated annealing processes, for both cpDNA and nrDNA, were employed to optimally allocate the twenty geographic samples into groups (considering *K* from 2 to 8). The optimal number of groups was selected according to

the highest F_{CT} (differentiation among groups) value. The genetic structure was further examined by an analysis of molecular variance (AMOVA) (Excoffier et al., 1992), implemented in Arlequin version 3.1 (Excoffier et al., 2005), using the whole species, where populations were grouped according to variety and to SAMOVA groups. BAPS version 5.3 (Bayesian Analysis of Population Structure) (Corander et al., 2008) was employed to analyze 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 optimal K cluster population partition was characterized by the highest marginal log-likelihood. We performed 10 algorithm repetitions for each K between 1 and 20.

The population expansion hypothesis was tested with different approaches for cpDNA and nrDNA sequence data sets. Two groups of neutrality tests were computed: (1) Tajima's D (1989) and Fu and Li's (1993) F^* and D^*, considering the frequency of mutation (segregating sites); and (2) Fu's (1997) F_S, based on the haplotype distribution. In addition, mismatch distributions were simulated under the sudden-demographic expansion and the spatial-demographic expansion models. All the tests were performed using Arlequin (Excoffier et al., 2005) and DNAsp (Rozas and Rozas, 1999) software, considering seven groups of populations, including the whole species, populations grouped according to variety, and four groups of different areas and geographic regions: Atlantic Forest south and southeast – AFSSE, Atlantic Forest northeast – AFNE, Amazonian basin – AB and Andean and Central America forests – ACAF. The Atlantic forest was divided into two geographical groups because many studies have reported a disjunction between the southern and northern Atlantic forest (Andrade et al., 2009; Cardoso et al., 2000; Lira et al., 2003; Novaes et al., 2010; Salgueiro et al., 2004).

3. Results

3.1. Patterns of variability in cpDNA and ITS sequences

The *psbA–trnH* and *trnL–trnF* intergenic spacers varied from 363 to 416 bp and 448 to 449 bp, respectively. The *psbA–trnH* intergenic spacer, which exhibits considerable nucleotide polymorphism, including a micro-inversion event, is characterized by a reverse-repeated region of 35 bp flanked by a 14 bp palindromic sequence and insertions and deletions. A 16 bp duplication region was observed in Bahia state (Atlantic forest) populations. The micro-inversion, the duplication region and each indel were considered to be a single mutational event. The *trnL–trnF* spacer was characterized by two indels and three substitutions. The amplification of the *matK* gene produced a 1500-bp fragment with 14 polymorphic sites. The total combined matrix comprised 2322 sites, of which 28 positions were variable and nine harbored gaps. Twenty-one haplotypes were observed by combining cpDNA data: thirteen haplotypes were within *S. parahyba* var. *parahyba* populations and eight were within *S. parahyba* var. *amazonicum*. The haplotype diversity (h) ranged from 0 to 0.571, and the nucleotide diversity (π) from 0 to 0.00088 in the 20 populations. The total haplotype and nucleotide diversities were 0.907 and 0.00239, respectively (Table 1). The highest haplotype diversity was observed in populations SP2 and ES from the Atlantic forest (four haplotypes each) and MT and AM2 from the Amazonian forest (three and four haplotypes, respectively). The populations SP1, MG, RJ1 and RJ2 exhibited two haplotypes, whereas the others were monomorphic (Table 1 and Fig. 2a). The full ITS sequences, including ITS1+5.8-S+ITS2, were 625 bp in length, comprising of 227 and 181 bp for ITS1 and ITS2, respectively. Four haplotypes (Fig. 3a) and 12 polymorphic sites, 10 originating from comparing the two varieties lacking insertions or deletions, were detected at the ITS. The four populations SP2, RJ1, RJ2 and EC were polymorphic, whereas the remaining populations were monomorphic (Table 1). The haplotype diversity (h) ranged from 0 to 0.600, and the nucleotide diversity (π) from 0 to 0.0096 in the 20 populations (Table 1). The total haplotype and nucleotide diversities were 0.694 and 0.00826, respectively.

3.2. Haplotype and population structure relationships

Similar topologies were obtained from the two different network approaches used to infer the relationships among the cpDNA and nrDNA haplotypes, and only the median-joining network is shown (Fig. 2b and Fig. 3b, respectively). H1 was the most frequent haplotype, occurring in 7 of the 20 populations in cpDNA, and Hn1 occurred in 9 out of the 19 populations in nrDNA (Fig. 2a and Fig. 3a). Most haplotypes were observed in only one population, and no haplotypes were shared between the two varieties (Fig. 2a and Fig. 3a).

According to BAPS, cpDNA and nrDNA are optimally partitioned into eight and four genetically structured groups, respectively. The cpDNA groups are displayed in Fig. 2b and Data S1 in the Supporting Information, and the nrDNA groups are presented in Fig. 3b and Data S2 in Supporting Information. The SAMOVA analyses using cpDNA allowed for the identity of eight population groups with $F_{CT} = 0.771$ ($P < 0.0001$) and no population clustering of the two varieties (Data S3 in Supporting information). The groups identified were consistent with the geographic population locations: (I) SC, PR1, PR2, SP1, MG, RJ2 (haplotypes 1, 5, 6); (II) SP2, RJ1 (haplotypes 1–4, 7); (III) RJ3, ES, BA3 (haplotypes 5, 8–11); (IV) BA1 (haplotype 19); (V) BA2 (haplotype 20); (VI) MT, AM1, EC (haplotypes 12–14, 18); (VII) AM2 (haplotypes 14–17) and (VIII) CO, CR, HO (haplotype 21). SAMOVA using nrDNA indicated the presence of distinct groups of genetically defined sampling areas. Considering $K = 2$, the populations are separated according to the two varieties (groups: SC, PR1, PR2, SP1, SP2, RJ1, RJ2, RJ3, ES, BA1, BA2, BA3 vs. AM1, AM2, MT, EC, CO, CR, HO; $F_{CT} = 0.954$). With $K = 3$, an additional partition was identified that subdivided the first group into two areas, with an F_{CT} value of 0.960. With $K = 4$, the F_{CT} increased to 0.979 and remained stable at $K = 5$ to $K = 8$, where the F_{CT} values ranged from 0.977 to 0.981 (Data S4 in Supporting Information). Therefore, we hypothesized the presence of four geographical groups: two in the Atlantic forest comprising SC, PR1, PR2, SP1, SP2, RJ1, BA1, BA2 vs. RJ2, RJ3, ES and BA3, one in the Amazonian rain forest comprising AM1, AM2 and MT and one comprising EC, CO, CR and HO.

AMOVA analysis revealed that 85.13% and 97.71% ($P < 0.0001$) of the total variation was due to differences among the cpDNA and nrDNA populations, respectively (Table 2). When the populations were grouped by taxonomic variety, the cpDNA showed most variation among populations within varieties (69.95%, $P < 0.0001$; Table 2). In contrast, for the nrDNA, AMOVA revealed that most of the variation (95.48%) was due to differences between the two varieties, where 3.34% of the variance was attributed to differences among populations within the varieties ($P < 0.0001$; Table 2). When the populations were grouped according to the SAMOVA groups, most of the variation was observed among groups (68.72% and 97.86 in cpDNA and nrDNA, respectively). The F_{ST} values calculated for each population pair ranged from 0 to 1.00 for both cpDNA and nrDNA, and most estimated values were significant ($P < 0.0001$, Data S5 in Supporting information). The majority of the non-significant pairwise F_{ST} values were observed among populations within the varieties, and non-significant values between the two varieties were observed only among the PR1, AM1 and HO populations for cpDNA (Data S5 in Supporting informa-

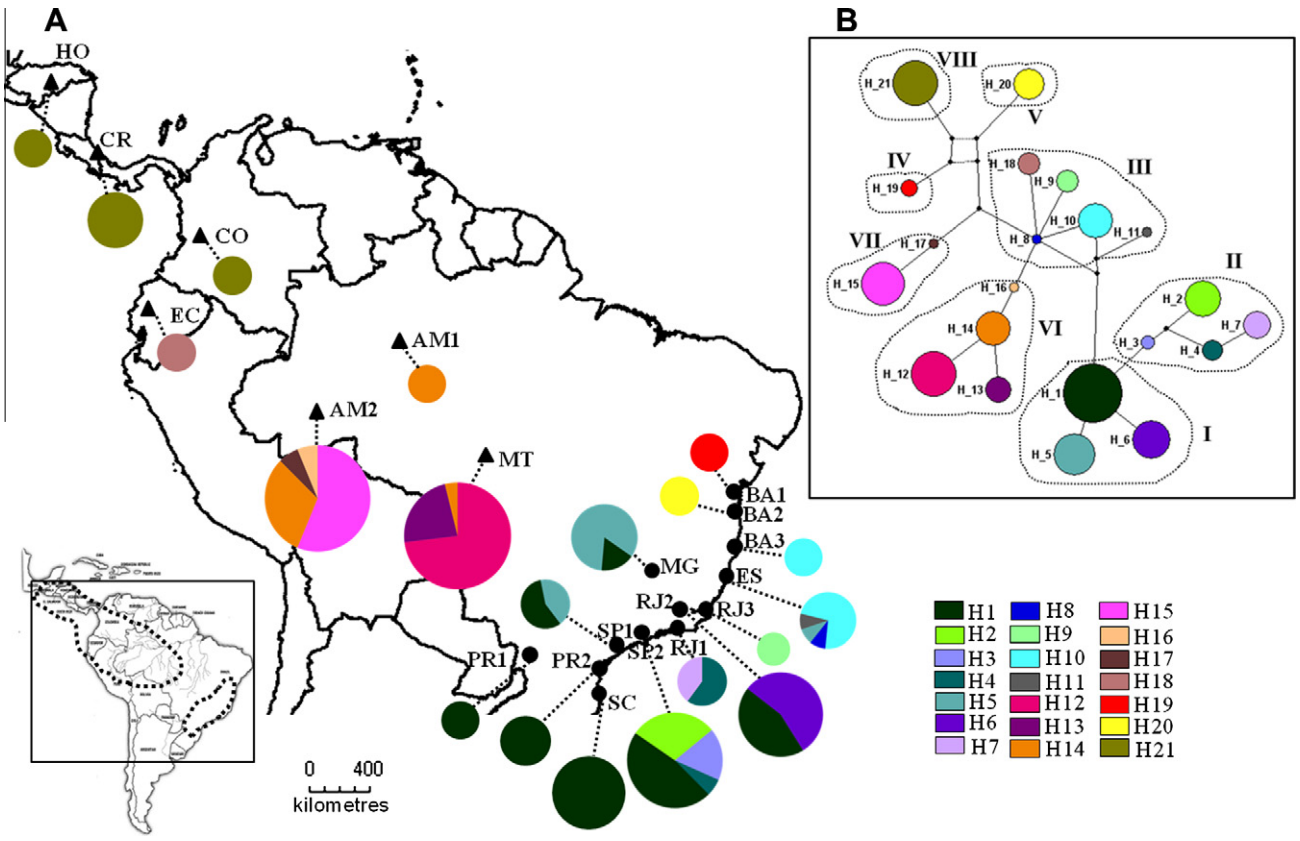

Fig. 2. Distribution of cpDNA haplotypes (A) and a median-joining network (B) for the *S. parahyba* populations. Circle size is proportional to sample size, and colors represent the different haplotypes, as shown in the key. The arrow circles on the network delimit the eight lineages identified using BAPS (Bayesian Analysis Population Structure) analysis.

tion). For the cpDNA data, we estimated a G_{ST} value (0.809, $P < 0.05$), which is significantly smaller than the N_{ST} value (0.947) and is indicative of a phylogeographic structure. In contrast, when the nrDNA was examined, the G_{ST} value (0.873) did not differ significantly from the N_{ST} value (0.848). Neutrality tests revealed significant positive values in several individual population groups and considering all populations (Table 3). The observed mismatch distributions for cpDNA haplotypes were multimodal for all groups except for the AFSSE group. For the nrDNA haplotypes, the mismatch distributions were unimodal for all the groups unless all populations were analyzed together (Table 3).

4. Discussion

4.1. Geographic patterns of genetic diversity

Based on a large collection covering most of the species range and populations from both recognized varieties (Barneby, 1996), high levels of haplotype and nucleotide variation in many populations were observed. The *Schizolobium parahyba* genetic diversity was not randomly distributed in space, and different patterns were observed using the cpDNA and nrDNA markers across the sampling area. High levels of genetic diversity, including the presence of many divergent haplotypes and lineages, were observed in *S. parahyba* var. *parahyba* populations from the southeast Atlantic forest (SP1, SP2, RJ1, RJ2 and ES populations) and in *S. parahyba* var. *amazonicum* populations from the Amazonian forest (MT and AM2 populations). These results suggest the presence of two genetic diversity centers for *S. parahyba*, one in the southeast Atlantic forest, corresponding to *S. parahyba* var. *parahyba*, and the other in the Amazonian basin, corresponding to *S. parahyba* var. *amazonicum*. Conversely, low genetic diversity levels were observed in the populations from the southern Atlantic forest (SC, PR1, and PR2, belonging to var. *parahyba*) and from Central America (CR, HO) and Colombia (CO) (plants belonging to var. *amazonicum*). Whereas populations from the southern Atlantic forest shared the nuclear and plastid haplotypes Hn1 and H1, Central American and Colombian populations shared the nuclear and plastid haplotypes Hn4 and H21, respectively.

The low genetic diversity and the presence of fixed haplotypes in these two regions may be the result of a founder effect. Interestingly, we observed a pattern of expansion toward the south in Atlantic forest, since the observed mismatch distribution for cpDNA and nrDNA haplotypes was unimodal (patterns consistent with expansion) when the populations from Atlantic forest south and southeast were analyzed separately. This result suggests an expansion due to a more recent establishment of these forests and are consistent with a range expansion and re-colonization of the southern portion of the forest by migrants from the southeast Brazilian Atlantic forest. These observations are in agreement with those found to *Passiflora actinia* and *P. Elegans* (Lorenz-Lemke et al., 2005) and *Vriesea gigantean* (Palma-Silva et al., 2009) in the Atlantic forest, which also have reported expansion toward the south. Fossil pollen records confirmed a replacement of forests by grasslands during the Last glacial Maximum in the southern Atlantic forest (Behling, 2001; Carnaval and Moritz, 2008).

The low levels of intra-population genetic diversity detected in several populations, where 11 of the 20 analyzed populations were monomorphic, coupled with the high levels of population differentiation, may be due to their small population sizes and high degrees of spatial isolation, suggesting low gene flow between populations over an extended period.

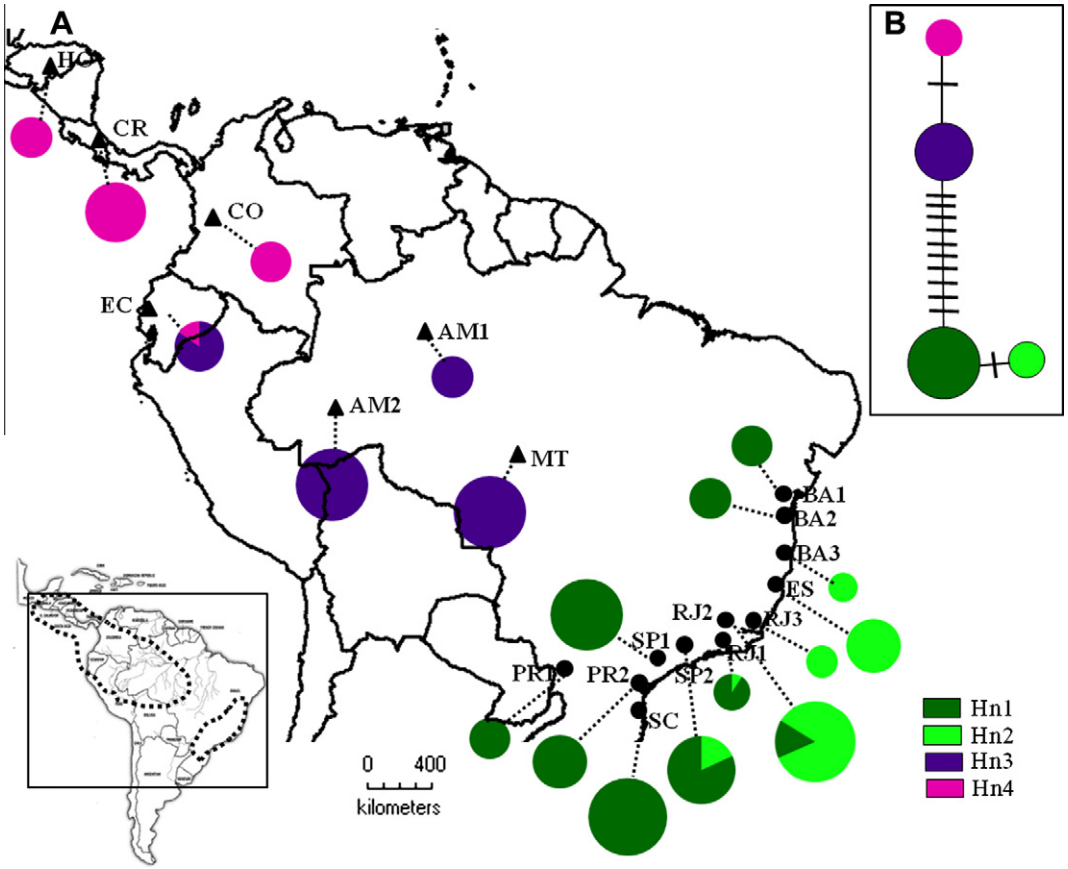

Fig. 3. Distribution of nrDNA haplotypes (A), phylogenetic analysis and a median-joining network (B) for *S. parahyba* populations. Circle size is proportional to sample size, and colors represent the different haplotypes, as shown in the key.

Table 2
Analyses of Molecular Variance (AMOVA) based on the nrDNA (ITS) and cpDNA (*psbA–trnH+trnL–trnF+matK*) sequences.

Source of variation	d.f	Sum. of squares	Variance components	Variation percentage	Fixation indices
ITS (all locations)					
Among populations	18	323.36	2.67	97.71	F_{ST}: 0.977
Within populations	110	6.90	0.062	2.29	
ITS (varieties)					
Among varieties	1	302.48	5.08	95.48	F_{CT}: 0.954
Among populations within varieties	17	20.87	0.17	3.34	F_{ST}: 0.988
Within Populations	110	6.90	0.06	1.18	F_{SC}: 0.739
ITS (SAMOVA groups)					
Among regions	3	320.98	3.56	97.86	F_{CT}: 0.978
Among populations within regions	15	2.37	0.01	0.41	F_{ST}: 0.982
Within populations	110	6.90	0.06	1.72	F_{SC}: 0.193
cpDNA (all locations)					
Among populations	19	399.10	2.14	85.13	F_{ST}: 0.851
Within populations	178	66.60	0.37	14.87	
cpDNA (varieties)					
Among varieties	1	74.13	0.45	16.34	F_{CT}: 0.163
Among populations within varieties	18	324.97	1.91	69.95	F_{ST}: 0.862
Within populations	178	66.60	0.37	13.70	F_{SC}: 0.836
cpDNA (SAMOVA groups)					
Among regions	7	349.28	1.86	68.72	F_{CT}: 0.687
Among populations within regions	12	49.82	0.48	17.50	F_{ST}: 0.862
Within populations	178	66.60	0.37	13.77	F_{SC}: 0.559

4.2. Phylogeographic and demographic patterns

The analyses of the cpDNA and nrDNA sequences of the *S. parahyba* populations revealed a high level of inter-population genetic differentiation (G_{ST} = 0.809, N_{ST} = 0.947 and G_{ST} = 0.873, N_{ST} = 0.848, respectively), where the presence of a significant phylogeographic structure was verified for cpDNA ($G_{ST} < N_{ST}$, $P < 0.05$) but not for nrDNA ($G_{ST} \approx N_{ST}$, $P > 0.05$). The presence of a strong population structure using cpDNA markers was also reported in other leguminous tree species, e.g. *Hymenaea coubaril* (Ramos

Table 3
Summary of demographic expansion tests performed in southern and south-eastern Atlantic forests (AFSSE), northeast Atlantic forest (AFNE), Amazonian basin (AB), and Andean and Central America forests (ACAF) for the var. *parahyba* and var. *amazonicum* groups and all the populations. Significant values in bold.

Parameter	AFSSE	AFNE	AB	ACAF	Var. parahyba	Var. amazonicum	All populations
cpDNA							
Tajima's *D*	0.414	1.294	**2.229**	0.915	−0.035	2.146	0.443
Fu and Li's (1993) *F*′	1.230	**1.639**	**1.766**	1.407	1.291	2.094	1.609
Fu and Li's (1993) *D*′	1.370	**1.478**	1.165	1.333	**1.770**	**1.550**	**1.961**
Fu's (1997) F_S	0.330	**6.845**	3.198	**7.357**	1.858	**6.289**	1.737
Mismatch distribution	Unimodal	Non-unimodal	Non-unimodal	Non-unimodal	Non-unimodal	Non-unimodal	Non-unimodal
ITS							
Tajima's *D*	1.303	0.425	–	−1.164	1.501	1.282	**3.429**
Fu and Li's (1993) *F*′	0.868	0.740	–	−1.611	0.855	0.950	**2.590**
Fu and Li's (1993) *D*′	0.516	0.732	–	−1.499	0.504	0.550	1.457
Fu's (1997) F_S	1.772	0.689	–	−0.794	1.806	1.788	**14.105**
Mismatch distribution	Unimodal	Unimodal	–	Unimodal	Unimodal	Unimodal	Non-unimodal

et al., 2009) and *Dalbergia nigra* (Ribeiro et al., 2010) that thrive in the Atlantic Forest. The large genetic differentiation among *S. parahyba* populations illustrated by the cpDNA data can be related to the poor seed dispersal system of this species, which is mediated by wind and gravity and therefore do not allow a long distance dispersal of the large seeds of *S. parahyba*. In addition, *S. parahyba* has a predilection to disturbance-prone settings, such as large forest gaps and floodplains, and does not survive in the shade (Poorter and Hayashida-Oliver, 2000), which may keep populations isolated from each other. This may be an additional explanation of the high genetic differentiation detected in this species (Hewitt, 1996). On the other hand, nrDNA did not show the presence of a phylogeographic structure with haplotypes distributed over very large areas within each variety niche, suggesting extensive and effective gene flow mediated by pollen within each variety. However, gene flow between the two varieties is very restricted.

The genetic patterns found to *S. parahyba* throughout its distribution suggest that Atlantic and Amazonian forest populations of *S. parahyba* were once fragmented by dry corridor formed by the Cerrado and Caatinga ecoregions, which form potential barriers to *S. parahyba* gene flow between these two wet forest, and have not merged again. The Brazilian cerrado (savanna) covers 2 million km^2 representing 23% of the total area of the country. It is an ancient ecoregion displaying high biodiversity (Ratter et al., 1997) and represents a potential barrier to gene flow. However, evidence from the Late Quaternary fossil record indicates the presence of rainforest in areas of northeast Brazil where semi-arid vegetation flourishes today, which implies that the Amazon and Atlantic forests were more strongly linked once in the past (Oliveira et al., 1999, Behling et al., 2000). It is also illustrated by the occurrence of a number of disjunct plant taxa (Fiaschi and Pirani, 2009) between Amazonian and Atlantic forests. The fact that *S. parahybam* is not found in gallery forests, in central Brazil, suggests that this network of wet forests was not suitable for connecting disjunct populations of this species, therefore increasing their level of isolation. In contrast, it seems that for some wet forest tree taxa the dry formations of central Brazil did not pose a significant barrier for migration. In the genus *Inga* (Fabaceae), for example, Amazon and Atlantic forest taxa share highly similar DNA sequences, which suggest recent diversification (Richardson et al., 2001). The overall lack of phylogenetic geographical structure combined with low genetic differentiation observed in *Inga* may result from significant gene flow across the riparian forests in central Brazil, where *Inga* species are frequently found.

The Andean uplift may have been a potential barrier to gene flow between Central American/Colombian and Amazonian forest populations (*cis–trans*-Andean population separation). *S. parahyba* populations may have experienced extended periods of genetic isolation, since this species does not grow at altitudes above 700 m. In addition, our preliminary analysis suggests that *Schizolobium* genera arose about 15 My (8.4–23.0 My) (Data S6 in Supporting information). Several molecular studies of species complexes or populations distributed on either side of the Andean divide, such as rainforest trees (Dick et al., 2003; Trenel et al., 2007), butterflies (Brower, 1994), frogs (Weigt et al., 2005) and snakes (Zamudio and Greene, 1997), observed levels of cross-Andean genetic divergence consistent with the timeframe of the major Andean orogeny (Moritz et al., 2000).

Although our *S. parahyba* cpDNA results illustrated population from Ecuador forests clustering with Amazonian populations, and our BAPS analysis revealed that the Ecuadorean population clustered with populations from the southeast Atlantic forest, contemporary gene flow among them could not be confirmed. Similar findings were observed for the BA1 and BA2 populations from the northeast Atlantic forest and for the CO, CR and HO populations from Colombia and Central America, which are closely related in the network analysis (Fig. 2). This probably has caused the low differentiation observed between the two varieties at cpDNA (16.3%) sequences. In fact, after excluding these populations, the differentiation value increased to 34.4%. Despite this relationships observed in the network analysis, these populations are very divergent, with many mutation events and exclusive haplotypes, suggesting absence of gene contemporary flow. The cpDNA results found in this study differ from those observed in *Hymaneae coubaril* and *H. stignocarpa* tree species, which showed low differentiation and haplotypes shared between these species (Ramos et al., 2009). The authors suggested that the vicariant species were subjected to the same impacts during the Quaternary climatic fluctuations and that the shared haplotypes and genetic similarity were due to the existence of ancestral polymorphisms and/or hybridization (Ramos et al., 2009). Here, we suggest the establishment of dry formations in central Brazil, which caused a major breakup in a former continuous wet forest distribution and may explain the disjunct pattern observed for both *S. parahyba* varieties and the low gene flow between them, since *S. parahyba* does not occur in this area.

5. Conclusions

Our results delineated the different patterns that guide *S. parahyba* evolution. The phylogeographic and demographic patterns suggest that episodes of genetic isolation and dispersal have shaped the genetic diversity of this species. Additionally, we have shown that the variation pattern of cpDNA (maternally inherited) and nrDNA (biparentally inherited) markers provide different insights into the phylogeographic structure and gene flow in *S. parahyba*. This comparative cpDNA and nrDNA marker analysis may contribute to the elucidation of the dynamics responsible for both

ancient and more recent events that have shaped the current distribution of genetic variability in Neotropical plants. Furthermore, the peculiarities of this species compared to other plant groups illustrate the complex evolutionary history of the Neotropical species.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.06.012.

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