Trees
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ORIGINAL ARTICLE

Genetic variability of *Araucaria angustifolia* in the Argentinean Parana Forest and implications for management and conservation

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Abstract

Key message **Genetic variability of *Araucaria angustifolia* populations in Argentina was moderate-to-low and reduced by logging. Some studied populations and the plantation are valuable gene pools for conservation and management.**

Abstract The main forces shaping genetic variability of woody species in fragmented forest are the geographical distribution and demographic history of populations. We conducted molecular analyses to evaluate how these factors have affected *Araucaria angustifolia* genetic variability in the Argentinean Parana Forest and to identify valuable gene pools for conservation and management purposes. Using 706 polymorphic AFLP (Amplified Fragment Length Polymorphism) markers, we analyzed nine native populations with different logging history and one plantation (312 individuals) of an uncertain origin. Average genetic diversity for the native populations was moderate-to-low (He = 0.128) in accordance with their marginal location within *Araucaria*'s range. In general, genetic diversity of populations decreases from east to west with increasing distances from the main area of species distribution on southern Brazil. Logging may have been responsible for further reduction of genetic variability in the more intensely exploited populations of the southern region and in some private fields. The moderate genetic differentiation among populations ($\Phi_{PT} = 0.080$) suggests an increase in the genetic structure of remnant populations because of fragmentation. UPGMA and Bayesian analyses agreed with the geographic location of populations. Populations from the southern Provincial Parks at *Araucaria*'s range edges grouped and differed genetically more from other populations. The highest genetic diversity of the plantation (He = 0.155) suggests that its individuals could have originated from seeds collected from different and/or highly variable sources of Brazil and the northeast of Argentina.

Keywords *Araucaria angustifolia* · Genetic variation · Geographical distribution · Logging history · Conservation · Management

Introduction

The genetic variation of forest species is a complex outcome of ecological characteristics, evolutionary history, and human impact. As a species spreads from its center of origin throughout evolutionary history, its genetic diversity decreases probably because of genetic drift processes during migration towards more recently colonized areas (Crisci et al. 2000; Martin and McKay 2004). This explains the lower genetic diversity associated with marginal location in several trees of economic value (Ferreira de Souza et al. 2009; Marchelli et al. 2010; Inza et al. 2012; Soldati et al. 2013).

In addition, forest fragmentation owing to human activity may lead to changes in genetic variability. As selective extraction occurs, population size and connectivity among

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remnant stands decrease, with serious consequences on their reproductive and genetic systems. Gene flow becomes more restricted and crosses between individuals become uncommon. Thus, populations may be at risk because of loss of genetic diversity and increases in genetic structure, genetic drift, and inbreeding processes, which affect individual's long-term survival (Young and Boyle 2000; Lowe et al. 2005; de Lacerda et al. 2013; Finger et al. 2014).

The Parana Forest is the largest ecoregion of the Atlantic forest; it extends throughout the south of Brazil, east of Paraguay, and the province of Misiones in the northeast of Argentina (Chebez and Hilbert 2003, Placci and Di Bitetti 2005). Spread of agriculture and unsustainable management of forest species have led to its fragmentation and degradation; therefore, its biodiversity has been threaten, and today, this forest occupies only 6–7% of its original area (Placci 2000; Rau 2005; SAyDS 2007). However, the interest in conservation has increased, and in Argentina, where this interest is more pronounced, the remnant forest reaches 50%. In contrast, the remnant forest in Brazil and Paraguay is only 3 and 10%, respectively (Holz and Placci 2003).

Araucaria genus (Araucariaceae) fossils have been registered throughout the world, but, today, the genus is present only in Australia and South America (Kranitz et al. 2014). *Araucaria angustifolia* (Bert.) O. Ktze (Paraná pine) is endemic to South America and the most important species of the Parana Forest owing to economic, ecological, and cultural interests. This species is a pioneer, long-lived, dioecious, and outcrossing large (20–30 m) conifer with soft resistant timber that is easy to work (Mac Donagh and Rivero 2005). *A. angustifolia* is naturally distributed in the eastern Parana Forest region (18– 30°S and 41–54°30′W; 500–900 m.a.s.l.) (Hueck 1953, Fig. 1) and southern Brazil is the main area of species distribution with the greatest genetic diversity (Ferreira de Souza et al. 2009). During the late Pleistocene (126,000–11,700 years ago, Cohen et al. 2013), the rainy climate, which is suitable for *A. angustifolia*, favored the formation of an ombrophilous nucleus in this region that has been expanded and contracted through different subsequent geological phenomena over time (Silveira Wrege et al. 2009). Although *A. angustifolia* had covered about 200,000 km^2 in the early twentieth century, overexploitation has severely fragmented and reduced its natural distribution (Auler et al. 2002).

Today, the Paraná pine inhabits mainly in the three southernmost states of Brazil (Paraná, Santa Catarina, and Rio Grande do Sul) and more sparsely in southeastern Brazil (São Paulo, Rio de Janeiro, and Minas Gerais), northeastern Argentina, and eastern Paraguay (Di Bitetti et al. 2003; Sebbenn et al. 2003). In Argentina, small patches of west marginal distribution of the species are restricted to the northeastern of the Misiones province. Until the 70 s, forest industry had almost exclusively used this species under policies of encouraging logging; which led to native species degradation. In 1986, authorities declared it a Provincial Natural Monument (Provincial Law 2380). However, selective extraction has continued and led to a highly disturbed landscape with old and often sick trees (Bertolini 2000,

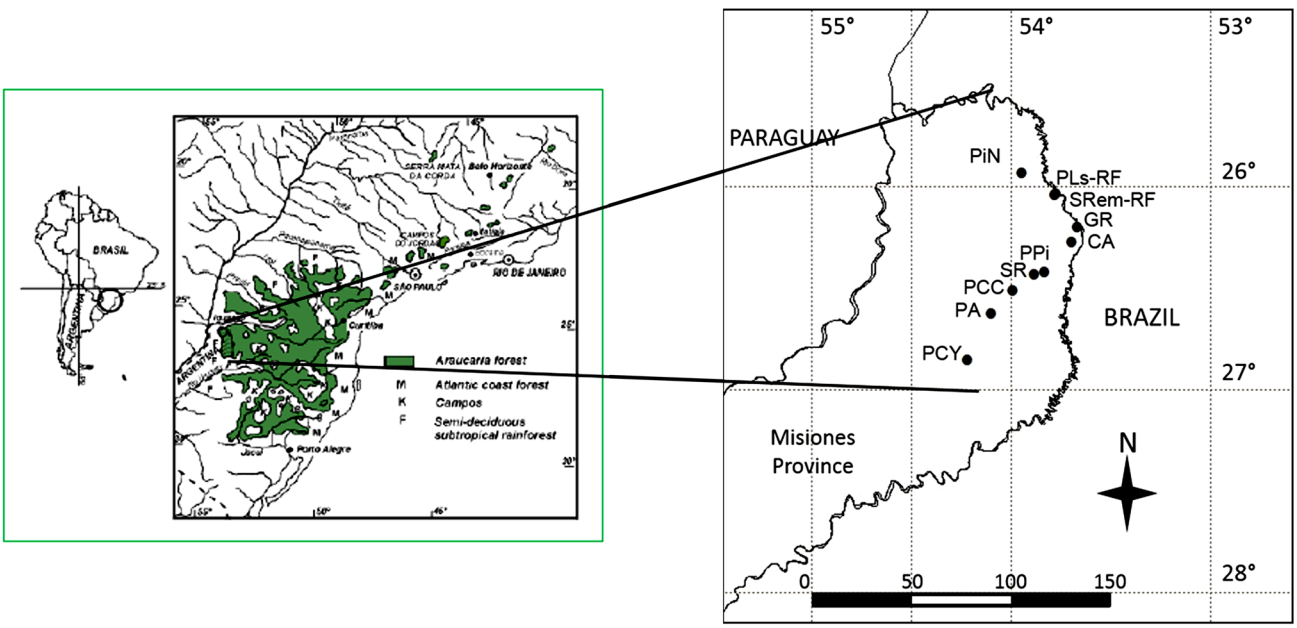

Fig. 1 Natural distribution of *Araucaria angustifolia* (Bert.) O. Ktze. in south Brazil and northeastern Argentina (Rau 2005 according to Hueck 1966, modified) and geographic location of nine native populations and one plantation sampled in northeastern Misiones (Argentina). Population codes are shown in Table 1

Chebez and Hilbert 2003; Rau 2005). Today, *A. angustifolia* is a 'critically endangered' species (IUCN 2018).

Intraspecific genetic diversity is essential to respond to environmental changes and to design conservation guidelines (Bittencourt 2007; Silveira Wrege et al. 2009; Gallo 2013). Furthermore, genetic diversity is critical to avoid extinction of endangered species. Understanding the impact of forest fragmentation on genetic variability is essential for conservation, sustainable management, and for domestication and breeding plans (Bittencourt 2007; Stefenon et al. 2009; Inza et al. 2012). Several researchers have reported that logging has put *A. angustifolia*'s genetic diversity at risk. However, their studies have focused only on Brazilian populations (Auler et al. 2002; Stefenon et al. 2007; Ferreira de Souza et al. 2009; Bittencourt and Sebbenn 2009). Recently, Stefenon et al. (2009) suggested that *A. angustifolia* plantations could be an important source of genetic diversity for conservation. In this sense, the Reserve Forest of Campo Anexo Manuel Belgrano (CAMB; ~ 2,000 ha) of the National Institute of Agricultural Technology (INTA) in San Antonio (Misiones) has been protecting their plantations since 1948 (Fernández et al. 2005).

By assessing AFLP markers, we studied the genetic variation of remnant *A. angustifolia* populations and one plantation in Argentina. Geographical distribution and fragmentation are discussed as forces shaping population genetic variability. The main goal was to determine which populations represent valuable gene pools for conservation and management of this species.

Materials and methods

Plant material

Healthy young leaves from 312 adult *A. angustifolia* trees randomly chosen were collected from nine native populations and one planted forest in their natural distribution area in the northeastern province of Misiones, Argentina (Table 1; Fig. 1). The sample size (*N*) of native populations (20–31 individuals) and plantation (77 individuals) was selected according to the extent of each stand. Trees were GPS (Geographic Position System) georeferenced and a minimum distance (80–100 m) was established to avoid sampling from closely related individuals (Gillies et al. 1999). Four of the native populations are from Provincial Parks (protected areas) and four from private fields (unprotected areas). In the CAMB, one native population, which was called Remnant Forest, and one extensive plantation (448 ha) were sampled. The CAMB protects *A. angustifolia* native remnants and one plantation originated from seeds of different origins (Brazil and other unknown origins of Misiones) (Pinazo and Fassola 2016 pers. com.) as an *in situ* conservation and *ex situ* seed-producing area. The leaves were dried in silica gel, disrupted with Tissue Lyser (Qiagen), and kept at − 20 °C until use.

DNA extraction and AFLP generation

Total DNA was extracted from dried leaves (~ 70 mg) with the DNeasy 96 Plant Kit (Qiagen) following commercial specifications. AFLP protocol and thermal cycling conditions were performed according to Khan et al. (2000) and Vos et al. (1995), respectively. Selective amplifications using primers with three additional nucleotides were obtained from

Table 1 Number of sampled individuals and geographical location of *Araucaria angustifolia* native populations and one plantation in northeastern Misiones (Argentina)

Population	Code	*N*	Department	Lat. (S)	Long. (W)	Alt. (m)	Population status (PA, UA)
Plantation-RF	PLs-RF	77	Gral Manuel Belgrano	26°01′	53°46′	534	PA, reserve multiple use
Piñalito Norte	PiN	21	Gral Manuel Belgrano	25°55′	53°56′	436	UA, private fields
Remnant Forest -RF	SRem-RF	24	Gral Manuel Belgrano	26°02′	53°46′	543	PA, reserve multiple use
Gramado	GR	28	Gral Manuel Belgrano	26°11′	53°39′	699	UA, private fields
Campiñas de Américo	CA	28	Gral Manuel Belgrano	26°16′	53°41′	794	UA, private fields
PP El Piñalito	PPi	27	San Pedro	26°25′	53°49′	719	PA, provincial park
Santa Rosa	SR	20	San Pedro	26°26′	53°52′	645	UA, private fields
PP Cruce Caballero	PCC	31	San Pedro	26°30′	53°59′	606	PA, provincial park
PP Araucaria	PA	25	San Pedro	26°37′	54°05′	548	PA, provincial park
PP Caá Yarí	PCY	31	Guaraní	26°51′	54°12′	465	PA, provincial park

The status of populations is indicated according to level of protection (PA, protected area; UA, unprotected area)

RF forest reserve, *PP* provincial park, *N* sampled size, *PA* protected area, *UA* unprotected area

pre-amplifications on high-quality DNA (150 ng) previously digested by two restriction endonucleases (*Pst*I and *Mse*I). The three most clear and polymorphic combinations were used, *Pst*I + CAG/*Mse*I + ATA, *Pst*I + CTA/*Mse*I + ATA, and *Pst*I + CTA/*Mse*I + ATC), which coded P49M43, P59M43, and P59M44, respectively. More details of enzyme and primer selection are described in Aguirre's work (2014). AFLP primers were fluorescently labeled and fragments were detected in a capillary automatic sequencer (ABI 3130XL, Applied Biosystems). The electropherogram analyses were performed using GeneMapper v.3.7 software (Applied Biosystems). Only polymorphic loci were considered and an additional visual check was performed to correct mislabeled peaks. Fragments between 75 and 500 bp (> 50 peak height) were scored as 1 (homozygous and heterozygous states) or 0 (null alleles) for the presence or absence of each peak, respectively, to generate a binary data matrix.

Data analysis

Genetic diversity

Average value of genetic diversity (He, Nei 1973) over all sampled trees was computed as the estimator for the genetic diversity of the species. The He was calculated from allele frequencies of AFLP data by square root procedure (Bonin et al. 2007) and corrected by population size (unbiased He) using the GenAlEx 6.3 software (Peakall and Smouse 2006). In addition, total genetic diversity (Ht) was calculated with AFLP-SURV 1.0 (Vekemans 2002) excluding loci with null allele frequency below 3/N, where N is the sample size (Lynch and Milligan 1994).

Genetic diversity for each population was estimated with He, percentage of polymorphic loci (PPL), and number of exclusive loci (EL) indexes with GenAlEx 6.3. Three approaches were followed for the analyses. One included all the sampled individuals ($N = 312$) of all native populations and the plantation. A second approach included all sampled individuals from the native populations and part of the plantation individuals. The sample size of the plantation was randomly reduced to a number more comparable to the native populations (25), thus giving 260 individuals in all. Four replications were conducted, and average genetic diversity indexes and standard deviations (SD) were estimated. Finally, the third approach only included native populations ($N = 235$).

Genetic structure and relationships

Genetic variation within and among populations was examined by an analysis of molecular variance (AMOVA, Excoffier et al. 1992) using the GenAlEx 6.3 software. Initially, the native populations and plantation were included (a: *all samples*). Population differentiation was described by the Φ_{PT} statistic and tested from 999 random permutations. In addition, the partition of Ht within and among populations was estimated with AFLP-SURV 1.0 software, and overall and pairwise population F_{ST} indexes (analogue to Φ_{PT}) were calculated (Vekemans 2002) with 1000 permutations tests to establish significance of genetic differentiation. For this first approach, historical gene flow (N_m) was obtained indirectly from F_{ST} according to $N_m = (1 - F_{ST})/4\ \alpha F_{ST}$, where N_m is the number of migrants per generation, and α is a correction factor that depends on the number of the sampled populations (Crow and Aoki 1984). In a second approach (b: *excluding plantation*), Φ_{PT} was estimated to evaluate genetic differentiation among native populations. Finally, in a third approach (c: *native populations and plantation groups*), Φ_{PT} was calculated to compare two groups corresponding to all studied native populations (taken as a whole) and the plantation.

Bayesian analysis was performed to identify the most probable number of genetic clusters (K) and population assignment using Structure 2.3.4 software (Pritchard et al. 2000; Falush et al. 2007). The analysis was performed under admixture model and correlated allele frequencies with 400,000 generations after a burn-in period of 60,000. Six replicates per each proposed K value ($K = 1$ to $K = 11$) were set. We used ΔK value (Evanno et al. 2005) estimated with Structure Harvester (Earl and von Holdt 2012) to obtain the true K value, because the likelihood of data (ln $X|K$) increased progressively without showing a maximum value. First, this analysis was conducted for all populations (a: *all samples*). Subsequently, the plantation and native populations were analyzed separately to further investigate the genetic structure of the data set.

A clustering analysis of populations derived from Nei's genetic distance (Nei 1978, GenAlEx 6.3) was performed in the first approach (a: *all samples*) based on the unweighted pair group method by arithmetic means (UPGMA) and a dendrogram was constructed with NTSYS 2.0 software (Rohlf 1998). Statistical support (r) was achieved by Mantel test with 1000 random permutations.

Results

Genetic diversity

The three AFLP primer combinations generated 706 polymorphic loci over the 312 *A. angustifolia* individuals. In detail, P59M43, P59M44, and P49M43 had 274, 235, and 197 polymorphic loci, respectively. The species genetic diversity (He) estimated over all samples was 0.122 (SD = 0.002, 0.108–0.155) and the analogous Ht was 0.151. The genetic diversity of the plantation was higher than that

of all native populations according to all indexes (Table 2, first line). He, PPL, and EL were 0.155, 80.2, and 32, respectively. For the native populations, GR and SRem-RF were the most diverse populations with a He of 0.133 and 0.134 and PPL of 57.8 and 53.8, respectively. In addition, GR showed the highest EL (22). In contrast, PCC, PA, and PCY populations from the protected areas together with PiN and CA populations from the private fields displayed a lower genetic diversity, with He and PLP ranges from 0.108 to 0.114 and 45.2 to 48.9, respectively. However, PCY and PPi had lower EL than GR, with values of 14 and 13, respectively, but higher than the rest of the populations.

With a reduced sampled size (25 individuals) from the plantation, the genetic diversity for all populations provided similar general results (Table 2, second line). Although He and PLP of the plantation decreased, when compared to the first approach, this group still showed the highest values (0.152 and 62.0, respectively), followed by SRem-RF and GR. Similarly, although He and PLP of PCC, PA, PCY, PiN, and CA slightly increased, these populations still showed the lowest values. In this approach, GR had the highest EL (27).

Table 2 Genetic diversity indexes for native populations and one plantation of *A. angustifolia* in the Argentinean Parana Forest using AFLP markers (*N*, sampled size; He, mean expected heterozygosity; PPL, percentage of polymorphic loci; EL, number of exclusive loci)

Population	*N*	He	PPL	EL
PLs-RF	77	0.155	80.2	32
	25	0.152 (0.005)	62.0 (1.8)	12 (3)
PiN	21	0.114	45.2	5
	21	0.119 (0.001)	47.2 (0.3)	6 (1)
SRem-RF	24	0.134	53.8	6
	24	0.140 (0.001)	56.2 (0.4)	10 (1)
GR	28	0.133	57.8	22
	28	0.139 (0.001)	60.3 (0.4)	27 (1)
CA	28	0.112	48.9	5
	28	0.117 (0.001)	51.0 (0.3)	9 (1)
PPi	27	0.115	50.1	13
	27	0.120 (0.001)	52.3 (0.3)	15 (1)
SR	20	0.129	49.7	2
	20	0.134 (0.001)	51.9 (0.3)	4 (1)
PCC	31	0.110	46.0	3
	31	0.115 (0.001)	48.1 (0.3)	8 (2)
PA	25	0.110	46.6	8
	25	0.115 (0.001)	48.6 (0.3)	9 (1)
PCY	31	0.108	46.2	14
	31	0.113 (0.001)	48.2 (0.3)	17 (1)

The first line corresponds to the analysis with all the individuals from native populations and the plantation. The second line displays the analysis from native populations with all the individuals and the plantation with sample size reduced to 25 randomly chosen individuals (average values and standard deviations from four replications are shown). The code for each population is in Table 1

When the native populations were studied separately, He ranged from 0.117 to 0.145, with a mean value of 0.128. These results are similar to those of the first approach (including the plantation). Both the GR and SRem-RF were the most diverse populations, whereas the three populations from the southern protected Parks were the least diverse. The populations of PCY and PPi again showed high EL values (Table 3).

Genetic structure and relationships

The AMOVA results with all native populations and the plantation (a: *all samples*) revealed a moderate and significant genetic differentiation among populations ($\Phi_{PT} = 0.080$; $p \leq 0.001$). However, most of the genetic variation (92%) was distributed within populations (Table 4). The native populations studied separately (b: *excluding plantation*) yielded a slightly increased Φ_{PT} (0.090; $p \leq 0.001$). In the third approach (c: *native populations and plantation groups*), we detected a low but significantly higher genetic differentiation between these two groups ($\Phi_{PT} = 0.03$, $p \leq 0.001$). The Lynch & Milligan's restriction analysis provided similar results. The Ht of 0.151 partitioned within and among populations yielded an F_{ST} value of 0.060; in addition, a considerable historical gene flow among *A. angustifolia* populations was evident ($N_m = 3.5$). Pairwise population F_{ST} indexes (Table 5) revealed a lower genetic differentiation between the plantation and native populations of SRem-RF, SR, and GR, and between the populations of the four Provincial Parks, which were also genetically more different than the rest of the populations. The highest and lowest F_{ST} were between SRem-RF and PPA and between CA and PiN, respectively.

The Bayesian clustering analysis suggested a population structure at $K = 6$. Another lower ΔK peak appeared at $K = 10$. Following Pritchard et al.'s criteria (2000), we

Table 3 Genetic diversity indexes for native populations of *A. angustifolia* in the Argentinean Parana Forest using AFLP markers (*N*, sampled size; He, mean expected heterozygosity; PPL, percentage of polymorphic loci; EL, number of exclusive loci)

Population	*N*	He	PPL	EL
PiN	21	0.124	48.8	6
SRem-RF	24	0.145	58.1	13
GR	28	0.143	62.4	30
CA	28	0.121	52.8	11
PPi	27	0.124	54.1	15
SR	20	0.139	53.7	4
PCC	31	0.119	49.7	11
PA	25	0.119	50.3	9
PCY	31	0.117	49.8	19

The code for each population is in Table 1

Table 4 Analysis of molecular variance (AMOVA) according to three different approaches

Comparative assessment	Source of variation	*df*	Variance component	% of total variance	Φ statistics	*p* value
a: all samples	Among populations	9	4.091	8	$\Phi_{PT}=0.08$	≤0.001
	Within populations	302	46.762	92		
b: excluding plantation	Among populations	8	4.640	9	$\Phi_{PT}=0.09$	≤0.001
	Within populations	226	44.764	91		
c: native populations and plantation groups	Among groups	1	1.402	3	$\Phi_{PT}=0.03$	≤0.001
	Within groups	310	49.829	97		

a all samples, *b* excluding plantation, and *c* native populations and plantation groups

df degrees of freedom

Table 5 Pairwise population differentiation (F_{ST} values) for native populations and one plantation of Argentinean *Araucaria angustifolia* estimated from AFLP markers

	PLs-RF	SRem-RF	CA	PiN	GR	SR	PPi	PCC	PCY	PPA
PLs-RF	0.000									
SRem-RF	0.023	0.000								
CA	0.055	0.094	0.000							
PiN	0.044	0.074	0.004	0.000						
GR	0.027	0.043	0.039	0.026	0.000					
SR	0.029	0.066	0.050	0.047	0.032	0.000				
PPi	0.039	0.059	0.060	0.045	0.054	0.051	0.000			
PCC	0.047	0.070	0.087	0.070	0.065	0.062	0.007	0.000		
PCY	0.056	0.088	0.065	0.044	0.065	0.062	0.014	0.030	0.000	
PPA	0.076	0.113	0.079	0.071	0.083	0.075	0.047	0.062	0.048	0.000

The code for each population is in Table 1

selected $K=6$ to explain the *A. angustifolia* population structure, because it was the smallest *K* capturing the major structure (Fig. 2). The populations were assigned to cluster 1 from a range of 11% (PPA) to 44% (PPC) of individuals. Cluster 2 mainly grouped a subgroup of PLs-RF trees (23%) and half of SRem-RF (51%), whereas cluster 3 contained the other subgroups of PLs-RF (31%), SR (38%), and GR (22%). Most individuals from CA (65%) and PiN (53%) and almost 20% from GR and SR were within cluster 4. Cluster 5 was characteristic of trees from the Provincial Parks (PPi, 34%; PCC, 39%; PCY, 40% and PPA, 71%). Finally, cluster 6 was present at a low proportion in all populations (1–12%). No additional information was obtained when we studied the plantations and native populations separately (data not shown). This result suggests that no greater structuring level is taking place.

Despite the low Nei's genetic distances among populations (0.007–0.031), the UPGMA dendrogram showed two main groups ($r=0.69$). The first group with two subgroups corresponds to populations from the CAMB (PLs and SRem) and private fields and a more distant second group with populations from the Provincial Parks (Fig. 3). These results are consistent with the genetic differentiation among populations from F_{ST} pairwise and the clusters identified by the Bayesian model.

Discussion

By assessing 706 AFLP primer combinations, we studied the genetic variability of Argentinean native populations of *Araucaria angustifolia* from protected and unprotected areas as well as of an extended plantation. This set of markers is higher than in similar studies in Brazil. Stefenon et al. (2007) and Ferreira de Souza et al. 2009's groups used 166 and 673 AFLP loci from one and six primer combinations, respectively. Although the adequate number is not defined, assessments with more than 250 markers could counteract the bias that may occur from dominant markers (Kremer et al. 2005). However, a higher number is more appropriate for species with large genome such as *A. angustifolia*.

Genetic diversity

The genetic diversity of *A. angustifolia* was moderate-to-low for all sampled trees (Ht = 0.151, He = 0.122) and for native populations when assessed separately (He = 0.128). These values are lower than those reported for Brazilian natural populations in studies that used AFLP (Ht = 0.30,

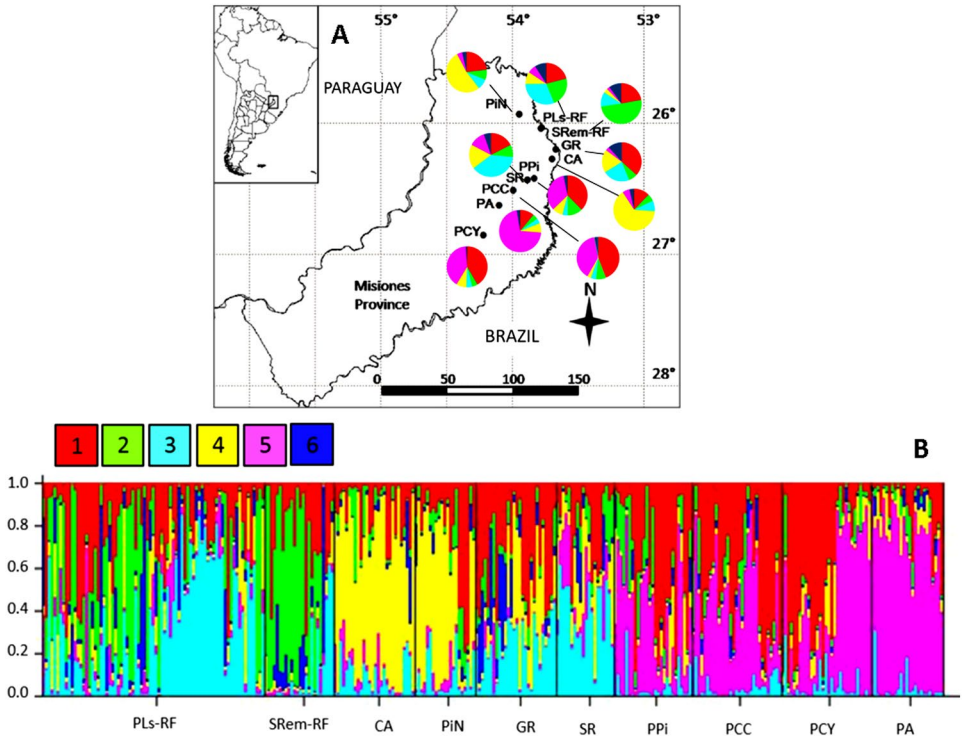

Fig. 2 Geographic distribution of genetic groups of *Araucaria angustifolia* populations and one plantation inferred from Bayesian analysis ($K=6$) (**A**). Circles represent the percentages of assignment of populations to clusters. Black dots indicate the exact location of populations. Genetic structure with individual genetic composition detail (**B**). Each individual is represented by a vertical bar

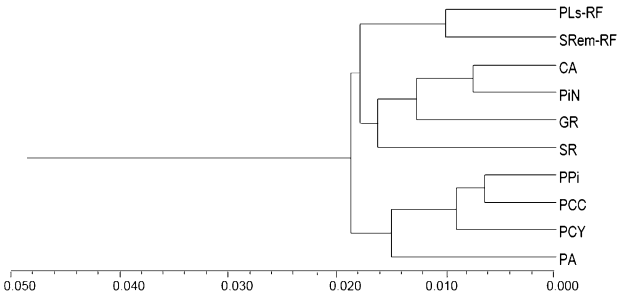

Fig. 3 UPGMA cluster analysis based on Nei's genetic distance among nine native populations and one plantation of *Araucaria angustifolia* in northeastern Argentina

Stefenon et al. 2007; Ht = 0.27; Ferreira de Souza et al. 2009) and RAPD (Ht = 0.26, Medri et al. 2003). They are also lower than those expected for long-lived perennial outcrossing species that are dispersed through gravity or attached to animals such as *A. angustifolia* (He = 0.16–0.27, Nybom 2004). However, these results are consistent with the low genetic diversity reported for Araucariaceae (Hamrick and Godt 1989). In contrast, *A. cunninghamii* (Pye et al. 2009) and *A. bidwilli* (Pye and Gadek 2004) from Australia and the other *Araucaria* species from Argentina, *A. araucana* (Bekessy et al. 2002), presented high genetic diversity. However, these studies covered the whole natural distribution of those *Araucaria*'s species, whereas our study covered a small area at the edges of the *A. angustifolia*'s range.

Geographical distribution and population demographic history play an important role in determining genetic variability of species (Hamrick et al. 1992; Stefenon et al. 2009). Remnant populations of *A. angustifolia* in northeast Argentina occur at low density as small isolated patches at the western edge of its natural distribution (Rau 2005). In this study, the populations decreased their genetic diversity from east to west, with increasing distance from the main area of species distribution (southern Brazil). Most diverse populations of Gramado and Remnant Forest are located nearer to Brazil, whereas less diverse populations, such as Piñalito Norte and Provincial Parks of Cruce Caballero, Caá Yarí and Araucaria, are at the edges of the *Araucaria*'s range. We expected this result because of the occurrence of genetic drift associated with founder effects or population bottlenecks during migration processes (Newton et al. 1999). Furthermore, it is consistent with haplotype studies in chloroplast DNA, which suggested that Argentinean *A. angustifolia* populations would have migrated from glacial refuges of southern Brazil (Ferrero Klabunde 2012). According to this, *A. angustifolia*'s palynological data suggest the existence of species refuges in protected valleys in southern and central Brazil (Ledru et al. 1998; Behling et al. 2004) and a migration process in north–south direction throughout the Paraná, Santa Catarina, and Rio Grande do Sul states (Stefenon et al. 2007). Similarly, Marchelli et al. (2010) associated the genetic diversity loss in western *A. araucana* populations of Patagonia (Argentina) to a recolonization process from the eastern. In addition, Inza et al. (2012) explained

the low genetic diversity of *C. angustifolia* populations in Argentina owing to an edge effect.

In agreement with our results, Ferreira de Souza et al. (2009) reported low-to-moderate genetic diversity levels of isolated populations on the northernmost extreme of *A. angustifolia* distribution in southeastern Brazil, with a slightly higher Ht (0.16). On the other hand, they detected the highest genetic diversity in the more continuous area of the species distribution in southern Brazil (Ht = 0.23–0.27) in accordance with Stefenon et al. 2007 (Ht = 0.21–0.31). This finding was attributed to a faster and more intense recovery of the effective population size after the post-glacial colonization owing to more benign weather conditions (Stefenon et al. 2008a).

Logging history could also explain genetic diversity in Argentinean *A. angustifolia* populations (Mac Donagh and Rivero 2005). *A angustifolia* exploitation in Argentina began in the southern distribution range in Misiones in 1940 and resulted in exhaustion of native stands by 1970 (Rau 2005). Some remnant populations from this area, included in our study, are small parks and/or recently created areas with legal protection and, therefore, they are degraded at different intensities (Bertolini 2000). Particularly, the Cáa Yarí Park is within the Yabotí Biosphere Reserve that protects a longer continuous forest path (SAyDS 2007). This may explain higher exclusive bands detected there. Northeast populations, closer to the main distribution area of the species in Brazil, show different diversity probably owing to different logging histories. In Piñalito Norte and Campiñas de Américo's populations, we found lower diversity probably because of its long disturbance history. Similarly, Ragonese and Castiglione (1946) reported low density of trees in Piñalito Norte attributed to logging. With respect to Campiñas de Américo, this area has been intensely exploited for more than 80 years. In our study, Remnant Forest and Gramado showed the highest diversity, which can be explained by a lower forest disturbance. Indeed, Remnant Forest is the oldest protected area and Gramado has the densest stands of the province (Ragonese and Castiglione 1946; Fernández et al. 2005). However, if these areas remain unprotected, the gene pool persisting in these populations will decline according to the different future scenarios predicted for the province by Izquierdo et al. (2011).

Evolutionary history and forest fragmentation are two forces shaping the genetic variability of *A. angustifolia*. The initial levels of genetic variability that depend on location are modified in fragmented landscapes (Degen et al. 2006). First, gene flow decreases with a subsequent loss of low-frequency alleles because of a direct tree removal or initial genetic drift processes. This is consistent with lower values of exclusive loci detected in more disturbed populations of private fields. Accordingly, using microsatellite markers, Bittencourt and Sebbenn (2009) observed loss of rare alleles in fragmented populations in comparison to continuous *A. angustifolia* populations in Brazil. Second, if forest disturbance is constant or intense, a decrease in heterozygosity may occur with higher risk of local population extinction (Young and Boyle 2000; Bittencourt and Sebbenn 2007; Inza et al. 2012). These processes could explain the lower genetic diversity in *A. angustifolia* populations with long logging history in Argentina. In a study of *A. araucana* in Argentina and Chile, Bekessy et al. (2002) associated a reduced genetic variation with highly fragmented populations. By comparing exploited and conserved forests of *A. angustifolia*, Sousa et al. (2004) found that selective logging reduced effective population size and heterozygosity. Similarly, Auler et al. (2002) detected low diversity and inbreeding in improperly managed and degraded populations. Although AFLP are neutral, they are more likely linked to adaptive characters because of their greater genome coverage; therefore, genetic diversity loss may lead to a serious compromise of the viability of disturbed populations (Jump and Peñuelas 2007; Bittencourt and Sebbenn 2009).

Genetic structure and relationships

Most of the genetic variation detected within the populations is consistent with that expected for long-lived perennial, woody, and outcrossing species and particularly for gymnosperms (Hamrick et al. 1992; Nybom 2004). The fact that Φ_{PT} (0.08) is slightly higher than F_{ST} (0.06) agrees with other studies (Mariette et al. 2001; Inza et al. 2012). The slight increase of Φ_{PT} (0.09) with the native populations alone may be associated with the exclusion of the plantation, because the plantation showed a low genetic differentiation (3%) with the native populations. This index was lower than those found on natural Brazilian populations studied by AFLP (10% by Stefenon et al. 2007; 19 and 12% by Ferreira de Souza et al. 2009) and SSRs (13% by Stefenon et al. 2007). These results may be explained by distance. The Argentinean populations are separated by no more than 100 km, whereas Brazilian populations are 600–1000 km apart. Despite the similar explored range (~ 150 km) in a study of *A. araucana* populations throughout Chile and Argentina (Bekessy et al. 2002), the genetic differentiation detected by RAPDs was higher (12.8%) than for the populations in our study. However, Bekessy et al. (2002) detected clustering populations across the Andes.

Although the estimated historical gene flow (Nm = 3.5) was adequate, the moderate genetic differentiation among populations suggests some restriction of pollen flow because of forest fragmentation in remnant populations in Argentina. This finding agrees with the results reported for this species in Brazil (Auler et al. 2002; Bittencourt and Sebbenn 2009; Stefenon et al. 2009).

The results from the pairwise population differentiation (F_{ST} pair's values), UPGMA (from Nei's genetic distances), and Bayesian analyses are in accordance with the geographic location of the studied populations and their logging history. Despite the short distance of Argentinean sampled populations, genetic differentiation increased with geographical distance in accordance with other studies (Stefenon et al. 2007; Ferreira de Souza et al. 2009). The intensely logged populations from the southern Provincial Parks at *Araucaria*'s range edges grouped and were genetically more differentiated from other populations. The plantation better grouped with the more diverse and closely populations of SRem-RF. On the other hand, the populations that were genetically more different (SRem-RF and PPA) were geographically distant to each other. The lowest genetic differentiation between the highly logged populations of CA y PiN could be associated with a genetic erosion process. The clustering composition of populations would be linked to a distant localization from the main center of distribution in Brazil and to their exploitation history. Thus, the highly logged populations of Campiñas de Américo, Piñalito Norte, and Provincial Parks were assigned mainly to only one or two clusters. The different cluster composition of Campiñas de Américo and Piñalito Norte with respect to Provincial Parks may be associated with a genetic erosion process during migration events from Brazil. However, further studies including Brazilian populations should be performed to ensure this. In contrast, the higher diversity in the protected Remnant Forest and lower disturbed Gramado populations, which are the nearest to Brazil, was consistent with their assignment to more clusters.

Plantation

The plantation showed higher genetic diversity (He = 0.155), exclusive loci (32), and lower genetic differentiation (3%) compared to native populations (He = 0.108–0.134 and EL = 2–22). This result suggests that the plantation could have come from seeds collected in different or highly variable sources of the Argentinean Paraná Forest and Brazil. This is consistent with some plantation compartments (Pinazo and Fassola 2016 pers. com.) registering seeds from local region (near the CAMB and southern edge of Misiones) and Brazil (near to Gramado and Campiñas de Américo in Argentina).

Bayesian and UPGMA results indicated that the local material could come mainly from the Remnant Forest of CAMB and private fields of Gramado and Santa Rosa. We were unable to confirm the use of seeds from the southern edge of Misiones and Brazil, because we did not include these populations in this study. These AFLP results are consistent with the previous studies from microsatellites (SSRs) in the Reserve Forest of CAMB (Sarasola et al. 2011). In contrast, our results differ to data obtained with AFLP from southern Brazilian populations that reported higher genetic diversity (Hj = 0.240) of plantations (Stefenon et al. 2008b). This difference may be because these seeds were collected in the main region of the *A. angustifolia* distribution. In the Brazilian study, no significant genetic differences were evident between the plantations and native populations. However, in accordance to our work, several researchers suggested that the admixture of seeds from several natural populations can lead to increased diversity (Sebbenn et al. 2003; Stefenon et al. 2008b).

Implications for conservation and conclusions

Knowledge of genetic variation is decisive for adequate management and conservation to ensure the continuity of *A. angustifolia*'s gene pool (Finkeldey and Hattemer 2007; Silveira Wrege et al. 2009). Several AFLP studies have helped conservationists to guide protection strategies for threatened native forest species (Cavers et al. 2004; De la Torre et al. 2008; Inza et al. 2012). For instance, in Argentina, a pattern of molecular variability of *Nothofagus nervosa* led to reformulate its protection status in a reserve area in Patagonia (Gallo et al. 2009).

Today, *A. angustifolia* conservation is implemented in few, often small, protected areas in Brazil (Stefenon et al. 2009) and Argentina (SAyDS2007). The Paraná pine is at higher risk in Argentina because of its difficult natural regeneration. Pollen falls at short distances and temperature increase reduces its viability (Fassola et al. 1999; Del Fueyo et al. 2008; Latorre et al. 2013). Increased temperatures would be aggravated under the climate change predicted for the region (Silveira Wrege et al. 2009). Moreover, the species regeneration depends on the opening of large gaps, which are not habitual conditions in small-scale disturbed areas owing to selective logging, or protected areas with dense forests (Souza et al. 2008).

However, the Argentinean *A. angustifolia* remnant can still be saved, as it is legally protected. The Reserve Forest of CAMB is under sustainable management and conservation (Forestry Act No. 26,331) (Goya et al. 2012). The plantation with the highest variability and with some exclusive loci is a valuable gene pool in *ex situ* conservation and breeding programs throughout seed production. Furthermore, native populations with fewer genetic groups in the plantation could supply new material to enhance its current genetic pool.

The greatest efforts for conservation of native populations should be mainly focused on populations from Gramado and Remnant Forest of CAMB because of their high variability. The conservation status of the species should be improved, and the Provincial Park areas should be extended and reorganized to preserve populations with exclusive loci

and genetic differentiation at edges of the *Araucaria*'s range that may have an adaptive value.

We suggest general guidelines for *in situ* and *ex situ* strategies to safeguard remnant *A. angustifolia*. That is to (1) improve connectivity among fragments by corridors of gene flow from new/increased protected areas; (2) collect seeds from more variable and/or differentiated areas; (3) evaluate species introduction with seed orchards in cooler areas towards higher latitudes to increase pollen viability; (4) promote management measures to enhance natural regeneration; and (5) take restoration actions to increase effective size and avoid genetic drift in private and protected areas.

Author contribution statement NZ, SLT, and LFF conceived the study, conducted and carried out sample collection, and contributed to design and interpretation of data analysis. NCA carried out DNA extraction, primer combination screening, and AFLP genotyping of two primer combinations, and contributed to explain, and interpreted results. MVI performed AFLP genotyping of one primer combination, analyzed and interpreted the results, and wrote the manuscript. NMP collected samples and provided information about logging history of populations. HEF contributed with information on the natural life history of *A. Angustifolia* and assisted in developing general guidelines for species use and conservation. All authors participated in reviewing and improving the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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