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Genetic diversity in populations of *Maytenus dasyclada* (Celastraceae) in forest reserves and unprotected Araucaria forest remnants

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ABSTRACT

Understanding the genetic structure and diversity of plants is fundamental to their conservation and permits their sustainable use by local communities. The genus *Maytenus* (Celastraceae) is composed of plants possessing pharmacological and antioxidant properties. However, the genetic and economic properties of the species *M. dasyclada*, a typical species of Araucaria forests in Brazil and Uruguay, have been little studied. In this work, the genetic structure and diversity of natural populations of *M. dasyclada* located in unprotected and preserved forest remnants were investigated using RAPD and isozymes markers. The results demonstrated that in areas of preservation, populations of *M. dasyclada* possess a relatively high degree of polymorphism and high values for Na, Ne, Shannon index, He and Ho, indicating high genetic variability. Moreover, these protected populations are very close to each other and potentially experience significant gene flow. The results presented here highlight the relevance of preservation areas for the conservation of *M. dasyclada*, and that populations inhabiting these areas could serve as a genetic source for the recovery of populations in regions where genetic diversity has been lost.

Keywords: conservation, forest reserves, genetic diversity, *Maytenus dasyclada*, molecular markers

Introduction

Many tropical countries, including Brazil, have employed extractive exploration with native species, sometimes resulting in their disappearance from natural ecosystems. Extinction of these species even at a local scale results in loss of important genetic information and, consequently, reduced capacity for ecosystem recuperation. Knowledge of the genetic structure and diversity of plants is fundamental for their conservation and permits their use by local communities (Nass *et al*. 2012; Asma *et al*. 2016). In these sense, investigations of genetics in native plants with ecological and economical potential can inform both extractive activities and efficient forest management (Piotti *et al*. 2013).

Genetic variability is the basis for species conservation. Knowledge of the genetic structure of plants is essential for the establishment of effective conservation actions, particularly for species in which knowledge is lacking, such as tropical trees (Pautasso 2009). Molecular or biochemical genetic markers are widely used in studies of genetic

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variability both within and among species (Rutledge *et al.* 2000; Amin *et al.* 2012; Cruz *et al.* 2016). RAPD (Random Amplified Polymorphic DNA) is a molecular marker that allows detection of polymorphism without previous knowledge of genetic sequences, and is commonly used in plant population studies (Ahmed *et al.* 2012; Zhang *et al.* 2013; Tiwari & Shrivastava 2016). Isozymes are biochemical markers used in the investigation of genetic diversity in natural populations (Kaya & Bilben 2012; Saini & Yadav 2013), allowing analysis of genotype frequencies, coefficients of diversity genetic and heterozygosis, and estimation of possible derivations of Hardy-Weinberg´s equilibrium (Alfenas *et al.* 1998). The combination of RAPD and isozymes is an effective wat to evaluate genetic diversity in plant populations for which there is limited knowledge of the genetic profile.

The genus *Maytenus* belongs to the family Celastraceae, which includes 55 genera and 850 species distributed among tropical and subtropical countries. There are 77 *Maytenus* species in the Brazilian flora (Carvalho-Okano 1992), some of which have pharmacological and antioxidant properties (Corsino *et al.* 2003; Pereira *et al.* 2005; Tiberti *et al.* 2007; Santos-Oliveira *et al.* 2009; Cansian *et al.* 2015; Santoyo *et al.* 2015; Bevenutti *et al.* 2016).

Among other Brazilian *Maytenus* species, *Maytenus dasyclada*, known as "coração-de-bugre", is a conservation concern. This species is poorly studied, however it is known that populations are declining in the natural flora, especially because it is a bush species which is commonly removed to "clean" forest and urban areas. Although not currently on the list of endangered species, *M. dasyclada* is typical species of Araucaria forest and is in danger of extinction due to forest fragmentation (Higuchi *et al.* 2012).

In this work, the genetic structure and diversity in natural populations of *M. dasyclada* were studied in unprotected and protected (institutionalized areas of preservation; Federal Reserve) forest remnants. Seven *M. dasyclada* populations were chosen in southern Brazil for comparison using RAPD and isozymes markers, with the goal of determining whether habitat fragmentation generated by anthropogenic activity has already caused perceptible effects at the population level (i.e., by reducing the index of genetic diversity). We hypothesized that populations located in protected forest remnants would be in a better state of genetic conservation than are populations located in unprotected areas.

Materials and methods

This study was conducted in Rio Grande do Sul and Santa Catarina states in southern Brazil (Fig. 1). *Maytenus dasyclada* Mart. were collected at seven sampling sites (N= 60 individuals). Three of the sampling sites lie in the Floresta Nacional de Passo Fundo (FLONA) forest reserve, with each site differing in landscape physiognomy and thus considered as a distinct population, as follows: native forest

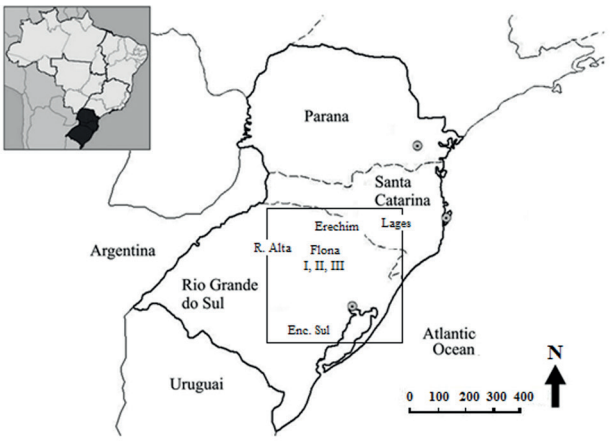

Figure 1. Location of the study area in southern Brazil.

site (FLONA I); site with *Pinus* spp. (FLONA II); and site with *Araucaria angustifolia* (Bertol.) (FLONA III). Descriptive data of the seven sample sites are presented in Table 1. These data demonstrate very similar landscape features among the Erechim, Ronda Alta and Passo Fundo regions, and different landscape features in the Lages and Encruzilhada do Sul regions.

RAPD analyses

The freshly collected plants were kept in liquid nitrogen during the transport stored in a laboratory freezer at -85 °C until RAPD and isozymes analysis.

For RAPD, genomic DNA was extracted as described in Mossi *et al.* (2009) with minor modifications. The foliar tissues were macerated in liquid nitrogen, mixed with CTAB buffer (2 % CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.2 % 2-mercaptoethanol) and incubated at 65 °C for 30 min. Proteins were eliminated with chloroform: isoamyl alcohol (24:1), and samples were washed once with ethanol absolute and once with 70 % ethanol. The DNA pellet was then re-suspended in TE buffer (10 mM TrisHCl pH 8.0, 1 mM EDTA). DNA quality was assessed using spectrophotometry (260 nm/ 280 nm) and visualization in agarose gel (0.8 %).

RAPD reactions were performed following Williams *et al.* (1990). Briefly, PCR mixtures contained 40 ng of DNA, 1.5 U Taq DNA polymerase, specific primers (0.2 mM), MgCl2 (3 mM), Triton (0.25 mM), and Taq Buffer (Gibco BRL). The cycle program was as follows: initial denaturation (92 °C for 3 min) followed by 40 cycles of 92 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min, with a final extension step of 72 °C for 3 min. We then tested 30 primers using OPB, OPF, OPW, and OPY kits (Operon Technologies Inc.), and obtained the best amplification results with OPB 11, OPF 01, OPF 09, OPF 10, OPF 12, OPH 12, OPW 04, OPW 08, OPW 12, OPY 10, OPY 16, OPY 17 and OPY 18.

Table 1. Characterization of the collection sites of *Maytenus dasyclada* and Matrix of geographic distances (Km) (A) and altitude differences (m) (B) in populations of *Maytenus dasyclada* sampling in Rio Grande do Sul and Santa Catarina (Brazil).

Characterization of the collection sites					
Local	Vegetation description	Coordinates	Fragment size (ha)	Number of samples	Average altitude (m)
A - FLONA I Passo Fundo, RS	Native Forest - Reserve - mixed rain forest	28°17'14'' S 52°11'26'' O	365.4	10	720
B - FLONA II Passo Fundo, RS	*Pinus* spp. - Reserve	28°17'32'' S 52°11'36'' O	278	10	700
C - FLONA III Passo Fundo, RS	*Araucaria angustifolia* - Reserve	28°17'44'' S 52°11"37'' O	391	10	702
D - Erechim, RS	Not protected forest remnant - mixed rain forest	27°45'37" S 52°13'20" O	12.5	10	730
E - Ronda Alta, RS	Not protected forest remnant - mixed rain forest	27°49'41" S 52°49'18" O	30	5	600
F - Lages, SC	Not protected forest remnant - grasslands and rain forest montana	27°51'19" S 50°10'33" O	103.06	5	990
G - Encruzilhada do Sul, RS	Not protected forest remnant - pampas and mixed rain forest fragments	30°32'46" S 52°30'18" O	7.8	10	400

Matrix of geographic distances (Km) (A) and altitude differences (m) (B)												
	FLONA I		FLONA II		FLONA III		Erechim		R. Alta		Lages	
	A	B	A	B	A	B	A	B	A	B	A	B
FLONA I	0	0										
FLONA II	0.7	20	0	0								
FLONA III	0.7	18	0.4	2	0	0						
Erechim	60	2	60	22	60	20	0	0				
R. Alta	80	128	80	108	80	110	59	130	0	0		
Lages	208	262	208	282	208	280	206	260	264	390	0	0
Enc. Sul	250	328	250	308	250	310	310	330	302	200	375	590

After amplification, DNA fragments were submitted to electrophoresis (240 min, 90V) in agarose gel (1.4 %) containing ethidium bromide (0.5 mg/mL) and photographed using a transilluminator digital system (GEL-PRO Imager, Media Cybernetics, Silver Spring, MD). The data were classified in relation to the presence or absence of specific bands, generating a matrix for analysis using the program PAST 2.0. The genograms of similarity (UPGMA) were constructed using the Jaccard coefficient. Principal component analyses (PCA) were carried out and Shannon index was calculated using PAST 2.0. The apparent number (Na) and effective number (Ne) of alleles, observed (Ho) and expected (He) heterozygosis, Shannon Index (Nei 1973), genetic distances (Nei 1972), and analyses of molecular variance (AMOVA) were analyzed using GenAlEX 6.4 (Peakall & Smouse 2009).

Isozyme assays

Enzyme extractions were performed using approximately 1 g of leaf tissue per mL of extraction solution 1 (see Alfenas *et al.* 1998), adding approximately 150 mg of PVP-40 during grinding to remove phenolic compounds and increase extract stability. The extract was then subjected to vertical electrophoresis (220 V, 40 mA) in a biphasic polyacrylamide gel formed by stacking phase (5 % polyacrylamide in Tris-HCl, pH 6.8) and running phase (12 % polyacrylamide in Tris-HCl, pH 8.8) (Alfenas *et al.* 1998).

Eleven enzymatic systems were tested, with six of them active in *M. dasyclada* and generating 15 loci that were able to be interpreted: ADH – alcohol dehydrogenase (E.C. 1.1.1.1; 3 loci); EST – esterase (E.C. 3.1.1.1; 1 locus); GDH – glucose dehydrogenase (E.C. 1.1.1.47; 3 loci); PO – peroxidase (E.C. 1.11.1.7; 2 loci); SDH – sorbitol dehydrogenase (E.C. 1.1.1.14; 3 loci); SOD – superoxide dismutase (E.C. 1.15.1.1; 3 loci). Results were evaluated based on the presence, distinctness, number, and location of bands for each enzymatic system.

Isozyme data were analyzed using GenAlEX 6.4, which generated allelic frequencies, genetic diversity index (percentage of polymorphic loci (P), apparent number of alleles (Na), effective number of alleles (Ne), observed heterozygosis (Ho), expected heterozygosis (He), and Shannon Index), Wright's genetic divergence (Fst), gene flow (Nm), and Nei's genetic distances. Analyses of molecular variance (AMOVA), principal components analysis (PCA),

and linear regressions were carried out using the same program.

Results

The RAPD assay resulted in 115 bands, with 88.70 % polymorphic and 11.30 % monomorphic. We obtained on average eight polymorphic bands per primer versus only 1 monomorphic. The *M. dasyclada* populations differed with respect to polymorphisms present (Tab. 2). Higher rates were observed in FLONA III (78.88 %) and Encruzilhada do Sul (74.41 %), followed by Lages (61.42 %). Ronda Alta and Erechim showed similar rates (41.79 % and 44.87 %, respectively).

The genogram originated by RAPD (Fig. 2) showed clusters of plants belonging primarily to the same populations. However, different populations could be approximated by generating two major groups: i) FLONA I, FLONA II and Erechim; and ii) FLONA III and Ronda Alta. Similarity between all plants ranged from 0.47 to 0.90, pointing to high genetic diversity. Separation between the studied populations becomes more evident in the PCA (Fig. 3). FLONA I and FLONA III strongly overlap, while FLONA II is only partially superposed with these two populations. Although Lages and Encruzilhada do Sul are related (Fig. 2), they compose two separate populations (Fig. 3).

Among the 15 isozymes that were active in *M. dasyclada*, about 58.76 % were polymorphic and 41.24 % monomorphic. Higher rates of polymorphic loci were observed in the FLONA populations (73.33 % and 66.67 % for FLONA II and FLONA I/FLONA III, respectively) and lower rates were observed in Erechim (40 %) and Lages (33.33 %). Regarding genetic equity, values for *M. dasyclada* populations were low with the exception of FLONA I (Tab. 3).

The isoenzymatic markers identified loci with two to six alleles. The number of apparent alleles (Na) ranged from 1.667 (FLONA II) to 1.067 (Lages), while number of effective alleles (Ne) ranged from 1.202 to 1.475 (Tab. 4). For these two parameters (Na and Ne), FLONA populations presented the highest values. Shannon index values were also higher in populations from FLONA sites.

Analysis of isozyme data resulted in observed heterozygosity (Ho) being higher than expected (He) in all study populations (Tab. 4). Plants collected in the three FLONA sites and Ronda Alta had higher Ho than other groups.

Analysis of genetic divergence using the Wright index (Fst) (Wright 1951) for isozyme data revealed that the Lages population is the most distant from all others *M. dasyclada* populations, while the FLONA I, FLONA II and FLONA III are very close. Ronda Alta and Erechim also have low genetic divergence (Tab. 5). Gene flow (Nm) is partially in accordance with divergence data, as it demonstrates high degree of similarity between the three FLONA populations, the Erechim and Ronda Alta populations, and the Encruzilhada do Sul and Ronda Alta populations (Tab. 5). According with Wright (Wright 1931), because Nm < 1 all the other populations compared in this study are in genetic isolation. Despite being a dominant marker, the RAPD data were not used for analyses of heterozygosity, divergence, and gene flow.

The AMOVA results indicated that 34 % and 43 % of the genetic variability is inter-populational for RAPD and isoenzymatic data, respectively, while 66 % and 57 % of the variability was intra-populational. The PCA based on isozyme data showed a superposition between the FLONA populations, the group formed by Erechim/Ronda Alta, and the group formed by Encruzilhada do Sul/ Lages (Fig. 4).

Nei´s genetic distances were calculated using RAPD and isozymes data (Tab. 6). We observed a positive correlation between the Nei's distances obtained by isozyme and RAPD data ($p = 0.010$, $R2 = 0.33$). The values of Nei's genetic distance with the RAPD data were more similar among populations than were values from isozyme data. The RAPD analysis indicates greater genetic distances in the populations of Ronda Alta, Lages and Encruzilhada do Sul, particularly in relation to FLONA II and FLONA III. The isozyme data indicates large genetic distances, primarily between Lages and Encruzilhada do Sul in relation to FLONA I, II, and III.

The Lages population has the largest genetic distance in relation to all others except Encruzilhada do Sul, which exhibits short distance from Lages (0.134). The expected lower genetic distance among the three populations of FLONA was best demonstrated by Nei's genetic distance values from isozyme data.

Analysis of isoenzymatic data revealed positive correlations between Nei´s genetic distance and geographic distances of the *M. dasyclada* populations ($p = 0.031$, $R2 = 0.3091$), as well between Nei´s genetic distances and altitude differences ($p = 0.008$, $R2 = 0.4192$). No significant correlation between Nei´s distance and altitude or geographic distances was found using RAPD data.

Table 2. Variation in the polymorphism degree between *Maytenus dasyclada* populations analyzed by RAPD.

	Polymorphic bands	Monomorphic bands	% of Polymorphism
FLONA I	53	36	59.55
FLONA II	53	41	56.38
FLONA III	71	19	78.88
Erechim	35	43	44.87
Ronda Alta	28	39	41.79
Lages	43	27	61.42
Encruzilhada do Sul	64	22	74.41

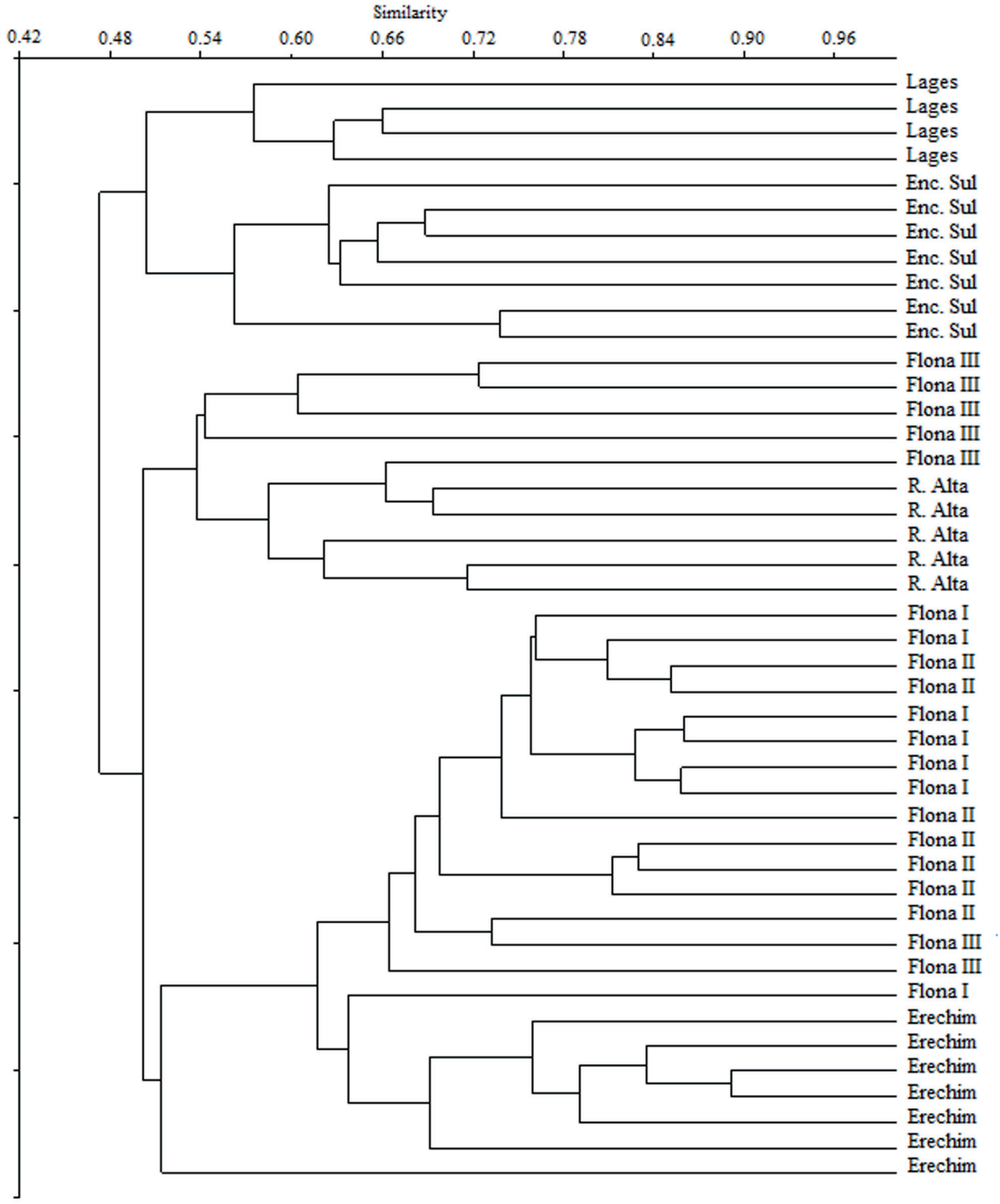

Figure 2. Genogram of seven *Maytenus dasyclada* populations collected in Rio Grande do Sul and Santa Catarina (Brazil) based on RAPD markers.

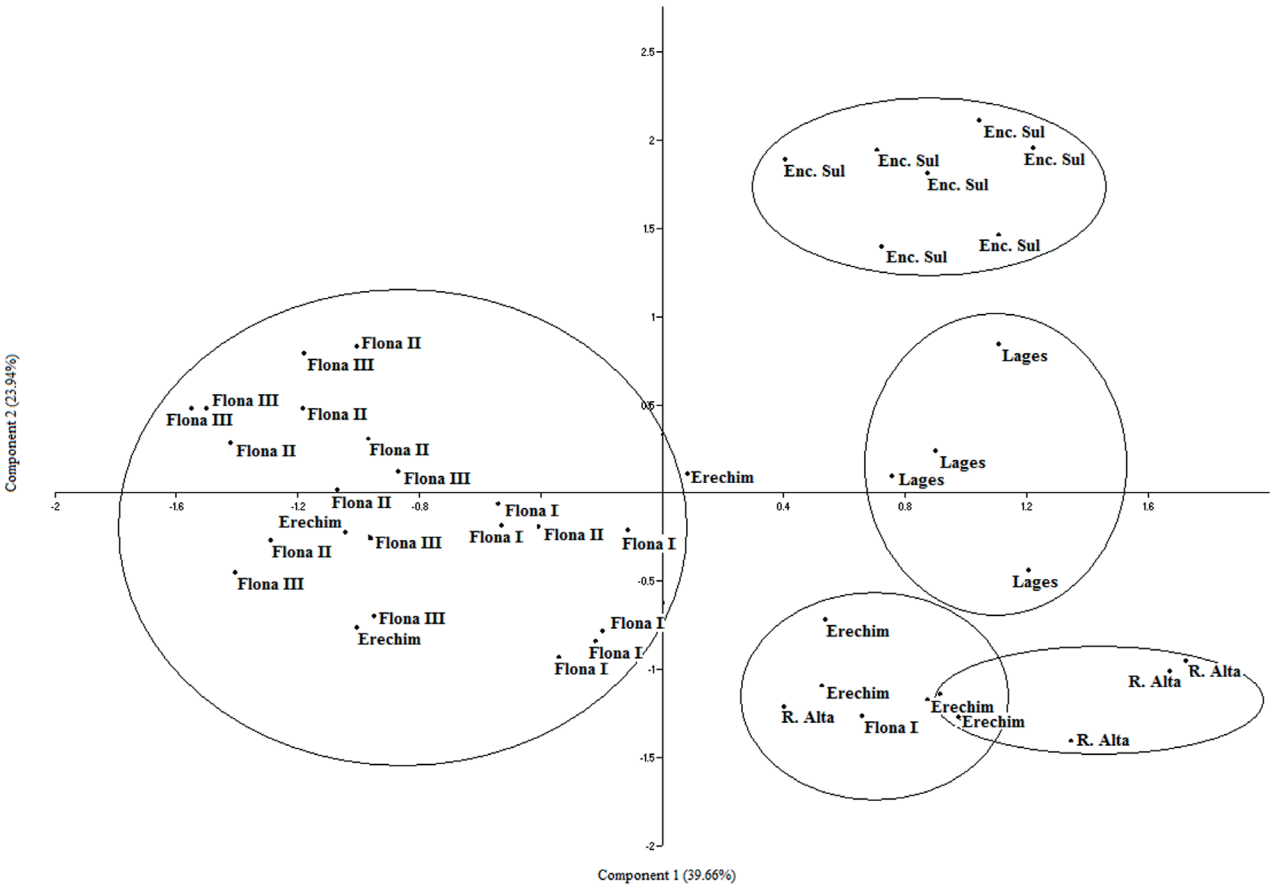

Figure 3. Principal component analyses (PCA) based on RAPD markers in *Maytenus dasyclada* populations collected in Rio Grande do Sul and Santa Catarina (Brazil).

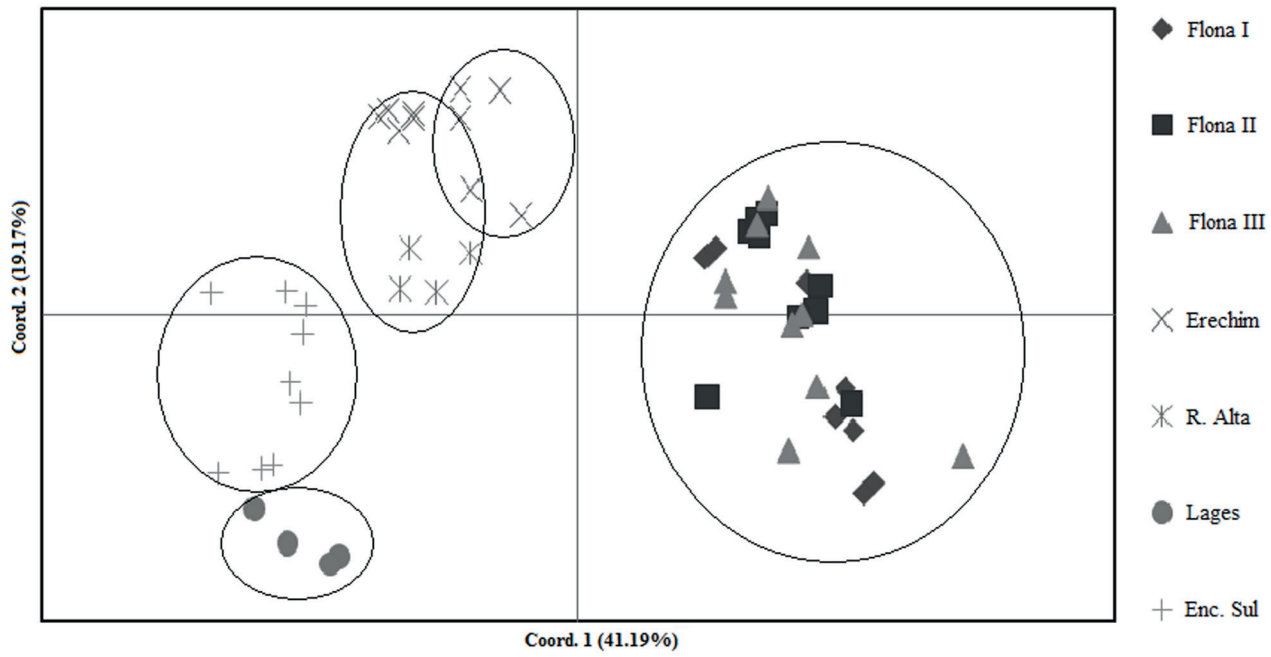

Figure 4. Principal component analyses (PCA) based on isozyme markers in *Maytenus dasyclada* populations collected in Rio Grande do Sul and Santa Catarina (Brazil).

Table 3. Frequency of polymorphic loci and genetic equity in populations of *Maytenus dasyclada* based in isozymes data.

Isozyme data	FLONA I	FLONA II	FLONA III	Erechim	R. Alta	Lages	Enc. Sul
Polymorphism (%)	66.67	73.33	66.67	40.00	53.33	33.33	46.67
Genetic equity (%)	53.33	26.66	33.33	13.33	26.66	20.00	13.33

Table 4. Number of apparent (Na) and effective (Ne) alleles, expected (He) and observed (Ho) heterozygosity and Shannon index in populations of *Maytenus dasyclada*, sampling in Rio Grande do Sul and Santa Catarina (Brazil) based in isozymes data.

Isozyme data	FLONAI	FLONAII	FLONAIII	Erechim	R.Alta	Lages	Enc. Sul
Na	1.600	1.667	1.600	1.400	1.533	1.067	1.400
Ne	1.475	1.431	1.388	1.202	1.389	0.957	1.244
He	0.288	0.280	0.260	0.120	0.217	0.123	0.181
Ho	0.304	0.295	0.276	0.127	0.242	0.136	0.192
Shannon	0.412	0.411	0.381	0.184	0.315	0.181	0.268

Table 5. Genetic divergence (Fst) and flow gene (Nm) index in populations of *Maytenus dasyclada*, sampling in Rio Grande do Sul and Santa Catarina (Brazil) based in isozymes data.

	FLONAI		FLONAII		FLONAIII		Erechim		R. Alta		Lages	
	Fst	Nm	Fst	Nm	Fst	Nm	Fst	Nm	Fst	Nm	Fst	Nm
FLONAII	0.111	1.996	0.000	0.000								
FLONAIII	0.158	1.332	0.120	1.837	0.000	0.000						
Erechim	0.257	0.725	0.212	0.932	0.252	0.742	0.000	0.000				
R. Alta	0.277	0.653	0.231	0.834	0.273	0.665	0.139	1.550	0.000	0.000		
Lages	0.467	0.285	0.425	0.338	0.470	0.282	0.518	0.232	0.442	0.315	0.000	0.000
Enc. Sul	0.346	0.473	0.307	0.564	0.360	0.444	0.294	0.601	0.184	1.110	0.394	0.385

Table 6. Values of Nei´s genetic distance in populations of *Maytenus dasyclada* based in RAPD and isozyme data.

RAPD data	FLONAI	FLONAII	FLONAIII	Erechim	R. Alta	Lages
FLONAII	0.112	0.000				
FLONAIII	0.157	0.091	0.000			
Erechim	0.100	0.129	0.172	0.000		
Ronda Alta	0.160	0.267	0.324	0.098	0.000	
Lages	0.162	0.212	0.255	0.129	0.159	0.000
Enc. Sul	0.183	0.239	0.257	0.132	0.169	0.139
Isozyme data	**FLONAI**	**FLONAII**	**FLONAIII**	**Erechim**	**R. Alta**	**Lages**
FLONAII	0.025	0.000				
FLONAIII	0.072	0.033	0.000			
Erechim	0.209	0.166	0.213	0.000		
Ronda Alta	0.294	0.236	0.294	0.069	0.000	
Lages	0.513	0.478	0.571	0.342	0.243	0.000
Enc. Sul	0.362	0.333	0.418	0.220	0.101	0.134

Discussion

The genus *Maytenus* is a group of plants with pharmacological and antioxidant properties (Corsino *et al*. 2003; Pereira *et al*. 2005; Tiberti *et al*. 2007; Santos-Oliveira *et al*. 2009; Cansian *et al*. 2015; Santoyo *et al*. 2015; Bevenutti *et al*. 2016). *Maytenus dasyclada* in particular is typical species of Araucaria forest that occurs only in Brazil and Uruguay (Carvalho-Okano & Leitão-Filho 2004), for which knowledge is lacking about its genetic properties and potential economic value. In this work, the structure and genetic diversity of *M. dasyclada* was investigated in seven natural populations of Rio Grande do Sul and Santa Catarina (Brazil), using RAPD and isozymes markers.

The two markers analyzed pointed to relatively high degree of polymorphism in FLONA populations, and moderate degree of polymorphism in Erechim and Ronda Alta. In the Lages and Encruzilhada do Sul populations, isozyme analysis revealed a very low degree of polymorphism compared to RAPD analysis (Tabs. 2, 3). The RAPD marker generated different groups of populations both in the genogram and the PCA (Figs. 2, 3), as well as for groups formed in the isozyme PCA (Fig. 4). PCAs from both RAPD and isozyme data confirmed the grouping tendency among the FLONA populations shown in the genogram. Differences in these two markers are well described in the literature. Royo & Itoiz (2004) and Aguiar *et al*. (2015) argue that RAPD

has greater discrimination capacity between individuals of the same species compared to isozymes, although the two markers lead to similar taxonomic clusters.

Parameter values (Na, Ne, Shannon index, He and Ho) for *M. dasyclada* collected in FLONA (I, II and III) indicate higher genetic variability in these populations (Silva *et al*. 2016) (Tab. 4). Moreover, the FLONA populations are very close to each other and potentially experience significant gene flow (Tab. 5), and may even be considered as a single population due to geographical proximity (e.g., as was suggested by Mossi *et al*. (2009) for *M. ilicifolia*). In contrast, the Lages population overall seems to have less variability (Tab. 4). The Lages population was the also most distant in relation to the other groups, with which there is no gene flow (Tab. 5). We do, however, speculate that these lower values could have been caused by the low number of specimens sampled this population (n = 4).

Lower Nei´s distance values were observed between FLONA samples (Tab. 6). This result generally agrees with results from Sahyun (2007) in a study of three *M. aquifolium* populations, in which they encountered distance values ranging from 0.034 to 0.961. Bessega *et al*. (2000) also reported positive correlations between geographic distance, altitude, and genetic distances obtained with isoenzymatic data that were absent in the analysis of RAPD data. Greater geographic or altitudinal distances resulted in greater genetic distances in all cases except Lages and Encruzilhada do Sul. The lack of correlation in these populations may be partly explained by the low number of plants collected in the Lages population. Low sample size may also justify the result of genetic flow between Ronda Alta and Encruzilhada do Sul populations, which should be low, due to geographic distance. However, Bessega (1997) also observed high levels of genetic similarity among *P. glandulosa* and other South American species, despite geographical isolation.

FLONA is a forest reserve, and the elevated indices of variability and polymorphism in *M. dasyclada* populations is indicative of a good state of genetic conservation. The percentage of polymorphisms was also high in FLONA II, an area that has been reforested with *Pinus* spp. These results together demonstrate the importance of preserved lands for the maintenance of native plants. On the other hand, sites with lower indices of polymorphism, especially in Ronda Alta, Lages and Erechim, have experienced loss of genetic diversity. This loss of diversity is likely related to habitat fragmentation (Pautasso 2009). This is potentially reversible through introduction of genetically distinct individuals such as those from FLONA I, II or III populations, as they form a cluster of plants belonging primarily to the same populations (Fig. 2) and do not overlap with the other analyzed groups (Figs. 3, 4).

In forest trees, high genetic diversity is better maintained within populations than among them (Hamrick 2004; Scotti *et al*. 2016), and results from the current study *M. dasyclada* are in concordance with this notion. This characteristic could be due to having effective pollen dispersal, which facilities gene flow (Kremer *et al*. 2012). Plants in the genus *Maytenus* are highly sought after by pollinators such as bees, ants, and other small insects, and seeds undergo zoochoric dispersal by birds and small monkeys (Lorenzi 2002; Catharino *et al*. 2005; Zipparro *et al*. 2005; Carvalho 2010). *Maytenus dasyclada* in preserved areas may have increased rates of pollination and seed dispersal, as the animals that carry out these services benefit from the same habitat protection. In these sense, conditions in the FLONA Preservation Park may facilitate maintenance of greater genetic diversity in *M. dasyclada*.

Implications for conservation

The low genetic diversity of many *M. dasyclada* populations in remaining unprotected forest could lead to potential risk of extinction, primarily due to continued fragmentation of the Atlantic forest (with particular emphasis on Araucaria forest).

The results of current study point to the relevance of preservation areas for conservation of *M. dasyclada*. Studies around the word have reinforced the importance and needs of adequate management of protected areas for conserving plant genetic resources (Koskela *et al*. 2013; Maxted 2003; Volis 2016), particularly for tropical plants (Pautasso 2009; Howe 2014). Knowledge of ecological and genetic characteristics of plant species may inform novel approaches for conservation planning, and may aid in identification of areas that best complement the established natural reserves (Prado *et al*. 2010; Silva *et al*. 2016). This study showed that although the Ronda Alta population showed a low degree of polymorphism (Tabs. 3, 6), it did present indicators of general variability, with parameter values approaching those of FLONA populations (Tab. 4). In these sense, Ronda Alta could be a target to future policies that aim to enlarge the *M. dasyclada* preservation area. In general, we emphasize the high rates of observed heterozygosity, higher than expected population heterozygosity (including in non-protected areas), and high structuring of populations, as these results indicate strong genetic diversity which must be preserved for the conservation this species.

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