Brazilian Journal of Botany (2020) 43:139–145
https://doi.org/10.1007/s40415-020-00586-8

ORIGINAL ARTICLE

Development and transferability of microsatellite markers for a complex of *Aspidosperma* Mart. & Zucc. (Apocynaceae) species from South American Seasonally Dry Tropical Forests

Patrícia Aparecida Messias[1] · Fábio Matos Alves[2] · Fábio Pinheiro[2] · Anete Pereira De Souza[2] · Ingrid Koch[2]

Received: 13 September 2019 / Revised: 26 November 2019 / Accepted: 23 January 2020 / Published online: 2 March 2020

Abstract
In this study, we developed and characterized microsatellite for *Aspidosperma pyrifolium* Mart. & Zucc., considered a species complex due to its morphological variation and disjunct distribution. This complex is predominant in the "Caatinga" of Northeast Brazil, but also occurs in semi-deciduous seasonal forests of central Brazil, in Chaco areas of the southwestern Brazil, Bolivia and Paraguay. These vegetations are included in the concept of Seasonally Dry Tropical Forests (SDTFs). SDTFs are considered one of the most endangered forests in the world due to intense anthropic pressure and high endemism. Concerning endemic species of SDTFs, little is known about the genetic structure of *A. pyrifolium* and the effects of disjunct distribution on their gene flow and genetic diversity. In this study, 16 polymorphic and four monomorphic microsatellites were characterized in 82 *Aspidosperma pyrifolium* individuals from three populations in Brazil's Northeast. The transferability resulted in an average of seven markers, amplified in 11 other species of the genus (*A. brasiliense* A.S.S. Pereira & A.C.D. Castello, *A. illustre* (Vell.) Kuhlm. & Pirajá, *A. inundatum* Ducke, *A. multiflorum* A.DC., *A. nobile* Müll.Arg., *A. polyneuron* Müll.Arg., *A. quebracho-blanco* Schltdl., *A. ramiflorum* Müll.Arg., *A. rigidum* Rusby, *A. schultesii* Woodson and *A. subincanum* Mart.). These markers can be used to estimate genetic parameters and help us to better understand the boundaries within *A. pyrifolium* complex. Their use can also be extended for conservation purposes of *A. pyrifolium* populations and consequently of SDTFs. Moreover, they may be applied in studies concerning other species of the genus.

Keywords *Aspidosperma pyrifolium* · "Caatinga" · Chaco · SDTF · SSR

1 Introduction

The Seasonally Dry Tropical Forests (SDTFs) are deciduous and semi-deciduous forests that occur in fertile soils, with pH ranging from neutral to basic and a precipitation average of less than 1800 mm per year, reaching less than 100 mm per month in the driest periods, which last 3–6 months (Murphy and Lugo 1986; Gentry 1995; Pennington et al. 2009; Dryflor 2016). They are widely distributed throughout the South America tropical region, occurring from "Caatingas" of Brazilian Northeast to the Chaco region in Northern of Argentina, Paraguay and Bolivia plus some areas in the dry Andean valleys (Prado and Gibbs 1993) and small spots in the Cerrado and Amazon biomes (Prado and Gibbs 1993; Pennington et al. 2000; Dryflor 2016).

SDTFs conservation has been historically neglected. These forests suffer from intense human occupation and use, which contributes for this type of system be recognized as one of the most threatened in the world. Currently, only 10% of their original extension remains and very few protected areas are established (Miles et al. 2006; Dryflor 2016). The Brazilian "Caatinga", a biome with great potential for ecosystem services, had 46% of its area deforested due to agriculture and illegal logging and only 7.8% is currently under protection of Conservation Units (Brasil 2018). The small portion of the Chaco biome located in Brazilian territory (the majority is in Argentina, Paraguay and Bolivia) is not protected by any Conservation Units. By 2008, 36% of its original coverage had already been destroyed, mainly by

✉ Patrícia Aparecida Messias
pabioufscar@gmail.com

[1] Postgraduate Program in Biological Sciences (Botany), Department of Botany, Institute of Bioscience, University of State São Paulo "Júlio de Mesquita Filho", Botucatu, SP 18618-689, Brazil

[2] Plant Biology Department, Institute of Biology, University of Campinas, Campinas, SP 13083-862, Brazil

extensive cattle raising (Alves 2014). Moreover, each biome fragment is virtually unique since up to 73% of their tree and shrub species seems to be exclusive (Dryflor 2016).

Aspidosperma pyrifolium Mart. & Zucc., popularly known in Northeast Brazil as "Pau-Pereiro, Pereiro or Pereiro-de-Saia" (Marcondes-Ferreira 1988) is regarded as a Caatinga environmental indicator (Veloso et al. 1991), and it is one of the endemic species of SDTFs in South American continent. It has a disjunct geographical distribution with most of its populations in the "Caatinga" (Northeast of Brazil) and others in Semi-deciduous Seasonal Forest areas of central Brazil and Chaco (Mato Grosso do Sul, Paraguay and Bolivia) (Woodson 1951; Marcondes-Ferreira 1988). There is a close relation between the morphological variation of this species and its geographic distribution; hence, it is treated here as the "*A. pyrifolium* complex" (Grube and Kroken 2000). It is an interesting species complex to have its genetic diversity evaluated once these results can bring arguments that contribute to decision making in conservation policies of both species complex and the forests where it occurs (Conner and Hartl 2004). Besides that, knowing the genetic diversity can also support decisions concerning species delimitation within the complex and elucidate part of their demographic histories. In this sense, informative genetic markers such as microsatellites are very powerful tools to estimate genetic diversity of natural populations. Microsatellites are highly polymorphic, show codominant inheritance and high mutation rates and, the best, are inexpensive and easily developed and genotyped (Alves et al. 2014; Chen et al. 2017; Lee et al. 2017).

In this study, we developed and characterized microsatellite markers for *Aspidosperma pyrifolium* and tested its interspecies transferability within the genus, in order to support studies on genetic diversity, population structure, phylogeography and conservation of both species and SDTFs.

2 Materials and methods

Sampling and DNA extraction – Leaf samples from three natural populations of *A. pyrifolium* populations were collected in the Northeast region of Brazil: 30 individuals from Aiuabá (AI), and 27 from Crateús (CR), both in the state of Ceará, and 25 individuals from Cabrobó (CA), state of Pernambuco (Fig. 1).

The vouchers were deposited in the herbarium of the Institute of Biosciences of Botucatu (BOTU). Genomic DNA

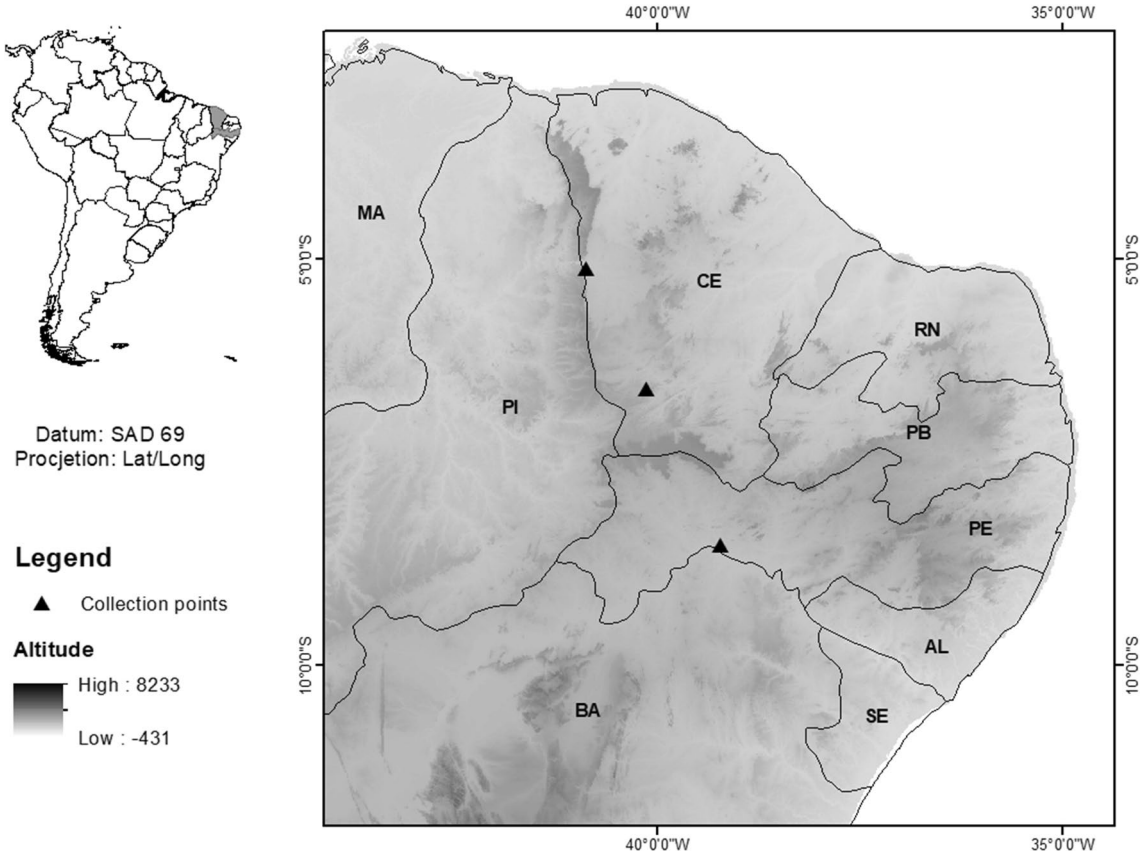

Fig. 1 Localities of the populations collected for the characterization of the microsatellite markers

was extracted using 20 mg dry leaves on silica, following the modified protocol proposed by Doyle (1991). Quantification was performed with the Nanodrop spectrophotometer.

Another 11 species of the genus were tested for transferability: *A. brasiliense* A.S.S. Pereira & A.C.D. Castello, *A. illustre* (Vell.) Kuhlm. & Pirajá, *A. inundatum* Ducke, *A. multiflorum* A.DC., *A. nobile* Müll.Arg., *A. polyneuron* Müll.Arg., *A. quebracho-blanco* Schltdl., *A. ramiflorum* Müll.Arg., *A. rigidum* Rusby, *A. schultesii* Woodson and *A. subincanum* Mart. ("Appendix 1"). Uniform extraction and quantification procedures were performed in one individual of each selected species.

Genomic library building and primer design – The microsatellite library was built following the modified protocol of Billotte et al. (1999). DNA was digested using *Afa I* enzyme (10u μL^{-1}), ligated to Rsa21 (10 µM) and Rsa25 (10 µM) adapters, and then amplified and purified. The PCR's product was enriched using magnetic beads and hybridized with $(CT)_8$ and $(GT)_8$ oligonucleotides. The selected fragments were amplified and quantified. Cloning of the fragments was performed with the pGem-T vector. The competent cells with the selected fragments were subjected to transformation through XL1-BLUE electroporation and cultivated in agar. Clones were selected in an Elisa plaque. Plasmid extraction was performed, and then the sequencing kit was added.

Chromas software v.2.6.5 was used for primer design (Technelysium—https://technelysium.com.au/wp/chromas/), while *Chromatogram Explorer* (Heracle BioSoft 2015) was employed to remove the adapters and perform sequence trimming. The clone consensus sequence was generated in *CAP3* (Huang and Madan 1999). *VecScreen* (NCBI—https://www.ncbi.nlm.nih.gov/tools/vecscreen/about/) was used to search for vector segments, and *BLAST* (NCBI—https://blast.ncbi.nlm.nih.gov/Blast.cgi) to check for material contamination. Microsatellites were identified using *SSRIT* software (Temnykh et al. 2001), while primers were designed in *Primer3 Plus* (Untergasser et al. 2012).

Microsatellite validation and characterization – The concentration of the genomic DNA used for amplification was 5 ng μl^{-1}. Polymerase chain reaction (PCR) employed 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM dNTPs, 0.2 mM dNTP, 10 mg ml^{-1} BSA, 0.5 mM of each primer, 1U Taq DNA (Invitrogen, Carlsbad, California, USA) and 15 ng DNA. The final volume of each reaction was 12 µl (Mili-Q Water). The temperature cycle used in the PCR was: initial denaturation at 94 °C for 3 min, followed by 34 cycles at 94 °C for 1 min, 60 °C for 1 min, 72° C for 1 min, and final elongation at 72 °C for 8 min. The PCR was first performed at 60 °C, and those that failed were tested at a temperature of 55 °C. Amplification was evaluated in 2% agarose gel. Genotyping was performed using vertical electrophoresis, with a 6% polyacrylamide gel; band staining was done with silver nitrate. We used 10 bp DNA Ladder (Invitrogen, Carlsbad, CA) to estimate fragment sizes.

For the characterization of the primers, we calculated expected (H_e) and observed (H_o) heterozygosity, using GDA software (Lewis and Zaykin 2000), and polymorphism information content (PIC), using Molkin v.3.0 software (Gutiérrez et al. 2005). Hardy–Weinberg equilibrium (HWE), fixation index (F) and linkage disequilibrium (LD) were estimated using GENEPOP v. 4.2 software (Rousset 2008). Free NA was employed to calculate Null allele frequency (NA) (Chapuis and Estoup 2007). The Bonferroni correction was applied to all P values (Rice 1989).

3 Results

We sequenced 48 clones and among which 22 had SSR. We were able to draw 31 primer pairs. All were successfully amplified, resulting in 16 polymorphic and four monomorphic markers (Apy_08, Apy_18, Apy_19 and Apy_20) for *A. pyrifolium*. Motifs were for the most part dinucleotides, divided into: nine simple, five perfect compounds, and four imperfect compounds. Two trinucleotides also occurred. Primer length ranged from 20 to 25 bp, and the amplified products ranged from 105 (Apy_13) to 290 bp (Apy17) (Table 1).

Transferability resulted in an average of seven amplified markers for the tested species. The markers Apy_01, Apy_02, Apy_05, Apy_07 and Apy_16 were amplified in all 11 species, while Apy_08, Apy_14 and Apy_17 failed to amplify. The species with the highest amplification rates were *A. schultesii* and *A. subincanum*, both with 16 markers, and the species with the lowest amplification was *A. illustre*, with seven ("Appendix 1").

The number of alleles found for *A. pyrifolium* ranged among loci from 2 and 15, PIC values ranged from 0.115 to 0.910, (H_o) ranged from 0 to 1 and H_e ranged from 0.081 to 0.924, with mean H_o values of 0.612, 0.607 and 0.540, and mean H_e values of 0.657, 0.661 and 0.619, for the AI, CR and CA populations, respectively (Table 2). The fixation index (F) ranged among loci from −0.600 and 1, and a mean of 0.069, 0.082 and 0.129 for the AI, CR and CA populations, respectively. Deviation of Hardy–Weinberg equilibrium was observed in some populations in the Apy_02, Apy_09, Apy_11, Apy_12, Apy_14, Apy_15, and Apy_17 loci (P value = 0.003, Bonferroni-corrected). Evidence of null alleles occurred only in the Apy_02 loci in the AI and CA populations, and in the Apy_14 in the AI and CR populations. Linkage disequilibrium (LD) was not significant for any loci (P value = 0.000417, Bonferroni correction).

Table 1 Primers developed for *Aspidosperma pyrifolium* Mart. & Zucc.

Marker	Primer sequence (5′–3′)	Motif	Ta (°C)	Range size (bp)	GenBank
Apy_01	F: TCTCCCTCGTTACTCCGTAT R: TCCTTCCTTACCCTCTGTGA	$(GT)_8$	60	157	MK425611
Apy_02	F: GCTGGTAGTGAAGGGACAGG R: TCATTGAACTTCCCCCTGCC	$(GT)_6$ $(TG)_5$ $(GA)_{11}(CGGA)_3$	55	278	MK425612
Apy_03	F: TCCAGCTGGGAATGAGTTTT R: CGAAGGCAAGAAAATGACGT	$(AG)_{32}$	60	260	MK425613
Apy_04	F: TGTTCTCTTCTCCTCGCTTC R: CAAACTCGCCATTACTGCAA	$(TG)_8$	60	241	MK425614
Apy_05	F: GGCTTTTTACACCAAAACCT R: GTATGGAATTTTGGTGGGGC	$(CT)_{19}$	60	176	MK425615
Apy_06	F: ACAGTAGTGATATTTGCGCT R: AAGGCTGTTAATGCTACCCA	$(CT)_{25}$	60	249	MK425616
Apy_07	F: CCGATCGAAGAGGATGTAGG R: CCACTGTGTCATACTTTGGC	$(CT)_{10}$ $(CA)_{11}$	60	257	MK425617
Apy_08	F: TCTTGTGACGAAGGTTCACA R: TTGACTTGAGCAGACAACCT	$(TG)_7$	55	238	MK425618
Apy_09	F: CTTCTGCAATCTTAAGAAGGG R: CGGCAACCTGAAAATGTGAT	$(GT)_{10}(G)_{10}$	60	179	MK425619
Apy_10	F: GCAGCTGGAGAATTTTGCACT R: ATGTCCAGGGGGTTGTGTAT	$(CT)_9$ $(TC)_{10}$	60	237	MK425620
Apy_11	F: TAACGAGGCCCCACAATAGC R: CCTGTGATGCAAGGGAAGGT	$(GT)_{10}$… $(GA)_{15}$ $(A)_9$	60	249	MK425621
Apy_12	F: GCTGGTCTTGTGGGACTTGA R: TCCTAGCCCGTTCTCATCCA	$(TGA)_4$… $(GT)_3$	60	257	MK425622
Apy_13	F: ACCTTTTGCTTGCTTTAGAAAAAGT R: CACGGCTTCCAGACTGTCTT	$(TG)_{10}$	55	105	MK425623
Apy_14	F: AGCAATTCAACATCCAAGCA R: AGATCTTTTTCGCACTCTCTC	$(GT)_9$	60	211	MK425624
Apy_15	F: AGAGTCTATTTTTAATCTCTGTCCA R: TCCACTTCTAAACCAGGCCA	$(TG)_{11}$	60	282	MK425625
Apy_16	F: GCGTAGTTTTAAGATCTTTTTCGCA R: GGGATGGTAAGAAGAGGGCG	$(CT)_3(CA)_9$	60	177	MK425626
Apy_17	F: TGCTTGTTTCCATGGTTGCT R: CCTAAGGGTTCAGGGCGATT	$(CT)_4$… $(CT)_3$… $(CT)_4$	55	290	MK425627
Apy_18	F: TGCCATTTAGGAGGAATCAATG R: TGAACCCTGTGAATTGCGTG	$(AG)_4$… $(AG)_4$	60	226	MK425628
Apy_19	F: TCAAGTTTTGCAATTACCTGCC R: TCCGGATGTTGCAGAGCTAC	$(TGA)_3$	60	178	MK425629
Apy_20	F: GAAACAACTCTCTGCACGGG R: AATTCACGACCCCAGGAG	$(ATC)_3$	60	151	MK425630

4 Discussion

The recovery of microsatellites in the sequenced clones had great results, with a success rate of about 65%, much higher than the 2.3% average plant recovery ratio (Zane et al. 2002). Primer amplification and transferability were also successful, with all primers synthesized for *Aspidosperma pyrifolium* individuals successfully amplified, and as much as 16 primers for other species of the genus.

Among the 16 polymorphic markers of *Aspidosperma pyrifolium* populations, PIC pointed out that 81.2% markers developed have high polymorphism, according to Chen et al. classification (2017). This result agrees with Bhargava and Fuentes (2010), who associated more repetition with higher mutation rates. Some locus indicated excess heterozygotes, with higher

Table 2 Genetic parameters for each locus in *Aspidosperma pyrifolium* populations

LOCUS	AI ($N=30$)						CR ($N=27$)						CA ($N=25$)						
	A	H_o	H_e	F	NA	HW	A	H_o	H_e	F	NA	HW	A	H_o	H_e	F	NA	HW	PIC
APY_01	2	**0.166**	0.155	−0.074	0	1	2	0	0.088	1	0.142	0.023	2	0	0.102	1	0.152	0.028	0.115[a]
APY_02	6	0.416	0.775	0.468	0.203*	0.000**	4	0.444	0.582	0.242	0.061	0.227	4	0.238	0.6	0.609	0.235*	0.001**	0.677[c]
APY_03	12	0.666	0.818	0.188	0.07	0.015	13	0.826	0.877	0.059	0.025	0.24	14	**1**	0.908	−0.102	0	0.924	0.865[c]
APY_04	15	0.814	0.888	0.084	0	0.037	12	0.666	0.83	0.201	0.104	0.051	12	0.6	0.761	0.215	0.094	0.103	0.875[c]
APY_05	5	**0.724**	0.69	−0.049	0	0.004	6	**0.894**	0.648	−0.394	0	0.014	7	0.59	0.693	0.15	0.007	0.514	0.653[c]
APY_06	13	**0.966**	0.881	−0.097	0	0.3	15	0.857	0.903	0.052	0	0.837	14	0.818	0.924	0.117	0.034	0.156	0.910[c]
APY_07	10	0.533	0.658	0.193	0.083	0.077	10	0.681	0.801	0.152	0.073	0.374	11	0.608	0.807	0.25	0.103	0.009	0.742[c]
APY_09	4	**0.633**	0.575	−0.103	0	0.056	5	0.555	0.65	0.15	0.017	0.114	5	0.391	0.723	0.464	0.182	0.000**	0.623[c]
APY_10	9	0.666	0.703	0.052	0.053	0.076	7	0.652	0.764	0.149	0.077	0.022	9	0.75	0.828	0.096	0.053	0.085	0.782[c]
APY_11	10	0.655	0.783	0.166	0.079	0.095	13	0.8	0.905	0.118	0.024	0.001**	8	0.625	0.843	0.262	0.103	0.04	0.908[c]
APY_12	3	0.166	0.409	0.597	0.158	0.000**	3	0.35	0.619	0.441	0.153	0.000**	2	0.125	0.19	0.349	0.084	0.203	0.383[b]
APY_13	4	**0.9**	0.648	−0.398	0	0.004	3	**0.608**	0.491	−0.244	0	0.171	4	**0.666**	0.507	−0.321	0	0.317	0.505[c]
APY_14	4	0.269	0.671	0.604	0.244*	0.000**	4	0.133	0.531	0.755	0.269*	0.000**	5	0.619	0.632	0.022	0.031	0.566	0.621[c]
APY_15	5	**0.827**	0.695	−0.194	0	0.000**	5	0.772	0.764	−0.011	0.006	0.003**	5	**0.75**	0.676	−0.111	0	0.013	0.711[c]
APY_16	5	0.633	0.681	0.072	0.026	0.139	7	**0.72**	0.647	−0.114	0.026	0.119	6	**0.791**	0.635	−0.252	0	0.396	0.603[c]
APY_17	2	**0.766**	0.494	−0.565	0	0.002**	2	**0.76**	0.48	−0.6	0	0.004	2	**0.083**	0.081	−0.022	0	1	0.370[b]
Mean		0.612	0.657	0.069				0.607	0.661	0.082				0.54	0.619	0.129			

Aiuabá = AI, Crateús = CR, and Cabrobó = CA; N is the sample size; A is the total number of alleles; H_o is the observed heterozygosity; H_e is the heterozygosity expected; in bold, H_o values greater than H_e; F is the fixation index; NA is the null allele frequencies; HW is the Hardy–Weinberg equilibrium test; PIC is the polymorphic information content

*Values above 20%

**Significant values using Bonferroni correction (P value is 5% = 0.003125)

[a]Values of low polymorphism (PIC < 0.25)

[b]Values of moderate polymorphism (0.25 ≤ PIC < 0.50)

[c]High polymorphism values (PIC ≥ 0.50)

H_o values in comparison with H_e (Apy_01, Apy_05, Apy_06, Apy_09, Apy_15 and Apy_17). The excess of heterozygotes was also confirmed by *F* values significantly lower than zero for Apy_15 and Apy_17 loci in AI population and Apy_15 locus in CR population. In contrast, *F* values were significantly greater than zero for Apy_02, Apy_12, and Apy_14 loci in AI population, Apy_11, Apy_12, and Apy_14 in CR population, and Apy_02 and Apy_09 in CA population, indicating inbreeding.

The evidence of null alleles in Apy_02 and Apy_14 merits attention, as these were significant in the HWE due to the low value of H_o and high F_{IS}. However, we cannot fail to point out that amplification errors may lead to false null alleles (Foucault et al. 1996). In conclusion, we highlight that the success of the amplifications seen here, as well as the high polymorphism and good indicators used in the descriptive statistics of primer sets for the studied species, show that these new markers have great potential as tools for future studies involving diversity, genetic structure and conservation of their populations and, consequently, for SDTFs. In addition, they may be used in studies on other species of the genus.

Acknowledgements To the Coordination for the Improvement of Higher Education Personnel (CAPES) for funding this work by means of a scholarship provided to PAM. To the Support Fund for Teaching, Research and Extension (FAEPEX—Process No. 519.292) for granting funding for sequencing of library clones and field trips to the Northeast. To *Espaço da Escrita – Pró-Reitoria de Pesquisa – UNICAMP* by English review. To the employees of the Aiuaba Ecological Park for their help during collections. To Ana Carolina Castello Devides for her assistance during routine work in the Laboratory of Molecular Systematics. To Danilo Augusto Sforça and Aline da Costa Lima Moraes for their assistance during routine work in the LAGM.

Authors' contribution PAM contributed to study design, field sample collection, laboratory procedure execution, data analysis, and writing of the manuscript. FMA contributed to assistance with laboratory procedures, data analysis, and writing of the manuscript. FP and IK contributed to study design and writing of the manuscript. APS was involved in supervision of laboratory procedures, methodological support and laboratory equipment provision.

Appendix 1

See Table 3.

Table 3 Transferability of microsatellites to species of the genus *Aspidosperma*

	Apy_01	Apy_02	Apy_03	Apy_04	Apy_05	Apy_06	Apy_07	Apy_08	Apy_09	Apy_10
A. multiflorum	+	+	+	+	+	+	+	–	–	+
A. brasiliense	+	+	+	+	+	+	+	–	+	–
A. nobile	+	+	–	–	+	–	+	–	+	–
A. polyneuron	+	+	+	+	+	–	+	–	+	+
A. quebrabro-blanco	+	+	–	–	+	–	+	–	+	–
A. ramiflorum	+	+	+	+	+	–	+	–	–	–
A. inundatum	+	+	+	+	+	–	+	–	+	–
A. rigidum	+	+	+	+	+	+	+	–	+	+
A. schultesii	+	+	+	+	+	+	+	–	+	+
A. illustre	+	+	–	+	+	–	+	–	–	–
A. subincanum	+	+	+	+	+	+	+	–	+	+
Total	11	11	8	9	11	5	11	0	8	5

	Apy_11	Apy_12	Apy_13	Apy_14	Apy_15	Apy_16	Apy_17	Apy_18	Apy_19	Apy_20	Total
A. multiflorum	+	+	–	–	–	+	–	+	+	–	13
A. brasiliense	+	+	–	–	–	+	–	+	+	–	13
A. nobile	+	–	+	–	–	+	–	+	+	–	10
A. polyneuron	+	–	+	–	–	+	–	–	+	+	12
A. quebrabro-blanco	+	–	+	–	–	+	–	+	+	–	10
A. ramiflorum	+	–	–	–	–	+	–	+	–	–	9
A. inundatum	+	–	+	–	+	+	–	+	+	–	13
A. rigidum	+	+	–	–	–	+	–	+	+	+	14
A. schultesii	+	+	–	–	+	+	–	+	+	+	16
A. illustre	–	–	–	–	–	+	–	+	–	–	7
A. subincanum	+	+	+	–	–	+	–	+	+	+	16
Total	10	5	5	0	2	11	0	10	9	4	

References

Alves FM (2014) Population genetics analysis of *Prosopis rubriflora* Hassler ("Espinheiro") and *Prosopis ruscifolia* Griseb. ("Algaroba") (Leguminosae, Mimosoideae) in remnants of Brazilian Chaco. Thesis, State University of Campinas, Institute of Biology, Campinas, SP

Alves FM, Zucchi MI, Azevedo-Tozzi AMG et al (2014) Characterization of microsatellite markers developed from *Prosopis rubriflora* and *Prosopis ruscifolia* (Leguminosae—Mimosoideae), legume species that are used as models for genetic diversity studies in Chaquenian areas under anthropization in South America. BMC Res Notes 7:1–6. https://doi.org/10.1186/1756-0500-7-375

Bhargava A, Fuentes FF (2010) Mutational dynamics of microsatellites. Mol Biotechnol 44:250–266. https://doi.org/10.1007/s12033-009-9230-4

Billotte N, Lagoda PJL, Risterucci AM et al (1999) Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits (Paris) 54:277–288

Brasil (2018) Caatinga. In: Ministério do Meio Ambient. http://www.mma.gov.br/biomas/caatinga. Accessed 16 Feb 2018

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631. https://doi.org/10.1093/molbev/msl191

Chen FF, Ma CY, Yan LP et al (2017) Isolation and characterization of polymorphic microsatellite markers for the chub mackerel (*Scomber japonicus*) and cross-species amplification in the blue mackerel (*S. australasicus*). Genet Mol Res 16:1–7

Conner JK, Hartl DL (2004) A primer of ecological genetics. Sinauer Associates, Sunderland, MA

Doyle J (1991) DNA protocols for plants. In: Hewitt GM, Johnston AWB, Young JPW (eds) Molecular techniques in taxonomy. NATO ASI series (series H: cell biology), vol 57. Springer, Berlin

Dryflor (2016) Plant diversity patterns in neotropical dry forests and their conservation implications. Science 80:1383–1388. https://doi.org/10.1126/science.aaf5080

Foucault F, Praz F, Jaulin C et al (1996) Experimental limits of PCR analysis of (CA)n repeat alterations. Trends Genet 7:450–452

Gentry HA (1995) Diversity and floristic composition of neotropical dry forests. In: Bullock S, Mooney H, Medina E (eds) In seasonally dry tropical forests. Cambridge University Press, Cambridge, pp 146–194

Grube M, Kroken S (2000) Molecular approaches and the concept of species and species complexes in lichenized fungi. Mycol Res 104:1284–1294

Gutiérrez JP, Royo LJ, Álvarez I et al (2005) Molkin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. J Hered 96:718–721

Heracle BioSoft (2015) Chromatogram explorer. http://www.dnabaser.com/download/chromatogram-explorer/. Accessed 11 Mar 2017

Huang X, Madan A (1999) CAP3: a DNA sequence assembly program. Genome Res 9:868–877

Lee MY, Moon HC, Jeon HS et al (2017) Characterization of 33 microsatellite markers and development of multiplex PCR for yellow-throated marten (*Martes flavigula*). Genet Mol Res 16:1–8. https://doi.org/10.4238/gmr16039854

Lewis P, Zaykin D (2000) Genetic data analysis (GDA): computer program for the analysis of allelic data (software). http://en.bio-soft.net/dna/gda.html

Marcondes-Ferreira W (1988) Aspidosperma Mart., nom. cons. (Apocynaceae): estudos taxonômicos. Universidade Estadual de Campinas, Campinas, SP, p 431

Miles L, Newton AC, DeFries RS et al (2006) A global overview of the conservation status of tropical dry forests. J Biogeogr 33:491–505. https://doi.org/10.1111/j.1365-2699.2005.01424.x

Murphy PG, Lugo AE (1986) Ecology of tropical dry forest. Annu Rev Ecol Syst 17:145–155. https://doi.org/10.1146/annurev.es.17.110186.000435

NCBI. BLAST. https://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed 10 Mar 2017

NCBI. VecScreen. https://www.ncbi.nlm.nih.gov/tools/vecscreen/. Accessed 10 Mar 2017

Pennington RT et al (2000) Neotropical seasonally dry forests and quaternary vegetation changes. J Biogeogr 27:261–273. https://doi.org/10.1046/j.1365-2699.2000.00397.x

Pennington RT, Lavin M, Oliveira-Filho A (2009) Woody plant diversity, evolution, and ecology in the tropics: perspectives from seasonally dry tropical forests. Annu Rev Ecol Evol Syst 40:437–457. https://doi.org/10.1146/annurev.ecolsys.110308.120327

Prado DE, Gibbs PE (1993) Patterns of species distributions in the dry seasonal forests of South America. Ann Mo Bot Gard 80:902. https://doi.org/10.2307/2399937

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225. https://doi.org/10.1111/j.1558-5646.1989.tb04220.x

Rousset F (2008) GENEPOP'007: a complete re-implementation of the gene pop software for Windows and Linux. Mol Ecol Resour 8:103–106

Technelysium. Chromas v.2.6.5—DNA sequencing software. https://technelysium.com.au/wp/chromas/. Accessed 10 Mar 2017

Temnykh S, DeClerck G, Lukashova A et al (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452

Untergasser A, Cutcutache I, Koressaar T et al (2012) Primer3—new capabilities and interfaces. Nucleic Acids Res 40:115

Veloso HP, Rangel Filho ALR, Lima JCA (1991) Classificação da vegetação brasileira adaptada a um sistema universal. IBGE, Departamento de recursos Naturais, Rio de Janeiro

Woodson RE (1951) Studies in the Apocynaceae. VIII. A interim revision of genus *Aspidosperma* Mart. & Zucc. Ann Mo Bot Gard 38:119–206

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16. https://doi.org/10.1046/j.0962-1083.2001.01418.x