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RESEARCH

Genetic diversity and population structure of *Aechmea distichantha* (Bromeliaceae), a widely geographically distributed species in South America

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Abstract

Aechmea distichantha Lemaire (Bromeliaceae) is an epiphytic, rupicolous or terricolous bromeliad, with a wide geographical distribution in the Cerrado, Chaco, and Atlantic Forest phytogeographic domains in South America. In this study, a plastidial DNA region and ten nuclear microsatellite markers were used to estimate the genetic diversity and population structure of nine populations of *Aechmea distichantha* from Brazil. Our results revealed that *A. distichantha* has low-to-moderate plastidial genetic diversity and moderate-to-high nuclear genetic diversity. In addition, a high genetic structure was observed among the *A. distichantha* populations in both genomes, suggesting restricted gene flow via seed and pollen. The high genetic differentiation found among *A. distichantha* populations in different geographical locations might be a consequence of its mixed reproductive system and restricted gene flow. The findings of the present study, with the unique genetic composition of most populations, suggests that in situ conservation is the most appropriate protection measure for these plant populations.

Keywords Atlantic Forest · Brazil · Bromeliad · Cerrado · Chaco · Microsatellite markers

Introduction

The Bromeliaceae family (Poales, 3726 species; Gouda et al. coun. updated) is the largest of the 37 families of angiosperms found exclusively in the Neotropics (Givnish et al. 2014). Its species have undergone recent adaptive radiation, which makes the family an interesting model for studying phylogeographic patterns, reproductive isolation barriers, and diversification in the Neotropics (Givnish et al. 2011; Palma-Silva and Fay 2020).

Aechmea distichantha Lem. (Fig. 1) is a bromeliad with a wide geographical distribution occurring in regions of deciduous, semi-deciduous and ombrophilous forests, in the Cerrado, Chaco, and Atlantic Forest phytogeographic domains, in Brazil, and in the northern region of Argentina, Bolivia, and Paraguay (Smith and Downs 1979; Martinelli et al. 2008; Versieux et al. 2018; Barberis et al. 2020). The species is an epiphytic, rupicolous or terricolous plant with well-developed phytotelma and short stolons (Smith and Downs 1979; Scrok and Varassin 2011; Alvarez et al. 2018). This species reproduces both sexually and asexually (clonal reproduction; Smith and Downs 1979; Scrok and Varassin 2011; Freire et al. 2018), being self-compatible with a

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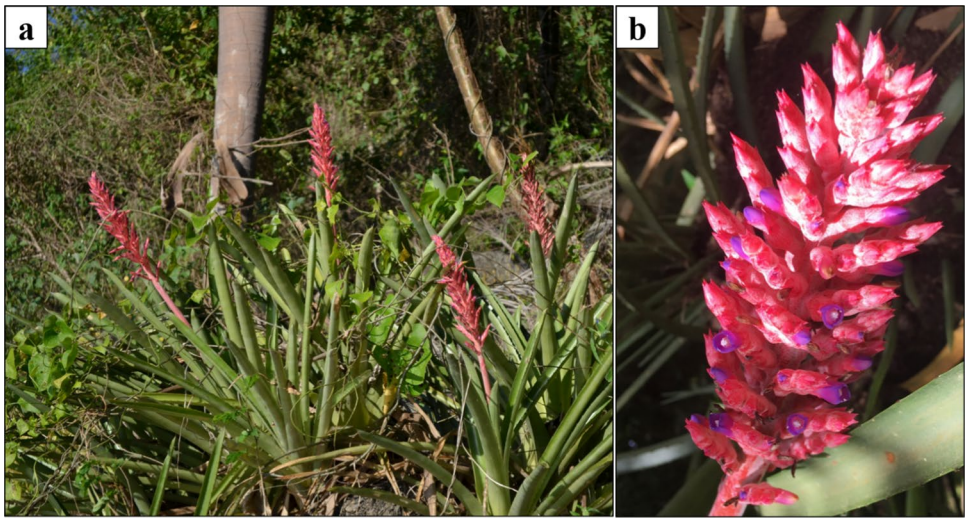

Fig. 1 Individuals of *Aechmea distichantha* from Fecho dos Morros population, Porto Murtinho city, Mato Grosso do Sul state, Brazil. **a** Flowering adult individuals; **b** inflorescence detail. Photographs: FMR Godoy

mixed reproductive system (Scrok and Varassin 2011). The pollinators of *A. distichantha* are hummingbirds, bees, and butterflies (Scrok and Varassin 2011; Freire et al. 2018). Its inflorescence is prevailing pink except for the blueish petals (Smith and Downs 1979; Scrok and Varassin 2011), conferring it an ornamental appeal. Indeed, the species has been used as an ornamental plant, and since it is not commercially produced, it undergoes some level of predatory exploitation due to illegal extraction (Santa-Rosa et al. 2013), which could lead to local extinction of such populations in the future.

The phytogeographic domains in which *A. distichantha* occurs (Atlantic Forest, Cerrado, and Chaco) show great biodiversity, despite that, these three phytogeographic domains have been threatened with habitat loss and high degree of fragmentation (Myers et al. 2000; Ganem et al. 2013; Tomas et al. 2015). For instance, only 12.4% of the original coverage of the Atlantic Forest in Brazil remains intact (SOS Mata Atlântica and INPE 2019). In the Cerrado, despite the existence of laws that protect fauna, flora, and resources such as soil and water, more than 8% of the area has already changed (Alho and Martins 1995; Myers et al. 2000), and only 1.6% is in conservation units (Oliveira and Marquis 2002). The humid Brazilian Chaco region has also suffered from intensive deforestation, especially in the last few decades, only 13% of its original vegetation remained intact (Tomas et al. 2015).

Habitat fragmentation can lead to the subdivision of natural populations, affecting genetic variation, leading to loss of heterozygosity and increased inbreeding, in addition to contributing to the extinction of populations (Brito 2009; Frankham et al. 2019). Studies of the diversity and genetic structure of natural populations allow us to propose actions that can reduce the genetic-demographic consequences of fragmentation (Kettle 2014). For this reason, the investigation of these aspects in natural populations is extremely important for the development of conservation and management strategies, especially for rare and endangered species, with few and/or small populations (Rao and Hodgkin 2002; Frankham et al. 2010, 2019; Hoban et al. 2020, 2021).

There are many ways to study the genetic structure of populations and verify the degree of variability that exists. Plastid DNA (cpDNA) sequences have been very useful to study the diversity and genetic structure of natural populations, as well as to obtain estimates of phylogenetic relationships between different plant species (Turchetto-Zolet et al. 2012; Fava et al. 2021). Plastid DNA is maternally transmitted in most angiosperms, including Bromeliaceae (Ennos 1994; Wagner et al. 2015). This molecule tends to evolve at a very slow pace in relation to the rearrangement of genes and primary sequences, in addition to not undergoing recombination (Hartl and Clark 2010). Furthermore, with sequencing efforts, sufficient genetic variation can be found for phylogenetic approaches in individuals within a particular species (Avise 2009). Among the various molecular markers currently available, microsatellite or SSR (Simple Sequence Repeats) markers also are shown to be a powerful tool for the analysis of genetic diversity in natural plant populations (Vieira et al. 2016; Allendorf 2017). Microsatellites are codominant markers, usually isolated from non-coding and species-specific regions. These markers can be used to help elucidate several questions related to taxonomy, paternity, genetic structure of populations, comparison between species, mating system, gene flow, ecological specialization, and colonization capacity of populations (Boneh et al. 2003; Vieira et al. 2016; Allendorf 2017).

In the past two decades, SSR markers have been successfully used in studies regarding genetic diversity, gene flow, population structure, and hybridization for at least 47 bromeliad species, which still represents only ca. 1.3% of the Bromeliaceae species (see supplementary Table 1 for details). Here, we used one plastidial DNA region and ten

microsatellite loci to estimate the genetic diversity and population structure of *A. distichantha*. We aimed to (1) evaluate the genetic diversity across populations and (2) infer the genetic structure of natural populations of *A. distichantha.* The findings of the present study may be utilized in conservation efforts on this species.

Material and methods

Population sampling and DNA extraction

We sampled 137 individuals from *A. distichantha*, from nine populations distributed in fragments of Cerrado, Chaco, and Atlantic Forest, from 20 to 2160 m elevation (Table 1; Fig. 2). The distance between these populations ranged from 27 to 1410 km. The ATSP, ITRJ, and IGRJ populations currently occur in conservation units, whereas the FMMS, RCMS, CAMG, LOPR, SBSC, and IRRS populations are unprotected (see Table 1 for population codes). To avoid errors in taxonomic identification, only flowering individuals were collected. To avoid sampling specimens from the same clonal origin, the plants were collected at a minimum distance of 10 m. Sampling consisted of collecting a fragment of leaf from each individual, cutting it into small pieces, and storing it in silica gel for dehydration until laboratory procedures. Total genomic DNA was extracted as described by Tel-Zur et al. (1999). DNA extractions were quantified on 1.5% agarose gel, stained with GelRed (Biotum, Hayward, California, USA), and compared with λ phage DNA.

Molecular markers, sequencing and genotyping

We used one cpDNA region and ten nuclear microsatellite loci (nrSSR) to investigate the genetic diversity and population structure of *A. distichantha*. The *mat*K gene was selected based on the extent of its polymorphism and was amplified and sequenced in 50 individuals from nine populations of *A. distichantha* (Tables 1, 2). For the amplification and sequencing of the *mat*K gene, we used the matK5 F (Crayn et al. 2000), BROM1 R, BROmatK 860 F (Schulte et al. 2005), and trnK2 R primers (Johnson and Soltis 1995).

The *mat*K gene was amplified by the polymerase chain reaction (PCR) using a Veriti 96-Well Thermal Cycler (Applied Biosystems), in a total volume of 30 µL containing 10 ng of DNA template, 1X GoTaq buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP mix, 1 mM forward and reverse primers, and 0.5 U GoTaq DNA polymerase (Bioline, London, UK). PCR was conducted using the following parameters: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 48 °C for 30 s, 72 °C for 30 s, and a final extension of 10 min at 72 °C. The PCR products were sent to Macrogen (Seoul, Korea) for both forward and reverse sequencing. The sequences were visualized, edited, and manually verified using Geneious version 10.2.3 (http://www.geneious.com, Kearse et al. 2012). Sequence alignment followed the MUSCLE algorithm with default parameters and was manually checked for ambiguous alignments. Mononucleotide repeats were removed because of uncertain homology, and indels longer than one base pair were recorded as single characters. Sequence data of both plastid regions of *mat*K were concatenated for subsequent analyses. The *A. distichantha* sequences generated in this study were deposited in GenBank (accession numbers MZ224176 - MZ224225, MZ224226 - MZ224275).

We analysed ten microsatellite loci from 137 individuals from eight sampled populations of *A. distichantha* (Tables 1, 2). Ten microsatellite markers have been previously designed for other bromeliads species: *Aechmea caudata* Lindm. (Ac55; Goetze et al. 2013), *A. coelestis* É.Morren (Ao06; Abondanza 2012), *Ananas comosus* (L.) Merr. (Acom_71.3 and Acom_82.8; Wöhrmann and Weising

Table 1 Information on the nine sampled populations of *Aechmea distichantha* from Brazil

Population codes	Locality/state	Site	Lat S	Long W	PD	Altitude (*m*)	Voucher
FMMS	Porto Murtinho/MS	Fecho dos Morros	21°27′	57°55′	CH	140	COR15747
RCMS	Porto Murtinho/MS	Retiro Conceição Farm	21°42′	57°53′	CH	80	COR15775
ATSP	Atibaia/SP	Pedra Grande	23°10′	46°31′	CE	1324	UEC48720
CAMG	Caldas/MG	Pedra Branca	21°57′	46°22′	AF	1334	COR17551
ITRJ	Itatiaia/RJ	Parque Nacional de Itatiaia	22°22′	44°43′	AF	2160	COR17550
IGRJ	Angra dos Reis/RJ	Ilha Grande	23°09′	44°08′	AF	20	RBR44218
LOPR	Londrina/PR	Campo das Pedras	23°38′	51°5′	AF	712	MBM16275
SBSC	São Bento do Sul/SC	Parque Florestal do SAMAE	26°14′	49°21′	AF	827	FURB49381
IRRS	Iraí/RS	Parque Municipal de Águas Termais de Iraí	27°11′	53°14′	AF	237	HAS36072

PD Phytogeographic domains; States: *MS* Mato Grosso do Sul, *SP* São Paulo, *MG* Minas Gerais, *RJ* Rio de Janeiro, *PR* Paraná, *SC* Santa Catarina, *RS* Rio Grande do Sul; Phytogeographic domains: *CH* Chaco, *CE* Cerrado and *AF* Atlantic Forest

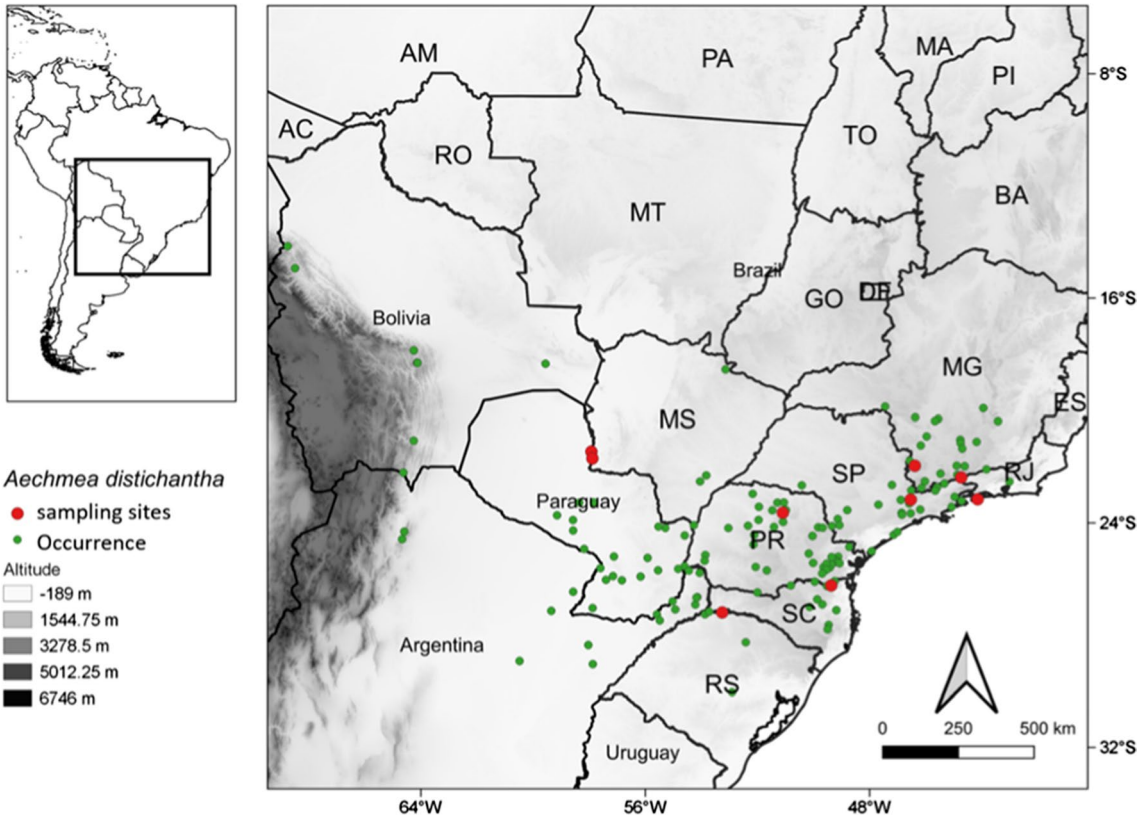

Fig. 2 Current geographical distribution of *Aechmea distichantha* and locations of the sampled populations of this study. The green circles represent the species occurrence sites and each red circle represents a sampling location for the populations (identified by their specific initial, for abbreviations, see Table 1). The records of the species occurrence points were obtained from the online databases of GBIF (https://www.gbif.org/) and SpeciesLink (http://splink.cria.org.br/)

Table 2 The estimated diversity indexes for plastid DNA (cpDNA = *mat*K) and ten nuclear microsatellites (nrSSR) of *Aechmea distichantha*

Population	cpDNA						nrSSR						
	N	S	*h*	π	NH	Haplotypes	N	A	A_P	R_S	H_O	H_E	F_{IS}
FMMS	3	1	0.667	0.0004	2	5;6	19	50	7	2.54	0.458	0.528	0.136*
RCMS	1	0	1.000	0.0000	1	6	6	35	5	2.99	0.605	0.648	0.075
ATSP	6	4	0.800	0.0011	4	1;2;3;4	22	75	16	3.48	0.526	0.696	0.250*
CAMG	8	3	0.464	0.0005	3	1;12;13	18	65	16	3.16	0.492	0.612	0.155*
ITRJ	7	0	0.000	0.0000	1	10	17	33	3	2.35	0.232	0.461	0.306*
IGRJ	7	13	0.667	0.0038	3	7;8;9	15	65	16	3.52	0.490	0.731	0.358*
LOPR	8	2	0.250	0.0003	2	6;14	20	64	14	3.09	0.499	0.644	0.210*
SBSC	3	0	0.000	0.0000	1	15	–	–	–	–	–	–	–
IRRS	7	0	0.000	0.0000	1	5	20	35	2	2.46	0.439	0.539	0.214*
TOTAL	50	23	0.428	0.0007	15	–	137	53	–	2.95	0.468	0.607	–

N Number of samples, *S* number of polymorphic sites, *h* haplotype diversity, π nucleotide diversity, *NH* number of haplotypes, *A* number of alleles, A_P private alleles, R_S allelic richness, H_O observed heterozygosity, H_E expected heterozygosity and F_{IS} inbreeding coefficient. For population abbreviation names, see Table 1

*Inbreeding coefficient (F_{IS}) differed significantly from the Hardy–Weinberg equilibrium (HWE) at $^*P < 0.001$

2011), *Orthophytum ophiuroides* Louzada & Wand. (Op30 and Op77A; Aoki-Gonçalves et al. 2014), *Pitcairnia albiflos* Herb. (PaD07 and PaZ01; Paggi et al. 2008), *P. geyskesii* L.B.Sm. (Pit5; Sarthou et al. 2003), and *Vriesea gigantea*

Gaudich. (VgC01; Palma-Silva et al. 2007). The loci were previously tested and optimized for cross-amplification in *A. distichantha* by Godoy et al. (2019). The SSR loci amplifications were conducted according to Godoy et al. (2019).

The PCR products were verified on a 1.5% agarose gel stained with GelRed and subsequently genotyped in a 3500xL DNA Analyzer automated sequencer (Applied Biosystems) with standard-size GeneScan 500 LIZ (Applied Biosystems). The number and size range of the alleles were determined using GeneMaker software version DEMO (SoftGenetics, State College, Pennsylvania, USA).

Molecular data analysis: plastidial DNA analysis

For cpDNA, we used the software Arlequin version 3.5 (Excoffier et al. 2005) to estimate haplotype (h), nucleotide (π) diversity (Nei 1987), number of haplotypes (NH), and number of polymorphic sites (S). Polymorphisms in the *mat*K marker sequences were used to construct different haplotypes. A haplotype network was built based on the cpDNA, using Network software version 4.6.1.1 (available at http://www.fluxusengineering.com), and the median-joining method (Bandelt et al. 1999) was used to estimate the evolutionary relationships between haplotypes.

We examined the genetic structure of the populations using "Clustering with linked loci" implemented in Bayesian clustering analysis (BAPS) 6.0 (Corander et al. 2013). To determine the most probable number of genetic groups (K), we performed ten algorithm repetitions for each K, from 1 to 9, using default software parameters. We evaluated the population structure using F_{ST} values calculated using the Arlequin software. We also carried out an analysis of molecular variance (AMOVA) to examine the partitioning of plastid genetic diversity within and between populations using the Arlequin software with 10,000 permutations.

Nuclear microsatellite analysis: genetic diversity and population structure

We estimated the number of alleles (A), private alleles (A_P), allelic richness (R_S), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) (Weir and Cockerham 1984), using the programs Arlequin, FSTAT version 2.9.3.2 (Goudet 1995), and Microsatellite analyzer (MSA) 4.05 (Dieringer and Schlötterer 2003). Departures from the Hardy–Weinberg equilibrium (HWE) were identified in GenePop, Web version 4.2 (Raymond and Rousset 2006).

AMOVA was performed to examine the partition of nuclear genetic diversity within and between populations in Arlequin with 10,000 permutations. To investigate the occurrence of population structure, we performed a Bayesian clustering algorithm implemented in Structure software, version 2.3.3 (Pritchard et al. 2000). For each K (from 1 to 10), we performed 10 replicates, using the admixture model, assuming independent allele frequencies, with a burn-in period of 100,000 and a run length of 500,000 to confirm the stabilization of summary statistics (Pritchard et al. 2000). We determined the most likely number of populations, K, by using the ΔK method described by Evanno et al. (2005), in Structure Harvester version 0.6.94 (Earl and von Holdt 2012).

To determine whether divergence among populations is an effect of isolation by distance, we tested the correlation between geographical and genetic distance matrices ($F_{ST}/(1-F_{ST})$ with a standardized Mantel test (Sokal and Rohlf 1995) using GenePop Web. Recent migration events were estimated in BayesAss 3.04 (Wilson and Rannala 2003). Samples were run for 1.0×10^8 interactions with a 10% burn-in, sampling every 1,000 interactions and using a 60% increase for the allele frequencies and mixing parameters of inbreeding coefficients. In order to assess whether there has been a recent reduction in the size of populations of *A. distichantha*, we used the Bottleneck software version 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999). Significance of the results was determined using the Wilcoxon one-tailed test with 10,000 iterations (Cornuet and Luikart 1996). The SMM models "stepwise mutation model" and TPM "two-phase model" were used because they are more suitable for microsatellite markers—with 95% single-step mutations, 5% multiple-step mutations, and variance among 12 steps (Piry et al. 1999).

Pollen versus seed flow

To estimate the relative contribution of pollen versus seed flow to total gene flow, we applied the following formula from Ennos (1994):

$$\text{Pollen flow/seed flow} = \frac{\left(\frac{1}{F_{ST(b)}}-1\right)-2\left(\frac{1}{F_{ST(m)}}-1\right)}{\left(\frac{1}{F_{ST(m)}}-1\right)}$$

where F_{ST}(b) and F_{ST}(m) are the levels of population differentiation calculated using biparentally (nrSSR) and maternally (cpDNA) inherited markers, respectively. The calculation of the pollen/seed flow ratio presented here assumes that maternal inheritance of plastid DNA is a rule in *A. distichantha*, as in most other angiosperms (Ennos et al. 1999).

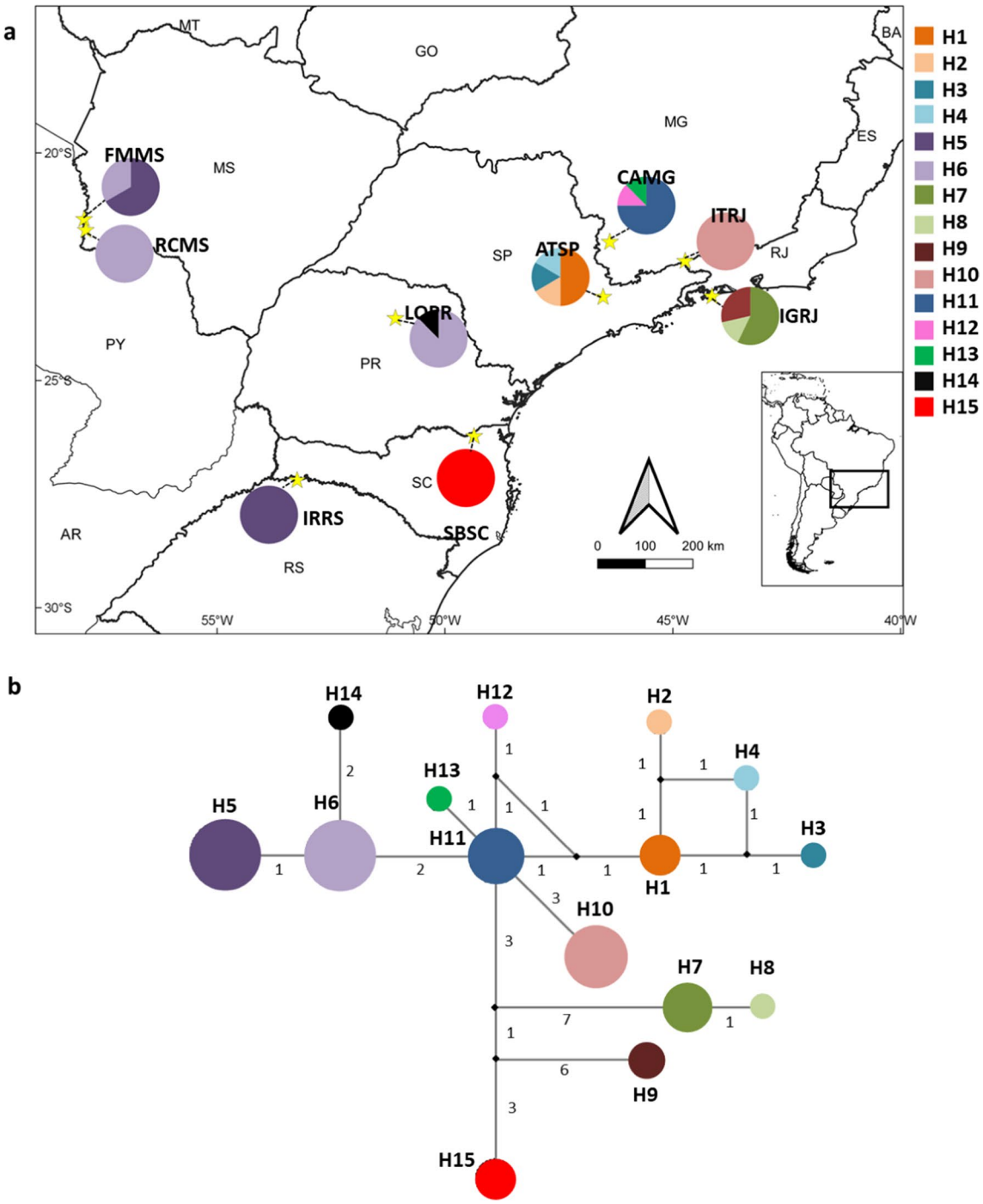

Fig. 3 Haplotypes of cpDNA (gene *mat*K) are identified by numbers and colours. **a** Haplotypes present in the populations of *Aechmea distichantha* analysed in this study, each population is represented by a star (for abbreviations, see Table 1); **b** Median-joining network of the found haplotypes and the number of mutations between them, the size of the circles is proportional to their frequency

Results

Plastid genetic diversity and population structure

Amplification of the *mat*K gene resulted in sequences with a length of 1580 bp, with 23 polymorphic sites (nine transitions, nine transversions, and five indels). Fifteen haplotypes were found in the 50 analysed *A. distichantha* individuals, ranging from one to four per population (Table 2; Fig. 3a). Haplotypic diversity ranged from zero (in three populations) to 1.000, and nucleotide diversity ranged from zero (in four populations) to 0.0038 (Table 2).

The cpDNA network built with the "median-joining" method revealed the relationship between the 15 haplotypes observed in the natural populations of *A. distichantha* (Fig. 3b). Most haplotypes were separated by one mutational step; however, some haplotypes were separated by more mutational steps, such as H6 and H14 (two mutational steps), H10 and H11 (three mutational steps), and H7 and H15 (seven mutational steps). The network showed that H11 was the central haplotype and was present only in the CAMG population. H5 and H6 haplotypes were the most frequent, and the only haplotypes shared between different populations: FMMS and IRRS shared the H5 haplotype, and FMMS, RCMS, and LOPR shared the H6 haplotype (Fig. 3b).

Bayesian clustering analysis (BAPS) of cpDNA sequences revealed a high genetic structure with six clusters ($K=6$) in the nine sampled populations of *A. distichantha* (Fig. 4a). The defined groups recovered the patterns observed in the haplotype network, in which the most distant haplotypes were grouped (Fig. 3b). AMOVA showed that 80.25 of the genetic variation occurred due to differences between populations, indicating a significant and high genetic structure ($F_{ST}=0.80$, $P<0.001$; Table 3) for the plastidial genome.

Table 3 Analysis of molecular variance (AMOVA) using cpDNA (*mat*K) and ten nuclear microsatellites (nrSSRs)

Source of variation	Percentage of variation	F_{ST}	*P* value
cpDNA			
Among populations	80.25	0.80	$P<0.001$
Within populations	19.75		
nrSSR			
Among populations	24.77	0.24	$P<0.001$
Within populations	75.22		

Nuclear genetic diversity and population structure

The sampled populations of *A. distichantha* showed moderate to high levels of genetic diversity at the ten microsatellite loci (Table 2). The number of alleles per population ranged from 33 (ITRJ) to 75 (ATSP), and the allelic richness from 2.35 (ITRJ) to 3.51 (IGRJ). Two to 16 private alleles were found in the sampled populations—two in the IRRS population, three in the ITRJ, five in the RCMS, seven in the FMMS, and 14 in the LOPR population. Three populations had 16 private alleles (ATSP, CAMG, and IGRJ;

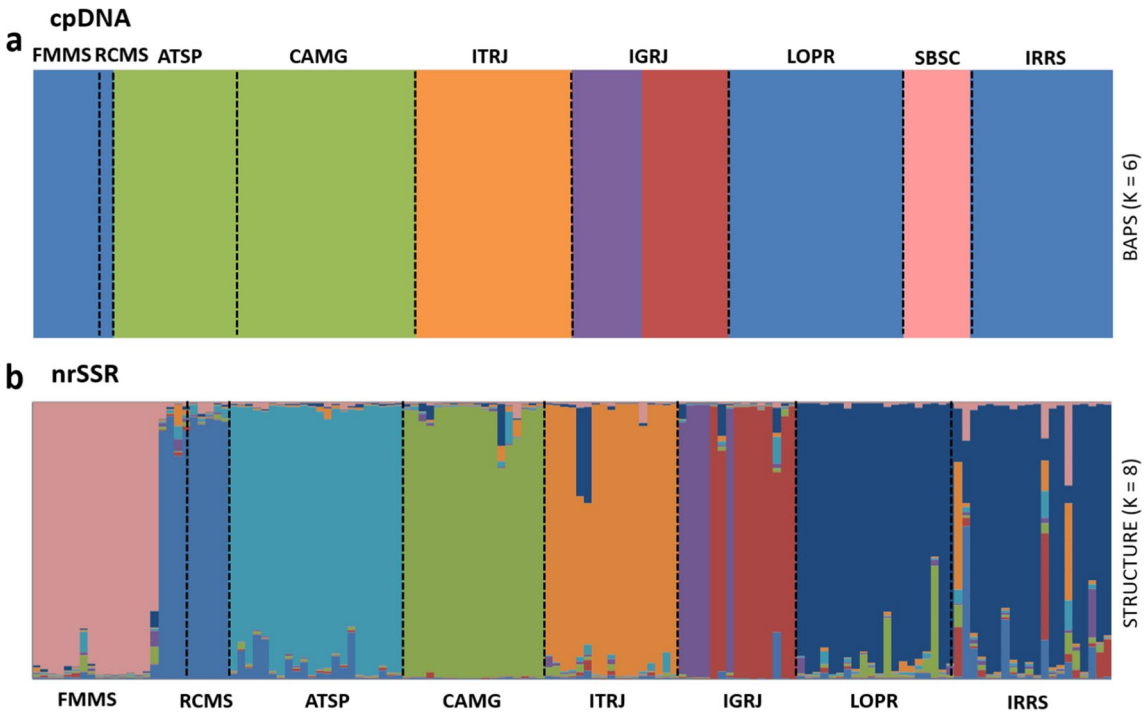

Fig. 4 Clustering analyses of genomic variation across the range of *Aechmea distichantha* populations. **a** Indicates the results for BAPS, with optimal partition of six clusters; **b** indicates the results for STRUCTURE, with optimal $K=8$. Individuals were arranged by population. Distinct colours represent distinct genetic clusters (for abbreviations, see Table 1)

Table 2). The observed and expected heterozygosity ranged from 0.232 to 0.605, and 0.461 to 0.731, respectively. With the exception of the RCMS population, all the other seven populations deviated significantly from the Hardy–Weinberg Equilibrium, showing a deficit of heterozygotes, with the inbreeding coefficient (F_{IS}) ranging from 0.075 to 0.358 (Table 2). Gene flow posterior probabilities rates, estimated in BayesAss, indicated no contemporary migration events among populations, except for a small percentage (14%) of ATSP migrants found in the RCMS population (Table 4).

AMOVA for nrSSR (Table 3) showed a high genetic structure ($F_{ST} = 0.24$, $P < 0.001$), revealing that the largest proportion of genetic variation was due to differences within populations (75.22%) rather than between populations (24.77%) (Table 3). Bayesian analysis of structure identified eight genetic groups ($K = 8$; Figs. 4b, 5), and a major genetic group was observed for most populations, indicating low gene flow among populations. Some individuals of the FMMS population showed a high probability of belonging to a group other than that represented by their locality (the predominant group in RCMS), which can probably be attributed to migrants between these two populations. The IGRJ population presented two genetic groups, whereas the populations geographically close to the LOPR and IRRS were grouped, demonstrating gene flow between them (Fig. 4b). However, the Mantel test was not significant ($r2 = 0.0046$, $P = 0.059$), showing no association between genetic and geographical distances, indicating the absence of isolation by distance among the sampled locations. According to the TPM and SMM models, no significant increase in heterozygosity was found in the Wilcoxon (Bottleneck) tests, indicating that the populations of *A. distichantha* did not experience a recent or strong bottleneck event.

Pollen versus seed flow

Based on the estimated F_{ST} values obtained from the molecular analysis of variance among populations—plastid (0.80) and nuclear (0.24)—we estimated the ratio of pollen and seed flow, which was 10.66, suggesting that gene flow via

Table 4 Estimates of the distribution of recent migration rates, calculated with BayesAss. For population abbreviation names, see Table 1

Source population	Recipient population							
	FMMS	RCMS	ATSP	CAMG	ITRJ	IGRJ	LOPR	IRRS
FMMS	**0.892**	0.0124	0.0294	0.0123	0.0168	0.0124	0.0123	0.0124
RCMS	0.0443	**0.6907**	**0.1455**	0.0239	0.0239	0.0239	0.0238	0.024
ATSP	0.0112	0.0111	**0.9217**	0.0113	0.0114	0.0111	0.0111	0.0111
CAMG	0.0128	0.0129	0.0129	**0.9082**	0.0131	0.0128	0.0145	0.0129
ITRJ	0.0136	0.0134	0.0138	0.0148	**0.9031**	0.0138	0.0139	0.0135
IGRJ	0.0144	0.0147	0.0146	0.0145	0.0145	**0.8979**	0.0145	0.0149
LOPR	0.0119	0.0119	0.0119	0.0143	0.0119	0.0119	**0.9127**	0.0134
IRRS	0.0122	0.0119	0.013	0.0145	0.015	0.0123	0.0157	**0.9054**

Bold values represent the proportion of non-migrant individuals in a population (values > 10%)

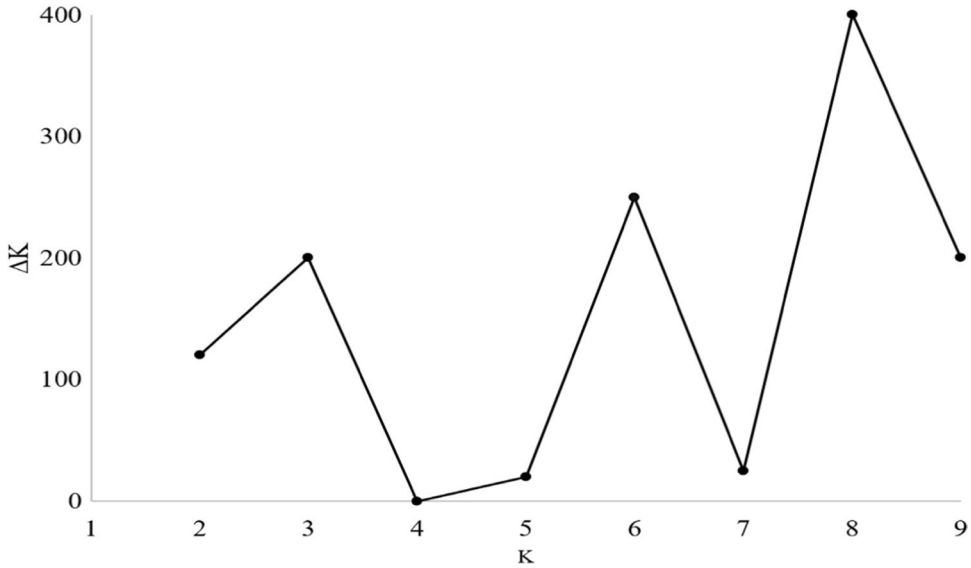

Fig. 5 Magnitude of ΔK from structuring analysis as a function of K (mean ± SD over 10 replicates), calculated according to the ΔK method, proposed by Evanno et al. (2005), for *Aechmea distichantha* nuclear microsatellite data. The modal value of these distributions indicates the true K or the highest level of structuring, here, there are eight genetic clusters

pollen in *A. distichantha* was more than tenfold greater than that via seeds.

Discussion

In this study, we investigated the genetic diversity and population structure of *A. distichantha*, a species with a wide geographical distribution, in the Cerrado, Chaco, and Atlantic Forest phytogeographic domains. Genetic analysis based on plastidial DNA revealed low-to-moderate genetic diversity within the populations. For the nuclear genome, our study showed moderate-to-high genetic diversity within populations. A high genetic structure was observed among the populations of *A. distichantha* for both genomes and gene flow via pollen was ten times more efficient than via seeds.

Genetic diversity

The genetic diversity indices from low to moderate (cpDNA) and from moderate to high (nrSSR) in the populations of *A. distichantha* may be related to its life history including sexual and asexual reproductive system, which can be considered advantageous in different situations, especially in species occupation and permanence in different environments (Karasawa 2009; Gütschow-Bento et al. 2010; Scrok and Varassin 2011). Furthermore, cpDNA is maternally transmitted in most angiosperms, including Bromeliaceae (Ennos 1994; Wagner et al. 2015). This molecule tends to evolve at a very slow pace in relation to the rearrangement of genes and primary sequences, in addition to not undergoing recombination. Several bromeliads of the genus *Aechmea* also show this pattern: *A. calyculata* (É.Morren) Baker (Goetze et al. 2016), *A. blumenavii* Reitz, *A. caudata*, *A. comata* Baker, *A. kleinii* Reitz, *A. winkleri* Reitz (Goetze et al. 2017), *A. kertesziae* Reitz (Goetze et al. 2018), *A. nudicaulis* Griseb. (Meireles and Manos 2018).

The moderate-to-high genetic diversity in *A. distichantha* (nrSSR) also suggests that its populations have not yet been affected by habitat fragmentation or intense illegal removal, and that genetic diversity within populations is not strongly affected by genetic drift, although the phytogeographic domains in which the species occurs—Cerrado, Chaco and Atlantic Forest—have suffered a long history of natural fragmentation (Ribeiro et al. 2009; Ganem et al. 2013; Tomas et al. 2015).

All populations, except RCMS, deviated significantly from HWE (Table 2) because of the high proportion of homozygotes, probably due to both self-fertilization and biparental inbreeding. *Aechmea distichantha* is a self-compatible species (Scrok and Varassin 2011; Freire et al. 2018) with a mixed reproductive system; that is, the species exhibits sexual reproduction with facultative self-fertilization (Scrok and Varassin 2011), which may enhance crossing between relatives.

Genetic structure and gene flow

We found a high genetic structure among the populations of *A. distichantha* for cpDNA and nrSSR ($F_{ST} = 0.80$ and 0.24, respectively), with most populations presenting only a single or few exclusive haplotypes (Fig. 3a, Table 3). These results suggest low gene flow and genetic connectivity among populations, which agrees with the absence of contemporary migration events between populations, as shown in the BayesAss analysis, evidencing high differentiation among populations. The high genetic structure and moderate haplotypic diversity suggest low gene flow for both markers, and consequently, limited dispersion of both pollen and seeds. Habitat fragmentation may be associated with reduced gene flow (Maciel et al. 2019). *Aechmea distichantha*, which occurs in environments that have suffered a long history of natural fragmentation (Ribeiro et al. 2009; Ganem et al. 2013; Tomas et al. 2015), showed reduced gene flow among its populations and, consequently, high genetic structure. These population structure values have already been described for other bromeliads of mixed mating systems (Barbará et al. 2009; Palma-Silva et al. 2009; Dantas-Queiroz et al. 2021; Mota et al. 2020).

A high plastidial genetic structure was also observed with the BAPS analysis, suggesting the existence of six genetic groups and demonstrating a high subdivision of *A. distichantha* populations (Fig. 4a). Similarly, the results from the Bayesian analysis revealed eight nuclear genetic groups and a low degree of admixture between the groups, also indicating a high population genetic structure (Fig. 4a). Western populations (FMMS, RCMS, LOPR, and IRRS) were grouped, whereas Eastern populations presented different genetic groups for the plastidial genome. Some populations in the West, although geographically distant from each other, shared the same genetic group (Fig. 4). This was also confirmed by the absence of isolation by distance between the populations of *A. distichantha*, detected by the Mantel test ($r^2 = 0.0046$, $P = 0.059$). We found haplotypes and private alleles in all populations (Table 2), corroborating the high genetic structure and low gene flow among populations, as noted by the low number of migrants per generation between populations (Barton and Slatkin 1986; Szpiech and Rosenberg 2011). It was not possible to detect recent or strong bottleneck events in *A. distichantha*, reflecting a constant size of the studied populations over time. The absence of a recent bottleneck, high genetic structure among populations, and high levels of genetic diversity found in *A. distichantha* suggest that the populations were founded by genetically diverse individuals. Similar results have been reported

for other bromeliads, including *A. kertesziae* (Goetze et al. 2018), *Pitcairnia flammea* Lindl. (Mota et al. 2020), *Vriesea incurvata* Gaudich. (Aguiar-Melo et al. 2019), and *V. reitzii* Leme & A.F.Costa (Soares et al. 2018).

Our analysis revealed that, in *A. distichantha,* gene flow via pollen is more than tenfold greater than that via seeds (10.66), which indicates restricted seed dispersion and shows the importance and efficiency of its pollinators. This pattern is commonly seen in bromeliads (Palma-Silva et al. 2009; Paggi et al. 2010; Goetze et al. 2018; Vicente-Silva et al. 2022). *Aechmea distichantha* presents zoochory seed dispersal, being carried out mainly by birds, as in most species of the genus *Aechmea* (Bonnet and Queiroz 2006; Lenzi et al. 2006; Goetze et al. 2018). The inefficiency in seed dispersal may have made it difficult to maintain the gene flow between their populations, something also observed in *A. kertesziae*, in which the high genetic structure found showed evidence of seed dispersal barriers (Goetze et al. 2018). This pattern differs from that found in a similar study with *V. incurvata*, in which a high gene flow between populations was reported, being equally effective through pollen and seeds, in this case, the anemochoric seed dispersal may have favoured the long-distance dispersal events, facilitating gene flow among populations, and keeping their connection with a high number of migrants (Aguiar-Melo et al. 2019). Several aspects of species biology, as well as ecological relationships and environmental conditions, can interfere in the gene flow both via pollen and seeds, thus, this issue should be better investigated in Bromeliaceae. Studies of the reproductive biology of *A. distichantha* have shown that hummingbirds, bees, and butterflies can pollinate the species. Although self-compatible, this species also has great reproductive success, especially when exposed to sun light and in individuals with larger inflorescences (Scrok and Varassin 2011; Freire et al. 2018). Thus, its pollinating agents play a fundamental role not only in gene dispersal but also in the formation of viable seeds, contributing to the maintenance of moderate to high levels of genetic diversity in the species.

The FMMS and RCMS populations of *A. distichantha* are located in humid Chaco, Western Brazil, on the border with Paraguay. This region is considered to be one of the most threatened ecoregions in Brazil, as native vegetation has been heavily replaced by pastures cultivated for livestock (Tomas et al. 2015). Recent estimates show a great reduction in the original vegetation, with only 13% remaining (Tomas et al. 2015). Therefore, species associated with this environment may present a restricted and fragmented distribution accompanying Chaco Forest remnants, as is the case for *A. distichantha*. The CAMG, IGRJ, ITRJ, SBSC, and IRRS populations were found in mixed rainforests, the LOPR population was found in semi-deciduous forests, and the ATSP population was found in semi-deciduous seasonal forests. The populations occur in different phytogeographic domains, but their distribution is not related to the groups formed by nuclear and plastid genome analysis. In addition, no apparent geographical barrier isolates the groups and the observed disjunction may reflect a historical pattern of variance, as was also observed in populations of *A. calyculata* (Goetze et al. 2016).

Conservation implications

Data on genetic diversity and population structure can contribute to the planning of effective conservation actions to guarantee the population persistence (Frankham et al. 2010, 2019). In the case of *A. distichantha*, the use of data from plastid and nuclear DNA markers is essential for conservation programmes, since it is threatened by potential predatory exploitation—due to its ornamental value (Santa-Rosa et al. 2013)—and by the fragmentation and loss of its natural habitat (Cerrado, Chaco, and Atlantic Forest; Ribeiro et al. 2009; Ganem et al. 2013; Tomas et al. 2015).

The ATSP, CAMG, and ITRJ populations have 16 private alleles each, therefore, they deserve special attention in any conservation measures. A greater diversity and frequency of haplotypes occur in these populations, which may be related to the fact that two of them are currently found in conservation units (Fig. 2, Table 3). Some studies have shown that populations that occur within protected areas tend to have higher rates of genetic diversity, as is the case for the Dicksoniaceae species, for which Montagna et al. (2012) found a greater genetic diversity inside conservation units of *Dicksonia sellowiana* Hook. populations, evidencing the importance of the units, both for conservation of the genetic diversity and for research on the topic of plant genetic resources use and conservation. Considering the obtained results, we suggest monitoring and in situ conservation of ATSP, CAMG and ITRJ populations, to maintain the observed genetic diversity, mainly considering the high population structure.

Conclusions

Our results showed that the populations of *A. distichantha* still retain moderate to high levels of genetic diversity, but with high genetic structure. The fact that it is a species with a mixed reproductive system probably contributes to this diversity. The gene flow via pollen was ten times greater than via seed, showing the importance of pollinators who significantly contribute to the genetic connectivity of

populations and to the maintenance of the genetic diversity. Clonal vegetative propagation may also be responsible for preserving the genotypes in the populations. Regarding the conservation of *A. distichantha*, in situ monitoring and conservation of ITRJ and IRRS populations are recommended, mainly because they have low genetic diversity, which would be an impediment to adaptation in the face of future environmental changes.

Information on Electronic Supplementary Material

Online Resource 1. List of 50 bromeliad species studied using SSR markers for genetic diversity, gene flow, population structure, and hybridization analysis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00606-023-01841-7.

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Authors' contributions All authors contributed to the study conception and design. Material preparation, and data collection were performed by FMRG, LVS, and GMP. Analyses were performed by FMRG, LVS, MVDQ, and CPS. The first draft of the manuscript was written by FMRG, and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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