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RESEARCH ARTICLE

Genetic diversity and structure populations in *Annona deceptrix* (Westra) H. Rainer (Annonaceae), an endangered species from Ecuador

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Abstract *Annona deceptrix* is an endemic species found on the Ecuadorian coast, and its vulnerable status is attributed to the degradation of its ecological niches caused by human activities. This species exhibits desirable agronomic traits with potential use for genetic improvement. The purpose of this study was to unveil the genetic diversity and population structure of 106 individuals from 11 natural populations of *A. deceptrix* in the province of Manabí using 18 specific simple sequence repeat (SSR) markers. Overall, a moderate level of genetic diversity ($He = 0.445$) was found. The AD8, AD5, AD6, AD10 and AD1 markers were the most informative for *A. deceptrix*. Analysis of molecular variance (AMOVA) revealed that 65% of the total genetic diversity was within individuals and 11% of genetic variation was attributable to the differentiation among populations. Low fixation index ($Fst = 0$) and moderate gene flow ($Nm = 1.159$), thereby indicating no genetic differentiation among populations. In addition, a null correlation between geographic and genetic distances among populations ($R^2 = 0.007$; $p = 0.02$) was detected by the Mantel test, indicating no significant isolation by distance. These findings are novel and have a great impact to begin planning conservation strategies for *A. deceptrix* considering that it is an endemic species, threatened by anthropogenic activity and has received very little research attention. In this sense, we suggest that the strategies should focus on populations such as Humedad, Agua Blanca, and Tachina, since they were those with the greatest diversity and number of private alleles.

Keywords Annonaceae · Microsatellite · Heterozygosity · AMOVA · Conservation specie

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Introduction

Annonaceae is one of the most diverse families of tropical forest species containing between 110 and 200 genera and from 2300 to 2500 species (Leal and Paull 2023). This family comprises woody trees, shrubs and vines which are originally from tropical lowland Central and South America and the West

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Indies (Cautín and Agustí 2005). Within the Annonaceae family, the *Annona* genus presents species of commercial interest such as *A. cherimola* Mill. (cherimoya), *A. glabra* L. (pond apple), *A. muricata* L. (soursop), *A. reticulata* L. (custard apple), atemoya (a hybrid of *A. squamosa* and *A. cherimola*) and *A. squamosa* L. (sweetsop or sugar apple), which are the main species cultivated due to their edible fruits (Leal and Paull 2023). Additionally, species of this genus has excellent nutritional and pharmacological properties for human health through direct consumption as fresh fruit or in processed foods (Al Kazman et al. 2022). However, a large number of threatened Annona species have not been genetically characterized (Awachare et al. 2018).

Ecuador is recognized as one of the countries with the highest biodiversity and density of endemic plant species worldwide (Joppa et al. 2013). In this sense, the country has around 113 species belonging to the Annonaceae family, among which 63 species have different uses (De la Torre et al. 2008). *Annona deceptrix* (Westra) H. Rainer commonly known as 'chirimoya de monte' or 'anonilla' is an endemic tree of the province of Manabí and other nearby cities in Ecuador (Pico-Mendoza et al. 2020). This species is distributed throughout the humid remnants in the coastal zone of the country. There are populations within the 'Machalilla' National Park and in the deciduous forests of the 'Chongón Colonche' Mountain range, located between Guayas and Esmeraldas provinces at an altitude of up to 850 m above sea level (Muriel 2011). Moreover, isolated populations have been observed in the northern area of the province of Manabí, specifically, at the 'Pata de Pájaro' hill located in the Pedernales canton (Fig. 1).

Annona deceptrix presents interesting botanical and agronomic traits such as the shape and size of its leaves, white to yellowish flowers, and fruits with different shapes (elongated and conical), texture (smooth and rough) and sweetness (Pico-Mendoza et al. 2024). However, due to anthropogenic activities associated with deforestation and agricultural expansion (Muriel 2011), and some intrinsic characteristics such as a low germination capacity of its seeds under natural conditions (Pico-Mendoza et al. 2020), this species has been reported as Vulnerable according to the IUCN Red List of Threatened Species in 2018 (Erkens 2021). Therefore, it is necessary to evaluate its genetic diversity and structure to establish management plans that will allow the species and its diversity to be conserved.

Most information available for the *Annona* genus is developed for commercial species such as *A. cherimolla*, *A. muricata*, and *A. squamosa*, which have been widely evaluated in terms of their morphological, agronomic and genetic diversity (Brisibe et al. 2017; Leal and Paull 2023). The lack of information on *A. deceptrix* limits our knowledge about it agronomic and nutritional properties. Furthermore, genetic research on this species would improve the evaluation of the risk of extinction and develop management strategies that guarantee its conservation (Garner et al. 2020). These investigations would allow the identification of populations with high genetic diversity and genotypes that have valuable nutritional and agronomic traits for genetic improvement of *Annona* species.

Polymerase chain reaction (PCR)-based molecular markers such as microsatellites or simple sequence repeats (SSR) have been widely used in many crops because they are considered a powerful molecular tool for the characterization of genetic variability and population structure of the species (Väli et al. 2008). Recently, a total of 22 SSR markers were developed

Fig. 1 Morphological variability in isolated populations of *Annona deceptrix* fruits from the 'Pata de Pájaro' hill located in the Pedernales canton

for *A. deceptrix*, which proved to be reproducible and suitable as molecular markers for genetic research in *Annona* species (Pico-Mendoza et al. 2024). Therefore, the objective of the current study was to evaluate the diversity and genetic structure of natural populations of *A. deceptrix,* distributed at Manabí province, using specific SSR markers for conservation purposes of this valuable endangered endemic species.

Materials and methods

Plant material

A total of 116 tree of *A. deceptrix* were collected from 11 sites distributed at Manabí province, including the administrative Regions of Pacoche (9) in the Canton of Manta; Agua Blanca (11), Ayampe (8), and Cerro El Mate (17) in the canton of Puerto Lopez; El Barro in the canton of Jipijapa; Tachina (23), Tachina Alto (6), Nalpe (11), Humedad (13), Vite (8) and Guacucal (6) in the canton of Pedernales. Each collection site was geographically using the GPS information, except for the location of Guacucal whose information was lost (Table 1).

Genomic DNA extraction

A sample of ~ 200 mg of young leaves of *A. deceptrix* was placed in 2.0 mL tubes and frozen in liquid nitrogen. Leaf samples were macerated with the Precellis and Cryollys systems (Bertin Technologies, Montigny-le-Bretonneux, France), and centrifuged at 6000 rpm in three cycles of 30 s each. Total genomic DNA extraction of all samples was performed using the cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle 1990). The quantity and quality of isolated DNA were determined using a Nanodrop and agarose gel electrophoresis. Eighteen SSR loci were evaluated according to Pico-Mendoza et al. (2024) and the PCR was carried out using the following steps: denaturation at 94 °C for 5 min; specific annealing temperature (Ta) for each primer pair (Table 2) for 45 s, extension at 72 °C for 45 s. This cycle was repeated 30 times, then a final extension at 72 °C. PCR products were separated by capillary electrophoresis in an ABI 3130xl Prism Genetic Analyzer with POP-7 polymer (Life Technologies, Foster City, California, USA). Allele sizes were calculated with GeneMapper software v 4.0 (Applied Biosystems, Foster City, California, USA).

Table 1 Information on *Annona deceptrix* populations sampled in the province of Manabí, Ecuador

Cantons of Manabí	Populations	N	Latitude	Longitude
Manta	Pacoche	9	1°08′29″S	80°87′23″W
Puerto López	Agua Blanca	11	1°35′00″S	80°42′28″W
	Ayampe	8	1°40′34″S	80°48′08″W
	El Mate	17	1°34′13″S	80°43′36″W
Jipijapa	El Barro	4	1°18′27″S	80°40′51″W
Pedernales	Tachina	23	0°02′03″N	80°00′04″W
	Tachina Alto	6	0°01′44″N	79°59′52″W
	Nalpe	11	0°02′33″N	79°58′31″W
	Humedad	13	0°00′50″N	79°58′45″W
	Vite	8	0°01′74″N	79°58′45″W
	Guacucal	6		

Statistical analysis

Monomorphic SSR, and those SSR loci and genotypes containing 20% or more of missing values were discarded from the subsequent analyses using the 'poppr' (Kamvar et al. 2014) and 'adegenet' (Jombart 2008) packages implemented in R Studio software. The genetic diversity parameters such as mean number of alleles (*Na*), the mean effective number of alleles (*Ne*), expected heterozygosity (*He*), observed heterozygosity (*Ho*), number of private allele (*Npa*), percentage of polymorphic loci (*PPL*), fixation index (*Fst*), inbreeding coefficient (*Fis*), and gene flow (*Nm*) were estimated for each locus and population using GenAlEx Version 6.5 (Peakall and Smouse 2012) and 'hierfstat' package (Goudet 2005).

To determine the genetic structure, analysis of molecular variance (AMOVA) was applied to estimate the variance components within and between populations in GenAlEx Version 6.5 (Peakall and Smouse 2012). The population structure of the *A. deceptrix* genotypes was evaluated by principal component analysis (PCA) implemented in 'ade4' (Chessel et al. 2004) and 'adegenet' (Jombart 2008) and visualized with 'ggplot2' (Wickham 2010) packages. Additionally, an unrooted tree was constructed to show the genetic relationship among the *A. deceptrix* genotypes. It was implemented using the function

Table 2 Information of the 18 pairs of simple sequence repeat (SSR) loci for *Annona deceptrix*

Locus	Primer sequences (5′–3′)	Repeat motif	Product size	size (bp)	Dye	Ta (°C)	GenBank
AD01	F: TCATGTTGTGGATGTGGAGC	(AG) 7	318	200–213	NED	60.8	ON469949
	R: TCTTTGGGAACCATCAGACC						
AD02	F: TTCACTGCATGCCATAGAAGA	(AAAC)5	139	324–343	NED	60.75	ON469950
	R: GTAATGGCCCGGAAGAGTATC						
AD03	F: TGAAGATTTCTGTATTCCATGGTT	(AT)6	145	135–146	FAM	60.6	ON469951
	R: TCTTGCCCGTGGTACTCAAT						
AD04	F: AAACGTAAGCAGACGCCAAC	(AC)6	201	152–163	FAM	61	ON469952
	R: AAGATTCCGACCCAACACAG						
AD05	F: AAGAGAGGCAGAAATCACGG	(AG)14	172	326–353	PET	61.35	ON469953
	R: CGATGAGACTGAGGTGGTCA						
AD06	F: GCGCAAATTGTGAGAAATGA	(AAC)7	113	196–216	FAM	59.95	ON469954
	R: GCCTCCACGGAAATTGTTT						
AD08	F: CTCTGACCAATGTCAACGGAT	(AG)7	165	357–370	PET	60.75	ON469956
	R: ATTCTGCAGGACAATCCCAA						
AD09	F: TCGTTTCTTCTATTTATCCCGAA	(AGC)7	206	145–165	PET	59.85	ON469957
	R: GCCGGGAGACTTGATTCTTT						
AD10	F: TCCATCTGTTCTGCTTGACG	(AG)6	273	248–259	VIC	62	ON469958
	R: ACCAGACGAAGAAGGCTTGA						
AD11	F: GGAGTTCCCTCAAAGAAGGG	(AAAAC)5	290	228–252	FAM	63	ON469959
	R: GCTGTCTTCCTGAGGCACTT						
AD13	F: CAGCAGTCTGATTGTCGGAA	(AAGC)5	234	306–325	VIC	61	ON469961
	R: GCGAGTCATGAAAGCTTGG						
AD15	F: TTTGCTGGCATTTGCTCTAC	(AAAAC)5	293	70–94	FAM	60	ON469963
	R: GAATTGTCTGCAGAACCACAAA						
AD16	F: GGAACAGCAGTCTTCTTGGC	(AGAT)6	290	271–294	NED	61	ON469964
	R: CTTGTTGGATGCGGACAAT						
AD17	F: GGTGGTGGAAATTGGTTGTC	(AGC)5	188	120–140	VIC	61.15	ON469965
	R: TCGAACCAACACCATGAGTC						
AD18	F: CGAGAGATATGCAGCATCCA	(AAG)5	233	153–167	VIC	62.65	ON469966
	R. TGACCTCCCATGCACCTAGT						
AD19	F: ACCACGAGGCAGGTCAGTT	(AGC)9	205	154–180	FAM	61.55	ON469967
	R: CACCAACAATGGTGTTCTCG						
AD21	F: ACCTCCAGTTGAGAAGAGCG	(AAGC)5	225	129–143	PET	62.15	ON469969
	R: CTGCGTGGGTCTGTGACTAA						
AD22	F: AAGTAGTACCGGTCAGAGAGCG	(AGCG)6	292	213–236	NED	59.85	ON469970
	R: GTTGGATTATTGAAACGCGG						

Ta annealing temperature

aboot implemented in the 'poppr' package (Kamvar et al. 2014) based on Nei's genetic distance with 1,000 bootstrap replicates. The resulting tree was visualized with function *plot.phylo* in the 'ape' package (Paradis and Schliep 2019). Moreover, the population structure was inferred using a Bayesian-based clustering method in Structure v2.3.4 (Pritchard et al. 2000). An admixture ancestry model with correlated allele frequencies assuming no prior information of population origin was used. Ten independent runs for each putative number of subpopulations (*K*), ranged from K = 1 to 11, were performed with a burn-in period of 100,000 steps followed by 1,000,000 MCMC iterations. The optimal *K* value was determined in

Structure Harvester (Earl and vonHoldt 2012) using the ad hoc statistic ΔK (Evanno et al. 2005). Finally, a Mantel test was performed to determine isolation by distance (IBD) between populations of *A. deceptrix* in GenAlEx Version 6.5 (Peakall and Smouse 2012).

Results

Screening of SSR primers in *A. deceptrix*

In this study, a total of 18 SSR markers were successfully amplified in 116 samples of *A. deceptrix*. After applying quality filters on raw data, ten samples were discarded because they presented more than 20% missing data (Fig. 2). Therefore, a total of 106 *A. deceptrix* individuals were evaluated with the 18 SSR markers in the subsequent analyses.

Genetic diversity

The 18 SSR markers were polymorphic and generated a total of 186 alleles across the 106 individuals of *A. deceptrix*. The number of total alleles (*Ta*) per locus ranged from 5 to 25 in the markers AD18 and AD8, respectively, with an average of 10.3 alleles per locus. In general, the AD8 locus was the one that presented the highest diversity parameters ($Ho = 0.777$; $He = 0.810$; $Ne = 4.423$), while the AD18 locus was the one that presented the lowest diversity ($Ho = 0.070$; $He = 0.190$; $Ne = 1.324$). Among the SSR markers evaluated the AD8, AD5, AD6, AD10 and AD1 were the most informative for *A. deceptrix*. The genetic differentiation coefficient (*Fst*) varied from 0.102 to 0.354 in the loci AD11 and AD9, respectively, with an average value of 0.207. On contrary, the gene flow (*Nm*) at loci AD11 ($Nm = 2.196$) and AD9 ($Nm = 0.455$) presented the highest and lowest value, respectively. All diversity parameters evaluated at the locus level are shown in Table 3.

At population level, El Barro was the population with the lowest number of alleles per locus ($Na = 1.444$) and number of effective alleles ($Ne = 1.404$). While Tachina was the population with the highest *Na* (7.500) and *Ne* (3.577). Overall, the number of alleles per locus and number of effective alleles was of 3.654 and 2.367, respectively. A total of 89 private alleles (*Npa*) were identified in the 11 populations by 18 SSR markers, except for Pacoche and El Barro where no *Npa* were identified. The highest number of private alleles was found in the Tachina ($Npa = 36$), followed by Agua

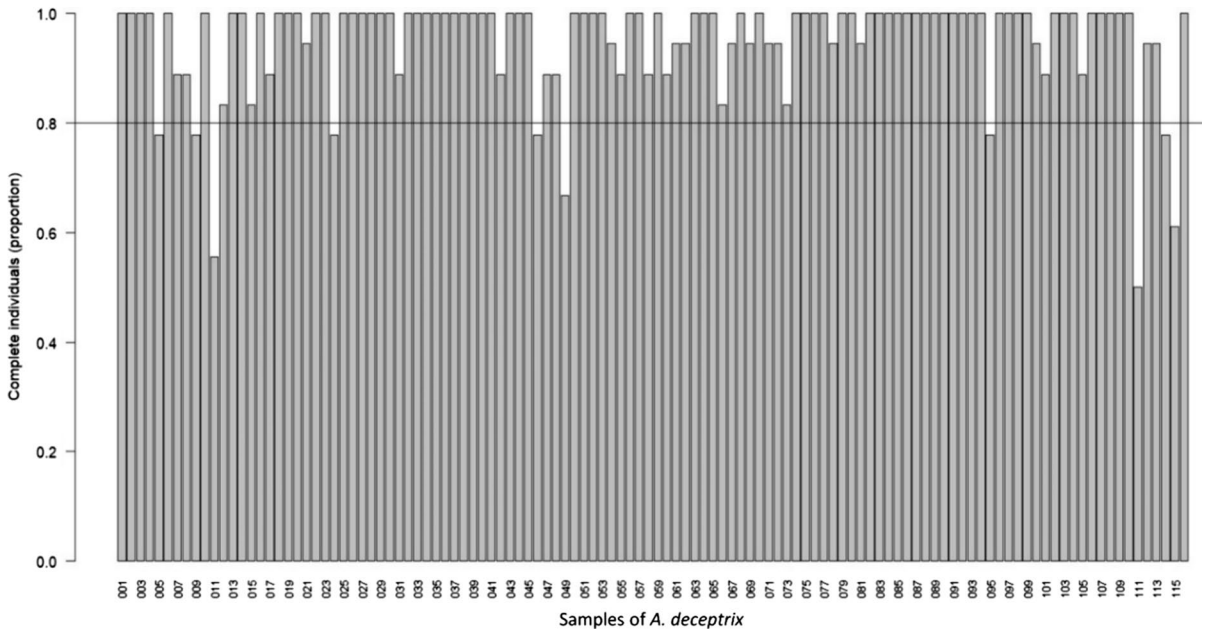

Fig. 2 One hundred and sixteen genotypes of *Annona deceptrix* with percentage of missing genotype data. Individuals with more than 20% (proportion < 0.8) were discarded

Table 3 Summary of genetic statistics and F-statistics of each locus in the 106 *Annona deceptrix* genotypes

Locus	*Ta*	*Na*	*Ne*	*Ho*	*He*	*Fis*	*Fst*	*Nm*
AD1	17	4.545	2.858	0.485	0.700	0.209	0.282	0.637
AD2	6	2.455	1.671	0.235	0.317	0.217	0.256	0.727
AD3	7	2.545	1.467	0.087	0.271	0.666	0.133	1.627
AD4	6	2.182	1.333	0.098	0.200	0.488	0.135	1.604
AD5	18	6.455	4.447	0.625	0.796	0.146	0.160	1.311
AD6	14	5.545	3.639	0.634	0.768	0.065	0.186	1.096
AD8	25	7.000	4.423	0.777	0.810	−0.048	0.146	1.466
AD9	6	2.818	1.927	0.340	0.386	0.060	0.354	0.455
AD10	17	5.455	3.791	0.719	0.763	−0.025	0.185	1.099
AD11	7	3.273	1.969	0.507	0.479	−0.160	0.102	2.196
AD13	15	3.636	2.188	0.619	0.512	−0.298	0.119	1.857
AD15	7	2.364	1.362	0.142	0.187	0.202	0.162	1.293
AD16	7	2.727	1.646	0.334	0.353	−0.012	0.345	0.475
AD17	7	3.000	1.971	0.385	0.428	0.042	0.242	0.784
AD18	5	1.727	1.324	0.070	0.190	0.613	0.182	1.124
AD19	8	3.182	2.321	0.546	0.510	−0.151	0.280	0.644
AD21	6	3.091	1.964	0.490	0.502	−0.062	0.111	2.002
AD22	8	3.818	2.321	0.406	0.531	0.187	0.347	0.470
Mean	10.333	3.657	2.368	0.417	0.483	0.119	0.207	1.159

Ta number of total alleles, *Na* number of different alleles, *Ne* number of effective alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, *Fis* inbreeding coefficient, *Fst* genetic differentiation, *Nm* gene flow

Blanca ($Npa = 14$), and Humedad ($Npa = 13$) populations. The observed heterozygosity (*Ho*) varied from 0.352 to 0.519 in the Ayampe and Guacucal, respectively. The expected heterozygosity (*He*) was highest in Humedad with a value of 0.649, followed by Agua Blanca ($He = 0.623$), and Tachina ($He = 0.616$). The lowest *He* was observed in El Barro with a value of 0.194. In general, the level of genetic diversity in the 106 *A. deceptrix* sample was moderate ($He = 0.445$). The percentage of polymorphic loci (*PPL*) was of 100% in Agua Blanca, Tachina, Nalpe, and Humedad, while El Barro presented the lowest number of *PPL* with a value of 38.89%. Finally, El Barro was the population with the lowest inbreeding ($Fis = -0.667$) and genetic differentiation ($Fst = -0.848$) coefficients, while the population Tachina presented the highest values of $Fis = 0.391$ and $Fst = 0.373$. All diversity parameters evaluated at the population level are shown in Table 4.

Table 4 Summary statistics of genetic diversity for all loci for each population of *Annona deceptrix*

Canton	Population	N	*Na*	*Ne*	*Npa*	*Ho*	*He*	*Fst**	*Fis*	*PPL*
Manta	Pacoche	7	2.389	1.932	0	0.409	0.374	−0.041	0.024	77.70%
Puerto López	Agua Blanca	10	4.944	3.412	14	0.472	0.623	0.287	0.331	100%
	Ayampe	7	2.722	1.908	6	0.352	0.36	−0.023	0.047	77.70%
	El Mate	17	2.778	1.915	3	0.363	0.361	0.01	0.037	72.20%
Jipijapa	El Barro	2	1.444	1.404	0	0.361	0.194	−0.848	− 0.667	38.80%
Pedernales	Tachina	23	7.500	3.577	36	0.426	0.616	0.373	0.391	100%
	Tachina Alto	6	2.500	1.859	4	0.374	0.343	−0.109	−0.038	77.70%
	Nalpe	11	5.444	2.674	6	0.457	0.575	0.236	0.277	100%
	Humedad	12	5.389	3.303	13	0.474	0.649	0.303	0.339	100%
	Vite	8	2.722	1.990	3	0.377	0.406	0.065	0.123	88.80%
	Guacucal	3	2.389	2.073	4	0.519	0.398	−0.342	−0.188	72.20%
	Mean	–	3.654	2.367	–	0.416	0.445	−0.008	0.061	82.30%

N sample size, *Na* number of different alleles, *Ne* number of effective alleles, *Npa* number of private alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, *Fst* genetic differentiation, *Fis* inbreeding coefficient, *PPL* percentage of polymorphic loci

*Negative values were considered as zero

Genetic differentiation and genetic structure

The analysis of molecular variance (AMOVA) revealed that a large part of the genetic variation occurred within individuals (65%), while 11% of genetic variation was attributable to the differentiation among populations (Table 5). The fixation index (*Fst*) was 0, indicating no genetic differentiation among populations. Pairwise estimates of *Fst* calculated between pairs of populations are shown in the Fig. 3. El Barro population presented the greatest differentiation (*Fst*) with the Ayampe (*Fst* = 0.372), Tachina Alto (*Fst* = 0.358), and Pacoche (*Fst* = 0.351) populations, which come from the Puerto López, Pedernales, and Manta cantons, respectively. In general, the least differentiation was found between

Table 5 Analysis of molecular variance (AMOVA) within and between different natural populations of *Annona deceptrix* from Manabí Province, Ecuador

Source	df	Sum of squares	Mean squares	Est. Var	Variation%	*Fst*
Among populations	10	180.248	18.025	0.635	11%	
Among individuals	95	592.941	6.241	1.302	23%	
Within individuals	106	385.500	3.637	3.637	65%	0.114*
Total	211	1158.689		5.574	100%	

df degrees of freedom, *Est. Var.* estimated variance, *Significant

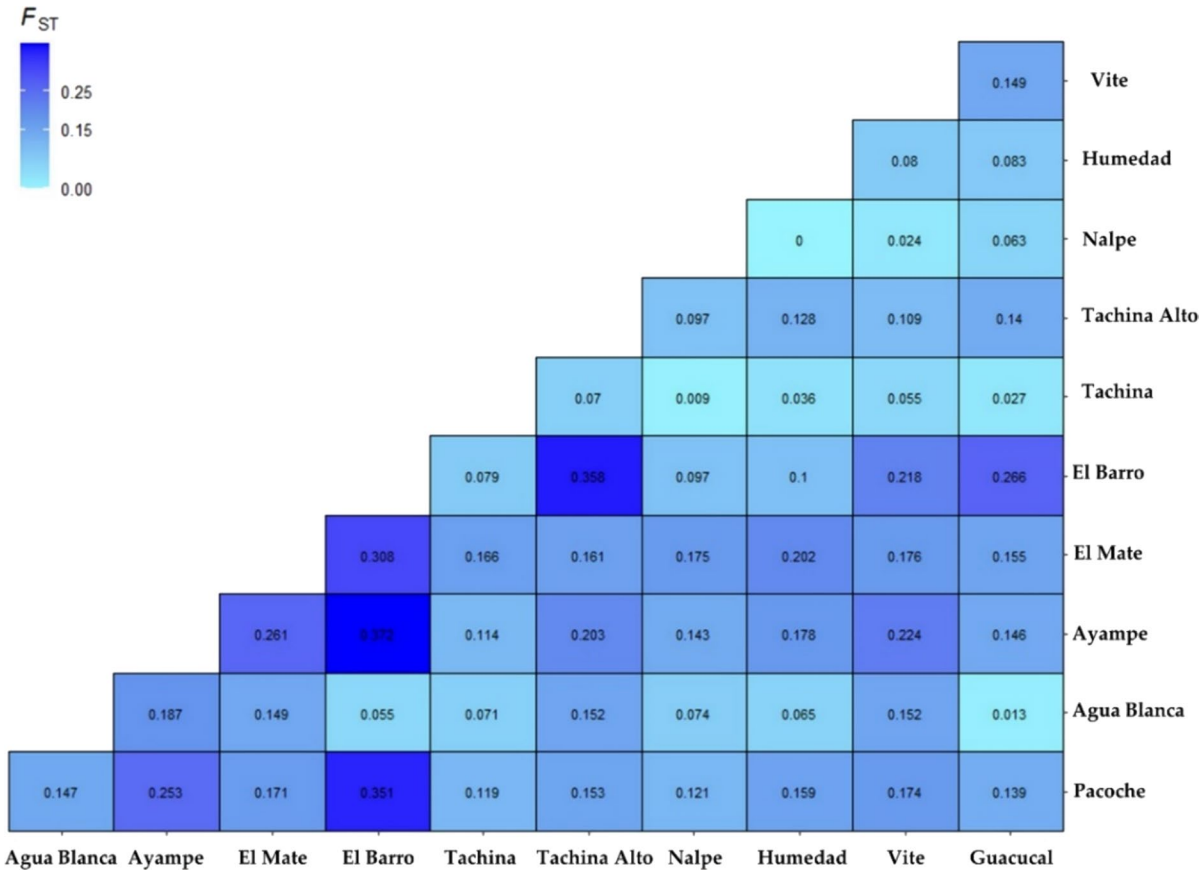

Fig. 3 Pairwise differentiation index among 11 populations of *Annona deceptrix*

populations belonging to the canton of Pedernales. In this sense, the pairs of populations Nalpe and Humedad ($Fst = 0$) and Nalpe and Tachina ($Fst = 0.009$) were the least differentiated. This result is in accordance with the Mantel test conducted for *A. deceptrix* populations, where a null correlation between geographic and genetic distances among populations ($R^2 = 0.007$; $p = 0.02$) was detected, indicating no significant isolation by distance (IBD) between populations (Fig. 4).

The principal component analysis (PCA) also showed a low differentiation between *A. deceptrix* populations, where the first principal component (PC) explained 25.6% of the variation, while the second PC explained 8.3%. This result indicated that the 11 natural populations can be grouped into two genetic clusters. In one cluster, individuals from the 11 populations were grouped, while in the second cluster, individuals from the populations of Agua Blanca (3), Tachina (5), Nalpe (2), and Humedad (4) were grouped (Fig. 5). Result is in agrees with the Bayesian analysis, where the most probable number of subpopulations was $K = 2$ (Fig. 6A and B). Finally, the genetic relationship between the *A. deceptrix* individuals from different populations can be grouped into three cluster based on genetic distance, where some individuals from the Agua Blanca, Tachina, Nalpe and Humedad populations were the most genetically distant in accordance with the structure and PCA results (Fig. 6C).

Discussion

SSR primers in *A. deceptrix*

SSR markers have been widely used to carry out research of genetic diversity due to their hyper-variability, multiallelic nature, reproducibility, relative abundance, extensive genome coverage, high polymorphism, and co-dominant inheritance (Kalia et al. 2011). The 18 SSR markers used in this study had a high percentage of polymorphic loci (82.3%) in the *A. deceptrix* populations, confirming that the SSR markers developed by Pico-Mendoza et al. (2024) are useful to evaluate genetic diversity in *A. deceptrix.* These results are also in consonance with those reported by Saitwal et al. (2022), where a total of 20 SSR were evaluated in different *Annona* genotypes from Maharashtra, India, and produced 83.72% of polymorphic loci. In addition, a 95.0% of polymorphic loci was reported in populations of *A. senegalensis* using 10

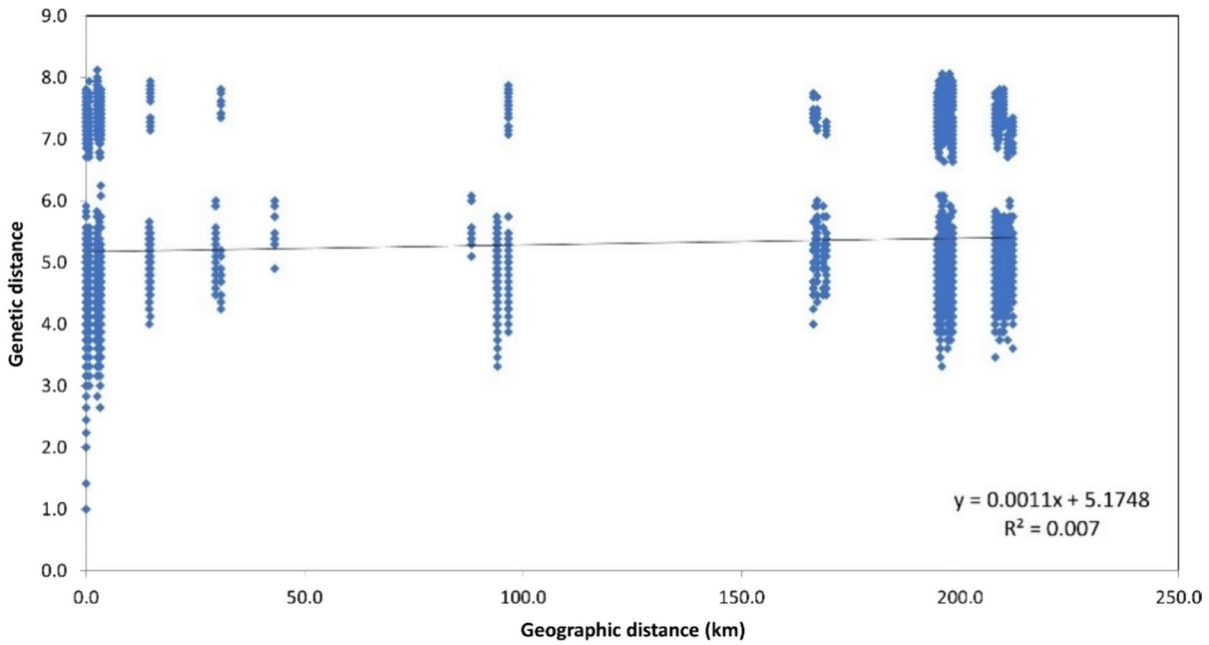

Fig. 4 Correlation analysis between geographic and genetic distance of 11 populations of *Annona deceptrix*

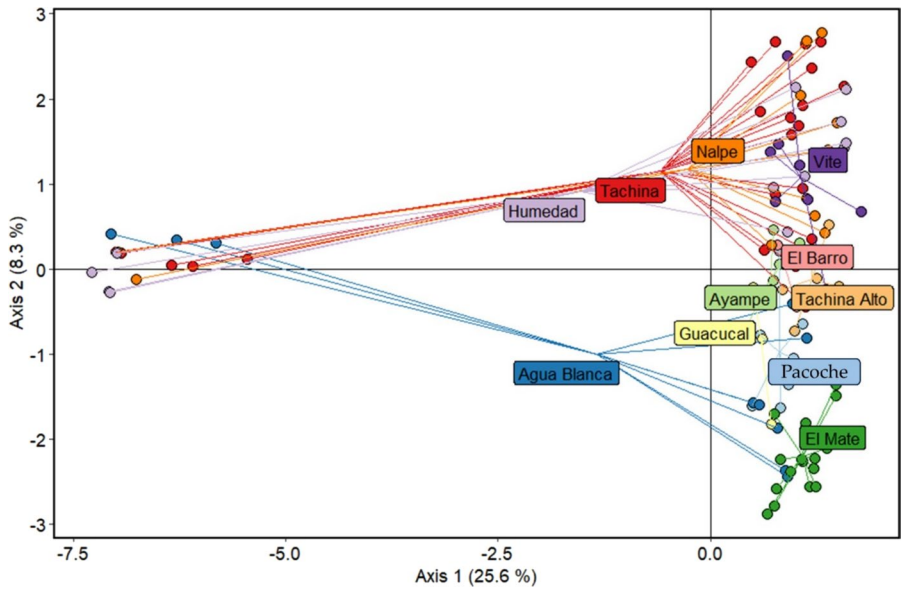

Fig. 5 Principal component analysis (PCA) of *Annona deceptrix* populations. The first and second axis explained 25.6% and 8.3% of the total genetic variance, respectively

SSR markers (Donhouedé et al. 2023). In our current study, the AD5, AD6, AD8, AD10 and AD1 markers were the most informative for *A. deceptrix*. Consequently, considering the high rate of transferability of SSR markers between related species, these primers could be useful for the evaluation of evaluate genetic diversity in other understudied *Annona* species, without the need to develop species-specific markers.

Genetic diversity of *A. deceptrix*

Ecuador contains a unique set of species and ecosystems, many of them endemic to the country (Cuesta et al. 2017). However, due to different sources of anthropogenic threat, many of these endemic species are expected to become extinct or threatened (Brooks et al. 2002) in the future. *A. deceptrix* is an endemic and endangered tree distributed throughout the humid remnants in the coastal zone of Ecuador (Pico-Mendoza et al. 2020). Ecosystems near coastal areas are considered fragile and with high rates of disturbance, being considered priority conservation areas (Cuesta et al. 2017). In Manabí, a coastal province of Ecuador, there is a high deforestation rate (6159 ha/year), which causes a high degree of threat to its native flora, including *A. deceptrix*. Despite this reality, there are no programs aimed at the conservation of threatened species. In this sense, to evaluate the genetic diversity of *A. deceptrix* populations is an important prerequisite to determine the management strategies that would guarantee its conservation.

To carry out effective conservation strategies, it is essential to understand the spatial patterns of the genetic diversity of the target species (Escudero et al. 2003), allowing the identification of populations with greater and lesser diversity, and thus, creating conservation strategies that optimize the use of available genetic resources (van Zonneveld et al. 2012). Among the populations of *A. deceptrix*, the greatest expected (*He*) and observed (*Ho*) heterozygosity was reported for Humedad ($He = 0.649$; $Ho = 0.474$), followed by Agua Blanca ($He = 0.623$; $Ho = 0.472$), and Tachina ($He = 0.616$; $Ho = 0.426$). In a previous work, individuals of *A. deceptrix* from Tachina (12), Agua Blanca (10), and Humedad (18) were evaluated with 22 SSR markers, whose *He* and *Ho* values were like those obtained in this study (Pico-Mendoza et al. 2024). Our results indicated that the *A. deceptrix* populations collected have a moderate level of genetic diversity, showing that the expected and observed heterozygosity of *A. deceptrix* was $He = 0.445$ and $Ho = 0.416$, respectively. This is similar to what has been reported in other *Annona* species, where a moderate level of genetic diversity ($He = 0.470$) was found in 14 populations of *A. squamosa* distributed in the Mexican states of Yucatán and Quintana Roo using isozyme markers (Salazar et al. 2010). Moreover, in *A. senegalensis* populations occurring in Western and Southern

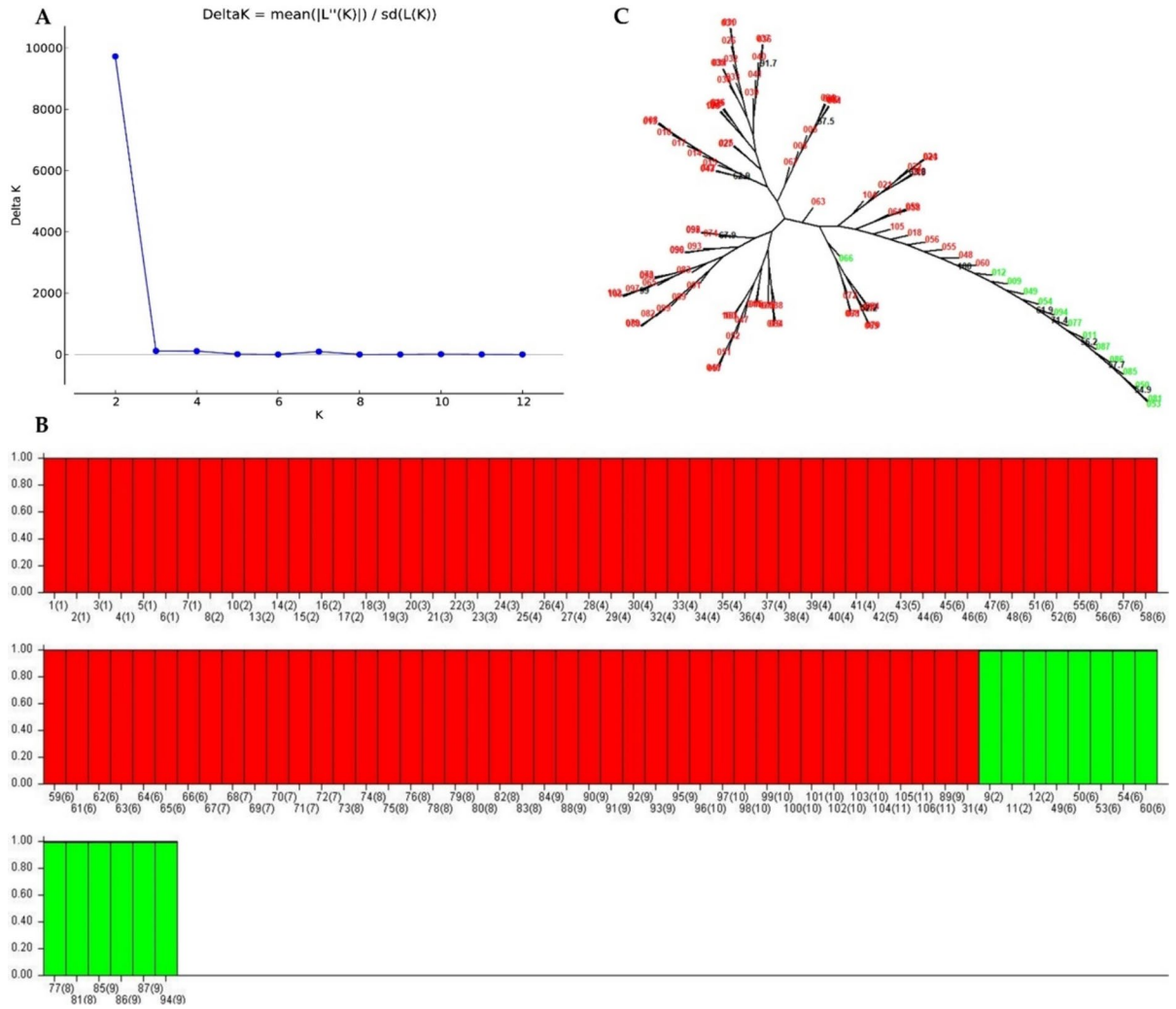

Fig. 6 Genetic structure of 106 genotypes from 11 *Annona deceptrix* populations inferred by Bayesian clustering and genetic distance. **A** Plot of mean posterior probability Ln (K) values varying of number of subpopulations (*K*) from 1 to 11. **B** The estimated membership probability (Q) for each genotype. **C** Unrooted neighbor joining tree of 106 genotypes of *A. deceptrix*. The color of the names is according to the result obtained by the structure analysis, where red and green corresponds to cluster 1 and cluster 2, respectively

Africa a high level of genetic diversity (*He* = 0.56) was found (Donhouedé et al. 2023). However, these values are higher than those reported in other populations of *Annona* localized in Brazil. For example, in four natural populations of *A. crassiflora* from northern Minas Gerais State, the genetic diversity evaluated with RAPD markers was *He* = 0.31 (Cota et al. 2011). In *A. squamosa*, populations from the Brazilian states of Maranhão and Piauí had a low genetic diversity (*He* = 0.222) using ISSR markers (Sá et al. 2022). Our findings suggests that the anthropogenic effect on the reduction in population size has affected the genetic variation of the species to a lesser extent.

Genetic differentiation and structure in *A. deceptrix*

The analysis of molecular variance (AMOVA) revealed that only 11% of the total genetic variation in *A. deceptrix* occurred among populations, while 65% resided within individuals. The low differentiation coefficient (*Fst* = 0) and the moderate gene flow

($Nm = 1.159$) indicated that there is no genetic differentiation between populations. However, the markers AD9, AD22, AD16, AD1, AD19, and AD2 have a high power of discrimination between populations ($Fst > 0.25$). The level of population differentiation reported in this study for *A. deceptrix* was contrary to what has been reported in other *Annonas* species such as *A. squamosa* (Nagori et al. 2018; Sá et al. 2022), *A. muricata* (Brisibe et al. 2017; Lira-Ortiz et al. 2022), *A. senegalensis* (Donhouedé et al. 2023), where populations are, generally, genetically differentiated according to its distribution and geographic distance. This result may be due to the negative relationship between the number of alleles per locus and the estimates of *Fst* values, resulting in a decrease in the power to discriminate between populations (O'Reilly et al. 2004). Furthermore, the result may be influenced by the population size used in this study, since a number of ~ 20–25 individuals (Danusevičius et al. 2016) per population or at least 10% of the individuals from populations are recommend for genetic research (Sinclair and Hobbs 2009; Costa et al. 2015).

Although anthropogenic activity has affected tropical forests since ancient times (van der Sande et al. 2019), our results suggest that the fragmentation of the coastal habitat in the province of Manabí has been below the fragmentation threshold which causes loss of genetic diversity and population structure (Cruzan 2001; Bacles and Jump 2011). Part of the data in the current study is supported by the Mantel test where no isolation by distance was detected between *A. deceptrix* populations, indicating that the reduction in population size due to anthropogenic activity has not greatly affected the genetic variation of the species. However, due to the intensive increase in land use change, especially during recent decades, the conservation of threatened species (mainly endemic) should be a high priority (Gibbs et al. 2010; Cuesta et al. 2017; Erkens et al. 2023). For the purposes of conservation of *A. deceptrix*, the strategies should focus on populations such as Humedad, Agua Blanca, and Tachina, since they were the populations with the greatest diversity and number of private alleles, which are good candidates for conservation because high levels of genetic variation are expected to increase the potential of populations to respond to selection and maintain the health of individuals (Kalinowski 2004). In addition, these populations are interesting for genetic improvement purposes because there is a greater probability of finding genotypes with characteristics of commercial interest than in populations with low genetic diversity (Frankel et al. 1995; van der Sande et al. 2019).

Conclusion

The SSR markers developed for *A. deceptrix* were informative and allowed evaluating the genetic diversity of 11 natural populations distributed in the province of Manabí, Ecuador. A moderate level of genetic diversity and no differentiation between populations was found. However, the large number of private alleles reported could indicate that populations are experiencing significant reductions in size. Therefore, these results have a great impact on the conversation policies of *A. deceptrix* considering that it is an endemic and threatened species. In this sense, it is suggest that the strategies should focus on populations such as Humedad, Agua Blanca, and Tachina, since they were the populations with the greatest diversity and number of private alleles, doing conservation activities such as habitat conservation, in situ and ex situ conservation, genetic banks, with the intention of safeguarding the genetic variability evaluated.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by José Pico-Mendoza, Luis Madrid, Eduardo Morillo and Juan Flor. The first draft of the manuscript was written by Basilio Carrasco and Osvin Arriagada and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data sets generated and/or analyzed during the current study are not publicly available due to future work being planned. But they are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest.

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