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When native meets exotic: Uncovering hybridization between Cuban and introduced mahogany using chloroplast and nuclear microsatellites

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Abstract The introduction of closely related species can threaten the genetic integrity of endemic taxa through hybridization and introgression. In Cuba, *Swietenia macrophylla* was widely planted in the 1970s for its fast growth and high-value timber, raising concerns about its potential genetic impact on the native *S. mahagoni* and the emergence of hybrids. This study investigates the genetic consequences of introducing *S. macrophylla* to Cuba by analysing 357 individuals from five populations, including two naturally regenerated *S. mahagoni* populations, a pure *S. macrophylla* remnant, and two mixed stands containing both species and morphologically intermediate forms. The two predominant cpDNA haplotypes were shared across all five populations, whereas one mixed stand exhibited the highest diversity, with nine chloroplast DNA haplotypes—suggesting that its establishment involved seeds from multiple sources. cpDNA markers didn't reveal clear species-level differentiation. In contrast, nuclear microsatellites indicated moderate genetic differentiation (mean F_{ST}= 0.15±0.02) and substantial within-population variability, with expected heterozygosity (He) ranging from 0.46 to 0.85. At the same time, *S. mahagoni* showed a higher number of alleles (N_a = 12.4–13.3) and expected heterozygosity (H_e = 0.73–0.78) than the remnant *S. macrophylla* population (N_a = 5.75; He = 0.59), likely reflecting the smaller size of the existing *S. macrophylla* plantation in Cuba. Based on discriminant analysis of principal components and STRUCTURE results, the two parental species were clearly separated into distinct genetic clusters, while putative hybrid individuals were mainly concentrated in the two mixed stands. Leaf morphological and molecular classifications were highly concordant in parental populations (≈91%), confirming the phenotypic identifiability of *S. mahagoni* and *S. macrophylla*. The estimated proportion of hybrids varied: STRUCTURE (7.8–15.1%), whereas NewHybrids (36.1–39.2%). These findings provide a genetic framework for conservation-oriented management of native tree species, which is a tool for implementing long-term genetic monitoring and consolidating certified germplasm banks to safeguard the native Cuban mahogany.

Keywords: *Swietenia,* Genetic diversity, introgression, Extinction vulnerability

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Introduction

In the Americas, the overexploitation of natural mahogany (*Swietenia* spp.) populations throughout history, coupled with the scarcity of commercial plantations, has led to stark reductions in supply and driven up prices in domestic and international markets (Anderson 2004; Chinchilla et al. 2020). Since the 18th century, trade liberalization — such as England's elimination of customs duties in 1721 — has promoted intensive transatlantic trade. This has led to near commercial extinction of *Swietenia mahagoni* in the Caribbean, followed by increased extraction to *S. macrophylla* in Central and South America (FAO 2000; Anderson 2004). This exploitation has led to significant fragmentation and reduction of the natural habitats of these species, which have been classified as endangered by CITES since 2002. As a result, the long-term availability and conservation of the genetic resources of mahogany species is compromised (CITES 2002; Barbosa Filho et al. 2016). This scenario reflects the historical primacy of the economic and cultural value of mahogany as a luxury wood over ecological conservation objectives (Anderson 2004; Lamb 2011).

Although other timber species are harvested in the tropical regions of the American continent, mahogany is fundamental to the realization of sustainable forest management in these regions, being used as a core species for silviculture and forest regulation (Navarro-Martínez et al. 2018). *S. macrophylla*, together with *S. humilis* and *S. mahagoni*, make up the genus *Swietenia*. Introducing closely related exotic species can compromise the genetic integrity of endemic species through processes such as hybridization and gene introgression. In Cuba, concerns have been raised about the potential impact of the introduced species *S. macrophylla* on the native species *S. mahagoni*, including the formation of hybrids that could affect genetic diversity and local adaptation.

Mahogany is one of the most remarkable cases of genetic erosion in a tropical tree species (Lemes et al. 2003; André et al. 2008). In addition to deforestation and land use change, climate change will lead to a decoupling between forest populations and the climate suitable for them (Garza-López et al. 2016). Genetic conservation and sustainable logging strategies depend on genetic diversity and effective population size. Intensive logging with low minimum diameters and short harvesting cycles can affect the genetic structure of remaining populations, compromising sustainable timber production (Sebbenn et al. 2008).

Genetic structure emerges from the interaction between a species' mating system and the dispersal kernels of both pollen and seeds; together, these processes generate the spatial arrangement of genetic variation observed in natural populations. Investigating mating patterns and the dispersal of pollen and seeds in commercial timber species is therefore critical for designing sustainable management and conservation strategies, because restricted gene flow or highly localized seed dispersal can produce strong spatial genetic structure and elevate inbreeding risk (Sebbenn et al. 2012). When mahogany populations show low genetic diversity, they become more susceptible to inbreeding depression, emergent pests and diseases, and reduced adaptive capacity — outcomes that undermine reproductive success and natural regeneration (André et al. 2008). Therefore, management and improvement programmes must prioritise not only productivity and resistance, but also the maintenance of effective population size and connectivity to ensure long-term resilience (Alves et al. 2020).

Previous studies of mahogany species in the region have been limited and unable to comprehensively assess genetic diversity and population structure using nuclear and chloroplast molecular markers (Quiala et al. 2022). Therefore, it is crucial to characterize the genetic diversity and potential hybridization between the two species to inform conservation and sustainable management strategies.

This study aimed at investigating the genetic consequences of introducing *S. macropylla* into Cuba. We characterized, (1) the genetic diversity and population structure of *Swietenia*

species complex in Cuba, and (2) the relationship between morphological classification and genetic assignment was examined. It is anticipated that the findings of this study will provide a genetic framework to support the conservation and sustainable management of the native Cuban species *Swietenia*, offering key insights for the advancement of conservation forestry and the preservation of tropical genetic resources in Cuba.

Material and methods

Study populations

The study was conducted in the province of Sancti Spíritus, Cuba, where five mahogany populations were sampled: two naturally regenerated populations of *S. mahagoni*, one remnant plantation of *S. macrophylla*, and two mixed stands. A total of 357 adult trees were evaluated: 80 individuals from each naturally regenerated population of *S. mahagoni*, 90 from each mixed stand, and all existing individuals from the remnant *S. macrophylla* plantation (Table 1) (Figure 1). For the chloroplast analysis eight adult trees were randomly selected from each population. Consequently, the total number of individuals analysed was 80 (Table 1). A minimum distance of 5–10 m was maintained between sampled trees, whenever possible, to reduce the likelihood of collecting closely related individuals.

Table 1 Sampled populations in Cuba.

Ab	Region	Pop	Sp	N	Nc	Ft	Latitude/Longitude	Altitude (m)
SG-A	Guira	Stand A	*S. mahagoni*	80	16	Ne	21.78942/-79.64634	60
SM-B	Modelo	Stand B	*S. mahagoni*	80	16	Ne	21.89687/-79.41949	5
SC-C	Colon	Stand C	*S. macrophylla*	17	16	Pl	21.91925/-79.44355	15
MB-D	Banao	Stand D	Mixed stand	90	16	Pl	21.82337/-79.56849	30
ME-E	Emigdio	Stand E	Mixed stand	90	16	Pl	21.91972/-79.35641	35

Note: Abbreviation (Ab). Region of the sample in Sancti Spíritus, Cuba. Population (Pop). Species (Sp). Sample size (N). Sample size for chloroplast analysis (Nc). Forest type (Ft): Ne (naturally regenerated populations) and Pl (Plantation).

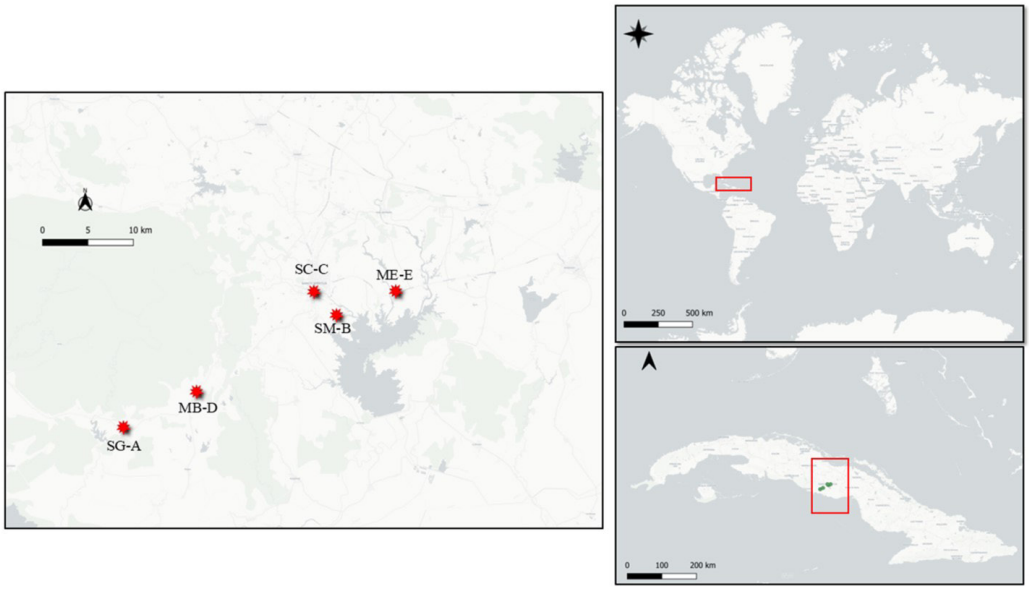

Figure 1 Geographical location of the studied stands in Cuba (for the abbreviations, see Table 1).

The two naturally regenerated populations of *S. mahagoni* correspond to former natural Cuban mahogany forests that were heavily deforested and subsequently exploited for agricultural purposes (Modelo site, where the land was completely ploughed after cessation of farming) and livestock grazing (Guira site). Natural forest regeneration began between 1993 and 1995, following the end of productive land use. Currently, both populations are over 30 years old, with an average height of approximately 9.94 m.

S. macrophylla was introduced to Cuba between 1969 and 1970 through a collaborative project with the Food and Agriculture Organization of the United Nations (FAO), aimed at evaluating its performance and potential for reforestation programs. Initial nurseries were established in the provinces of Matanzas and Ciego de Ávila. However, due to natural events and urban expansion, these early seedbeds and the resulting seedlings have mostly disappeared. For the present study, a remnant plantation established during that period—originating from nursery-produced seeds—was selected. This stand is approximately 55 years old, with an average height of 16.47 m, and was planted using a 3 × 3 m spacing.

The two mixed stands were established between 1982 and 1984. Prior to planting at the Banao site, the land had been used for various crops and was later converted into a forest plantation. Manual soil preparation, including hole digging, was carried out with a 3 × 2.5 m planting frame, mainly aimed at timber production. The Emigdio site was previously used as pasture, and the same soil preparation was applied; however, a 3 × 3 m spacing was used, focusing more on species development and conservation. Seedlings for both sites originated from seeds collected in the locality of Colón, selected for their fast growth and high wood quality. These plantations are currently around 42 years old, with an average height of 15.74 m.

DNA extraction, amplification, and sizing

Leaflets were collected from 2 populations of *S. mahagoni* (SG-A and SM-B), a remnant of *S. macrophylla* (SC-C) and two mixed stands (MB-D and ME-E) covering the range of the species in Sancti Spíritus, Cuba (Table 1, Fig. 1). The material was stored in a deep freezer at temperatures of -60°C until it was used for DNA extraction. Genomic DNA was extracted from biological specimens using the CTAB method described by (Doyle & Doyle 1987) with minor modifications. DNA was quantified using NanoDrop 8000 (Thermo Scientific, Wilmington, USA, 2008) and the extracted DNA was stored at -20°C.

Chloroplast microsatellites analysis

Chloroplast DNA variation was assessed using three universal chloroplast microsatellite markers (ccmp4, ccmp7 and ccmp10 (Weising & Gardner, 1999). DNA was diluted (1:30) before PCR, which was performed in a volume of 15 µl with 1 x PCR buffer, 25 mg $MgCl_2$, 200 µM of dNTPs, 1 unit of Taq polymerase and 0.2 µM of each primers, adding 2 µl of genomic DNA. Amplifications were carried out in a Corbett Palm-Cycler CG1-96 (initial denaturation for 15 min at 95^{0}C, after by 30 to 35 denaturation cycles of 60 sec at 94^{0}C, followed by an extension of 60 sec at 72^{0}C and a final extension at 72^{0}C for 20 min) with 30 cycles for ccmp4 and 35 cycles for ccmp7 and ccmp10, following a denaturation, annealing and extension protocol. Fluorescently stained primers were used for genotyping: Cy5/blue for ccmp7 and ccmp10, and Cy5.5/Green for ccmp4. The amplified fragments were analyzed on a GenomeLab GeXP system (Beckman Coulter) with an internal size standard (CEQ 400). Polymorphisms were identified from the different lengths of the detected microsatellite fragments.

Nuclear microsatellites analysis

Eight nuclear microsatellites (Sm01, Sm31, Sm32, Sm40, Sm45, Sm46, Sm47 and Sm51) were used (Lemes et al. 2002) (Table 2). Two multiplex reactions were performed for PCR amplification: multiplex I-Sm01, I-Sm31, I-Sm32 and I-Sm40; multiplex II-Sm45, II-Sm46, II-Sm47 and II-Sm51. The PCR reaction was carried out in a total volume of 11 µL (both multiplexes), containing 7.5 µL of

Table 2 Characteristics of the 8 microsatellite loci (Lemes et al. 2002).

SSR locus	Repeat	P.S (5'-3')	P.C (µM)	A.Rs (pb)	A	Ho	He	Q	I
Sm01	(AG)19	5'-GCGCGATTGATTGACTTC-3' 5'-GCGCTTAGCATTATTCTCC-3'	1.25	261–295	17	0.65	0.80	0.65	0.06
Sm31	(AG)31	5'-CTTCTAATGTTCTGATGCCTG-3' 5'-AGCAACTCGTGAGGAATTTAC-3'	2.0	80–138	25	0.85	0.94	0.87	0.09
Sm32	(AG)20	5'-CACCTTATGTACACCACACAG-3' 5'-GAAGGAGACACCAGCAATC-3'	2.0	146–184	15	0.74	0.91	0.81	0.02
Sm40	(AG)19	5'-TGCTACTGTCAAGAGTGTAT-3' 5'-GACAAACATGTACCACAAG-3'	2.0	120–146	13	0.69	0.75	0.56	0.10
Sm45	(AG)21	5'-CCTTATGTTCACCACACAGTA-3' 5'-GAGACACCAGCAATCCAG-3'	1.25	140–178	15	0.89	0.91	0.81	0.02
Sm46	(AG)20	5'-GCAGTACTCGCCTATCTTCA -3' 5'-TGAGAACTGCAGAATCCTTT-3'	2.0	190–226	17	0.83	0.88	0.77	0.03
Sm47	(AG)24	5'-GCCATTGGTCTCAATCTTAC-3' 5'-GGAAGAGTCTTAGAACACAG-3'	2.0	114–150	11	0.73	0.84	0.69	0.05
Sm51	(AG)22	5'-GCAATTTCCAGAAGAAACC-3' 5'-CTGTAGGCGATAACAATCAG-3'	2.0	138–182	19	0.86	0.88	0.76	0.03

Note: SSR locus name, repeat, forward and reverse primer sequences (P.S), primer concentration (µM), allelic size range (bp), number of alleles detected per locus (A), observed heterozygosity (H_o), expected heterozygosity (H_e), probability of paternity exclusion (Q), probability of genetic identity (I), and GenBank accession number.

Qiagen Multiplex PCR Master Mix 2×, 0.3 µL of direct sequence of marker, 0.3 µL of reverse sequence, 1.2 µL of RNase-free water and 2 µL of DNA. The PCR profile consisted of 15 min initial denaturation at 95°C, followed by 30 cycles of 1 min denaturation at 94°C, a 30 s annealing step at 55°C, a 1 min elongation step at 72°C and a 20 min final extension step at 60°C. The amplified PCR products were diluted in RNase-free water. The amplified PCR products were diluted and analyzed on a GemoneLab GeXP genetic analyzer using the Frag-3 method and the Size Standard 400.

Data analysis

cpSSR data analysis

The PERMUT cpSSR programme was used (Pons & Petit 1996) to calculate chloroplast DNA diversity parameters, including h_S (haplotype diversity within populations), h_T (total haplotype diversity), G_{ST} (differentiation between populations based on unordered alleles) and R_{ST} (differentiation between populations based on ordered alleles).

An analysis of molecular variance (AMOVA) was performed with GenAlEx 6.5 (Smouse & Peakall 2012) to estimate the distribution of genetic variation between and within populations, using 999 permutations for randomization tests. Genetic distances between pairs of populations were calculated with HAPLOTYPE ANALYSIS© 1.05 (Eliades et al. 2019), based on the method of molecular evolutionary genetic (Nei 1987).

SPAGEDI 1.5 software (Hardy 2003) was used to assess the presence of a phylogeographic pattern by permutation of allelic sizes. If the observed T_{SR} exceeds the T_{SR} after permutations, the existence of a phylogeographic pattern can be inferred. Relationships between cpSSR haplotypes were analyzed using the median linkage network algorithm (Bandelt et al. 1999) NETWORK 5.0, which calculates mutational steps and considers haplotype frequency. To assess phylogenetic relationships between populations, an UPGMA network was constructed using the PAST software (Hammer et al. 2001), based on the Euclidean pair-group algorithm. In addition,

one thousand bootstrapped matrices of pairwise genetic distances were generated (Nei 1987) with the same programme. The association between pairwise genetic distance (Nei 1987) and geographical distances (GGD) was assessed using the Mantel test (Mantel 1967) using GenAlEx 6.5 software. Geographical distances were calculated from UTM coordinates of sampled individuals in each population evaluated, generating a pairwise geographic distance matrix used in the analysis.

Nuclear data analysis

a. Population structure analysis

As the presence of null alleles can reduce the accuracy of individual assignment in genetic structure analyses, we evaluated their frequency using the MICROCHECKER programme (Van Oosterhout et al. 2004; Carlsson 2008). The evaluation using MICROCHECKER indicated that null alleles were not present at high frequencies in most loci, with estimates generally below 0.20–0.25 according to the different methods applied. Only one locus showed an estimate above 0.30, suggesting the possible presence of null alleles in that specific marker. However, as the observed frequencies were generally low to moderate, it was decided to include all loci in the population structure analyses using STRUCTURE v.2.3.4 software (Pritchard et al. 2000).

The programme STRUCTURE v.2.3.4 was configured to perform three independent runs, each with a burn-in period of 500000 iterations, followed by 10^5 iterations for each value of K (between 1 and 5), with 10 repetitions. The mixture model with correlated allele frequencies was used, with no a priori information on the geographical origin of the individuals. The mixture model was chosen for its ability to allow for the mixed ancestry of individuals, and the use of correlated allele frequencies was chosen for its potential to optimize the detection of population structures or closely related species. The optimal number of genetic clusters was determined by comparing the values of log probability (ln Pr(X|K)) and using the Evanno method (ΔK) (Evanno et al. 2005) on the STRUCTURE Selector v.0.6.94 platform (Li & Liu 2018).

The graphical representation was performed on the STRUCTURE Selector v.0.6.94 platform using CLUMPAK (Kopelman et al. 2015) with the ΔK recommended on the platform. Individuals were classified according to their membership coefficients (q), which were obtained in STRUCTURE. Those with $q \geq 0.90$ in a group were considered pure and those with intermediate values ($0.10 < q < 0.90$) were considered to be of mixed ancestry. This included individuals with high ancestry proportions within the intermediate range (0.61–0.89), who were categorized as introgressive forms (Curtu et al. 2007).

Subsequently, the Bayesian model-based programme NEWHYBRIDS (Anderson & Thompson 2002) was utilized to calculate the posterior probability that individuals belonged to one of six categories: (1) pure mahogany, (2) pure macrophylla, (3) first-generation hybrids, (4) second-generation hybrids, (5) mahogany backcrosses, and (6) macrophylla backcrosses. The present analysis focuses on first- and second-generation hybrids, which are instrumental in detecting hybridization between species. The assignments were made without a similar priori distribution. The analysis was performed thrice, with 100,000 sweeps and a burn-in period of 50,000. Individuals were assigned to a given class using a conservative posterior probability threshold of ≥ 0.90. To assess the robustness of hybrid detection, a relaxed threshold of ≥ 0.80 was also applied. Individuals assigned to the F2 category were interpreted as introgressed genotypes. The prior information on pure parental individuals obtained from the previous STRUCTURE analysis was included in the analysis. The results of the probability of assignment by population obtained in NEWHYBRIDS and the previous STRUCTURE result (ΔK=3) were visualized using stacked bar graphs, adapting the classic style of STRUCTURE graphs.

b. Genetic diversity and differentiation

The standard indices of genetic diversity were estimated for each population using GenAlEx v.6.5 (Peakall & Smouse 2006; Smouse & Peakall 2012). These indices included the number of alleles per locus (Na), the number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and the fixation index (F_{ST}). Furthermore, the results were corroborated with GENEPOP v.4.8 (Raymond & Rousset 1995; Rousset 2008) in order to guarantee the consistency of the estimators obtained. In order to evaluate the effect of possible hybrids, the analyses were repeated, excluding individuals classified as potentially hybrid by NewHybrids under two assignment thresholds: 0.10–0.90 and 0.20–0.80 (Anderson & Thompson 2002; Vähä & Primmer 2006; Curtu et al. 2007) the exclusion of these individuals did not result in a substantial alteration of the observed patterns of diversity and differentiation.

The genetic differentiation between populations was assessed using pairwise F_{st} estimates, Nei's genetic distances, and molecular variance analysis (AMOVA) in GenAlEx v.6.5. For AMOVA, Wright's FST was calculated as the ratio of variance among populations to total variance. In addition, a standardized F_{ST} was computed by dividing the observed FST by its maximum possible value ($F_{ST,max}$), to account for the dependence of F_{ST} on within-population heterozygosity. The differences between ecotypes were evaluated using discriminant analysis of principal components (DPCA) in R (RStudio 2025). In accordance with the previously published morphological classification (Coca et al. 2024), an evaluation was conducted to ascertain the concordance of said classification with the nuclear genetic assignment obtained in the present study. Individuals whose morphological category coincided with the species assigned by the nuclear genotypes (membership thresholds 0.10–0.90 and 0.20–0.80) were designated as 'concordant'. Once the morphological and nuclear categories had been determined, we proceeded to quantify the concordance between both approaches through the percentage of coincidence, reported on a general scale and stratified by population.

Results

cpSSR data analysis

The analysis identifies 11 unique cp DNA haplotypes defined by the markers ccmp10, ccmp4 and ccmp7 (Table 3), distributed across five populations. The most frequent haplotype is 2, present in all populations with 49 individuals, followed by haplotype 6, with 15 individuals. ME-E (mixed stand) is the most diverse, representing 9 out of the 11 haplotypes, while the other populations share less genetic variation and are concentrated in a few common haplotypes. The markers ccmp10 and ccmp4 are mostly constant, with ccmp7 contributing the most variability.

Table 3 Observed chloroplast microsatellite in *Swietenia* ssp. in Cuba.

Haplotype#	ccmp10	ccmp4	ccmp7	Population	N
H01	110	121	128	ME-E	2
H02	110	121	129	All	49
H03	110	121	130	MB-D	1
H04	110	121	148	ME-E	5
H05	110	121	149	ME-E	2
H06	110	122	129	All	15
H07	110	122	133	MB-D	1
H08	110	123	129	SG-A, ME-E	2
H09	110	123	130	ME-E	1
H010	111	121	130	ME-E	1
H011	111	121	133	ME-E	1

Note: N: number of individuals. The numbers under the loci represent allele length in nucleotides. Abbreviations are defined in Table 1.

In total, 11 alleles were observed at three cpSSR loci across all 80 individuals in the 5 populations of *Swietenia* spp. The loci, ccmp4 and ccmp7 showed the largest number of alleles, ccmp7 present the highest value for Ne (1.80), meanwhile ccmp4 shows the highest values for I (0.59) and H (0.39). The meanings I and H values were (0.36 and 0.20) (Table 4).

The number of alleles and effective number of alleles was highest in ME-E (Na = 3.67; Ne = 2.57) when compared with the SM-B (mixed stand) and SC-C (*S. macrophylla*) with the lowest Na = 1.33; and SC-C with Ne = 1.09 (Table 5). The I ranged from 0.13 (SC-C) to 0.93 (ME-E).

The average genetic diversity (h) of the three analyzed cpSSR loci in the populations of *Swietenia* spp. in Cuba was 0.20. The results showed that the three cpSSR loci used in this study had high polymorphism in the populations of *Swietenia* spp. in Cuba.

The cpSSR analysis of 80 mahogany individuals identified 11 haplotypes, of which eight were singletons (72.73%). Among populations, ME-E exhibited the highest haplotypic distinctiveness, harboring six private haplotypes (haplo-1, -4, -5, -9, -10 and -11), while SG-A (*S. mahagoni*), SM-B (*S. mahagoni*) and SC-C lacked unique haplotypes (Table 6).

Table 4 Diversity indices for each cpSSR marker across *Swietenia* individuals.

ID	Na	Ne	I	H
ccmp4	2.40	1.59	0.59	0.36
ccmp7	2.40	1.80	0.43	0.20
ccmp10	1.20	1.06	0.08	0.04
Mean	2.00	1.48	0.36	0.20
SD	0.35	0.25	0.12	0.06

Note: Observed number of alleles per locus (Na); Mean number of effective alleles (Ne); Shannon´s Information index (I); gene diversity (H); Standard deviation (SD).

Table 5 Genetic diversity of *Swietenia* populations in Sancti Spíritus based on chloroplast microsatellite loci.

Pop.	N	N_a	N_e	I	h
SG-A	16	1.67 (±0.68)	1.22 (±0.22)	0.24 (±0.24)	0.13 (±0.13)
SM-B	16	1.33 (±0.33)	1.29 (±0.29)	0.22 (±0.22)	0.16 (±0.16)
SC-C	16	1.33 (±0.33)	1.10 (±0.09)	0.13 (±0.13)	0.07 (±0.07)
MB-D	16	2.00 (±0.58)	1.24 (±0.13)	0.32 (±0.16)	0.18 (±0.09)
ME-E	16	3.67 (±1.20)	2.57 (±1.10)	0.93 (±0.38)	0.47 (±0.17)
Mean		2.00 (±0.35)	1.48 (±0.25)	0.36 (±0.12)	0.20 (±0.07)

Note: Population (Pop.); Observed number of alleles per locus (N_a); Mean number of effective alleles (N_e); Shannon´s Information index (I); Haploid diversity (h); ±standard errors in parentheses; Overall mean (Mean). Abbreviations are defined in Table 1.

Table 6 Observed chloroplast microsatellite in *Swietenia* ssp. in Cuba.

Pop.	A	P	N_E	R_H	H_{CP}	D^2_{sh}
SG-A	3	0	1.66	2.00	0.43	0.24
SM-B	2	0	1.88	1.00	0.50	0.17
SC-C	2	0	1.28	1.00	0.23	0.08
MB-D	4	2	1.71	3.00	0.44	1.09
ME-E	9	6	6.10	8.00	0.89	69.68
Means	4.00	1.60	2.53	3.00	0.50	14.25

Note: Population (Pop.); Number of haplotypes (A); Number of private haplotypes (P); Effective number of haplotypes (N_E); Haplotypic richness (R_H); Haplotypes diversity (H_{CP}); Mean genetic distance between individuals (D^2_{sh}). Abbreviations are defined in Table 1.

Haplotype-2 and haplotype-7 are common in the 5 populations studied (Figure 2). The frequency of haplotype-2 is the highest in SC-C (28,66%) (Table 3). Haplo-8 is common in two populations of the five populations studied. ME-E had the highest number of haplotypes 9 (haplo-1; haplo-2; haplo-4, haplo-5, haplo-6, haplo-8, haplo-9, haplo-10, haplo-11). Different values of haplotype diversity (H_{CP} = 0.23-0.89) were observed in all populations studied, with ME-E having the highest haplotype diversity (Table 6). The effective number of haplotypes ranged from 1.28 to 6.10 and the mean genetic distance between individuals ranged from 0.08 (SC-C) to 69.68 (ME-E). The average genetic distance between individuals (D^2_{sh}) was incorporated as a complementary measure of intrapopulation variability, since it incorporates both frequency and differences in allele size (Goldstein et al. 1995; Slatkin 1995).

The values of genetic distance between populations ranged from 0.019 (SG-A/MB-D) to 0.777 (MB-D/ME-E) (Table 4). Hierarchical analysis of molecular variance (AMOVA) showed that the variation between populations accounted for 41% of the total variance. The AMOVA performed within populations showed greater differentiation between populations (PhiPT = 59%, p > 0.05) (Table 7).

Figure 2 shows the distribution of the haplotypes found in each population evaluated. Figure 3 shows spatial genetic structure distribution of the chloroplast DNA in each population studied. ME-E had the highest number of haplotypes detected with a total of 9. The native species exhibits a greater level of biodiversity in comparison to the haplotypes of the introduced species. Concurrently, the most significant diversity in haplotypes was identified

Table 7 Hierarchical analysis of molecular variance (AMOVA).

Source of variation	df	SS	VC	Var%	p
Among populations	4	950.95	13.65	41	
Within populations	75	1455.75	19.41	59	p >0,05
Total	79	2406.70	33.06	100	

Note: Degrees of freedom (df); Sum of Squares (SS) and Variance Components (VC); Var%: percentage of variation.

Figure 2 Geographic distribution of the 11 common chloroplast DNA in 5 *Swietenia* populations (abbreviations are defined in Table 1) (for the abbreviations, see Table 1) studied in Cuba.

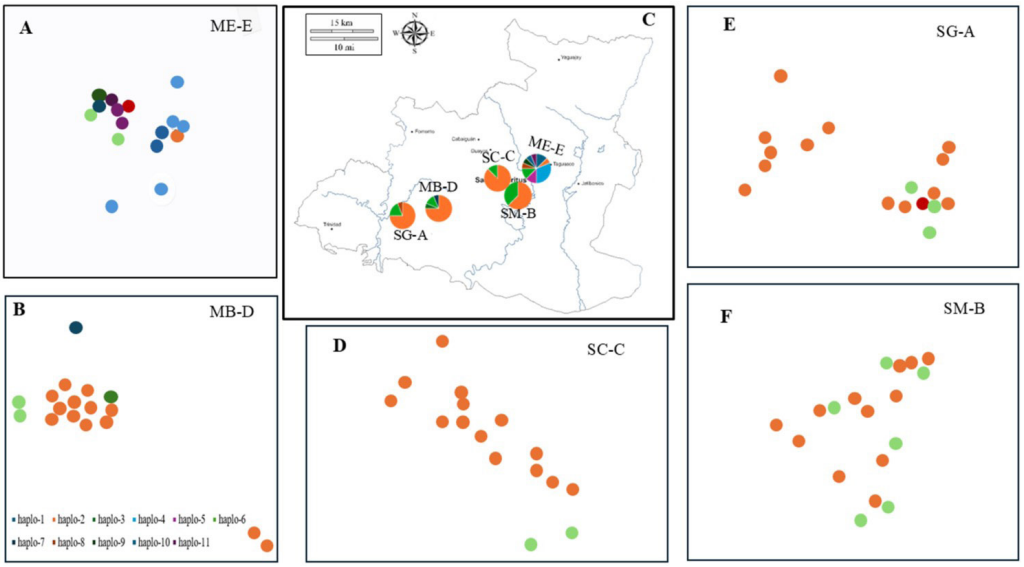

Figure 3 Spatial Genetic Structure distribution of the chloroplast DNA in each population studied in Cuba. Populations abbreviations are defined in Table 1. *S. macrophylla* (Hon), *S. mahagoni* (Cub) and intermediate forms (mix). The classification of the subjects according to species was based on morphology (Coca et al. 2024).

in mixed stands, constituting 81.81% (ME-E) and 36.36% (MB-D). Chloroplast markers did not show clear diagnostic differences between species; however, they revealed appreciable variability in maternal haplotypes among the populations evaluated. In this context, ME-E presented the greatest diversity of chloroplast haplotypes, in accordance with the distribution observed in Figure 3.

A comparison of the two Mantel analysis results reveals significant discrepancies in the magnitude of the correlation and the significance of the relationships evaluated. In the initial analysis, the correlation between the geographical and population distance matrices was found to be moderate to strong ($r = 0.6001$), with a highly robust significance ($p = 1 \times 10^{-4}$). This finding suggests that geographic differences are closely related to differences between populations, indicating a clear pattern of spatial structuring that reflects the influence of geographic distance.

In contrast, the second analysis, which assesses the relationship between geographical distances and gene diversity, shows a much weaker correlation ($r = 0.06171$) and only marginal significance ($p = 0.0428$). While the correlation is statistically significant, the magnitude of the correlation indicates that geographical distance has a very limited impact on gene diversity, and the result barely exceeds the significance thresholds defined by the null model ($Q_{95} = 0.0581$). In summary, while geographic distance exerts a marked influence on population structuring, its relationship with gene diversity is much more tenuous, suggesting that the latter may be influenced by factors additional to or independent of geography, such as gene flow, selection or historical events.

Nuclear data analysis

The eight nuclear SSR markers evaluated in 357 *Swietenia* individuals demonstrated moderate to high genetic diversity (Table 8). The number of alleles per locus (Na) ranged from 8.6 to 17.2 (mean = 11.88 ± 0.76), and the effective number of alleles (Ne) ranged from 2.31 to 6.91 (mean = 4.89 ± 0.41). The observed heterozygosity (H_o) ranged from 0.35 to 0.75, while the expected heterozygosity (H_e) ranged from 0.46 to 0.85, with an average F_{ST} of 0.15 ± 0.02. This indicates moderate genetic differentiation between loci and adequate variability within the population.

The genetic diversity of *Swietenia* populations in Cuba exhibited notable variations between populations (Table 9). The number of alleles observed per locus (Na) ranged from 5.75 ± 1.07 in SC-C to 16.38 ± 1.24 in ME-E, with an overall average of 11.88 ± 0.76. The effective number of alleles (Ne) ranged from 3.30 ± 0.66 (SC-C) to 7.53 ± 1.07 (ME-E), reflecting differences in allele frequency distribution between populations. The Shannon information index (I) ranged from 1.23 ± 0.18 in SC-C to 2.20 ± 0.18 in ME-E, indicating that the latter population is genetically more complex. The observed heterozygosity (H_o) ranged from 0.45 ± 0.11 in SC-C to 0.64 ± 0.06 in SG-A, while the expected heterozygosity (H_e) fluctuated from 0.59 ± 0.10 in SC-C to 0.82 ± 0.05 in ME-E. The results demonstrate that the populations exhibit adequate genetic variability, with ME-E being distinguished by its heightened genetic richness and complexity.

Table 8 Diversity indices for each nuclear SSR marker across 357 *Swietenia* individuals.

ID	N_a	N_e	I	H_o	H_e	F_{ST}
sm01	10.20	4.74	1.68	0.52	0.74	0.14
sm31	8.60	2.31	1.02	0.35	0.46	0.23
sm32	12.40	5.07	1.86	0.75	0.78	0.01
sm40	11.60	5.27	1.74	0.43	0.70	0.20
sm45	17.20	6.91	2.27	0.58	0.85	0.08
sm46	11.00	3.92	1.66	0.59	0.71	0.19
sm47	11.40	5.47	1.88	0.59	0.79	0.10
sm51	12.60	5.43	1.81	0.72	0.74	0.15
Mean	11.88	4.89	1.74	0.57	0.72	0.15
SD	0.76	0.41	0.09	0.04	0.03	0.02

Note: Observed number of alleles per locus (N_a); Mean number of effective alleles (N_e); Shannon´s Information index (I); Observed heterozygosity (H_o); expected heterozygosity (H_e); the fixation index (F_{ST}); Standard deviation (SD).

Table 9 Genetic diversity of *Swietenia* populations based on nuclear microsatellite loci.

Population	N	N_a	N_e	I	H_o	H_e
SG-A	80	12.38 (±1.15)	5.32 (±0.73)	1.91 (±0.15)	0.64 (±0.06)	0.78 (±0.04)
SM-B	80	13.25 (±1.74)	4.72(±0.77)	1.77 (±0.17)	0.55 (±0.09)	0.73 (±0.04)
SC-C	17	5.75 (±1.07)	3.30 (±0.66)	1.23 (±0.18)	0.45 (±0.11)	0.59 (±0.10)
MB-D	90	11.64 (±0.80)	3.76 (±0.62)	1.59 (±0.15)	0.62 (±0.08)	0.69 (±0.05)
ME-E	90	16.38 (±1.24)	7.53 (±1.07)	2.20 (±0.18)	0.58 (±0.08)	0.82 (±0.05)
Overall mean		11.88 (±0.76)	4.89 (±0.41)	1.74 (±0.09)	0.57 (±0.04)	0.72 (±0.03)

Note: Observed number of alleles per locus (N_a); Mean number of effective alleles (Ne); Shannon´s Information index (I); Observed heterozygosity (H_o) expected heterozygosity (H_e); ±standard errors in parentheses. Abbreviations are defined in Table 1.

Table 10 Summary of genetic diversity and population differentiation indices for all individuals and excluding potential hybrids under two assignment thresholds.

Statistic	All individuals	Excluding hybrids 0.10–0.90	Excluding hybrids 0.20–0.80
Ho	0.562	0.562	0.5649
Hs	0.6998	0.6998	0.7067
Ht	0.832	0.832	0.8324
Dst	0.1323	0.1323	0.1257
Htp	0.8651	0.8651	0.8639
Dstp	0.1653	0.1653	0.1572
Fst	0.159	0.159	0.151
Fstp	0.1911	0.1911	0.1819
Fis	0.1969	0.1969	0.2006
Dest	0.5506	0.5506	0.5358

Note: The abbreviations used in the Statistic column are defined as follows: Ho, observed heterozygosity; Hs, expected heterozygosity within populations; H_t, total heterozygosity among populations; D_{st}, genetic differentiation among populations ($H_t - H_s$); H_{tp}, weighted total heterozygosity; D_{stp}, weighted genetic differentiation; FST, Wright's fixation index among populations; F_{STP}, F_{ST} weighted following Weir & Cockerham (1984); F_{IS}, Wright's inbreeding coefficient within populations; and Dest, Jost (2008) differentiation estimator.

Table 11 AMOVA and genetic differentiation parameters for five Swietenia populations in Sancti Spíritus.

Source of variation	df	SS	VC	Var%	p
Among populations	4	68.930	0.099	3	
Within populations	709	2563.020	3.615	97	p >0,05
Total	713	2631.950	3.714	100	

Note: Degrees of freedom (df); Sum of Squares (SS) and Variance Components (VC); Var%: percentage of variation.

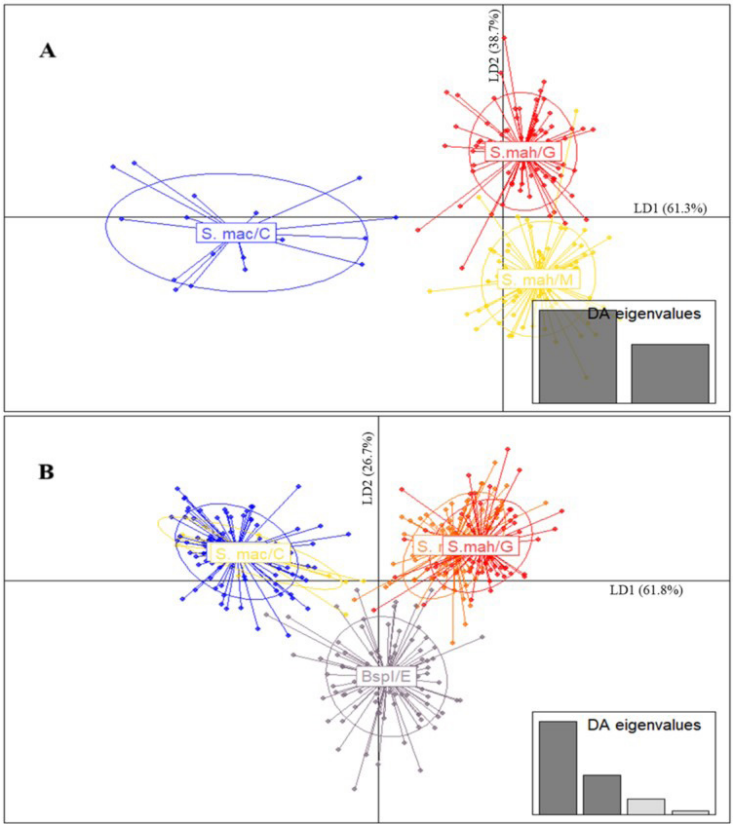

Figure 4 Principal component discriminant analysis (PCD) based on nuclear genetic data: (A) pure species of *Swietenia* and (B) all sampled locations, including mixed populations. In panel A, the first two linear discriminant functions (LD1 and LD2) explain 61.3% and 38.7% of the discriminant variance, respectively. In panel B, LD1 and LD2 explain 61.8% and 26.7% of the discriminant variance. Each point represents an individual, and the colours indicate species or population groups. Red and orange correspond to populations of *S. mahagoni*; yellow to the remnant of *S. macrophylla*; and grey and blue to mixed plantations.

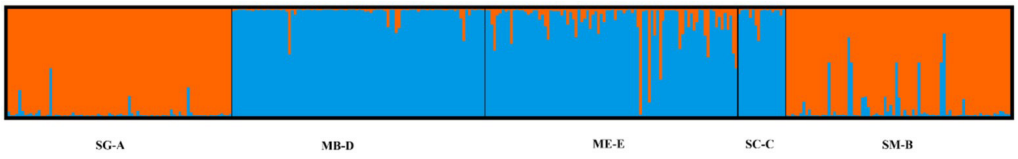

Figure 5 Clustering obtained for two genetics clusters (k=2). Population codes are as follows: SG-A (*S. mahagoni*), MB-D (mixed stand), ME-E (mixed stand), SC-C (*S. macrophylla* remnant), and SM-B (*S. mahagoni*). Abbreviations are defined in Table 1.

The low diversity values observed in the SC-C are closely related to the small size of the remnant, as all existing individuals in the area were evaluated. Therefore, this lower genetic variability reflects the population's limited demographic condition and not a deficiency in the sampling or the markers used. Taking together, these results reveal contrasting patterns of genetic diversity between species and population types (natural, remnant or plantation), as well as between different locations.

As illustrated in Table 10, the genetic diversity and population differentiation indices for all individuals and groups, excluding potential hybrids (thresholds 0.10–0.90 and 0.20–0.80), are presented. The values of H_o, H_s, H_t, D_{st}, H_{tp}, D_{stp}, F_{IS}, F_{ST}, F_{STP}, and D_{est} were found to be highly comparable across the three groups, thereby suggesting that the exclusion of potential hybrids did not result in any significant alterations to the prevailing patterns of genetic diversity and structure.

Analysis of molecular variance (AMOVA) revealed that most of the genetic variation in *Swietenia* spp is found within populations (97%), with only 3% found between populations (Table 11). The Fst was low but significant (0.027, P = 0.001), indicating a weak but significant genetic structure between the sampled sites. However, when standardized by its maximum possible value ($F_{ST,max}$ = 0.096), the adjusted F′ST reached 0.278, suggesting a moderate relative level of population differentiation.

Discriminant Analysis of Principal Components (DAPC) (Figure 4A) revealed distinct genetic differences between SC-C and the two S. *mahagoni* populations (SG-A and SM-B), particularly along the first discriminant axis. The *S. mahagoni* populations were closer to each other with slight overlap, indicating a closer genetic relationship. When all locations were included (Figure 4B), the ME-E population was positioned between *S. mahagoni* and *S. macrophylla*, with scattered and broad clouds.

The STRUCTURE analysis with K=2 (Figure 5) demonstrated significant genetic divergence between populations: individuals from populations SG-A and SM-B were predominantly allocated to cluster 2 (0.970 and 0.919), while populations MB-D, ME-E, and SC-C were primarily assigned to cluster 1 (0.974, 0.884, and 0.954, respectively). The allele frequency exhibited a moderate divergence (D=0.1195) between the clusters, while the average heterozygosity within each group was found to be comparable (0.807 and 0.792). The F_{ST} values (0.0713 and 0.0911) confirmed the differentiation detected.

A Bayesian population structure analysis was performed using STRUCTURE software, based on nuclear marker data. The optimal number of genetic groups (K) was estimated using the Evanno method (Evanno et al. 2005). The results showed that the value of LnP(K) increased progressively from K = 1 to K =5, with the rate of increase decreasing from K = 4 onwards. The highest ΔK value, however, was obtained for K = 3 (ΔK = 747.01), accompanied by a mean log-likelihood value of LnP(K) = − 10562.86 with a low standard deviation of ±0.87, indicating a stable and well-defined solution (Table 12; Figure 6 A and B).

Table 12 Estimation of the optimal number of genetic groups (K) based on Evanno's method.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln′(K)	Ln″(K)	ΔK
1	10	-12775.88000	0.34577	NA	NA	NA
2	10	-11444.91000	8.98659	1330.97000	448.92000	49.95444
3	10	-10562.86000	0.86948	882.05000	649.52000	747.01897
4	10	-10330.33000	29.16669	232.53000	24.26000	0.83177
5	10	-10073.54000	3.86816	256.79000	NA	NA

Note: Mean log-likelihood values (Mean LnP(K)) and their standard deviation (Stdev LnP(K)) for each value of K (number of groups), based on three replications (Reps) per K.

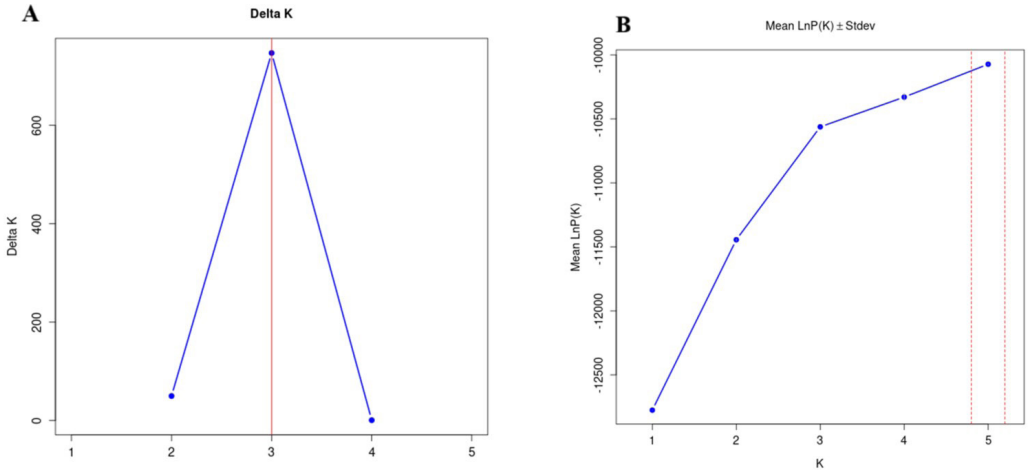

Figure 6 Highest ΔK value (A) and (B) Mean LnP(K).

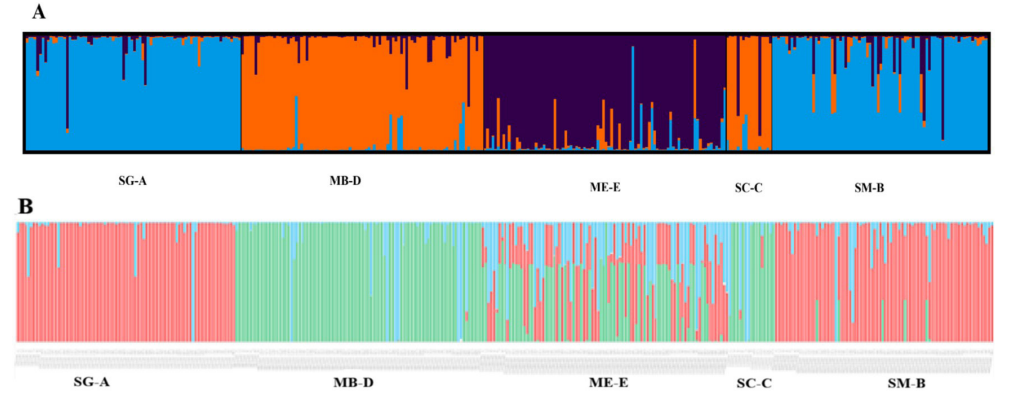

Figure 7 Clustering obtained for three genetics clusters. (A) created using the ΔK selected by the Evanno method and (B) based on the results obtained by the Newhybrids software. Where SG-A (*S. mahagoni*), MB-D (mixed stand), ME-E (mixed stand), SC-C (*S. macrophylla* remnant), and SM-B (*S. mahagoni*). Abbreviations are defined in Table 1.

The STRUCTURE analysis with K=3 (Figure 7A), which was identified as the optimal value according to the ΔK method (Evanno et al. 2005), demonstrated a more clearly defined genetic differentiation between populations. Populations SG-A and SM-B were predominantly assigned to cluster 3 (0.937 and 0.874, respectively), while populations MB-D and SC-C were primarily grouped in cluster 1 (0.915 and 0.847), and population ME-E exhibited a stronger relationship with cluster 2 (0.891). The divergence in allele frequencies between clusters was moderate to high (0.1213–0.1920), with average heterozygosity values of 0.7877, 0.6642, and 0.8246 for clusters 1, 2, and 3, respectively. The F_{ST} values ranged from 0.0653 to 0.2255, thereby supporting the hypothesis of genetic differentiation between the detected groups.

The STRUCTURE analysis with K=3 differentiated three clusters, assigning populations SG-A and SM-B to cluster 3, populations MB-D and SC-C to cluster 1, and population ME-E to cluster 2. The NewHybrids analysis corroborated this categorization, classifying populations SG-A and SM-B predominantly as pure *S. mahagoni*, populations MB-B and SC-C as pure *S. macrophylla*, and population ME-E as exhibiting a substantial proportion of hybrids (Figure 7 B).

Comparison between morphology and genetics

We compared morphological identification with (i) genetic assignment based on nuclear microsatellites and (ii) Bayesian categorization of hybrids (NewHybrids). At the first threshold ($q \geq 0.90$), the mean agreement between methods per population was 64.9% (Morph–Nuclear), 64.3% (Morph–NH) and 76.0% (Nuclear–NH). With the second threshold ($q \geq 0.80$), agreement increased slightly to 67.2%, 67.0% and 72.8%, respectively, indicating that the overall patterns are robust to the assignment criteria (Tables 13A and 13B).

Both populations of the native species (SG-A and SM-B) showed high concordance under both thresholds ($q \geq 0.90$: 95–97.5% in SG-A; 80–93.75% in SM-B; $q \geq 0.80$: 95–98.75% and 85–91.25%, respectively). SC-C showed high Morph–Nuclear agreement (88.24% → 94.12% when moving to $q \geq 0.80$), but less agreement with NewHybrids (64.71% → 70.59%). In the mixed stands, the discordance was marked: MB-D exhibited low concordance in comparisons involving morphology (Morph–Nuclear 48.89% → 46.67%; Morph–NH 50% → 47.78%), while between the two genetic approaches the agreement was high (Nuclear–NH 77.78% → 82.22%). ME-E showed the higher disagreement (Morph–Nuclear 10% → 7.78%; Morph–NH 34.44% → 36.67%; Nuclear–NH 34.44% → 17.78%), consistent with extensive hybridization and limitations of phenotypic identification in mixed stands (Tables 13A and 13B).

Table 13A Concordance (%) between pairs of methods by population under $q \geq 0.90$. The percentages of agreement for Morph–Nuclear, Morph–NH, and Nuclear–NH are shown in the population studied.

Population	Morph–Nuclear	Morph–NH	Nuclear–NH
SG-A	95	92.5	97.5
SM-B	82.5	80	93.75
SC-C	88.24	64.71	76.47
MB-D	48.89	50	77.78
ME-E	10	34.44	34.44

Note: Abbreviations are defined in Table 1.

Table 13B Concordance (%) between pairs of methods by population under $q \geq 0.80$. The percentages of agreement for Morph–Nuclear, Morph–NH, and Nuclear–NH are shown in the population studied.

Population	Morph–Nuclear	Morph–NH	Nuclear–NH
SG-A	96.25	95	98.75
SM-B	91.25	85	88.75
SC-C	94.12	70.59	76.47
MB-D	46.67	47.78	82.22
ME-E	7.78	36.67	17.78

Note: Abbreviations are defined in Table 1.

Table 14 Overall classification counts by method. Numbers of parental genotypes (*S. mahagoni*, *S. macrophylla*) and hybrids identified by morphology, nuclear assignment ($q \geq 0.90$; $q \geq 0.80$) and NewHybrids ($q \geq 0.90$; $q \geq 0.80$).

Taxon	Morph	Nuclear (0.10-0.90)	Nuclear (0.20-0.80)	NewH (0.10-0.90)	NewH (0.20-0.80)
S. mahagoni	220	143	152	139	145
S. macrophylla	54	160	177	78	83
Hybrid	83	54	28	140	129

As context, the overall classification between "parental" and "hybrid" differed between methods (Table 14). For genetic assignments, individuals with q-values ≥ 0.90 were considered confidently assigned to a parental species, whereas individuals with q-values between 0.10–0.90 were classified as hybrids; a more relaxed threshold (0.20–0.80) was also explored to account for admixed genotypes. Morphological identification classified a total of 274 parental individuals (220 *S. mahagoni* and 54 *S. macrophylla*) and 83 individuals with intermediate characters. In contrast, nuclear assignments identified 54 hybrids under the threshold q ≥ 0.90 (303 parental) and 28 hybrids with q ≥ 0.80 (329 parental), reflecting a conservative estimate of hybridization. For its part, NewHybrids detected a considerably higher number of hybrids, with 140 individuals for q ≥ 0.90 and 129 individuals for q ≥ 0.80. The highest concordance was observed between the two genetic approaches (Nuclear–NH), and the greatest discrepancies were concentrated in mixes stand, providing evidence that morphological classification alone is insufficient and that genetic assignment is essential for identifying mixed genotypes. Across the dataset, hybridization is generally considerable: approximately 36% to 39% according to NewHybrids (129-140/357), with nuclear assignments providing a conservative lower bound of 8% to 15%.

Discussions

The cpSSR results are consistent with patterns of local isolation and the presence of partially differentiated genetic pools. In contrast, the nuclear markers revealed high intrapopulation variability together with localized signals of genetic admixture. Moreover, estimates of recent migration indicate limited but asymmetric gene flow. This asymmetry was inferred from the admixture proportions (q values) obtained through nuclear assignments and NewHybrids: most admixed individuals showed greater ancestry from *S. mahagoni* than from *S. macrophylla*, and hybrids were concentrated in mixed plantations, suggesting directional introgression from *S. mahagoni* into mixed and remnant plots rather than reciprocal gene exchange.

cpSSR analysis

The cpSSR data revealed moderate chloroplast diversity in *Swietenia* populations in Cuba (mean h = 0.20), suggesting limited maternal gene flow between stands. The relatively high polymorphism observed at the *ccmp4* and *ccmp7* loci indicates that these markers are informative for detecting differentiation at the population level within the genus. However, given that chloroplast loci are completely linked and sampling per population was modest (n = 8), we interpret this polymorphism as informative at the level of maternal haplotypes and population differentiation, but not as independent evidence of interspecific hybridization. The contrasting diversity values between ME-E (N_a = 3.67, N_e = 2.57) and SC-C (N_a = 1.33, Ne = 1.09) suggest that genetic structure may be strongly influenced by differences in origin and management history, with ME-E likely reflecting a more heterogeneous or introgressed population and SC-C a more homogeneous one. Similar patterns of unequal chloroplast diversity have been described for *S. macrophylla* in fragmented landscapes (Lemes et al. 2010; Lowe et al. 2003) supporting the idea that limited seed-mediated dispersal contributes to the maintenance of localized maternal lineages.

Chloroplast DNA analyses in *S. macrophylla* have also revealed strong regional differentiation and the persistence of geographically restricted haplotypes across Amazonian and Central American populations (Lemes et al. 2010). These findings indicate that the dispersal of seeds is spatially constrained, leading to the formation of distinct maternal lineages through isolation and historical fragmentation. The cpSSR patterns observed in *S. mahagoni* in Cuba align with this scenario, providing the first evidence of such chloroplast structure within Antillean populations of the genus. This result highlights

the need to conserve seed sources that represent the existing haplotypic diversity, as maternal lineage loss could reduce the adaptive potential of reforested and remnant stands.

At the same time, a study in seven natural populations of *S. macrophylla* in the Brazilian Amazon showed high genetic diversity (13-27 alleles per locus, H_E = 0.781) and moderate but significant differentiation between populations (F_{ST} = 0.097; R_{ST} = 0.147), with isolation by distance. Four populations showed significant inbreeding. The findings highlight the need to conserve multiple populations in diverse habitats to protect the genetic diversity of the species (Lemes et al. 2003).

Complete chloroplast genomes are essential for developing high-resolution phylogenetic trees and analyzing evolutionary relationships with greater precision (Sobreiro et al. 2020). High chloroplast haplotype diversity has been reported for tropical tree species occurring in non-overexploited and non-fragmented forests, including *Dalbergia monticola* (Andrianoelina et al. 2009), and similar patterns have been observed in *Swietenia* spp. The levels of genetic variation in this species are in the range of other neotropical trees, such as *Manilkara zapota* (H_o = 0.45) and *Simarouba amara* (H_o = 0.53), showing similar genetic diversity in timber species (Thompson et al. 2015).

Research on *S. macrophylla* species reflects the importance of cpSSR studies for understanding genetic diversity and population differentiation (Lemes et al. 2011). In *S. macrophylla*, the research revealed high differentiation in Amazonia (F_{ST}=0.91) and moderate diversity in Central America (F_{ST} = 0.36), with implications for timber certification (Lemes et al. 2010).

ME-E stood out for its high genetic diversity, with 9 haplotypes (6 private), a haplotypic diversity value (H_{CP}) of 0.89, and the highest average genetic distance between individuals (D^2_{sh} = 69.68), pointing local evolutionary processes. In contrast, SG-A, SM-B and SC-C populations showed low genetic diversity, with only 2-3 haplotypes, no private haplotypes, and reduced H_{CP} (0.23-0.50) and D^2_{sh} (0.08-0.24) values, suggesting possible bottlenecks or low intra-population variability. The presence of shared haplotypes (haplo-2 and haplo-7) in the five populations indicates some historical gene flow, although limited. The high diversity of chloroplast haplotypes observed in ME-E is more consistent with the incorporation of multiple maternal seed origins than with recent hybridization processes. Since chloroplast markers reflect strictly maternal inheritance and are insensitive to pollen-mediated gene flow, high values of cpDNA diversity in planted or managed systems are usually associated with mixtures of provenances, multiple introductions, or historical establishment practices, rather than with interspecific hybridization (Petit et al. 2005; Ennos, 1994).

Meanwhile, another study of eight populations of *S. macrophylla* in Mesoamerica revealed significant genetic differences (F_{ST} and R_{ST}) and a marked phylogeographic structure. Pacific populations from Costa Rica and Panama were genetically distinct from the others, which were grouped into two clades: Mexico-Belize-Guatemala and South Atlantic Nicaragua-Costa Rica. The correlation between geographic distance and genetic divergence highlights the influence of isolation by distance and a complex biogeographic history in the region (Novick et al. 2003).

In research on the genetic diversity of species of the family *Meliaceae*, different approaches have been applied to analyze chloroplast DNA (cpDNA). For example, in a study on *S. macrophylla*, These markers allowed a detailed analysis of the genetic diversity and variation of the species, contributing to the study of its genetic structure (Cavers et al. 2007). In contrast, a study on *Khaya senegalensis*, another species of the same family, involved an analysis of three cpDNA loci in 503 individuals from a wide geographical area spanning from Senegal to Uganda. This analysis identified 14 haplotypes but showed low geographic differentiation (G_{ST} = 0.096). The results show a weak genetic structure and high genetic diversity in western populations, where all haplotypes were concentrated, while central-eastern populations had only two cosmopolitan haplotypes and lacked low-frequency haplotypes (Sexton et al. 2015).

Nuclear analysis

Population Genetic Structure of Swietenia spp.

Genetic distance and AMOVA analyses reveal significant genetic structure among *Swietenia* spp. populations studied in Sancti Spíritus, Cuba. Genetic distance between populations ranged from 0.019 (SG-A/MB-D) to 0.777 (MB-D/ME-E), highlighting the high differentiation between ME-E and other populations, which coincides with their high number of private haplotypes. The AMOVA showed that 41% of the genetic variation is due to differences between populations, while 59% is attributed to variation within populations, with a moderate to high rate of genetic differentiation (PhiPT = 0.59, $p > 0.05$). This comparatively high between-population component should be interpreted with caution, as the analysis includes populations representing two closely related species (*S. mahagoni* and *S. macrophylla*). Therefore, it is likely that part of the observed differentiation reflects interspecific divergence rather than restricted gene flow between populations of the same species. This suggests limited gene flow between populations, possibly influenced by geographical or reproductive barriers. Figures 2 and 3 reinforce this interpretation by showing a heterogeneous haplotype distribution, where ME-E emerges as a key genetic reservoir with the highest haplotypic richness (9 haplotypes).

In 2012, three key studies on *S. macrophylla* provided valuable information for its management and conservation. The first analysed spatial genetic structure (SGS) and pollen and seed movement in an overexploited population in the Bolivian Amazon, finding pollen and seed immigration; where observed heterozygosity was significantly lower and fixation index higher in seedlings ($H_o = 0.697$, $F = 0.068$) than in adults ($H_o = 0.761$, $F = -0.023$), with significant SGS up to 150 m, indicating that close individuals are relatives (Sebbenn et al. 2012). The third developed Single Nucleotide Polymorphism (SNP) markers for the identification of *Swietenia*, differentiating it from other interchangeable timber species, and proposing an accessible method for the certification of timber with degraded DNA. These advances support the sustainable management and conservation of the species (Höltken et al. 2012).

However, in the Yucatan Peninsula and southern Veracruz, Mexico, research was carried out to evaluate the genetic variation and structure of six fragmented populations of *S. macrophylla*, using four nuclear microsatellites. Moderate genetic variation ($H_E = 0.600$), high inbreeding ($F_{IS} = 0.309$) and low but significant genetic structure ($F_{ST} = 0.095$) were found. The analysis identified three distinct groups and suggested that habitat fragmentation has affected genetic variation, supporting the hypothesis of recent expansion and founder effect, with possible dispersal routes through the Caribbean or the Gulf of Mexico slope (Trujillo-Sierra et al. 2013).

Furthermore, research was carried out in Santa Rosa National Park, Costa Rica, on this species in five successional plots with different times since disturbance. Using microsatellites, 21 alleles were identified, with higher diversity in early successional sites. Genetic differentiation between populations was low ($F_{ST} = 0.063$), indicating extensive gene flow. Adult genetic diversity does not fully explain seedling genetic diversity, highlighting key implications for tropical dry forest conservation (Céspedes et al. 2003).

The genetic variability of *S. macrophylla* was analysed in different regions of South America using microsatellites (SSR) in populations from Loreto, Ucayali, Madre de Dios (Perú) and six provinces of Ecuador. In Perú, a high allelic diversity was found, with 155 alleles in total, although genetic differentiation was weak between locations, with a slight difference between populations from Loreto and those from Ucayali and Madre de Dios (Pajuelo Romero 2021). In Ecuador, genetic diversity was low, with a deficit of heterozygosity and moderate genetic differentiation ($\Phi_{ST} = 8\%$) (Limongi et al. 2022). Both studies highlight the need for strategies to increase genetic diversity in natural populations, such as appropriate selection of seed trees and crossing between genetic groups.

The integrated analyses employed in this study (STRUCTURE and NewHybrids) enable direct response to the study's objectives. STRUCTURE

identified K=3 as the most informative solution (ΔK by Evanno) (Evanno et al. 2005), delineating three clusters with consistent assignments by population; NewHybrids, meanwhile, classified a substantial proportion of ME-E individuals as hybrids, indicating that the signal of a 'third cluster' in STRUCTURE corresponds largely to a set of individuals of hybrid origin rather than to a pure parental unit. This methodological combination (structure + hybrid classification) strengthens the interpretation of local introgression as a factor generating the observed structure (Anderson & Thompson 2002).

The STRUCTURE analysis at K = 3 revealed two clearly differentiated clusters corresponding to *S. mahagoni* and *S. macrophylla*, along with a third cluster representing hybrid individuals concentrated in mixed stands. This pattern suggests localized but ongoing gene introgression between the native and introduced species. Similar genetic structures have been reported in other *Swietenia* species, where overlapping flowering periods and shared pollinators facilitate interspecific hybridization (Lemes et al. 2002, 2011; Novick et al. 2003).

The presence of a distinct hybrid cluster also parallels observations in other tree genera, such as *Quercus*, *Eucalyptus*, where secondary contact between congeners has produced stable hybrid zones (Ramos-Ortiz et al. 2015; An et al. 2017; Burge et al. 2019; Pfeilsticker et al. 2022). In such cases, hybrids often act as bridges for gene flow, potentially influencing adaptive variation and the genetic structure of parental populations. The concentration of admixed individuals in the Emigdio and Banao stands indicates that hybridization in Cuban mahogany is spatially restricted but may increase, highlighting the importance of monitoring reproductive interactions between native and introduced *Swietenia* taxa to preserve the genetic integrity of *S. mahagoni*.

The discrepancy between morphological classification and genetic analyses observed in this study is indicative of the intricacies of the studied population. Whilst the prevailing morphology indicated the dominance of a *S. mahagoni* pure species (ME-E), the outcomes of STRUCTURE (k = 2 and k = 3) and NewHybrids demonstrated that the population is predominantly constituted by hybrids, thereby unveiling genetic substructures that are imperceptible at the phenotypic level. These findings emphasize the necessity of employing a combination of morphological and molecular approaches to accurately interpret population structure in scenarios of historical or contemporary hybridization.

The detection of *S. macrophylla* × *S. mahagoni* hybrids in the ME-E population is consistent with historical and technical reports in the region. When both species are planted or appear in proximity, hybridization is frequent and intermediate forms have been described on Caribbean islands, including records in Cuba and Puerto Rico. The empirical results obtained in this study demonstrate that the introduction of *S. macrophylla* can act as a source of gene flow to remnants of *S. mahagoni*, producing hybrid cohorts that are detectable by neutral markers. Such observations are consistent with those documented in regional studies, which have shown that the process of hybridization is more prevalent between species when they co-occur (Bakewell-Stone 2023; Coca et al. 2024).

With regard to the genetic diversity that was observed, the heterozygosity values and the differences between clusters are consistent with those of previous studies on *S. macrophylla*. These studies demonstrate relatively high levels of variability and local differentiation, which are dependent on the history of exploitation and management (e.g. plantations, germplasm movements). This finding indicates that the genetic heterogeneity observed within the clusters may be attributable to both contributions from introduced material and the historical structure of the parental populations in their respective areas of origin. This interpretation is consistent with microsatellite-based studies of *Swietenia* in Mesoamerica (Novick et al. 2003).

Genetic diversity and differentiation

The eight nuclear SSR markers evaluated in 357 individuals showed moderate to high levels of genetic diversity (mean $N_a \approx 11.88$; mean $H_e \approx 0.72$) and moderate overall population

differentiation (F_{ST} ≈ 0.15). These values are consistent with previous studies on *S. macrophylla* that have reported comparable levels of variability at regional and local scales, attributable to its reproductive biology (high outcrossing) and the history of exploitation and management of the resource in the Neotropical region. In particular, studies based on microsatellites and other markers have documented relatively high heterozygosity and variable spatial structure in natural and managed mahogany populations (Lemes et al. 2003, 2011).

Analysis of molecular variance (AMOVA) revealed that the vast majority of genetic variation is found within populations (97%), with only 3% found between populations. This result is supported by a low but significant fixation index (F_{ST}) of 0.027, with a P-value of 0.001. This pattern—predominance of intrapopulation variation and low proportion between populations—is consistent with what is expected for woody, long-lived species with a predominantly allogamous reproductive system, where most neutral variation is concentrated within populations while spatial differentiation tends to be low to moderate. Consequently, the distribution of variation observed in this study is consistent with the extant literature on tropical tree genetics (Hamrick et al. 1992; SHENG et al. 2005).

The high levels of genetic variation within the population observed with nuclear markers can be explained in part by hybridization and introgression between *S. mahagoni* and *S. macrophylla*, especially in mixed stands. Hybridization can increase allelic richness and heterozygosity within populations by combining divergent genetic backgrounds, even when overall population differentiation remains low (Bakewell-Stone 2023). This process is consistent with the mixing patterns detected by nuclear assignment and NewHybrids analyses, and contrasts with the stronger structure observed for chloroplast markers, which reflect more restricted seed-mediated gene flow.

A marked contrast is evident in diversity estimates per population. ME-E exhibits the greatest genetic richness and complexity (higher N_a and N_e, greater I and H_e), while SC-C has notably low values. The low diversity observed in SC-C can be explained by two factors. Firstly, the small remnant size, as all existing individuals were analyzed. Secondly, demographic processes such as genetic drift and bottlenecks. These processes are well known to reduce variability and increase inbreeding in small, fragmented remnants. Conversely, the elevated diversity observed in ME-E indicates the contribution of heterogeneous genetic material, potentially originating from either a larger native effective population or from historical/recent inputs of foreign germplasm (e.g., seeds/seedlings from diverse sources). The relationship between population size, drift, and loss of variability has been thoroughly documented, and it provides a comprehensive explanation for the observed disparity between these extremes (Hensen & Oberprieler 2005; Frankham 2005).

Multivariate analysis (DAPC) placed ME-E in an intermediate position between the genetic clusters attributed to *S. mahagoni* and *S. macrophylla*, showing scattered clouds that indicate high variability and mixing. This pattern is consistent with the hypothesis that the cluster sampled in ME-E includes material from different sources, which would explain the intermediate distribution in the discriminant space. The DAPC supports the interpretation of the existence of admixture/intrusion in ME-E and is consistent with the results of STRUCTURE and NewHybrids (Lowe et al. 2003; Jombart et al. 2010).

The comparison of indices with and without the exclusion of potential hybrids (thresholds 0.10–0.90 and 0.20–0.80) demonstrated highly comparable values for H_o, H_s, H_t, F_{ST}, and other estimators. This finding suggests that, despite the presence of a significant proportion of hybrid individuals, their exclusion does not result in a substantial alteration to the overall estimates of diversity and structure within the sampled set. In practical terms, this suggests that the presence of hybrids is localized and does not dominate the total variability of the sample. However, their local impact may be relevant to the genetic integrity of specific remnants.

As demonstrated in previous studies (Rajora & Zinck 2021), the elimination of hybrids exerts a comparatively negligible influence on global metrics. However, its impact on interpretations at the population/local level is significant.

The patterns observed here replicate findings from studies on *Swietenia* in Mesoamerica and Amazonia: (Lemes et al. 2002, 2003, 2011; Novick et al. 2003) documented relatively high levels of nuclear diversity and complex spatial structures associated with demographic history and management, while local-scale studies have described low differentiation between populations but the presence of fine structure and gene flow at the local scale. Our results, which combine moderate-high diversity, predominance of intrapopulation variation, and local signs of mixing, fit consistently within this regional framework.

Comparison between morphology and genetics

In pure stands, the coincidence between morphological identification and nuclear assignment was high and consistent, supporting the diagnostic reliability of morphology, showing more than 91% correct classification. This pattern is consistent with evidence that, in contexts with a defined population structure and restricted gene flow at the local scale, morphology accurately captures the divergence between parental lineages and, therefore, recovers genetic assignments with high precision. In *S. macrophylla*, SSR/AFLP studies have shown fine genetic structure, moderate differentiation, and limited seed dispersal, a background that favours congruence between phenotype and genotype in unmixed stands (Lemes et al. 2002; Novick et al. 2003).

This result is consistent with theory and empirical evidence showing that, in scenarios with low admixture and well-differentiated parents, assignment methods (STRUCTURE) and Bayesian hybridisation models (*NewHybrids*) tend to converge in the identification of parental individuals, while diverging on subtle hybrid classes when introgression increases (Anderson & Thompson 2002; Vähä & Primmer 2006).

Simultaneously, our results show systematic discrepancies between morphological classification and genetic assignments, with average concordances per population of 64.9–67.2% for Morph–Nuclear and 64.3–67.0% for Morph–NewHybrids ($q \geq 0.90$ and $q \geq 0.80$, respectively), while the highest agreement was observed between the two molecular approaches (Nuclear–NH: 72.8–76.0%).

The magnitude of hybridization in the sample set was substantial: NewHybrids estimates ~36–39% (129–140/357, according to $q \geq 0.80$ or $q \geq 0.90$), while nuclear assignments place a conservative lower bound of 8–15% (28–54/357). This result is consistent with reviews documenting the limitations of morphology in detecting hybridization, since hybrids of *Swietenia* species sometimes exhibit dominant characteristics of *S. mahagoni* or *S. macrophylla* (Marquetti et al. 1975). In *S. macrophylla*, fine-scale genetic structure and restricted gene flow have been described in Central American populations, conditions that facilitate the retention of lineages and the formation of local genetic gradients that are difficult to detect with morphology alone. Consequently, it is recommended to integrate molecular markers for a solid diagnosis (López-Caamal & Tovar-Sánchez 2014).

Conclusions

Chloroplast DNA markers did not show clear diagnostic differences between species, probably due to the few markers used. Nuclear DNA markers revealed hybridization between *S. mahagoni* and *S. macrophylla*, concentrated in mixed stands. Despite localized hybridization, the native species *S. mahagoni* maintains a differentiated population structure, suggesting that its genetic integrity is not currently compromised. In morphologically pure populations, morpho-molecular concordance was high (~85–95%), confirming morphological identifiability in pure stands; in contrast, in mixed stands, certain discrepancies consistent with hybrid cohorts were observed, which justifies integrating genetic tools for reliable classification. This highlights the need to integrate and complement these classifications with genetic tools in mixed

stands for conservation and management programs to safeguard the genetic identity of *S. mahagoni*, reinforcing long-term genetic monitoring and consolidating certified germplasm banks. More broadly, this study shows how human-mediated introductions, even for forestry purposes, can reshape the genetic landscape of native taxa, reinforcing conservation genetics as a key tool for the sustainable management of tropical tree species.

Conflict of interest

The authors declare no financial or personal interests could influence the work presented in this paper.

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Data availability

Data supporting the findings of this study are included in the manuscript.

Author contribution statement

ALC designed the study, LIRC conducted the fieldwork and prepared the draft manuscript for publication. EC assisted in collecting and analysing the data. ALC and EC supervised the study at all stages and contributed to the draft manuscript. ALC and EC reviewed the entire draft manuscript for improvement. We are pleased to announce that all authors have read and approved the final manuscript

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