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Leaf morphology and genetic diversity in the *Swietenia* species complex in Cuba

SUMMARY

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INTRODUCTION

The aim of this research is to contribute to our understanding of the dynamics, composition and structure of forest communities in a context of rapid global change, in which factors such as climate change, land-use changes and the intensification of natural and human-induced disturbances are significantly altering the functioning of ecosystems. In this regard, the study of forest communities is essential for identifying vulnerable species and populations, anticipating future scenarios and generating scientific foundations to guide conservation and sustainable management strategies.

In tropical ecosystems, these changes are exacerbated by external pressures such as intensive agricultural practices, illegal or industrial logging, forest fires and the ongoing loss of biodiversity, which compromise the forests' ability to accumulate biomass, store carbon and maintain their ecological functions. Within this context, the Meliaceae family, which is of great importance in tropical forests, and in particular the genus *Swietenia*, are of significant interest from an ecological, economic and conservation perspective. The species *S. macrophylla* and *S. mahagoni* have historically been heavily exploited due to the high quality of their timber, which has contributed to their population decline and their inclusion on the list of endangered species in CITES Appendix II.

Added to this are reports of hybrids and individuals exhibiting intermediate forms between *S. mahagoni* and *S. macrophylla* in various locations across the Caribbean and other regions, raising further questions about the genetic integrity of the populations, particularly in Cuba, where *S. macrophylla* was introduced during the second half of the 20th century as part of a collaborative project with the FAO to assess the productive capacity of this species under local conditions. Given that both species are phylogenetically close and reproductively compatible, natural hybridisation is biologically plausible in sites where they coexist.

However, the actual extent of this process, as well as its potential effects on population dynamics, genetic diversity and the genetic integrity of *S. mahagoni*, remain largely unknown. This lack of information limits the formulation of effective conservation and management measures. Therefore, this research focuses on the integrated assessment of morphological and genetic variability, as a basis for elucidating these processes and providing solid evidence for the understanding and conservation of these species.

CHAPTER 1. BACKGROUND TO THE STUDY

1. 1. Genus *Swietenia*

The genus *Swietenia* (Meliaceae) comprises three Neotropical tree species: *S. mahagoni*, *S. macrophylla* and *S. humilis*, which are distributed from southern Florida and the Caribbean to Central and South America (Danquah et al., 2019). These species are characterized by pinnate compound leaves, winged seeds and a timber of high commercial value known as mahogany (Khare, 2007) (Khare, 2007). Under favourable conditions, they reach reproductive maturity at around 12 years of age (Weaver, 1997).

The exploitation of *Swietenia* species dates back several centuries, driven by the exceptional quality of their timber, widely valued for its durability, dimensional stability and aesthetic properties. This harvesting pressure has led to a progressive decline in their natural populations, as well as habitat fragmentation and degradation (Leao et al., 2018). In response to these threats, the international trade in mahogany has been regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), adopted in 1973 with the aim of preventing overexploitation resulting from global trade (Wardell, 2023).

Despite the solid scientific basis supporting the need to protect these species, economic interests have hindered their inclusion on the CITES lists (S. F. Oldfield, 2013). *S. macrophylla* was finally listed in Appendix II in 2003, following decades of international debate, whilst *S. mahagoni* already had a history of vulnerability linked to overexploitation (Rodan & Blundell, 2003). However, illegal logging continues to account for a significant proportion of total harvesting (Blundell, 2004).

Inclusion in CITES has led to progress in trade regulation and the development of traceability systems, although significant challenges remain, such as the accurate identification of timber and the differentiation between species. In this regard, techniques such as near-infrared spectroscopy (NIRS) and the use of molecular markers have been proposed as complementary tools to improve traceability and trade control (de Palacios et al., 2020). However, the effectiveness of these measures depends largely on the strengthening of databases on population status, ecological dynamics and genetic variability of the species (S. Oldfield, 1988). In this context, the present study focuses on *S. mahagoni* and *S. macrophylla*, species of particular relevance in Cuba, where they coexist in mixed stands.

1.1.1. *S. mahagoni* and *S. macrophylla*

The species *S. mahagoni* and *S. macrophylla* are key components of tropical forest ecosystems and have historically been harvested for the high value of their timber. Although they share general characteristics of the genus, they differ in their geographical distribution, ecology and response to environmental conditions.

S. mahagoni, known as Cuban mahogany, is a smaller species, generally reaching up to 20 m in height, and is found in the Caribbean, southern Florida and some regions of Central America (Figure 1.1) (Gilman & Harchick, 2014; He et al., 2020). It grows mainly in dry and sub-humid tropical forests, showing remarkable tolerance to different soil types, although it prefers calcareous soils (Whitmore, 2003).

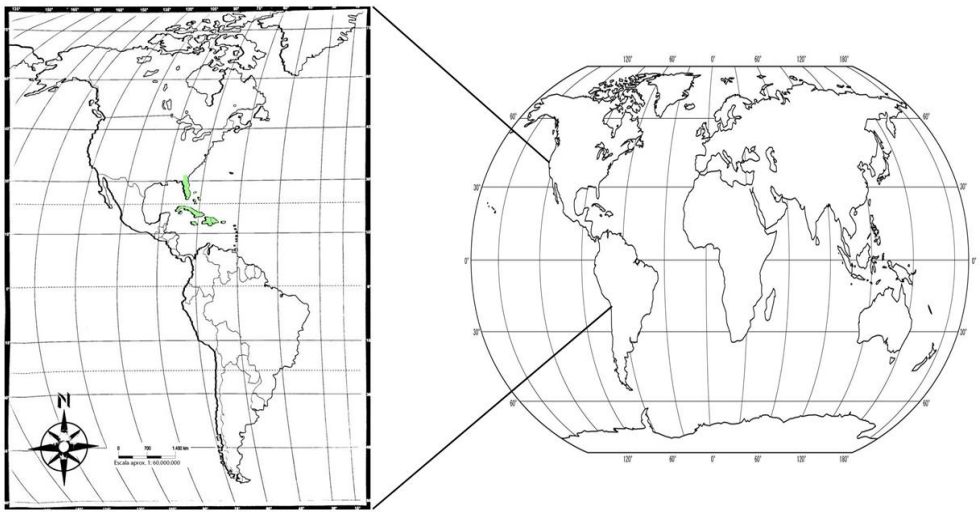

Figure 1.1. Natural distribution of *S. mahagoni* (He et al., 2020).

For its part, *S. macrophylla*, or large-leaved mahogany, has a much wider distribution, ranging from Mexico to the Amazon basin (Figure 1.2), and can reach heights of over 40 m (Paiva, 2011). This species is mainly associated with tropical humid and sub-humid forests, with higher water requirements and greater ecological plasticity (Grogan et al., 2011).

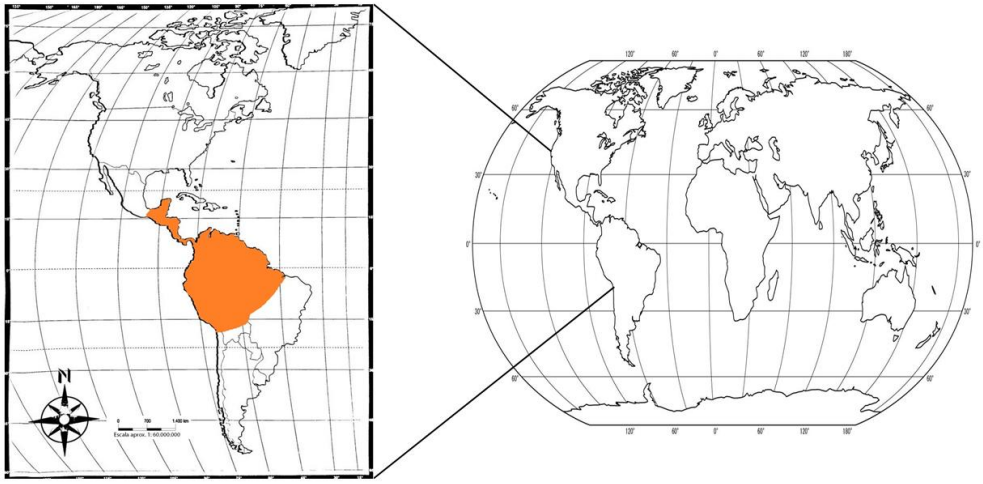

Figure 1.3. Natural distribution *S. macrophylla* (Grogan & Barreto, 2005; Sampayo-Maldonado et al., 2021).

Both species have been used extensively in the timber industry, particularly in the manufacture of fine furniture, construction, musical instruments and specialist joinery (Francis, 1995; Puentes et al., 2002). However, selective logging has had negative impacts on the genetic structure of their populations, favouring the elimination of individuals with desirable phenotypic characteristics (Braga et al., 2011).

The coexistence of *S. mahagoni* and *S. macrophylla* in certain regions has led to processes of natural hybridization, documented in the Caribbean and other tropical areas (Ewel & Whitmore, 1973; Nelson et al., 2020). These hybrids, commonly referred to as intermediate forms (MIF), exhibit morphological characteristics intermediate between both parental species, particularly in leaves and fruits (Helgason et al., 1996).

It has been observed that these hybrids may exhibit adaptive advantages, such as higher growth rates compared to the parental species, especially under favourable environmental conditions (Evaristo et al., 2016). Furthermore, they combine functional traits of both species, such as the wood quality of *S. mahagoni* and the vigorous growth of *S. macrophylla* (Timyan, 1979).

Despite their ecological importance and silvicultural potential, the genetic basis of these hybrids remains poorly studied, which limits our understanding of their role in population dynamics and the genetic integrity of the parent species.

1.2. Leaf morphology studies

Leaf morphology is a fundamental tool for taxonomic identification and the study of intra- and interspecific variability in forest species (Jennings et al., 2001). In the genus *Swietenia*, the leaves are compound, alternate and generally pinnate, with leaflets that can vary in number, size and shape (Yadav et al., 2015).

The morphological differences between *S. mahagoni* and *S. macrophylla* are particularly evident in their leaves. *S. mahagoni* has smaller leaves (10–30 cm) with 2 to 5 pairs of ovate leaflets, whereas *S. macrophylla* develops larger leaves (up to 45 cm) with 3 to 6 pairs of larger, asymmetrical leaflets (Collado López, 2006; Khare, 2007). These differences reflect adaptations to contrasting environmental conditions, such as water availability and solar radiation.

Leaf morphological variability is also influenced by ecological factors, with changes in the size and shape of leaflets observed depending on the type of habitat (Puentes et al., 2002). This plastic trait makes leaves sensitive indicators of environmental adaptation and potential markers for the identification of hybrid forms.

Various studies have highlighted the usefulness of leaf morphometric traits such as rachis length (RL), number of leaflets (NL), petiole length (PL), leaflet length (LL) and width, and number of veins (NV), for species differentiation and the detection of genetic variability (Styles & Khosla, 1976; Pennington et al., 2009). However, systematic analyses of these characteristics in natural populations remain limited.

1.3. Genetic diversity in *Swietenia* species

Genetic diversity is an essential component for the adaptation and survival of forest species in the face of environmental changes (Degen & Sebbenn, 2014). In the case of *Swietenia*, various studies have documented high levels of genetic diversity in natural populations, particularly in those unaffected by logging (Lemes, 2000).

However, habitat fragmentation and selective logging have been associated with reductions in genetic diversity and increases in levels of inbreeding (S. S. de Oliveira, 2018). These processes may compromise the long-term viability of populations, affecting their adaptive capacity.

In terms of genetic structure, moderate differentiation has been observed between populations, frequently associated with patterns of distance-based isolation (Lemes et al., 2003; Novick et al., 2003). Furthermore, the reproductive system of these species is characterized by a high rate of outbreeding, although there is evidence of inbreeding among related individuals in fragmented populations (Gillies et al., 1999).

The use of molecular markers, such as microsatellites (SSRs), plastid DNA and SNPs, has enabled a more in-depth analysis of genetic variability, gene flow and population structure (E. J. Oliveira et al.,

2006; Höltken et al., 2012). These approaches have proven to be key tools for genetic conservation, timber certification and the sustainable management of forest resources.

Despite advances in the study of *S. macrophylla*, the genetic information available for *S. mahagoni* is considerably null. Furthermore, studies on hybrids between the two species are scarce, despite their importance for understanding processes of gene flow, introgression and species delimitation.

In Cuba, the introduction of *S. macrophylla* during the 20th century and its coexistence with *S. mahagoni* provide a unique setting for the study of these processes. However, the lack of precise records regarding the origin of the genetic material and the decline of the original populations represent significant constraints on research.

In this context, there is a clear need for integrated approaches that combine morphological and genetic analyses, with the aim of characterizing existing variability, identifying potential hybridization processes and generating relevant information for the conservation and sustainable management of these species.

1.4. Purpose of the study

The overall objective of the study was to contribute to a comprehensive understanding of the morphological and genetic variability of the genus Swietenia in Cuba, with a view to supporting conservation, sustainable management and reforestation strategies for these species, in accordance with the guidelines established by CITES.

The specific objectives were:

Objective 1:

Differentiate between the two species of Swietenia and intermediate morphological forms using leaf morphological traits.

Objective 2:

Evaluate chloroplast DNA variation in pure and mixed stands of S. macrophylla and S. mahagoni.

Objective 3:

Analyze the nuclear genetic diversity and level of hybridization degree between S. macrophylla and S. mahagoni.

CHAPTER 2. Materials and Methods

2.1. Study area

The selection of the study area was based on historical records and the current availability of populations of the species of interest. Based on the information reported by Puentes et al. (2002), the historical presence of natural populations of *S. mahagoni* was identified in the province of Sancti Spíritus, Cuba (Figure 2.1). In parallel, an exhaustive search was conducted in different regions of the country with the aim of locating populations of *S. macrophylla*, particularly in areas where nurseries were established during its introduction in the 1960s and 1970s. However, no individuals were found in most of these areas, due to factors such as inadequate management, illegal logging, climatic events and urbanisation processes, etc...

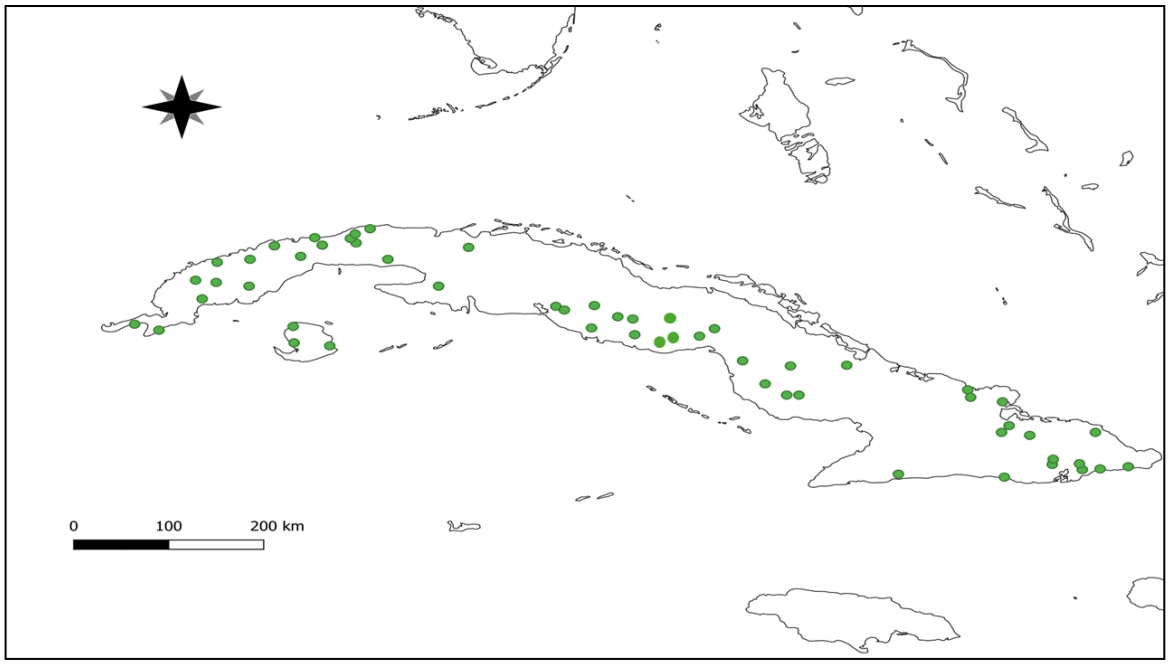

Figure 2.1. Historical natural distribution of *S. mahagoni* populations in Cuba (Puentes et al., 2002).

Finally, a remnant population of *S. macrophylla* was identified in Sancti Spíritus, where natural populations of *S. mahagoni* and mixed stands containing both species and intermediate individuals were also observed. This coexistence made the region an ideal setting for the study. The study area is located in the province of Sancti Spíritus (21.9381207 N; −79.5267729 W), in central Cuba, and included five sites: two naturally established populations of *S. mahagoni* (Guira and Modelo), a remnant stand of *S. macrophylla* (Colón) and two mixed stands (Banao and Emigdio). The region has a hot tropical climate with marked seasonality, characterized by a dry season from November to April and a rainy season from May to October, influenced by tropical and extratropical atmospheric systems (INSMET, 2026).

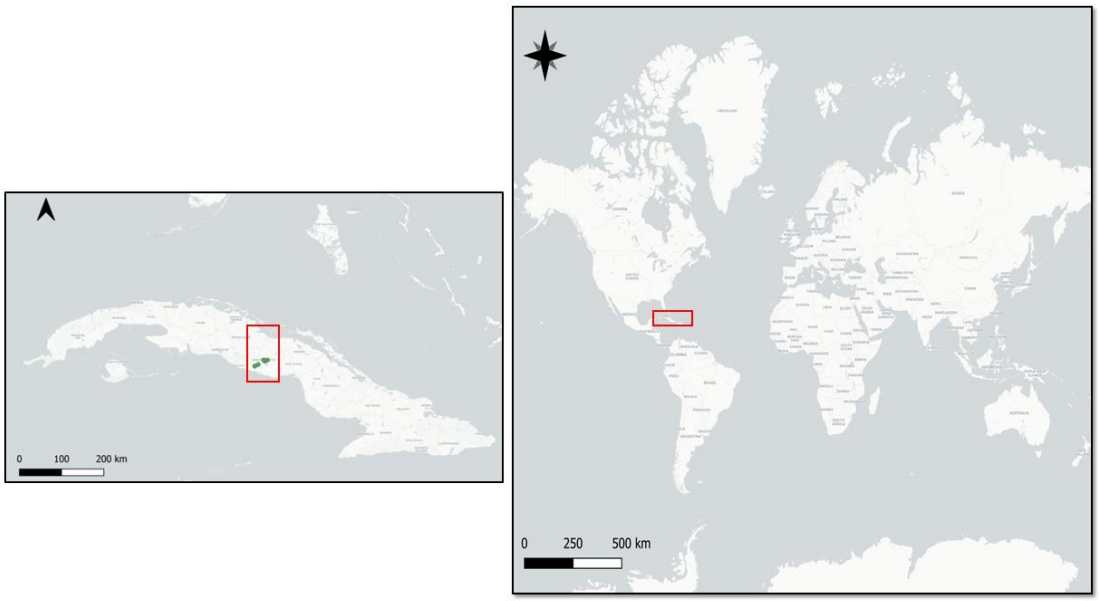

Figure 2.2. Geographical distribution of the study area in Cuba.

The natural stands of *S. mahagoni* are found in forests that were previously deforested and converted to agricultural (Modelo) and livestock farming (Guira) activities. Natural regeneration began between 1993 and 1995, resulting in current stands that are over 30 years old, with an average height of 9.94 m. Meanwhile, the remaining *S. macrophylla* stems from a plantation established between 1969 and 1970 as part of a project with the FAO, and is approximately 55 years old with an average height of 16.5 m.

The mixed stands at Banao and Emigdio were established between 1982 and 1984 in areas previously used for agriculture and grazing, respectively. Both have an average age of 42 years and an average height of 15.8 m. Individuals with intermediate characteristics have been identified in these stands, suggesting the occurrence of natural hybridization between *S. mahagoni* and *S. macrophylla.*

The total study area covered more than 6 ha. A total of 357 adult individuals were assessed, distributed across the five populations, with sample sizes of 80 individuals in each natural population of *S. mahagoni*, 90 individuals in each mixed stand and 17 individuals in the remnant of *S. macrophylla* (Table 2.1). Eight individuals per population were selected for chloroplast analysis. During sampling, carried out between March and May 2023, a minimum distance of 5–10 m was maintained between individuals to reduce the likelihood of sampling related trees.

Table 2.1. Selected individuals by population.

Ab	Region	Pop	Sp	N	Nc	Latitude/Longitude
SG-A	Guira	Stand A	*S. mahagoni*	80	16	21.78942/-79.64634
SM-B	Modelo	Stand B	*S. mahagoni*	80	16	21.89687/-79.41949
SC-C	Colon	Stand C	*S. mac*rophylla	17	16	21.91925/-79.44355
MB-D	Banao	Stand D	Mixed stand	90	16	21.82337/-79.56849
ME-E	Emigdio	Stand E	Mixed stand	90	16	21.91972/-79.35641

Note: Abbreviation (Ab). Region of the sample in Sancti Spíritus, Cuba. Population (Pop). Species (Sp). Sample size (N). Sample size for chloroplast analysis (Nc).

Leaf samples were collected and stored at –60 °C until DNA extraction. Genomic DNA was extracted following the CTAB protocol of Doyle & Doyle (1987), with modifications, and its concentration was determined by spectrophotometry (NanoDrop 8000), after which it was stored at –20 °C.

2.2. Leaf Morphometrics Analysis

For each individual, three leaves were selected from the eastern part of the canopy, with the aim of minimising biases associated with differences in light exposure and photosynthetic capacity (Gillies et al., 1999; Apostol et al., 2017). A total of 771 leaves were analyzed.
Sampling was carried out taking into account the vertical stratification of the canopy (upper, middle and lower), selecting only mature and fully developed leaves in order to reduce variability associated with developing tissues. All measurements were taken on fresh leaves immediately after collection.
The following morphometric traits were assessed: rachis length (RL), number of leaflets (NL), petiole length (PL), length (LL) and width (LW) of the leaflets, and number of veins (NV) (Figure 2.3). Based on these data, a Morphological Descriptor Analysis (MDA) was performed, following criteria established in the literature (Montiel et al., 2020; Cocuzza et al., 2021).

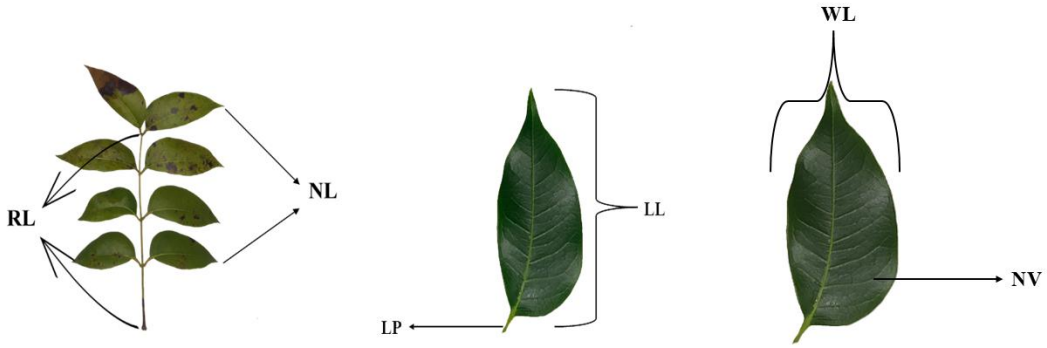

Figure 2.3. Graphical representation of the evaluated trait (Rodriguez et al., 2024).

Statistical analysis was carried out using IBM SPSS Statistics v20.0 (2011) and STATISTICA v8.0 (StatSoft, 2008). Student's t-test was applied to assess differences between species, after first verifying the homogeneity of variances using Levene's test; where this assumption was not met, Welch's test was used. Furthermore, an analysis of variance (ANOVA) and a discriminant analysis (DA) were performed to differentiate between species and identify possible hybrids. Finally, a cluster analysis (CA) was applied to assess the relationships between populations.

2.4. Genetic Analysis

2.4.1. Chloroplast DNA Analysis

Chloroplast DNA variation was assessed using three universal microsatellites (ccmp4, ccmp7 and ccmp10) developed by (Weising & Gardner (1999). Amplification was carried out by PCR using fluorescent primers, and the resulting fragments were separated by capillary electrophoresis.

PCR reactions were carried out in a 15 µl volume under standard conditions, and the amplified products were analysed on a GenomeLab GeXP system (Beckman Coulter). Polymorphisms were determined based on variations in fragment size.

Genetic diversity parameters (hS, hT, G_{ST} and R_{ST}) were estimated using the PERMUT programme (Pons & Petit, 1996). Genetic structure was assessed using AMOVA in GenAlEx 6.5 (R. P. P. Smouse & Peakall, 2012), whilst genetic distances were calculated following Nei (1987). Phylogeographic structure was analysed using SPAGEDI (Hardy, 2003), and relationships between haplotypes were reconstructed using median-joining networks (Bandelt et al., 1999).

Relationships between populations were represented using a UPGMA dendrogram (Hammer et al., 2001), with bootstrap support of 1000 replicates. Finally, the correlation between genetic and geographical distances was assessed using the Mantel test (Mantel, 1967).

2.4.2. Nuclear DNA Analysis

Nuclear genetic diversity was analysed using eight specific microsatellites (SSRs) (Lemes et al., 2002), organized into two multiplex reactions (Table 2.2). Data quality was assessed using MICROCHECKER (Van Oosterhout et al., 2004), which detected low frequencies of null alleles; consequently, all loci were retained.

Table 2.2. 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 (Ho), expected heterozygosity (He), probability of paternity exclusion (Q) and probability of genetic identity (I).

The population structure was analysed using STRUCTURE v2.3.4 (Pritchard et al., 2000), employing a mixture model with correlated allele frequencies. K values between 1 and 5 were evaluated, with multiple replicates, and the optimal number of groups was determined using the ΔK method (Evanno et al., 2005). Individuals were classified according to their membership coefficients (q), with those having q ≥ 0.90 considered pure.

The NEWHYBRIDS programme (Anderson & Thompson, 2002) was used to assign individuals to genetic categories (parental, F1, F2 and backcrosses), applying probability thresholds of ≥ 0.90 and ≥ 0.80.

Genetic diversity was assessed using standard indices (He, Ho, FIS), and genetic differentiation was estimated using FST and AMOVA. Additional analyses were carried out excluding hybrid individuals, with no significant changes observed in the overall patterns.

Finally, spatial genetic structure (SGS) was analyzed using multilocus spatial autocorrelation (Smouse & Peakall, 1999), estimating the Sp statistic (Vekemans & Hardy, 2004) to quantify the intensity of genetic structure at a small scale.

CHAPTER 3: Leaf morphological analysis

3.1. Description of leaf morphological traits

Morphological comparisons between *S. mahagoni* and *S. macrophylla* revealed statistically significant differences ($p \leq 0.05$) in the six leaf variables analyzed. The rachis length was significantly greater in *S. macrophylla*, with an increase of 79% compared to *S. mahagoni*. Similarly, the number of leaflets showed a marked difference between the two species, with compound leaves having fewer leaflets in *S. mahagoni* and more in *S. macrophylla*, with a relative difference of 39.49% between species.

Likewise, the remaining morphometric traits of the leaflets—petiole length, leaflet length, and leaflet width—showed significantly higher mean values in *S. macrophylla*, with increases of 35.29%, 70.99%, and 65.65%, respectively, compared to *S. mahagoni*. In terms of the number of veins per leaflet, *S. macrophylla* also showed higher values, exceeding *S. mahagoni* by 39.27%.

3.2. Analysis of morphological variation between taxonomic groups

The assessment of leaf morphometric traits in *Swietenia mahagoni* and *S. macrophylla* revealed statistically significant differences between the two species ($p \leq 0.05$), as shown in Figure 3.1. The greatest discrepancies were observed in rachis length (RL), with an absolute difference of 34.39 cm, and in leaflet length (LL), with 11.16 cm. These were followed by leaflet width (LW), with 4.11 cm, and the number of veins (NV), with 9.17. Meanwhile, the number of leaflets (NL) and petiole length (PL) showed differences of 4.84 and 0.21 cm, respectively. In relative terms, these variations corresponded to increases of 78.88% (RL), 43.85% (NL), 31.05% (PL), 71.01% (LL), 65.66% (LW) and 39.08% (NV), confirming a consistent morphological differentiation between the two species.

Principal component analysis (PCA), performed exclusively on data from pure populations of *S. mahagoni* and *S. macrophylla* (Figure 3.1A), showed that the first component explained 78.28% of the total variation and was primarily determined by leaflet length (LL) and rachis length (RL). The second component explained 10.32% of the variation and was mainly associated with petiole length (PL), revealing a clear pattern of separation between the two species in the multivariate space.

Meanwhile, the discriminant analysis (Figure 3.1B), applied solely to the two species, identified leaflet length (LL) as the variable with the greatest discriminant power ($p < 0.05$), with a partial Wilks' lambda value of 0.85. From this analysis, the discriminant function Root 1 = 581.9 – 79.39·LL was obtained, which allowed for effective differentiation between *S. mahagoni* and *S. macrophylla*. Based on the graphical representation of this function, an intermediate range between –1.4 and –4 was defined, corresponding to intermediate morphological forms (MIF), a criterion that was subsequently used for the classification of individuals in mixed stands.

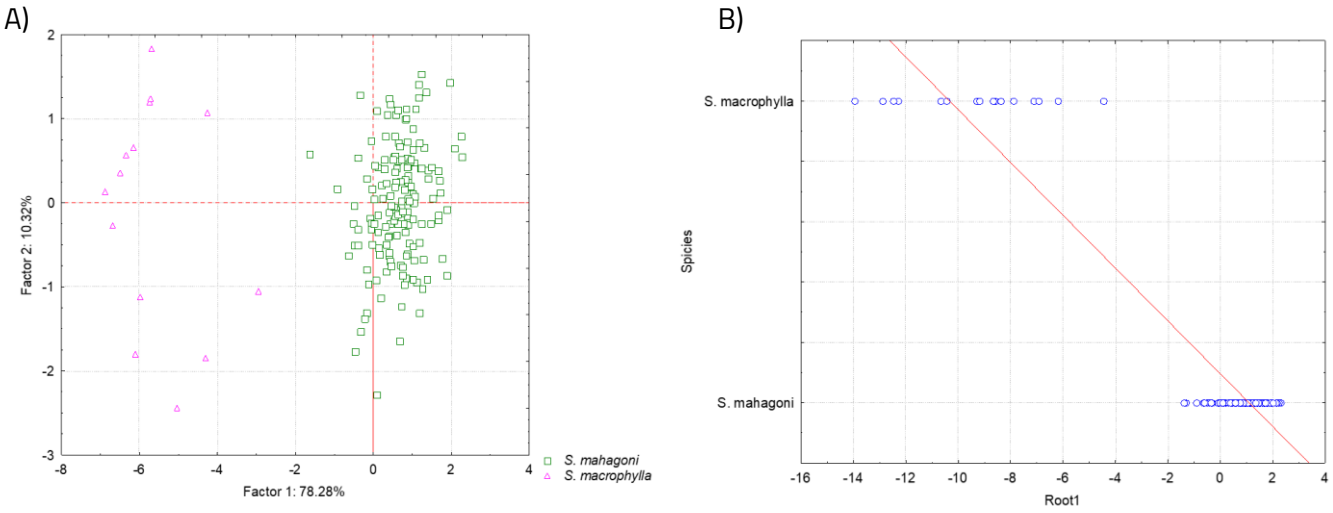

Figure 3.1. A) Principal Component Analysis and B) Discriminant analysis only with the two pure species *S. mahagoni* and *S. macrophylla* (Rodriguez et al., 2024).

The application of the previously obtained discriminant function allowed the individuals present in the mixed stands to be classified. In the mixed stand at Emigdio (Figure 3.2), out of a total of 90 individuals evaluated, 56 were assigned to *S. mahagoni*, 2 to *S. macrophylla* and 32 were classified as intermediate morphological forms (MIF). Meanwhile, in the mixed stand in Banao (Figure 3.3B), of the 90 individuals analyzed, 8 corresponded to *S. mahagoni*, 46 to *S. macrophylla*, and 39 were identified as MIF.

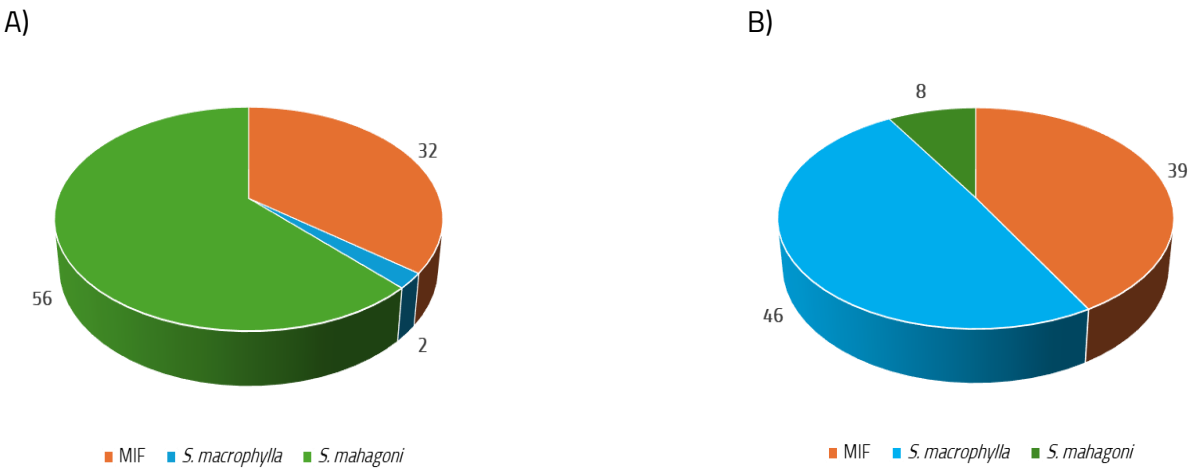

Figure 3.2. Classification of the individuals of each mixed stands A) Emigdio and B) Banao

Once the classification of individuals into *S. mahagoni*, *S. macrophylla* and MIF had been established based on the first discriminant analysis, a new Principal Component Analysis (PCA) was performed incorporating the three categories (Figure 3.3A). In this analysis, Factor 1 explained 69.56% of the total variation and was dominated mainly by rachis length and leaflet length, while Factor 2 explained 15.69% of the variation and was associated primarily with petiole length. Subsequently, a new Discriminant Analysis was performed using the leaf morphometric parameters of *S. mahagoni*, *S. macrophylla*, and the previously identified MIF. The highest Wilks' lambda values (0.14–0.21) were recorded in four of the six variables analyzed, specifically leaflet length, number of veins, leaflet width and petiole length. However, the partial Wilks' lambda analysis indicated that the leaflet length variable made the greatest contribution to overall discrimination, with a value of 0.67, which is consistent with the criterion established by Fisher (1936), according to which lower lambda values reflect greater discriminatory power.

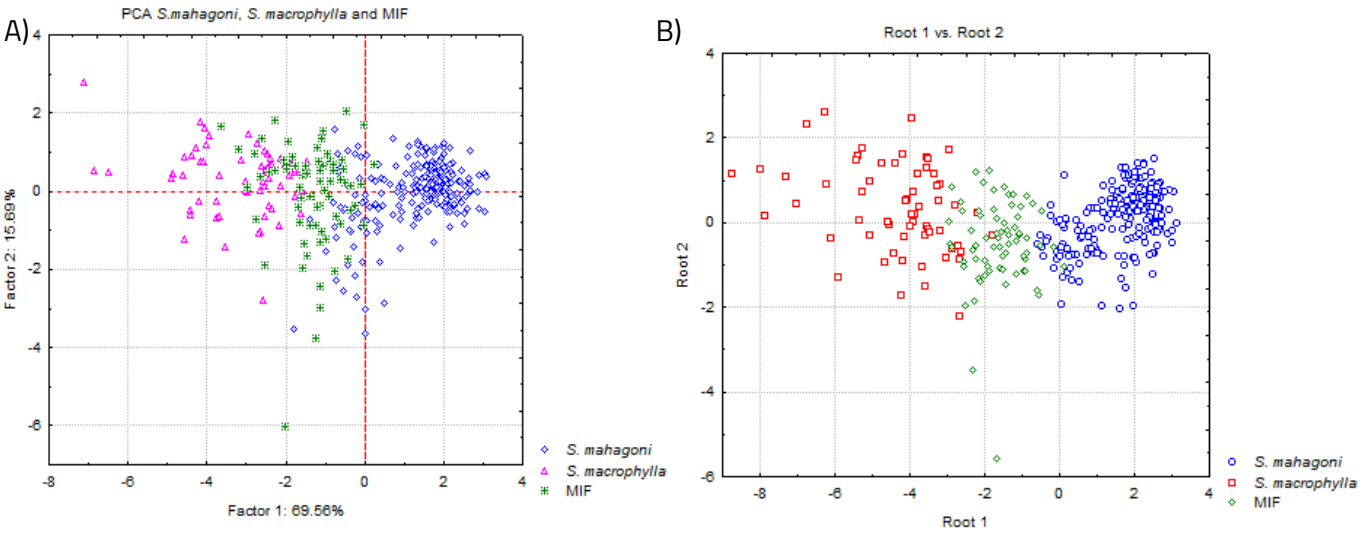

Figure 3.3. A) PCA and B) DA with the two species and the MIF (Rodriguez et al., 2024)

Two discriminant functions were obtained from this analysis. The first function was expressed as: Root 1 = Root1: 375.62-69.95*LL+8.32*NV-13.25*LW+146.46*PL, while the second function was defined as: Root 2 = 337.68+82.54*LL-21.10*NV-164.18*LW-48.51*PL . The Root 1 function explained 98.61% of the total variance and allowed for a clear differentiation of *S. mahagoni* from the rest of the groups. This function was characterized by negative coefficients associated with leaflet length and width, and positive coefficients linked to the number of veins and petiole length. Root 2 mainly distinguished intermediate morphological forms, which had negative values in this function, in contrast to both parental species, which had positive values.

The graphical representation of the discriminant functions (Figure 3.3B) showed a clear separation between the groups. Individuals of *S. mahagoni* were located in the quadrant defined by positive values in both functions, according to the means of the canonical variables, while *S. macrophylla* was characterized by negative values in Root 1 and positive values in Root 2. In contrast, the intermediate

morphological forms were predominantly grouped in the region defined by negative values in both functions, reflecting their intermediate position based on leaf morphological characteristics.

During the first field sampling, a total of 357 individuals were recorded, of which 160 corresponded to *S. mahagoni* from two naturally established populations, 180 individuals belonged to two mixed stands, and 17 individuals corresponded to the remnant of *S. macrophylla*. After applying discriminant analysis based on the main leaf morphometric characteristics, the classification matrix showed a high predictive capacity, achieving a correct assignment percentage of 94.96% when considering the four variables with the greatest discriminatory power (Table 3.2).

As a result of this procedure, the final classification included 220 individuals assigned to *S. mahagoni*, 54 to *S. macrophylla*, and 83 to MIF. Classification errors were low and differentiated between groups, with values of 2.68% for S. mahagoni, 14.52% for S. macrophylla, and 4.23% for MIF, confirming the robustness of the discriminant analysis for the morphological identification of species and their intermediate forms.

3.3. Clustering of populations according to leaf morphometric traits

The populations corresponding to both species, together with the mixed stands, were initially grouped into a primary cluster. At this first level of grouping, there is a clear separation between *S. mahagoni* and *S. macrophylla*, which shows an adequate level of discrimination between the populations of both species and the mixed stands. This differentiation is based on five of the six morphological characters evaluated with discriminating power (RL, PL, LL, WL, and NN), which showed statistically significant differences ($p \leq 0.05$). As a result, consistent segregation was obtained between the populations of the two mahogany species and the mixed plantations.

CHAPTER 4: Chloroplast DNA diversity

4.1. Chloroplast DNA haplotypes

The analysis revealed a total of 11 chloroplast DNA haplotypes, identified using the ccmp10, ccmp4, and ccmp7 markers (Table 4.1), distributed across the five studied populations. Haplotype H02 was the most common, present in all populations and represented by 49 individuals, followed by H06 with 15 individuals (Table 4.1). The ME-E mixed-stand population exhibited the highest haplotype diversity, containing 9 of the 11 detected haplotypes, whereas the other populations displayed lower genetic variability, with most individuals concentrated in a few dominant haplotypes. Regarding marker behavior, ccmp10 and ccmp4 showed minimal variation, while ccmp7 contributed most to the observed haplotype differentiation

Table 4.1. Observed chloroplast microsatellite in *Swietenia* ssp. in Cuba (Rodriguez et al. 2026).

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 2.1

Across the five *Swietenia* populations studied (n = 80), across the three cpSSR loci, a total of eleven alleles was observed. The loci ccmp4 and ccmp7 exhibited the greatest allelic richness, with ccmp7 showing the highest effective number of alleles (Ne = 1.80). Meanwhile, ccmp4 displayed the highest information index (I = 0.59) and haplotype diversity (H = 0.39). Average values for I and H were 0.36 and 0.20, respectively, indicating a moderate level of chloroplast genetic variation within the analyzed populations.

The ME-E mixed plantation exhibited the highest values for both the number of alleles (Na = 3.67) and the effective number of alleles (Ne = 2.57) among the populations analyzed. In contrast, the SM-B mixed stand and the SC-C (*S. macrophylla*) population showed the lowest allelic richness (Na = 1.33), with SC-C also displaying the lowest Ne (1.09) (Table 4.2). The genetic information index (I) followed a similar trend, ranging from 0.13 in SC-C to 0.93 in ME-E, highlighting the higher chloroplast genetic diversity present in the ME-E mixed plantation relative to the other populations.

Table 4.2. Genetic diversity of *Swietenia* populations in Sancti Spíritus based on chloroplast microsatellite loci (Rodriguez et al. 2026)

Population	N	Na	Ne	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)
Overall mean		2.00 (±0.35)	1.48 (±0.25)	0.36 (±0.12)	0.20 (±0.07)

Note: Observed number of alleles per locus (Na); Mean number of effective alleles (Ne); Shannon´s Information index (I); Haploid diversity (h); ±standard errors in parentheses. Abbreviations are defined in Table 2.1.

The mean genetic diversity (h) across the three cpSSR loci in Cuban *Swietenia* populations was 0.20, reflecting a notable level of chloroplast variation. Overall, these results indicate that the three cpSSR markers exhibited a high degree of polymorphism within the populations studied.

The cpSSR analysis of 80 mahogany individuals revealed 11 haplotypes, eight of which were unique (72.73%). At the population level, the ME-E mixed plantation exhibited the highest haplotype uniqueness, containing six private haplotypes (haplo-1, -4, -5, -9, -10, and -11). In contrast, the SG-A (*S. mahagoni*), SM-B (*S. mahagoni*), and SC-C populations lacked exclusive haplotypes (Table 4.3), indicating lower chloroplast differentiation at these locations.

Table 4.3 Diversity indices of cpSSR haplotypes for 5 *Swietenia* spp. populations (Rodriguez et al. 2026).

Population	A	P	Ne	Rh	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: Number of haplotypes (A); Number of private haplotypes (P); Effective number of haplotypes (Ne); Haplotypic richness (Rh); Haplotypes diversity (H_{CP}); Mean genetic distance between individuals (D^2_{sh}). Abbreviations are defined in Table 2.1.

Haplotypes 2 and 7 were broadly distributed among the five populations studied (Figure 4.1). Notably, haplotype 2 was most common in the SC-C population, representing 28.7% of sampled individuals. Haplotype 8, on the other hand, was found in only two of the five populations. The ME-E mixed plantation displayed the highest haplotype richness, with nine haplotypes identified (haplo-1, -2, -4, -5, -6, -8, -9, -10, and -11), indicating substantial chloroplast variability within this population. Haplotype diversity (H_{CP}) values ranged from 0.23 to 0.89 across the populations, with ME-E again showing the highest value (Table 4.3). The effective number of haplotypes varied between 1.28 and 6.10, while the mean genetic distance among individuals spanned from 0.08 in SC-C to 69.68 in ME-E. To provide a more comprehensive assessment of intrapopulation variation, the average genetic distance between individuals (D^2_{sh}) was also calculated, incorporating both haplotype frequencies and allele size differences (Goldstein et al. 1995; Slatkin 1995).

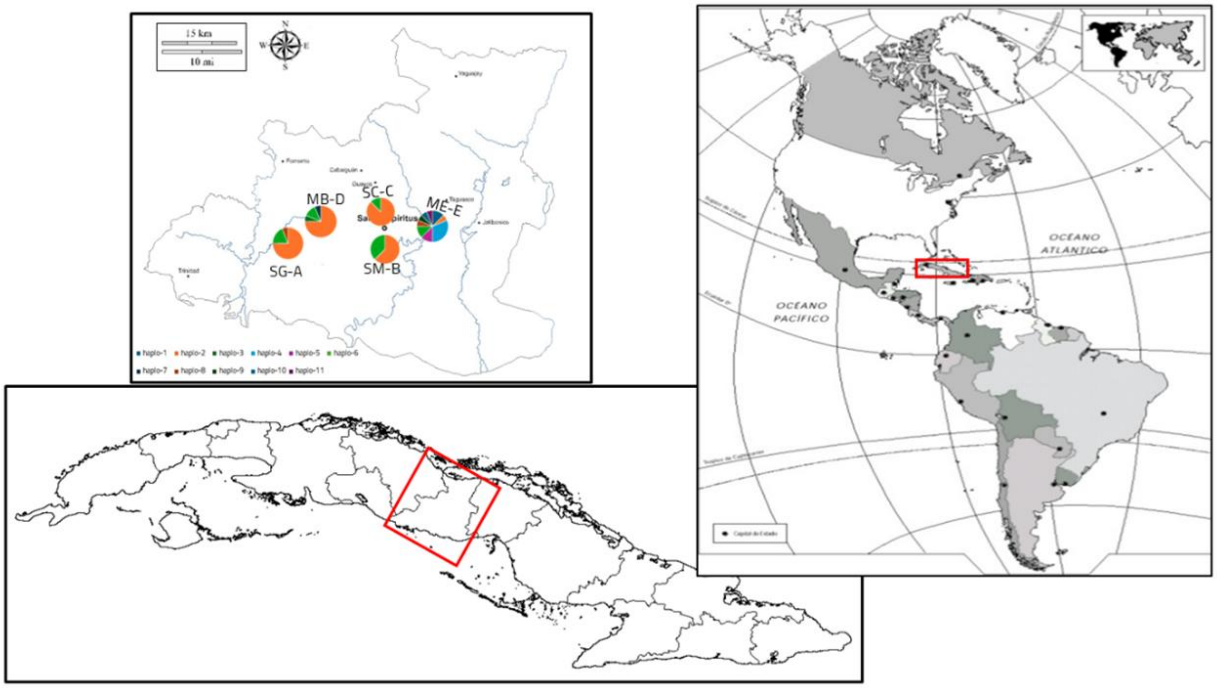

Figure 4.1. Geographic distribution of the 11 common chloroplast DNA (for the abbreviations, see Table 2.1)

Genetic distances between populations ranged from 0.019, observed between SG-A and MB-D, to 0.777 for the pair MB-D and ME-E, reflecting varying levels of chloroplast divergence among the sites. Hierarchical Analysis of Molecular Variance (AMOVA) revealed that 41% of the total variation was attributable to differences between populations. Within-population AMOVA indicated even greater differentiation among populations, with PhiPT = 59% ($p > 0.05$).

Figure 4.2 shows the spatial distribution of chloroplast DNA variation across the populations studied. The ME-E population exhibited the greatest haplotype richness, with nine haplotypes detected. Overall, native species displayed higher genetic diversity compared to the introduced species. The highest haplotype diversity was concentrated in mixed plantations, representing 81.8% in ME-E and 36.4% in MB-D of all haplotypes identified. While the chloroplast markers could not fully distinguish species, they revealed substantial variation in maternal haplotypes among populations. Once again, ME-E stood out as the population with the most pronounced chloroplast diversity, consistent with the spatial pattern shown in Figure 4.2.

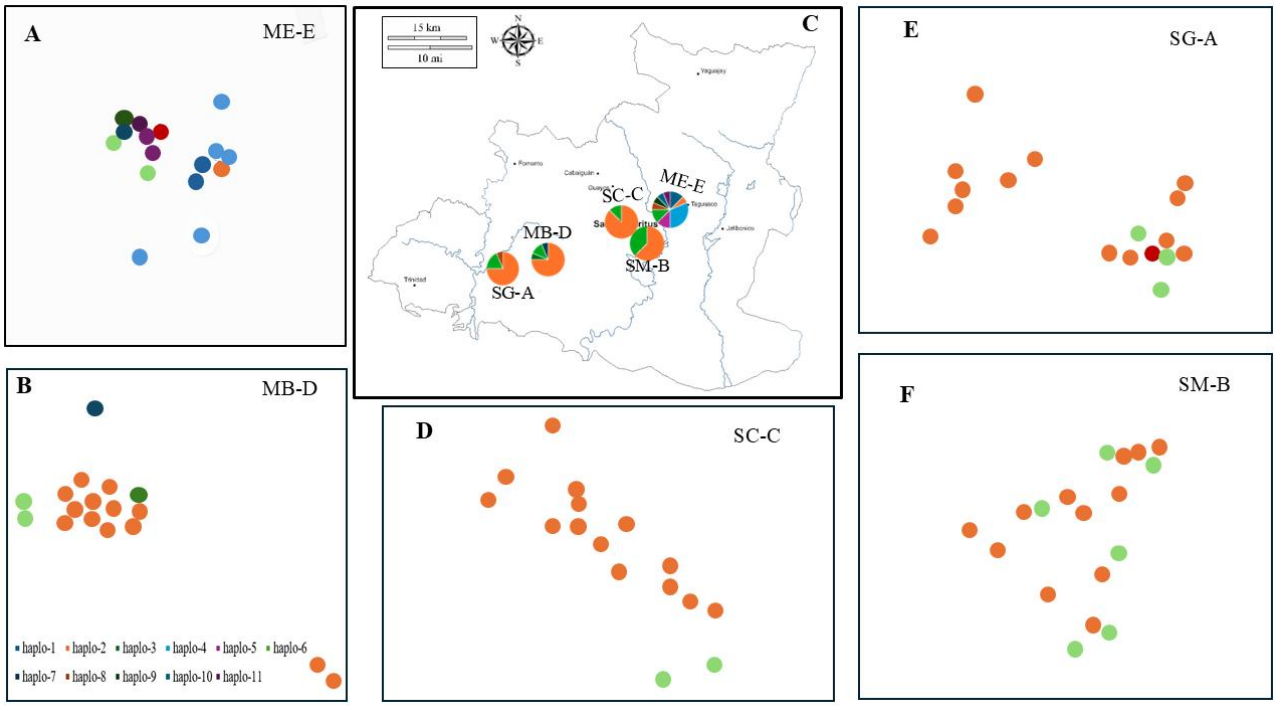

Figure 4.2. Spatial Genetic Structure distribution of the chloroplast DNA in each population studied in Cuba. Populations abbreviations are defined in Table 2.1 (Rodriguez et al. 2026). The classification of the subjects according to species was based on morphology (Rodriguez et al. 2024).

The two Mantel analyses showed notable differences in both the strength of correlation and the statistical support of the relationships examined. In the first analysis, a moderate-to-strong correlation was observed between the geographic distance and population distance matrices ($r = 0.6001$), with very high statistical significance ($p = 1 \times 10^{-4}$). This indicates that geographic separation is strongly linked to population differentiation, reflecting a clear pattern of spatial structuring consistent with isolation by distance.

By contrast, the second analysis, which assessed the relationship between geographic distance and genetic diversity, showed a very weak correlation ($r = 0.06171$) with marginal significance ($p = 0.0428$). While the p-value suggests a statistically detectable association, the low correlation coefficient indicates that geographic distance has minimal impact on genetic diversity, barely exceeding the significance threshold set by the null model ($Q95 = 0.0581$).

Overall, these results suggest that, although geographic distance strongly influences population structure, its effect on genetic diversity is much weaker. This implies that other factors—such as gene flow, selective pressures, or historical events—may play a more important role in shaping the observed genetic variation.

CHAPTER 5: Nuclear genetic diversity, population structure and hybridization signals

5.1. Genetic diversity

The eight nuclear SSR markers assessed in 357 *Swietenia* individuals revealed moderate to high genetic diversity. Allelic richness per locus (Na) ranged from 8.6 to 17.2, with a mean of 11.88 ± 0.76, while the effective number of alleles (Ne) varied between 2.31 and 6.91 (mean = 4.89 ± 0.41). Observed heterozygosity (Ho) ranged from 0.35 to 0.75, and expected heterozygosity (He) ranged from 0.46 to 0.85. The mean F_{ST} across loci was 0.15 ± 0.02, indicating moderate genetic differentiation, alongside substantial intrapopulation genetic variability.

Genetic diversity among *Swietenia* populations in Cuba varied notably across sites (Table 5.1). The number of alleles per locus (Na) ranged from 5.75 ± 1.07 in SC-C to 16.38 ± 1.24 in ME-E, with an overall mean of 11.88 ± 0.76. Similarly, the effective number of alleles (Ne) spanned 3.30 ± 0.66 (SC-C) to 7.53 ± 1.07 (ME-E), reflecting differences in allele frequency distribution among populations. The Shannon information index (I) ranged from 1.23 ± 0.18 in SC-C to 2.20 ± 0.18 in ME-E, indicating higher genetic complexity in the latter. Observed heterozygosity (Ho) varied from 0.45 ± 0.11 (SC-C) to 0.64 ± 0.06 (SG-A), while expected heterozygosity (He) ranged from 0.59 ± 0.10 (SC-C) to 0.82 ± 0.05 (ME-E).

Table 5.1. Genetic diversity of *Swietenia* populations in Sancti Spíritus based on nuclear microsatellite loci (Rodriguez et al., 2026).

Population	N	Na	Ne	I	Ho	He
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 (±0.1)
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		11.88	4.89 (±0.41)	1.74 (±0.09)	0.57 (±0.04)	0.72 (±0.03)

mean		(±0.76)				

Note: Observed number of alleles per locus (Na); Mean number of effective alleles (Ne); Shannon´s Information index (I); Observed heterozygosity (Ho) expected heterozygosity (He); ±standard errors in parentheses. Abbreviations are defined in Table 2.1.

Overall, these results indicate that the analyzed populations retain substantial genetic variability, with ME-E showing the highest genetic richness and complexity among the sites evaluated. The lower diversity observed in the SC-C population is largely attributable to the small size of this remnant, as all individuals were sampled. Thus, the reduced genetic variability reflects demographic constraints rather than limitations of the sampling or markers. These findings highlight contrasting patterns of genetic diversity across species, population types (natural, remnant, or plantation), and geographic locations.

Analysis of molecular variance (AMOVA) showed that most of the genetic variation in *Swietenia* populations is found within populations (97%), with only 3% explained by differences among populations. The genetic differentiation coefficient (F_{ST}) was low but statistically significant (F_{ST} = 0.027; P = 0.001), indicating a weak population structure across the sites studied. When standardized relative to its theoretical maximum (F_{ST}max = 0.096), the F'ST index reached 0.278, reflecting a moderate level of population differentiation.

Discriminant Analysis of Principal Components (DAPC) (Figure 5.1A) revealed clear genetic separation of the SC-C population from the two *S. mahagoni* populations (SG-A and SM-B), primarily along the first discriminant axis. The *S. mahagoni* populations clustered closely, with slight overlap, indicating higher genetic similarity. Considering all populations together (Figure 5.1B), ME-E occupied an intermediate position between *S. mahagoni* and *S. macrophylla*, with individuals broadly and diffusely distributed across the discriminant space.

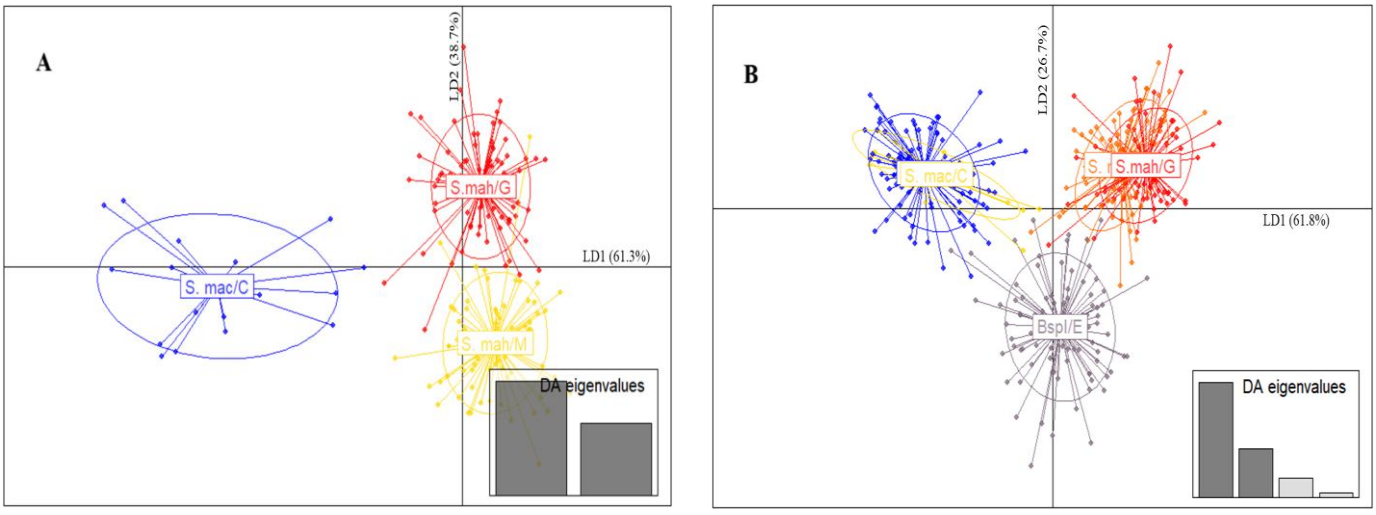

Figure 5.1. Discriminant principal component analysis: (A) pure species and (B) all the locations evaluated (Rodriguez et al., 2026).

5.2. Population structure and hybridization

Bayesian analysis of population structure using STRUCTURE with K = 2 (Figure 5.2) revealed marked genetic differentiation between the groups analyzed. Individuals belonging to populations 1 and 5 showed a predominant assignment to the orange colour (cluster 2), with high membership coefficients (0.970 and 0.919, respectively). In contrast, populations 2, 3, and 4 were mainly associated with the blue colour (cluster 1), with assignment values of 0.974, 0.884, and 0.954. The allele frequency divergence between the two clusters was moderate (D = 0.1195), while the average heterozygosity within each cluster was comparable, at 0.807 and 0.792, respectively.

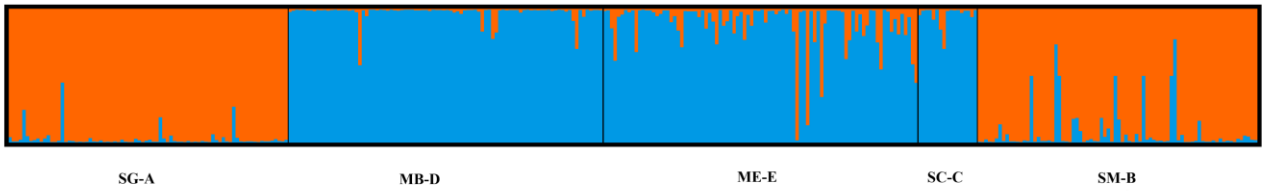

Figure 5.2. Clustering obtained for two genetics clusters (k=2). Population codes are as follows: SG-A (*S. mahagoni*), MB-D (mixed stand), ME-E (mixed stand), SC-C (*S. macrophylla* remnant), and SM-B (*S. mahagoni*) (Rodriguez et al., 2026). Abbreviations are defined in Table 2.1.

The FST values (0.0713 and 0.0911) corroborate the genetic differentiation observed between the clusters. Furthermore, a high level of admixture evident in the Emigdio mixed stand (ME-E), where a methodological discrepancy has been observed: 56 individuals that were classified as *S. mahagoni* based on leaf morphology are predominantly associated with genetic cluster 1 (*S. macrophylla*). By contrast, the mixed stand at Banao (MB-D) shows greater concordance, with *S. macrophylla* predominating morphologically and genetically. This discrepancy may suggest that leaf morphology is not always sufficient to detect admixture or hybridization in mixed stands, as leaf traits may overlap between the parental species, even when genetic analysis reveals a different pattern.

Bayesian analysis of population structure using STRUCTURE and nuclear markers allowed inference of the optimal number of genetic clusters (K) via the Evanno method (Evanno et al. 2005). The LnP(K) values increased progressively from K = 1 to K = 5, though the rate of increase slowed from K = 4 onward. The highest ΔK was observed at K = 3 (ΔK = 747.01), with a mean likelihood of LnP(K) = −10562.86 and a low standard deviation (±0.87), indicating a stable and robust solution. These results support the presence of three primary genetic groups among the analyzed populations (Figure 5.3A–B).

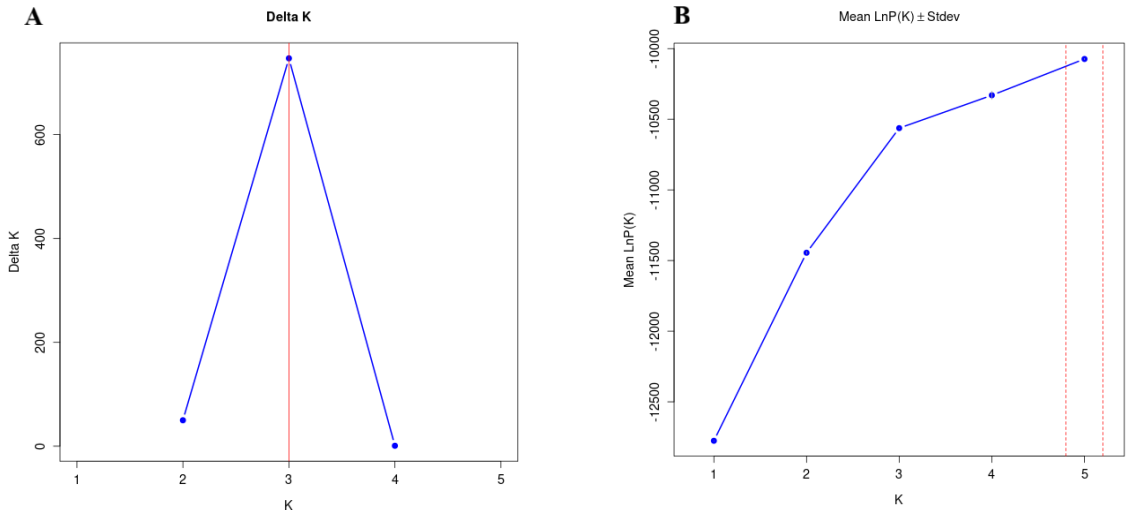

Figure 5.3. Highest ΔK value (A) and (B) Mean LnP(K) (Rodriguez et al., 2026).

The STRUCTURE analysis at K = 3 (Figure 5.4A), identified as optimal using the ΔK method (Evanno et al. 2005), revealed clear genetic differentiation among the populations. Populations 1 and 5 were predominantly assigned to cluster 3 (blue), with membership coefficients of 0.937 and 0.874, respectively. Populations 2 and 4 were mainly associated with cluster 1 (orange) (0.915 and 0.847), whereas population 3 showed stronger affiliation with cluster 2 (violet) (0.891). Allele frequency divergence between clusters was moderate to high, ranging from 0.1213 to 0.1920. Mean heterozygosity for clusters 1, 2, and 3 was 0.7877, 0.6642, and 0.8246, respectively. F_{ST} values ranged from 0.0653 to 0.2255, confirming significant genetic differentiation among the identified groups.

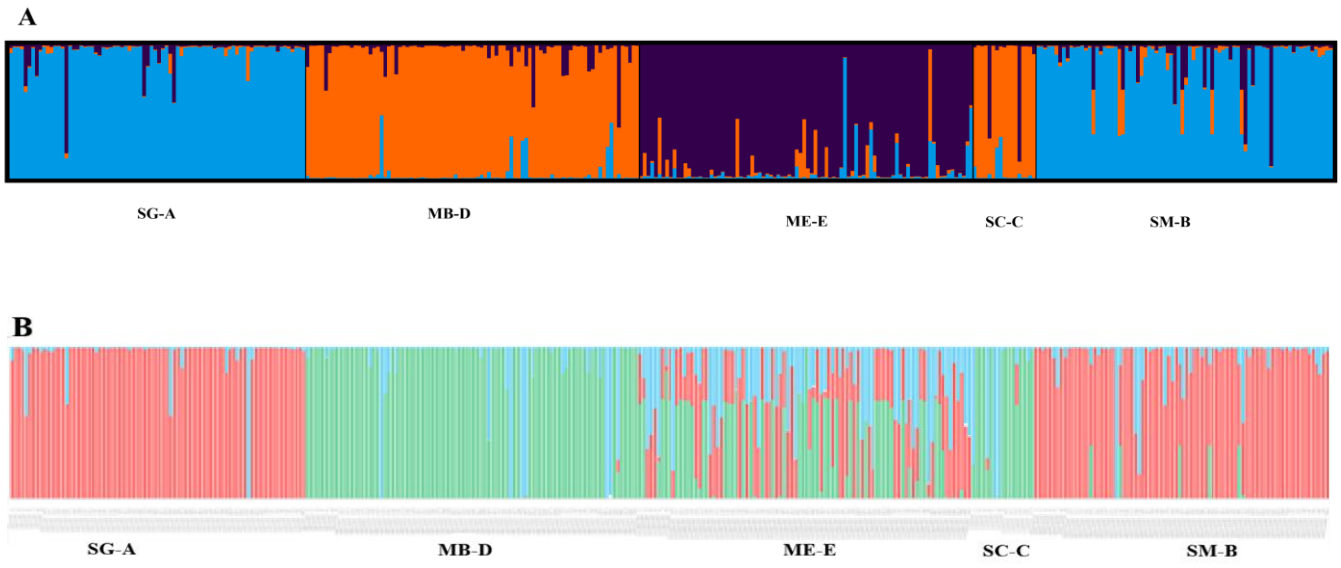

Figure 5.4. Clustering obtained for three genetics clusters. (A) created using the ΔK selected by the Evanno method and (B) based on the results obtained by the Newhybrids software. Where SG-A (*S. mahagoni*), MB-D (mixed stand), ME-E (mixed stand), SC-C (*S. macrophylla* remnant), and SM-B (*S. mahagoni*) (Rodriguez et al., 2026). Abbreviations are defined in Table 2.1

The STRUCTURE analysis at K = 3 distinguished three genetic clusters: populations 1 (SG-A) and 5 (SM-B) were assigned to cluster 3 (blue), populations 2 (MB-D) and 4 (SC-C) to cluster 1 (orange), and population 3 (ME-E) to cluster 2 (violet). These results aligned with NewHybrids analyses, which confirmed that populations 1 and 5 were predominantly S. mahagoni and populations 2 and 4 were mainly S. macrophylla, while also detecting some admixed individuals. This indicates that, although each population is largely associated with one parental species, complete genetic homogeneity is absent. In contrast, ME-E exhibited a high proportion of hybrid individuals (Figure 5.4B).

5.3. Comparative analysis between leaf morphology and genetics assignment

Morphological identification was compared with (i) genetic assignment based on nuclear microsatellites and (ii) Bayesian hybrid classification using NewHybrids. At the stricter threshold ($q \geq 0.90$), the average agreement per population was 64.9% for Leaf Morphology–STRUCTURE, 64.3% for Leaf Morphology–NewHybrids, and 76.0% for STRUCTURE–NewHybrids. Using a more relaxed threshold ($q \geq 0.80$), agreement slightly increased to 67.2%, 67.0%, and 72.8%, respectively. These findings suggest that overall assignment patterns are consistent and robust across different classification criteria.

Both native populations (SG-A and SM-B) exhibited strong concordance across assignment methods at both thresholds. For $q \geq 0.90$, agreement ranged from 95–97.5% in SG-A and 80–93.75% in SM-B, while at $q \geq 0.80$ values remained high (95–98.8% and 85–91.3%, respectively). The SC-C population showed strong agreement between morphological identification and nuclear assignment (88.2%), increasing to 94.1% at the $q \geq 0.80$ threshold; however, concordance with NewHybrids was noticeably lower, rising only from 64.7% to 70.6%.

In mixed plantations, the discordance between methods was more pronounced. MB-D showed low levels of concordance in comparisons involving leaf morphology (Leaf Morphology–STRUCTURE: 48.9% → 46.7%; Leaf Morphology–NewHybrids: 50% → 47.8%), while the concordance between both genetic approaches was high (STRUCTURE–NewHybrids: 77.8% → 82.2%). The ME-E population exhibited the highest degree of discrepancy between methods (Leaf Morphology–STRUCTURE: 10% → 7.8%; Leaf Morphology–NewHybrids: 34.4% → 36.7%; STRUCTURE–NewHybrids: 34.4% → 17.8%), which is consistent with a scenario of introgession and extensive hybridization. This highlights the limitations of relying solely on phenotypic identification in mixed stands, where morphological traits may not fully capture underlying genetic variation, especially in the presence of hybrids or introgressed individuals.

Overall, the classification of individuals as 'parental' or 'hybrid' revealed notable differences depending on the method applied (Table 5.2). In genetic assignments, individuals with q ≥ 0.90 were considered confidently assigned to a parental species, while those with intermediate values (0.10–0.90) were treated as hybrids. A more permissive threshold (0.20–0.80) was also evaluated to account for genotypes showing varying levels of introgression.

Table 5.2. Overall classification counts by method. Numbers of parental genotypes (*S. mahagoni*, *S. macrophylla*) and hybrids identified by leaf morphology, STRUCTURE assignment (q ≥ 0.90; q ≥ 0.80) and NewHybrids (q ≥ 0.90; q ≥ 0.80) (Rodriguez et al., 2026)

	Leaf Morph	STRUCTURE (0.10-0.90)	STRUCTURE (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

Morphological identification classified 274 individuals as parental (220 *S. mahagoni* and 54 *S. macrophylla*) and 83 as intermediates morfological forms. By contrast, nuclear assignments identified 54 hybrids at q ≥ 0.90 (303 parental) and 28 hybrids at q ≥ 0.80 (329 parental), reflecting a conservative estimate of hybridization. NewHybrids, however, detected substantially more hybrids—140 at q ≥ 0.90 and 129 at q ≥ 0.80.

The highest concordance occurred between the two genetic approaches (STRUCTURE–NewHybrids), while the greatest discrepancies appeared in mixed stands, underscoring the limitations of relying solely on leaf morphology for identifying hybrids. These results indicate that hybridization is widespread in the studied system, with NewHybrids estimating 36–39% of individuals as hybrids, whereas nuclear assignments provide a more conservative lower bound of 8–15%.

5.4. Fine-Scale Spatial Genetic Structure

The spatial autocorrelation correlograms showed very low values for the genetic correlation coefficient in both populations analyzed (Figure 5.5). In the SG-A population, the coefficient was slightly positive in the first distance classes (25–75 m) and negative in intermediate classes (100–125 m), with some isolated deviations approaching the limits of the 95% confidence intervals. In the SM-B population, the coefficient fluctuated around zero in all distance classes evaluated (10–70 m) and remained within the confidence intervals, indicating an approximately random distribution of genotypes and the absence of detectable genetic clustering at the spatial scale analyzed.

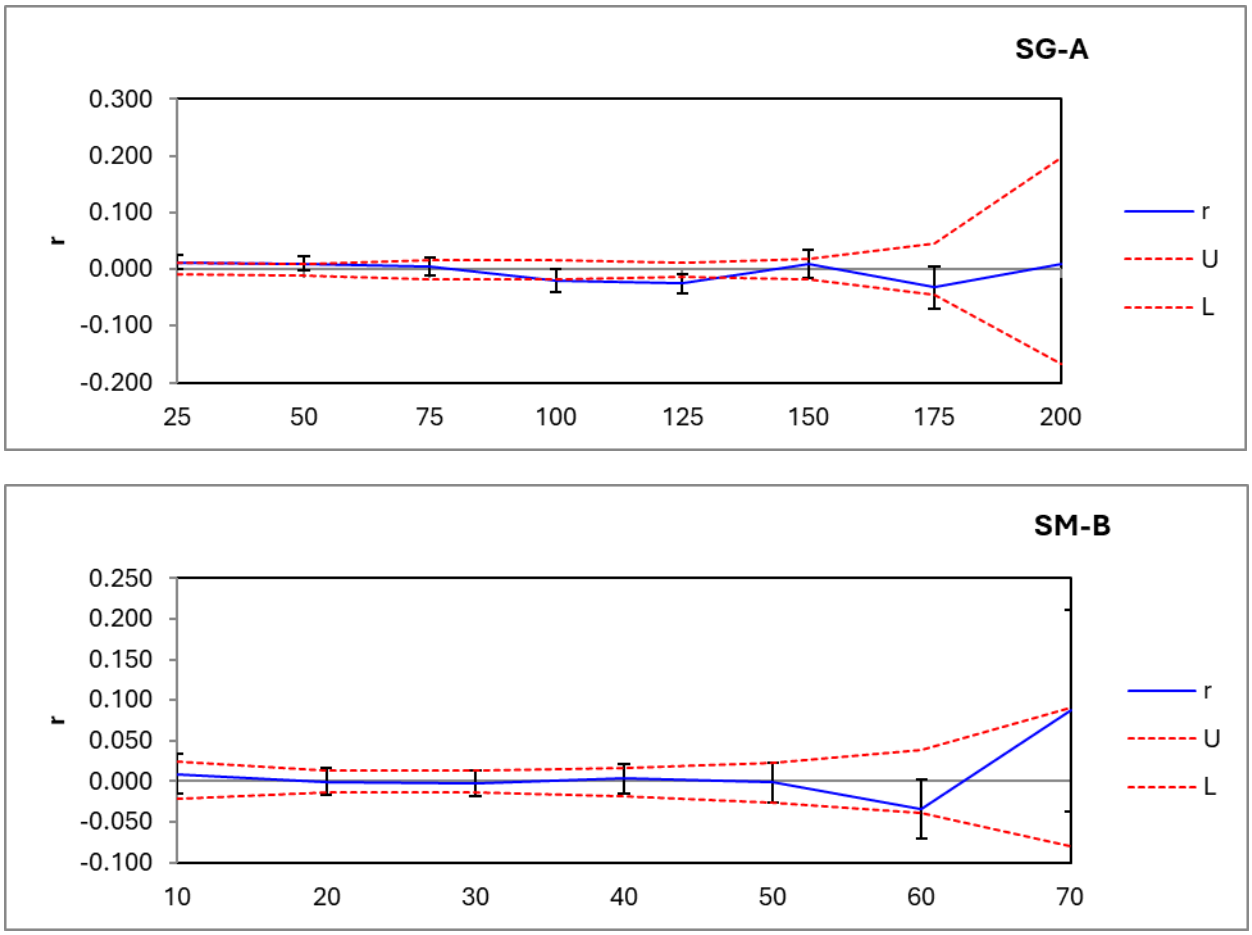

Figure 5.5. Multilocus spatial genetic correlograms of genetic and geographical distance in two populations of *S. mahagoni*. The y-axis is the genetic correlation coefficient (r), and the x-axis is the distance class (m); confidence intervals (95%) were calculated using permutation tests (red lines) and bootstrapped 95% confidence error bars around r are also shown (Rodriguez et al., 2025).

In SG-A, the kinship coefficient in the first distance class was low and positive (F_1 = 0.0074), while in SM-B it was practically zero ($F_1 \approx 0$). The kinship between the closest individuals (F_1) was close to zero in both populations (SG-A: 0.0074; SM-B: –0.0002). Consistently, the slope of the regression of the kinship coefficient as a function of the logarithm of distance was weakly negative and of low magnitude (SG-A: bF = –0.0109 ± 0.0205; SM-B: bF = –0.0111 ± 0.0207), indicating a barely perceptible decrease in kinship with increasing distance.

Consequently, the intensity of the spatial genetic structure was equally low in both populations, with virtually identical Sp values (SG-A: 0.0110; SM-B: 0.0111). An Sp value of ≈ 0.011 in both populations indicates the presence of a weak spatial structure; however, global permutation tests applied to the regression slopes were not significant (two-tailed test, p > 0.5), so no consistent fine spatial genetic structure] (FSGS) was detected at the scale analyzed.

CHAPTER 6. CONCLUSIONS. CONTRIBUTION TO THE FIELD AND RELEVANCE. DISSEMINATION OF RESULTS

6.1. Conclusions

The results obtained allow for an integrated assessment of the morphological and genetic variability of *Swietenia* in Cuba, as well as the relative usefulness of the different approaches employed. The conclusions are presented below, organised according to each of the specific objectives set out in this research.

Regarding leaf morphology:

- **Taxonomic differentiation based on leaf traits:** Consistent and statistically significant differentiation was found between the species *S. mahagoni* and *S. macrophylla* in all of the leaf morphometric variables evaluated, demonstrating that the traits of *S. macrophylla* are markedly larger. This confirms and validates the practical usefulness of these leaf traits for establishing a clear morphological distinction between pure populations of both species.
- **Identification of leaflet length as a key discriminatory trait:** Through multivariate analysis, it was determined that leaflet length is the morphometric trait with the greatest power to discriminate between the two species. The integration of this and other variables into mathematical functions proved to be a highly effective methodology for separating individuals and structuring classification models.
- **Confirmation and clasification of Intermediate Morphological Forms (IMF):** The application of discriminant functions in mixed stands (Emigdio and Banao) allowed for the conclusive confirmation of the existence of Intermediate Morphological Forms. These individuals exhibit a transitional leaf morphology that is geometrically located and grouped in an intermediate region with respect to the two parental species.
- **High predictive reliability and population segregation:** The multivariate classification model developed proved to be highly robust, achieving a predictive accuracy of almost 95% in the initial morphological assignment of individuals to their respective groups. This, supported by a cluster analysis that showed clear segregation between pure species and mixed stands, provides a highly reliable methodological tool for the identification and management of pure populations. However, for comprehensive taxonomic and genetic monitoring—particularly in mixed stands subject to introgression—this morphological tool should be integrated with molecular analyses, as leaf traits alone cannot fully resolve complex hybrid genotypes.

Regarding chloroplast DNA analaysis:

- **Identification of moderate genetic variation without discrimination at the species level:** Analysis of chloroplast DNA in populations of *Swietenia* spp. revealed a moderate level of genetic variation and the existence of 11 haplotypes. Although the markers used (particularly ccmp4 and ccmp7) detected considerable genetic variability, it was concluded that these are not sufficient to establish unequivocal or diagnostic discrimination between species.
- **Mixed plantations as reservoirs of genetic diversity:** Mixed stands exhibited the highest levels of chloroplast genetic diversity. In particular, Emigdio mixed stand was identified as the main reservoir of diversity, as it contained nine of the eleven haplotypes detected and had the highest number of unique haplotypes (six). This pattern contrasts markedly with the limited differentiation and lower allelic richness observed in the pure populations of the native species and of the introduced species. In this context, the 'Emigdio' mixed stand, by harbouring a significant proportion of the total haplotype diversity, constitutes a valuable resource for the genetic conservation and sustainable management of these species.
- **Predominantly intrapopulation genetic structuring:** The evaluation of the genetic structure of the populations indicates that most of the genetic variation is within the stands. Molecular variance analysis (AMOVA) confirmed this dynamic, determining that 59% of the total variance is explained by differences within populations, compared to 41% attributable to divergence between the different locations evaluated.
- **Influence of management history and seed origin on chloroplast structure:** Although statistical analyses (Mantel test) revealed a link among geographical and genetic distances based on chloroplast haplotypes, this pattern reflects the taxonomic distribution of the sampled forest stands (native species versus introduced remnants) and the history of forest management, rather than a natural evolutionary process of isolation by distance. The marginal influence of geography on haplotype diversity levels suggests that maternal genetic variability in these populations is strongly conditioned by human-mediated factors, such as the initial provenance of the introduced germplasm, historical fragmentation and the mixing of different seed sources during the establishment of mixed stands.

Regarding nuclear DNA anlysis:

- **High intrapopulation genetic diversity and conservation of integrity in native species:** Analysis using nuclear microsatellite markers revealed moderate to high levels of genetic variability, with most of this variation (97%) concentrated within the populations. It was found that the natural populations of *S. mahagoni* maintain adequate genetic diversity and preserve their integrity, as they were identified predominantly as pure species with minimal evidence of introgression. It is worth noting that the Guira population (SG-A) exhibited the highest degree of genetic purity, with a level of admixture that was virtually negligible compared with the

Modelo population. For their part, mixed stands (especially the ME-E population) serve as the main reservoirs of allelic richness and complexity due to crossbreeding.

- **Clear population differentiation and high prevalence of hybridization:** The evaluation of the population structure confirmed the existence of well-defined genetic groups that allow pure parental species to be separated from hybrid forms. Multivariate and Bayesian analyses (STRUCTURE and NewHybrids) demonstrated that hybridization is a widespread phenomenon in these forest systems, reaching estimated proportions of up to 36–39%. Considering the cross-species transferability limitations of the SSR markers used, this represents a robust, yet conservative estimate of the complex introgression occurring in mixed stands, genetically positioning these individuals in a clear intermediate transition zone.
- **High taxonomic concordance in pure species and morphological insufficiency in mixed stands**: The comparative analysis revealed a high degree of agreement (with similarity values ranging from 85% to over 98%) when traditional morphological identification was compared with genetic assignment in the populations of the parental species. However, in mixed stands, discrepancies were detected and a drop in concordance was observed (reflecting the pronounced phenotype-genotype decoupling observed particularly in Emigdio population). This demonstrates that, although leaf morphology is a highly valid tool in isolated populations, it is insufficient for classifying individuals in scenarios of extensive admixture, making the use of molecular tools indispensable for identifying complex hybrid genotypes.
- **Absence of fine-scale genetic structure (FSGS) and positive implications for conservation**: Spatial autocorrelation analysis at the intrapopulation level in natural populations of *S. mahagoni* revealed a virtually random genotypic distribution, with no evidence of significant genetic clustering at the local level. The very weak correlation found between the kinship of individuals and their geographical distance allows us to conclude that there is no significant fine-scale spatial genetic structure within the stands. Consequently, these predominantly pure stands (specifically the Guira population) are consolidated as ideal seed collection, sources for reforestation programs, as they guarantee the obtaining of diverse, robust genetic material with minimal levels of kinship.

Overall, this thesis demonstrates that morphological identification is adequate for pure populations of *Swietenia* in Cuba, but that reliable detection of hybridization in mixed stands requires the complementary use of molecular genetic tools to overcome the pronounced phenotype-genotype uncoupling. The integration of morphological and molecular approaches provides a solid basis for the design of conservation strategies, the establishment of representative germplasm banks, and the implementation of long-term reforestation and genetic monitoring programs, in line with CITES guidelines and the preservation of the genetic identity of *S. mahagoni*.

6.2. Contribution to the field and relevance

The main original contribution of this doctoral thesis lies in the integration of leaf morphometric analyses with nuclear and chloroplast molecular markers to evaluate species differentiation and hybridization between *S. mahagoni* and *S. macrophylla* in Cuba.

This research offers the first empirical demonstration of localized hybridization between both species in mixed stands, detected using nuclear microsatellite markers and Bayesian modelling, and demonstrates that such hybridization has not compromised the genetic structure or identity of the native species *S. mahagoni*. It also establishes that leaf morphology is a reliable tool for taxonomic identification in isolated populations, but is insufficient on its own in contexts of interspecific mixing due to a pronounced phenotype-genotype uncoupling.

Furthermore, it demonstrates that naturally established populations of *S. mahagoni* maintain high levels of genetic diversity and a weak spatial genetic structure, positioning them as key genetic reservoirs and potential sources of seeds for restoration programmes. Taken together, the results provide a replicable morpho-molecular framework for the genetic conservation of tropical forest species subject to human introductions.

6.3. Future directions

Considering the critical conservation status of both species in Cuba—where both *S. mahagoni* and *S. macrophylla* are endangered, but the latter still bears commercial pressure under regulations—the findings of this research pose new challenges and opportunities. Future lines of forestry research should be geared towards ensuring the protection of native resources, optimising the sustainable use of resources and establishing rigorous science-based controls. Based on the morpho-molecular framework developed, the following future directions are proposed:

- **Assessment of the productive potential of hybrids to mitigate logging pressure:** The confirmation that hybridisation is a widespread phenomenon in mixed stands (reaching estimated proportions of up to 39% according to Bayesian analyses) opens up a strategic avenue for reforestation with productive approaches. Future studies should evaluate the long-term performance of these interspecific hybrids in plantations such as ME-E. If hybrid vigour (heterosis) is confirmed, resulting in higher growth rates or resilience, the promotion of these mixed stands for purely commercial purposes could satisfy market demand, diverting and alleviating the logging pressure that currently threatens the relict populations of both parent species.
- **Transition to genomic approaches (NGS) and study of contemporary gene flow:** To fully understand the dynamics of introgression, it is necessary to scale up the resolution of current molecular markers to Single Nucleotide Polymorphisms (SNPs). In addition, detailed studies on reproductive ecology in sympatry (phenological overlap, pollinator networks and dispersal distances) should be promoted. This high-resolution genomic and ecological information will

allow us to model contemporary gene flow and predict the future risk of genetic contamination from commercial plantations to strictly protected natural patches.

- **In situ conservation strategies:** Once it has been confirmed that the natural populations of *S. mahagoni* (SG-A and SM-B) retain their taxonomic purity and lack a fine-scale spatial genetic structure (FSGS), they should be legally declared and protected as 'Certified Seed Gardens' and untouchable genetic reserves, serving as primary source for future ecological restoration programmes.

6.4. Dissemination of results

The findings generated throughout this doctoral research were communicated through three peer-reviewed publications and shared at six international scientific events.

Publications derived from the research material developed in this thesis:

1. **Rodriguez Coca L.I.**, Ciocîrlan, E., BOGGIANO, A. G. T., FERNÁNDEZ, L. A. D., ÉVORA, J. F. L., Codrean, C., & Curtu, A. L. (2024). Leaf-based characterization of intermediate forms between Cuban and Honduran mahogany. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *52* (1), 13731-13731. https://doi.org/10.15835/nbha52113731(I.F: 1.3; Q2)
2. **Rodriguez Coca L.I.**, Ciocîrlan E., Curtu A.L. 2026. When native meets exotic: Uncovering hybridization between Cuban and introduced mahogany using chloroplast and nuclear microsatellites. *Annals of Forest Research*. 69(1): 21-44. https://doi.org/10.15287/afr.2026.4354 (I.F: 2.9; Q1)
3. **Rodriguez Coca L.I.**, Ciocirlan, E., & Curtu, A. L. (2025). From Abandoned Lands to Genetically Robust Stands: Nuclear Genetic Diversity and Fine Scale Spatial Genetic Structures in Cuban Mahogany (Swietenia mahagoni). *Bulletin of the Transilvania University of Brasov. Series II: Forestry, Wood Industry, Agricultural Food Engineering*, 53-64. https://doi.org/10.31926/but.fwiafe.2025.18.67.2.4

Participation in international scientific conferences and events:

1. Liuder Isidoro RODRÍGUEZ COCA, Elena CIOCIRLAN, Alexandru Lucian CURTU. Leaf morphological traits distinguish between Cuban native species of mahagoni and the Hondurian mahagony. 6th Edition of the International Conference of the Integrated Management of Environmental Resources Conference Suceava-Romania. 23-24 November 2023, Suceava Romania.
2. Liuder Isidoro RODRÍGUEZ COCA, Elena CIOCIRLAN, Alexandru Lucian CURTU. Leaf morphological traits distinguish between Putative Hybrids Between Cuban and Hondurian mahagony species. Doctoral Conference DoCo 2024. Transilvania University of Brasov. 26-27 June 2024, Brasov, Romania.
3. Liuder Isidoro RODRÍGUEZ COCA, Elena CIOCIRLAN, Alexandru Lucian CURTU. GENETIC DIVERSITY AND POPULATION STRUCTURE OF CUBAN FOREST SPECIES. International competition "My 3 minutes PhD" at Université de Pau et des Pays de l'Adour. 1-5 July 2024, Pau, France.

4. Liuder Isidoro RODRÍGUEZ COCA, Elena CIOCIRLAN, Alexandru Lucian CURTU. From roots to genes: Unraveling the genetic diversity of Cuban mahogany through PCR and DNA analysis. International Summer School, Bioanalytical (Tele)Monitoring for Life Sciences - Medicine, Food Control, Environmental Monitoring. Transilvania University of Brasov. 13-21 September 2024, Brasov, Romania.
5. Liuder Isidoro RODRÍGUEZ COCA, Elena CIOCIRLAN, Alexandru Lucian CURTU. Chloroplast DNA variation and genetic differentiation in Swietenia species. 11th International Symposium Forest and Sustainable Development 2024. 17-18 October 2024, Brasov, Romania.
6. Liuder Isidoro RODRÍGUEZ COCA, Elena CIOCIRLAN, Alexandru Lucian CURTU. Investigating the genetic effects of introducing Honduran mahogany (*Swietenia macrophylla*) into Cuba: implications for the conservation of the native species of mahogany. Third Biannual EVOLTREE International Conference. 18-21 November 2025, Madrid, Spain.

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