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ARTICLES

Assessing genetic diversity of *Dalbergia stevensonii*: an endangered species in Guatemala

Evaluación de la diversidad genética de *Dalbergia stevensonii*: una especie amenazada en Guatemala

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SUMMARY

Owing to extensive overexploitation and continuous habitat loss in recent decades, *Dalbergia stevensonii* has become endangered in Central America and its place of origin. Genetic diversity is essential for ensuring the long-term survival of the species and is vital in breeding programs. However, knowledge of the genetic diversity of *D. stevensonii* is limited, as no study has been conducted to estimate the population variability in Guatemala. This study is the first to determine the genetic diversity and structure of wild populations of *D. stevensonii* in Guatemala using molecular markers. The genetic diversity of 90 wild *D. stevensonii* trees from six populations located in their natural range is currently being evaluated. Genetic diversity was moderate, with an average of 5.83 alleles per locus, a mean Shannon index value of 1.42, and observed and expected heterozygosity of 0.32 and 0.37, respectively. AMOVA indicated that only 6 % of the genetic variation was between populations, and genetic distance values (pairwise F_{ST}) suggested moderate differentiation between populations. STRUCTURE and DAPC analyses revealed the existence of three clusters for the 90 samples, while the genetic differentiation among populations is attributed more to genetics than to geographic distance. Conservation of the genetic diversity of *D. stevensonii* is critical to ensure its long-term survival, and the success of breeding programs focused on improving the traits and characteristics of the species, such as growth rate, wood quality, and resistance to pests and diseases. These findings provide a crucial genetic basis for conservation, management, and restoration of this endemic species.

Keywords: conservation, indigenous trees, population structure, microsatellites, habitat fragmentation.

RESUMEN

Debido a la sobreexplotación y continua pérdida de hábitats en las últimas décadas, *Dalbergia stevensonii* está en peligro de extinción en América Central, su lugar de origen. La diversidad genética es un factor esencial para garantizar la supervivencia de la especie a largo plazo y es vital en los programas de mejoramiento. Sin embargo, el conocimiento sobre la diversidad genética de *D. stevensonii* es limitado, ya que no se ha realizado ningún estudio para estimar la variabilidad de las poblaciones en Guatemala. Este es el primer estudio realizado en Guatemala para determinar la diversidad genética y la estructura de las poblaciones silvestres de *D. stevensonii* utilizando marcadores moleculares. Actualmente, se está evaluando la diversidad genética de 90 árboles silvestres de *D. stevensonii* procedentes de seis poblaciones situadas en su área de distribución natural. La diversidad genética es moderada, con una media de 5,83 alelos por locus, un valor medio del índice de Shannon de 1,42 y una heterocigosidad observada y esperada de 0,32 y 0,37 respectivamente. El análisis AMOVA mostró que solamente el 6 % de la variación genética se presenta entre poblaciones, y los valores de distancia genética (F_{ST} por pares) sugieren una diferenciación moderada entre poblaciones. Los análisis STRUCTURE y DAPC revelaron la existencia de tres conglomerados para las 90 muestras, mientras que las diferenciaciones genéticas entre poblaciones se atribuyeron más a la distancia genética que a la geográfica. La conservación de la diversidad genética de *D. stevensonii* es fundamental para garantizar su supervivencia a largo plazo y el éxito de los programas de mejoramiento centrados en la mejora de los rasgos y características de la especie, como la tasa de crecimiento, la calidad de la madera y la resistencia a plagas y enfermedades. Estos hallazgos proporcionan una base genética crucial para la conservación, gestión y restauración de esta especie endémica.

Palabras clave: conservación, árboles autóctonos, estructura poblacional, microsatélites, fragmentación del hábitat.

INTRODUCTION

Dalbergia is an exclusively diploid genus with 2n = 20 chromosomes (Hiremath *et al*. 2004). Honeybees, beetles, and butterflies are typical pollination agents that require crosspollination (Vasudeva and Sareen 2009). A crucial and widespread regeneration strategy for *Dalbergia* species in tropical dry forests is sprouting. The capacity for sprouting is integral to the management systems, including plantations, of select *Dalbergia* species. Notable examples of species exhibiting robust sprouting are *D. sissoo* Roxb. *ex* DC., *D. stevensonii* Standl., and *D. cochinchinensis* Pierre (Matin *et al*. 2006). *D. stevensonii* Standl, a member of the diverse pantropical family Fabaceae, is a forest species of high economic importance because of its excellent wood quality and is found in broadleaf evergreen swamp forests in southern Belize and adjacent areas of Guatemala and Mexico (CITES 2013). However, according to the abundance inventory of *D. stevensonii (*FNPV 2016), between 1991 and 2012, there was a 30.86 % reduction in the cover of this species in Guatemala. The heartwood of this rosewood is incredibly hard, dense, and resilient, making it ideal for making ornaments, musical instruments, carvings, luxury wood to decorate, fine furniture, and cabinetry (Hassold *et al*. 2016). Due to its high commercial value, *D. stevensonii* has been overexploited for a long time and is included as "critically endangered" in the IUCN (International Union for Conservation of Nature) red list (Martínez-Salas *et al*. 2021) and also protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Nevertheless, many species are still being targeted for illegal logging (Espinoza *et al*. 2015, Vardeman and Runk 2020). China, one of the world's largest buyers of tropical hardwoods and rosewood furniture, receives most of the world's harvested rosewood (Barrett *et al*. 2010).

As a result of heavy exploitation and illegal trafficking of its timber, the species has become rare, and only a limited number of individuals are found in parts of its original, highly fragmented habitat in the remaining forests of northern Guatemala, specifically in a tiny area called the Franja Transversal del Norte (FTN) and a small zone in the Peten department. Moreover, the susceptibility of species to climate change is evidenced by reduced flowering and fruit and seed formation under adverse conditions, posing another threat to their survival (Herrera-Sosa *et al*. 2016). Therefore, a comprehensive study is urgently required to obtain information on the levels and patterns of genetic variation in *D. stevensonii* in Guatemala. This information is essential for establishing an effective conservation program.

Molecular markers are often used to elucidate genetic variation in tree species (Nybom 2004). However, in *D. stevensonii*, no studies have used DNA molecular markers. Unlike other molecular markers, microsatellite markers (simple sequence repeats, SSR) are ideal for studying the genetic composition of wild populations because of their co-dominant nature and high variability (Powell *et al.* 1996, Fregene *et al*. 2003). The use of microsatellite markers to analyze the genetic diversity of *D. stevensonii* may provide a valuable tool for conserving and protecting this threatened species. In addition, SSR marker development has been innovated by transcriptome-based next-generation sequencing (RNA-seq), especially for species without a reference genome. (Dai *et al.* 2015, Huang *et al.* 2016, Taheri *et al.* 2018). This approach has been applied to SSR identification, development, and association studies in many tree species (Dervishi *et al*. 2018, Dong *et al.* 2018, Li *et al.* 2018).

Therefore, this study aims to assess the genetic diversity of wild *D. stevensonii* populations and determine the endangered and fragmented status of this species. The results of this study provide valuable genetic information for the conservation of *D. stevensonii*.

METHODS

Plant material. Utilizing the occurrence records of *D. stevensonii* in Guatemala, as documented in the abundance inventory of the species (FNPV 2016), fifteen trees were sampled at each of the following locations: Ixcán, Cahabón, Cobán, Fray (FTN area), and Sayaxché and Poptún (Peten Department) (figure 1). Leaves were collected from 15 *D. stevensonii* individuals at each location. Selected trees were sampled at a distance of > 30 m (Gutiérrez *et al*. 2015) to avoid kinship between trees. Five young leaves were collected from each specimen in polyethylene bags and transported to Prague, Czech Republic, for genetic analysis at the Czech University of Life Sciences Prague (CZU) Molecular Genetics Laboratory.

DNA extraction. Genomic DNA was extracted using the CTAB 2X protocol (CTAB 2 %, NaCl 5M, Tris HCl 1M, EDTA 0.5M, PVP 1 %, β mercaptoethanol 0.2 %) described by Doyle and Doyle (1987). To verify the integrity of the obtained DNA, 0.5 % agarose-TAE gels were used. The isolated DNA was eluted in 100 µL of elution buffer (TE 1X) and deposited at -20 °C until use. A Quantus™ fluorometer (Promega, Madison, WI, USA) was used to evaluate the quality and quantity of the extracted DNA.

DNA amplification. Since there are no reports of specific SSR markers for *D. stevensonii*, we used 12 polymorphic SSR markers initially developed for *D. hupeana* Hanse because they show high transferability to other *Dalbergia* species, such as *D. cochinchinensis* Pierre, *D. cultrata* T.S.Ralph, *D. sisso* Roxb. *ex* DC., *D. oliveri* Gamble *ex* Prain, *D. polyadelpha* Prain, *D. mimosoides* Franch and *D. yunnanensis* Franch (Li *et al.* 2021). PCR amplifications were performed in 20 µL reaction volumes as follows: 10 µL of 2X Taq MasterMix (Promega, Madison, WI, USA), approximately 50 ng of DNA, 5 pmol of reverse primer,

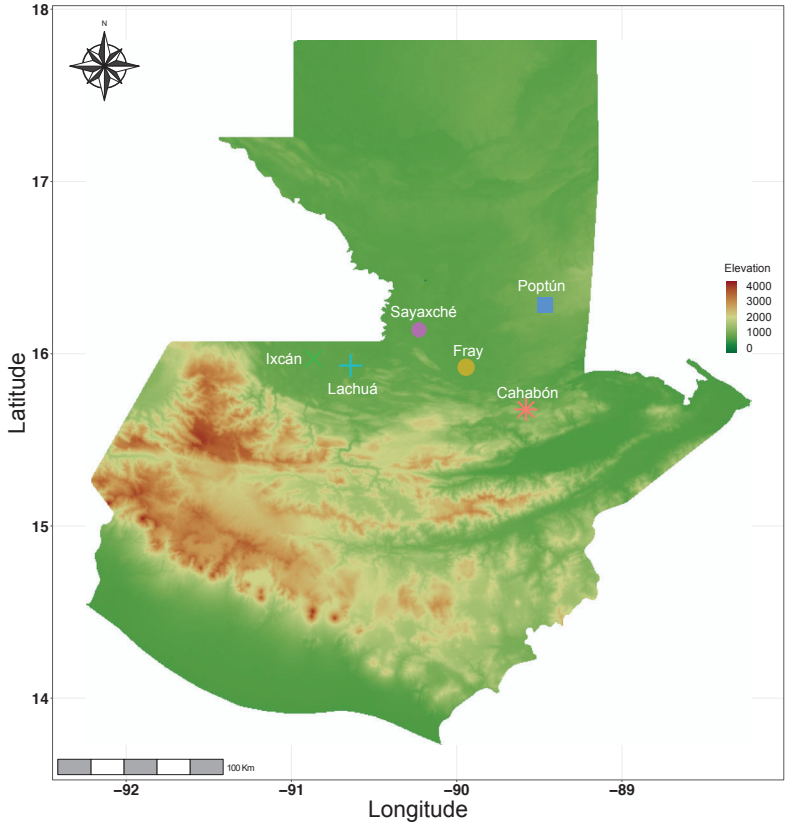

Figure 1. Map of Guatemala, displaying the geographical location of sampled *D. stevensonii* populations.
Mapa de Guatemala con la ubicación geográfica de las poblaciones de *D. stevensonii* muestreadas.

and 5 pmol of direct primer with the 5′ ends labeled with fluorescent dyes (FAM, PET, VIC, or NED), and sterile double-distilled water added to 20 µL. PCR was performed with a decreasing temperature ramp profile (touchdown) with an initial denaturation at 94 °C for 5 min; 10 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s, with a 1 °C decrease in alignment temperature for each cycle; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 10 min. PCR products were detected using an ABI 3500 capillary electrophoresis analyzer (Applied Biosystems, Waltham, MA, USA) with a GeneScan-500LIZ size standard (Applied Biosystems, Waltham, MA, USA). Fragments were genotyped for presence/absence at each locus, and allele sizes were scored using GeneMaker v2.2.0 (SoftGenetics LIC, State College, PA, USA) and manually double-checked to reduce genotyping error.

Genetic diversity analysis. The program GenoDive v.3.05 (Meirmans, 2020) was used to calculate essential statistics such as the number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), index of genetic differentiation (F_{ST}), and inbreeding coefficient (F_{IS}) to evaluate genetic diversity per locus and population. The Shannon information index (I) and private allele listing were performed using the Poppr library (Kamvar *et al.* 2014). GenoDive was used to perform the Hardy-Weinberg equilibrium test.

Genetic differentiation analysis. Genetic differentiation of populations was determined using analysis of molecular variance (AMOVA) implemented in the Poppr software. Covariance components were used to calculate fixation indices and to determine gene flow and differentiation between populations. A randomization test with 1,000 permutations was used to determine significance. The amount of gene flow (Nm) between populations was estimated using the formula $Nm = [(1/ F_{ST}) - 1]/4$. To assess isolation by distance, the Mantel test was performed using the ade4 package on genetic and geographic distance matrices with 10,000 permutations. Pairwise population differentiation was analyzed using the F_{ST} index with 1,000 permutations in the mmod package.

Population structure analysis. The genetic structure of the *D. stevensonii* samples was explored using hierarchical cluster analysis. The poppr package was used to calculate Nei's genetic distance and apply hierarchical UPGMA clustering. To further examine the structure of the populations, a Bayesian clustering method was run using

STRUCTURE v2.3.4 (Pritchard *et al.* 2000) with 10,000 steps and 100,000 MCMC iterations for cluster numbers from 1 to 10. The optimal K value was estimated using the methods of Evanno (Evanno *et al.* 2005) and Puechmaille (Puechmaille 2016). The results were analyzed and visualized using the STRUCTURE SELECTOR web server. STRUCTURE population assignment was confirmed using DAPC analysis (Jombart *et al*. 2010). The adegenet package and Bayesian information criterion (BIC) values were used to select the optimal number of gene clusters. Clusters were visualized using scatter plots and bar graphs. They were also confirmed with DAPC using a priori information and cross-validation (Xval.dapc).

RESULTS

Genetic diversity. The 12 SSR loci presented a number of alleles per locus ranging from 3.83 to 9.17, with an allele frequency range of 0.01 - 0.99 (table 1). The highest number of alleles (9.17) was detected at the Dhup185 locus, which also had the highest effective number of alleles (Ne, 5.76), expected heterozygosity (He, 0.80), Shannon diversity (I, 1.90), and polymorphic information content (PIC, 0.60). In terms of overall PIC, both Dhup185 and Dhup89 were highly informative, with PIC values above 0.60, whereas Dhup14 and Dhup70 were less informative, with PIC values below 0.25, and the remaining 8 loci were moderately informative, with PIC values between 0.25 and 0.50. The mean genetic differentiation index (F_{ST}) was 0.39, ranging from -0.11 (Dhup78) to 0.68 (Dhup191). Except for Dhup61 and Dhup78, all other loci showed significant deviations from the Hardy-Weinberg equilibrium in the 90 *D. stevensonii*.

Among the six populations, the percentage of polymorphic loci (PPL) ranged from 65.45 % to 89.75 % (table 2). The highest PPL was found in the Lachuá population, which also had the highest number of alleles (Na = 7.25). The Cahabón population, which had the lowest PPL, had the lowest Na (4.50). In total, seven private alleles were identified among the analyzed populations: three belonging to Lachuá, two in Ixcán, and one each in Fray and Sayaxché. The observed heterozygosity (Ho) ranged from 0.22 (Cahabón) to 0.41 (Lachuá), and the expected heterozygosity (He) ranged from 0.25 (Cahabón) to 0.46 (Lachuá), with a mean of 0.32 and 0.37, respectively. In addition, the Lachuá population, which had the highest genetic diversity (He, 0.46), also showed the highest Shannon diversity value (1.70).

Genetic differentiation. AMOVA and pairwise F_{ST} analyses were performed to explore the genetic variations between populations. The results showed that only 6 % of the total genetic variation occurred between populations and 39 % of the intrapopulation variation was due to the heterozygosity of individuals within each population (table 3). The overall F_{ST} was very small (0.06, table 3), and the overall gene flow was 2.89 (Nm) estimated among all populations (table 1). In addition, the pairwise

Table 1. Diversity statistics of the 12 SSR loci in 90 samples of *D. stevensonii.*

Estadísticas de diversidad de los 12 loci SSR en 90 muestras de *D. stevensonii*.

Locus	Na	Ne	I	Ho	He	PIC	F_{IS}	F_{ST}	Nm	P_{HWE}
Dhup14	4.00	3.23	1.23	0.33	0.69	0.24	0.50	0.49	1.70	0.005**
Dhup61	5.50	3.58	1.41	0.70	0.72	0.45	0.00	0.01	2.14	0.727ns
Dhup64	6.67	4.17	1.59	0.32	0.70	0.55	0.57	0.58	2.40	0.027*
Dhup70	3.83	1.77	0.79	0.13	0.45	0.23	0.67	0.71	4.47	0.000***
Dhup78	6.67	4.64	1.61	0.86	0.74	0.35	-0.10	-0.11	4.09	0.751ns
Dhup89	6.67	4.96	1.66	0.27	0.70	0.61	0.65	0.67	2.04	0.000***
Dhup90	5.83	3.65	1.47	0.58	0.76	0.54	0.18	0.14	2.23	0.021*
Dhup181	5.67	3.95	1.46	0.42	0.75	0.44	0.42	0.46	4.65	0.000***
Dhup183	5.67	2.56	1.16	0.40	0.53	0.34	0.24	0.24	1.56	0.000***
Dhup185	9.17	5.76	1.90	0.44	0.80	0.69	0.44	0.48	2.27	0.000***
Dhup191	5.33	3.41	1.38	0.24	0.69	0.32	0.65	0.68	4.69	0.000***
Dhup194	5.00	3.66	1.39	0.39	0.77	0.33	0.45	0.40	2.45	0.000***
Mean	5.83	3.78	1.42	0.44	0.68	0.45	0.39	0.39	2.89	

Na - number of observed alleles; Ne - number of effective alleles; I - Shannon index, Ho - observed heterozygosity; He - expected heterozygosity; F_{IS} - inbreeding index; F_{ST} - index of genetic differentiation; Nm - gene flow estimated from F_{ST} Nm = [(1/ F_{ST}) - 1]/4; PHWE - *P*-value for deviation from Hardy-Weinberg equilibrium: ns not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

F_{ST} values ranged from 0.013 to 0.108 (table 4). The highest level was between the Ixcán and Fray populations (0.108), whereas the lowest was between Poptún and Cahabón (0.013).

Population structure. An approach based on an admixture model was used to evaluate the population structure of 90 *D. stevensonii*. The optimal number of clusters (K) of the investigated populations was three and four according to the delta K and MedMedK values obtained from the STRUCTURE SELECTOR website (figure 2A and 2B). Based on K = 3, a graphical representation of the estimated membership coefficients of each individual is displayed in figure 2C and 2D. Each color shows the proportion of each individual's membership, represented by a vertical line, to the three or four clusters. Individuals with a probability

Table 2. Summary of the different population diversity statistics of *D. stevensonii* averaged over the 12 SSR loci.
Resumen de los diferentes estadísticos de diversidad poblacional de *D. stevensonii* promediados sobre los 12 loci SSR.

Population	m a.s.l.	N	Na	Ne	Ap	I	Ho	He	F	F_{IS}	P_{HWE}	PPL
Ixcán	133	15	6.58	4.01	2	1.54	0.39	0.42	0.44	0.48	**	88.50
Lachuá	185	15	7.25	5.08	3	1.70	0.41	0.46	0.50	0.39	**	89.75
Fray	170	15	5.50	3.14	1	1.22	0.40	0.39	0.34	0.37	**	80.55
Sayaxché	125	15	5.42	3.59	1	1.36	0.27	0.40	0.28	0.45	**	77.54
Poptún	543	15	5.75	3.76	0	1.41	0.24	0.29	0.40	0.35	**	77.54
Cahabón	259	15	4.50	3.10	0	1.30	0.22	0.25	0.39	0.24	**	65.45
Mean			5.83	3.78	-	1.42	0.32	0.37	0.39	0.38		79.88

m a.s.l. - meter above sea level; Na - number of different alleles; Ne - number of effective alleles; Ap - number of private alleles; I - Shannon index, Ho - observed heterozygosity; He - expected heterozygosity; F - fixation index; F_{IS} - inbreeding index; P_{HWE} - *P*-value for deviation from Hardy-Weinberg equilibrium: ns not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. PPL - percentage of polymorphic loci.

Table 3. Analysis of molecular variance (AMOVA) for six populations of *D. stevensonii.*
Análisis de la varianza molecular (AMOVA) para seis poblaciones de *D. stevensonii.*

Source of variation	df	Sum of Squares	Mean Squares	Variance component	% total variance	F_{ST}	F_{IS}
Among pops	5	74.139	14.828	0.290	6 %		
Within pops	84	514.233	6.122	1.789	39 %		
Among individuals	90	229.000	2.544	2.544	55 %		
Total	179	817.370		4.62	100 %	0.063	0.413

Table 4. Pairwise genetic differentiation (F_{ST}) among the six populations.
Índice de diferenciación genética por pares (F_{ST}) entre las seis poblaciones.

Population	Ixcán	Lachuá	Fray	Sayaxché	Poptún	Cahabón
Ixcán	0.000	0.001*	0.001*	0.001*	0.001*	0.001*
Lachuá	0.048	0.000	0.001*	0.001*	0.001*	0.001*
Fray	0.108	0.107	0.000*	0.005*	0.001*	0.001*
Sayaxché	0.051	0.061	0.037	0.000	0.001*	0.002*
Poptún	0.082	0.093	0.094	0.070	0.000	0.033*
Cahabón	0.031	0.056	0.052	0.035	0.013	0.000

Values below the diagonal indicate the magnitude of differentiation (F_{ST}); values above the diagonal indicate the probability value. * Significance at *P*-value < 0.05.

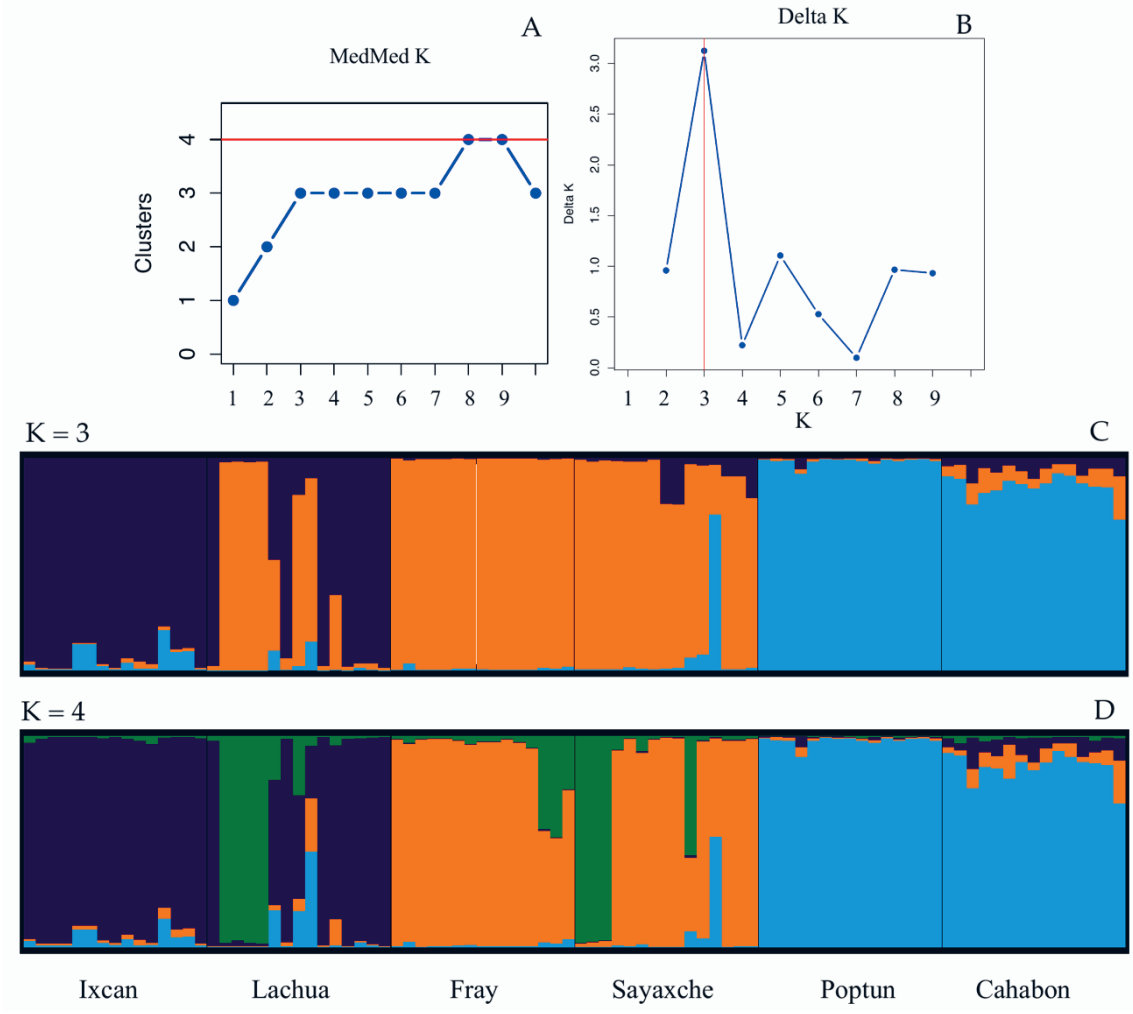

Figure 2. STRUCTURE analysis results for 90 individuals of *D. stevensonii* based on data from 12 microsatellites. Cluster number (K) estimation using (A) MedMed K and (B) Delta K methods. Estimated genetic structure of the six populations based on STRUCTURE analysis with cluster number (K) of three (C) and four (D). Each graph's color represents a different cluster, and the black segments separate the populations.

Resultados del análisis STRUCTURE para 90 individuos de *D. stevensonii* basados en datos de 12 microsatélites. Estimación del número de conglomerados (K) utilizando los métodos (A) MedMed K y (B) Delta K. Estructura genética estimada de las seis poblaciones basada en el análisis STRUCTURE con un número de conglomerados (K) de tres (C) y cuatro (D). En cada gráfico, cada color representa un conglomerado diferente y los segmentos negros separan las poblaciones.

greater than 0.75 were considered pure, while individuals with a probability less than 0.75, admixture. When k = 3, the purple cluster predominates in Ixcán. This grouping included 17 individuals with 14 pure and 3 mixed individuals, while the orange cluster included most individuals from Fray and Sayaxché. Likewise, Poptún and Cahabón were composed entirely of individuals from the blue cluster, whereas Lachuá presented a mixture of purple and orange clusters (figure 2C and 2D).

Discriminant analysis of principal components (DAPC) identified a similar structure using a priori sampling information, indicating the formation of 3 clusters (I: Ixcan-Lachuá; II: Fray-Sayaxché; III: Poptún-Cahabón) (figure 3A and 3B).

The DAPC analysis implemented the k-means clustering method (figure 4) and was consistent with the population assignment of STRUCTURE when no a priori sampling information was used. To describe the data, 35 principal components accounting for 77.6 % of the total variance and 2 discriminant functions were retained. The find.cluster function detected three clusters associated with the lowest Bayesian information criterion (BIC) value (figure 4A). The cross-validation analysis confirmed these results. The DAPC (figure 4B) showed three clusters, with the linear discriminant separating cluster 1 (on the left) from clusters 2 and 3 (on the right). Cluster 2 was the largest (40 individuals), followed by cluster 1 (27 individuals) and finally cluster 3, with 23 individual

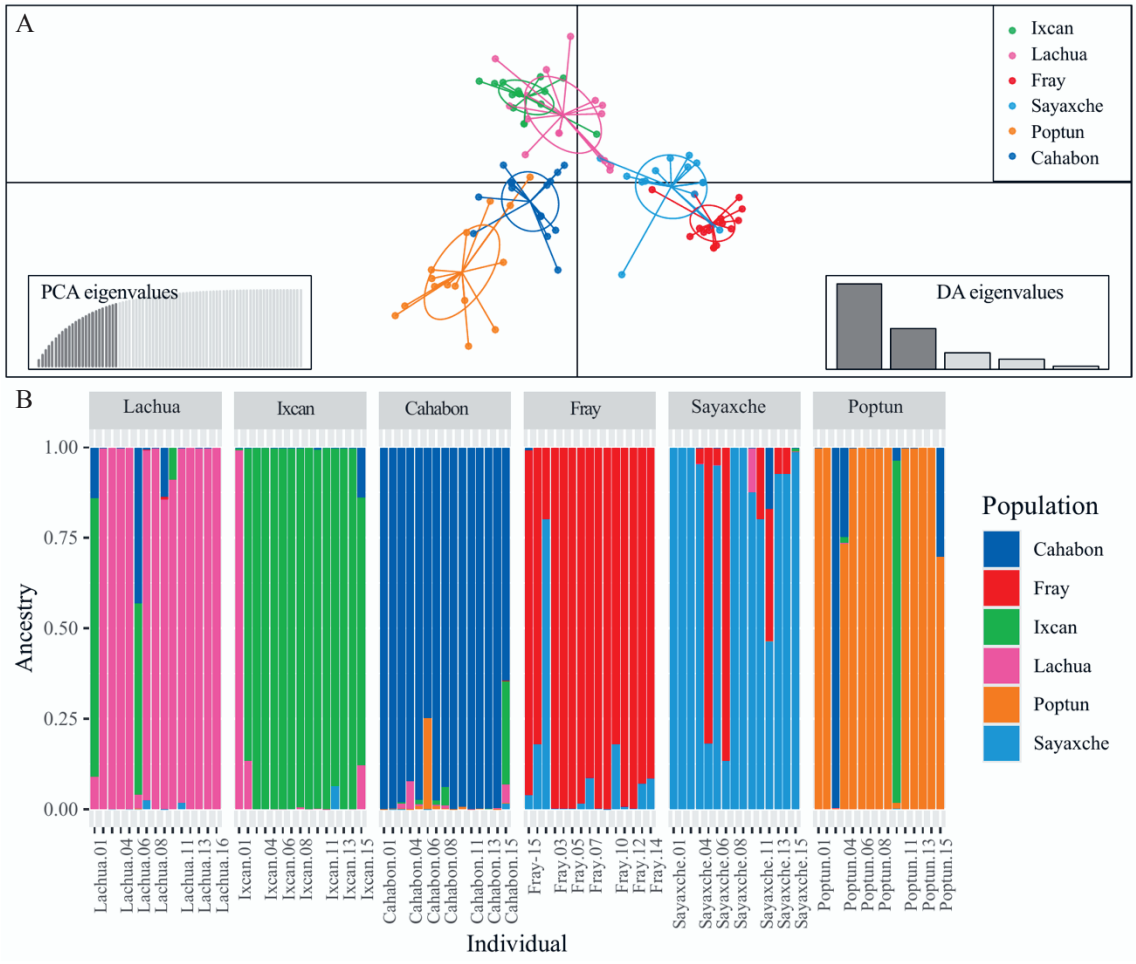

Figure 3. Population genetic structure, obtained from a DAPC analysis, of 90 individuals of *D. stevensonii* from six wild populations. (A) Scatterplot of the first two discriminant functions. (B) Bar graph of the DAPC analysis where each individual is represented by a vertical colored line. The same color in different individuals indicates that they belong to the same group.

Estructura genética poblacional, obtenida a partir de un análisis DAPC, de 90 individuos de *D. stevensonii* provenientes de seis poblaciones silvestres. (A) Diagrama de dispersión de las dos primeras funciones discriminantes. (B) Gráfico de barras del análisis DAPC en donde cada individuo está representado por una línea vertical de color. El mismo color en diferentes individuos indica que pertenecen al mismo grupo.

samples. When ancestry assignment was performed for K = 3, no clear separation of the populations was observed (figure 4C).

A pairwise F_{ST} matrix was used for principal coordinate analysis (PCoA). The first and second axes explain 42.42 % and 35.36 % of the variance in the molecular data, respectively (figure 5A). The PCoA clearly distinguished three groups: Ixcán and Lachuá populations were grouped as cluster I, Sayaxché and Fray were grouped as cluster II, and the other two populations (Poptún and Cahabón) were grouped as cluster III. In addition, the UPGMA dendrogram tree showed similar results, based on the unbiased genetic distance of Nei among the investigated populations (figure 5B).

Subsequently, Mantel tests were performed between pairwise F_{ST} matrices and geographic and genetic distances. The results showed that the genetic differences between the studied populations were attributed more to genetic distance (Rxy: 89.3 %, $P < 0.05$) than to geographic distance (Rxy: 15.1 %, $P < 0.05$). Thus, there was neither a clear structuring based on geographic origin nor predominant isolation by distance among the populations.

DISCUSSION

Identification of genetic diversity is crucial for the long-term succession of a species and plays an important role in breeding programs. However, our knowledge of the genetic diversity of *D. stevensonii* is limited. To date, there have been no published studies that have used genetic markers to estimate population genetic variability in this species.

The diversity of genetic material is often related to various factors such as geographic range, population size, longevity, mating system, morphological evaluation, mi-

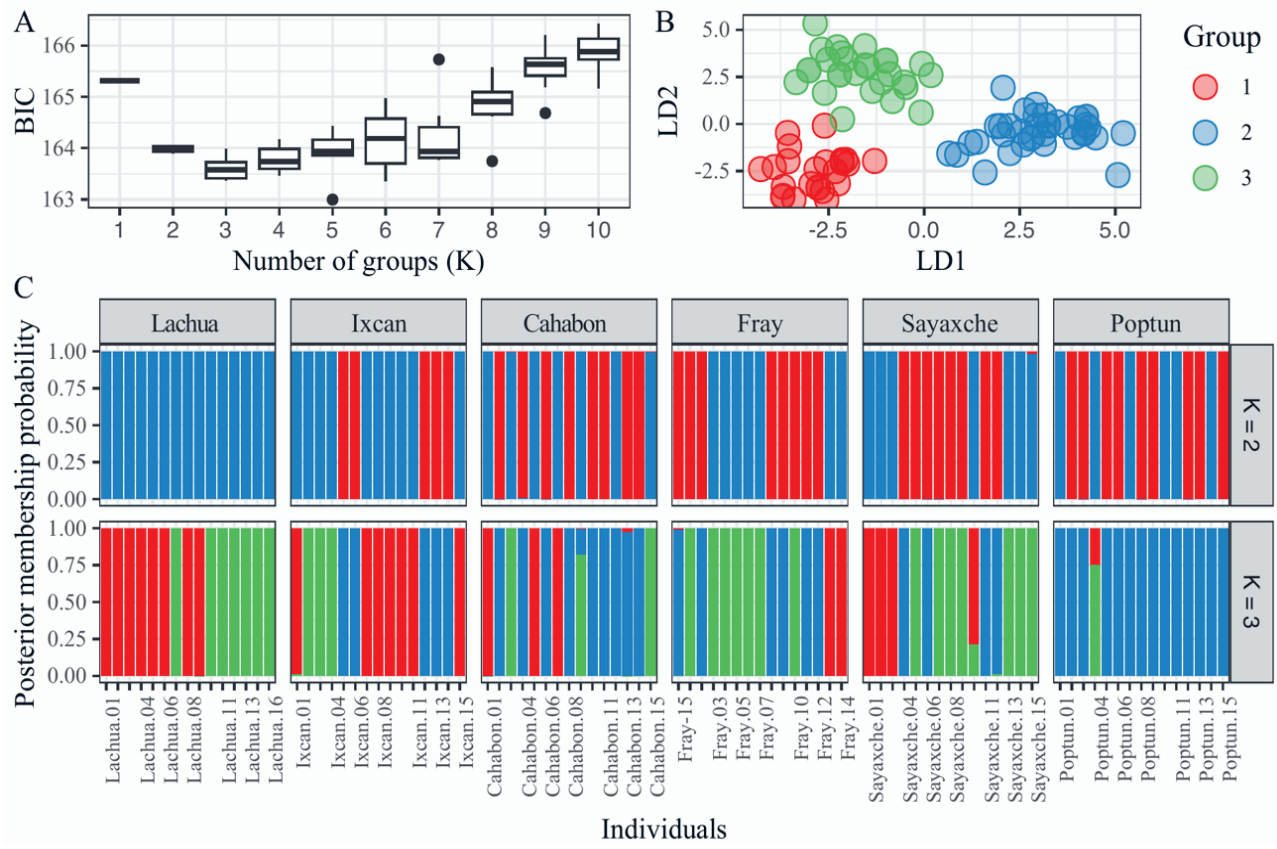

Figure 4. A) BIC to infer the most probable number of genetic groups (K = 3). B) DAPC scatterplot of the 90 individuals of *D. stevensonii* grouped into 3 genetic groups. C) Barplot representation of the DAPC results. The probabilities of assignment to each genetic group are presented with different colors representing the genetic groups. Assignment probabilities at K = 2 and K = 3 are shown.

A) BIC para inferir el número más probable de grupos genéticos (K = 3). B) Diagrama de dispersión DAPC de los 90 individuos de *D. stevensonii* agrupados en 3 grupos genéticos. C) Representación de diagrama de barras de los resultados de DAPC. Las probabilidades de asignación a cada grupo genético se presentan con diferentes colores que representan los grupos genético. Se muestran las probabilidades de asignación a K = 2 y K = 3.

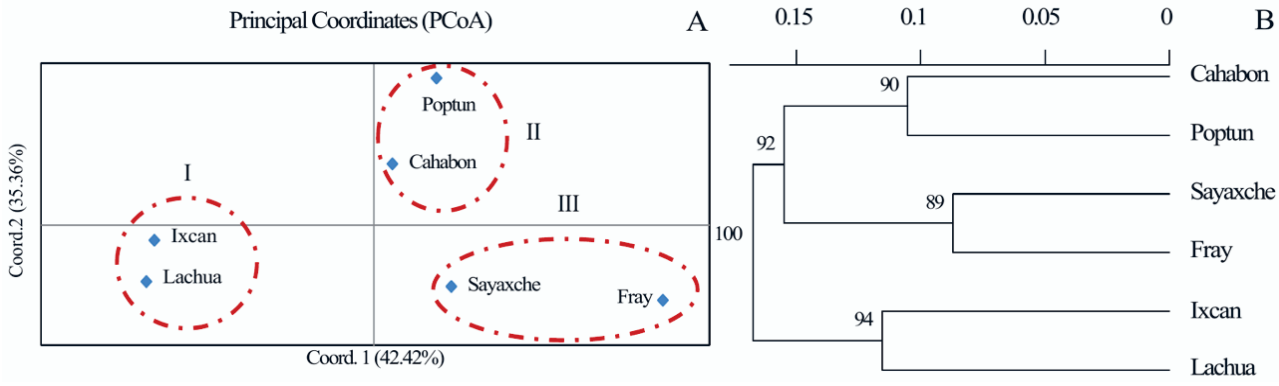

Figure 5. Relationships among the six wild populations of *D. stevensonii* in Guatemala. (A) Principal coordinate analysis (PCoA) based on F_{ST} pairwise comparison. The first principal coordinate explained 42.42 % of the variation. The second principal coordinate explained 35.36 % of the variation. (B) UPGMA tree based on Nei's genetic distance between six populations of *D. stevensonii*. Values at the nodes represent the statistical bootstrap support of 1,000 iterations.

Relaciones entre las seis poblaciones silvestres de *D. stevensonii* en Guatemala. (A) Análisis de coordenadas principales (PCoA) basado en la comparación por pares F_{ST}. La primera coordenada principal, explicó el 42,42 % de la variación. La segunda coordenada principal, explicó el 35,36 % de la variación. (B) Árbol UPGMA basado en la distancia genética de Nei entre sies poblaciones de *D. stevensonii*. Los valores en los nodos representan el soporte estadístico bootstrap de 1.000 iteraciones.

gration, and balance of selection in wild plant species (Ferrer *et al.* 2004, X. Li *et al.* 2018). A higher level of genetic diversity is often considered an indication of better adaptation to the environment (Gadissa *et al.* 2018). However, the genetic diversity of *D. stevensonii* is determined to be of medium level through observed and expected heterozygosity values of 0.32 and 0.37, respectively. Previous studies on forest species have consistently shown that species with limited or endemic distributions tend to possess significantly lower genetic diversity than those with more widespread distributions (Hamrick *et al.* 1992, Gichira *et al.* 2017, Zhong *et al.* 2019). This condition is due to smaller populations, geographic isolation, and reduced gene flow, resulting in limited gene exchange and an increased risk of inbreeding (Tóth *et al.* 2019).

This situation is particularly evident in *D. stevensonii*, whose natural distribution is restricted to small regions of Guatemala. The genetic diversity values were lower than those of tropical tree species with a wider distribution, such as *Swietenia macrophylla* King (5 SSR markers, Ho = 0.41, He = 0.78) (Alcalá *et al*. 2014), *Cedrela odorata* L. (7 SSR markers, Ho = 0.78, He = 0.87) (Paredes-Villanueva *et al*. 2019), and *Tectona grandis* L. (23 SSR markers, Ho = 0.90, He = 0.94) (Maisuria *et al.* 2022).

Interestingly, the genetic diversity of *D. stevensonii* is even lower than that of some rare and endemic tree species, such as *Boswellia papyrifera* (Caill.) Hochst (He = 0.69) (Addisalem *et al.* 2016), *D. cochinchinenesis* (He = 0.55), *D. oliveri* (He = 0.75) (Hartvig *et al.* 2018), *D. odorifera* T.C. Chen (He = 0.37) (Liu *et al*. 2019). Similar results were observed for *Ottelia acuminata* (Gagnep.) Dandy (He = 0.35, endemic to southwestern China) (Zhai *et al.* 2018) and *Dipterocarpus alatus* Roxb. *ex* G.Don (He = 0.22, endemic to southeastern Vietnam) (Vu *et al.* 2019). *D. stevensonii* has even lower genetic diversity than rare and endemic tree species, possibly because of human activities such as logging and agriculture, leading to a reduction in its population size. This reduction may have triggered genetic drift and random fluctuation in allele frequencies, which can cause a loss of genetic diversity in smaller populations. Inbreeding could also play a role in reducing genetic diversity, as it occurs when close relatives mate and inherit the same alleles from both parents (Charlesworth and Willis 2009). These combined factors contributed to the lower genetic diversity of *D. stevensonii.*

The specie *D. stevensonii* populations are currently highly fragmented, with each subpopulation consisting of only a few individuals, and large trees are rarely observed. This condition is attributed to unsustainable logging practices and illegal activities, which are mainly influenced by increasing demand in the Chinese market, resulting in deforestation and habitat loss of *D. stevensonii* in Guatemala. This situation is consistent with the idea that species with restricted distributions tend to have lower genetic diversity, mainly because of the overexploitation of their resources. Species with cross-mating systems tend to have low differentiation between populations and high variation within populations (Hartvig *et al.* 2018, Li X *et al.* 2018). AMOVA analysis of *D. stevensonii* showed that most of the genetic variation occurred within populations (94 %), while only 6 % occurred among populations, which is lower than in other *Dalbergia* species (0.236 in *D. cochinchinensis*, 0.126 in *D. oliveri*) (Hartvig *et al.* 2018). Genetic differentiation among populations is affected by gene flow and genetic drift (Schaal *et al.* 1998).

The 12 SSR markers in the present study indicated an overall gene flow of 2.89 (table 1). This moderate gene flow can reduce the effects of genetic drift, decrease genetic variation among populations, and increase diversity within populations. However, despite gene flow, fragmentation of the original populations is a determining factor in the rapid reduction in the genetic diversity of the species, which can have severe consequences in terms of vulnerability to pests and diseases and adaptability to climate change. This observation has also been reported in other species, such as *Acer miaotaiense* (Li X *et al.* 2018) and *Plectranthus edulis* Agnew (Gadissa *et al.* 2018). Genetic drift cannot be ruled out despite high gene flow, as population sizes are so small that any reduction may result in genetic drift.

Pairwise F_{ST} suggested that moderate genetic differentiation was found among the wild populations of *D. stevensonii*, based on a range of 0.013 to 0.107 (table 4). The highest level of differentiation (0.107) was observed between the Lachuá and Fray populations, which were 100 km apart, consistent with the idea that long-term isolation may limit gene flow between populations (Li N *et al.* 2018). Slightly higher values have been reported in *D. nigra* populations, suggesting high genetic differentiation and strong isolation (Barreto *et al*. 2023). In contrast, *D. latifolia* Roxb. and *D. sissoides* Wight & Arn. exhibit lower genetic differentiation values, indicating a higher level of gene flow between them (Vasudevan *et al*. 2023). However, the level of differentiation between Poptún and Cahabón was only 0.013, despite being separated by 90 km. STRUCTURE model analysis and DAPC, UPGMA, and PCoA analyses suggested that the 90 *D. stevensonii* trees could be divided into three clusters: cluster I consisted of Ixcán and Lachuá populations, cluster II comprised Poptún and Cahabón, and cluster III consisted of Sayaxché and Fray populations. Remarkably, individuals within cluster two were sampled from populations at higher altitudes, indicating that genetic differentiation may be attributed, at least in part, to adaptation to specific altitude conditions.

Genetic differentiation among populations was positively related to geographic distance (15.1 %) and genetic distance (89.3 %), as determined by Mantel tests, with genetic distance being more influential than geographic distance. No clear geographic-based structures or signs of isolation by distance were found, and the current population structure of *D. stevensonii* is believed to be more influenced by human activities.

To avoid the loss of the genetic diversity of *D. stevensonii* due to all the threats it faces, *ex situ* conservation strategies are considered the best solution. These strategies involve preserving and safeguarding the species outside their natural habitat. One approach is the establishment of seed banks or germplasm repositories, where seeds or plant tissues of *D. stevensonii* are collected and stored under controlled conditions (Frascella *et al*. 2022). This helps maintain the genetic diversity of the species and serves as a backup in the case of habitat destruction or population decline. Another *ex situ* strategy is the establishment of arboreta or living collections, where *D. stevensonii* are grown in dedicated gardens or botanical institutions (Kasso and Balakrishnan 2013). These collections provide opportunities for research, education, and public awareness of the species and its conservation. Additionally, efforts can be made to promote the cultivation and sustainable management of *D. stevensonii* in agroforestry systems, which not only supports conservation but also provides alternative income sources for local communities.

CONCLUSIONS

Native populations of *D. stevensonii* in Guatemala have moderate genetic diversity, a moderate degree of heterozygosity deficiency, low genetic differentiation, and a small population size. This situation is mainly due to extreme human activities, particularly excessive logging. The Guatemalan government took several measures to address this problem, including the promotion of *D. stevensonii* to the list of threatened species by the Consejo Nacional de Áreas Protegidas, prohibition of its exploitation, and establishment of national reserves for *in situ* conservation, such as the Laguna de Lachuá national reserve. Despite these efforts, the population size of *D. stevensonii* continues to decrease owing to illegal logging. We recommend implementing *ex situ* strategies to avoid loss of genetic diversity and increase genetic variability through crossbreeding.

AUTHOR CONTRIBUTIONS

JARC and BL participated in the conceptualization and funding acquisition; JARC conducted the data analysis. Field sampling was conducted by JARC, JEBS, CEVG, and MH. Preliminary writing of the manuscript was undertaken by JARC, AM, HDD, MK, and SB. All authors have contributed to the integration and revision of the final version of the manuscript.

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