



Genetic diversity and differentiation of *Ternstroemia huasteca* (Pentaphylacaceae) in Eastern Mexico: insights for its conservation

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Received: 8 August 2025 / Accepted: 17 November 2025 / Published online: 15 December 2025
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Abstract

Ternstroemia huasteca is an endangered tree species endemic to the southern Sierra Madre Oriental in Mexico, primarily confined to tropical montane cloud forests. Two population groups of this species are known: one in the Sierra Gorda region, spanning Querétaro and the northern part of Hidalgo, and another occurs in the Zacualtipán region of Hidalgo, extending into the Huayacocotla region of Veracruz. The conservation status of these populations and their degree of genetic diversity remain unknown. Given that habitat conservation forecasts are not optimistic and *T. huasteca* is already threatened, it is urgent to generate information about the conservation status, specifically regarding the genetic diversity of montane tree species. We genetically evaluated *T. huasteca* across its distribution range using a robust set of 16 microsatellite markers. Our results reveal relatively high genetic diversity (mean $H_O = 0.64$; mean $H_E = 0.64$) and moderate genetic differentiation ($F_{ST} = 0.142$). Genetic clustering revealed a clear separation between the Sierra Gorda and Zacualtipán populations, with substructure within the Zacualtipán group. We also detected a significant isolation-by-distance pattern, suggesting that gene flow decreases with increasing geographic distance. We found evidence of inbreeding and low genetic diversity in one population under silvicultural management. Our findings highlight the urgent need for conservation strategies that enhance connectivity and genetic diversity to ensure the long-term viability of montane forest species, given the ongoing anthropogenic pressures and climate change.

Keywords Conservation genetics · Microsatellites · SSRs · Tropical montane cloud forest

Introduction

Assessing genetic diversity and structure in threatened species is crucial for designing effective conservation strategies, especially in fragmented ecosystems. Tropical montane cloud forests in Mexico are highly fragmented and host many endemic tree species that are at risk, yet their genetic diversity is often unknown. *Ternstroemia huasteca* B.M.Barthol. (1988) is a tree species endemic to the Sierra Madre Oriental in Mexico, where it is distributed and limited to its southernmost region, the Carso Huasteco, within the tropical montane cloud forest (TMCF) and oak and pine-oak forests at altitudes ranging from 900 to 2200 m asl (Luna-Vega et al. 2004, 2010). Given that these forests are threatened by severe land-use change (Jiménez-García and Peterson 2019), *T. huasteca* has been listed as a vulnerable species in the IUCN Red List (Fuentes et al. 2020) and it is regarded as endangered according to The Red List of

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Mexican cloud forest trees (González-Espinosa et al. 2011), and to the evaluation of Luna-Vega et al. 2022.

Mexican tropical montane cloud forest (TMCF) is patchy and primarily associated with narrow altitude ranges and specific slope orientations (shady, and moist slopes). This distribution pattern arises from historical climate changes, such as the Pleistocene glacial cycles (Ramírez-Barahona et al. 2013), and the complex landscape heterogeneity of the Sierra Madre Oriental (Williams-Linera 2002). The narrow environmental requirements of the TMCF render it vulnerable to climate change, and it is projected to lose 68% of its area by 2080, under climate change scenarios (Ponce-Reyes et al. 2012).

Moreover, the rampant change in land use has not only fragmented but also reduced the distribution area of TMCF (Ponce-Reyes et al. 2013), including the habitat of *T. huasteca*. The current distribution of *T. huasteca* consists of small, isolated patches located in the Carso Huasteco region, where two groups of populations can be identified (Fig. 1): one in the Sierra Gorda (Querétaro and northern Hidalgo states), and the second in eastern Hidalgo and the Huayacocotla region of Veracruz (Zacualtipán group). Between them lies a distribution gap of about 50 km (in a straight line). These regions are deemed critical or medium priority for conservation (Toledo-Aceves et al. 2011).

Population genetics studies on tree or tree-like plant species from TMCF –such as *Alsophila firma* (Baker) (Ramírez-Barahona and Eguiarte 2014), *Chiranthodendron pentadactylon* (Hernández-Langford 2020), *Dicksonia*

navarrensis (Reyes-Ortiz et al. 2022), *Liquidambar styraciflua* (Ruiz-Sánchez et al. 2014), *Magnolia* sect. *Macrophylla* (Chávez-Cortázar et al. 2021), *Magnolia schiedeana* (Rico et al. 2023), *M. tamaulipana* (García-Montes et al. 2022), and *Podocarpus matudae* (Ornelas et al. 2010)—demonstrate the existence of isolation by distance across large distribution areas, low to moderate genetic diversity, low gene flow, moderate to high genetic/phylogeographic structure, and, in most cases, low effective population sizes and inbreeding.

Some of these examples are compatible with the Pleistocene refugia hypothesis, which posits that during glacial periods, some forests were continuous (with little demographic change and ongoing gene flow), but during interglacials, they became fragmented and isolated. In genetic terms, populations that once formed a single, continuous distribution would become differentiated from one another by accumulating mutations and decreasing gene flow (Ramírez-Barahona and Eguiarte, 2013, and references therein). Additionally, studies on *M. tamaulipana* (García-Montes et al. 2022), *Oreopanax xalapensis* (Vera-Maloof et al. 2019), and *Pinus chiapensis* (Del Castillo et al. 2011) suggest that long-term habitat stability and fragmentation dynamics have shaped present-day genetic structure in tropical montane cloud forest species.

Based on the species' distribution, fragmented habitat, and limited population sizes, we hypothesize that (1) *T. huasteca*'s fragmented populations might exhibit reduced genetic diversity, particularly in smaller populations, and (2)

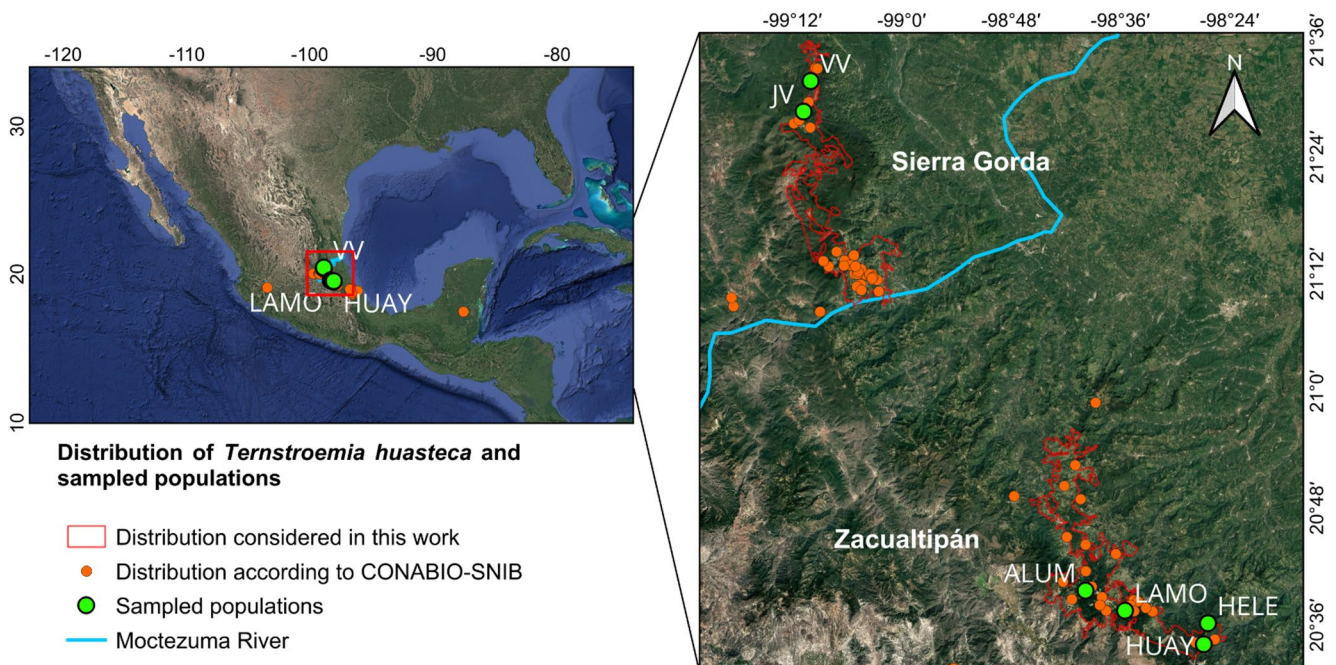


Fig. 1 Distribution area of *T. huasteca* according to CONABIO/Enciclovida accessions (orange bullets) and this work (red polygon). Sampled populations for this work are shown as green bullets

there would be significant genetic structure due to limited gene flow, specifically between the populations separated by known biogeographic barriers. As no genetic evaluation has been conducted on *T. huasteca* before this study, we aimed to: (1) evaluate genetic diversity and differentiation across its distribution range, (2) analyze the spatial genetic structure of its populations, particularly testing for Isolation by Distance (IBD) patterns, and (3) discuss the implications of our findings for the conservation of *T. huasteca*, considering its vulnerable status and fragmented habitat.

Materials and methods

Tissue collection and DNA isolation

We collected young leaves in silica gel from 108 individuals of *T. huasteca* across most of its distribution range, except for some missing locations from southern Sierra Gorda and northern Hidalgo due to public order conditions. The only available distribution for *T. huasteca* is that of CONABIO/Enciclovida. However, these records show significant incongruences: several accessions from tropical, low-elevation areas far outside the Sierra Madre Oriental, suggesting misidentification or a lack of taxonomic consensus. Therefore, in the absence of a detailed distribution study, we considered in this work only individuals within the tropical montane cloud forest of the Sierra Madre Oriental (Fig. 1) and matching the morphological descriptions of Bartholomew (1988). Individuals were collected at least 20 m apart from one another. We ground the dried leaf tissue in 2 mL microtubes using a Retsch Mill (MM400). DNA was isolated using the CTAB protocol (Doyle and Doyle 1987).

Genotyping

We selected a subset of 17 microsatellite markers developed by Alvarado-Sizzo et al. (2023) that had been successfully tested in *T. huasteca* and other *Ternstroemia* species. The microsatellite markers were combined into four multiplex PCR reactions, each containing four or five fluorescently labelled oligos. PCR reactions were prepared according to the instructions of the Platinum Direct PCR Universal Master Mix (Thermo-Fisher, USA) manufacturer's instructions, except for oligo concentrations, which were adjusted based on each marker product's fluorescence intensity (RFU). The annealing temperature was set at 60 °C, as all the primers were designed around this value. Fragment analysis was conducted at Psomagen Inc. (Maryland, USA), and genotyping was achieved using the Microsatellite plugin (v. 1.4.7) of Geneious Prime 2022 (Dotmatrix, NZ). Allele

scoring was performed manually, following the guidelines in the supplementary material of Selkoe and Toonen (2006).

Genetic analysis

Null alleles, Hardy-Weinberg equilibrium, and linkage disequilibrium

We assessed the presence and frequency of null alleles using the Brookfield (1996) method implemented in the R PopGenReport v.3.0.7 package. Deviations from Hardy-Weinberg equilibrium (HWE) were calculated for each locus and separately for each locality. Additionally, we calculated HWE across all samples using the 'hw.test' function in the package pegas v.1.165, with 1000 Monte Carlo permutations. The alpha levels for determining statistically significant deviations from Hardy-Weinberg proportions and independent genotype assortment were adjusted using the false discovery rate (FDR) approach developed by Benjamini and Hochberg (1995), applying an alpha level of 0.05. The Benjamini-Hochberg method (1995) corrected *p*-values for multiple comparisons. To assess linkage disequilibrium, we employed the index of association (*I_a*) and an adjusted statistic independent of locus number ($\bar{r}_d$) (Agapow and Burt 2001). These measures compare the observed variance in pairwise distances between individuals to the variance expected under linkage equilibrium. Both coefficients were calculated using the R package poppr v.2.9.3. (Kamvar et al. 2014, 2015). The significance of *I_a* and $\bar{r}_d$ was evaluated using a permutation test with 999 replicates, shuffling genotypes at each locus. The number of individuals (*N*) and the number of multilocus genotypes (MLGs) were calculated using poppr v.2.9.3 (Kamvar et al. 2014) as well as the number of alleles (*A*), and the expected (*H_E*) and observed (*H_O*) heterozygosities using hierfstat v.0.5–11 (Goudet 2005). We estimated rarefied allelic richness using the 'allel.rich' function of PopGenReport v.3.0.7 (Adamack and Gruber 2014). Population inbreeding coefficients (*F_{IS}*) were calculated for each population using hierfstat v.0.5–11. We applied the HWE tests described above to determine whether *F_{IS}* values differed significantly from zero in any population (after FDR correction). All the referred packages belong to the R Core Team environment (2024).

Genetic structure and isolation by distance

We conducted genetic clustering using STRUCTURE 2.3.4 (Pritchard et al. 2000) under the "No Admixture" model. We ran 1,000,000 MCMC iterations (100,000 as burn-in) for each value of *K* from *K*=2 to *K*=8, with ten replicates per *K*. The most likely number of clusters was determined using Evanno's method (Evanno et al. 2005) in the

program “Structure harvester” (Earl and VonHoldt 2012). Once we obtained the first K assignment and considering that STRUCTURE is known to be biased toward detecting the primary genetic partitions at $K=2$ (Janes et al. 2017), we conducted a second run using the same parameters to explore population substructure. The STRUCTURE clustering results were processed in CLUMPP (Jakobsson and Rosenberg 2007) to summarize the ten replicates, and the clustering graphs were generated in StructuRly (Crisuolo and Angelini 2020). Additionally, we performed a Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010) in the R package adegenet (Jombart et al. 2008).

To assess isolation by distance (IBD) among all the populations and between the populations of the Zacualtipán group, a Mantel test (Mantel 1967) was conducted. The Sierra Gorda localities could not be analyzed separately because the Mantel test requires at least three populations to compute multiple pairwise distances and assess statistical significance. Geographic distances, derived from coordinates and converted to Euclidean distances, were calculated using the ‘dist’ function from the stats package (v.4.3.1) in R. Genetic distances were represented by a linearized pairwise F_{ST} matrix ($F_{ST}/1-F_{ST}$). The Mantel test (Mantel 1967) was conducted using the ‘mantel rtest’ function from the ade4 package v.1.7–22 (Thioulouse et al. 2018), and scatterplots were subsequently created with the adegenet package v.2.2.10 (Jombart et al. 2008). We calculated the paired F_{ST} among populations considering localities as populations. We also conducted a hierarchical Molecular Analysis of Variance (AMOVA) in the R package poppr to quantify the distribution of genetic variation within and among populations and between the two regions (Sierra Gorda and Zacualtipán population groups). The significance of the variance components was tested using 999 permutations.

Results

Null alleles, Hardy-Weinberg equilibrium, and linkage disequilibrium

The dataset indicated that each locus contained at least 70% of the data. Nonetheless, four samples with over 40% missing data were excluded, leaving 104 samples for further analysis. The Tli182 locus displayed high null allele frequencies in five of the six populations and was subsequently

Table 1 Basic genetic diversity parameters

Pop	<i>n</i>	H_O	H_E	Ar	F_{IS}
ALUM	23	0.63	0.71	2.7	0.12
HELE	19	0.6	0.64	2.56	0.04
HUAY	6	0.56	0.36	1.73	−0.61
LAMO	14	0.66	0.68	2.63	0.02
JV	20	0.7	0.73	2.78	0.02
VV	22	0.69	0.7	2.68	−0.01
Mean	17	0.64	0.64	2.51	−0.04

N =No. of individuals, H_O =Observed heterozygosity, H_E =Expected heterozygosity, Ar=Allelic richness, F_{IS} =Inbreeding coefficient. ALUM: Alumbres, Hidalgo; HELE: Helechaes, Veracruz; HUAY: Huayacocotla, Veracruz; LAMO: La Mojonera, Hidalgo; JV: Joya Verde, Querétaro; VV: Valle Verde, Querétaro

discarded. Although some loci, such as Tli162 and Tli107, exhibited null allele frequencies exceeding 10%, these varied across populations, so all markers except Tli182 were retained for subsequent analyses. None of the loci demonstrated significant linkage disequilibrium after Bonferroni correction for multiple comparisons. Hardy–Weinberg disequilibrium was significant in four loci and two populations (Supplementary Table 1).

Genetic diversity and differentiation

All 16 markers were highly polymorphic, and we did not identify any clones. Across all populations, the mean H_O and H_E were 0.64. The Huayacocotla (HUAY) population exhibited the lowest observed heterozygosity ($H_O=0.56$) and expected heterozygosity ($H_E=0.36$), whereas the Joya Verde (JV) population displayed the highest diversity ($H_O=0.70$ and $H_E=0.73$). The inbreeding index ranged from −0.61 in HUAY to 0.12 in Alumbres (ALUM), with intermediate values of −0.01 in Valle Verde (VV) and 0.02 in both La Mojonera (LAMO) and JV (Table 1). The overall F_{ST} was 0.15, and AMOVA indicated that most variation (73.77%) occurred within populations, whereas 12.25% occurred among populations. The remaining 13.97% is attributed to differences between the Sierra Gorda and Zacualtipán groups (Table 2). The lowest pairwise differentiation among populations was observed between the two Sierra Gorda populations ($F_{ST}=0.026$). In contrast, the small population HUAY showed the highest differentiation values compared to the Sierra Gorda group ($F_{ST}=0.269–0.263$). Within the Zacualtipán group, HUAY was also highly differentiated ($F_{ST}=0.19–0.234$) from the other populations, whereas

Table 2 Analysis of molecular variance (AMOVA), including fixation indices

Source	df	Sum of Squares	Mean Square	Estimated variance	% Estimated variance	Fixation indices	<i>p</i> -value
Between Regions	1	87.642	87.642	1.205	13.979	$F_{CT}=0.140$	0.056
Between samples Within Regions	4	93.904	23.476	1.056	12.254	$F_{ST}=0.142$	0.001
Within samples	98	622.92	6.356	6.356	73.767	$F_{IS}=0.262$	0.001
Total	103	804.466	7.81	8.617	100		

differentiation among the other Zacualtipán populations was moderate ($F_{ST}=0.073$ to 0.115) (Fig. 2).

Genetic clustering

STRUCTURE analysis revealed two main genetic groups ($K=2$) according to Evanno's method (Fig. 3a, b). One genetic group comprised the two localities from the Sierra Gorda group (JV, VV), while the other encompassed the four locations of the Zacualtipán group, representing each a genetic group (ALUM, HELE, HUAY, LAMO). To investigate finer-scale structure, we performed a separate STRUCTURE analysis on just the Zacualtipán group populations. In this subset, STRUCTURE identified $K=4$

as the best-supported partition, indicating that, at our sampling scale, each locality functions as a distinct genetic group (Fig. 3c, d). The DAPC results were congruent with the STRUCTURE findings: it identified that the two Sierra Gorda localities mostly overlapped and four clusters for each Zacualtipán group location, with slight overlap among them (Fig. 4).

We analyzed the relationship between genetic differentiation ($F_{ST}/(1-F_{ST})$) and geographic distance (log-transformed) to assess patterns of isolation-by-distance (IBD) across all *T. huasteca* populations. The Mantel test yielded a moderate but significant relationship ($r=0.483$, $r^2 = 0.233$, $p=0.024$). These findings suggest that genetic distances increase with geographic distances, consistent with the

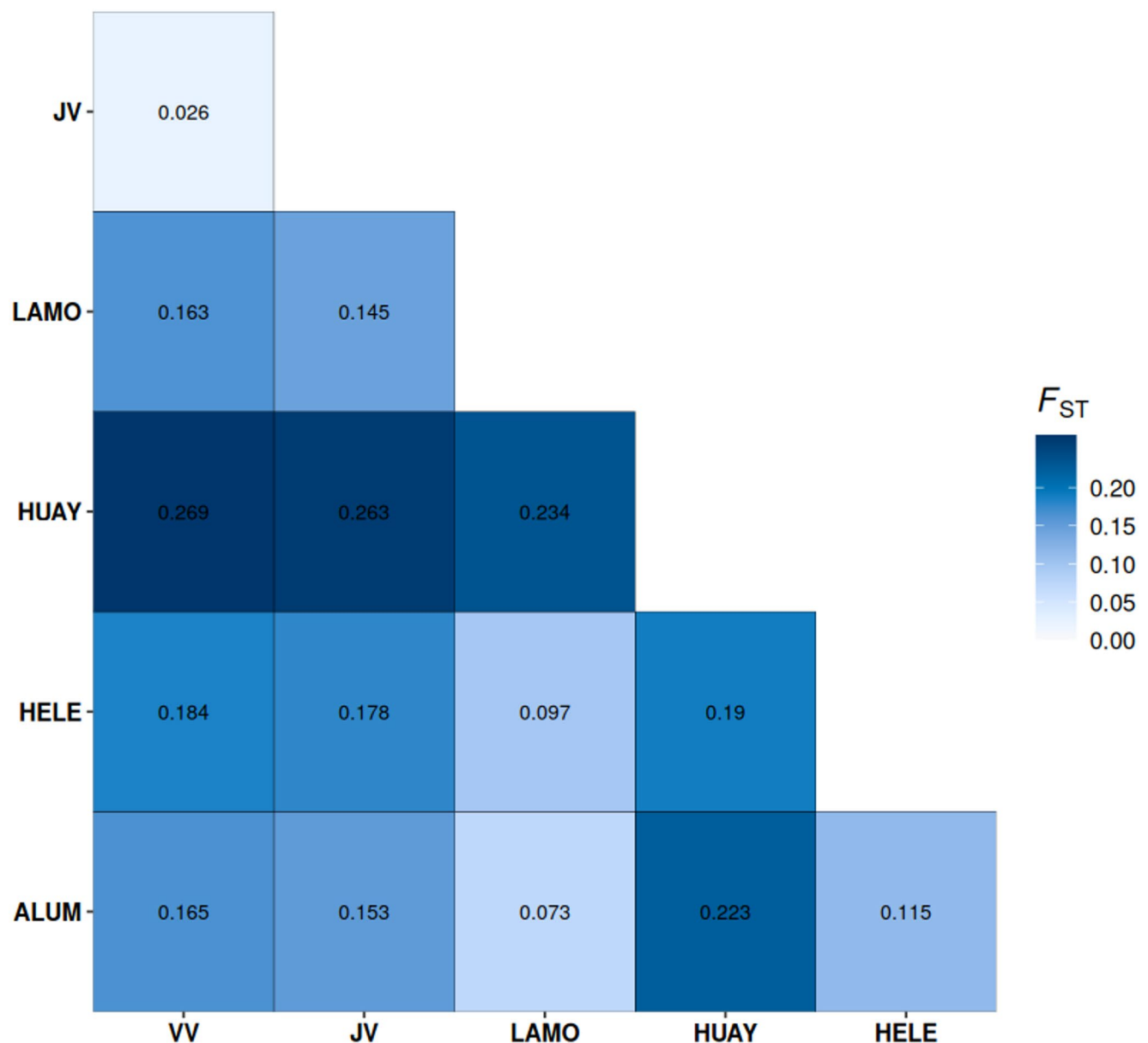


Fig. 2 Paired F_{ST} values among populations of *T. huasteca*

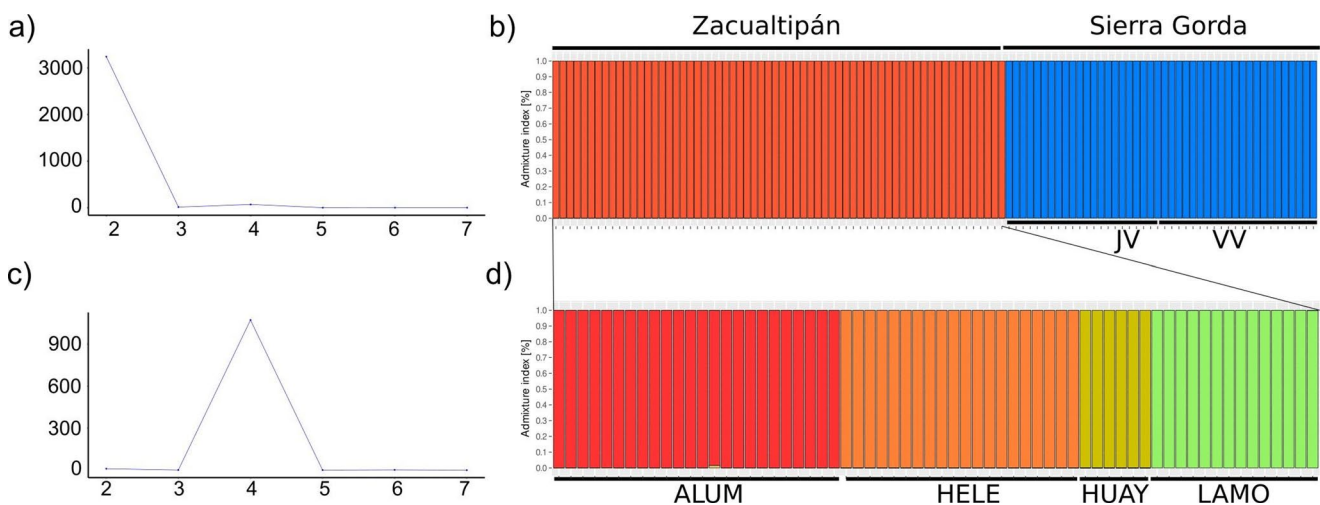


Fig. 3 Genetic Clustering generated in STRUCTURE showing (a) Evanno's ΔK including all samples, (b) $K=2$ individual assignment for all samples, (c) Evanno's ΔK of the Zacualtipán group, (d) $K=4$ individual assignment for the Zacualtipán group. ALUM: Alumbres,

Hidalgo; HELE: Helechales, Veracruz; HUAY: Huayacocotla, Veracruz; LAMO: La Mojonera, Hidalgo; JV: Joya Verde, Querétaro; VV: Valle Verde, Querétaro

expected IBD pattern where more geographically distant populations exhibit greater genetic differentiation (Fig. 5a). In contrast, within the Zacualtipán populations, the regression analysis showed a weak positive trend, with geographic distance having little explanatory power for genetic differentiation (Fig. 5b). Similarly, the Mantel test produced a low correlation coefficient ($r=0.084$) and a non-significant p -value ($p=0.39$), indicating no clear evidence of an isolation by distance pattern for these populations.

Discussion

Genetic diversity and differentiation

Despite *T. huasteca*'s restricted range and fragmented habitat, we found that it maintains relatively high genetic diversity (average $H_E = 0.64$). This level of genetic diversity is higher than that reported for other threatened Pentaphragmaceae, such as *Euryodendron excelsum* (average $H_E = 0.64$) and *Ternstroemia gymnanthera* ($H_E = 0.330\text{--}0.495$) (Cui and Su 2015), which were also evaluated using microsatellites. It is also comparable to the diversity of *Ternstroemia lineata* ($H_E = 0.676$), the only Mexican species that has been genetically analyzed to date (from a single, fragmented population in western Mexico; Alvarado-Sizzo et al. 2023). Research on tropical montane cloud forests suggests that complex topography and environmental heterogeneity may be associated with high genetic diversity (Ramírez-Barahona and Eguiarte 2013; Jaramillo-Correa et al. 2009). In the case of *T. huasteca*, the relatively high genetic diversity might indicate historically large population sizes or gene flow that preserved genetic variation, which is positive from

a conservation perspective (Willi et al. 2022). We noted that *T. huasteca* flowers are hermaphrodites; if the species is primarily outcrossing, this reproductive strategy would promote heterozygosity and genetic diversity (Hamrick and Loveless 1990). Investigating the breeding system and pollination biology of *T. huasteca* (e.g., presence of self-incompatibility) could clarify how its mating system contributes to the observed genetic diversity.

Only the HUAY population, comprising just six individuals, displayed low diversity values ($H_E = 0.36$) and evidence of inbreeding ($F_{IS} = -0.61$), indicating an excess of heterozygotes relative to Hardy-Weinberg expectations. This could be a founder-effect artifact or a result of sampling remaining, possibly unrelated individuals (e.g., Baloux 2004; Lin et al. 2024). Alternatively, genotyping fewer individuals can sometimes overestimate heterozygosity estimates if rare alleles are captured by chance. Regardless, HUAY's low genetic diversity and very small size indicate it is at high risk. Apart from HUAY, we did not detect any significant inbreeding in the other populations since F_{IS} were close to zero. This pattern suggests that population size is an important factor influencing genetic diversity in *T. huasteca*, as documented in other cloud forest trees such as *Alsophila firma* (Ramírez-Barahona and Eguiarte 2014), *Oreopanax xalapensis* (Vera-Maloof et al. 2019), and *Pinus chiapensis* (Del Castillo et al. 2011).

Genetic differentiation is moderate ($F_{ST} = 0.142$). The AMOVA confirmed that most of the variation (73.77%) occurs within populations, consistent with outcrossing as a breeding system (Hamrick and Loveless 1990). 12.25% of the variation is attributable to differences among populations within the two regions, and 13.98% is explained by differences between the Sierra Gorda and Zacualtipán

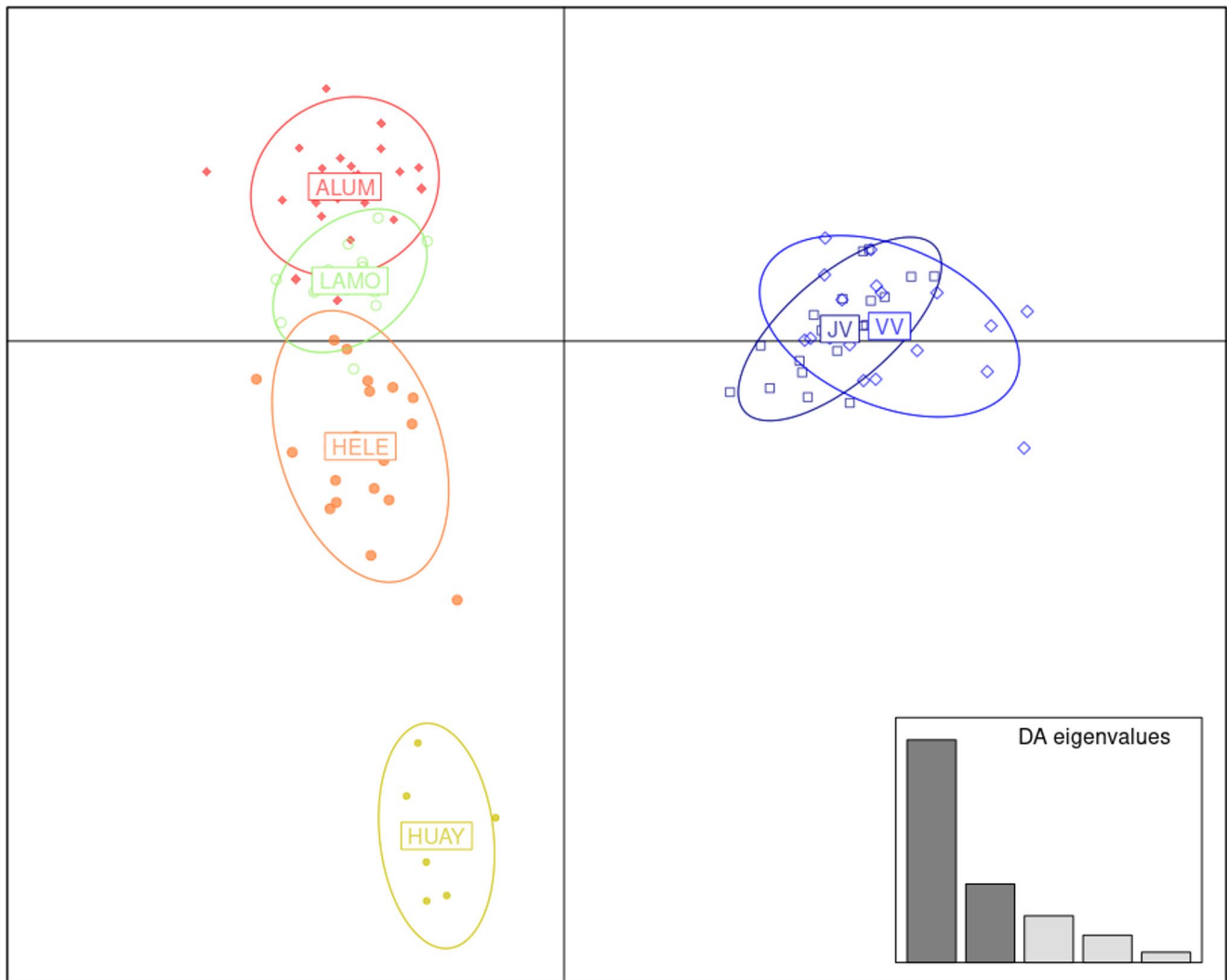


Fig. 4 Discriminant Analysis of Principal Components (DAPC). ALUM: Alumbres, Hidalgo; HELE: Helechales, Veracruz; HUAY: Huayacocotla, Veracruz; LAMO: La Mojonera, Hidalgo; JV: Joya

Verde, Querétaro; VV: Valle Verde, Querétaro. The colours for each cluster are the same as those used to represent the genetic groups in the STRUCTURE analysis

regions. This partitioning suggests that gene flow, historically or currently, has not been completely disrupted. Biotic dispersal agents likely play a role in maintaining connectivity (Hamrick and Loveless 1986). Although the specific pollinators of *T. huasteca* have not been documented. Entomophilous pollination is a viable option, as several South American *Ternstroemia* species are insect-pollinated (Bittrich et al. 1993). Likewise, once the fruits of *T. huasteca* ripen, they display red, shiny seeds—traits commonly associated with bird dispersal (Wenny et al. 2016). Bird-mediated seed dispersal has been reported in Mexican tropical mountain cloud forests (Pérez-Cadavid et al. 2018). These factors, insect pollination and bird dispersal, may together help explain the observed distribution of genetic variation.

The differentiation values indicate a clear genetic structure: the two Sierra Gorda populations form one distinct

genetic group separate from the Zacualtipán populations. Our Mantel test and regression analyses demonstrated a significant isolation-by-distance effect across all populations, indicating that genetic differentiation increases with geographic distance. The ≈ 50 km gap separating Sierra Gorda from Zacualtipán has likely led to limited gene flow between these regions over time, resulting in their moderate genetic divergence. However, this may be biased by the gap itself and the limitations of our sampling. At smaller spatial scales within the Zacualtipán region, we did not detect a significant IBD pattern, as reported in other cloud forest species such as *Magnolia* (Chávez-Cortázar et al. 2021; García-Montes et al. 2022; Rico et al. 2023). The high differentiation of the HUAY population (even relative to other Zacualtipán populations) underscores that when a population becomes extremely isolated or reduced in size, genetic

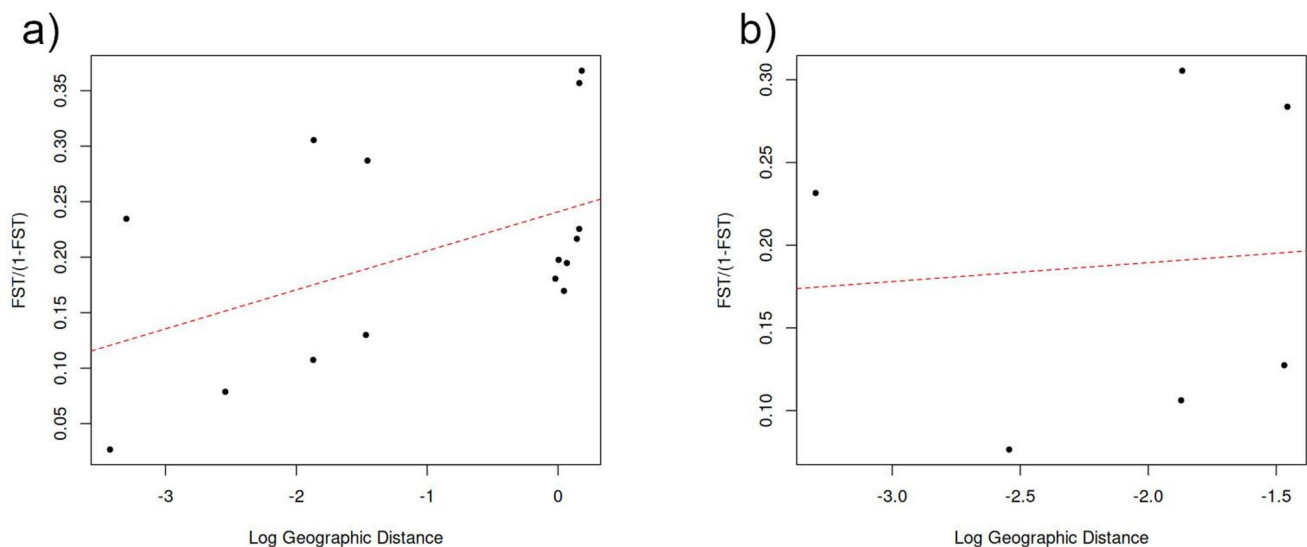


Fig. 5 Scatterplot showing the results of the Mantel test: (a) IBD across the entire data set; (b) IBD in the Zacualtipán population group

drift can quickly lead to divergence or loss of genetic diversity, as observed.

Genetic clustering

The concordant STRUCTURE and DAPC clustering patterns demonstrate that *T. huasteca* has a hierarchical genetic structure: a primary division between the Zacualtipán and Sierra Gorda groups, with a substructure among populations within the larger Zacualtipán region. Such a regional structure is not uncommon in cloud forest species. For example, in *Magnolia* sect. *Macrophylla* populations also show strong genetic isolation, to the point that highly isolated populations have been described as distinct species (Chávez-Cortázar et al. 2021). In our case, although the genetic divergence between Sierra Gorda and Zacualtipán populations is significant, we interpret it as the result of extended geographic isolation rather than evidence of incipient speciation. The pattern can be understood by examining both historical and current factors. It is plausible that past climatic fluctuations (e.g., Pleistocene glacial-interglacial cycles) contributed to the long-term isolation of *T. huasteca* populations in refugial areas, as has been suggested for other tropical montane cloud forest taxa (Ornelas et al. 2013; Ramírez-Barahona and Eguiarte 2013), but testing this for *T. huasteca* would require explicitly testing historical demographic hypotheses (e.g., coalescent methods and/or paleodistribution modeling). The observed genetic gap is consistent with the Moctezuma River basin, which lies between the Sierra Gorda and Zacualtipán regions and has been identified as a historical biogeographic barrier in the Sierra Madre Oriental (Luna-Vega et al. 1999). This barrier, together with the

considerable distance, likely limited gene flow, resulting in the distinct genetic clusters observed currently.

Taxonomic status and conservation implications

The high differentiation between the Zacualtipán and Sierra Gorda groups warrants further investigation into whether these represent distinct evolutionary lineages, but our data alone are not sufficient to support any taxonomic revision. We suggest future work, such as molecular phylogenetic analysis or a detailed morphological examination to determine whether the Sierra Gorda group deserves recognition as a separate taxonomic entity. From a conservation perspective, our findings highlight two relevant points: first, if *T. huasteca* is managed as a single species, the significant genetic divergence between the Zacualtipán and Sierra Gorda groups demands that each region's populations should be treated as separate units. The effective isolation between groups could make each group more vulnerable to inbreeding and long-term local genetic drift. Alternatively, if future research confirms that the Sierra Gorda and Zacualtipán groups are separate species, then each group's geographic range and population size would be even smaller than previously considered. In that scenario, both lineages would face an increased risk of extinction. Second, since we observed inbreeding and low genetic diversity in the population under silvicultural management (HUAY), our results indicate that even forested environments may not be enough to support *T. huasteca* populations in the long term, highlighting the importance of considering non-timber species alongside forestry management strategies. Finally, we consider that regardless of the taxonomic framework, our results will assist in re-evaluating the conservation status of *T. huasteca*.

by providing insights into the genetic structure and diversity of a representation of its distribution, including populations that are already showing evidence of genetic erosion.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10592-025-01742-x>.

Acknowledgements H. A-S thanks the Dirección General del Personal Académico (DGAPA), Universidad Nacional Autónoma de México (UNAM) for the Postdoctoral Grant 2022–2024. The authors express gratitude to Luis Roberto Martínez Beiza and Julio César Ramírez Martínez for their support in the field, to Roberto Pedraza from Sierra Gorda A.C. for access and logistics in various locations within Sierra Gorda, state of Querétaro, and to Gabriela Saucedo and Ejido La Mojonera for the access permit to the El Hayal reserve in the State of Hidalgo.

Author contributions All of the authors listed made substantial contributions to the manuscript and qualify for authorship, and no authors have been omitted. H. A-S. conducted the research, collected the samples, performed lab work and genetic analysis, analyzed the data, and drafted the manuscript. E. A-M. performed genetic analysis, analyzed the data, and reviewed the manuscript critically. O. A-A. collected the samples, reviewed the data, and critically reviewed the manuscript. G. R. reviewed the data and manuscript critically. I. L-V. reviewed the data and the manuscript critically and directed revisions.

Funding This research was funded by the grant PAPIIT IN219424 (DGAPA-UNAM). Author H. A-S. has received a postdoctoral grant from DGAPA-UNAM (2022–2024).

Data availability The genotype data used in all the analyses are available with this submission in the supplementary file (Supporting_Data sheet); in accordance with Springer Nature research data policy.

Declarations

Data archiving statement The genotype data used in all the analyses are available with this submission in the supplementary file.

Competing interests The authors declare no competing interests.

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