Structure, genetic diversity, and bottlenecks in *Taxus globosa* populations: A nearctic endangered species with fragmented distribution.

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Abstract

Taxus globosa is a conifer native to the Nearctic region, inhabiting isolated mountainous areas of Mexico's Sierra Madre Oriental. Its limited distribution and fragmented habitats raise concerns about its genetic diversity. To address this, we assessed genetic diversity and structure across six populations ranging from Nuevo León to Oaxaca using microsatellite markers and methods such as analysis of molecular variance (AMOVA) and Bayesian assignment analysis. The results indicated significant genetic structuring and reduced genetic diversity from north to south, with low observed heterozygosity (Ho = 0.18 ± 0.03) and high inbreeding levels (F_{is} = 0.75 ± 0.04). Four main genetic groups were identified, and the Hidalgo population showed significant differentiation. All populations demonstrated evidence of recent reductions, especially in Veracruz and Oaxaca, likely due to silvicultural activities. These findings highlight the need for conservation strategies to protect *T. globosa*'s genetic diversity and suggest that ex situ cultivation practices should be implemented for future reforestation efforts.

Keywords: Habitat fragmentation, genetic differentiation, microsatellite markers, latitudinal gradient, distribution relics, timber extraction.

Introduction

Colonizing plants experience a gradual and complex migration process shaped by various ecological and evolutionary factors (Pianka, 2011). As these species migrate, they face adaptive challenges that affect their ability to maintain genetic diversity. Such challenges include natural selection, the founder effect, and inbreeding. These factors can increase genetic differentiation while reducing genetic variability by eliminating less fit alleles. Consequently, the genetic structure of these populations is expected to be influenced by latitude, with greater divergence occurring at distances further from their original range (Martin & McKay, 2004).

Understanding plant colonization is complex in areas with environmental heterogeneity, such as eastern Mexico, which is known for its geological diversity and ecological variability. Many Nearctic species have become isolated here in "sky islands," high mountain areas with unique microhabitats that have preserved northern species since the last Ice Age (McLaughlin, 1994; Rico et al., 2021). These montane cloud forests exhibit lineage divergence, reduced genetic variability, and divergent traits due to genetic drift and natural selection (Love et al., 2023). Unfortunately, these ecosystems are now threatened by habitat loss and climate change pressures.

Mountain cloud forests are some of the world's highest biomass ecosystems. While global deforestation rates for these forests are 1.1 %, Mexico exceeds 3 %. Projections suggest that by 2030, about 3,608 hectares of these forests in central Mexico will be converted to pasture (Leija-Loredo et al., 2018). Moreover, climate change risks Nearctic species by challenging their genetic variability and long-term survival, leading to genetic loss and differentiation. The extent of these impacts varies based on species traits, such as their lifespan, whether they are woody, possess adaptations that facilitate their dispersal, and whether they can propagate by cloning (Alsos et al., 2012; Jiménez-García & Peterson, 2019) Additionally, habitat loss from human activities further jeopardizes the survival of Nearctic species in the Neotropics, as seen with some yew tree species (*Taxus* spp.).

Generally, mountain trees are found in fragmented and isolated habitats (Aguirre-Planter et al., 2000). This disruption is

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because mountainous geography creates natural barriers, in addition to those generated by fragmentation, that impede gene flow and impoverish genetic diversity (Cruz-Salazar et al., 2023). Such genetic impoverishment limits the trees' ability to adapt to climate change. It can increase the risk of gene loss (rare and common alleles) by up to 35 % in isolated populations compared to those with geographical connections (Juan, 2023). Generally, these trees are of Nearctic origin, and their ability to disperse is limited at lower latitudes, as seen with yew species around the world.

Yew trees (*Taxus* spp.) are valuable for producing paclitaxel (Taxol), a cancer-fighting compound first found in the bark of the Pacific yew (*Taxus brevifolia*) (Wani et al., 1971). Since industrial production of Taxol is difficult (Kikuchi & Yatagai, 2003), natural resources are heavily relied on (Denis et al., 1988; Schippmann, 2001; Takeya, 2003), which could lead to the overexploitation of yew species and population declines, putting them at risk of extinction (Gao et al., 2007; Schippmann, 2001). This situation can lead to genetic erosion and an increase in inbreeding (Zhang et al., 2009). To address these issues, it is important to evaluate the distribution of genetic resources, understand the spatial organization of genetic variation, and assess the impacts of fragmentation and isolation on gene flow.

Taxus globosa, or Mexican yew (tejo in Spanish), is a Nearctic tree protected by the Mexican government (SEMARNAT, 2010). Its range encompasses humid areas from central Nuevo León and Tamaulipas to southern Honduras, but is characterized by sparse populations inhabiting in humid microclimates on steep slopes. Research on this species has mainly focused on its distribution and habitat characteristics (Zavala-Chávez et al., 2001; Zavala-Chávez, 2002; Soto-Hernández et al., 2011; López-Upton & García-Martí, 2015; Muñoz-Gutiérrez et al., 2019). To date, no genetic studies have been carried out on *T. globosa*. It is only accounted for by *T. yunnanensis* (Miao et al., 2008), *T. wallichiana* (Yang et al., 2009), *T. baccata* (Gargiulo et al., 2019), and some related species (Lewandowski et al., 1995; Collins et al., 2003; Hilfiker et al., 2004a, b).

This study evaluated the genetic diversity and structure of *T. globosa* using microsatellite markers from six populations across a latitudinal gradient in Mexico. We aim to enhance understanding of the species' genetic distribution, predicting strong genetic structure and reduced diversity, especially in southern populations.

Material and Methods

Study area

The study locations were distributed along the Mexican Sierra Madre Oriental in Mexico, in the states of Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, Veracruz, and Oaxaca, where there were previous records of *T. globosa* collection (López-Upton & García-Martí, 2015; Muñoz-Gutiérrez et al., 2019, Figure 1, Table 1).

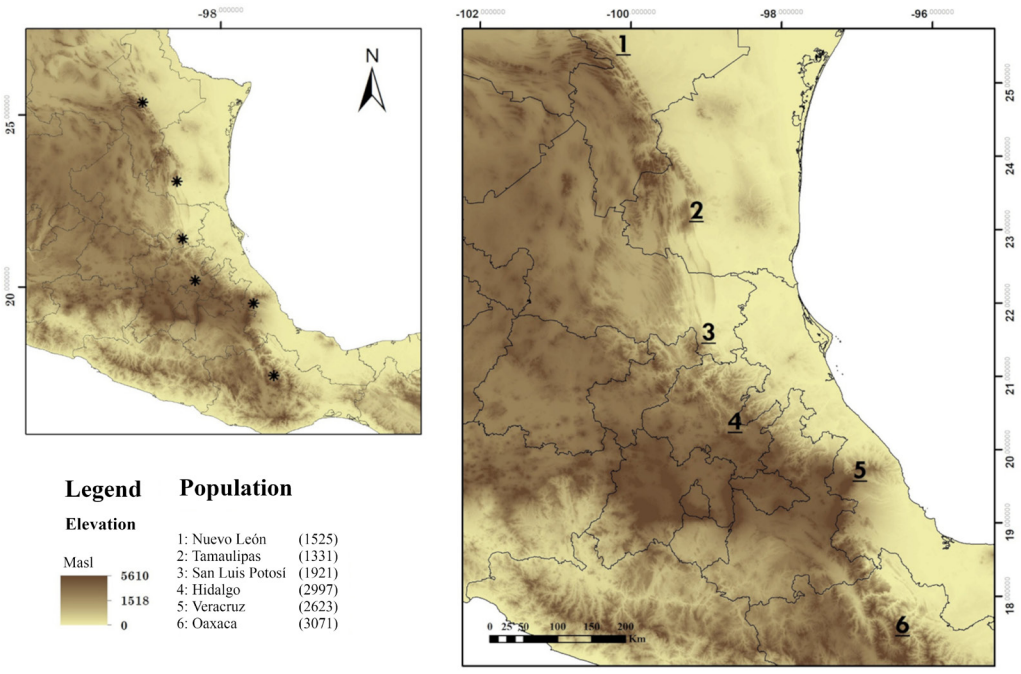

Figure 1
A distribution map of the six populations of *Taxus globosa* sampled in this study. These populations are located along the Mexican Sierra Madre Oriental.

Table 1
Sampled populations of the *Taxus globosa* in this work.
MCF: mountain cloud forest, AB: Abies forest, A-QF: Abies-Quercus forest, PF: Pinus forest, P-QF: Pinus-Quercus forest.

ID	State	Longitude	Latitude	Elevation	Vegetation type
1	Nuevo León	–100.1969	25.36475	1525	AB, A-QF
2	Tamaulipas	–99.229	23.05803	1331	MCF, PF, AB
3	San Luis Potosí	–99.06522	21.40017	1921	MCF
4	Hidalgo	–98.71331	20.18503	2997	AB
5	Veracruz	–97.06032	19.518086	2623	AB, PF, P-QF
6	Oaxaca	–96.493	17.41172	3071	AB, A-QF

Sampling

Leaf tissue was collected from 20 individuals in each population, for a total of 120 trees from all populations. Permit No. SGPA/DGVS/4240/18 was granted for the collection. The leaves were cleaned with 70 % alcohol to eliminate DNA from other organisms and were transported in airtight plastic bags with silica gel to reduce moisture. They were then stored in a freezer at –20 °C in the Genetics Laboratory of the Biological Research Center of the Autonomous University of the State of Hidalgo.

DNA extraction

For DNA extraction, the protocol of Doyle & Doyle (1987) was used with modifications to eliminate *Taxus*'s high concentration of secondary metabolites. 0.5 g of dry tissue was macerated with liquid nitrogen until a fine powder was obtained and 1 mL of extraction buffer was added (CTAB-PVP 2 % -Tris-HCl 100 mM pH 8, NaCl 1.4 M, EDTA 20 mM pH 8, and 1 µL of 2-β-mercaptoethanol) to continue mashing. The mixture was centrifuged at 800 rpm for 8 min. The precipitate was obtained and washed up to three times with the extraction buffer and then incubated at 37 °C for 1 h with 10 µL of RNAse (1 mg·mL–1). Next, 10 µL of proteinase K (10 mg·mL–1) was added and incubated again at 60°C for 1 h. Subsequently, 600 µL of chloroform:phenol:isoamyl alcohol (25:24:1) and 250 µL 2 % NaCl were added. and the mixture shaken at 300 rpm for 1 h at 25 °C.

Finally, the mixture was centrifuged at 10,000 rpm for 10 min, and the supernatant was recovered and precipitated with 2/3 parts of the final volume (300–500 µL) of cold isopropanol, allowing it to settle for 12–24 h, at –20 °C. After 12 h, it was centrifuged at 12,000 rpm for 10 min, the supernatant was removed without losing the precipitated product, and 1 ml of cold absolute ethanol was added, and the mixture was centrifuged at 14,000 rpm for 10 min. Then, the precipitated product was removed from ethanol and allowed to dry to be resuspended in 50 µL of sterile distilled water or TE buffer (10mM Tris-HCl, 1mM EDTA Na2).

The genetic material was quantified by spectrophotometry in a MAPADA nano spectrophotometer (MAPADA instruments, China). Each purified and quantified sample was stored in a freezer at –20°C to prevent degradation of the genetic material. DNA integrity was checked on 1 % agarose gels and stained with 10 % ethidium bromide; electrophoresis was performed at 90V for 90 min to compare the quantification readings.

Microsatellite amplification

Fifteen microsatellites reported by Dubreuil et al. (2008); Miao et al. (2008) and Yang et al. (2009) for *Taxus wallichiana*, *T. yunnanensis*, and *T. baccata* (Table 2) were evaluated; each primer was concentrated at five µM. The reaction mixture for PCR was 10–50 ng/µl of genomic DNA from the extracted samples (1.5 µl); adding 1.6 µl of MgCl2 (25 mM), 0.7 µl of forward primer, 0.7 µl of reverse (5 µM), 0.3 µl of dNTP's (10 mM), 2.4 µl of buffer (5×), 0.5 µl of dH2O and 0.3 µl (1U) of Promega® TaqDNA polymerase, for a total of 8 µl per reaction tube.

The PCR technique was carried out in a Thermo Scientific Artik endpoint thermal cycler, adjusting the annealing temperature and the number of cycles to 94 °C for 9 min, 35 cycles of: 55 s at 94 °C, 50 s at 58–63 °C (Table 2), 55 s at 72 °C, and the final extension at 72 °C for 7 min. The PCR products were visualized in 15 % acrylamide gels run at 90 V for 90 min and stained with ethidium bromide at 10 mg/ml concentration.

Data analysis

The gels obtained were photographed in order to later observe the amplified samples. The photographs were analyzed using the GelAnalyzer program (Lazar & Lazar, 2017), capturing the size of the base pairs and the intensity of each individual's bands. With this information, a database was built to carry out the statistical analyses.

The size of the amplified samples was standardized by Poisson frequency distribution with resampling to standardize the repeat size (García-Montes et al., 2020). Subsequently, the frequencies and heterozygosity were corrected by null allele analysis with the INEST 2.2 program (Chybicki & Burczyk, 2009). With these corrected data, the following genetic variability indicators were calculated: percentage of

Table 2.
Microsatellites used in the present study are based on those reported for Taxus wallichiana, Taxus yunanensis, and Taxus baccata. Reported by Dubreuil et al. (2008); Miao et al. (2008) and Yang et al. (2009). * In parentheses after the primer name, GenBank accession numbers are provided; F: Forward (sequence in 5´-3´ direction) and R: Reverse (complementary sequence); Ta: optimal alignment temperature reported in the literature; Bp: base pairs (range of sequences expected by literature reports and observed in the present study).

Primer	Sequence (5´-3´)	Ta (°C)	Bp (expected)	Bp (observed)
TG41 (FJ839824)	F: CTCTTAGTTGTGAAGGCAATG R: ATGAGTGGGCCATGAAAT	59°	107-135	93-159
TG118 (HM630366)	F: GAAGGGATAGCCGACATTATT R: AGCCTCGCAGTCCACAAAGTC	59°	107-135	119-250
TG144 (HM630365)	F: TATCCCACATTTAGCATTAG R: ATAGAGCCGACCCATTCA	63°	108-118	110-198
TB01 (AB029370)	F: TGGGAGAGCAGAGCAGTGATTTAT R: ACTGAGTGGTACGGTTGGTTGG	56°	138-144	390-544
TW01 (AY959321)	F: CTCCACCAATTCCCCACTTACCA R: TCCTTCCAAGCAATTTCGTCTCC	63°	405-420	399-470
TS07 (AB029370)	F: CTCCACCAATTCCCCACTTACCA R: TCCTTCCAAGCAATTTCGTCTCC	54°	239-245	161-337
TS09 (HM630370)	F: TGCTTTTGGGAAATGTTGTG R: CGAAAAAGGTACCATGGAAAT	57°	200-242	116-341
Tax23 (EF012580	F: TCAGCCTTATTCGAGTTTTC R: TGAAGTAGCTTTGCTTGTGA	58°	153-183	106-186
Tax26 (EF012581)	F: TCCTCAAATGTTAACCGAGT R: GGAAGTTCATTCTTTCATGC	58°	208-284	170-240
Tax31 (EF012582)	F: CAAATGAGGGGTAGGACTAT R: TGCACACATCTATCTACATTTTC	58°	207-260	140-298
Tax36 (EF012583)	F: CAGAGTCTTTTGGGCTTCTA R: CCTTGGAATCTTTTCCTTTT	63°	126-262	98-167
Tax60 (EF012584)	F: TACATGGCCTTTTGGATTTA R: GGGTTTACACCCATAAACAA	52°	110-178	85-186
Tax86 (EF012585	F: CCCTAGGGTTGGTGGAATTT R: TGTGGGAATCCATTTAAGCA	58°	152-304	145-295
Tax92 (EF012586)	F: GCCTATTTCGAAACCATAGA R: GTGATGGAACTGCCATATCT	58°	160-280	134-257
Tax362 (HM630370)	F: TGCTTTTGGGAAATGTTGTG R: CGAAAAAGGTACCATGGAAAT	61°	85-119	63-90

polymorphic loci (P %), average number of alleles per locus (A), adequate number of alleles per locus (N_e), Shannon index (I), observed and expected heterozygosity (*HO* and *HE*), inbreeding coefficient (F_{is}), and Hardy-Weinberg equilibrium (HW). These values were calculated with the GenAlEx 6.5 program (Peakall & Smouce, 2006).

The population's genetic structure was estimated using four methods: 1) AMOVA (Analysis of Molecular Variance) estimates genetic variation among populations and calculates F-statistics hierarchically (F_{st}, F_{is}, F_{it}). The null hypothesis ($F_{st} = 0$) suggests no genetic difference between populations, indicating they are part of a single gene pool with minimal sampling effects (Peakall & Smouse, 2006). 2) Neighborhood-joining trees use Nei genetic distances to assess population relationships based on allele frequency similarities. This method creates a clustering tree evaluated through a 1000-step bootstrap process in Past v. 4.05 (Hammer et al., 2021) and assesses mean distances between clusters. 3) Bayesian assignment analysis identifies exclusive genetic groups. We used a 100,000-step, 100,000 burns, with an admixture correlate alleles model, and 10 repetitions for each k, from 2 to 12 groups. This method, conducted with STRUCTURE v 2.3.3 (Pritchard et al., 2000; Falush et al., 2003; 2007), determines the number of genetic groups based on shared allele probabilities, maximizing individual differences. 4) Discriminant Analysis of Principal Components (DAPC) is a multivariate approach that identifies related genetic groups. It integrates PCA to find significant variance directions and then applies discriminant analysis (DA) to maximize group separation. DAPC operates independently of Hardy-Weinberg equilibrium and linkage assumptions (Jombart et al., 2010); we utilized Statistica v. 10 to perform this analysis (StatSoft, 2011).

A bottleneck analysis was performed to assess recent reductions in effective population size. This test examines the deviation from drift-mutation equilibrium, which is observed in a substantial increase in the number of heterozygous genotypes for each locus, generated by Bayesian iterations up to 10,000 steps. For this test, the two-phase mutation model (TPM) was used; it is intermediate between the infinite islands model (IIM) and the stepwise mutation model (SMM). The iteration result was compared with that expected in the equilibrium model by a Wilcoxon test using the Bottleneck v.1.2.02 program (Cornuet & Luikart, 1996; Piry et al., 1999). This analysis also detects the lack of heterozygous genotypes, a decrease attributable to a population increase based on inbreeding, which is considered an ancestral bottleneck, in addition to being able to analyze each locus separately. The comparison at each locus depends on the differences between the calculated heterozygosity and the expected heterozygosity in the two-phase mutation model under the assumption of drift-mutation equilibrium (HE−HEQ = DH), divided by the standard deviation (SD) of the heterozygosity calculated by the iteration at 10,000 steps.

RESULTS

Genetic diversity

The corrected data showed that five (Tax23, Tax31, Tax36, Tax60 and TB01) of the fifteen analyzed loci had null alleles, which when identified were eliminated to recalculate the genetic variability indicators. Overall, 19.33 ± 0.16 individuals per population amplified for all the probed markers. Across the species, the mean number of alleles per locus was 5.42 ± 0.26, the effective number of alleles per locus was 4.05 ± 0.2, the Shannon genetic diversity index was 1.4 ± 0.06, and the observed heterozygosity was 0.18 ± 0.03. The expected heterozygosity was 0.68 ± 0.02, with an overall inbreeding coefficient of 0.75 ± 0.04. These attributes are differentially expressed among populations (Table 3).

The highest inbreeding coefficient was found in populations in the south of the distribution; Oaxaca, Veracruz, and Hidalgo, and the lowest coefficient was found in Nuevo Leon. None of the populations are in Hardy-Weinberg equilibrium. The population with the most exclusive alleles was San Luis Potosi with ten, with average frequencies of 0.23 ± 0.05. The next highest populations in number of exclusive alleles were Oaxaca and Tamaulipas, with three each, with average frequencies of 0.33 ± 0.16 for Oaxaca and 0.31 ± 0.19 for Tamaulipas (Table 3). No exclusive alleles were found in Veracruz.

Genetic structure

The molecular variance analysis showed significant differences between populations (F_{st} = 0.21, $p < 0.001$), within populations (F_{is} = 0.74, $p < 0.001$), and between individuals (F_{it} = 0.8, $p < 0.001$). Hence, the number of migrant individuals per generation is low (Nm = 0.94). The pairwise analysis showed that the population from Hidalgo is the most divergent, followed by the population from Oaxaca, and the most similar were San Luis Potosí and Tamaulipas (Table 4). Even with these similarities, all populations are significantly different.

According to the Nei distances in the neighbor-joining clustering model, divergences between populations indicate the formation of two groups: Hidalgo as one group and the rest of the populations as the other. Within the second group, San Luis Potosí maintains the most significant distance from three subgroups, one to the north (Nuevo León and Tamaulipas) and the other two in the south (Veracruz and Oaxaca) (Figure 2 A).

The Bayesian assignment analysis without a priori origin (adMIXTURE maximum mixture model) finds no divergence between Nuevo León and Tamaulipas or between Veracruz and Oaxaca, generating only four genetic groups (Figure 2B). The most significant variation is within San Luis Potosí, followed by Hidalgo, which constitutes an intermediate pattern between neighbor-joining and AMOVA. This can be contrasted with an assignment model for K = 6, representing the assignment with a priori origin (Figure 2 C, D).

The discriminant function analysis of principal components (DAPC) indicates the highest divergence in Hidalgo.

However, there is a notable similarity between San Luis Potosí and Tamaulipas. On the X axis, which explains the most variance, Tamaulipas and Nuevo León, positioned at the extremes of the X axis, and San Luis Potosí and Oaxaca, closer to the midpoint of the axis, are similar. This pattern aligns with the expectations from Fst and the neighbor-joining clustering. However, it does not match the findings from the Bayes assignment analysis, which is more balanced between the X and Y axes of the DAPC (Figure 3).

Table 3
Genetic diversity indicators for the six *Taxus globosa* populations evaluated. N: number of amplified individuals, Na: average number of alleles per locus, Ne: effective number of alleles per locus, I: Shannon genetic diversity index, Ho: observed heterozygosity, He: expected heterozygosity, Fis: inbreeding coefficient, HW: Chi square for the Hardy-Weinberg equilibrium test, *: significant difference with an alpha less than 0.001.

ID	Population	N	Na	Ne	I	Ho	He	Fis	HW
1	**Nuevo León**	20	5.13 ± 0.59	4.06 ± 0.56	1.36 ± 0.14	0.27 ± 0.09	0.67 ± 0.06	0.61 ± 0.12	53.2*
2	**Tamaulipas**	20	5.4 ± 0.54	4.12 ± 0.46	1.45 ± 0.1	0.28 ± 0.08	0.72 ± 0.03	0.62 ± 0.11	56.8*
3	**San Luis Potosí**	20	7.07 ± 0.67	5.1 ± 0.47	1.7 ± 0.1	0.21 ± 0.05	0.78 ± 0.02	0.74 ± 0.06	86.6*
4	**Hidalgo**	20	3.93 ± 0.75	3.07 ± 0.55	0.97 ± 0.2	0.11 ± 0.05	0.48 ± 0.09	0.81 ± 0.08	59.1*
5	**Veracruz**	20	6.33 ± 0.43	4.29 ± 0.351	1.58 ± 0.06	0.11 ± 0.04	0.75 ± 0.01	0.85 ± 0.05	87.6*
6	**Oaxaca**	16	4.67 ± 0.47	3.63 ± 0.34	1.32 ± 0.1	0.09 ± 0.06	0.69 ± 0.03	0.88 ± 0.07	50.7*

Table 4
Paired molecular analysis of variance. The values of Fst (differentiation coefficient) are shown on the diagonal and below the number of migrant individuals per generation (Nm), in all cases $p < 0.001$

ID	Nm\Fst	Nuevo León	Tamaulipas	San Luis Potosí	Hidalgo	Veracruz	Oaxaca
1	Nuevo León		0.12	0.21	0.31	0.18	0.23
2	Tamaulipas	1.88		0.17	0.29	0.13	0.20
3	San Luis Potosí	0.94	1.19		0.28	0.12	0.15
4	Hidalgo	0.54	0.62	0.66		0.29	0.33
5	Veracruz	1.14	1.66	1.75	0.62		0.13
6	Oaxaca	0.85	1.00	1.40	0.51	1.66	

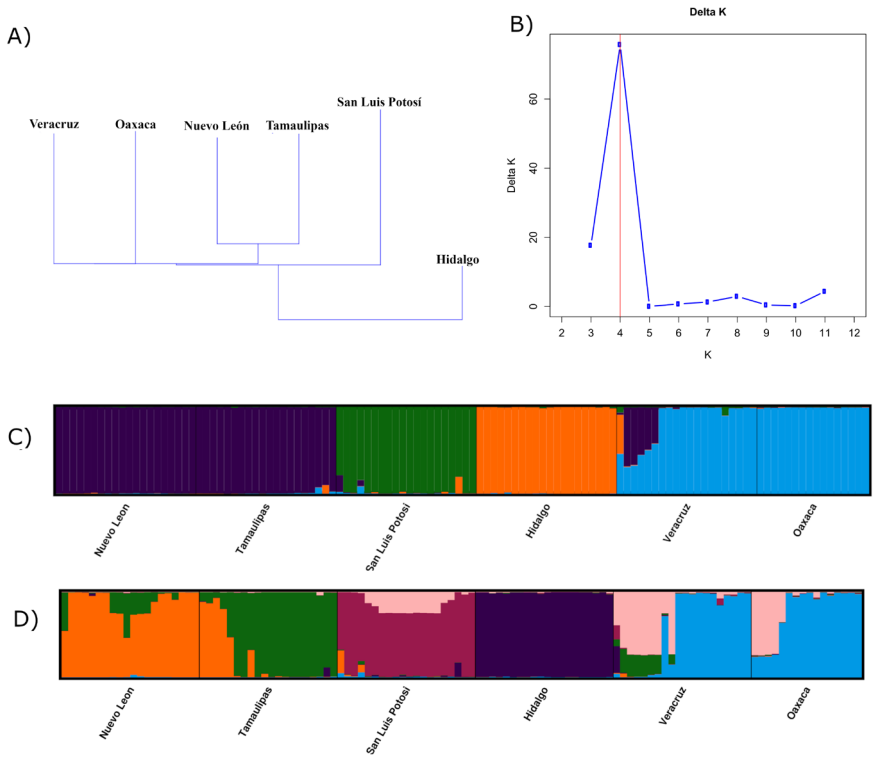

Figure 2
A) Clustering analysis by Nei genetic distances using the neighbor-joining algorithm. A 1000-step bootstrap supports the tree branches; B) Bayesian assignment analysis of individuals, Evanno et al. (2005) plot; C) Clustering diagram for K = 4; D) Clustering diagram for K = 6 corresponding to the original populations analyzed.

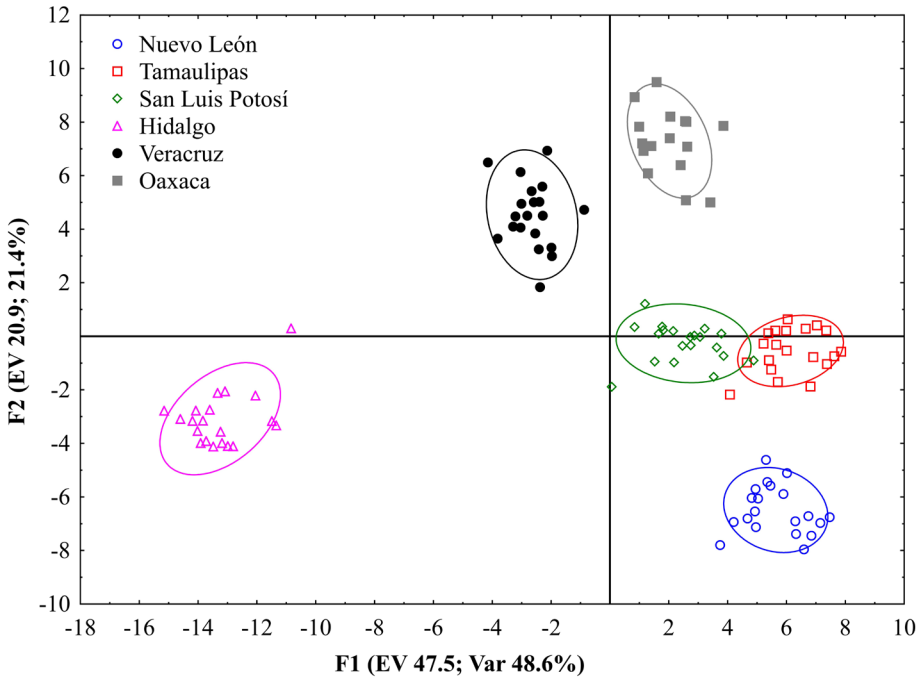

Figure 3.
Discriminant Analysis of Principal Component for genetic variation among the *Taxus globosa* populations analyzed.

The bottleneck analysis found that all populations have an excess heterozygosity under the TPM model of mutation-drift equilibrium. However, the magnitude of this population reduction was lower in Hidalgo, considering the number of loci with an excess of heterozygotes. On the other hand, Veracruz does not seem to have a significant bottleneck according to the Wilcoxon test. However, according to the difference test, there is a significant excess of heterozygosity, so it is assumed that this is the most recent reduction event of all (Table 5). In the rest, population reductions are similar.

Table 5. Bottleneck analysis for the *Taxus globosa* populations analyzed. The data were obtained using the TPM model.

ID	State	Loci with excess He	Wilcoxon p	Difference test
1	**Nuevo León**	8.71	0.0003	T = 3.377, p = 0.0004
2	**Tamaulipas**	8.89	0.00002	T = 3.82, p = 0.00007
3	**San Luis Potosí**	8.98	0.00005	T = 3.429, p = 0.0003
4	**Hidalgo**	6.81	0.002	T = 2.7, p = 0.003
5	**Veracruz**	8.86	0.054	T = 1.97, p = 0.02
6	**Oaxaca**	8.68	0.00002	T = 3.913, p = 0.00005

Discussion

Genetic diversity

Our study of *Taxus globosa* provides insights into the diversity and genetic structure of the species across a latitudinal gradient in Mexico. Our findings indicate that genetic diversity among the studied populations exhibits a significant geographic pattern, with southern populations showing lower variability. This trend aligns with observations in other species with fragmented distributions in mountainous regions , such as *Abies religiosa*, *A. hidalgensis*, *Pinnus hartwegii*, and *Quercus rugosa*, which also display reduced genetic diversity in southern or more isolated populations due to historical-ecological barriers and disturbances (Ledig et al., 2000; Valencia-Ávalos, 2004; Alsos et al., 2012; Sáenz-Romero et al., 2016; Love et al., 2023; Rosales-Islas & Octavio-Aguilar, 2023). In previous studies, random amplified polymorphic DNA (RAPD), intersimple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP) data (Mohapatra et al., 2008, 2009; Saikia et al., 2000; Zhang et al., 2009) have been used to investigate the genetic diversity of *T. wallichiana*, *T. yunnanensis*, and *T. baccata*. In all cases, the dominant nature of these genetic markers may lead to an underestimation of the recessive allele frequency in a population, causing bias in the estimation of genetic diversity and differentiation (Nybom, 2004).

Notably, our results reveal that the genetic structure does not conform to the serial pattern suggested by the stepping-stone model, which typically describes latitudinal genetic structure based on stepwise colonization with bottlenecks occurring at each colonization event (Nieto-Blázquez et al., 2021). Numerous hypotheses have been proposed to explain the latitudinal diversity gradient, but a consensus on the underlying causes remains elusive. While many of these hypotheses focus on genetic richness, microevolutionary processes (such as stepping-stone), and ecosystem history, the significant impact of human activity and climate change on biodiversity loss cannot be overlooked (Zhang et al., 2022).

We know that micro-evolutionary processes, such as demographic history, natural selection, and gene flow, influence genetic diversity within and between populations. Additionally, closely related species tend to exhibit similar levels of genetic diversity (Carvalho et al., 2019). In this context, the observed (Ho) and expected (He) heterozygosity of *T. globosa* populations suggest a general reduction in genetic diversity, with a high inbreeding coefficient (Fis = 0.75). This pattern has also been observed in other closely related species, such as *T. baccata* and *T. wallichiana*, where population isolation and habitat fragmentation have reduced genetic diversity and increased inbreeding (Hilfiker et al., 2004b; Zhang et al., 2009).

Populations from San Luis Potosí, Tamaulipas, and Nuevo León are characterized by greater genetic diversity. However, San Luis Potosí is notable for its number of exclusive alleles, suggesting that this population is more stable or experiences lower fragmentation pressure than the others. Similar findings have been reported in *T. yunnanensis*, where populations less affected by fragmentation retain significant genetic diversity (Miao et al., 2008).

Habitat fragmentation impacts plant allelic richness and genetic diversity (measured as expected heterozygosity) differently across various life forms. Long-lived trees, for instance, may show a decline in allelic richness while maintaining high genetic diversity (González et al., 2020), akin to the patterns observed in our *T. globosa* populations in Tamaulipas and Nuevo León. Additionally, the time since disturbance plays a role, emphasizing the need for targeted conservation efforts for populations at risk of losing genetic diversity, such as those in Veracruz and Oaxaca. Thus, based on our results, San Luis Potosí would be the population least affected by fragmentation.

Genetic structure

The AMOVA reveals significant differences between populations (Fst = 0.21, $p < 0.001$), indicating low migration between them (Nm = 0.94). The strong genetic differentiation found in the Hidalgo population is comparable to that reported in T. wallichiana, where geographical barriers have formed genetically distinct lineages (Gao et al., 2007). Despite the four analyses being based on different principles, Hidalgo consistently shows divergence across all four.

It is important to delimit the scope and limitations of each methodological approach to understand the apparent discrepancies between the genetic structure results. AMOVA divides the total variance within and between sites, similar to an analysis of variance. The Nei's distances, used in the neighbor-joining (NJ) tree analysis, group individuals based on the frequencies of all alleles present in the populations. This approach does not differentiate between adaptive and non-adaptive alleles, nor does it consider geographic origin. Instead, it relies solely on average distance to construct the NJ tree. One advantage of this method is that it maximizes the variation between populations. In contrast, Bayesian assignment through resampling evaluates the likelihood that two individuals belong to the same group. These groups are created by randomly removing individuals and markers, leading to a dynamic probabilistic assignment process each time a new group is formed. If an individual changes groups due to removing a marker or another individual, their relationship becomes less intense. Consequently, factors such as geographic origin and adaptation can significantly influence the calculation of this probability. Finally, DAPC focuses on the total multivariate orthogonal distances between individuals to form clusters in a linear approach (Jombart et al., 2010; Peña-Malavera et al., 2014; Miller et al., 2020). Given the geographic distances between the analyzed populations of *T. globosa*, an independent allocation approach seems the most appropriate for addressing the issue. However, hierarchical models (which use independent allocation) and non-hierarchical models (which utilize random allocation) are employed to minimize bias.

Bayesian assignment analysis (hierarchical model) confirms the existence of at least four main genetic groups. Notably, populations from Hidalgo and San Luis Potosí exhibit more significant genetic divergence. This finding is consistent with studies on *T. baccata*, which have shown that populations from different regions possess distinct genetic structures due to limited gene flow (Dubreuil et al., 2008). Furthermore, a reduction in genetic diversity is observed towards the south of the latitudinal gradient, aligning with patterns seen in other Nearctic species, such as *Pinus hartwegi*i, *Pseudotsuga menziesii* and *Abies religiosa* (Aguirre-Planter et al., 2000; Cruz-Nicolás et al., 2019; Juan, 2023). This trend suggests that habitat fragmentation and climate conditions have restricted genetic exchange, thereby favoring processes of genetic drift. An additional fact in this regard is that the populations in Hidalgo and San Luis Potosí are found in natural reserves, conserved to a certain extent, but isolated from other patches of similar vegetation accompanied by species such as *Abies religiosa*. Thus, even though these sites are not being fragmented nor do they have anthropogenic disturbances, they could be considered true sky islands. However, the population in Tamaulipas is also found in a protected reserve, but the existing non-regulated exploitation is directed against this species, which could generate a bottleneck in the medium term. In Veracruz and Oaxaca, the situation is entirely different, since the populations are found within areas of forest exploitation of different intensities. The Veracruz way of forestry uses a model that eliminates non-exploitable species, which result in monospecific stands of *Pinus* spp., which is consistent with our result showing an intense bottleneck in this zone. While Oaxaca focuses on selective logging of timber trees and preserves and protects species such as *T. globosa*, unfortunately young plants are often crushed during tree extraction, limiting recruitment and creating random deaths that increase the genetic effect of bottlenecks.

Bottleneck analysis indicates that most populations have excess heterozygotes, suggesting that they have experienced population reduction events. Similar patterns have been observed in *T. wallichiana*, which are attributed to logging and habitat fragmentation (Mohapatra et al., 2009). Besides the isolation that promotes inbreeding, fragmentation causes a random loss of genetic diversity, and can start a path aligned with the extinction vortex model. When populations decline to the brink of extinction, they enter this vortex, where the small size of the population increases genetic drift, causes loss of genetic diversity, and results in the fixation of maladaptive alleles. This hinders their ability to adapt and leaves them at low densities, making them particularly vulnerable to extinction due to demographic stochasticity (Nordstrom et al., 2023).

The results highlight the need for conservation strategies for *T. globosa*, focused on maintaining gene flow between populations and protecting key habitats. Microsatellite markers have enabled the identification of nuanced genetic differences, which could contribute to the design of reforestation programs and germplasm banks (Gargiulo et al., 2019).

Conclusion

This study shows that *T. globosa* populations exhibit a genetic structure across the distribution of the species in Mexico, with a decline in genetic diversity toward southern regions. Such diversity reduction patterns coincide with areas affected by anthropogenic disturbances such as forestry activities, where less genetic diversity and intense population reduction were found in more human-affected places. Comparisons with other species within the *Taxus* genus support the hypothesis that environmental alterations and isolation have significantly influenced the genetic diversity of this species. The findings of this research could provide informative guidance for developing effective conservation strategies to ensure the survival of this endangered conifer.

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