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INSTITUTO DE CIENCIAS BÁSICAS E INGENIERÍA

DOCTORADO EN CIENCIAS EN BIODIVERSIDAD Y CONSERVACIÓN

Caracterización morfológica, genética y demográfica de *Taxus globosa* Schlecht (Taxaceae) en poblaciones de la Sierra Madre Oriental, México.

T E S I S

QUE PARA OBTENER EL GRADO DE
DOCTOR EN CIENCIAS EN BIODIVERSIDAD Y CONSERVACIÓN

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ABREVIATURAS

°C	Grados Celsius
µl	Microlitros
µM	Micromolar
AMOVA	Análisis de varianza molecular
BMM	Bosque Mesófilo de Montaña
CIB	Centro de Investigaciones Biológicas.
cm	Centímetros
CONABIO	Comisión Nacional para el Conocimiento y Uso de la Biodiversidad
DA	Análisis discriminante
DAP	Diámetro a la altura del pecho
DAPC	Análisis discriminante de componentes principales
DE	Desviación estándar
DF	Grados de Libertad
DH	Diferencia de heterocigosis
dH_2O	Agua Destilada Estéril
DNA	Ácido Desoxirribonucleico
dNTP	Dinucleótidos Trifosfato
EV	Eigenvalues; raíz propia, valores propios
F	Índice de Fijación
F_{IS}	Coeficiente de Endogamia Dentro de las Poblaciones
F_{IT}	Coeficiente de Endogamia Entre Poblaciones
F_{ST}	Coeficiente de Diferenciación Poblacional
ha	Hectáreas
H_E	Heterocigosis Esperada
H_{EQ}	Heterocigosis en el Equilibrio
H_O	Heterocigosis Observada
$\hat{H}_T$	Heterocigosis total
HW	Equilibrio de Hardy-Weimberg
I	Índice de Shannon

I.I.M.	Modelo de islas infinitas
IBD	Aislamiento por Distancia
INEGI	Instituto Nacional de Estadística, Geografía e Informática
IPCC	Panel Intergubernamental sobre Cambio Climático
ISSR	Secuencias Repetidas Intergénicas Simples
km	Kilómetros
km^2	Kilómetros cuadrados
LnP(D)	Logaritmo Natural de la Probabilidad de Divergencia
M	Molar
mg	Miligramo
MgCl$_2$	Cloruro de Magnesio
min	Minutos
ml	Mililitro
mm	Milímetros
mM	Milimolar
MS	Cuadrados Medios
m s. n. m.	Metros Sobre el Nivel del Mar
N	Norte
ng	Nanogramos
N-J	Neighbour-Joining
Nm	Número de migrantes por generación
nm	Nanómetros
NOM	Norma Oficial Mexicana
O	Oeste
pb	Pares de bases
PCA	Análisis de componentes principales
PCR	Reacción en Cadena de la Polimerasa
QTL	Locus de Caracteres Cuantitativos
S	Segundos
SEMARNAT	Secretaría del Medio Ambiente y Recursos Naturales
SJ	San José

SMM	Modelo de mutación paso a paso
SMO	Sierra Madre Oriental
SNP	Polimorfismo de nucleótidos simples
SS	Suma de cuadrados
SSR	Secuencias Simples Repetidas
Ta	Temperatura de Alineamiento
TPM	Modelo de Mutación de Dos Fases
U	Unidades Enzimáticas
UICN	Unión Internacional para la Conservación de la Naturaleza
UNESCO	Organización de las Naciones Unidas para la Educación, la Ciencia y la Cultura
V	Volts
VE	Varianza Explicada

CAPÍTULO I

Introducción general

1   INTRODUCCIÓN

El género *Taxus* (Taxaceae) se distribuye casi en su totalidad por el hemisferio norte. La existencia de cuatro especies de *Taxus* es ampliamente reconocida en el continente americano (Cope, 1998). En el área más septentrional de América del Norte, *T. canadensis* habita en la costa atlántica de Estados Unidos y Canadá, mientras que *T. brevifolia* se encuentra en la costa del Pacífico. *T. floridana*, una especie que muestra una distribución muy restringida en lugares específicos dentro del estado de Florida (Redmond, 1984). Finalmente, la especie considerada como objeto de este trabajo, *T. globosa* Schltdl. es una de las especies menos estudiadas desde el punto de vista biológico, genético y demográfico que habita en la zona más austral (Zamudio, 1992), donde se localiza de manera escasa bajo el dosel de bosques mixtos de coníferas, generalmente en cañadas o laderas de exposición húmeda ubicados en México y al norte de Centroamérica (Estrada *et al*. 2006).

Esta especie, mejor conocida como tejo mexicano, tiene una distribución discontinua en México a lo largo de la Sierra Madre Oriental y en las montañas de Oaxaca y Chiapas. El conocimiento sobre la distribución real del Tejo Mexicano es más difuso y el tamaño de sus poblaciones es mucho menor en la región biogeográfica Neotropical. Hay numerosos registros con tan solo 30-40 individuos por población en Sierra Juárez en Oaxaca. Se han encontrado ejemplares de herbario de *T. globosa* en bosques nubosos pertenecientes a Los Altos de Chiapas (Contreras-Medina *et al*. 2011).

Este tejo también vive en los bosques húmedos de Guatemala (Standley & Steyermark, 1958) ubicados en la Reserva de la Biosfera Las Minas, la Sierra de los Cuchumatanes y la Cadena Volcánica. En El Salvador, núcleos muy pequeños se ubican residualmente en áreas remotas cerca de la cima del Cerro del Pital a 2670 msnm (Silba, 1983). A pocos kilómetros hacia el oeste, en Honduras, la especie habita en el Parque Nacional Santa Bárbara, además de varias localidades dentro del Parque Nacional Celaque, lugar considerado como el límite más austral de su distribución global (Contreras-Medina *et al*. 2011).

Taxus globosa es un árbol que no supera los 15 m de altura y 60 cm de diámetro de tronco; por lo que es una especie más pequeña que *T. baccata*, y más emparentada con el resto de especies americanas del litoral oriental, como la arbustiva *T. canadensis*. Sus acículas son más brillantes, alargadas y acuminadas en el ápice, con un color verde oscuro

más visible a lo largo de las áreas del nervio medio y los márgenes inferiores. El arilo y la semilla son el sello distintivo de *T. globosa*, estos son más redondeados y comprimidos; por eso, en 1838, Diederichvon Schlechtendal la describió como *Taxus baccata* var. *globosa* (Shemluck *et al*. 2003). La especie crece preferentemente en suelos ácidos, bien drenados, ricos en materia inorgánica. El rango altitudinal de distribución fluctúa de 1.100 a 2.960 m. La mayor altitud se encuentra en los bosques nubosos de Oaxaca superando los 2600 msnm que es la máxima altitud registrada (Charco, 2003).

Taxus globosa crece en fondos protegidos y sombreados de cañones que muestran agua estancada permanente o estacional; la presencia de la especie depende de que el dosel superior filtre una gran cantidad de la radiación solar directa. La especie necesita una precipitación anual superior a los 800 mm pero que puede superar los 2,500 mm. Por ejemplo, en bosques del Estado de Hidalgo, el tejo habita en un clima templado y subhúmedo bajo un dosel de *Abies religiosa* (Kunth) Schltdl. y Cham. Está cubierta protectora reduce significativamente las tasas de evapotranspiración dentro del bosque. En esta zona, las precipitaciones oscilan entre 1,000 y 1,200 mm excluyendo la precipitación horizontal que debería aumentar considerablemente la cantidad total. La temperatura media anual en esos bosques bien conservados es de 14°C (Zamudio, 1992).

Los bosques de tejo mexicano se caracterizan por su distribución dentro de un gradiente latitudinal amplio y discontinuo. Están aislados por áreas más bajas y cálidas, incluidas las áreas semidesérticas. Por lo general, estas formaciones registran una alta precipitación orográfica, un clima templado y una alta complejidad florística que varía incluso entre sitios cercanos (Rzedowski, 1978). Por ejemplo, Luna-Vega *et al*. (1994) describe estos bosques como comunidades vegetales típicas que existen en zonas templadas, donde hay un elemento florístico subordinado de naturaleza subtropical.

Las formaciones estructurales que coexisten con el tejo en México son bosques mixtos de pino-encino: *Pinus pseudostrobus*, *P. ayacahuite*, *P. patula*, *Arbutus xalapensis*, *Quercus crassifolia*, *Q. germana*, *Q. rysophylla*, *Q. laurina* y *Tilia mexicana* entre muchos otros y bosques de abetos (*Abies religiosa* y *A. vejarii*). El tejo también se presenta en bosques nublados junto a especies pertenecientes a este tipo de formaciones como *Ostrya virginiana*, *Liquidambar styraciflua*, *Cornus disciflora* por nombrar algunas especies de amplia distribución (Luna-Vega *et al*. 1994; Shemluck *et al*. 2003; García-Aranda *et al*.

2011). En los bosques montanos de Mesoamérica (ubicados en el sur de México, Guatemala, Honduras y El Salvador), la especie se asocia típicamente con Pinus ayacahuite y Abies guatemalensis (Rosito, 1999).

Debido a lo escaso de su densidad y el aislamiento de sus poblaciones, es de suponer que existe una marcada variación morfológica determinada por el ambiente, además, es poco probable que exista un flujo genético a lo largo de la distribución, por lo que la diferenciación morfológica y genética se esperaría que fuese elevada. Estas son las preguntas que se evalúan en este trabajo. Esta información permitirá establecer un marco evolutivo para la especie y estimar el panorama actual de conservación para el Tejo Mexicano.

2 ANTECEDENTES
2.1 Familia *Taxaceae*
Las coníferas (Pinophyta) son especies de árboles o arbustos dioicos o monóicos, con corteza rugosa o lisa en placas grandes y gruesas, con fisuras o en tiras largas y delgadas. En el mundo, existen entre 546 y 670 especies de esta división de plantas (Eckenwalder, 2009; Farjon, 2010), distribuidas en 6 familias y 71 géneros. En México se distribuyen 94 especies de coníferas, clasificadas en cuatro familias (*Cupressaceae, Pinaceae, Podocarpaceae y Taxaceae*) y 10 géneros (Gernandt y Pérez-de la Rosa, 2014), lo que representa sólo 0.4% del total de especies de plantas vasculares (23,314) en el país (Villaseñor & Ortiz, 2014; Villaseñor, 2016).

En México la mayor riqueza de coníferas se concentra en el noroeste, principalmente en los estados de Durango, Jalisco y Chihuahua, con 32, 31 y 29 especies, respectivamente. En el noreste, en la Sierra Madre Oriental, los estados con mayor número de especies son Coahuila con 28, y Nuevo León e Hidalgo con 24 cada uno. En los estados de Hidalgo, Oaxaca, Puebla y Veracruz, se concentra una alta riqueza de géneros de coníferas, en cada uno de ellos se distribuye una o más especies de *Abies*, *Cupressus*, *Juniperus*, *Pinus*, *Pseudotsuga*, *Podocarpus*, *Taxodium* y *Taxus* (Gernandt & Pérez de la Rosa, 2014).

2.2 El género *Taxus*
Taxus pertenece a la familia Taxaceae (gimnospermas) y comprende entre siete y doce especies que se distribuyen en el hemisferio norte, en América, Asia y Europa (Zavala-Chávez et al. 2001). En América se presentan cuatro especies: *T. brevifolia, T. canadienses, T. floridiana* y *T. globosa,* las cuales se encuentran distribuidas de manera restringida (Soto-

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Hernández *et al*. 2011). En México, se conoce una especie representativa y endémica, *Taxus globosa*, conocida coloquialmente como tejo mexicano, granadillo, romerillo palmira o tlascal y al igual que las distintas especies de *Taxus* es utilizada comúnmente como leña, para producir carbón o como ornamento (Zamudio, 1992; Zavala-Chávez, 2001).

2.3   Generalidades de *Taxus globosa*

Es una de las cuatro especies de *Taxus* existentes en América (Cope, 1998), es escasa a pesar de tener poblaciones biológicamente viables; además de que crece en hábitats restringidos, a los cuales está peculiarmente adaptada y ello genera que también sea una especie ecológicamente poco conocida (Zavala-Chávez, 2001). Fue descrita en 1838 por el botánico alemán Diederich Franz Leonhard Von Schlechtendal, el material que colecto provenía de la localidad del Guajolote, Real del Monte, Hidalgo, México (Soto *et al*. 2000; Shemluck *et al.* 2003). En su descripción Schlechtendal considera a *T. globosa* como una especie muy similar a la especie europea *T. baccata*; sin embargo, *T. globosa* se distingue por sus hojas más largas y delgadas, agudas, claramente falcadas y por la semilla ovoide más gruesa (Zamudio, 1992; Shemluck *et al.* 2003). Hay una gran variedad de características que se utilizan para distinguir a *T. globosa* del resto de las especies pertenecientes al género como, la longitud y en ancho de la hoja, color de la hoja, color de las ramas, presencia de una quilla dorsal en la hoja, forma del ápice de la hoja, disposición de los brotes, forma de los brotes en escala y forma de brotes enteros (Shemluck *et al*. 2003).

Las poblaciones de *T. globosa* se desarrollan bajo condiciones ambientales muy particulares, en altitudes entre 1000 y 2950 m, en sitios donde la temperatura promedio fluctúa entre 14.5 y 15.4 °C y la humedad relativa es alta (entre 15 y 30%), ya sea por la precipitación pluvial o por la presencia de neblinas frecuentes (García y Castillo, 2000; Muñoz-Gutiérrez *et al*. 2009; Zavala-Chávez, 2001; Zavala-Chávez *et al*. 2001; López-Herrera, 2008). Los árboles del tejo mexicano forman parte del estrato arbóreo en bosques maduros (García-Aranda *et al*. 2011); las poblaciones generalmente tienen una distribución discontinua, con pocos individuos y se desarrollan en lugares sombríos y húmedos: hondonadas de montañas, cañadas, barrancas, laderas con pendientes de entre 40° y 60°; en laderas con exposición norte-noreste (Contreras-Medina *et al*. 2011; Zavala-Chávez *et al*. 2001).

Son arboles perennifolios, dioicos de entre seis y 12 m de altura; tronco erecto, de 30 a 40 cm de diámetro, con múltiples ramificaciones que forman una copa redondeada y la corteza es escamosa, de color café claro (Zamudio, 1992). Las hojas se distribuyen en espiral arregladas en forma dística, son aplanadas y ligeramente falcadas; presentan ápice agudo; base angosta; pecíolo corto; márgenes ligeramente involutos; una sola vena media engrosada, son de color verde oscuro en el haz, más claras en el envés, y con hileras de estomas en el envés (Figura 1A; Villareal & Estrada, 2012). En el caso de los árboles femeninos presentan brotes portadores de un óvulo terminal en un eje axilar con escamas dispuestas helicoidalmente (Figura 1B), el cual, al madurar, se cubre con una estructura carnosa de color rojizo denominada arilo (Figura 1C). Se sabe que el arilo es comestible pero la semilla es venenosa; es atractivo y comestible para aves, que transportan el fruto, se comen el arilo y desechan la semilla, participando en su dispersión. Además de las aves, se desconoce qué otras especies de fauna silvestre participan en la dispersión de semillas y/o son depredadores de estructuras reproductoras, o si la producción de éstas es suficiente para mantener e incrementar la población (Zavala-Chávez *et al.* 2001; Muñoz-Gutiérrez *et al.* 2019). Los árboles masculinos presentan pequeños estróbilos formados por cinco o hasta nueve sacos polínicos (Figura 1B).

En general, se sabe que el ciclo reproductivo de *T. globosa* es anual, inicia de octubre a noviembre y culmina de septiembre a noviembre del siguiente año. Sin embargo, el mes de floración varía dependiendo del sitio, de diciembre a marzo o de febrero a mayo; la polinización es entre febrero y marzo; y la fructificación entre mayo y agosto en algunas localidades del norte de México, y de julio a noviembre en localidades del centro del país. Los datos de campo indican que la regeneración de las poblaciones puede ser tanto a partir de semillas como de rebrotes de la base del tallo. No obstante, se desconocen los detalles de la diferenciación de las estructuras reproductoras y de la fertilización de la ovocélula, además de la fenología completa de la especie (Zavala-Chávez *et al.* 2001; Ramírez-Sánchez *et al.* 2011).

Las semillas carecen de alas, son ovoides, de 5-7 mm de largo por 4 mm de ancho, de color café, con el ápice apiculado, cubiertas por un arilo carnoso de color rojizo. La producción de semillas es limitada, se producen entre 6 y 15 semillas maduras por árbol, y su germinación está limitada por la presencia de un embrión subdesarrollado, esto indica una

latencia de tipo morfológico, lo que dificulta aún más la germinación (Muñoz-Gutiérrez *et al*. 2009; Soto-Hernández *et al*. 2011; Zavala-Chávez, 2002).

Figura 1. Estructuras características de *Taxus globosa*. A: Forma de las hojas, B: Brotes axilares femeninos, C: Arilo. (Fotos originales: LL-C).

En Hidalgo, se usa la madera de *T. globosa* como leña, postes para cerca y en la construcción de casas; además, durante la semana santa se fabrican ramos con las hojas y ramas. En otros estados del país se emplea como curtiente y para elaborar carbón (García-Aranda *et al*. 2011; Zamudio, 1992).

2.4   Distribución geográfica

Los individuos de *Taxus* se distribuyen a lo largo de las zonas templadas del hemisferio norte: Asia y América del Norte, en altitudes que van de los 1500 a 3000 m (Hartzell, 1991; Figura 2). En Asia *Taxus wallichiana* se localiza en Afganistán, Tíbet, China, India, Filipinas y Birmania; *T. cuspidata* en Japón, Corea y Manchuria y *T. chinensis* al este de China (Zavala-Chávez, 2001). En Europa predomina *Taxus baccata* desde las islas británicas, en la península Escandinava, hasta la cuenca del río Amur en Rusia (Hartzell, 1991; Figura 2).
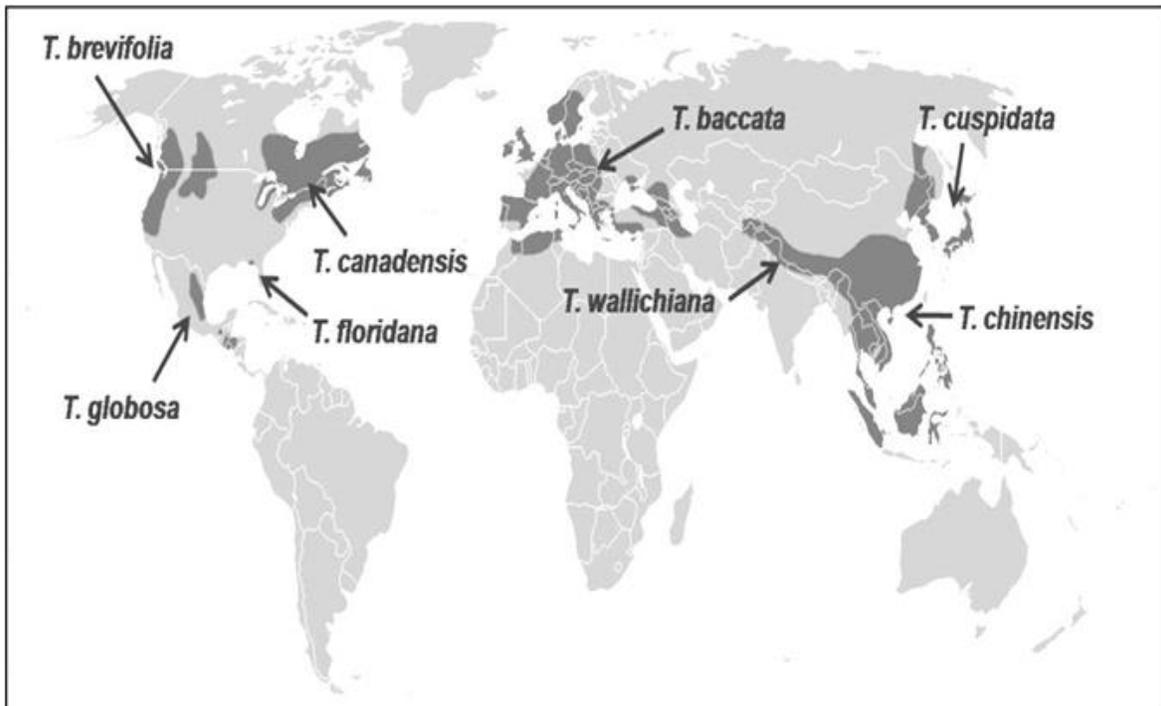
Figura 2. Distribución mundial del género *Taxus* (Tomado de Hartzell, 1991).

En América del Norte, *Taxus brevifolia* se distribuye desde el norte de California hasta la cuenca del Río Snake en Alaska; *Taxus floridiana* en el Noroeste de Florida; *Taxus canadienses* desde Manitoba hasta el sureste de Virginia así como en Indiana y en el Norte de Dakota y *Taxus globosa*, se localiza desde la parte central del estado de Nuevo León y Tamaulipas, pasando por la cuenca del Golfo y el Eje Neovolcánico Transversal en México, hasta Guatemala y el Sur de Honduras en Centroamérica (Soto *et al*. 2000; Yánez, 2000; Zavala-Chávez, 2001; Figura 2).

En México su distribución incluye 16 sitios en Nuevo León, 12 en Tamaulipas, 9 en Veracruz, 16 en Oaxaca, 1 en San Luis Potosí, 1 en Puebla, 8 en Querétaro, 3 en Chiapas y 9 en Hidalgo (López & García, 2015, Figura 3), en el Centro de América se encuentran cuatro sitios en Guatemala, uno en el Salvador y cuatro en Honduras; el intervalo latitudinal es de 25°30 norte a 14°21 norte y el intervalo altitudinal es de 3333 m en la porción Sur, en Guatemala, a 1050 m en Tamaulipas (Shemluck *et al*.

2003).

Figura 3. Distribución de *Taxus globosa* en México (Tomado de López & García, 2015).

2.5 Estudios previos con *Taxus globosa* en México

Hace apenas unos años se inició el estudio de *T. globosa* con el fin de conocer, en el mediano plazo, su hábitat, el estado actual de sus poblaciones y mecanismos de propagación, así como los principales aspectos relacionados con la síntesis del taxol, el cual es un compuesto recientemente aprobado como agente anticancerígeno por la Oficina de la Administración de Fármacos y Alimentos en Estados Unidos (Soto *et al*. 2000). Sin embargo, no existe hasta ahora ningún estudio a escala regional en el que se analice la variación morfológica, genética y demografía de las poblaciones de *T. globosa*, a lo largo de su área de distribución en México. Aquí podemos observar los trabajos realizados en los últimos años con *T. globosa* (Cuadro 1).

Cuadro 1. Estudios biológicos, ecológicos, morfológicos, genéticos y demográficos sobre *Taxus globosa* en México.

Autor y Año	Título del estudio
Zavala-Chávez *et al*. 2001.	El romerillo (*Taxus globosa* Schltdl.): biología, dificultades y perspectivas de su uso.
Contreras-Medina *et al*. 2010.	Representatividad de *Taxus globosa* Schltdl. (Taxaceae) en las áreas naturales protegidas de Mesoamérica.
García-Aranda *et al*. 2011.	Modelación en Maxent para la generación del área potencial de distribución de *Taxus globosa* Schltdl. en México.
García-Aranda *et al*. 2012.	Clasificación de nueve sitios de bosque mixto de coníferas con presencia de *Taxus globosa* en la Sierra Madre Oriental, Nuevo León y Tamaulipas, México.
López-Upton, J., & Garcia-Martí, X. 2015.	*Taxus globosa* Schltdl. (Taxáceas). Distribución y Diagnóstico de un tejo en peligro de extinción.
Muñoz-Gutiérrez *et al*. 2019.	Revisión sobre la distribución y conservación de *Taxus globosa* Schltdl. (Taxaceae) en México.
Antúnez, P., 2021.	Influencia de la fisiografía, el suelo y el clima en *Taxus globosa.*
Ramírez-Sánchez *et al*. 2011.	Variación morfológica de semillas de *Taxus globosa* Schltdl. provenientes de dos regiones geográficas de México.
Chávez, F. Z., 2002	Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México.

3 JUSTIFICACIÓN

Taxus globosa presenta una distribución restringida, adaptada particularmente a hábitats húmedos y protegidos, presentándose de manera discontinua desde el norte de México hasta Honduras y con muy pocos individuos en sus poblaciones; lo cual nos indica que es una especie vulnerable debido a la creciente destrucción de su hábitat. Esto afecta la dinámica de las pocas poblaciones existentes y puede ocasionar su extinción (Zamudio, 1992). De igual forma, el aislamiento geográfico entre sus poblaciones indica que el intercambio genético es limitado, lo que pone en riesgo su variabilidad genética y conservación a largo plazo. En la legislación mexicana (NOM-059-ECOL-2010), se le refiere como una especie "rara", sujeta a protección especial (Pr), y en la Lista Roja de especies Amenazadas de la IUCN se

considera en la categoría de bajo riesgo, principalmente por su área de distribución, superior a 2000 km^2. Es posible que la supervivencia de algunas de las poblaciones del tejo mexicano dependa de la conservación del BMM en Mesoamérica, el cual se considera como amenazado por diversas causas, principalmente relacionadas con las actividades humanas (Muñoz-Gutiérrez *et al.* 2009; Muñoz-Gutiérrez *et al*. 2019).

Existen algunos estudios sobre variación morfológica encaminados a la descripción de la especie (Zavala-Chávez *et al*. 2001; Ramírez-Sánchez *et al*. 2011) y otros trabajos en los que se utilizaron marcadores genéticos en las especies *Taxus yunnanensis* (Miao *et al*. 2008), *T. wallichiana* (Yang *et al*. 2009) y *T. baccata* (Gargiulo *et al*. 2019). Sin embargo, no existe hasta ahora ningún estudio a escala regional en el que se analice la variación morfológica y genética de las poblaciones de *T. globosa*, a lo largo de su área de distribución en México. Por ello, es importante generar información cuantitativa básica sobre el estado actual de sus poblaciones en varios aspectos: morfológicos, genéticos y demográficos, entre otros, que permitan a corto plazo, realizar propuestas para el uso, manejo y conservación de esta especie.

4   OBJETIVOS
4.1   Objetivo general
Evaluar la variación morfométrica, genotípica y poblacional de *Taxus globosa* Schlecht (Taxaceae), a lo largo de la Sierra Madre Oriental, México; para definir la relación entre estas características y el estado de conservación de las poblaciones que garanticen un uso sostenible ante su potencial biotecnológico.

4.2   Objetivos particulares
- ❖ Estimar el grado de variación morfológica foliar de las poblaciones de *T. globosa* a lo largo de su área de distribución en la Sierra Madre Oriental, México, para determinar la plasticidad de la especie.
- ❖ Evaluar la diversidad y estructura genética de las poblaciones de *T. globosa* mediante el uso de marcadores codominantes SSR's, para conocer la conectividad entre las poblaciones como un posible factor de riesgo.
- ❖ Determinar el grado de perturbación en seis poblaciones de *T. globosa*, por medio de parámetros estandarizados; para establecer cuáles de ellas se encuentran más afectadas y delimitar el estado de conservación general para la especie.

❖ Establecer parámetros demográficos relacionados con la viabilidad de las poblaciones, mediante análisis de tablas de vida estáticas; para identificar las categorías del ciclo de vida susceptibles de manejo o conservación y las áreas con mayor amenaza.

5 REFERENCIAS

Barrales-Cureño HJ, & Soto-Hernández RM. 2012. Taxoides: metabolitos secundarios del árbol del tejo (*Taxus* spp.). Revista Chapingo. Serie ciencias forestales y del ambiente, 18(2), 207-218. DOI:10.5154/r.rchscfa.2011.02.017

Charco J. 2007. El tejo en el norte de África. El tejo en el Mediterráneo Occidental (Serra L, ed). Conselleria de Territori i Habitatge. Ministerio de Medio Ambiente de España. CAM. pp. 177-185. Charco J. (2007). El tejo en el norte de África. El tejo en el Mediterráneo Occidental (Serra L, ed). Conselleria de Territori i Habitatge. Ministerio de Medio Ambiente de España. CAM. pp. 177-185. DOI: 10.3959/2009-9.1

Contreras-Medina R, & Luna I. 2001. Presencia de *Taxus globosa* Schlecht. (Taxaceae) en el estado de Chiapas, México. *Polibotánica*, (12), 51-55.

Contreras-Medina R, Luna-Vega I, & Ramírez-Martínez JC. 2011. Representatividad del tejo mexicano (*Taxus globosa* Schltdl., Taxaceae) en las áreas naturales protegidas de Mesoamérica. *Spanish Journal of Rural Development,* 2(2), 51-60. DOI: 10.5261/2011.ESP2.06

Cope EA. 1998. *Taxaceae: the genera and cultivated species*. The Botanical Review, 64, 291-322. DOI: 10.1007/BF02857621

Eckenwalder JE. 2009. Conifers of the world: the complete reference. Timber Press, Portland. 720 p.

Estrada E, Salazar CG, Villarreal JA, García JP, & González MA. 2006. Cuantificación de *Taxus globosa* en el Noreste de México. *In The Southwestern Naturalist. 53rd. Annual Meeting*, Colima, Colima, México. pp. 136-137.

Farjon A. 2010. *A Handbook of the World's Conifers.* Leiden: Brill Academic Publishers. 2 vols., 1111 p. DOI: 10.1163/9789047430629

García SF, & Castillo LP. 2000. Aspectos ecológicos de *Taxus globosa* Schlecht. En las Mesas de San Isidro, municipio de Río Verde, San Luis Potosí. *Biotam*, 11(3), 11-18.

García-Aranda MA, Estrada-Castillón AE, Jurado-Ybarra E, & González-Uribe DU. 2011. Análisis de once poblaciones naturales de *Taxus globosa* en la Sierra Madre Oriental. *Madera y bosques,* 17(1), 93-104.

Gargiulo R, Saubin M, Rizzuto G, West B, Fay MF, Kallow S, & Trivedi C. 2019. Genetic diversity in British populations of *Taxus baccata* L.: is the seedbank collection representative of the genetic variation in the wild? *Biological Conservation*, 233: 289-297. DOI: 10.1016/j.biocon.2019.01.014

Gernandt DS, & Pérez-de la Rosa JA. 2014. Biodiversity of Pinophyta (conifers) in México. *Revista mexicana de biodiversidad*, 85(supl.), S126-S133. DOI: 10.7550/rmb.32195

López-Herrera M. 2008. *Taxus globosa* Schltdl., una especie medicinal en el Parque Nacional El Chico, Hidalgo. Estudios biológicos en las áreas naturales del estado de Hidalgo. Ed. Universidad Autónoma del Estado de Hidalgo, Pachuca, México, pp. 63-68.

Vega IL, Cruz SO, & Ayala OA. 1994. Florística y notas biogeográficas del bosque mesófilo de montaña del municipio de Tlanchinol, Hidalgo, México. Anales del Instituto de Biología. *Serie Botánica*, 65(1), 31-62.

Miao YC, Su JR, Zhang ZJ, Li H, Luo J, & Zhang YP. 2008. Isolation and characterization of microsatellite markers for the endangered *Taxus yunnanensis*. Conservation Genetics, 9(6), 1683-1685.

Muñoz-Gutiérrez L, Vargas-Hernández JJ, López-Upton J, & Soto-Hernández M. 2009. Effect of cutting age and substrate temperature on rooting of *Taxus globosa*. *New forests*, 38(2), 187-196. DOI: 10.1007/s11056-009-9139-6

Muñoz-Gutiérrez L, Ramírez-Sánchez SE, & Velasco-García MV. 2019. Review on the distribution and conservation of *Taxus globosa* Schltdl. (Taxaceae) in México. *Revista mexicana de ciencias forestales*, 10(56), 65-84. DOI: 10.29298/rmcf.v10i56.479

Ramírez-Sánchez SE, López-Upton J, García de los Santos G, Vargas-Hernández JJ, Hernández-Livera A, & Ayala-Garay ÓJ. 2011. Variación morfológica de semillas de *Taxus globosa* Schltdl. provenientes de dos regiones geográficas de México. *Revista fitotecnia mexicana*, 34(2), 93-99. DOI: 10.35196/rfm.2011.2.93

Redmond AM. 1984. Ecología de la población de *Taxus floridana* (Nutt.), un árbol dioico del sotobosque (Tesis). Florida State University. 47 p.

Rosito Monzón JC. 1999. Estudio florístico de la comunidad del cipresillo (Taxus globosa Schlecht.) en los cerros Pinalón, Guaxabajá y Mululjá en la Sierra de las Minas Floristic study of cipresillo (*Taxus globosa* Schlecht.) community on Pinalón, Guaxabajá and Mululjá, on Sierra de las Minas (Tesis). Universidad de San Carlos de Guatemala, Guatemala (Guatemala). Facultad de Agronomía. Instituto de Investigaciones Agronómicas.

Rzedowski J. 1978. Vegetación de México. Limusa. México DF, México.

Shemluck MJ, Estrada E, Nicholson R, & Brobst SW. (2003). A preliminary study of the taxane chemistry and natural history of the Mexican yew, *Taxus globosa* Schltdl. *Boletín de la Sociedad Botánica de México,* 72, 119-127. DOI: 10.17129/botsci.1672

Silba J. 1983. An international census of the Coniferae. Phytologia Memoir. pp. 1-79.

Standley PC, & Steyermark JA. (1958). Podocarpaceae. Flora of Guatemala. *Fieldiana Botany*, 24, 1-478.

Soto M, Sanjurjo M, González MT, Cruz D, & Giral F. 2000. El tejo mexicano (*Taxus globosa* Sch.). Potencial de su aprovechamiento en taxol. CIENCIA *ergo-sum*, *Revista Científica Multidisciplinaria de Prospectiva*, 7(3), 277-279.

Soto-Hernández M, López J, Vargas-Hernández JJ, Muñoz-Gutiérrez L, & San Miguel R. 2011. Estado de conservación de *Taxus globosa* en México. *Spanish Journal of Rural Development*, 2(2), 61-68.

Villareal J. & Estrada E. 2012. Taxaceae. Flora Veracruzana, Fascículo 155. Instituto de Ecología, A. C., Centro de Investigaciones Tropicales. 15 p.

Villaseñor JL, & Ortiz E. 2014. Biodiversidad de las plantas con flores (División Magnoliophyta) en México. *Revista Mexicana de Biodiversidad*, 85, S134-S142. DOI: 10.7550/rmb.31987

Villaseñor JL. 2016. Checklist of the native vascular plants of Mexico. *Revista Mexicana de Biodiversidad*, 87(3), 559-902. DOI: 10.1016/j.rmb.2016.06.017

Wickremesinhe ER, & Arteca RN. 1994. Cultivos de suspensión de células de *Taxus*: optimización del crecimiento y producción de taxol. *Revista de fisiología vegetal*, 144 (2), 183-188.

Yánez EL. 2001. APUENTES DE Demografía. México: Universidad Autónoma de Chapingo, División de Ciencias Forestales. pp. 66-68.

Yang JB, Li HT, Li DZ, Liu J, & Gao LM. (2009). Isolation and characterization of microsatellite markers in the endangered species *Taxus wallichiana* using the FIASCO method. *HortScience,* 44(7), 2043-2045. DOI: 10.21273/HORTSCI.44.7.2043

Zamudio RS. 1992. Familia *Taxaceae*, En J. Rzedowski & G. Calderón (Eds.), *Flora del Bajío y de sus regiones adyacentes* (Fascículo 9). Instituto de Ecología, A.C., Centro Regional del Bajío. Pátzcuaro, Michoacán. 6 p.

Zavala-Chávez F. 2001. Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México. I: Población de adultos y algunas características del hábitat. *Ciencia Ergo Sum*, 8(2), 169-174.

Zavala-Chávez F, Soto-Hernández M, & Rodríguez-González T. 2001. El romerillo (*Taxus globosa* Schlecht.): biología, dificultades y perspectivas de su uso. *Revista Chapingo Serie Horticultura*, 7(1), 77-94. DOI: 10.5154/r.rchsh.1999.02.016

Zavala-Chávez F. 2002. Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México. II: Población de juveniles y algunos datos de semillas. *Ciencia Ergo Sum*, 9(2), 177-183.

CAPÍTULO II

Efecto del ambiente sobre la plasticidad foliar de *Taxus globosa* en la Sierra Madre Oriental, México: especie neártica de distribución restringida

Leaf plasticity, environmental variation, and latitudinal gradients: The case of *Taxus globosa*, a plant with restricted distribution

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6   ABSTRACT

***Background and Aims*:** Environmental variation can impact plant growth positively or negatively, especially on long-lived tree species with restricted ranges and low ecological tolerance. This study examines the leaf morphological variation of *Taxus globosa*, a threatened tree, focusing on how environmental factors affect its phenotypic plasticity using natural experimentations as fount of variability.

Methods: Six populations along a latitudinal gradient were analyzed for twelve foliar attributes and 19 environmental attributes. Morphological differences between populations were analyzed by serial PERMANOVAs. A factor analysis was used to extract environmental attributes correlated with morphological variation. A non-metric multidimensional scaling analysis was used to observe associations.

***Key Results*:** Significant differences were found among populations, explained mainly by temperature and precipitation. The petiole length/petiole thickness ratio was negatively correlated with temperature seasonality ($r^2 = -0.89$, $p = 0.033$). NMDS analysis indicated that northern populations were morphologically similar and associated with temperature attributes, while populations in the middle distribution clustered based on precipitation

during the driest month. The southern population was distinct, displaying smaller leaves and a positive correlation with rainfall.

***Conclusions*:** Our results show that morphological differences in the petiole are due to the influence of climatic and geographic factors, while differences in the leaf blade respond to local modifications, such as silvicultural practices. These findings highlight the need for further research on the genetic structure and adaptation of *T. globosa* to plan conservation strategies for its populations ubicated in fragmented habitats.

7   INTRODUCTION

Climate gradients influence local adaptation and phenotypic plasticity of trees, affecting their fitness (Atkin *et al.* 2006; Gárate-Escamilla *et al.* 2019). From germination to the senescent stage, trees respond to environmental changes through physiological modulation in the face of biotic and abiotic threats (Kijowska-Oberc *et al.* 2020; Sevik *et al.* 2021), leading to phenotypic plasticity as an adaptive strategy.

Phenotypic plasticity, the ability of a genotype to generate different phenotypes depending on the environment, is the primary way plants cope with environmental challenges (Bradshaw, 1965). Plasticity can be expressed differently at different growth stages, resulting in variations in the morphological properties of leaves in adult individuals depending on environmental attributes (Liu and Su 2016). Leaves, being highly plastic structures, reflect ecological variability. At higher elevations, they tend to be greater in mass, nitrogen concentration, and carbon isotopic composition, while their area decreases, although this variation is less pronounced in woody plants (Midolo *et al.* 2019). These patterns enhance plant efficiency under intense radiation, low temperatures, and low precipitation, trends that parallel those observed along latitudinal gradients.

Large-scale variations, such as those across a latitudinal gradient, reveal species' adaptive trade-offs and biogeographic history. For instance, in *Arabidopsis thaliana* 'Arabidopsis', leaf area and nitrogen content plasticity are greater in central than in marginal populations along its distribution range (Estarague *et al.* 2022), supporting the idea that the center of the ecological niche offers optimal conditions for maintaining trait variation

(Maciel-Mata *et al.* 2015). In contrast, environmental stress at the margins of the species distribution tends to homogenize morphological variation and differentiate peripheral populations.

However, present-day environmental changes are occurring at a rate that many plants cannot compensate for either physiologically or genetically, increasing the risk of local extinction, particularly in species with restricted distribution or relict populations (Kristensen *et al.* 2020; Ramírez-Amezcua *et al.* 2016). Understanding and predicting these effects requires assessing response type, the influence of environmental fluctuation, and population resilience to stress (Kristensen *et al.* 2020). While phenotypic plasticity is key to rapid adaptation, it can also hinder evolutionary responses by redistributing phenotypic variation and weakening natural selection. Additionally, not all plastic responses are beneficial, so evaluating their effects over time and in different environments is essential to better understanding their role in adaptive evolution (Fox *et al.* 2019).

Taxus "Yew" is a Nearctic-affinity genus belonging to the family Taxaceae, comprising 13 species distributed across the Northern Hemisphere (Cope 1998; Möller *et al.* 2013). In America, four species are recognized: *T. canadensis* in northeastern USA and central-eastern Canada, *T. brevifolia* along the Pacific coast, *T. floridana* restricted to Florida, and *T. globosa*, which inhabits cloud forests from Mexico to Central America. The latter species is confined to ravines and humid exposed slopes, forming small, scattered populations (Zamudio 1992; López-Upton and Garcia-Martí 2015).

Given its restricted and limited distribution ranges, low environmental tolerance, and reproductive constraints (Cope 1998; García-Aranda *et al.* 2011; López-Upton and Garcia-Martí 2015), *T. globosa* represents an ideal model for examining how phenotypic plasticity mediates survival in challenged environments. This study aims to evaluate the morphological variation of *T. globosa* leaves across the Sierra Madre Oriental and to identify the environmental factors associated with its phenotypic plasticity. We hypothesize that morphological variation in northern populations will be more functional and more conserved compared to the original populations, due to their proximity to the species' Nearctic origin. However, given the rapid habitat changes, this plasticity may primarily reflect responses to local environmental stress rather than adaptive selection, potentially disrupting the inheritance of advantageous traits.

8   MATERIAL AND METHODS

8.1   Study specie

Taxus globosa is irregularly distributed across the Sierra Madre Oriental. It is a small tree with sporadic reproductive events, typically yielding fewer than 50 seeds per female tree, and with long gaps between reproductive years. It thrives in acidic, well-drained soils rich in organic matter and requires over 70% relative humidity. Its elevation range is 1,100–2,960 meters above sea level. This species shares its habitat with various oak and conifer species in the montane cloud forest (Pérez-García and Mendoza 2002; Shemluck *et al.* 2003; Ramírez-Sánchez *et al.* 2011). Of the 29 populations reported in Mexico, 15 are well-preserved, two are in poor condition, and 12 are in decline, the best conditions being located in Nuevo León and Hidalgo. Many populations suffer from fragmentation, habitat loss, low reproduction, lack of recruitment, and land-use changes. The Guatemala, El Salvador, and Honduras populations are subject to similar threats (López-Upton and Garcia-Martí 2015).

8.2   Study area

Taxus globosa is found in various plant associations including cloud forests, *Pinus* forests, and *Quercus-Pinus* forests. In Chiapas, it grows on humid slopes within *Abies* and *Pinus* forests (Contreras & Luna 2001), while in Tamaulipas, it is located in El Cielo Biosphere Reserve. In Nuevo León, it is often in the canopy of *Quercus-Pinus* and *Picea-Abies* forests (García-Aranda *et al.* 2011). In Hidalgo, it thrives at high elevations in El Chico National Park, coexisting with species such as *Quercus glabrescens* and *Prunus serotina* (Zavala-Chávez 2001; Muñoz-Gutiérrez *et al.* 2019). At lower elevations, it occupies specific microenvironments within *Abies* and *Quercus* forests, sharing the canopy with various species. We selected six populations from Oaxaca to Nuevo León to represent the morphological variation of *T. globosa* (Table 1, Figure 1), based on previous reports (López-Upton and García-Martí 2015; Muñoz-Gutiérrez *et al.* 2019).

Table 1. Coordinates of the six populations studied for this work. (Data on vegetation type and associated genera were taken from López-Upton & García-Martí 2015, and Muñoz-Gutiérrez *et al.* 2019.)

ID	State	Municipality	X	Y	Elevation	Vegetation type	Associated trees
1	Nuevo León	Santiago	-100.196	25.36475	1525	AF, A-QF	*Abies, Quercus*
2	Tamaulipas	Gómez Farias	-99.229	23.05803	1331	MCF, PF, AF	*Abies, Garrya, Pinus*
3	San Luis Potosí	Xilitla	-99.0652	21.40017	1921	CF	*Abies, Pinus, Pseudotsuga, Quercus*
4	Hidalgo	Mineral del Chico	-98.7133	20.18503	2997	CF, P-GF	*Abies, Garrya, Pinus,*
5	Veracruz	Acajete	-97.0603	19.518086	2623	AF, PF	*Abies, Pinus*
6	Oaxaca	Ixtlán de Juárez	-96.493	17.41172	3071	AF, A-QF	*Abies, Quercus*

CF: coniferous forest, MCF: montane cloud forest, AF: *Abies* forest, A-QF: *Abies-Quercus* forest, PF: *Pinus* forest, P-GF: *Pinus-Garrya* forest. X: longitude, Y: latitude.
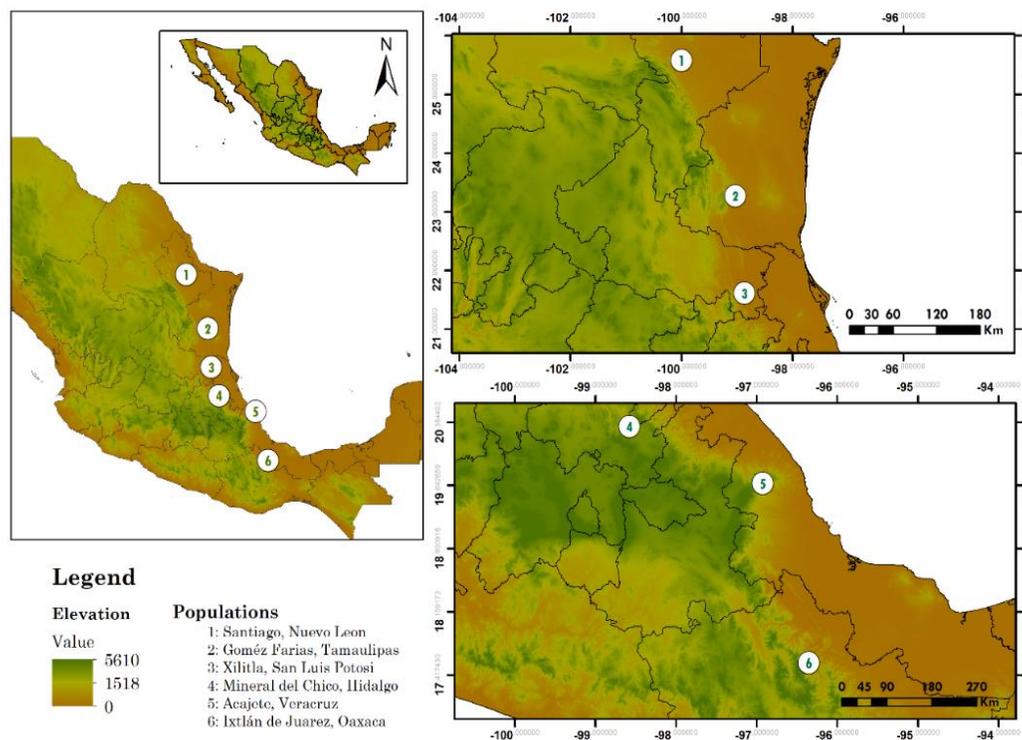
Figure 1. Map showing locations of the six studied populations of *Taxus globosa* in the Sierra Madre Oriental, Mexico.

8.3 Morphological evaluation

In all sampled sites, a fieldwork search was conducted until ten individuals were located. Due to the sparsity of their distribution, commonly a large area as much as 3 ha was searched. Ten mature leaves were collected from each individual, ensuring they were in good condition with no signs of damage or desiccation. This resulted in a total of 100 leaves per site. At each site, we recorded the GPS coordinates of each individual using a Magellan eXplorist 110 receiver. The quantitative traits evaluated were leaf length including the petiole (LL), leaf length excluding the petiole (LLEP), leaf width at the base (LWB), leaf width at mid-height (LWMH), leaf width at the apex (LWA), leaf thickness (LT), petiole length (PL), petiole width at mid-height (PWMH), and petiole thickness (PT). Additionally, we calculated proportional indicators (LT/LWMH, LL/LWMH, and PL/PT) to best represent leaf size variation. We selected these attributes based on the report by Strandby *et al*. (2009).

Each leaf was photographed on a 1-cm grid, and the measurements associated with each trait were estimated with a vernier caliper and the tpsDig version 2.16 program (Delgado *et al.* 2011). The shape of the leaf apex (SLA) was the only qualitative trait, but it was not included in the statistical analyses, as it was acute in all leaflets and therefore did not vary (Table 2, Figure 2).

Table 2. Morphological attributes of leaves evaluated in the populations of *Taxus globosa* along the Sierra Madre Oriental (modified from Strandby *et al.* 2009).

No	Attribute	Acronym
1	Leaf length (including petiole)	LL
2	Leaf length (excluding petiole)	LLEP
3	Leaf width at the base	LWB
4	Leaf width at mid-height	LWMH
5	Leaf width at the apex	LWA
6	Leaf thickness	LT
7	Petiole length	PL
8	Petiole width at mid-height	PWMH
9	Petiole thickness	PT
10	Leaf thickness/leaf width at mid-height	LT/LWMH
11	Leaf length (excluding petiole)/leaf width at mid-height	LLEP/LWMH
12	Petiole length/Petiole thickness	PL/PT
13	Shape of the leaf apex	SLA
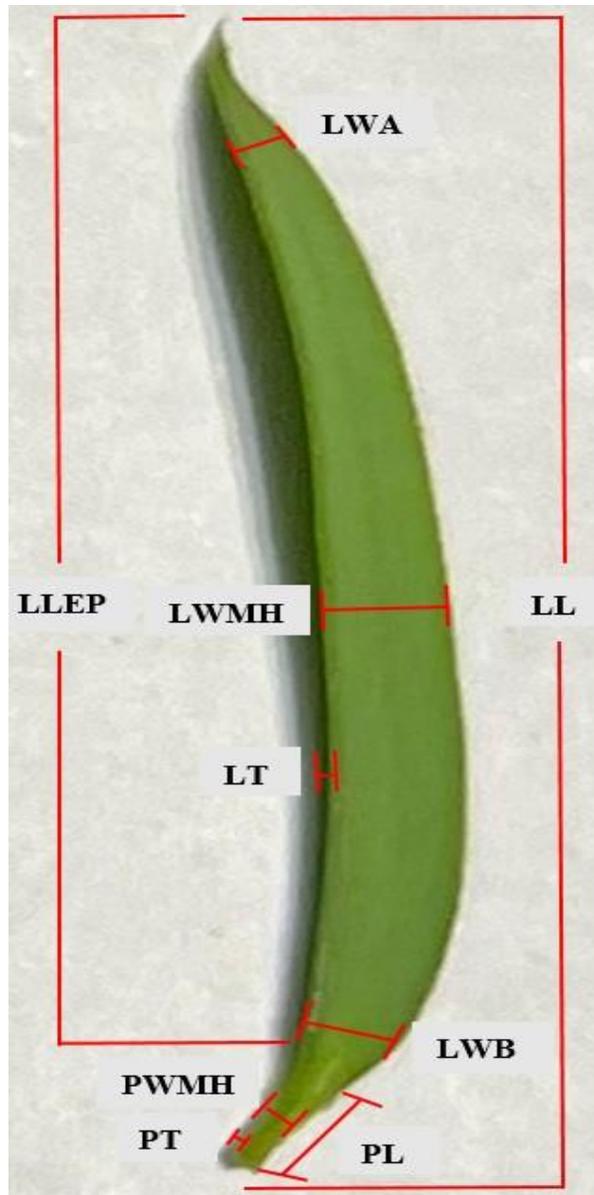
Figure 2. Illustration of the morphological traits evaluated in the populations of *Taxus globosa* along Sierra Madre Oriental.

8.4 Environmental Assessment

Using each site's georeferenced locations, we searched the WorldClim database (Fick & Hijmans 2017) to extract data on 19 environmental attributes, which were utilized to analyze the latitudinal variation explaining the observed morphological differences (Table 3).

Table 3. Environmental traits used to analyze variability among the Sierra Madre Oriental populations of *Taxus globosa* (Modified from Fick & Hijmans, 2017).

No.	Environmental trait	Key
1	Average annual temperatura	BIO1
2	Average diurnal range (Monthly average of maximum temperature)	BIO2
3	Isothermality	BIO3
4	Seasonality of temperatura	BIO4
5	Maximum temperature of the warmest month	BIO5
6	Minimum temperature of the coldest month	BIO6
7	Annual temperature range	BIO7
8	Average temperature of the wettest quarter	BIO8
9	Average temperature of the driest quarter	BIO9
10	Average temperature of the warmest quarter	BIO10
11	Average temperature of the coldest quarter	BIO11
12	Annual precipitation	BIO12
13	Precipitation of the wettest month	BIO13
14	Precipitation of the driest month	BIO14
15	Seasonality of precipitation	BIO15
16	Precipitation of the wettest quarter	BIO16
17	Precipitation of the driest quarter	BIO17
18	Precipitation of the warmest quarter	BIO18
19	Precipitation of the coldest quarter	BIO19

8.5 Statistical analysis

Both databases, morphological and environmental attributes, were orthogonalized using the formula $z = \frac{x_i - \mu}{\sigma}$, where x_i represents each value obtained, μ represents the arithmetic mean of the attribute over all populations, and σ is the standard deviation of each attribute (e.g., Galván-Hernández *et al.* 2021).

The populations were described by their morphological attributes (mean, standard deviation) to facilitate understanding of latitudinal variation. A Mardia test was performed to determine the feasibility of a linear multimetric analysis; however, the data were not multinormal. Therefore, nonparametric tests were used. To discover morphological

differences between populations, serial PERMANOVAs (9,999 permutations) were carried out to obtain only the attributes important for explaining variation (Anderson 2017). Since replicates were lacking for environmental data, a factor analysis with normalized varimax rotation was carried out, identifying attributes that explain climate variation between sites. We used the refined bases to calculate Euclidean distances between populations. With these new recalculated and reduced-dimensionality data, a Mantel test was performed between the environmental and morphological Euclidean distances. In addition, a Spearman's multiple correlation analysis was carried out (since the data were not multinormal) using the refined morphological and environmental variables in their original units, without unifying the data to Euclidean distances. This analysis enables univariate relationships among attributes to be observed.

A non-metric multidimensional scaling (NMDS) was carried out, unifying the refined matrices to find morphological similarities between the sites in relation to environmental variables. Due to the different natures of the morphological and environmental data, we selected the Gower distance, which can handle different types of variables; specifically binary, categorical, ordered, continuous, and semi-continuous variables (Gower 1971). All analyses were calculated using Past v4.03 (Hammer *et al.* 2001) and STATISTICA version 10 (StatSoft 2017) software.

9 RESULTS

The PERMANOVA showed that the populations differed significantly in the morphological attributes evaluated (F = 30.95, p = 0.0001). The minimal model to explain these differences recovered seven of the twelve attributes: PT, PL/PT, LWB, LLEP, PL, LL, and LWMH, in order of importance (Table 4).

Table 4. Serial PERMANOVA with description of morphological attributes by population. *Significant attribute for explaining total variation (p < 0.001).

Attribute	F	Nuevo León	Tamaulipas	San Luis Potosí	Hidalgo	Veracruz	Oaxaca
PT*	27.82	0.021 ± 0.005	0.027 ± 0.007	0.025 ± 0.005	0.024 ± 0.006	0.023 ± 0.006	0.016 ± 0.005
PL/PT*	28.87	6.279 ± 0.904	5.160 ± 1.107	6.712 ± 0.943	6.852 ± 1.511	6.304 ± 1.548	7.530 ± 2.289
LWB*	29.03	0.143 ± 0.135	0.138 ± 0.020	0.211 ± 0.030	0.199 ± 0.052	0.129 ± 0.020	0.109 ± 0.024
LLEP*	29.40	2.575 ± 0.235	2.653 ± 0.218	2.261 ± 0.269	2.242 ± 0.147	2.580 ± 0.394	2.238 ± 0.351
PL*	29.72	0.131 ± 0.025	0.132 ± 0.019	0.164 ± 0.018	0.161 ± 0.036	0.138 ± 0.020	0.111 ± 0.029
LL*	29.76	2.706 ± 0.256	2.785 ± 0.232	2.425 ± 0.275	2.403 ± 0.166	2.717 ± 0.407	2.348 ± 0.367
LWMH*	30.23	0.191 ± 0.021	0.198 ± 0.016	0.197 ± 0.038	0.199 ± 0.052	0.198 ± 0.027	0.150 ± 0.042
TOTAL	**30.95**						
LT	32.77	0.034 ± 0.035	0.029 ± 0.007	0.029 ± 0.006	0.029 ± 0.006	0.027 ± 0.006	0.025 ± 0.008
LWA	33.12	0.124 ± 0.120	0.122 ± 0.015	0.113 ± 0.010	0.112 ± 0.087	0.114 ± 0.018	0.092 ± 0.023
PWMH	33.28	0.033 ± 0.021	0.038 ± 0.021	0.029 ± 0.006	0.029 ± 0.007	0.036 ± 0.007	0.039 ± 0.008
LT/LWMH	33.66	0.182 ± 0.213	0.143 ± 0.026	0.209 ± 0.091	0.200 ± 0.236	0.137 ± 0.020	0.172 ± 0.062
LLEP/LWMH	34.15	14.09 ± 0.668	14.05 ± 0.066	16.28 ± 6.448	11.85 ± 5.918	13.68 ± 1.377	16.45 ± 4.443

The environmental factor analysis showed that only the BIO15 (seasonality of precipitation) and BIO18 (precipitation of the warmest quarter) layers did not contribute to variation between sites. Two factors accounted for up to 94% of the total site variance; the first relates to temperature attributes and the second to precipitation attributes (Table 5).

Table 5. Factor analysis with normalized varimax rotation by key in each *Taxus globosa* population. Only attributes important for explaining the variation among populations were included (in red).

Attribute	F1	F2	Nuevo León	Tamaulipas	San Luis Potosí	Hidalgo	Veracruz	Oaxaca
BIO1	0.98	-0.14	18.4	18.3	17.2	11.5	11.8	12.2
BIO2	0.92	-0.26	13.6	13.8	13.5	13	12.9	12.9
BIO3	-0.88	0.42	57	59	62	67	68	71
BIO4	0.89	-0.40	35.56	31.22	25.45	15.71	16.76	10.8
BIO5	0.98	-0.16	29.5	29.2	28	21.2	21.1	21.5
BIO6	0.98	0.14	5.9	6	6.4	1.9	2.4	3.5
BIO7	0.91	-0.39	23.6	23.2	21.6	19.3	18.7	18
BIO8	0.97	-0.22	22	20.8	19	12.2	12.7	12.5
BIO9	0.98	0.17	14.6	13.9	14.8	9.6	10.5	11.7
BIO10	0.97	-0.22	22.6	21.6	19.9	13.5	13.7	13.6
BIO11	0.99	0.01	13.6	13.9	13.6	9.4	9.4	10.7

BIO12	-0.15	0.95	804	970	1697	919	1275	1899
BIO13	0.13	0.99	190	198	411	163	259	348
BIO14	-0.24	0.92	14	15	34	18	30	27
BIO16	-0.13	0.96	403	510	888	440	682	982
BIO17	-0.10	0.99	56	60	112	56	94	102
BIO19	-0.39	0.87	59	60	127	74	126	175
Eigenvalue	12.42	5.49						
Explained variance	65.39	28.92						
Cumulated variande	65.39	94.31						

F1: correlation coefficients of attributes with factor 1. F2: correlation coefficients of attributes with factor 2. The unit used for temperature data was °C (degrees Celsius); for precipitation data, mm (millimeter).

According to the Mantel test, there is no significant correlation between the unified Euclidean distances for morphological and environmental attributes (Mantel test: $r^2 = 0.04$, Exp Rx = 2.37, Obs Rx = 1.01). But the multiple correlation analysis with raw data showed that the petiole length/petiole thickness relation (PL/PT) correlates negatively and significantly with seasonality of temperature (BIO4) ($r^2 = -{-}0.89$ p = 0.033). However, there are significant autocorrelations within both matrices (Figure 3).
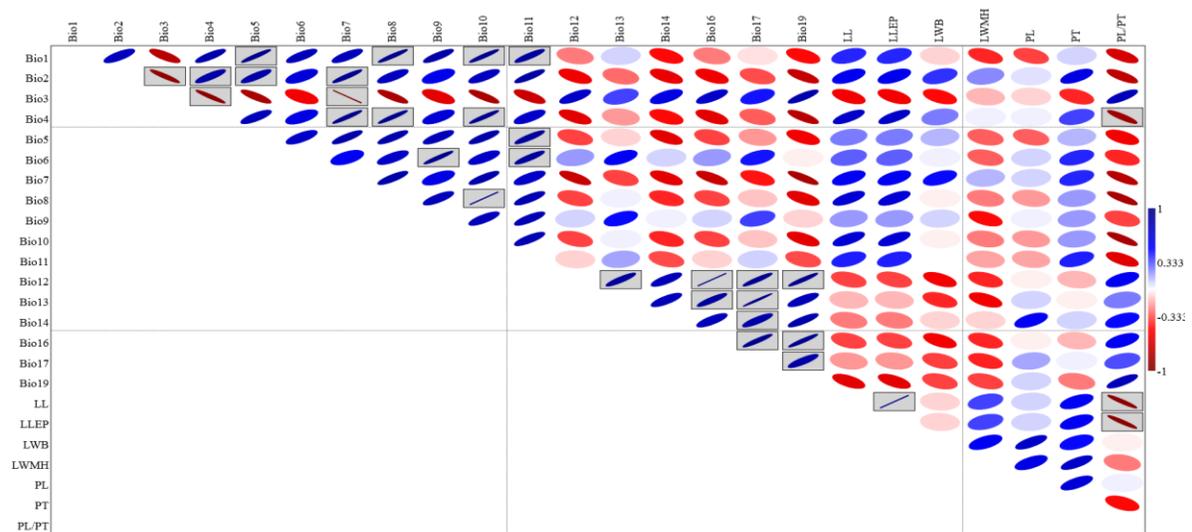
Figure 3. Spearman's multiple correlation analysis between environmental (refined by factor analysis with normalized varimax rotation) and morphological (refined by serial PERMANOVA) raw variables. Positive correlations are shown in blue, and negative correlations in red. Gray boxes indicate significant correlations.

The NMDS showed that the populations from Tamaulipas, Nuevo León, and Veracruz are morphologically similar, having large leaves, and being positively correlated with temperature-related environmental attributes. The San Luis Potosí and Hidalgo populations are morphologically comparable; their strongest correlation is precipitation in the driest month (Bio 14). Oaxaca was the most divergent population, with the smallest leaves for all attributes and a positive correlation with precipitation attributes. This ordering is robust because the correlations with the axes are significant and the stress of the regression model is zero (Figure 4).
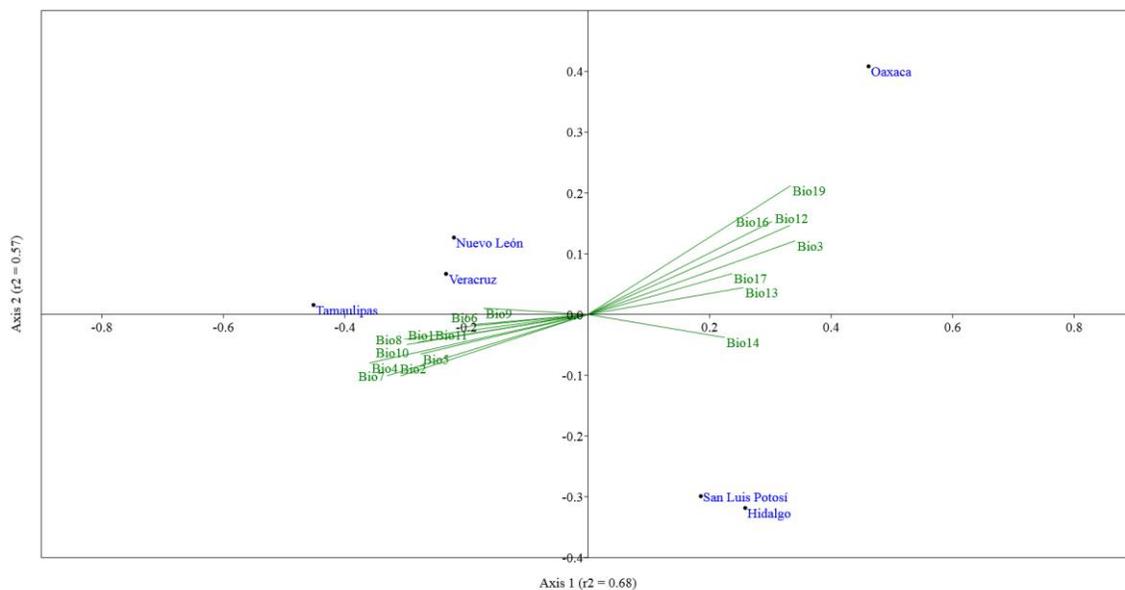
Figure 4. NMDS ordination analysis evaluating seven morphological measures and 17 environmental attributes unified by Gower distances from the original measure in the evaluated *Taxus globosa* populations.

10 DISCUSSION

In general, leaves of *Taxus globosa* tend to be larger in the north. This latitudinal morphological gradient counterintuitively may be related to the higher temperatures in the northern populations and greater precipitation in the south (NMDA). This pattern aligns with *T. globosa*'s Nearctic origin and suggests that the plant demonstrates enhanced efficiency in stressful environments, as proposed by Midolo *et al.* (2019). However, not all morphological traits respond in the same way or at the same magnitude, and some studied populations do

not always show consistent trends in temperature and precipitation (negative Mantel test). The variations in specific morphological characteristics and the local environments at the analyzed sites help us understand this plant's history of phenotypic plasticity.

10.1 Morphological attributes of leaf blade

The seven morphological traits explaining population variation are linked to two structures, the leaf blade (LWB, LLEP, LL, and LWMH) and petiole (PT, PL/PT, and PL). Variations in leaf blade characteristics reveal how plants adapt to environmental changes, enabling them to cope with water stress and extreme temperatures (Lambers *et al.* 2008). The size and shape of leaves are closely linked to carbon, water, and energy exchange between plants and their surroundings (Brito-Rocha *et al.* 2016). Moreover, leaf size strongly correlates with precipitation levels, while leaf shape shows plasticity in response to temperature changes. Specifically, leaf width decreases as temperature increases, and leaf size diminishes with decreasing precipitation (Li *et al.* 2020). However, it remains unclear whether these patterns represent a general trend, or how leaf size and shape may vary in response to environmental fluctuations. Also, species from various taxonomic and functional groups may respond differently to these ecological changes (Delgado *et al.* 2011; Brito-Rocha *et al.* 2016).

The morphological pattern of *Taxus globosa* shows that the longest leaves are found in Tamaulipas, Veracruz, and Nuevo León (northern populations, except Veracruz); the widest in Hidalgo and San Luis Potosí (middle distribution); and the shortest and narrowest in Oaxaca (southern population). Based on the pattern described earlier, only the populations of Hidalgo and San Luis Potosí align with what was expected (high precipitation in the driest month and low temperature, leading to large and broad leaves). This result suggests that these regions have the most suitable environmental conditions, which favor the plant's leaf variation. In contrast, the Oaxaca population is notable for its small leaves, even in high rainfall and low temperatures. The decrease in leaf size in Oaxaca suggests that microenvironmental factors could influence leaf development.

Also, the large leaves of Nuevo León and Tamaulipas do not follow the pattern proposed by Li *et al*. (2020) because they are found in areas of high temperature and low precipitation. However, the plant's Nearctic origin suggests that remnants of genetic and functional variation similar to the original plants should exist in the north (Cavender-Bares *et al*. 2015). Therefore, the Veracruz population is the only one not fitting into this grouping

due to its higher rainfall and intermediate temperature. Thus, Oaxaca and Veracruz exhibit unusual leaf morphology that deviates from the expected latitudinal pattern. This anomaly may be attributed to the microenvironmental variations linked to disturbances in both areas. These rapid changes hinder plants from fully expressing their adaptive phenotypic plasticity (Petit *et al.* 2003; García-Aranda *et al.* 2011). Previous research on *Taxus* species concluded that habitat fragmentation and discontinuous distribution can lead to patterns of morphological differentiation (not only foliar) similar to those observed in this study (Zavala-Chávez 2001; Ramírez-Sánchez *et al.* 2011).

These population anomalies break all the univariate correlations between leaf blade attributes and climate variables (Figure 3). However, the petiole length/width ratio (PL/PT) correlates negatively with temperature seasonality (BIO4).

10.2 Morphological attributes of petiole

Temperature seasonality (BIO4) is a bioclimatic attribute that measures temperature variability over time. It is calculated by dividing the standard deviation of monthly temperature by the average of monthly temperature (coefficient of variation) (Busby, 1991). This was the only climate trait correlated with a morphological attribute (PL/PT); the more significant the temperature variation, the shorter and thicker the petiole. Nuevo León, Tamaulipas, and San Luis Potosí experience the highest seasonal temperature variation with the shortest and thickest petioles, followed by Veracruz and Hidalgo. Oaxaca exhibits the most stable temperatures and the longest and thinnest petioles.

Petiole structure is significantly influenced by species-specific optimal conditions for temperature and precipitation (which define their ecological niches), plant height, and leaf area, regardless of phylogenetic inheritance (Bradshaw 1965; Lambers *et al.* 2008; Filartiga *et al.* 2022). Different species with similar thermal and hydrological requirements often develop similar anatomical structures in their petioles. For instance, in various tree and shrub species, larger leaves are typically found on taller trees that grow in more stable and productive environments. These leaves feature longer, round petioles primarily made of sclerenchyma. Additionally, they have larger vessels, fiber-rich areas between vascular bundles, and poorly defined phloem rays (Filartiga *et al.* 2022). This description applies to the Oaxacan population. In contrast, shorter trees and shrubs in cooler or drier habitats, such

as those in Nuevo León, Tamaulipas, and San Luis Potosí, generally have smaller, stiffer leaves.

Petiole and leaf blade structure variations can affect how plants adapt to extreme environmental conditions. Typically, maximum morphological divergence is observed at the extremes of a species' distribution, whether in latitude or elevation gradients. This divergence contributes to a certain degree of structural stability and morphological homogeneity in the middle of the gradient. In these cases, leaves show adaptive variations due to natural selection, largely unaffected by phylogenetic constraints and primarily influenced by climate challenges (Riordan *et al.* 2016; Zerey-Belaskri and Benhassaini 2016; Bijarpasi *et al.* 2019), as is the case of leaf blade variation of *Taxus globosa*.

In our situation, however, we see a gradient in petiole morphology that can be linked to latitudinal differences. This variation suggests a more effective adaptive response in the northern parts of the distribution, resembling a gradual colonization model (often referred to as a stepping-stone model). Further research is needed to explore whether the pattern of genetic variation is similar. We can say that climate significantly impacts leaf blades, while latitudinal gradients significantly influence petiole characteristics. This means that leaves are more adaptable to quick and localized climate changes, whereas petioles are more stable structures that respond to broader climate gradients.

Finally, we want to emphasize the intensive silvicultural activities in Veracruz and the semi-conservative forestry in Oaxaca. In these regions, timber extraction does not target *T. globosa* but focuses on the pine species that coexist with it. However, its populations are impacted by canopy removal and the creation of gaps, which alter microclimatic conditions, resulting in increased temperatures and sunlight exposure, which is particularly intense in Veracruz. These anthropogenic disturbances explain the climatic and morphological association with populations in the northern part of their distribution, where temperatures are higher and precipitation lower.

In contrast, in Oaxaca, logging efforts aim to preserve individuals of *T. globosa* and align them with their protected conservation status. Nevertheless, large pines can occasionally crush adult trees and seedlings when they are felled. In this case, the creation of gaps is less pronounced. Still, random removal of adult trees affects the species' ability to respond, evident in the reduced size of leaf blades in this area due to short times to foliar

regeneration. This adaptation is necessary to compensate for the rapid loss of branches and leaves. These observations are important for understanding the patterns of phenotypic plasticity in response to sudden and extensive local changes.

All results align with observations of the plant's genetic base, showing reduced genetic diversity from north to south, indicating low diversity and high inbreeding levels due to recent bottlenecks. Notably, the population decline in Oaxaca and Veracruz is severe, while the Hidalgo population exhibits the most significant morphological variation and genetic divergence. General trends are noted because genetic data remain unpublished.

11 CONCLUSION

Taxus globosa shows foliar variability across the Sierra Madre Oriental. In general, northern populations have larger leaves, supporting the hypothesis that they retain traits from their Nearctic origin. However, separating the variability in leaf blade from petiole attributes also points to phenotypic plasticity as a response to immediate environmental stress and climate changes.

Factor analysis reveals that geographic variation influences the attributes related to temperature and precipitation. However, a negative Mantel test between morphological and climate traits indicates that microenvironmental factors and habitat fragmentation break the latitudinal effect. For example, the Oaxaca population exhibits smaller leaves due to local changes, and broad leaves are present in the middle distribution populations (Hidalgo and San Luis Potosí), positively related to precipitation during the driest month. Additionally, human activities, such as forestry and forest management, impact phenotypic plasticity by altering the forest canopy and creating gaps that induce unexpected morphological changes.

In contrast, petiole structure analysis shows that it is influenced by temperature seasonality, with populations in areas of high temperature variability having shorter, thicker petioles, in a latitudinal gradient from north to south.

In sum, while northern populations of *T. globosa* retain traits of the petiole related to Nearctic origin, the leaf blade is shaped by environmental pressures and anthropogenic factors. This pattern underscores the need to consider both natural selection and immediate responses when evaluating species' adaptive capabilities with restricted distributions.

12 ACKNOWLEDGMENTS

13 DATA AVAILABILITY

The morphological and environmental databases are available to the reader by direct email to the corresponding author pablo_aguilar9900@uaeh.edu.mx.

14 REFERENCES

Anderson MJ. 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). *Wiley StatRef: Statistics Reference Online* 1-15. DOI: 10.1002/9781118445112.stat07841

Atkin OK, Loveys BR, Atkinson LJ, & Pons TL. 2006. Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Botany* 57: 267–281. DOI: 10.1093/jxb/erj029

Bijarpasi MM, Shahraji TR, & Lahiji HS. 2019. Genetic variability and heritability of some morphological and physiological traits in *Fagus orientalis* Lipsky along an elevation gradient in Hyrcanian forests. *Folia Oecologica* 46(1): 45-53. DOI: 10.2478/oecol-2019-0007

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13: 115-155.

Brito-Rocha E, Schilling AC, Dos Anjos L, Piotto D, Dalmolin AC, & Mielke MS. 2016. Regression models for estimating leaf area of seedlings and adult individuals of Neotropical rainforest tree species. *Brazilian Journal of Biology* 76: 983-989.

Busby JR. 1991. BIOCLIM – a bioclimate analysis and prediction system. *Plant Protection Quarterly* 6: 8-9.

Cavender-Bares J, González-Rodríguez A, Eaton DAR, Hipp AAL, Beulke A, & Manos PS. 2015. Phylogeny and biogeography of the American live oaks (*Quercus* subsection *Virentes*): A genomic and population genetics approach. *Molecular Ecology* 24(14): 3668-3687. DOI: 10.1111/mec.13269

Contreras-Medina R, & Luna I. 2001. Presencia de *Taxus globosa* Schlecht. (Taxaceae) en el estado de Chiapas, México. *Polibotánica* (12): 51-55.

Cope EA. 1998. Taxaceae: the genera and cultivated species. *The Botanical Review* 64(4): 291-322.

Delgado AC, Jain SE, Sánchez MSV, & Reyes PC. 2011. Cambios en la morfología floral y foliar en *Crataegus tracyi*: importancia de la asimetría fluctuante como indicador de estrés ambiental. *Biológicas Revista de la DES Ciencias Biológico Agropecuarias* 13(1): 44-49.

Estarague A, Vasseur F, Sartori K, Bastias CC, Cornet D, Rouan L, Beurier G, Exposito-Alonso M, Herbette S, Bresson J, Vile D, Violle C. 2022. Into the range: a latitudinal gradient or a center-margins differentiation of ecological strategies in *Arabidopsis thaliana*? *Annals of Botany* 129(3): 343-356. DOI: 10.1093/aob/mcab149

Fick SE, & Hijmans RJ. 2017. WorldClim 2: new 1‑ km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37(12): 4302-4315.

Filartiga AL, Klimeš A, Altman J, Nobis MP, Crivellaro A, Schweingruber F, & Doležal J. 2022. Comparative anatomy of leaf petioles in temperate trees and shrubs: The role of plant size, environment and phylogeny. *Annals of Botany* 129(5): 567-582. DOI: 10.1093/aob/mcac014

Fox RJ, Dibeksib JM, Schunter C, Ravasi T, & Gaitán-Espitia JD. 2019. Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactios of the Royal Society B: Biological Sciences* 374(1768): 20180174. DOI: 10.1098/rstb.2018.0174

Galván-Hernández DM, Octavio-Aguilar P, Lazcano-Cruz L, & Sánchez-González, A. 2021. Diferenciación morfológica y genética en poblaciones aisladas de haya mexicana *Fagus grandifolia* subsp. mexicana. *Revista de Investigación Forestal* 32 (5): 2169-2179.

Gárate-Escamilla H, Hampe A, Vizcaíno-Palomar N, Robson M, & Garzón MB. 2019 Range-wide variation in local adaptation and phenotypic plasticity of fitness-related traits in *Fagus sylvatica* and their implications under climate change. *Global Ecology and Biogeography* 28(9): 1336-1350. DOI: 10.1111/geb.12936

García-Aranda MA, Estrada-Castillón AE, Jurado-Ybarra E, & González-Uribe DU. 2011. Análisis de once poblaciones naturales de *Taxus globosa* en la Sierra Madre Oriental. *Madera y Bosques* 17(1): 93-104.

Gower JC. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857.

Hammer Ø, Harper DA, & Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Paleontolgia Electronica* 4(1): 9.

Kijowska-Oberc J, Staszak AM, Kaminnski J, & Ratajczak E. 2020. Adaptation of forest trees to rapidlyc changing climate. *Forest* 11(2): 123. DOI: 10.3390/f11020123

Kristensen TN, Ketola T, & Kronholm I. 2020. Adaptation to environmental stress at different timescales. *Annals of the New York Academy of Sciences* 1476(1): 5-22. DOI: 10.1111/nyas.13974

Lambers H, Chapin III FS, & Pons TL. 2008. *Plant Physiological Ecology*. 2nd edition. Springer Science and Business Media. DOI: 10.1007/978-0-387-78341-3

Li Y, Zou D, Shrestha N, Xu X, Wang Q, Jia W, & Wang Z. 2020. Spatiotemporal variation in leaf size and shape in response to climate. *Journal of Plant Ecology* 13(1): 87-96. DOI: 10.1093/jpe/rtz053

Liu W, & Su J. 2016. Effects of light acclimation on shoot morphology, structure, and biomass allocation of two *Taxus* species in southwestern China. *Scientific Reports* 6(1): 35384.

López-Upton J, & Garcia-Martí X. 2015. *Taxus globosa* Schltdl. (Taxaceae): Distribution and diagnosis of an endangered yew. *Earth* 4: 80-88. DOI: 10.11648/j.earth.s.2015040301.13

Maciel-Mata CA, Manríquez-Morán N, Octavio-Aguilar P, & Sánchez-Rojas G. 2015. Geographical distribution of the species: a concept review. *Acta Universitaria* 25(2): 3-9. DOI: 10.15174/au.2015.690

Midolo G, De Frenne P, Hölzel N, & Wellstein C. 2019. Global patterns of intraspecific leaf trait responses to elevation. *Global Change Biology* 25(7): 2485-2498. DOI: 10.1111/gcb.14646

Möller M, Gao LM, Mill RR, Liu J, Zhang DQ, Poudel RC, & Li DZ. 2013. A multidisciplinary approach reveals hidden taxonomic diversity in the morphologically

challenging *Taxus wallichiana* complex. *Taxon* 62(6): 1161-1177. DOI: 10.12705/626.9

Muñoz-Gutiérrez L, Ramírez-Sánchez SE, & Velasco-García MV. 2019. Review on the distribution and conservation of *Taxus globosa* Schltdl. (Taxaceae) in Mexico. *Revista Mexicana de Ciencias Forestales* 10(56): 65-84.

Petit RJ, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M, Mohanty A, Müller-Starck G, Demesure-Musch B, Palmé A, Martín JP, Rendell S, & Vendramin G. 2003. Glacial refugia: Hotspots but not melting pots of genetic diversity. *Science* 300 (5625): 1563-1565. DOI: 10.1126/science.1083264

Pérez-García B, & Mendoza A. 2002. Morfología vegetal neotropical. *Revista de Biología Tropical* 50: 893-902.

Ramírez-Amezcua Y, Steinmann VW, Ruiz-Sánchez E, & Rojas-Soto OR. 2016. Mexican alpine plants in the face of global warming: potential extinction within a specialized assemblage of narrow endemics. *Biodiversity and Conservation* 25: 865-885. DOI: 10.1007/s10531-016-1094-x

Ramírez-Sánchez SE, López-Upton J, García de los Santos G, Vargas-Hernández JJ, Hernández-Livera A, & Ayala-Garay ÓJ. 2011. Variación morfológica de semillas de *Taxus globosa* Schltdl. provenientes de dos regiones geográficas de México. *Revista Fitotecnia Mexicana* 34(2): 93-99.

Riordan EC, Gugger PF, Ortego J, Smith C, Gaddis K, Thompson P, & Sork V. 2016. Association of genetic and phenotypic variability with geography and climate in three southern California oaks. *American Journal of Botany* 103(1): 73-85. DOI: 10.3732/ajb.1500135

Sevik H, Cetin M, Ozel HB, Erbek A, & Zeren CI. 2021. The effect of climate on leaf micromorphological characteristics in some broad-leaved species. *Environment, Development and Sustainability* 23: 6395-6407. DOI: 10.1007/s10668-020-00877-w

Shemluck MJ, Estrada E, Nicholson R, & Brobst SW. 2003. A preliminary study of the taxane chemistry and natural history of the Mexican yew, *Taxus globosa* Schltdl. *Botanical Sciences* (72): 119-127. DOI: 10.17129/botsci.1672

Statsoft Inc. 2017. Statistica for Windows. Computer program manual. StatSoft, Inc. http://statistica.software.informer.com/13.0.

Strandby U, Christensen KI, & Sørensen M. 2009. A morphometric study of the *Abies religiosa–hickelii–guatemalensis* complex (Pinaceae) in Guatemala and Mexico. *Plant Systematics and Evolution* 280(1): 59-76. DOI: 10.1007/s00606-009-0164-x

Zamudio RS. 1992. Familia Taxaceae, Flora del Bajío y de sus regiones adyacentes, Fascículo 9. Instituto de Ecología, A. C., Centro Regional del Bajío. Pátzcuaro, Michoacán. 6 p.

Zavala-Chávez F. 2001. Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México. I: Población de adultos y algunas características del hábitat. *Ciencia Ergo Sum* 8(2): 169-174.

Zerey-Belaskri A, & Benhassaini H. 2016. Morphological leaf variability in natural populations of *Pistacia atlantica* Desf. subsp. *atlantica* along climatic gradient: new features to update *Pistacia atlantica* subsp. *atlantica* key. *International Journal of Biometeorology* 60: 577-589. DOI: 10.1007/s00484-015-1052-4

CAPÍTULO III

Variación genética de las poblaciones de *Taxus globosa* a lo largo de la Sierra Madre Oriental, México

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

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15 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 (Fis = 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.

16 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; Cruz-Salazar *et al*. 2023). This disruption is because

mountainous geography creates natural barriers, in addition to those generated by fragmentation, that impede gene flow and impoverish genetic diversity. Such genetic impoverishment limits the trees' ability to adapt to climate change. It can increase the risk of gene loss 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 essential 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), leading to the overexploitation of yew species and significant 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 crucial 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.

17 MATERIAL AND METHODS

17.1 Study area
The study locations were located 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).
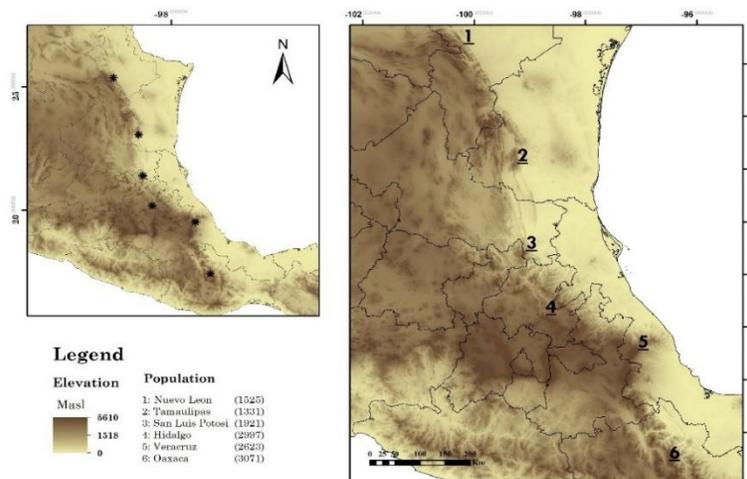
Figure 1. A distribution map of the six populations of *Taxus globosa* sampled in this study. These populations are located along the Mexican Sierra Madre Oriental.

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

17.2 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.

17.3 DNA extraction

For DNA extraction, the protocol of Doyle & Doyle (1987) was used with modifications to adequately 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 Na$_2$).

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.

17.4 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 (1.5µl); 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.

Table 2. Microsatellites used in the present study are based on those reported for *Taxus wallichiana*, *Taxus yunnanensis*, and *Taxus baccata*. Reported by Dubreuil *et al.* (2008); Miao *et al.* (2008) and Yang *et al.* (2009).

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

* 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).

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.

17.5 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 polymorphic loci (P%), average number of alleles per locus (A), adequate number of alleles per locus (Ne), Shannon index (I), observed and expected heterozygosity (HO and HE), inbreeding coefficient (Fis), 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 (Fst, Fis, Fit). The null hypothesis (Fst = 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*. 2001) and assesses mean distances between clusters. 3) Bayesian assignment analysis identifies exclusive genetic groups using a 100,000-step probabilistic model with 10 repetitions. 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.

18 RESULTS

18.1 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.

18.2 Genetic structure

The molecular variance analysis showed significant differences between populations ($Fst = 0.21$, $p < 0.001$), within populations ($Fis = 0.74$, $p < 0.001$), and between individuals ($Fit = 0.8$, $p < 0.001$). 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 2A).

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 2C, D).

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	
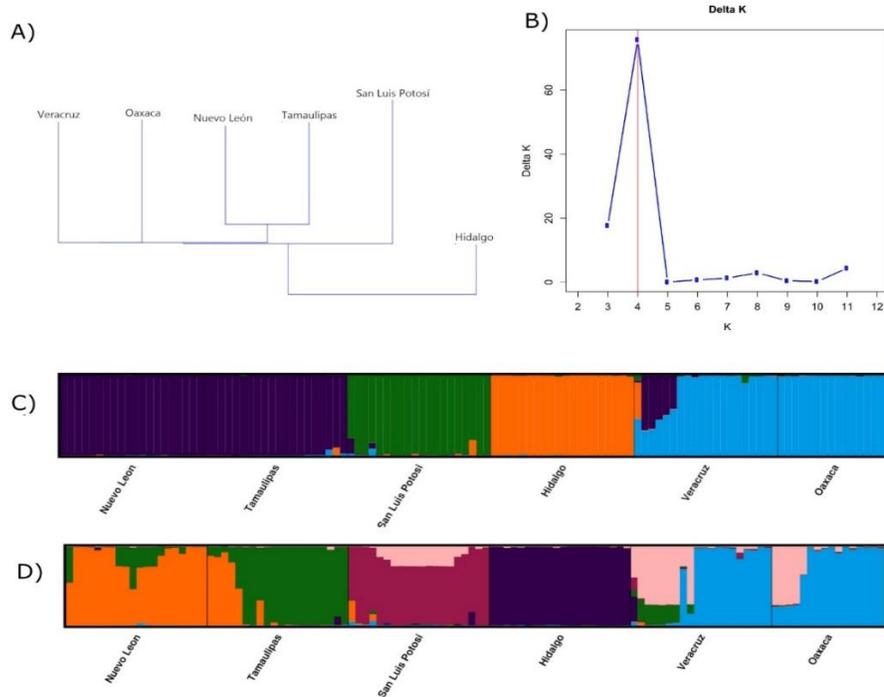
Figure 2. A) Clustering analysis by Nei genetic distances using the neighbor-joining algorithm. A 1000-step bootstrap supports the tree branches; B) Bayesian assignment analysis of individuals, Evanno *et al*. (2005) plot; C) Clustering diagram for K = 4; D) Clustering diagram for K = 6 corresponding to the original populations analyzed.
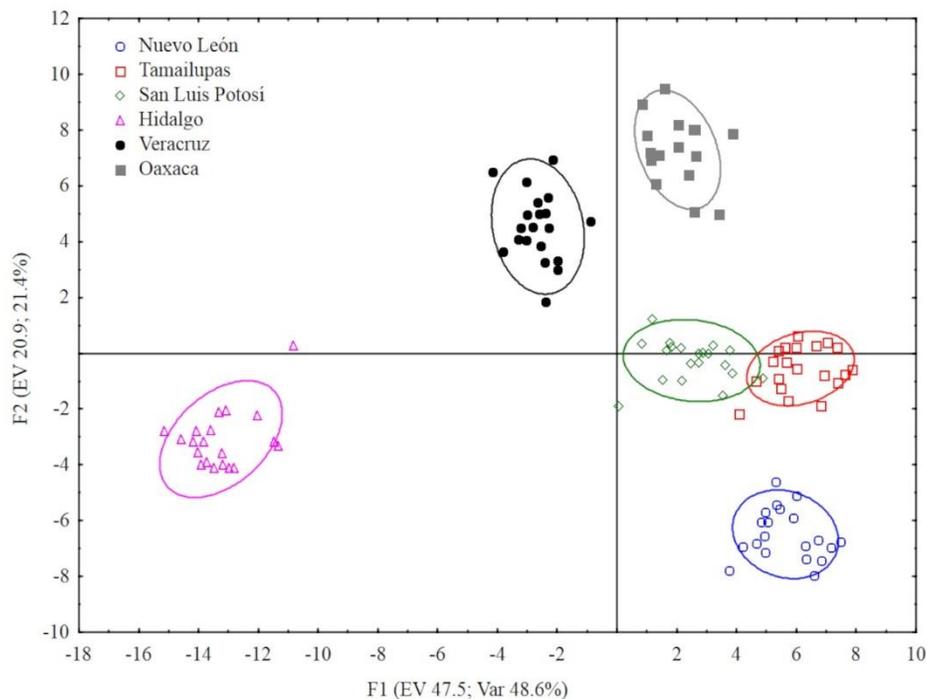
Figure 3. Discriminant Analysis of Principal Component for genetic variation among the *Taxus globosa* populations analyzed.

The 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).

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	0.00029	T = 3.377 p = 0.00037
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	0.0023	T = 2.7 p = 0.00347
5	Veracruz	8.86	0.0535	T = 1.97 p = 0.02443
6	Oaxaca	8.68	0.00002	T = 3.913 p = 0.00005

19 DISCUSSION

19.1 Genetic diversity

Our study of *Taxus globosa* provides important 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.

19.2 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 hartwegii, 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. globose*, 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). In addition to the isolation that exacerbates inbreeding, fragmentation leads to a random loss of genetic diversity, pushing local populations toward an extinction vortex. 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).

20 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 be essential for developing effective conservation strategies to ensure the survival of this endangered conifer.

21 ACKNOWLEDGEMENTS

22 REFERENCES

Alsos IG, Ehricj D, Thuiller W, Eidensen PB, Tribsch A, Schönswetter P, Lagaye C, Taberlet P, & Brochmann C. 2012. Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society B: Biological Sciences*, 279(1735), 2042–2051. DOI: 10.1098/rspb.2011.2363

Aguirre-Planter E, Furnier GR, & Eguiarte LE. 2000. Low levels of genetic variation within and high levels of genetic differentiation among populations of species of *Abies* from southern Mexico and Guatemala. *American Journal of Botany*, 87(3), 362–371. DOI: 10.2307/2656632

Carvalho YGS, Vitorino LC, de Souza UJB, & Bessa LA. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity,* 11(4), 62. DOI: 10.3390/d11040062

Chybicki IJ, & Burczyk J. 2009. Simultaneous estimation of null alleles and inbreeding coefficients. *Journal of Heredity,* 100(1), 106-113. DOI: 10.1093/jhered/esn088

Collins D, Mill RR, & Möller M. 2003. Species separation of *Taxus baccata*, *T. canadensis*, and *T. cuspidata* (Taxaceae) and origins of their reputed hybrids inferred from RAPD and cpDNA data. *American Journal of Botany,* 90(2), 175-182. DOI: 10.3732/ajb.90.2.175

Cornuet JM, & Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics,* 144(4),

2001-2014. DOI: 10.1093/genetics/144.4.2001

Cruz-Nicolás J, Vargas-Hernández JJ, Ramírez-Vallejo P, & López-Upton J. 2011. Diversidad genética y diferenciación de las poblaciones de *Pseudotsuga menziesii* (Mirb.) Franco en México. *Revista Fitotecnia Mexicana,* 34(4), 233-240. DOI: 10.35196/rfm.2011.4.233

Doyle JJ, & Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin,* 19, 11-15.

Dubreuil M, Sebastiani F, Mayol M, González-Martínez SC, Riba M, & Vendramin GG. 2008. Isolation and characterization of polymorphic nuclear microsatellite loci in *Taxus baccata* L. *Conservation Genetics,* 9(6), 1665-1668. DOI: 10.1007/s10592-008-9515-3

Evanno G, Regnaut S, & Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14(8), 2611-2620. DOI: 10.1111/j.1365-294X.2005.02553.x

Falush D, Stephens M, & Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics,* 164(4), 1567-1587. DOI: 10.1093/genetics/164.4.1567

Falush D, Stephens M, & Pritchard JK. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes,* 7(4), 574-578. DOI: 10.1111/j.1471-8286.2007.01758.x

Gao LM, Möller M, Zhang XM, Hollingsworth ML, Liu J, Mill RR, & Li DZ. 2007. High variation and strong phylogeographic pattern among cpDNA haplotypes in *Taxus wallichiana* (Taxaceae) in China and North Vietnam. *Molecular Ecology,* 16(22), 4684-4698. DOI: 10.1111/j.1365-294X.2007.03537.x

García-Montes MA, Rubio-Tobón CA, Islas-Barrios Y, Serrato-Díaz A, Figueredo-Urbina, CJ, Galván-Hernández DM, & Octavio-Aguilar P. 2020. The influence of anthropogenic disturbance on the genetic diversity of *Ceratozamia fuscoviridis* (Zamiaceae). *International Journal of Plant Sciences,* 181(5), 497-508. DOI: 10.1086/707108

Gargiulo R, Saubin M, Rizzuto G, West B, Fay MF, Kallow S, & Trivedi C. 2019. Genetic diversity in British populations of *Taxus baccata* L.: Is the seedbank collection representative of the genetic variation in the wild? *Biological Conservation,* 233, 289-297. DOI: 10.1016/j.biocon.2019.01.014

González AV, Gómez-Silva V, Ramírez MJ, & Fontúrbel FE. 2020. Meta-analysis of the differential effects of habitat fragmentation and degradation on plant genetic diversity. *Conservation Biology,* 34(3), 711-722. DOI: 10.1111/cobi.13422

Hammer Ø, Harper DAT, & Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* 4(1), 9 pp.

Hilfiker K, Gugerli F, Schütz JP, Rotach P, & Holderegger R. 2004. Low

RAPD variation and female-biased sex ratio indicate genetic drift in small populations of the dioecious conifer *Taxus baccata* in Switzerland. *Conservation Genetics,* 5(3), 357-365. DOI: 10.1023/B:COGE.0000031144.95293.1b

Hilfiker K, Holderegger R, Rotach P, & Gugerli F. 2004. Dynamics of genetic variation in *Taxus baccata*: local versus regional perspectives. *Canadian Journal of Botany,* 82(2), 219-227. DOI: 10.1139/b03-136

Jombart T, Devillard S, & Balloux F. 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics,* 11, 94. DOI: 10.1186/1471-2156-11-94

Juan EJ. 2023. Estructura genética de *Pinus hartwegii Lindl.* en el Parque Nacional La Malinche (Tesis de Maestría). Universidad Autónoma de Tlaxcala. Disponible en: repositorio.uatx.mx:8443/jspui/handle/DSyTI_UATx/719

Jiménez-García D, & Peterson AT. 2019. Climate change impact on endangered cloud forest tree species in Mexico. *Revista Mexicana de Biodiversidad,* 90, Article e902781. DOI: 10.22201/ib.20078706e.2019.90.2781

Kikuchi Y, & Yatagai M. 2003. The commercial cultivation of *Taxus* species and production of taxoids. In H. Itokawa & K.-H. Lee (Eds.), *Taxus: The Genus Taxus,* pp. 151–178. DOI: 10.4324/9780203381144-10

Lazar I, & Lazar I. 2017. Gel Analyzer 2010a: Freeware 1D gel electrophoresis image analysis software. Available at: www.gelanalyzer.com/index.html

Ledig FT, Hodgskiss PD, & Johnson DR. 2000. Genetic diversity and breeding structure of *Abies religiosa*, a Mexican endemic. *American Journal of Botany*, 87(3), 386-398. DOI: 10.2307/2656633

Leija-Loredo EG, Pavón NP, Sánchez-González A, Rodríguez-Laguna R, & Ángeles-Pérez G. 2018. Land cover change and carbon stores in a tropical montane cloud forest in the Sierra Madre Oriental, Mexico. *Journal of Mountain Science*, 15(10), 2136-2147. DOI: 10.1007/s11629-018-4937-y

Lewandowski A, Burczyk J, & Mejnartowicz L. 1995. Genetic structure of English yew (*Taxus baccata* L.) in the Wierzchlas Reserve: implications for genetic conservation. *Forest Ecology and Management*, 73(1-3), 221-227. DOI: 10.1016/0378-1127(94)03477-E

Li YL, & Liu JX. 2018. StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources*, 18(1), 176–177. DOI: 10.1111/1755-0998.12719

López-Upton J, & García-Martí X. 2015. *Taxus globosa* Schltdl. (Taxaceae). Distribution and diagnosis of an endangered yew. *Earth Sciences*, 4(3-1), 80-88. DOI: 10.11648/j.earth.s.2015040301.13

Love SJ, Schweitzer JA, Woolbright SA, & Bailey JK. 2023. Sky islands are a global tool for predicting the ecological and evolutionary consequences of climate change. *Annual Review of Ecology,*

Evolution, and Systematics, 54, 219-236. DOI: 10.1146/annurev-ecolsys-102221-050029

Martin PR, & McKay JK. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution*, 58(5), 938-945. DOI: 10.1111/j.0014-3820.2004.tb00428.x

McLaughlin SP. 1994. An overview of the flora of the Sky Islands, southeastern Arizona: diversity, affinities, and insularity. En L. F. DeBano, P. F. Ffolliott, A. Ortega-Rubio, G. J. Gottfried, R. H. Hamre, & C. B. Edminster (Eds.), *Biodiversity and management of the Madrean Archipelago: The Sky Islands of Southwestern United States and Northwestern Mexico.* United States Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station., pp. 60-70.

Miao YC, Su JR, Zhang ZJ, Li H, Luo J, & Zhang YP. 2008. Isolation and characterization of microsatellite markers for the endangered *Taxus yunnanensis*. *Conservation Genetics*, 9, 1683-1685. DOI: 10.1007/s10592-008-9532-2

Miller JM, Cullingham CI, & Peery RM. 2020. The influence of a priori grouping on inference of genetic clusters: simulation study and literature review of the DAPC method. *Heredity*, 125(5), 269-280.DOI:10.1038/s41437-020-0348-2

Mohapatra KP, Sehgal RN, Sharma RK, & Mohapatra T. 2008. Evaluation of genetic variation in West Himalayan yew and its associates using RAPD and AFLP markers. *Journal of Plant Biochemistry and Biotechnology*, 17, 161-165. DOI: 10.1007/BF03263278

Mohapatra KP, Sehgal RN, Sharma RK, & Mohapatra T. 2009. Genetic analysis and conservation of endangered medicinal tree species *Taxus wallichiana* in the Himalayan region. *New Forests*, 37, 109-121. DOI: 10.1007/s11056-008-9112-9

Muñoz-Gutiérrez L, Ramírez-Sánchez SE, & Velasco-García MV. 2019. Review on the distribution and conservation of *Taxus globosa* Schltdl. (Taxaceae) in Mexico. *Revista Mexicana de Ciencias Forestales*, 10(56), 65-84.

Nieto-Blázquez ME, Quiroga MP, Premoli AC, & Roncal J. 2021. Podocarpus in the paleogeographically complex island of Hispaniola: a stepping-stone colonization and conservation recommendations. *Diversity and Distributions*, 28(2), 214-226. DOI: 10.1111/ddi.13444

Nordstrom SW, Hufbauer RA, Olazcuaga L, Durkee LF, & Melbourne BA. 2023. How density dependence, genetic erosion and the extinction vortex impact evolutionary rescue. *Proceedings of the Royal Society B: Biological Sciences*, 290, Article 20231228. DOI: 10.1098/rspb.2023.1228

Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, 13(5), 1143-1155. DOI: 10.1111/j.1365-294X.2004.02141.x

Peakall ROD, & Smouse PE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1),

288-295. DOI: 10.1111/j.1471-8286.2005.01155.x

Peña-Malavera A, Bruno C, Fernández E, & Balzarini M. 2014. Comparison of algorithms to infer genetic population structure from unlinked molecular markers. *Statistical Applications in Genetics and Molecular Biology*, 13(4), 391-402. DOI: 10.1515/sagmb-2013-0006

Pianka ER. 2011. *Evolutionary Ecology* (7.ª ed., eBook). Harper & Row. 356 p, ISBN 978-0065012255.

Piry S, Luikart G, & Cornuet JM. 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, 90(4), 502-503. DOI: 10.1093/jhered/90.4.502

Pritchard JK, Stephens M, & Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959. DOI: 0.1093/genetics/155.2.945

Rico Y, León-Tapia MÁ, Zurita-Solís M, Rodríguez-Gómez F, & Vásquez-Morales SG. 2021. Influence of Pleistocene climatic oscillations on the phylogeography and demographic history of endemic vulnerable trees (section Magnolia) of the Tropical Montane Cloud Forest in Mexico. *PeerJ*, 9, e12181. DOI: 10.7717/peerj.12181

Rosales-Islas E, & Octavio-Aguilar P. 2023. Effect of forest management on the genetic diversity of *Abies hidalgensis*, a threatened species with restricted distribution. *Journal of Forest Science*, 69(5), 193-204. DOI: 10.17221/13/2023-JFS

Sáenz-Romero C, Rehfeldt GE, Duval P, & Lindig-Cisneros RA. 2016. Genetic variation of *Pinus hartwegii* in central Mexico and the influence of climate change. *Tree Genetics & Genomes*, 12(3), 48. DOI: 10.1007/s11295-016-0995-0

Saikia D, Khanuja SPS, Shasany AK, Darokar MP, Kukreja AK, & Kumar S. 2000. Assessment of diversity among *Taxus wallichiana* accessions from Northeast India using RAPD analysis. *Plant Genetic Resources News*, 121, 27-31.

Schippmann U. 2001. Medicinal Plants Significant Trade Study. CITES Project S-109. Plants Committee Document PC9 9.1.3 (rev.). Bundesamt für Naturschutz (BfN), Bonn, Germany.

SEMARNAT. 2010. *Protección ambiental – Especies nativas de México de flora y fauna silvestres – Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio – Lista de especies en riesgo*. Norma Oficial Mexicana NOM-059-SEMARNAT-2010. Diario Oficial de la Federación, Segunda Sección, 30 de diciembre de 2010. Disponible en: www.profepa.gob.mx/innovaportal/file/435/1/nom_059_semarnat_2010.pdf

Soto Hernández M, Sanjurjo M, González Garza MT, Cruz Vega D, & Giral González F. 2000. El tejo mexicano (*Taxus globosa* Sch.). Potencial de su aprovechamiento en taxol. *CIENCIA ergo-sum*, 7(3), 277-279.

Soto-Hernández M, López-Upton J, Vargas-Hernández JJ, Muñoz-Gutiérrez L, & San Miguel R.

2011. Estado de conservación de *Taxus globosa* en México. *Spanish Journal of Rural Development*, 2(2), 61-68. DOI: 10.5261/2011.ESP2.07

StatSoft Inc. 2011. STATISTICA, version 10 (data analysis software system). Tulsa, OK. Available at: www.statsoft.com

Takeya K. 2003. Plant tissue culture of taxoids. Taxus. London, UK, and New York, NY: CRC Press - Taylor and Francis, pp. 151-178.

Valencia-Ávalos S. 2004. Diversidad del género *Quercus* (Fagaceae) en México. *Boletín de la Sociedad Botánica de México*, 75, 33-53. DOI: 10.17129/botsci.1691

Wani MC, Taylor HL, Wall ME, Coggon P, & McPhail AT. 1971. Plant antitumor agents: VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *Journal of the American Chemical Society*, 93(9), 2325-2327. DOI: 10.1021/ja00738a045

Yang JB, Li HT, Li DZ, Liu J, & Gao LM. 2009. Aislamiento y caracterización de marcadores microsatélites en la especie en peligro de extinción *Taxus wallichiana* mediante el método FIASCO. *HortScience*, 44(7), 2043-2045. DOI: 10.21273/HORTSCI.44.7.2043

Zavala-Chávez F. 2001. Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México. I: Población de adultos y algunas características del hábitat. *Ciencia Ergo Sum*, 8(2), 169-174.

Zavala-Chávez F, Soto-Hernández M, & Rodríguez-González MT. 2001. El romerillo (*Taxus globosa* Schltdl.): Biología, dificultades y perspectivas de su uso. *Revista Chapingo Serie Horticultura*, 7(1), 77-94.

Zavala-Chávez F. 2002. Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México. II: Población de juveniles y algunos datos de semillas. *Ciencia Ergo Sum*, 9(2), 177-183.

Zhang Y, Song YG, Zhang CY, Wang TR, Su TH, Huang PH, Meng HH, & Li J. 2022. Latitudinal diversity gradient in the changing world: retrospectives and perspectives. *Diversity*, 14(5), 334. DOI: 10.3390/d14050334

Zhang XM, Gao LM, Möller M, & Li DZ. 2009. Molecular evidence for fragmentation among populations of *Taxus wallichiana* var. *mairei*, a highly endangered conifer in China. *Canadian Journal of Forest Research*, 39(4), 755-764. DOI: 10.1139/X09-003

CAPÍTULO IV

Análisis demográfico y de perturbación de las poblaciones de *Taxus globosa* a lo largo de la Sierra Madre Oriental, México

Disturbance, demography, and threats: Assessing *Taxus globosa* populations along the Sierra Madre Oriental, Mexico

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Running title

1. *Taxus globosa* under threat

2. Disturbance and demography of *Taxus globosa*

3. *Taxus globosa* populations in Mexico

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23 ABSTRACT

Background: *Taxus globosa* is a threatened endemic plant in Mexico. It has a restricted and discontinuous distribution in the Sierra Madre Oriental. Its population assessment classifies it as threatened, but risk factors for the species have not been evaluated.

Questions and/or Hypotheses: How does disturbance demographically affect *Taxus globosa* populations distributed throughout the Sierra Madre Oriental?

Study species: *Taxus globosa*

Study sites and years: States of Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, Veracruz, and Oaxaca. Years 2022, 2023, and 2024

Methods: Six populations located along a latitudinal gradient were assessed to analyze their demographic structure and the impact of anthropogenic disturbance on their populations. Five groups were identified based on three dasometric attributes, which were used to generate static life tables. In addition, indicators of anthropogenic disturbance were evaluated in each population to determine the disturbance index.

Results: Population growth rates (λ) show that the Veracruz and Tamaulipas populations have declining populations ($\lambda < 1$), while the Hidalgo, San Luis Potosí, and Oaxaca populations maintain positive growth. Disturbance analysis revealed that logging, human activities, and trails are the environmental modifications with the greatest impact on populations. A significant negative correlation ($rs^2 = -0.93$, $p = 0.02$) between logging and λ indicates that this activity is the main population-limiting factor.

Conclusions: Effective conservation of *T. globosa* should focus on protecting stable populations; regulating logging; and restoring disturbed habitats, which are primarily caused by logging.

*Keywords***:** *Taxus globosa*, demography, anthropic disturbance, population growth rate, life tables, threatened species

24 INTRODUCTION

Natural and anthropogenic disturbances significantly influence the population dynamics of plant species with restricted distributions and specific ecological requirements. Factors such as deforestation, overgrazing, and forest fires modify community structure, limit regeneration, and reduce functional and genetic diversity (Chen *et al*. 2024, Song *et al*. 2024, Wang *et al*. 2024). *Taxus globosa* is a gymnosperm endemic to Mexico. Its distribution is one of the most restricted in the world, as its habitat is very specific, scarce, and threatened (Cope 1998). It grows in sites with well-drained acidic soils and abundant organic matter, and commonly coexists with some broadleaf trees, mainly of the genus *Quercus*, as well as

species of the genera *Abies*, *Pinus*, and *Picea* (Zavala-Chávez 2001, Luna-Vega 2003). It is observed between 1000 and 3000 meters above sea level, and its preferred sites are small ravines where the average annual temperature ranges between 14.5 and 15.4 °C, often with fog, dew, and frost (Zavala-Chávez 2001). Its populations are made up of patches, strips, or isolated individuals in Mexico, Guatemala, El Salvador, and Honduras (Medina & Vega 2001, Shemluck *et al.* 2003).

This species has been classified as threatened due to habitat fragmentation, illegal logging, and land-use changes (CONABIO 2010). *T. globosa* populations have a demographic structure that is highly sensitive to changes in disturbance regimes, including fires, selective logging, and agricultural activities, which can alter recruitment, growth, and mortality patterns, as is the case with species that coexist with this tree (Zavala-Hurtado *et al.* 2003, Martínez-Ramos *et al.* 2016). The plant grows in threatened and limited plant communities such as cloud forests, *Abies* forests, and communities exploited for forestry such as pine and pine-oak forests, so these types of vegetation are in a critical state due to timber extraction, reduction in cover due to land use change, and climate change (Malda 1990, Leija-Loredo *et al.* 2018). In addition, it has been identified that the distribution of *T. globosa* has decreased drastically in recent decades (García-Aranda *et al.* 2011, 2012). For this reason, it has been classified in the "Endangered" category by the Red List of the International Union for Conservation of Nature (Thomas P. 2013), as well as in the "Subject to Special Protection" category according to Mexican Official Standard NOM-059-SEMARNAT-2010 (SEMARNAT 2010).

Demographic studies have shown that long-lived species with low regeneration rates, such as *T. globosa*, are particularly susceptible to disturbances that interfere with early life cycle phases, such as germination and seedling establishment (Erlichman *et al.* 2024). Given that its distribution area is one of the most threatened (López-Upton & García Martí 2015), it is necessary to identify the correlation between its survival and disturbance within its populations, since it is known that both environmental factors and changes caused by anthropogenic disturbance can modulate morphological, genetic, and ecological characteristics of the species (Antúnez 2021, Sevik *et al.* 2021). In this context, it is essential to assess population structure and regeneration patterns across disturbance gradients

throughout this tree's natural distribution in order to identify critical threats and propose effective conservation strategies.

Although demographic data are available for *T. globosa* populations in the Sierra Madre Oriental, they are limited to a few populations, specifically in the states of Nuevo León and Tamaulipas, the latter located within the El Cielo Biosphere Reserve (García-Aranda *et al.* 2011). Therefore, the objectives of this work are: 1) to establish demographic parameters related to population viability through the analysis of static life tables, in order to identify life cycle categories susceptible to management; 2) to determine the degree of disturbance associated with the six *T. globosa* study populations; 3) to measure the correlation between the demographic component and the degree of disturbance in order to identify specific threats to the species by population.

25 MATERIALS AND METHODS

25.1 Study área

The study localities are distributed along the Sierra Madre Oriental of Mexico, from north to south in Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, Veracruz and Oaxaca; states where there are previous records of collection (López-Uptón & García-Martí 2015, Muñoz-Gutiérrez *et al*. 2019, Figure 1, Table 1).

Table 1. *Taxus globosa* populations sampled in the present study. Data on vegetation type and associated genera are from López-Uptón & García-Martí 2015; Muñoz-Gutiérrez *et al*. 2019.

ID	State	Longitude	Latitude	Altitude	Vegetation Type	Associated Tree Genera
1	Nuevo León	-100.196	25.36475	1525	BA, BA-Q	*Abies, Quercus*
2	Tamaulipas	-99.229	23.05803	1331	BMM, BP, BA	*Abies, Garrya, Pinus*
3	San Luis Potosí	-99.0652	21.40017	1921	BC	*Abies, Pinus, Pseudotsuga, Quercus*
4	Hidalgo	-98.7133	20.18503	2997	BMM, BP-A	*Abies, Garrya, Pinus,*
5	Veracruz	-97.0603	19.518086	2623	BA, BP	*Abies, Pinus*
6	Oaxaca	-96.493	17.41172	3071	BA, BA-Q	*Abies, Quercus*

BC: coniferous forest, BMM: montane mesophilic forest, BA: Abies forest, BA-Q: Abies-Quercus forest, BP: Pinus forest, BP-A: Pinus-Garrya forest, BPE: Pinus-Quercus forest.
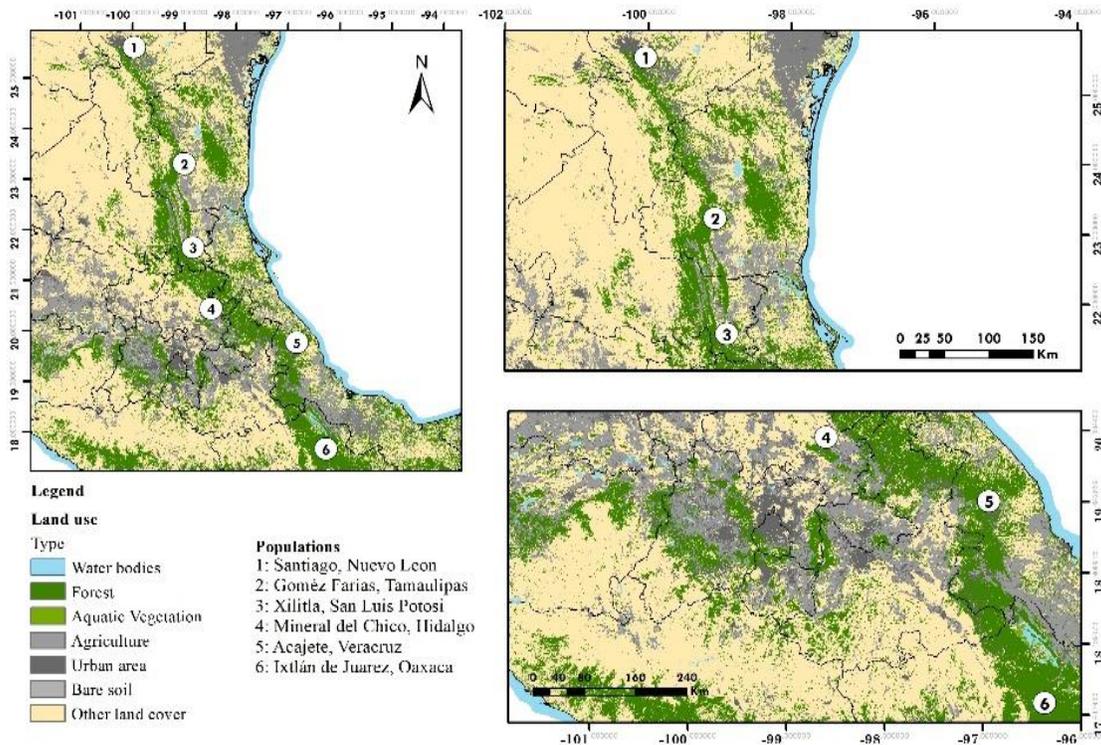
Figure 1. Location of the six populations of *Taxus globosa* that were used for the present study.

25.2 Demographic analysis

In the field, all individuals comprising each population were geographically located, and each one was measured for height, stem diameter at breast height, crown diameter (length and width), angle of insertion of the first branch, and the number of reproductive structures (female or male), using a tape measure, a clinometer, and a GPS receiver (Magellan$^{®}$ eXplorist$^{®}$ 110). These traits were entered into a cluster analysis using Euclidean distance measures, a Ward amalgamation model, and a 10,000-step bootstrap procedure to define the morphological groups into which the individuals would be classified. With this data, together with information on the reproductive component, a static life table (SLT) was constructed for each population.

The demographic features for the SLT were: i) lx: survival rate between classes, and ii) mx: average fertility of each class in reproductive age (Castillo-Lara *et al*. 2018). From these values, the reproductive rate R_0 ($\Sigma l_x m_x$) and generation time T_G ($\frac{\Sigma X x l x m x}{\Sigma l x m x}$) were derived, needed to calculate the per capita (intrinsic) population growth rate ($r \approx \frac{(\ln Ro)}{TG}$), which tells

us the approximate unweighted population increase from one generation to the next (Valverde *et al*. 2005). With these data, the finite population growth rate was calculated ($\lambda =$ $\exp^r$).

25.3 Disturbance analysis

Seven 20×1 m (140 m^2) transects were laid at each site. Each transect was subdivided into 1×1 m squares, on which nine disturbance variables were recorded, subdivided into three categories: human activities, livestock, and habitat deterioration (Table 2).

Table 2. Variables associated with disturbance that were evaluated in *Taxus globosa* populations (modified from Martorell & Peters 2005).

No	Variable	ID
1	Presence of cuts/machete cuts	PC
2	Human activity (crops, fences, trash, etc.)	HA
3	Human trails (sidewalks)	HT
4	Livestock trails (roads)	LT
5	Evidence of fire (campfires, logs)	EF
6	Cattle excrement	CE
7	Goat excrement	GE
8	Totally altered surface (roads, pavement, houses)	TAS
9	Timber extraction (stumps, sawmills)	TE

The percentage of disturbance (%D) in each population was calculated with the following formula:

$$\%D = \frac{\left[\dfrac{\text{área afectada}}{20\ m^2} \ x\ 7\ transectos\right]}{100}$$

The attributes per site were used as variables for a non-metric dimensional ordination (NMDS) based on Euclidean distances, which enabled differences between the conservation status of the sites to be detected. The centroid coordinates per group, obtained from the NMDS of the disturbance, were used to calculate a matrix of Euclidean distances between locations, which was correlated by a Mantel test with the absolute differences between population growth rates (λ), to establish whether there is an effect of disturbance on the evaluated demographic attributes. Because Euclidean distances pool information, the relationships between each disturbance variable and absolute differences in λ were explored

individually using non-parametric Spearman correlations. For all the analyses described, Past v.4.05 was used. (Hammer *et al*. 2001).

26 RESULTS
26.1 Demography
A total of 146 trees from six populations distributed from Nuevo León to Oaxaca were evaluated. Cluster analysis generated five groups of trees with distinct characteristics (Table 3).

Table 3. Morphological attributes of the trees evaluated in six Mexican populations of *Taxus globosa* (mean ± standard deviation).

	Overall plant length (cm)	Angle of first branch	Diameter at breast height (cm)	Fork height (cm)	Crown diameter 1 (cm)	Crown diameter 2 (cm)	Number of fruits
Group 1	37.70 ± 27.7	19.05 ± 17.6	0.59 ± 0.42	0.62 ± 2.5	33.22 ± 21.4	30.97 ± 19.7	0.28 ± 1.9
Group 2	114.82 ± 34.5	34.18 ± 16.7	1.22 ± 0.47	7.42 ± 16.5	99.93 ± 29	99 ± 30.3	0.40 ± 2
Group 3	247 ± 64.1	46.05 ± 20.8	2.45 ± 2	25.54 ± 24	198.11 ± 47.8	198.07 ± 47.2	4.39 ± 5.2
Group 4	404.22 ± 120.6	46.54± 25.6	5.92 ± 3.5	19 ± 30	325.94 ± 116.7	317.17 ± 126.6	5.44 ± 14.9
Group 5	682.50 ± 175.7	44.71 ± 23.8	19.17 ± 10.6	98.21 ± 60.6	737 ± 160.1	788.75 ± 253.9	20.63 ± 40.7

The traits that best explain the variation between groups are overall plant length (height), diameter at breast height, average crown diameter, and to a lesser extent, the angle of insertion of the first branch. Six plants in the lower categories (groups 1 and 2) bore fruit, so they were considered early individuals in the demographic analysis (6.5% of non-reproductive individuals).

The morphological structure within the populations showed that in Hidalgo and San Luis Potosí, there are no individuals from group 5, in Oaxaca and Hidalgo group 2 is overrepresented, and in Tamaulipas the distribution is homogeneous without a clear demographic trend (Figure 2). In Nuevo León, San Luis Potosí and Hidalgo, group 3 contains the majority of the fruits (21, 52 and 28 respectively), while in Tamaulipas and Veracruz only individuals from group 5 reproduce (15 and 7 respectively). In Oaxaca, reproductive females are precocious in groups 1 and 2, which suggests pruning cuts.

Overall, the species could be considered stable, since the average λ is 1.02 ± 0.3, so demographic variation appears to be caused by specific events within populations. However,

it is clear that there are significant differences in population structure across the Sierra Madre Oriental, particularly in Ro, TG, and both population growth rates (Table 4).

These groupings identified that the populations of Tamaulipas and Veracruz are in population decline, with reproductive events only in group 5. In contrast, the populations of San Luis Potosí, Hidalgo, and Oaxaca are increasing, with reproductive events starting in group 2.
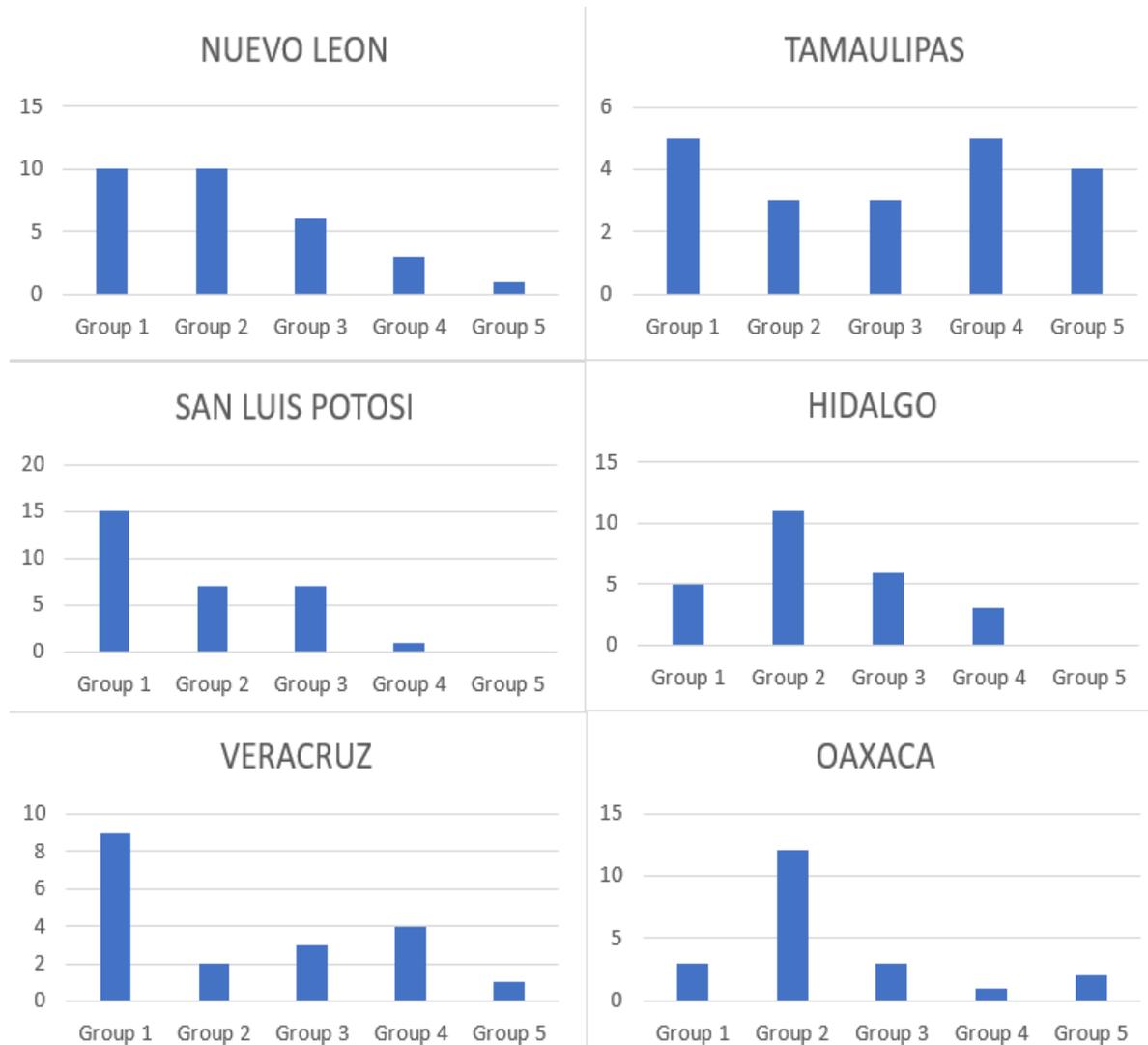

Figure 2. Morphological distribution within the evaluated *Taxus globosa* populations. Populations are ordered by latitude from north to south.

Table 4. Demographic parameters of population growth for *Taxus globosa* populations. Ro: net reproductive rate, TG: generation time (transition between size category groups until the first reproductive event), r: intrinsic population growth rate, λ: finite population growth rate.

Population	*Ro*	*TG*	*r*	*λ*
Nuevo León	1	3.14	0	1
Tamaulipas	0.4	5	-0.17	0.83
San Luis Potosí	2.53	3.02	0.30	1.35
Hidalgo	2	2.98	0.23	1.26
Veracruz	0.04	5	-0.60	0.54
Oaxaca	1.24	1.78	0.12	1.13

26.2 Disturbance

The indicators with the greatest impact on *Taxus globosa* populations are human activities (HA) and human-used trails (HT). However, by population, the greatest impact is found in Nuevo León, with up to 139 m^2 affected, followed by Veracruz, with 137 m^2, and Oaxaca, with 101 m^2 (Table 5).

Table 5. The indicators with the greatest impact on *Taxus globosa* populations are human activities (HA) and human-used trails (HT). However, by population, the greatest impact is found in Nuevo León, with up to 139 m^2 affected, followed by Veracruz, with 137 m^2, and Oaxaca, with 101 m^2 (Table 5).

ID	Nuevo León	Tamaulipas	San Luis Potosí	Hidalgo	Veracruz	Oaxaca	Sum
PC	3	32	2	5	24	14	80
HA	58	2	4	3	16	24	**107**
HT	33	8	4	4	25	30	**104**
LT	0	3	0	0	5	3	11
EF	3	0	0	0	3	4	10
CE	0	2	0	0	1	2	5
GE	0	2	0	0	2	2	6
TAS	27	3	0	0	18	9	57
TE	6	10	3	3	30	7	59
Sum	**130**	62	13	15	**124**	**95**	

Veracruz is the region with the greatest variation in disturbance levels, followed by Oaxaca. Virtually all of the environmental modification agents evaluated are present in these sites, albeit with varying areas. At the other extreme are San Luis Potosí and Hidalgo, both

in nature reserves, where several indicators were zero. Nuevo León and Tamaulipas, the latter located in a nature reserve, are in between. However, there are targeted impacts on certain trees, as the felling in El Cielo only affected *T. globosa* individuals without affecting other species, a situation not seen in any other study location.

Multidimensional non-metric analysis was able to explain the variation in the disturbance data (stress: $0.01 > 0.05$), especially along the first axis (axis 1: $R^2 = 0.74$, $p < 0.01$; axis 2: $R^2 = 0.52$, $p < 0.05$). This ranking showed that the Nuevo León population exhibits environmental modifications due to disturbances that are different from the rest and are associated with urbanization. These modifications include HA, HT, and TAS, which are characteristic of areas with adjacent houses and roads. This is not found in the other localities. Veracruz and Oaxaca share similarities in disturbance factors such as PC and HT. However, logging in Veracruz generates massive environmental changes, while in Oaxaca, although logging also takes place, the changes are moderate due to different exploitation models. San Luis Potosí and Hidalgo are very homogeneous and similar (Figure 3).
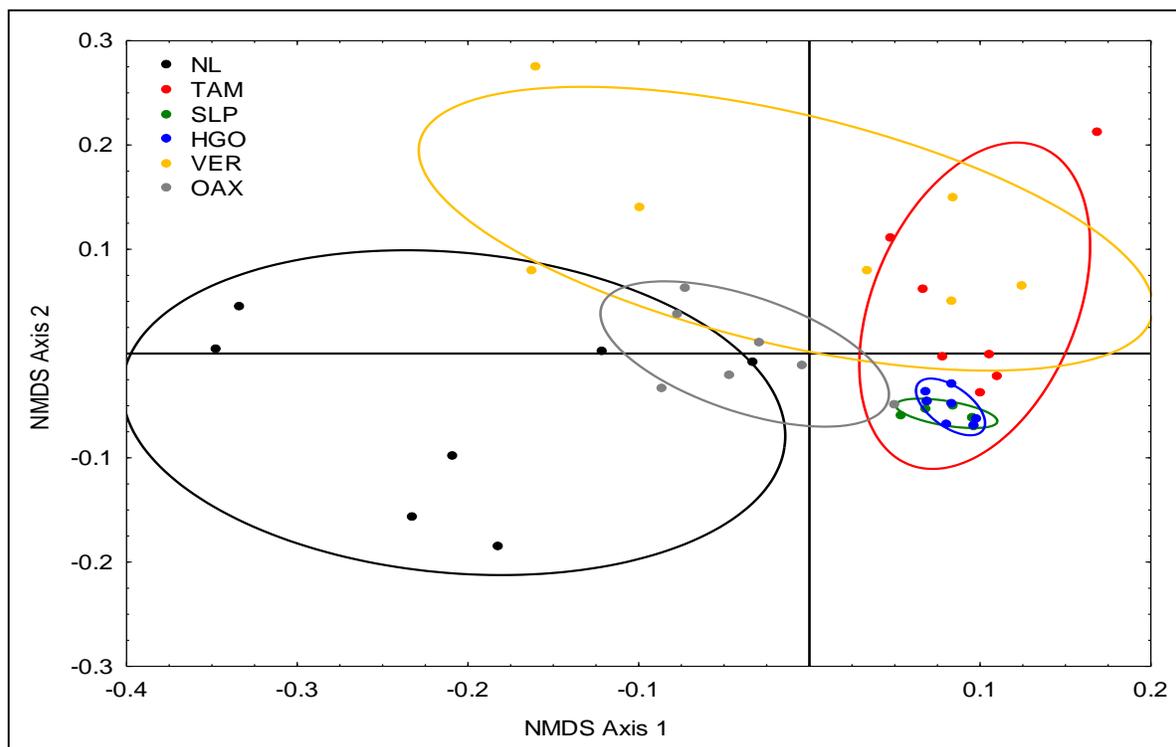

Figure 3. Multidimensional non-metric analysis for the disturbance attributes evaluated in the *Taxus globosa* populations assessed along the Mexican Sierra Madre Oriental.

The Mantel test did not show an association between population growth rate and Euclidean distances obtained by disturbance attributes ($R^2 = 0.008$, $p > 0.05$). However, when each disturbance attribute was evaluated separately, a significant inverse relationship was found between timber extraction and λ ($rs^2 = -0.93$, $p = 0.02$).

27 DISCUSSION

The population structure showed that the largest trees, those in group 5, are not common at all sites, which influences their persistence by affecting the species' reproductive potential, especially when disturbance eliminates vegetation cover. *Taxus globosa* populations are immersed in a heterogeneous environmental matrix associated with Nearctic communities such as the mountain cloud forest, *Pinus* forest, *Abies* forest, and pine-oak forest, among others. These types of associations have influenced their ability to adapt to environmental changes in recent years; this is coupled with the inherent demographic susceptibility of long-lived trees (Leija-Loredo *et al*. 2018, Erlichman *et al*. 2024).

Even so, there are persistent populations with a sufficient capacity for regeneration and positive population growth rates, but only when the vegetation matrix is maintained and under low anthropogenic influence, which is the case in two of the study populations in central Mexico. The rest show moderate to severe anthropogenic environmental modifications that affect the populations in diverse ways. Our results were clear, forestry is the main threat to this species.

27.1 Demography

From the cluster analysis, five morphological groups were identified, differentiated by height, diameter at breast height, average crown diameter and angle of insertion of the first branch. This improves the proposed classification based only on height and diameter (Zavala-Chávez 2001). It may be that tree shape reflects the state of conservation of the study sites, as is the case of the walnut (*Juglans pyriformis*), where forest exploitation ends up degenerating the forests, leaving only poorly shaped individuals or homogenizing the dasometric structure of the populations (Acosta-Hernández *et al*. 2011).

A notable finding in this study is the absence of large individuals (morphological group 5) in the *T. globosa* populations from Hidalgo and San Luis Potosí, despite these being considered among the most conserved and having positive population growth rates ($\lambda = 1.26$ and 1.35 respectively). This absence has ecological and conservation implications that should

be discussed. A first hypothesis is that this structure could reflect young populations, with recent recruitment events following a disturbance or change in environmental conditions. In fact, these populations reproduce from low size classes (group 2), which can be interpreted as a process of active regeneration. This pattern has been documented in other temperate forest tree species, such as *Abies religiosa*, where the dominance of juveniles has been interpreted as regeneration following disturbances (Martínez *et al*. 2013).

However, it is also possible that this structure reflects selective harvesting of large individuals, whether historical or contemporary. Even though these populations are found in protected areas, this does not guarantee the absence of anthropogenic pressure. The occasional harvesting of larger trees is common in species with medicinal or timber value, such as yew, and can alter population structure by eliminating the longest-lived individuals, which may not necessarily be the most fertile (Ticktin 2004), as early reproductive events are observed in managed populations. Another plausible explanation is the presence of diseases or differential mortality that disproportionately affect old or large trees. Fungal pathogens or accumulated environmental stress can induce collapse in larger cohorts, as has been reported for *Fagus sylvatica* and *Quercus robur* in Europe (Thomas *et al.* 2016).

Furthermore, in highly conserved environments with a closed overstory canopy, such as the cloud forests that *T. globosa* inhabits, competition for light can limit growth in height even in long-lived individuals. This phenomenon, characteristic of understory species, can result in short, yet very old trees (Lusk 2002). Therefore, the lack of tall individuals should not necessarily be interpreted as a lack of adults, but rather as an adaptation to intense shade conditions.

This study shows a significant differentiation in the population structure of *T. globosa* between the populations of Hidalgo and Oaxaca, despite the fact that both present a predominance of individuals from morphological group 2. In the case of Hidalgo, this dominance can be interpreted as a natural regeneration process, where most individuals are in juvenile stages with emerging reproductive capacity, which is consistent with a young and expanding population structure ($\lambda > 1$). This pattern is common in populations that have started their recovery after a period of disturbance or reduced pressure, as reported by Pérez-Harguindeguy *et al*. (2021) for *Podocarpus parlatorei* in the subtropical Andes, where intermediate size classes predominated in sites with less recent anthropogenic impact.

In Oaxaca, although group 2 also predominates, the context is different. Reproductive individuals were observed in very small morphological categories (groups 1 and 2), which suggests a process of induced reproductive precocity, probably as a result of human interventions such as repeated pruning or felling. This hypothesis is reinforced by considering that in woody evergreen species, repeated pruning can induce early flowering and changes in the phenological cycle, as has been demonstrated in *Quercus ilex* and *Olea europaea* in Mediterranean systems (Rufat *et al*. 2018, Munne-Bosch & Alegre 2004).

When comparing population growth rates (λ) of *T. globosa* in different regions, it is observed that while in San Luis Potosí, Hidalgo, and Oaxaca, positive rates ($\lambda > 1$) are recorded, indicating expanding populations with good regeneration conditions, globally the species maintains an average rate close to stability ($\lambda = 1.02 \pm 0.3$). This discrepancy suggests that fluctuations in growth rates are influenced by specific local factors. Studies in other tree species have shown similar results. For example, in *Oreomunnea mexicana*, population growth rates of $\lambda = 1.101 \pm 0.018$ were observed, reflecting the influence of local factors on the regeneration and survival of the species (González-Espinosa *et al*. 2019).

Reproductive events in small-sized individuals (groups 1 and 2) in the Oaxaca population represent an ecologically and evolutionarily important fact, as they provide options for reproduction that maximize species fitness in heterogeneous environments (Giesel 1976, Lloyd, 2008). This pattern can be interpreted as an adaptive strategy that increases reproductive success under specific environmental conditions or under management pressures such as selective pruning. In environments subject to disturbance, reproductive precocity can be advantageous, enabling individuals to leave offspring before mortality events occur (Obeso 2002).

This pattern is not unique to *T. globosa*; studies of other tree species in the region have documented similar behavior. For example, juvenile individuals of *Pinus oaxacana*, a species native to the Mixteca Alta region of Oaxaca, have been observed producing cones and seeds, suggesting an accelerated life cycle possibly in response to human pressure and marginal habitat conditions (Sáenz-Romero *et al*. 2012). In *Juniperus deppeana*, another Mexican conifer, it has been documented that frequent pruning in forest harvesting areas can induce earlier reproductive maturation in small individuals as a way of compensating for stress or mechanical damage (Flores-Tolentino *et al*. 2018). This phenomenon could also be

occurring in Oaxaca, where local exploitation of *T. globosa* can lead to atypical phenological responses. It is notable that precocity is greater in males than in females, which reinforces the theory of differential reproductive cost, with short- and long-term effects that mitigate limitations in pollen availability and seed shading by increasing the density of reproductive individuals in populations (Ohya *et al*. 2017).

Taken together, these cases support the hypothesis that the early reproduction observed in *T. globosa* in Oaxaca is not an anomalous behavior, but rather a plastic response that has been reported in other tree species under similar ecological or management conditions. This reproductive strategy should be considered in monitoring and management schemes, as it may indicate both regeneration potential and a sign of ecological stress.

27.2 Disturbance

Disturbances in *Taxus globosa* populations are primarily related to human activity remnants (HA) and human trails (HT), of which Nuevo León and Veracruz are the sites with the largest affected areas. This trend is consistent with that reported for *Abies religiosa* in Nevado de Toluca, where proximity to urban centers and road infrastructure has caused significant understory degradation and soil compaction (Sánchez-González & López-Mata 2011). For *T. globosa*, the sum of the recorded HA and HT values overall were 107 m² and 104 m², respectively, comparable with the levels of impact reported for temperate forests in areas with high recreational use.

Timber extraction (TE) was another important factor, especially in Veracruz, with a significant impact on population growth rate ($rs^2 = -0.93$, $p = 0.02$). This finding is similar to what has been documented in *Quercus rugosa*, where selective logging has been associated with reduced regeneration and altered population structure (Gómez-Aparicio *et al.* 2005). While in *T. globosa* the total TE was 59 m², in *Q. rugosa* a decrease in recruitment of up to 45% has been observed in sites with extractive intervention.

Populations located in nature reserves, such as San Luis Potosí and Hidalgo, showed the lowest levels of total disturbance (15 m² and 19 m², respectively), and were notable for the complete absence of several disturbance indicators. This is consistent with studies on *Pinus hartwegii* in Iztaccíhuatl–Popocatépetl National Park, where actively protected areas showed significantly less soil compaction and a lower incidence of logging (Cortés-Montaño

et al. 2012). This comparison reinforces the positive role of protected natural areas in the conservation of forest species.

Total soil modification (TSM) was particularly high in Nuevo León (27 m²), associated with adjacent urbanization. This situation has been previously documented for *Cedrela odorata* in Veracruz, where urban expansion has led to significant loss of forest cover and a decrease in natural recruitment (Pérez-De la Rosa *et al*. 2014). In both species, the presence of roads and buildings reduces the ecosystem's ability to sustain regenerative processes.

Although less frequent, the presence of livestock excrement (CE and GE) was also recorded. The total amount of CE and GE was 11 m². In studies with *Nectandra salicifolia* in the Sierra de Manantlán, grazing compaction has been shown to reduce germination and seedling establishment by up to 70%, as it alters soil structure and natural regeneration (Zamora-Crescencio *et al*. 2013). This suggests that the presence of livestock is an additional risk that should be considered in management plans.

Non-metric multidimensional analysis (NMDS) revealed marked differences between the evaluated populations. This was noted, for example, in Nuevo León, where urbanization factors are clearly associated with a distinct disturbance pattern. Similar studies have successfully used NMDS to describe disturbance patterns in plant communities. For example, Enoki *et al*. (2016) used NMDS to identify disturbance gradients in *Castanopsis* forests in Japan, finding that human components and microclimate alterations were determining factors in community structure. Bravo *et al*. (2014) used NMDS in tropical forests in southern Mexico to discriminate between areas degraded by livestock farming and those with active natural regeneration, demonstrating the usefulness of this method for assessing anthropogenic pressure at multiple scales.

These studies support the use of NMDS as a robust tool for interpreting complex ecological data and reinforce the interpretation of the patterns observed in *T. globosa*, where the axes of the analysis clearly reflect the disturbance gradients from relatively conserved environments (Hidalgo, San Luis Potosí) to areas highly modified by human activities (Nuevo León, Veracruz).

The Mantel test applied in this study did not show a significant association between population growth rate (λ) and Euclidean distances generated by disturbance attributes (R² =

0.008, p > 0.05). However, when the disturbance factors were examined separately, a strong negative relationship was identified between timber extraction and λ. This pattern has been reported in similar studies, such as in *Podocarpus parlatorei* in the Argentine Andes, where the Mantel test also did not yield significant overall relationships between environmental and demographic distances, but strong associations were detected when evaluating specific variables such as land use (Teich *et al*. 2005).

This study provides robust empirical evidence on the relationship between anthropogenic disturbance and population viability in *T. globosa*, and highlights the urgency of establishing conservation strategies that integrate demographic monitoring, regulation of extractive activities, and habitat restoration. These actions are crucial to ensuring the persistence of a species that, in addition to its ecological value, has biocultural and pharmacological importance (Wheeler & Hehnen 1993, Soto *et al.* 2000).

28 CONCLUSION

This study provides solid evidence of the variability in the population structure and demographic dynamics of *Taxus globosa* across its distribution in the Sierra Madre Oriental. Through morphological analysis and reproductive patterns in six populations, marked contrasts between regions were detected, demonstrating that local conditions and forest management history decisively influence the current status of the species.

The populations of San Luis Potosí, Hidalgo, and Oaxaca show positive growth rates (λ > 1), indicating population viability and active regeneration, with reproductive events even from early size categories. This finding is especially relevant in Oaxaca, where the presence of small reproductive individuals could be the result of both adaptive strategies and anthropogenic pressures (pruning, felling of old trees on individuals, targeted logging, etc.), a phenomenon that has been reported in other conifers under disturbed conditions.

In contrast, the populations of Veracruz and Tamaulipas show negative or zero growth rates and depend exclusively on large individuals for reproduction, indicating an aging structure and vulnerability to disturbance events or habitat loss.

Furthermore, this study provides evidence that *T. globosa* faces different levels of disturbance throughout its distribution, with human activities, urbanization, and logging being the most influential factors. Despite the apparent overall stability of the species (mean λ = 1.02 ± 0.3), the heterogeneity observed at the local level indicates that *T. globosa* should

not be considered ecologically stable throughout its distribution. Instead, regionally differentiated conservation strategies are required that recognize the value of expanding young populations and urgently address pockets of demographic decline.

Finally, this work highlights the importance of incorporating demographic and morphological approaches into conservation plans for threatened species, especially those with long life cycles and discontinuous distributions, such as *T. globosa*. Localized assessments allow for the detection of both opportunities and risks hidden beneath population averages, thus contributing to the design of more precise, informed, and sustainable management strategies.

29 ACKNOWLEDGMENTS

30 REFERENCES

Acosta-Hernández CC, Luna-Rodríguez M, Octavio-Aguilar P, Morales-Romero Z, Galindo-González J, Noa-Carrazana JC, Vázquez-Torres SM, & Iglesias-Andreu LG. 2011. Effect of forest harvesting on morphologicl variation of *Juglans pyriformis* Liebm. *Revista Chapingo Serie Ciencias Forestales y del Ambiente,* 17(3). 379-388. DOI: 105154/r.rchscfa2011.04.033

Antúnez P. 2021. Influence of physiography, soil and climate on *Taxus globosa*. *Nordic Journal of Botany,* 39(3). DOI: 10.1111/njb.03058

Bravo F, Martínez-Ramos M, & Bongers F. 2014. Assessing local ecological knowledge to understand forest dynamics and degradation: A case study from Chiapas, Mexico. *Forest Ecology and Management*, 334, 370-379. DOI: 10.1016/j.foreco.2014.09.016

Castillo-Lara P, Octavio-Aguilar P, & De-Nova JA. 2018. *Ceratozamia zaragozae* Medellín-Leal (Zamiaceae), an endangered Mexican cycad: New information on population structure and spatial distribution. *Brittonia, 70*(2), 155-165. DOI: 10.1007/s12228-017-9513-1

Chen X, Wang X, Song Y, & Chi Y. 2024. *A review of studies on the effects of anthropogenic disturbances on plant–soil–microorganism interactions in grassland ecosystems. Agronomy*, 14(12), 2890. DOI: 10.3390/agronomy14122890

CONABIO. 2010. *Ficha técnica de Taxus globosa Schltdl. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.* Available at: https://www.biodiversidad.gob.mx/

Cope EA. 1998. Taxaceae: The genera and cultivated species. *The Botanical Review,* 64(3), 291-322. DOI: 10.1007/BF02857621

Cortés Montaño C, Ángeles G, Martínez M, & Sánchez J. 2012. Impacto del uso turístico sobre el bosque de *Pinus hartwegii*. *Revista Mexicana de Ciencias Forestales*, 3(13), 29-44.

Enoki T, Ishii H, & Takahashi K. 2016. Tree community patterns in relation to topographic and disturbance gradients in a warm-temperate evergreen broad-leaved forest, southwestern Japan. *Ecological Research*, 31(3), 371-380. DOI: 10.1007/s11284-016-1343-7

Erlichman A, Sandell L, Otto SP, Aitken SN, & Ronce O. 2024. Planting long-lived trees in a warming climate: Theory shows the importance of stage-dependent climatic tolerance. *Evolutionary Applications,* 17(6), e13711. DOI: 10.1111/eva.13711

Flores-Tolentino M, González-Espinosa M, Ramírez-Marcial N, & Camacho-Cruz A. 2018. Efectos del aclareo y la poda sobre la reproducción de *Juniperus deppeana* en un bosque templado. *Revista Mexicana de Ciencias Forestales*, 9(49), 56-70. DOI: 10.29298/rmcf.v9i49.135

García-Aranda MA, Estrada-Castillón AE, Jurado-Ybarra E, & González-Uribe DU. 2011. Análisis de once poblaciones naturales de *Taxus globosa* Schltdl. (Taxaceae) en la Sierra Madre Oriental. *Madera y Bosques,* 17(1), 93-104. DOI: 10.21829/myb.2011.1711156

García-Aranda MA, Cantú-Ayala C, Estrada-Castillón E, Pando-Moreno M, & Moreno-Talamantes A. 2012. Distribución actual y potencial de *Taxus globosa* (Taxaceae) en México. *Journal of the Botanical Research Institute of Texas,* 6(2), 587-598.

Giesel JT. 1976. Reproductive strategies as adaptations to life in temporally heterogeneous environments. *Annual Review of Ecology and Systematics*, 7, 57-79.

Gómez-Aparicio L, Zavala MA, Bonet FJ, & Zamora R. 2005. Are pine plantations valid tools for restoring Mediterranean forests? *Forest Ecology and Management*, 217(1), 77-93. DOI: 10.1016/j.foreco.2005.05.010

González-Espinosa M, Ramírez-Marcial N, & Camacho-Cruz A. 2019. Demografía de *Oreomunnea mexicana* (Standl.) J. F. Leroy en el bosque de niebla de Sierra Juárez, Oaxaca. *Revista Mexicana de Biodiversidad*, 90, e902733. DOI: 10.22201/ib.20078706e.2019.90.2733

Hammer Ø, Harper DA. 2001. Past: paquete de software de estadística paleontológica para la educación y el análisis de datos. *Palaeontologia electronica,* 4(1). 1.

Leija-Loredo EG, Pavón NP, Sánchez-González A, Rodríguez-Laguna R, & Ángeles-Pérez G. 2018. Land cover change and carbon stores in a tropical montane cloud forest in the Sierra Madre Oriental, Mexico. *Journal of Mountain Science,* 15(10), 2136-2147. DOI: 10.1007/s11629-018-4937-y

Lloyd DG. 2008. Variation strategies of plants in heterogeneous environments. *Biological Journal of the Linnean Society*, 21(4), 357-385. DOI: 10.1111/j.1095-8312.1984.tb01600.x

López-Upton J, & García-Martí X. 2015. *Taxus globosa* Schltdl. (Taxaceae). Distribution and diagnosis of an endangered yew. *Earth Sciences,* 4(3-1), 80-88. DOI: 10.11648/j.earth.s.2015040301.13

Luna-Vega MI. 2003. *Taxus globosa*: Taxa of the mesophilic mountain forest of the Sierra Madre Oriental included in the Norma Official Mexicana. Disponible en: www.conabio.gob.mx/conocimiento/ise/fichasnom/Taxusglobosa00.pdf

Lusk CH. 2002. Leaf area accumulation helps juvenile evergreen trees tolerate shade in a temperate rainforest. *Oecologia*, 132(2), 188-196. DOI: 10.1007/s00442-002-0940-6

Malda G. 1990. Plantas vasculares raras, amenazadas y en peligro de extinción en Tamaulipas. *Biotam,* 2(2), 55-61.

Martínez E, Ramírez-Marcial N, & González-Espinosa M. 2013. Dinámica de regeneración de *Abies religiosa* en bosque perturbado del centro de México. *Revista Mexicana de Biodiversidad*, 84(2), 542-556. DOI: 10.7550/rmb.31683

Martínez-Ramos M, Anten NPR, & Ackerly DD. 2016. Defoliation and growth responses of tropical tree saplings from different successional stages: An integration of hydraulic architecture and leaf ecophysiology. *Tree Physiology,* 36(2), 208-220. DOI: 10.1093/treephys/tpv126

Martorell C, & Peters EM. 2005. The measurement of chronic disturbance and its effects on the threatened cactus *Mammillaria pectinifera*. *Biological Conservation,* 124 (2), 199-207. DOI: 10.1016/j.biocon.2005.01.025

Medina RC, & Vega IL. 2001. Presencia de *Taxus globosa* Schlecht. (Taxaceae) en el estado de Chiapas, México. *Polibotánica,* 12, 51-55.

Munne-Bosch S, & Alegre L. 2004. Die and let live: Leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology*, 31(3), 203-216. DOI: 10.1071/FP03236

Muñoz-Gutiérrez L, Ramírez-Sánchez SE, & Velasco-García MV. 2019. Review on the distribution and conservation of *Taxus globosa* Schltdl. (Taxaceae) in México. *Revista Mexicana de Ciencias Forestales,* 10(56), 65-84. DOI: 10.29298/rmcf.v10i56.479

Ohya I, Nanami S, & Itoh A. 2017. Dioecious plants are more precocious than cosexual plants: A comparative study of relative sizes at the onset of sexual reproduction in woody species. *Ecology and Evolution*, 7(15), 5660-5668. DOI: 10.1002/ece3.3117

Obeso JR. 2002. The costs of reproduction in plants. *New Phytologis*t, 155(3), 321-348. DOI: 10.1046/j.1469-8137.2002.00477.x

Pérez-De la Rosa D, López-Mata, & García E. 2014. Land-use change and recruitment of *Cedrela odorata*. *Ecological Engineering*, 62, 85-93. DOI: 10.1016/j.ecoleng.2013.10.014

Pérez-Harguindeguy N, Tecco PA, & Cingolani AM. 2021. Demographic structure and regeneration dynamics of *Podocarpus parlatorei* along disturbance gradients in subtropical montane forests. *Forest Ecology and Management*, 489, 119065. DOI: 10.1016/j.foreco.2021.119065

Rufat J, Arbonés A, & Villar, JM. 2018. Pruning intensity and flowering in *Quercus ilex*: impact on vegetative and reproductive responses. *Trees*, 32(4), 1105-1114. DOI: 10.1007/s00468-018-1694-7

Sáenz-Romero C, Pérez-Ruíz R, & Rehfeldt GE. 2012. Modeling distribution and growth of *Pinus oaxacana*, a threatened species, under current and future climates. *Forest Ecology and Management,* 282, 129-137. DOI: 10.1016/j.foreco.2012.07.039

Sánchez-González A, & López-Mata L. 2011. Human impact on the structure and regeneration of *Abies religiosa* forests. *Forest Ecology and Management*, 261(3), 501-509. DOI: 10.1016/j.foreco.2010.11.004

SEMARNAT. 2010. Secretaría De Medio Ambiente y Recursos Naturales. Protección Ambiental - Especies Nativas de México de Flora y Fauna Silvestres - Categorías de Riesgo y Especificaciones Para su Inclusión, Exclusión o Cambio - Lista de Especie en Riesgo. Norma Oficial Mexicana NOM-059- ECOL-2010. Diario Oficial, 2da Sección. Gobierno de México, México. Disponible en: www.dof.gob.mx/normasOficiales/4254/semarnat/semarnat.htm

Sevik H, Cetin M, Ozel HB, Erbek A, & Zeren CI. 2021. The effect of climate on leaf micromorphological characteristics in some broad-leaved species. *Environment, Development and Sustainability,* 23(4), 6395-6407. DOI: 10.1007/s10668-020-00877-w

Shemluck MJ, Estrada E, Nicholson R, & Brobst SW. 2003. A preliminary study of the taxane chemistry and natural history of the Mexican yew, *Taxus globosa* Schltdl. *Boletín de la Sociedad Botánica de México,* 72, 119-127. DOI: 10.17129/botsci.1672

Song Y, Wang X, Chen X, & Chi Y. 2024. *Forest disturbance and regeneration dynamics in montane ecosystems. Forest Ecology and Management*, 555, 121032. Available at: www.sciencedirect.com/science/article/abs/pii/S0378112724007187

Soto M, Sanjurjo M, González MT, Cruz D, & Giral F. 2000. El tejo mexicano (*Taxus globosa* Sch.). Potencial de su aprovechamiento en taxol. *CIENCIA ergo-sum, Revista Científica Multidisciplinaria de Prospectiva,* 7(3). Disponible en: www.redalyc.org/articulo.oa?id=10401910

Teich I, Lutz E, & Bucher EH. 2005. Effects of forest fragmentation on tree regeneration in the montane forest of southern Yungas, Argentina. *Biodiversity and Conservation,* 14(10), 2209-2220. DOI: 10.1007/s10531-004-7187-5

Thomas FM, Blank R, & Hartmann G. 2016. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forestry*, 89(3), 277-294. DOI: 10.1093/forestry/cpv043

Thomas P. 2013. *Taxus globosa*. The IUCN Red List of Threatened Species 2013: e.T30724A2795235. DOI: 10.2305/IUCN.UK.2013-1.RLTS.T30724A2795235.en

Ticktin T. 2004. The ecological implications of harvesting non-timber forest products. *Journal of Applied Ecology*, 41(1), 11-21. DOI: 10.1111/j.1365-2664.2004.00859.x

Valverde T, Cano-Santana Z, Meave J, & Carabias V. 2005. *Ecología y medio ambiente*. Pearson Educación.

Wang L, Zhang J, & Liu H. 2024. *Disturbance regimes and biodiversity patterns in temperate forest communities*. *Journal of Ecology*, 112(2), 387-401. DOI: 10.1111/1365-2745.14367

Wheeler NC, & Hehnen MT. 1993. Taxol: a study in technology commercialization. *Journal of Forestry,* 91(10), 15-18. DOI: 10.1093/jof/91.10.15

Zamora-Crescencio R, Rico-Gray V, Barrientos-Medina RC, Puc-Garrido EC, & Villegas P. 2013. Ganadería y regeneración de especies forestales. Revista Fitotecnia Mexicana, 36(4), 345-352.

Zavala-Chávez F. 2001. Análisis demográfico preliminar de *Taxus globosa* Schlecht en el Parque Nacional El Chico, Hidalgo, México (Tesis de Maestría). Universidad Autónoma del Estado de Hidalgo.

Zavala-Hurtado JA, Valverde T, & Martínez E. 2003. Population dynamics of the endangered long-lived shrub *Echeveria gibbiflora* in a disturbed habitat in Central Mexico. *Biological Conservation,* 109(3), 321-329. DOI: 10.1016/S0006-3207(02)00160-1

CAPÍTULO V

Discusión y conclusión general

31 DISCUSIÓN

En las plantas arbóreas, los patrones de morfología, diversidad genética y dinámica demográfica están estrechamente relacionados con las condiciones ambientales y el grado de perturbación de sus hábitats. La morfología foliar, por ejemplo, puede reflejar tanto adaptaciones a factores climáticos como temperatura y humedad, como respuestas inmediatas a microambientes alterados por la actividad humana (Li *et al*. 2020; Liu & Su, 2016). En el caso de *Taxus globosa*, se ha observado una notable variación en el tamaño y forma de las hojas a lo largo de un gradiente latitudinal, lo cual evidencia una plasticidad fenotípica posiblemente relacionada con su origen neártico y su distribución discontinua en ambientes de montaña.

Desde el punto de vista genético, *T. globosa* muestra una baja heterocigosis observada y altos niveles de endogamia, especialmente en las poblaciones del sur de su distribución. Estos patrones son consistentes con lo que ocurre en muchas especies relictas de hábitats fragmentados, donde el aislamiento y la limitada dispersión de semillas reducen el flujo génico y aumentan el riesgo de pérdida de variabilidad genética (Ledig *et al*. 2000; Petit *et al*. 2003). La fragmentación del paisaje también afecta negativamente la demografía de estas poblaciones, al reducir la densidad de individuos reproductivos y limitar la regeneración natural.

Adicionalmente, la perturbación antrópica, como la tala selectiva o la apertura de claros en el dosel, altera el microclima local y puede interrumpir tanto los procesos reproductivos como el crecimiento vegetativo. En *T. globosa*, estas alteraciones se reflejan en una disminución del tamaño foliar en ciertas regiones, así como en señales demográficas de cuello de botella y declive poblacional (Galván-Hernández *et al*. 2021).

Poblaciones del norte: Nuevo León y Tamaulipas

En la parte más septentrional de su distribución mexicana, *T. globosa* presenta en Nuevo León y Tamaulipas hojas de mayor tamaño, tanto en longitud como en algunas medidas de ancho y grosor del pecíolo. Estos atributos reflejan una posible conservación de rasgos adaptativos propios de su origen neártico, es decir, de ambientes templados húmedos y variables (Cavender-Bares *et al*. 2016). El tamaño foliar grande puede favorecer el intercambio de gases y la captación de luz en condiciones de competencia media y mayor estacionalidad térmica, como la registrada en estas localidades (BIO4).

Desde el punto de vista genético, estas poblaciones muestran una mayor diversidad genética y menor coeficiente de endogamia en comparación con las del centro y sur (Ho= 0.27 en ambas; Fis= 0.61; Tabla 1). Esta variabilidad sugiere una colonización más antigua o una mayor estabilidad poblacional en esta región, posiblemente como consecuencia de una menor presión antrópica o de mayor conectividad histórica (Midolo *et al*. 2019; Galván-Hernández *et al*. 2021). Sin embargo, aún se detectan señales de reducción poblacional, lo cual indica procesos de fragmentación en curso.

El pecíolo en ambas poblaciones es corto y grueso, lo cual se correlaciona negativamente con la estacionalidad térmica (BIO4), sugiriendo que estas estructuras podrían funcionar como adaptaciones estructurales a climas con grandes fluctuaciones térmicas. Este patrón ya ha sido documentado en otros árboles de zonas templadas como *Acer* y *Pinus* (Liu & Su, 2016; Filartiga *et al*. 2022).

Poblaciones del centro: San Luis Potosí e Hidalgo

Estas poblaciones muestran un comportamiento intermedio tanto en morfología como en genética, aunque con algunos contrastes relevantes. En Hidalgo, se observó una de las mayores altitudes de distribución (2997 msnm), lo cual está asociado a microclimas más fríos y húmedos, lo que puede explicar la anchura foliar significativamente mayor y el desarrollo de hojas anchas, particularmente en San Luis Potosí (AHM, AHB altos).

Genéticamente, la población de Hidalgo es la más diferenciada del resto, con alelos exclusivos y una estructura poblacional distinta. Este aislamiento podría explicarse por su ubicación en una zona montañosa con alto grado de fragmentación del hábitat, donde los efectos de deriva genética y endogamia se intensifican (Fis= 0.81; Tabla 1), coincidiendo con lo reportado en otras especies relictas como *Fagus grandifolia* subsp. mexicana (Galván-Hernández *et al*. 2021) o *Abies religiosa* (Ledig *et al*. 2000). Por el contrario, San Luis Potosí presentó el mayor número de alelos exclusivos (10), lo cual sugiere que podría funcionar como reservorio genético clave para la conservación.

Desde el punto de vista ambiental, ambas poblaciones se sitúan en zonas de transición ecológica con alta biodiversidad y diferentes tipos de vegetación (bosque mesófilo y bosques mixtos). La morfología foliar amplia podría relacionarse con una mayor precipitación en el mes más seco (BIO14), lo que favorece hojas de mayor superficie, como han demostrado estudios en otras coníferas mesófilas (Li *et al*. 2020).

Poblaciones del sur: Veracruz y Oaxaca

En las regiones más meridionales (Veracruz e Ixtlán de Juárez, Oaxaca), *T. globosa* presenta un patrón morfológico y genético que difiere notablemente de las demás poblaciones. A pesar de encontrarse en sitios con alta precipitación y menor estacionalidad térmica, estas poblaciones desarrollan hojas más pequeñas, tanto en longitud como en grosor, lo que contradice los modelos esperados basados en clima (Midolo *et al*. 2019). Este desacoplamiento puede explicarse por la influencia de perturbaciones locales y prácticas silvícolas intensas.

En Veracruz, la tala dirigida a especies de *Pinus* genera claros que modifican el microclima, aumentando la temperatura y reduciendo la humedad en el sotobosque. Este cambio ambiental abrupto parece inducir una expresión morfológica subóptima en *T. globosa*, como lo documentaron Petit *et al*. (2003) y Delgado *et al*. (2011), donde la plasticidad fenotípica se ve limitada por la rapidez del disturbio. Oaxaca, a pesar de tener un manejo más conservador, también sufre de afectaciones por caída de árboles adultos de otras especies, lo cual puede impactar en el tamaño foliar como respuesta adaptativa rápida.

Desde el punto de vista genético, estas poblaciones son las más erosionadas: presentan las menores heterocigosis observadas (Ho= 0.11) y los valores más altos de endogamia (Fis > 0.85; Tabla 1). Además, muestran señales claras de cuellos de botella recientes y ausencia de alelos exclusivos en el caso de Veracruz, lo que las coloca en una situación crítica para la conservación.

Tabla1. Indicadores porcentuales de los atributos morfológicos, genéticos, demográficos y de perturbación en poblaciones de *Taxus globosa* a lo largo de un gradiente latitudinal en México.

Población	Morfología foliar Longitud hoja / Ancho medio / Grosor pecíolo	Diversidad genética He / Ho / Fis / Alelos exclusivos	Demografía Plántulas / Juveniles / Adultos / Equilibrio H-W	Perturbación Área perturbada (m²) / Nivel de impacto
Nuevo León	16.9 / 2.3 / 0.54 Hojas largas y anchas, pecíolos cortos y gruesos	0.67 / 0.27 / 0.61 / 1	17 / 42 / 24 Mayor número de individuos reproductivos observados / Si	45. 01 / Perturbación moderada; bosque con dosel relativamente conservado
Tamaulipas	15.8 / 2.1 / 0.49 Hojas similares a Nuevo León; valores intermedios de grosor y longitud	0.72 / 0.28 / 0.62 / 3	19 / 33 / 21 Población muy parecida a Nuevo León / No	60.44 / Perturbación alta; claros dispersos y actividades humanas cercanas
San Luis Potosí	14.2 / 2.5 / 0.47 Hojas anchas y pecíolos intermedios	0.78 / 0.21 / 0.74 / 10 Mayor número de alelos exclusivos	23 / 49 / 27 Endogamia moderada; potencial fuente genética / No	37.59 / Perturbación moderada; fragmentación creciente
Hidalgo	13.5 / 2.4 / 0.53 Hojas anchas y cortas; variabilidad morfológica destacada	0.48 / 0.11 / 0.81 / 2	21 / 31 / 19 Alta diferenciación genética / No	19. 18 / Perturbación baja; algunas zonas bien conservadas, otras con manejo forestal
Veracruz	12.3 / 2.0 / 0.41 Hojas medianas a pequeñas y pecíolos cortos	0.75 / 0.11 / 0.85 / 0 Sin alelos exclusivos	10 / 16 / 13 Señales de cuello de botella reciente / No	72.27 / Perturbación muy alta; tala selectiva, apertura de claros, cambio microclimático
Oaxaca	11.1 / 1.8 / 0.38 Hojas más pequeñas y delgadas; pecíolos largos y delgados	0.63 / 0.11 / 0.83 / 1	8 / 12 / 11 Población pequeña; regeneración limitada / No	66. 55 / Perturbación alta; caída de árboles adultos, silvicultura semiconservativa

El análisis combinado de la variación morfológica, la estructura genética y los factores ambientales en *T. globosa* revela una compleja interacción entre plasticidad fenotípica, aislamiento poblacional y presiones climáticas, influida tanto por procesos históricos como por perturbaciones contemporáneas.

El análisis morfológico del pecíolo apoya esta interpretación. En Oaxaca, los pecíolos fueron significativamente más largos y delgados, lo que sugiere un patrón adaptativo a ambientes más estables térmicamente, pero posiblemente menos eficientes en condiciones de estrés hídrico o exposición solar excesiva (Filartiga *et al*. 2022).

La plasticidad morfológica observada (especialmente en la lámina foliar y el pecíolo) mostró patrones latitudinales esperados parcialmente, en donde las poblaciones del norte presentaron hojas más grandes y pecíolos más robustos, lo cual concuerda con estudios que han demostrado adaptaciones foliares a gradientes térmicos y de humedad en coníferas de distribución altitudinal o latitudinal (Liu *et al*. 2016; Li *et al*. 2020). Sin embargo, el presente trabajo difiere de estudios como los realizados por Midolo *et al*. (2019), quienes encontraron una fuerte correlación entre atributos foliares y variables climáticas en especies templadas,

ya que en *T. globosa* la correlación fue débil (r² = 0.04, p > 0.2), sugiriendo un papel más relevante de factores microambientales y de la historia evolutiva.

En cuanto a la estructura genética, los resultados son consistentes con lo documentado en otras especies relictas de origen neártico, como *Abies religiosa* (Ledig *et al*. 2000) y *Fagus grandifolia* subsp. *mexicana* (Galván-Hernández *et al*. 2021), que muestran alta diferenciación genética, baja heterocigosis observada y fuertes señales de endogamia. No obstante, este trabajo aporta evidencia más detallada mediante el uso de microsatélites codominantes, lo cual mejora la resolución respecto a estudios previos que utilizaron marcadores dominantes como RAPDs o ISSRs, los cuales tienden a subestimar la diversidad genética (Nybom, 2004; Gargiulo *et al*. 2019). Además, se identificó un patrón de disminución de la diversidad hacia el sur del gradiente latitudinal, lo que contrasta con algunos trabajos en especies boreotropicales como *Quercus rugosa*, donde no se observó dicha reducción (Peña-Ramírez *et al*. 2021).

Una contribución notable de este estudio es la evidencia de que la plasticidad fenotípica de *T. globosa* no siempre se alinea con su estructura genética. Las poblaciones de Hidalgo y San Luis Potosí mostraron alta variación morfológica pero no necesariamente la mayor diversidad genética, lo cual contrasta con estudios como el de Morales-Castañeda *et al*. (2023) en *Pinus hartwegii*, donde la mayor diversidad genética coincidía con una mayor amplitud fenotípica. Esto sugiere que, en *T. globosa*, los factores ambientales locales y la perturbación antrópica tienen un efecto diferenciador más fuerte y rápido que la historia evolutiva a nivel genético.

Por último, las diferencias morfológicas inesperadas en poblaciones altamente perturbadas, como las de Oaxaca y Veracruz, apoyan lo encontrado por Delgado *et al*. (2011) y Petit *et al*. (2003), quienes demostraron que la alteración del hábitat puede interrumpir la expresión adaptativa de rasgos morfológicos en especies de distribución discontinua. Las anomalías registradas en este estudio pueden deberse al manejo forestal y la fragmentación del dosel, que alteran las condiciones microclimáticas críticas para la expresión de rasgos funcionales.

En suma, este trabajo resalta la importancia de estudios integradores que combinen morfología, genética y demografía para entender la respuesta de especies amenazadas frente al cambio ambiental. El gradiente norte-sur en *T. globosa* evidencia una transición de

poblaciones con mayor diversidad genética y plasticidad morfológica adaptativa (norte) hacia poblaciones más fragmentadas, erosionadas y ambientalmente perturbadas (sur). Mientras que los rasgos foliares responden en parte al ambiente inmediato, la estructura del pecíolo refleja adaptaciones más conservadoras ligadas al gradiente térmico. La estructura genética altamente diferenciada, especialmente en Hidalgo y San Luis Potosí, sugiere refugios evolutivos o zonas de persistencia.

Esta heterogeneidad en la respuesta ecológica y genética indica que las estrategias de conservación deben diseñarse de forma regionalizada. Específicamente, deben considerarse acciones de conectividad genética en el norte, manejo forestal más estricto en el centro y restauración ecológica urgente en las poblaciones del sur.

32 CONCLUSIÓN

Los resultados integrados de los tres estudios permiten establecer que *Taxus globosa* es una especie que enfrenta un proceso avanzado de diferenciación morfológica y genética, asociado a su distribución discontinua en las montañas de la Sierra Madre Oriental. La variación observada en las hojas y pecíolos a lo largo del gradiente latitudinal, así como la estructura genética profundamente fragmentada, son reflejo de una historia evolutiva condicionada por la expansión postglacial, las limitaciones de su capacidad de dispersión y, más recientemente, por la creciente presión de las actividades humanas y los efectos del cambio climático.

La fuerte estructuración genética detectada, junto con los elevados niveles de endogamia y la baja heterocigosidad observada, indican que *T. globosa* se encuentra en una situación de vulnerabilidad genética crítica, especialmente en las poblaciones más meridionales, lo que reduce su capacidad adaptativa y eleva su riesgo de extinción local ante perturbaciones ambientales futuras (Frankham, 2005; Love *et al*. 2023).

Asimismo, la plasticidad fenotípica observada, particularmente en los caracteres foliares, pone de manifiesto la importancia de considerar las interacciones entre las condiciones microambientales y la historia de perturbación de cada sitio, pues estas pueden modular de manera significativa la morfología de la especie, independientemente de su carga genética (Atkin *et al*. 2006; Nicotra *et al*. 2010).

Por lo tanto, cualquier estrategia de conservación para *T. globosa* debe contemplar un enfoque integral que combine la protección de hábitats clave, el manejo forestal sostenible y el diseño de corredores ecológicos que faciliten el flujo génico y minimicen los efectos de la

fragmentación. Particular atención merece la conservación de las poblaciones septentrionales, por su mayor diversidad genética, y de las poblaciones del sur, por su singularidad genética y posible diferenciación local.

Finalmente, este conjunto de investigaciones subraya la relevancia de integrar estudios morfológicos, genéticos y ecológicos en la evaluación de especies de distribución restringida, particularmente aquellas sujetas a presiones ambientales y antrópicas crecientes, pues sólo una visión sistémica permitirá diseñar estrategias efectivas para su preservación a largo plazo.

33 REFERENCIAS

Atkin OK, Loveys BR, Atkinson LJ, & Pons TL. 2006. Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Botany* 57: 267–281. DOI: 10.1093/jxb/erj029

Cavender-Bares J, Ackerly DD, Hobbie SE, & Townsend PA. 2016. Evolutionary legacy effects on ecosystems: Biogeographic origins, plant traits and implications for management in the era of global change. *Annual Review of Ecology, Evolution, and Systematics,* 47(1), 433-462. DOI: 10.1146/annurev-ecolsys-121415-032229

Delgado AC, Jain SE, Sánchez MSV, & Reyes PC. 2011. Cambios en la morfología floral y foliar en *Crataegus tracyi*: importancia de la asimetría fluctuante como indicador de estrés ambiental. *Biológicas*, 13(1), 44-49.

Filartiga AL, Klimeš A, Altman J, Nobis MP. Crivellaro A, Schweingruber F, & del Prado ÁL. 2022. Structural traits of petioles in woody plants under contrasting climates. *Ecology and Evolution,* 12(6), e8941.

Frankham R. 2005. Stress and adaptation in conservation genetics. *Journal of Evolutionary Biology,* 18(4), 750-755. DOI: 10.1111/j.1420-9101.2005.00885.x

Galván-Hernández DM, Octavio-Aguilar P, Lazcano-Cruz L, & Sánchez-González A. 2021. Diferenciación morfológica y genética en poblaciones aisladas de haya mexicana *Fagus grandifolia* subsp. *mexicana*. *Revista de Investigación Forestal*, 32(5), 2169-2179. DOI: 10.21829/myb.2021.3252222

Gargiulo R, Luria CF, & Manafò M. 2019. A practical guide for microsatellite development and analysis in non-model plants. *Plant Methods*, 15, 24.

Ledig FT, Hodgskiss PD, & Johnson DR. 2000. Genetic diversity and breeding structure of *Abies religiosa*, a Mexican endemic. *American Journal of Botany*, 87(3), 386-398. DOI: 10.2307/2656622

Li Y, Chen Y, Yin W, Wang X, Liu H, & Tang Z. 2020. Leaf morphological responses to climate gradients in temperate forests. *Ecology and Evolution*, 10(10), 4504-4517. DOI: 10.1002/ece3.6230

Liu W, & Su J. 2016. Effects of light acclimation on shoot morphology, structure, and biomass allocation of two *Taxus* species in southwestern China. *Scientific Reports*, 6, 35384. DOI: 10.1038/srep35384

Love SJ, Schweitzer JA, Woolbright SA, & Bailey JK. 2023. Sky islands are a global tool for predicting the ecological and evolutionary consequences of climate change. *Annual Review of Ecology, Evolution, and Systematics*, 54, 219-236. DOI: 10.1146/annurev-ecolsys-102221-050029

Midolo G, De Frenne P, Hederová L, Hiiesalu I, Holub P, Kopecký M, & Maes SL. 2019. Global patterns of leaf traits responsive to climate. *Global Ecology and Biogeography*, 28(1), 33-45. DOI: 10.1111/geb.12860

Morales-Castañeda M, González-Rodríguez A, Aguirre-Planter E, & Ornelas JF. 2023. Landscape genetics of high-mountain pine species under anthropogenic pressure. *Forest Ecology and Management*, 543, 121961.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, & van Kleunen M. 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science,* 15(12), 684-692. DOI: 10.1016/j.tplants.2010.09.008

Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, 13(5), 1143-1155. DOI: 10.1111/j.1365-294X.2004.02141.x

Peña-Ramírez VM, González-Rodríguez A, Mendoza-Maya E, & Oyama K. 2021. Genetic diversity and structure of *Quercus rugosa* in fragmented landscapes of Central Mexico. *Tree Genetics & Genomes*, 17, 57.

Petit RJ, Aguinagalde I, De Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, & Vendramin GG. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, 300(5625), 1563–1565. DOI: 10.1126/science.1083264

34 Anexo 1. Permiso de Colecta

SEMARNAT
SECRETARÍA DE
MEDIO AMBIENTE
Y RECURSOS NATURALES

Oficio N° SGPA/DGVS/003509/18

Ciudad de México, a 25 de abril de 2018

DR. ARTURO SÁNCHEZ GONZÁLEZ
UNIVERSIDAD AUTÓNOMA DEL ESTADO DE HIDALGO
CENTRO DE INVESTIGACIONES BIOLÓGICAS
REYNA DE LOS BONDADOSOS No. 1556
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MINERAL DE LA REFORMA, HIDALGO
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En atención a la solicitud de Licencia de colecta científica o con propósitos de enseñanza, por proyecto, la cual fue recibida el 19 de abril de 2018, en la Dirección General de Vida Silvestre y se le otorgo la bitácora 09/K4-1008/04/18 y considerando que ha dado cumplimiento a los requisitos establecidos para efectuar investigación y colecta científica de flora y fauna silvestres en territorio mexicano y con fundamento en el Artículo 32 Bis fracciones I, III, XXII, XXXIX de la Ley Orgánica de la Administración Pública Federal; Artículo 19 fracción XXV y 32 fracción VI, XVIII, XXI, XXIV del Reglamento Interior de la Secretaría de Medio Ambiente y Recursos Naturales, publicado en el Diario Oficial de la Federación el 26 de noviembre de 2012; 79, 80 fracción I, 82, 83 y 87 párrafo cuarto de la Ley General del Equilibrio Ecológico y la Protección al Ambiente; Artículos 9°. Fracción XII, 97 y 98 de la Ley General de Vida Silvestre; 12, 123 Fracción IV y 126 del Reglamento de la Ley General de Vida Silvestre; Artículo 85, Artículo 88, fracciones I y II, Artículo 105, fracciones II y III del Reglamento la Ley General del Equilibrio Ecológico y la Protección al Ambiente; la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, protección ambiental-especies nativas de México de flora y fauna silvestres-categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-lista de especies en riesgo, la opinión técnica emitida por la Dirección General de Vida Silvestre **autoriza** la licencia de colecta científica o con propósitos de enseñanza en materia de vida silvestre por proyecto sobre especies o poblaciones en riesgo o sobre hábitat crítico, para desarrollar las siguientes actividades inherentes al proyecto de investigación denominado **"Estructura y composición de especies vegetales de la Sierra Madre Oriental, México"**, con el objetivo de:

- Colecta de hasta tres (03) muestras para herborizar incluyendo tejido foliar por especie y por localidad, de las siguientes especies: "helechos arborescentes, pezma" **Dicksonia sellowiana**, helechos, de las familias **Cyatheaceae** y **Dicksoniaceae** que se encuentren en riesgo de acuerdo con la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, "haya" **Fagus grandifolia subsp. Mexicana**, "flor de mayo, yoloxochitl" **Magnolia dealbata**, "oyamel" especie del género *Abies* que se encuentra en riesgo de acuerdo con la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, "cicadas" especies del género **Ceratozonia** que se encuentra en riesgo de acuerdo con la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, y "cedro rojo" **Cedrela odorata**.

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- Colecta de hasta dos (02) ejemplares por especie de plantas angiospermas que se encuentren en riesgo de acuerdo con la Norma Oficial Mexicana NOM-059-SEMARNAT-2010.

Las actividades se llevarán a cabo **en Tlanchinol, Tizapan, Medio Monte, Agua Blanca, la Sierra Hidalguense, Localidad La Mojonera, Localidad Medio Monte, Localidad El Gosco, Localidad Acomulco, Apantlazol, Chilijapa, Zotictla, Localidad Tlanchinol, Localidad Tepehuacán de Guerrero, Localidad Acaxochitlán, Ejido El Cerezo, Ejido Pueblo Nuevo, Ejido Xolostitla, Peñas Cargadas, Llano Grnade en Hidalgo, Cuetzalan y Zilacatipan en Puebla, Naupan, Localidad Huayacocotla y Localidad Volcán de Acatlán en Veracruz, corredor de montaña del Bosque Mesófilo de Montaña de la Sierra Madre Oriental, Reserva Estatal de la Biosfera el Cielo en Tamaulipas, Localidad Landa de Matamoros en Querétaro, Localidad Xilitla en San Luis Potosí, Reserva Ecológica Estatal Sierra de San Juan, y la Sierra de San Juan en Nayarit, fuera de Áreas Naturales Protegidas Federales.** La presente autorización tendrá una vigencia de **un (01) año** a partir de la emisión de la misma.

Las actividades se realizarán con el aval de la Universidad Autónoma del Estado de Hidalgo, con la colaboración de los CC, Biol. Adriana Gisela Hernández Álvarez, estudiante en el tercer semestre de la Maestría en Ciencias en Biodiversidad y Conservación, Biol. Marisol Gutiérrez Lozano, estudiante en el cuarto semestre de la Maestría en Ciencias en Biodiversidad y Conservación, M. en C. José Luis Reyes Ortiz, estudiante en el tercer semestre del Doctorado en Ciencias en Biodiversidad y Conservación, Dr. Pablo Octavio Aguilar, profesor-investigador del Laboratorio de Genética, Área Académica de Biología, UAEH, Biol. Norma Sarai Trigueros Gómez, estudiante en el primer semestre de la Maestría en Ciencias en Biodiversidad y Conservación, Biol. Luis Alberto Islas Tello, estudiante en el primer semestre de la Maestría en Ciencias en Biodiversidad y Conservación, Lorena Jael Soto Tapia, estudiante de séptimo semestre de la Licenciatura en Biología, **el titular y colaboradores deberán sujetarse obligatoriamente a las siguientes condiciones:**

1.- Cumplir con las disposiciones Administrativas, Fiscales y de Sanidad exigibles por las autoridades competentes en la materia, sean Federales, Estatales o Municipales, así como con las disposiciones establecidas en la Ley General del Equilibrio Ecológico y la Protección al Ambiente y su Reglamento y demás disposiciones legales aplicables.

2.- En todo momento el investigador será responsable de los impactos significativos que haya sobre las poblaciones de la flora o fauna silvestres y sus hábitats, por lo que deberá considerar el riesgo de perturbación del ecosistema, antes de su ejecución y no llevarlo a cabo si el riesgo es alto.

3.- Previo al inicio de las actividades de campo, deberá enviar obligatoriamente por escrito y utilizando cualquier medio su programa de trabajo a las Delegaciones Federales de la

Secretaría de Medio Ambiente y Recursos Naturales en el estado de **Hidalgo** 01 (771) 717 9416, **Nayarit** 01 (311) 215 4902, **Puebla** 01 (222) 229 9506, **Querétaro** 01 (442) 238 3410, **San Luis Potosí** 01 (444) 834 0635, **Tamaulipas** 01 (834) 318 5254 y **Veracruz** 01 (228) 841 6521, enviando copia del mismo a la Dirección General de Vida Silvestre. De igual manera, al término de dichas actividades lo notificará a esa Delegación Federal, enviando un reporte detallado por escrito.

4.- La totalidad del material colectado deberá destinarse exclusivamente a los fines específicos del proyecto, objeto de la presente autorización. Con base al Capítulo IV, Artículo 98 de la Ley General de Vida Silvestre, las muestras y ejemplares serán depositados en instituciones de la Universidad Autónoma del Estado de Hidalgo, Universidad Autónoma de San Luis Potosí, Instituto Tecnológico de Ciudad Victoria, Herbario MEXU de la UNAM, la titular de la autorización, asume la responsabilidad de remitir a esta Dirección General, copia de la(s) constancia(s) del(os) depósito(s) debidamente firmado(s), especificando la cantidad del material depositado

5.- Con base al Capítulo IV, Artículo 98 de la Ley General de Vida Silvestre y 126 del Reglamento de la Ley General de Vida Silvestre, el responsable del proyecto deberá someter a la consideración de la Dirección General de Vida Silvestre, en un plazo no mayor de 30 (TREINTA) días de concluida la vigencia de la presente, un informe que describa **detalladamente** las actividades realizadas, los resultados obtenidos, la problemática del área trabajada, las potenciales alternativas de solución y -en su oportunidad, la(s) publicación(es) y sobre tiros producto de la investigación.

6.- Queda estrictamente prohibido efectuar cualquier aprovechamiento de las especies de flora y fauna silvestres, cualquiera que sea su estatus, excepto lo aquí autorizado, así como realizar actividades en áreas naturales protegidas de México, sean Estatales o Federales, sin previa autorización.

7.- De acuerdo al Artículo 87 de la Ley General del Equilibrio Ecológico y la Protección al Ambiente al Capítulo IV, Artículo 97 de la Ley General de Vida Silvestre, esta autorización no ampara el aprovechamiento de los especímenes colectados para fines comerciales, ni de utilización en biotecnología.

Se recomienda que, durante sus actividades de campo, en el caso de encontrar ejemplares de especies listadas en la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, se notifique de ello (la especie, ubicación geográfica y la fecha) a esta Dirección General, en el informe de actividades antes mencionado.

La presente autorización es personal e intransferible y habrá de mostrarse a las Autoridades Federales, Estatales y Municipales cuantas veces lo soliciten. Así mismo y tomando en consideración lo establecido en el Artículo 87 de la Ley General de Vida Silvestre el titular de la presente deberá contar con el consentimiento previo, expreso e informado de los legítimos propietarios de la(s) tierra(s) donde pretende desarrollar el proyecto.

El incumplimiento de las condiciones aquí establecidas, dará origen a la instauración de un procedimiento administrativo ante la autoridad competente, para proceder a la cancelación de la autorización y a la aplicación de la legislación correspondiente, según sea el caso.

Notifíquese la presente resolución al **DR. ARTUTO SÁNCHEZ GONZÁLEZ**, CENTRO DE INVESTIGACIONES BIOLÓGICAS, DE LA UNIVERSIDAD AUTÓNOMA DEL ESTADO DE HIDALGO, por alguno de los medios legales previstos por el Artículo 35 y demás relativos y aplicables de la Ley Federal de Procedimiento Administrativo.

**ATENTAMENTE
EL DIRECTOR GENERAL**

LIC. JOSÉ LUIS PEDRO FUNES IZAGUIRRE

"Por un uso eficiente del papel, las copias de conocimiento de este asunto son remitidas vía electrónica".

C c p- C. Joel González Moreno. - Director General de Inspección de Vida Silvestre, Recursos Marinos y Ecosistemas Costeros, PROFEPA. - e-mail: vida_silvestre@profepa.gob.mx, jmejia@profepa.gob.mx.
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 C. Roberto Rodríguez Medrano. - Delegado Federal de la SEMARNAT en el Estado de Nayarit. - e-mail: roberto.medrano@nayarit.semarnat.gob.mx.
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 C. Diego Cobo Terrazas. - Delegado de la PROFEPA en el Estado de Veracruz. - e-mail: dcobo@profepa.gob.mx.
 C. Noe Ríos Somoza.- Coordinación de Asesores de la Subsecretaría de Gestión para la Protección Ambiental. - e-mail: noe.rios@semarnat.gob.mx.
 Subdirección de Comercio Nacional, Internacional y Otros Aprovechamientos.

Archivo General (09/K4-1008/04/18)
car.madian/colectacienif/ArturoSánchezGonzálezInvestigación-UAEH-ITBTC-T)SUNIVS(25-04-18)

35   Anexo 2. Protocolo modificado de Doyle & Doyle 1987

1. En un mortero moler alrededor de 0.5 g de tejido con nitrógeno líquido hasta obtener un polvo fino.

2. Agregar 1 ml de buffer CTAB-PVP 2X con 1 µl de β - mercaptoetanol y seguir moliendo. Recuperar en un microtubo de 2 ml.

3. Centrifugar a 8 000 rpm durante 8 min.

4. Eliminar el sobrenadante y suspender con 1 ml de CTAB-PVP 2X, mezclar en vortex hasta que la parte orgánica se desprenda y se mezcle con el CTAB-PVP 2X.

5. Centrifugar a 9,000 rpm durante 8 minutos, la parte orgánica debe quedar casi blanca y la fase acuosa verde intenso.

6. Eliminar el sobrenadante y suspender en 600 µl de CTAB-PVP 2X, agregar 10 µl de RNA´sa (10mg/ml), incubar a 37°C durante 1 hora.

7. Después, agregar 10 µl de Proteinasa k (10 mg/ml), incubar a 60 °C durante 1 hora.

8. Agregar 600 µl de cloroformo: octanol 24:1, agitar hasta homogeneizar y obtener una solución con aspecto lechoso.

9. Centrifugar a 10,000 rpm durante 10 min, el sobrenadante (parte de arriba) tiene que quedar transparente.

10. Trasladar el sobrenadante (600 µl) a un tubo nuevo, cuidando de no tomar la interfase.

11. Agregar 2/3 (400 µl) del volumen final de isopropanol helado para precipitar el DNA.

12. Las muestras se dejan reposar durante la noche a -20°C.

13. Centrifugar a 12,000 rpm durante 10 min. Decantar el sobrenadante sin perder la pastilla.

14. Limpiar el DNA agregando 1 ml de etanol 70% frío y centrifugar a 14,000 rpm durante 10 min.

15. Eliminar el sobrenadante (secar totalmente) y resuspender con 50 µl de agua ultra pura o dependiendo el tamaño de la pastilla.