

Resolving the *Magnolia pacifica* complex (Magnoliaceae): a coalescent-based approach reveals five distinct evolutionary lineages

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Abstract

Background and aims – Species delimitation using multilocus coalescent-based methods has provided new insights into evolutionary processes and species boundaries. In this study, we applied a multilocus, multispecies coalescent framework to investigate species limits and estimate divergence times within the *Magnolia pacifica* complex, a group of endemic species from western and north-western Mexico traditionally classified based on morphological and ecological traits without explicit statistical evaluation.

Material and methods – We obtained sequences of three chloroplast intergenic spacers (*trnT-trnL*, *rpl32-trnL*, *trnH-psbA*) and three nuclear genes (LFY, PHYA, AGT1) from 67 individuals representing all six described species and the closely related *Magnolia iltisiana*. Phylogenetic reconstructions were performed using Bayesian inference and maximum likelihood. Divergence times were estimated using the RelTime-ML algorithm. Species delimitation was conducted under the multispecies coalescent model using Bayesian Phylogenetics and Phylogeography (BPP) with algorithms A10 and A11.

Key results – Our analyses confirm the monophyly of the *M. pacifica* complex within *Magnolia* sect. *Magnolia* and integrate *M. iltisiana* into the complex. Divergence of the complex was dated to 1.27 million years ago. Species delimitation analyses revealed five separately evolving lineages: *M. pacifica* (including *M. talpana*), *M. iltisiana*, *M. pugana* (including *M. granbarrancae*), *M. tarahumara*, and *M. vallartensis*, and we formally synonymize *M. talpana* under *M. pacifica* and *M. granbarrancae* under *M. pugana*.

Conclusion – Pronounced morphological divergence has, in some cases, occurred within genetically cohesive lineages, highlighting a pattern of incipient differentiation. Our delimitation effectively resolves the *M. pacifica* species complex, providing a robust phylogenetic hypothesis and a refined taxonomic framework for understanding the diversification of Mexican magnolias and for guiding conservation priorities.

Keywords

chloroplast DNA, coalescent-based inference, molecular markers, nuclear DNA, phylogenetic inference, plant species complex

INTRODUCTION

Biodiversity conservation efforts rely substantially on taxonomy because most decisions and management plans are based on described species. Establishing taxonomic boundaries can be difficult in organisms that exhibit cryptic morphological variation, broad geographic distributions, or histories of introgression (Padial and De la Riva 2006; Prata et al. 2018). These challenges are particularly pronounced in groups of closely related species, where recent divergence and processes such as incomplete lineage sorting or introgression can obscure species boundaries, as commonly observed in species complexes (Ancona et al. 2022). Species description based only on morphology often fails to uncover cryptic species, leading to either underestimation of biodiversity or over-splitting lineages when phenotypic plasticity within a species is mistaken for genetic differences (Fujita et al. 2012; Freitas et al. 2020). Consequently, genetic data have become essential for species delimitation over the past few decades, providing critical insights into evolutionary relationships that are not always apparent from morphology alone (Shaffer and Thomson 2007; Coates et al. 2018; Rico and Gutiérrez Becerril 2019; López-Ramírez et al. 2024).

Advancements in DNA-based systematics have made molecular data widely used in taxonomic species delimitation (Carstens et al. 2013; Rannala 2015; Smith and Carstens 2022). The most commonly used criterion for molecular-based species delimitation is reciprocal monophyly (Knowles and Carstens 2007). However, a lack of monophyly is frequently observed in species of recent divergence due to the retention and stochastic sorting of ancestral polymorphisms (Maddison and Knowles 2006; Vieira et al. 2022). Alternatively, species delimitation based on coalescent approaches, such as the Multi-Species Coalescent (MSC) model, can effectively estimate species trees without relying on reciprocal monophyly (Yang and Rannala 2010). Therefore, coalescent models better describe the stochastic nature of the speciation process (Knowles and Carstens 2007; Yang 2015). The Multilocus MSC model uses DNA sequence data from multiple loci to infer more reliable species phylogeny (Wiens et al. 2010; Wang et al. 2014). Data from both chloroplast DNA (cpDNA) and nuclear DNA (nDNA) have been employed in several studies to determine the phylogeny of closely related plant species and to differentiate species under the MSC approach (Barrett and Freudenstein 2011; Ruiz-Sánchez 2015; Rico and Gutiérrez Becerril 2019; Lin et al. 2022; Vieira et al. 2022).

Magnolia L. is the largest and most diverse genus in the Magnoliaceae family, consisting of around 390 species (Aldaba Núñez et al. 2026). It includes two subgenera and 15 sections, and only four of them occur in the Neotropics: *Magnolia* sect. *Macrophylla* Figlar & Noot., *M.* sect. *Magnolia*, *M.* sect. *Cubenses* (Imkhan.) F.A.Aldaba, E.Martínez & Samain, and *M.* sect. *Talauma* (Juss.) Baill. (Wang et al. 2020; POWO 2024; Aldaba Núñez et al.

2026). *Magnolia* sect. *Magnolia* originated approximately 10 million years ago (Mya) (Veltjen et al. 2022) and is distributed in the south-eastern United States of America, Mexico, and Central America. It is characterized by having evergreen to partially deciduous leaves, with leaf undersides glaucous or not, stipules adnate to the base of the petiole (appearing to be free) except for one species with stipules adnate to most of the petiole; two ovules per carpel, and the stomata group of Baranova 5 (Baranova and Jeffrey 2000; Figlar and Nooteboom 2004). It comprises 49 taxa, distributed across Mesoamerica, Central America, Mexico, the United States of America, and the Caribbean, with some of these geographical regions overlapping (Aldaba Núñez et al. 2026). Twenty of these species (41%) have been described in the last two decades, primarily based on morphological characters and eco-geographical data (Vázquez-García et al. 2002, 2021, 2022, 2024; Archila et al. 2023; IPNI 2025; Jiménez et al. 2025). However, limited morphological variability, phenotypic plasticity, recent lineage divergence, and incipient speciation processes have hindered full taxonomic resolution within this section (Rico and Gutiérrez-Becerril 2019).

The heterogeneous landscape and climatic variability in Mexico, along with the pollination and seed dispersal capacity in *Magnolia*, and its specific requirements for germination and seedling establishment (Vásquez-Morales and Ramírez-Marcial 2019; Gallardo-Yobal et al. 2022; Bernal-Rodríguez et al. 2025), are key factors explaining the high degree of allopatry within the genus (Vázquez-García 1994, 1995; Vázquez-García et al. 2012). Geographical isolation can lead to divergent evolution; however, it does not always result in speciation (Wiens 2004). Recent population genetic studies of *Magnolia* species complexes have shown that the high levels of genetic differentiation among morphologically distinct populations do not necessarily support the recognition of different species (Rico and Gutiérrez Becerril 2019; Chávez-Cortázar et al. 2021).

The *Magnolia pacifica* species complex (*M.* sect. *Magnolia*) comprises six endemic species in west and north-western Mexico: *M. granbarrancae* A.Vázquez, Muñiz-Castro & A.T.Nuño, *M. pacifica* A.Vázquez (sensu stricto), *M. pugana* (Iltis & A.Vázquez) A.Vázquez & Carvajal, *M. talpana* A.Vázquez, Muñiz-Castro & A.S.Ortega, *M. tarahumara* (A.Vázquez) A.Vázquez, and *M. vallartensis* A.Vázquez & Muñiz-Castro (Vázquez-García 1994; Vázquez-García et al. 2002, 2012, 2013, 2021; Muñiz-Castro et al. 2020). All these species were described without a statistical framework, based on qualitative and quantitative morphological traits, ecological distinctions, and geographic data. They occur in isolated canyons or ravines of mountains across four biogeographic provinces (Sierra Madre del Sur, Trans-Mexican Volcanic Belt, Sierra Madre Occidental, and Pacific Lowlands) (Morrone et al. 2017), along latitudinal, continentality, and moisture gradients, with restricted gene flow between populations (Muñiz-Castro et al. 2020). Most natural populations

of these *Magnolia* species exhibit allopatric distribution and face severe habitat reduction due to threats such as land-use changes, soil degradation, over-harvesting, poor fruiting, diminished pollinators, seed predation, and a low rate of natural regeneration (Sánchez-Velázquez and García-Moya 1993; Cires et al. 2013; Rivers et al. 2016). The Red List of Magnoliaceae has classified *M. tarahumara* as Vulnerable, *M. pacifica* and *M. pugana* as Endangered, and *M. vallartensis* as Critically Endangered (Rivers et al. 2016). *Magnolia talpana* and *M. granbarrancae* have recently been categorized as Critically Endangered (Akanke and Muñoz-Castro 2023; Sara and Muñoz-Castro 2023), underscoring the importance of considering these species in biodiversity conservation efforts.

Phylogenetic hypotheses based on cpDNA and nDNA sequences suggest that *M. pacifica*, *M. tarahumara*, and *M. pugana* are closely related, forming a clade with *M. iltisiana* A.Vázquez within *M. sect. Magnolia* (Veltjen et al. 2022). Subsequently, Aldaba Núñez et al. (2024) performed a phylogenomic analysis that supported a close relationship among *M. pacifica*, *M. iltisiana*, *M. pugana*, and *M. vallartensis*. Building upon these findings, a recent phylogenomic study by Aldaba Núñez et al. (2026) reassessed the classification of Neotropical *Magnolia* using nuclear and plastid data, providing a robust, updated framework for the genus. Their analysis confirms the close relationship of *M. pacifica*, *M. iltisiana*, *M. pugana*, and *M. vallartensis*, within *M. sect. Magnolia*. Furthermore, a population genetic study by Muñoz-Castro et al. (2020) using ISSRs identified two main groups within the *M. pacifica* complex: *M. pacifica* – *M. vallartensis* and *M. pugana*. Within each group, two subgroups were observed; *M. pacifica* was distinct from *M. vallartensis* in the *M. pacifica* – *M. vallartensis* group, while two subgroups were identified within *M. pugana*. Subsequently, Vázquez-García et al. (2021) described *M. granbarrancae* and *M. talpana* based on morphological characters and the findings of Muñoz-Castro et al. (2020). Nevertheless, no previous phylogenetic or population genetic studies have included all the species described in the *M. pacifica* complex.

The present study aims to elucidate the phylogenetic relationships of the *M. pacifica* complex and to examine the evolution of separate lineages using a Bayesian coalescent-based species delimitation approach, following the General Lineage species concept proposed by De Queiroz (1998). We hypothesize that the *M. pacifica* complex is a monophyletic group within *M. sect. Magnolia*, and that the species described within the *M. pacifica* complex represent separately and independently evolving lineages.

MATERIAL AND METHODS

Study species and their distribution

This study focused on the *Magnolia pacifica* species complex, which comprises six species (*M. granbarrancae*, *M. pacifica*, *M. pugana*, *M. talpana*, *M. tarahumara*, and *M. vallartensis*), as well as *M. iltisiana* (Figs 1, 2). *Magnolia iltisiana* was chosen as an outgroup due to its morphological similarity and geographical proximity to the species within the *M. pacifica* complex (Vázquez-García 1994).

Morphologically, *Magnolia pacifica* s. str. and *M. talpana* are similar. They share ellipsoid glabrous fruits and elliptic to elliptic-lanceolate leaves. However, they differ in flower size, with *M. talpana* having smaller flowers and a compact pollination chamber involving the outer and inner whorls of petals, where the inner petals are nearly equal in size, unlike the varying sizes in *M. pacifica* (Vázquez-García et al. 2021). Additionally, *M. talpana* has pubescent peduncles, whereas *M. pacifica* has glabrous peduncles. Both species inhabit cloud forests, riparian forests, and ecotones with oak-pine forests, with *M. pacifica* found in north-western Jalisco and southern Nayarit, and *M. talpana* endemic to western Jalisco (Fig. 2). *Magnolia vallartensis* is characterized by smaller glabrous fruits with fewer carpels, broadly oblong to elliptic leaves with an obtuse apex, creamy-white flowers, and a smaller tree size (up to 15 m) (Vázquez-García et al. 2012). It is found in riparian forests, tropical sub-deciduous and subperennial forests, occurring in ecotones with tropical cloud forests at lower elevations in the coastal mountains of Puerto Vallarta and Cabo Corrientes (Fig. 2).

Magnolia pugana and *M. granbarrancae* are morphologically similar, with both species having glabrous fruits with glabrous peduncles and lanceolate to narrowly elliptic leaves. Unlike *M. pugana*, *M. granbarrancae* has smaller flowers and a tighter pollination chamber. Additionally, *M. granbarrancae* has smaller subglobose to broadly obovoid fruits with orange seeds, while *M. pugana* has oblongoid to ellipsoid fruits with scarlet red seeds. Both species thrive in seasonally dry climates, with *M. granbarrancae* strictly inhabiting gallery riparian forests surrounded by tropical seasonal dry forest endemic to the Rio Verde region, central Jalisco, and *M. pugana* inhabiting streams and springs surrounded by tropical seasonal dry forest and oak-pine forests in northern Jalisco and southern Zacatecas (Fig. 2).

Magnolia tarahumara exhibits a gradient of morphological characteristics across its broad latitudinal geographic distribution range. Its leaves vary from elliptic to lanceolate-elliptic, and its glabrous fruits range from small and rounded with few carpels to elliptical-oblongoid with more carpels, rarely showing pubescence. This species inhabits cloud forests from northern Nayarit to moist canyons with perennial streams, characterized by riparian vegetation and oak forests, in the southern part

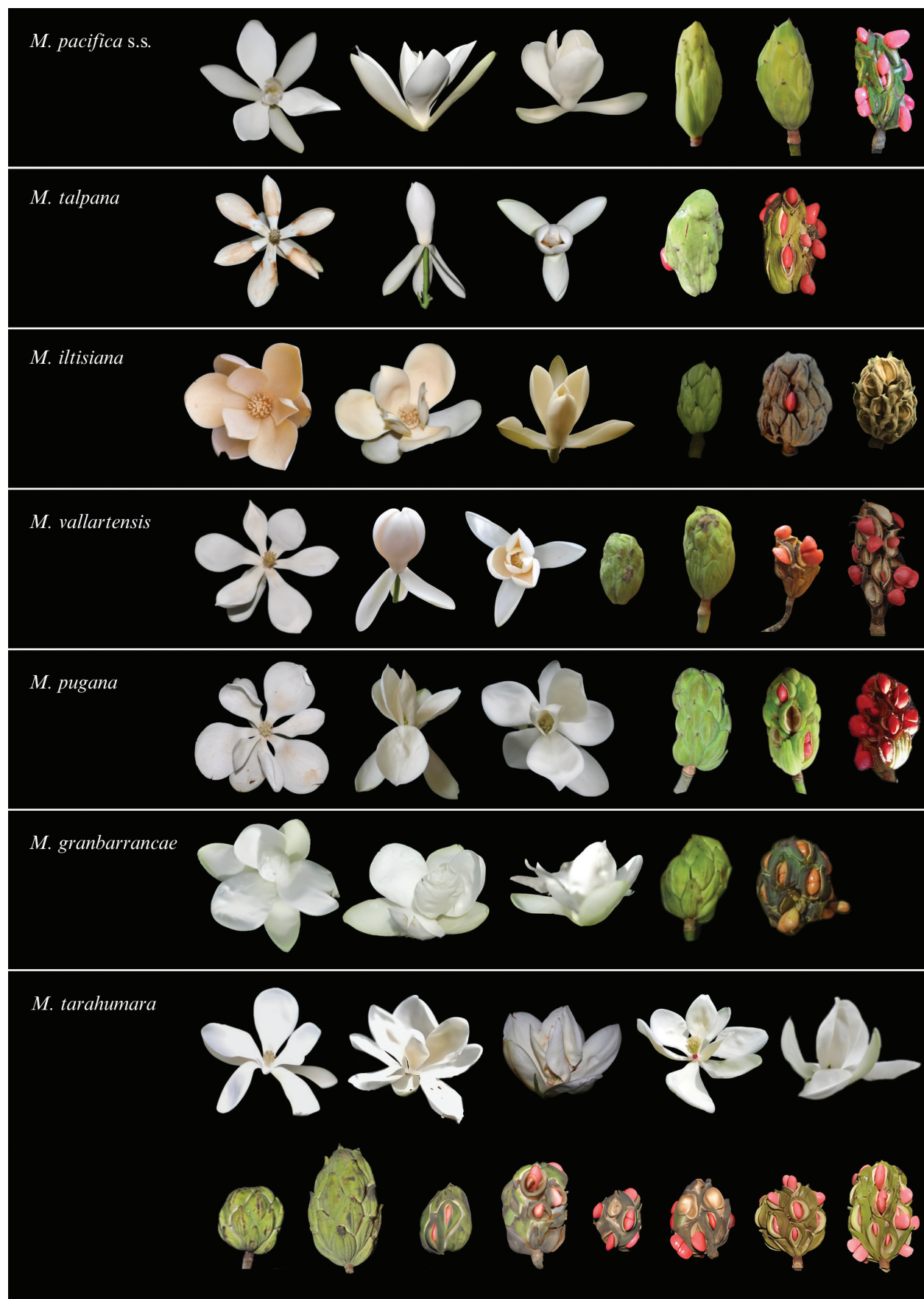


Figure 1. Morphological variation in flowers and fruits of the *Magnolia pacifica* species complex.

of Chihuahua and Sonora throughout the Sierra Madre Occidental (Fig. 2).

Magnolia iltisiana features elliptic to oblong-lanceolate leaves that are green and glabrous above, and pale green and pubescent beneath (Vázquez-García 1994). Its flowers are creamy-white to pale yellowish, and its fruits are sub-globose to oblong and densely yellowish pubescent. This species inhabits moist sub-deciduous cloud forests, primarily in ravines, ranging from south Jalisco to Michoacan (Fig. 2).

Plant material and DNA extraction

Plant material was collected in 25 localities throughout the *Magnolia pacifica* species complex range (Fig. 2; Table 1). Samples were stored in Silica Gel at -20°C until DNA extraction. Genomic DNA was extracted using a DNeasy® Plant Mini Kit (QIAGEN), and resultant concentrations were quantified using a NanoDrop™ One UV-Vis spectrophotometer.

PCR amplification, sequencing, and alignment

We performed PCR amplification of three chloroplasts (cpDNA) intergenic spacers: *trnT-trnL*, *rpl32-trnL* (Azuma et al. 2011; Rico and Gutiérrez Becerril 2019),

and *trnH-psbA* (Azuma et al. 1999; Kim and Suh 2013). Additionally, three low-copy nuclear Conserved Ortholog Set (COS) genes were amplified: LEAFY (LFY), Phytochrome A (PHYA), and Alanine-glyoxylate aminotransferase (AGT1). As described in previous studies (Mathews and Donoghue 1999; Nie et al. 2008; Kikuchi and Osone 2020), universal primers were used to amplify COS genes, which have proven useful in phylogenetic reconstructions of closely related species (Li et al. 2008; Zimmer and Wen 2013; Kikuchi and Osone 2020).

Amplifications were conducted in 18 μL reaction volumes, containing 150 ng of genomic DNA, 1X PCR buffer, 2–2.5 mM MgCl_2 , 0.2 mM dNTPs mix, 0.4 μM of each primer, and 0.8 U Taq polymerase (Invitrogen), using a PTC-100 thermal cycler (MJ Research, Inc.). PCR programs were tailored for each primer (Suppl. material 1). Amplification products were stained with GelRed (Biotium, Fremont, California) and separated by electrophoresis on 1.2% agarose gels in 1X TBE buffer, run at 80 V for approximately 1.5 h. The gels were then visualized and photographed under UV light using a D-Digit™ Gel Scanner (LI-COR, Bioscience).

Following visualization, PCR products were sequenced by the University of Arizona Genetics Core (Arizona,

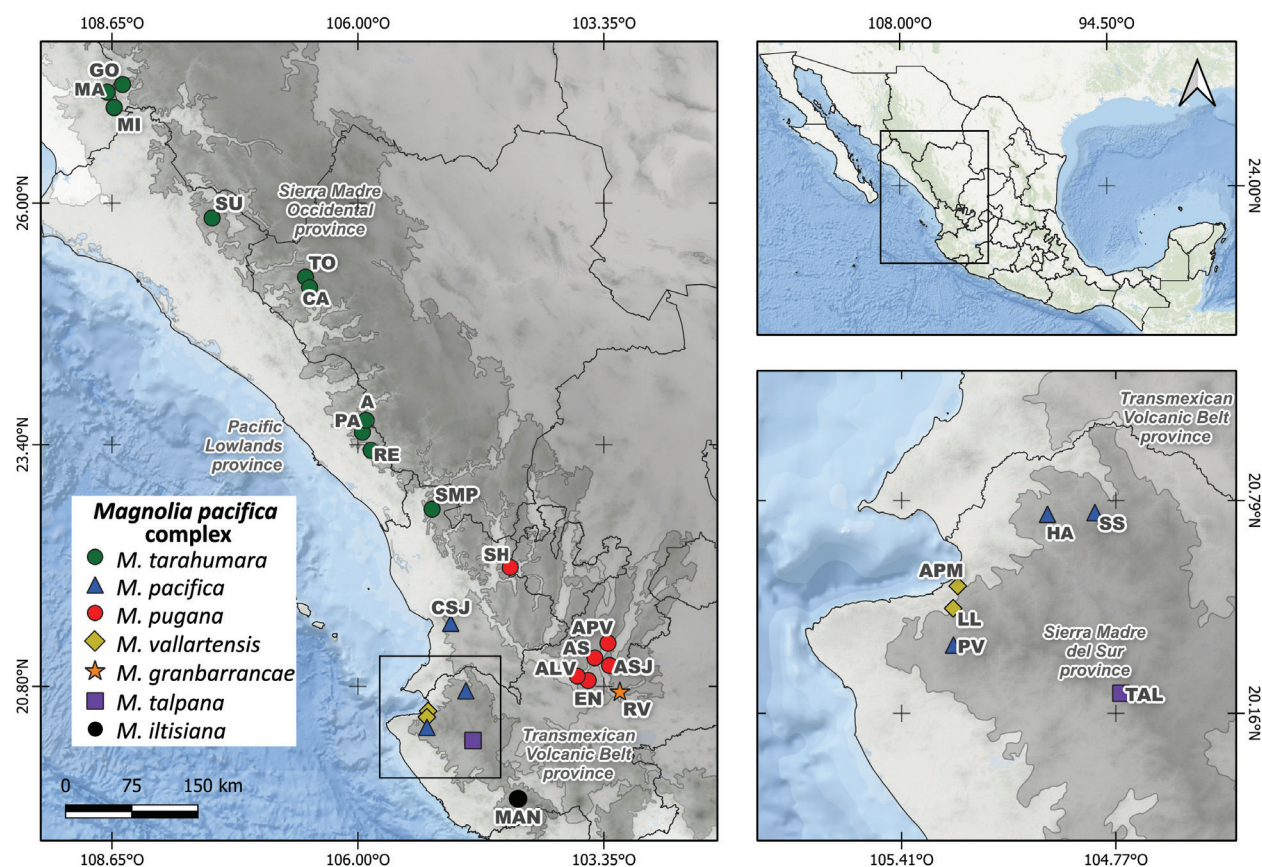


Figure 2. Sample locations of the *Magnolia pacifica* species complex. *Magnolia tarahumara* is represented by green dots, *M. pacifica* by blue triangles, *M. pugana* by red dots, *M. vallartensis* by yellow rhombi, *M. granbarrancae* by an orange star, *M. talpana* by a purple square, and *M. iltisiana* by a black dot. Location codes are provided in Table 1.

Table 1. Species, location, code, biogeographic province, and state of collected individuals. * indicates the type locality of the species. Biogeographical provinces sensu Morrone et al. (2017). State abbreviations: CHI = Chihuahua, DGO = Durango, JAL = Jalisco, NAY = Nayarit, SON = Sonora, SIN = Sinaloa, ZAC = Zacatecas. *n* = number of individuals collected. Elev. = elevation in m a.s.l.

Species	Location	Code	Biogeographic province	State	<i>n</i>	Latitude (N)	Longitude (W)	Elev.
<i>M. granbarrancae</i>	Rio Verde	RV*	Pacific Lowlands	JAL	5	20°44'3.516"N	103°11'2.976"W	1160
<i>M. iltisiana</i>	Las Joyas, Manantlán	MAN*	Sierra Madre del Sur	JAL	3	19°35'12.876"N	104°17'6.036"W	1850
<i>M. pacifica</i>	Cerro San Juan	CSJ	Sierra Madre Occidental	NAY	4	21°28'36.156"N	105°0'6.588"W	1480
	San Sebastián	SS*	Sierra Madre del Sur	JAL	5	20°45'50.436"N	104°50'7.836"W	1550
	Haraveri Botanical Garden	HA	Sierra Madre del Sur	JAL	2	20°45'23.940"N	104°58'26.328"W	770
	Provincia, Cabo Corrientes	PV	Sierra Madre del Sur	JAL	4	20°21'41.508"N	105°15'23.148"W	900
<i>M. pugana</i>	Arroyo La Virgen	ALV	Transmexican Volcanic Belt	JAL	3	20°48'49.968"N	103°34'58.368"W	1460
	Arroyo El Encanto	EN*	Transmexican Volcanic Belt	JAL	2	20°48'18.324"N	103°33'19.944"W	1470
	Arroyo San José	ASJ	Sierra Madre Occidental	JAL	2	21°1'50.376"N	103°15'10.188"W	1300
	Arroyo Sampurrón	AS	Sierra Madre Occidental	JAL	1	21°3'8.460"N	103°14'31.380"W	1260
	Palo Verde	APV	Sierra Madre Occidental	ZAC	3	21°15'49.428"N	103°18'18.468"W	1530
	Sierra Huichola	SH	Sierra Madre Occidental	JAL	1	22°3'13.752"N	104°19'3.072"W	1480
<i>M. talpana</i>	Talpa	TAL*	Sierra Madre del Sur	JAL	4	20°13'5.340"N	104°45'34.056"W	1340
<i>M. tarahumara</i>	Mirasol	MI	Sierra Madre Occidental	SON	2	27°1'19.092"N	108°37'0.876"W	960
	Las Magnolias	MA	Sierra Madre Occidental	SON	2	27°1'37.488"N	108°37'36.372"W	1210
	Gorogachi	GO	Pacific Lowlands	CHI	4	27°15'58.572"N	108°32'32.676"W	850
	Revolcaderos	RE	Sierra Madre Occidental	DGO	1	23°36'5.544"N	105°50'56.904"W	1990
	Mesa de los Alisos	A	Sierra Madre Occidental	SIN	1	23°34'56.892"N	105°51'29.556"W	2150
	El Palmito	PA	Sierra Madre Occidental	SIN	1	23°33'51.264"N	105°50'41.964"W	1910
	Canelas	CA	Sierra Madre Occidental	DGO	2	25°7'1.488"N	106°32'31.992"W	1410
	Topia	TO	Sierra Madre Occidental	DGO	1	25°12'19.368"N	106°34'3.468"W	1700
	Surutato	SU	Sierra Madre Occidental	SIN	4	25°50'25.332"N	107°34'12.900"W	1550
	Santa María Picachos	SMP	Transmexican Volcanic Belt	NAY	5	22°41'51.072"N	105°10'51.240"W	1420
<i>M. vallartensis</i>	Arroyo Palo María	APM*	Pacific Lowlands	JAL	4	20°31'55.956"N	105°14'44.988"W	350
	Arroyo Las Lajitas	LL	Pacific Lowlands	JAL	4	20°28'13.908"N	105°15'24.408"W	650

USA). Sequence quality was subsequently reviewed and edited using Sequencher v.4.1.4 (Gene Codes Corporation, Ann Arbor, MI, USA), while sequence alignments were manually constructed using PhyDE-1 (Müller et al. 2010). The resulting sequences were deposited in GenBank (Suppl. material 2).

For the nuclear COS genes, the genotypes were phased into haplotypes using DnaSP v.6.12.03 (Rozas et al. 2017) with the PHASE algorithm (Stephens et al. 2001; Stephens and Donnelly 2003). This process was applied to each gene using default MCMC settings, assuming no recombination within a gene. Additionally, we included extra sequences from GenBank (Suppl. material 3).

To determine the model of molecular evolution that best fits each marker, we applied corrected Akaike Information Criterion (AICc) in jModelTest v.2.1.6 (Darriba et al. 2012). Subsequently, a concatenated dataset comprising the cpDNA intergenic spacers and nDNA COS genes was conducted using Mesquite v.2.75 (Maddison and Maddison 2011).

Phylogenetic reconstruction

To determine whether the *Magnolia pacifica* complex forms a monophyletic group within *M. sect. Magnolia*, a phylogenetic analysis was conducted including 13 representative taxa from the section and eight individuals from the *M. pacifica* complex. Two species from *M. sect. Macrophylla* were included as outgroup to root the tree (Suppl. materials 2, 3). To further analyse the relationship and classification of the *M. pacifica* complex species, a focused reconstruction was conducted, including 67 individuals representing all sampled populations (Suppl. material 2), with *Magnolia macrophylla* Michx. retained as the outgroup. The outgroup selection was based on studies by Nie et al. (2008) and Dong et al. (2022). We employed two analytical methods: Bayesian inference (BI) and maximum likelihood (ML).

The BI analyses were run on the CIPRES Science Gateway (Miller et al. 2010) using MrBayes v.3.2.7 (Ronquist et al. 2012). Each run consisted of four Markov Chain Monte Carlo (MCMC) of 20,000,000 generations, with tree sampled every 1,000 generations. The first 25% of the sampled trees were discarded as burn-in. Posterior probabilities (PP) were calculated using a 50% majority-rule consensus of the retained trees after burn-in. The harmonic means of likelihood were estimated using the 'sump' command (Ronquist and Huelsenbeck 2003). MCMC chains were evaluated for each independent run in Tracer v.1.7.1 (Rambaut et al. 2018) to verify that the effective sample sizes (ESS) were > 200 (Drummond and Bouckaert 2015). The 50% majority-rule consensus tree was visualized in FigTree v.1.4.3 (Rambaut 2016). Concurrently, ML analyses were performed using RAxML (Randomized Accelerated Maximum Likelihood) via raxmlGUI 2.0 software (Edler et al. 2020). The parameter function was set to "ML + rapid bootstrapping" with 1000 replicates.

Divergence time estimation

To estimate divergence times, we constructed a ML tree based on 94 individuals (Suppl. materials 2, 3), including 78 ingroup and 16 outgroup samples. The ingroup comprised representative individuals of the *Magnolia pacifica* complex, including 23 individuals of *M. tarahumara*, 21 of *M. pacifica* s. str., four of *M. talpana*, 12 of *M. pugana*, eight of *M. vallartensis*, five of *M. granbarrancae*, and five of *M. iltisiana*. Additionally, 13 individuals from other taxa within *M. sect. Magnolia* were included as outgroups. Two taxa from *M. sect. Macrophylla* were also incorporated as outgroups, and *M. mexicana* DC. (*M. sect. Talauma*) was designated as the most external taxon to root the tree.

Divergence times were estimated along branches on the concatenated ML tree (comprising cpDNA intergenic spacers + nDNA COS genes) using the RelTime-ML algorithm in MEGA v11.0. This algorithm employs ML-optimized branch-length estimation and represents a robust statistical approach for divergence time inference (Mello 2018). To calibrate our findings, we utilized a normal distribution with a mean age of 32 Ma and a specified standard deviation for the root node of *M. sect. Macrophylla*. This calibration was based on the calibrated multi-species coalescent summary tree for Magnoliaceae by Veltjen et al. (2022). The summary tree was visualized using FigTree v.1.4.3.

Species delimitation

Species delimitation within the *Magnolia pacifica* complex was assessed under the multispecies coalescent (MSC) framework using Bayesian Phylogenetics and Phylogeography (BPP) v.4.2 (Yang 2015). BPP uses the MSC model to compare alternative species delimitation methods within a Bayesian framework using molecular data, without relying on reciprocal monophyly as a criterion for species recognition (Yang and Rannala 2010; Rannala and Yang 2013). This approach is particularly suitable for recently diverged lineages and gene tree discordance.

Species delimitation and species tree estimation were first conducted using algorithm A11, which jointly estimates the number of species and their phylogenetic relationships under a reversible-jump Markov Chain Monte Carlo (rjMCMC) framework. Analyses were based on 67 individuals of the *M. pacifica* complex (Suppl. material 2) and six loci, including three plastid intergenic spacers and three low-copy nuclear COS genes. Nuclear COS genes were treated as diploid, and phased haplotypes were used as input, while cpDNA loci were modelled as haploid. Locus-specific inheritance scalars were set to 1.0 for nuclear genes and 0.25 for plastid markers (Hey and Nielsen 2004). The species tree topology was allowed to vary during MCMC using nearest-neighbour interchange (NNI) and subtree-pruning-regrafting (SPR) moves, thereby enabling exploration of alternative species

delimitation models and topologies without assuming species monophyly a priori.

To further evaluate support for the species tree inferred under A11, a second analysis was conducted using algorithm A10, in which the species tree topology with the highest posterior probability obtained under the A11 analysis was fixed. Under this framework, rjMCMC moves were restricted to species delimitation models compatible with the fixed topology, while maintaining the same prior distributions for population size and divergence time parameters. This approach allowed for the estimation of posterior probabilities for internal nodes and lineage relationships conditional on the inferred species tree, providing an explicit assessment of support for relationships among delimited lineages.

RESULTS

Phylogenetic reconstruction

For both phylogenetic reconstructions, the molecular evolution models produced by jModelTest for cpDNA intergenic regions were: *trnH-psbA* = GTR + G, *trnT-trnL* = TPM1uf + G, *rpl32-trnL* = TVM + I; meanwhile, for the COS genes, models were: LFY = TPM1 + G, PHYA = TIM1ef + G, and AGT1 = HKY + I.

The final dataset used to test the monophyly of the *Magnolia pacifica* complex within *M. sect. Magnolia* included 23 samples; the ingroup comprised 13 representative taxa of *M. sect. Magnolia* and eight individuals of the *M. pacifica* complex, while the outgroup

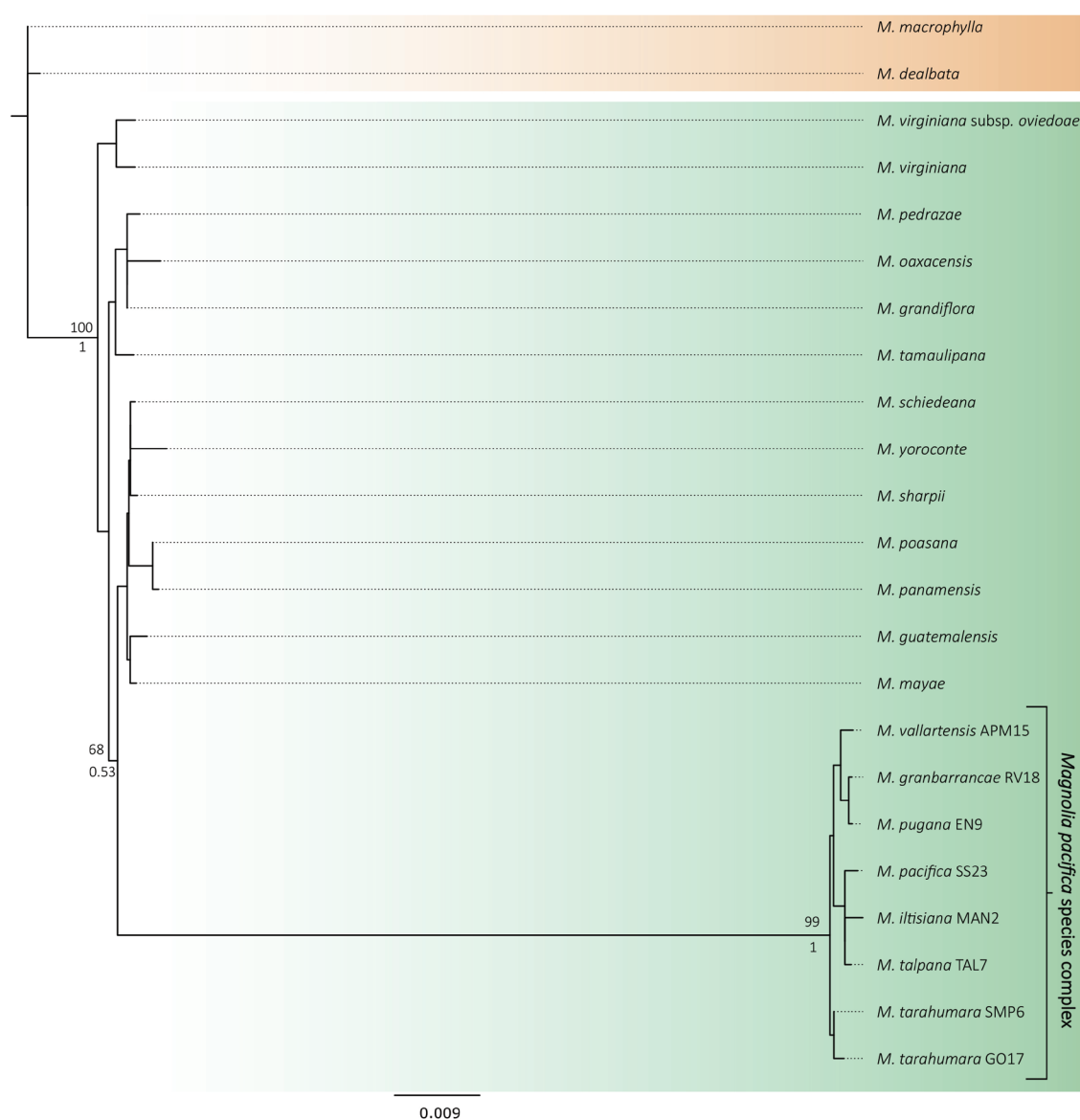


Figure 3. Maximum Likelihood phylogenetic tree of *Magnolia* sect. *Magnolia*. Branch support values are indicated as parametric bootstrap support (above) and Bayesian posterior probability (below). Clades are highlighted with coloured backgrounds: *M. sect. Macrophylla* (orange, outgroup), and *M. sect. Magnolia* (green, ingroup).

was composed of *M. macrophylla* and *M. dealbata* Zucc. from *M. sect. Macrophylla*. The number of aligned base pairs (bp) was 433 bp for *trnH-psbA*, 809 bp for *trnT-trnL*, 700 bp for *rpl32-trnL*, 797 bp for LFY, 965 bp for PHYA, and 1,109 bp for AGT1. The concatenated cpDNA + nDNA matrix was 4,818 bp in length and contained 413 polymorphic sites. Partitioned molecular reconstruction of the concatenated cpDNA + nDNA matrix using Bayesian inference (BI) and maximum likelihood (ML) retrieved similar topologies. The ML tree is depicted in Fig. 3. The phylogenetic tree showed *M. sect. Magnolia* is a monophyletic group rooted by *Magnolia dealbata* and *M. macrophylla* (both belonging to *M. sect. Macrophylla*). Within *M. sect. Magnolia*, the *M. pacifica* complex forms a

monophyletic group with high posterior probabilities (PP = 1) and parametric bootstrap support (BS = 99) values.

For the focused phylogenetic reconstruction of the *M. pacifica* complex, the final dataset consisted of 68 samples, including 67 ingroup and *M. macrophylla* as outgroup. The number of aligned base pairs (bp) was 430 bp for *trnH-psbA*, 788 bp for *trnT-trnL*, 702 bp for *rpl32-trnL*, 797 bp for LFY, 905 bp for PHYA, and 906 bp for AGT1. The concatenated cpDNA + nDNA matrix was 4,533 bp in length and contained 185 polymorphic sites. The partitioned molecular reconstruction of the concatenated cpDNA + nDNA matrix using ML and BI retrieved similar topologies. The ML tree is depicted in Fig. 4. The phylogenetic analysis recovered a moderately supported tree with four clades. Clade 1 (shown in yellow in Fig. 4):

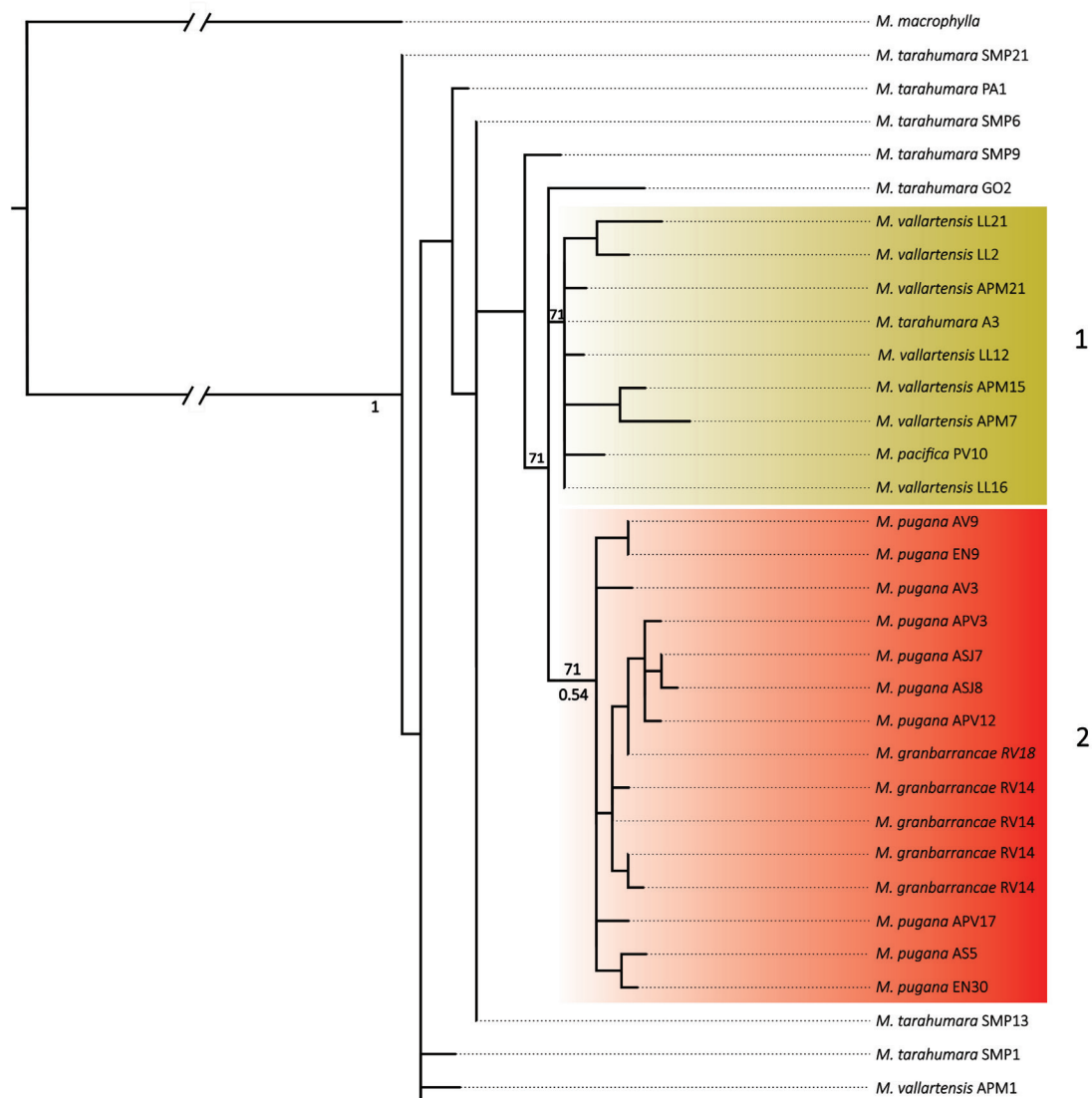


Figure 4. Maximum Likelihood phylogenetic tree of the *Magnolia pacifica* complex. Branch support values are indicated as parametric bootstrap support (above) and Bayesian posterior probability (below). Four main clades are colour-coded: Clade 1 (yellow) includes *M. vallartensis* (8/9 individuals), one *M. pacifica*, and one *M. tarahumara*; Clade 2 (red) comprises all individuals of *M. pugana* and *M. granbarrancae*; Clade 3 (blue) includes *M. pacifica* (13/14 individuals), *M. iltisiana*, and all individuals of *M. talpana*; Clade 4 (green) is formed by *M. tarahumara* (15/23 individuals). Uncoloured branches represent individuals outside these primary clades.

composed of seven out of eight *M. vallartensis* individuals, one individual of *M. pacifica*, and one individual of *M. tarahumara*. Clade 2 (shown in red in Fig. 4): composed of all individuals of *M. pugana* and *M. granbarrancae*. Clade 3 (shown in blue in Fig. 4): composed of all individuals of *M. pacifica*, *M. iltisiana*, and all individuals of *M. talpana*. Clade 4 (shown in green in Fig. 4): composed of 15 out of 23 individuals of *M. tarahumara*. The remaining individuals are outside the four clades.

Divergence time estimation

Based on the concatenated matrix (cpDNA intergenic spacers + nDNA COS genes) and the calibration applied in this study, the split of *M. sect. Magnolia* was estimated to have occurred around 25 Mya during the late Oligocene. The divergence of the species within the section was estimated at ~11 Mya (95% confidence interval: 9.75–11.31 Mya) during the late Miocene. Furthermore, the

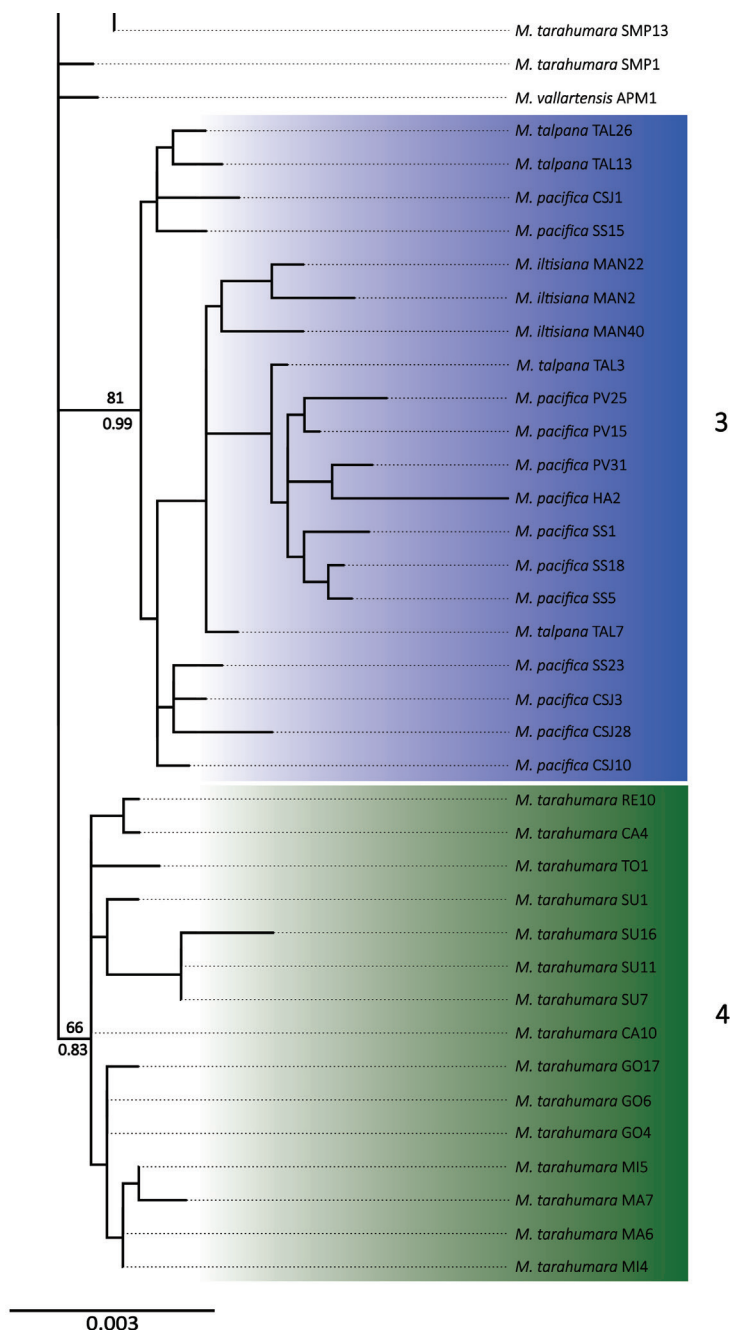


Figure 4 (continued). Maximum Likelihood phylogenetic tree of the *Magnolia pacifica* complex. Branch support values are indicated as parametric bootstrap support (above) and Bayesian posterior probability (below). Four main clades are colour-coded: Clade 1 (yellow) includes *M. vallartensis* (8/9 individuals), one *M. pacifica*, and one *M. tarahumara*; Clade 2 (red) comprises all individuals of *M. pugana* and *M. granbarrancae*; Clade 3 (blue) includes *M. pacifica* (13/14 individuals), *M. iltisiana*, and all individuals of *M. talpana*; Clade 4 (green) is formed by *M. tarahumara* (15/23 individuals). Uncoloured branches represent individuals outside these primary clades.

M. pacifica complex is grouped within *M. sect. Magnolia* with a split at ~7.5 Mya and a very recent divergence date of 1.27 Mya (95% confidence interval: 0.64–2.53 Mya) during the early Pleistocene (Fig. 5).

Species delimitation

Species delimitation analysis using the A11 algorithm in BPP (under the multispecies coalescent), strongly supports a model recognizing four independently evolving lineages: corresponding to *Magnolia tarahumara*, *M. iltisiana* – *M. pacifica* (i.e. *M. iltisiana* + *M. pacifica* + *M. talpana*), *M. pugana* (i.e. *M. pugana* + *M. granbarrancae*), and *M. vallartensis*. The most frequently sampled model (PP = 0.738) recovered a species tree topology where *M. tarahumara* is sister to a clade comprising *M. iltisiana* – *M. pacifica*, as well as *M. pugana* and *M. vallartensis*. Models recognizing fewer than four species received negligible support (PP ≤ 0.004), providing strong evidence against lineage collapse within the complex.

Complementarily, the A10 analysis, which evaluates species delimitation on a fixed guide tree, provided unequivocal support for the same four-species hypothesis (Fig. 6). The model in which all three internal nodes represent speciation events received a posterior probability of 1.0, while all alternative models were rejected.

The guide tree recovered *M. tarahumara* as the most divergent lineage, with *M. pugana* and *M. vallartensis* forming a strongly supported clade sister to *M. iltisiana* – *M. pacifica*, and all nodes showing posterior probabilities of 1.0. Together, these results indicate that, despite limited resolution in concatenated phylogenetic reconstructions,

multilocus coalescent-based analyses consistently support the recognition of four distinct evolutionary lineages within the *Magnolia pacifica* complex.

DISCUSSION

Under the General Lineage Species Concept and employing a coalescent approach, our study provides a phylogenetic framework that confirms the *M. pacifica* complex as a monophyletic group within *M. sect. Magnolia*. The phylogenetic analyses recover four well-supported clades (Figs 4, 6). However, integration of morphological and distributional data further distinguishes five distinct evolutionary lineages, as the *M. iltisiana* – *M. pacifica* clade contains two lineages that are morphologically and biogeographically distinct but not resolved as separate clades in the phylogenetic analyses (explained below). This framework offers a new perspective on species boundaries that informs both the taxonomy and conservation of this group.

Phylogenetic relationships

Here, we present the first phylogenetic analysis of all described species in the *M. pacifica* complex (*M. granbarrancae*, *M. pacifica*, *M. pugana*, *M. talpana*, *M. tarahumara*, and *M. vallartensis*). These species, along with *M. iltisiana*, were recovered as a monophyletic group within *M. sect. Magnolia*. This result is in line with previous phylogenetic and phylogenomic analyses involving certain species of the *M. pacifica* complex (Azuma et al. 2001; Kim et al. 2001; Nie et al. 2008; Wang

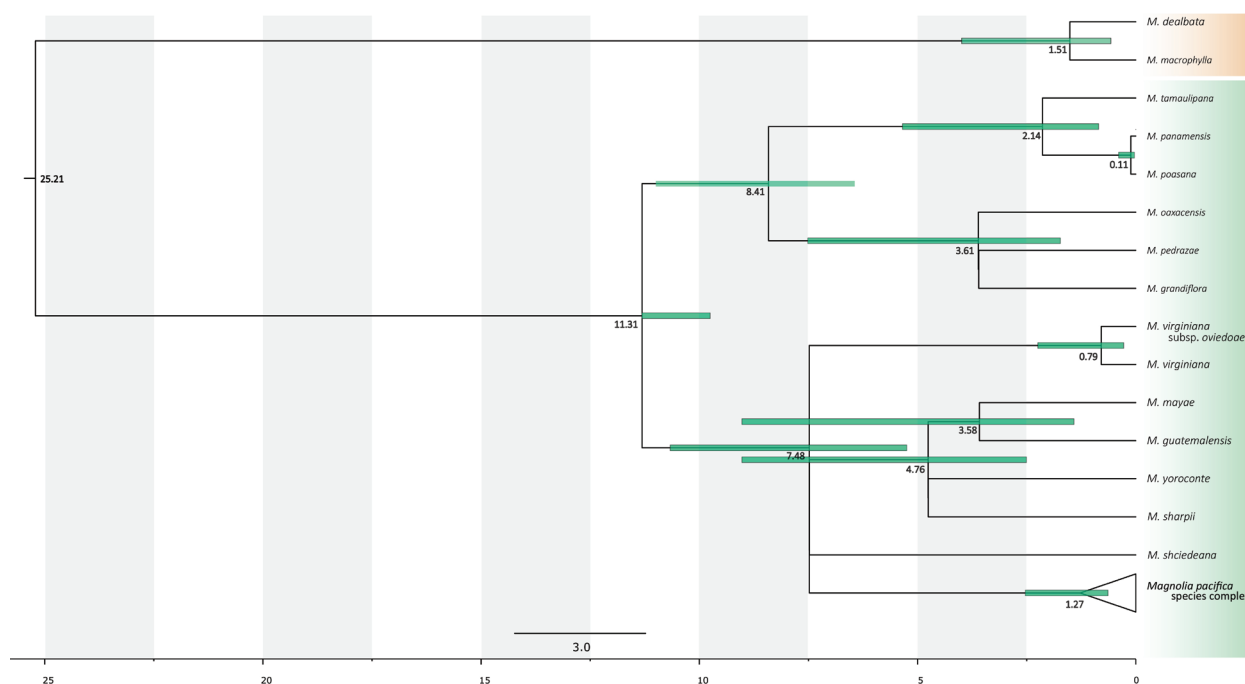


Figure 5. Calibrated time tree obtained using RelTime-ML analysis based on the concatenated sequences (cpDNA intergenic spacers + nDNA COS genes). Node labels indicate the parametric bootstrap support. Divergence dates for each node are shown in millions of years ago (mya). Green bars surrounding each node represent 95% confidence intervals for the divergence date estimates. The orange shadow highlights *Magnolia* sect. *Macrophylla*, while the green shadow indicates *Magnolia* sect. *Magnolia*.

et al. 2020; Rico et al. 2021; Veltjen et al. 2022; Aldaba Núñez et al. 2024). The placement of *M. iltisiana* within the *M. pacifica* complex is supported by its close relationship, as evidenced in previous phylogenetic reconstructions (Azuma et al. 2001; Kim et al. 2001; Nie et al. 2008; Veltjen et al. 2022; Aldaba Núñez et al. 2024, 2026). Moreover, these species exhibit morphological similarities (Fig. 1) and geographical proximity (Fig. 2).

The phylogenetic relationships within the complex remain somewhat inconclusive in this analysis. *Magnolia pacifica*, *M. talpana*, *M. tarahumara*, and *M. vallartensis* are polyphyletic, potentially due to introgression or incomplete lineage sorting. The inclusion of additional nDNA and cpDNA regions in future analyses could help further refine the phylogeny.

Divergence time estimation

In our study, we estimated the split of *M. sect. Magnolia* occurred around 25 Mya, with the divergence within the section happening approximately 11 Mya. These estimated times are consistent with previous research, which suggested an earlier split of *M. sect. Magnolia* around 30 Mya in the late Oligocene, and a later divergence of *M. sect. Magnolia* at approximately 10 Mya during the late Miocene (Dong et al. 2022; Veltjen et al. 2022; Guzmán-Díaz et al. 2025). Furthermore, the divergence of the

M. pacifica complex from the rest of *M. sect. Magnolia* is estimated to have occurred around 7.5 Mya, followed by rapid radiation around 1.27 Mya during the Calabrian age of the Pleistocene, the most recent period of repeated glaciations (Hewitt 2004). The Pleistocene climate fluctuations, combined with the highly heterogeneous mountainous environments in western and northwestern Mexico, likely created climatic and orographic barriers, promoting isolation and hindering gene flow among *Magnolia* populations (Gugger et al. 2011; Ornelas et al. 2013). This scenario could have facilitated the allopatric speciation of the *M. pacifica* complex. For instance, previous studies have suggested that climatic events have influenced the geographical distribution of species like *M. pedrazae* A.Vázquez and *M. schiedeana* Schltdl., leading to range shifts, expansions, and contractions (Rico et al. 2021). This hypothesis is supported by various phylogeographic studies in different plant species (Ruiz-Sánchez and Ornelas 2014; Ornelas et al. 2019; Ortiz-Rodríguez et al. 2020). Further research should consider incorporating population genetics processes (Maddison and Knowles 2006), such as those assessed by phylogeography, as well as incorporating additional nDNA and cpDNA regions or even phylogenomics. These approaches could provide further insights into the biogeographic and climatic factors influencing the speciation processes within the complex.

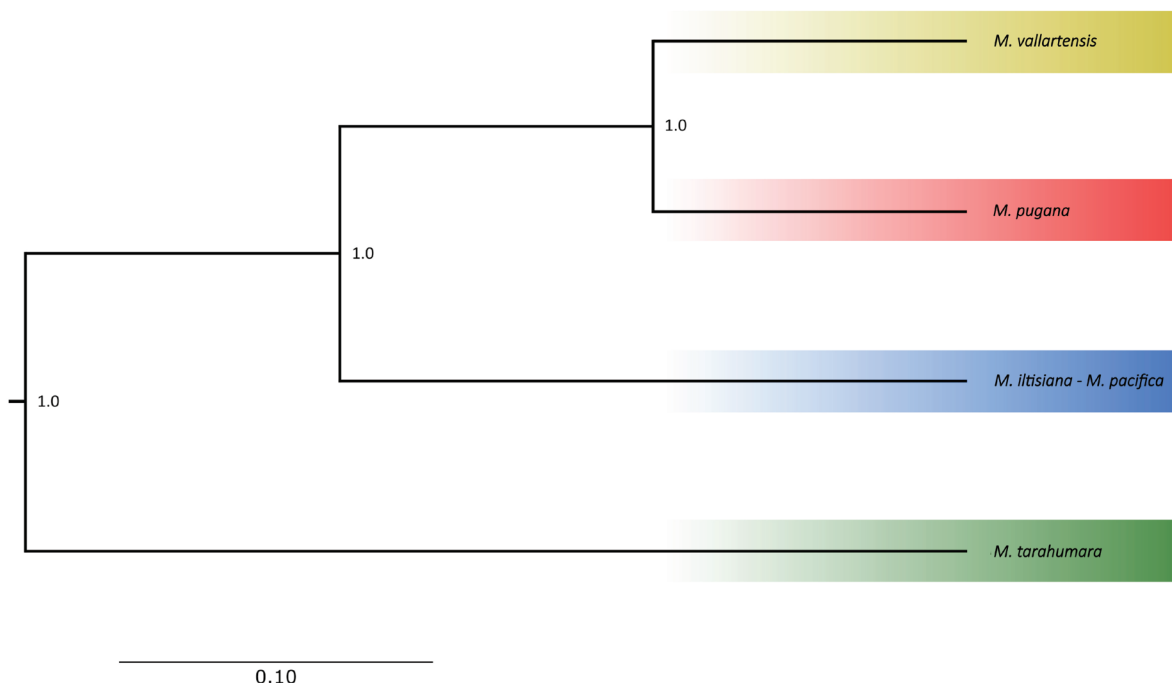


Figure 6. Species tree and Bayesian species delimitation (A10 analysis) for the *Magnolia pacifica* complex. The analysis recovered four independently evolving lineages: *M. tarahumara*, *M. iltisiana* – *M. pacifica* (including *M. talpana*), *M. pugana* (including *M. granbarrancae*), and *M. vallartensis*. Node labels indicate the posterior probability support for each delimited lineage.

Species delimitation

The *Magnolia pacifica* complex has undergone a complex and dynamic taxonomic history with repeated changes in species circumscription based on morphological, geographic, and ecological characteristics. Originally, *M. pacifica* was treated as a single, morphologically variable species comprising multiple subspecies with broad, partially overlapping geographic distributions, and closely associated with *M. iltisiana* due to their morphological similarity and geographic proximity (Vázquez-García 1994). Subsequent taxonomic revisions progressively elevated several of these lineages to species rank: *M. pugana*, *M. tarahumara*, *M. vallartensis*, *M. talpana*, and *M. granbarrancae*, based primarily on combinations of vegetative, floral, and fruit characters, as well as ecological and geographic differentiation without a formal statistical framework (Vázquez-García et al. 2002, 2012, 2013, 2021). However, the recognition of these taxa has remained challenging due to overlapping morphological variation and the recent divergence of lineages, raising long-standing questions regarding species boundaries and evolutionary independence within the complex. This taxonomic uncertainty provided the central motivation for applying a multispecies coalescent framework to explicitly test species limits and evolutionary relationships within the *M. pacifica* species complex using multilocus molecular data.

Under the multispecies coalescent model in BPP, we recovered four independently evolving lineages: *Magnolia iltisiana* – *M. pacifica* (i.e. *M. iltisiana* + *M. pacifica* + *M. talpana*), *M. pugana* (i.e. *M. pugana* + *M. granbarrancae*), *M. tarahumara*, and *M. vallartensis*, reflecting shared ancestry and distinct coalescent histories among populations.

The *Magnolia iltisiana* – *M. pacifica* lineage, includes individuals currently assigned to *M. iltisiana*, *M. pacifica*, and *M. talpana*. These populations are distributed along a latitudinal continuum across the mountainous regions of the Western Sierra Madre del Sur subprovince (Morrone 2017) and partially share ecological conditions, suggesting historical or ongoing gene flow among populations. Notably, *M. talpana* (TAL locality; Table 1) was originally included within *M. pacifica* (Vázquez-García 1994), and its subsequent recognition as a distinct species was based on consistent floral differences, including reduced flower size, a more compact inner petal whorl, and a differentiated pollination chamber (Vázquez-García et al. 2021). However, previous molecular evidence from six nuclear ISSR markers revealed only weak genetic differentiation between *M. talpana* and *M. pacifica* s. str. (Muñiz-Castro et al. 2020). Consistent with previous molecular evidence, our multispecies coalescent analyses support the interpretation that these taxa form a single, independently evolving lineage. The strong support for a single coalescent lineage (PP = 1.0 in the A10 analysis) indicates that, despite their phenotypic divergence, these populations have either diverged too recently for ancestral

polymorphism to have sorted completely across the six loci assayed or that low levels of gene flow have been sufficient to maintain genetic cohesion. However, it is possible that we are underrepresenting *M. iltisiana*, as we included individuals from only one population, located close to *M. pacifica*. *Magnolia iltisiana* differs morphologically from *M. pacifica* in having densely pubescent bracts, peduncular internodes, petioles, and flower buds, and yellowish-greenish velutinous to felted fruits, in contrast to the glabrous to rarely pubescent and usually completely glabrous fruits of *M. pacifica* (Vázquez-García 1994). Following Morrone (2017), these species inhabit different biogeographic districts within the Western Sierra Madre del Sur subprovince. While *M. pacifica* occurs in the Jalisco-Tuito district, *M. iltisiana* inhabits the Jalisco-Manantlan district. This biogeographic pattern has been observed in other plant species: *Abies jaliscana* (Martínez) Mantilla, Shalisko & A.Vázquez, *Daphnopsis mexiae* Nevling, *Juniperus jaliscana* Martínez, *Pinus jaliscana* Pérez de la Rosa, *Quercus cualeensis* L.M.González, *Q. tuitensis* L.M.González, occurring in the Jalisco-Tuito district; and *Abies flinckii* Rushforth., *A. religiosa* (Kunth) Schltdl. & Cham., *Beilschmiedia manantlanensis* Cuevas & Cochrane, and *Arachnothryx manantlanensis* (Lorence) Borhidi in the Jalisco-Manantlan district. This transition zone is considered a potential ecological barrier between adjacent areas due to differences in climatic conditions such as humidity and seasonal precipitation (Vázquez-García et al. 2010, 2014; Santiago-Alvarado et al. 2016). Their biological and taxonomic significance will require further evaluation using additional molecular data, as well as detailed morphological, geographic, and ecological evidence analysed within a statistical framework. Therefore, our results suggest the synonymization of *M. talpana* under *M. pacifica*, while *M. iltisiana* is herein maintained as a distinct species.

The *M. pugana* lineage includes individuals currently assigned to *M. pugana* and *M. granbarrancae*. The population of *M. granbarrancae* was previously considered part of *M. pugana*. Evidence from six nuclear ISSRs showed genetic differentiation among allopatric populations of *M. pugana* and revealed two subgroups (Muñiz-Castro et al. 2020). A portion of one subgroup (locality RV) was later described as *M. granbarrancae* based on morphological differences such as smaller flowers and fruits, fewer carpels, and orange seeds vs scarlet red. These distinctions led to the description of *M. granbarrancae* as a distinct species from *M. pugana* (Vázquez-García et al. 2021). However, our multispecies coalescent analysis reveals that this morphological and moderate genetic differentiation (as detected by dominant ISSR markers) does not correspond to a break in coalescent history. The model that recognizes *M. pugana* and *M. granbarrancae* as a single independently evolving lineage received unequivocal support (PP = 1.0, A10 analysis). This suggests that the observed differentiation likely represents either: 1) incipient divergence with insufficient time for complete lineage sorting across the

loci sampled, or 2) phenotypic divergence driven by local adaptation. Consequently, our genetic data do not support the recognition of *M. granbarrancae* as a distinct lineage from *M. pugana*. Based on these results, we propose the synonymization of *M. granbarrancae* under *M. pugana*.

We confirmed that *M. vallartensis* represents a separately evolving lineage, consistent with the morphological distinctiveness of *Magnolia* populations inhabiting the coastal mountains of the municipality of Puerto Vallarta (Vázquez-García et al. 2012). It is distinguished from *M. pacifica* by a combination of morphological characters: outer petals with a colourful abaxial base; leaves broadly oblong to elliptic (vs elliptic-lanceolate) and larger in size; fewer carpels; and more numerous petals. Furthermore, ecological evidence suggests that the tropical river canyons separating *M. vallartensis* and *M. pacifica* populations act as barriers to effective gene flow. Although birds and beetles can potentially disperse pollen and seeds across these canyons, the microclimatic conditions at lower elevations – characterized by tropical deciduous forest rather than cloud or evergreen forests – prevent seedling establishment. This creates an ecological filter that, despite long-distance dispersal events, results in genetic isolation and differentiation between the two species (Muñiz-Castro et al. 2020). These genetic differences may also be attributed to parapatric ecological differentiation (Nosil 2012), given that *M. vallartensis* and *M. pacifica* occur in distinct habitats. *Magnolia vallartensis* thrives in the ecotone of tropical montane cloud forest and tropical sub-evergreen forest at elevations ranging from 100 to 1,120 m a.s.l. experiencing a tropical maritime climate with higher relative humidity, mean annual temperature, and rainfall. In contrast, *M. pacifica* grows in montane cloud forests at elevations of 750 to 2,250 m a.s.l., in a temperate montane climate characterized by lower relative humidity, mean annual temperature, and precipitation. Additionally, these species differ in their flowering phenology: *M. vallartensis* exhibits no evident flowering seasonality, whereas *M. pacifica* shows a highly seasonal pattern (Dahua-Machoa 2018; Muñiz-Castro et al. 2020).

Our coalescent delimitation strongly supports *M. tarahumara* as a distinct evolutionary lineage (PP = 1.0, A10 analysis), despite its paraphyletic presentation in the concatenated phylogenetic tree. This apparent contradiction is a known phenomenon in species delimitation and can be explained by the effectiveness of multispecies coalescent models to distinguish between incomplete lineage sorting (ILS) and true evolutionary independence (Jiao et al. 2021). *Magnolia tarahumara* exhibits a broad latitudinal distribution spanning approximately 700 km along the Sierra Madre Occidental. It inhabits a variety of environments and elevations, ranging from cool, moist canyons with perennial streams and nearly evergreen riparian vegetation at lower elevations in the Pacific Low Lands (850 to 1200 m a.s.l.) in northern localities, to cloud forests at higher elevations

on the Sierra Madre Occidental (1400 to 2100 m a.s.l.) in the southern localities (Vázquez-García 1994). This lineage also displays a gradient of morphological traits, with small, rounded fruits and few carpels in the northern localities, transitioning to elliptical-oblongoid fruits with more numerous carpels in the southern localities. The southernmost part of its range, in northern Nayarit, is morphologically recognized as the “Huajicori group” (Vázquez-García et al. 2021), which appears genetically divergent in the concatenated phylogenetic analysis. The Huajicori group could represent an incipient divergence or a locally adapted population, but it has not yet crossed the threshold of evolutionary independence recognized by the coalescent model. Therefore, our data validate *M. tarahumara* as a separately evolving lineage.

Our species delimitation hypothesis results should be interpreted with caution considering both intrinsic characteristics regarding speciation (1–3), and methodological concerns (4–6): 1) speciation is not instantaneous, but rather a continuum process (De Queiroz 2007), 2) morphological characters may evolve faster than the formation of species, or may not accompany genetic divergence (Chan and Grismer 2019), 3) a divergence time as recent as 1.27 Mya may not be sufficient for the formation of separately evolving lineages (Hudson and Coyne 2002; Knowles and Carstens 2007), 4) small sample size can impact the accuracy of the BPP results, warranting cautious interpretations (Carstens et al. 2013; Chan and Grismer 2019), 5) it is argued that the MSC model reflects genetic structure, which includes both population structure within species as well as between species (Sukumaran et al. 2021), 6) BPP has been shown to over-delimit species (Leaché and Fujita 2010; Sukumaran et al. 2021). De Queiroz (2007) introduced the concept of a “grey zone” in which lineage separation and divergence are not yet fully complete, leading to conflicts in species concepts and making species boundaries difficult to define. Despite these important considerations, our strong statistical support from the MSC analyses provides a robust and testable hypothesis for species boundaries within the *Magnolia pacifica* complex. We therefore propose the recognition of five independently evolving lineages as the most coherent taxonomic framework, given the current morphological and molecular data: *Magnolia pacifica* (encompassing *M. pacifica* and *M. talpana*), *M. iltisiana*, *M. pugana* (encompassing *M. pugana* and *M. granbarrancae*), *M. tarahumara*, and *M. vallartensis*. This species delimitation has direct implications for conservation, as it redefines the evolutionary units to prioritize, ensuring that management efforts align with the genetic diversity of the group. Future studies employing independent lines of evidence, such as genomic-scale datasets, morphometric, ecological, expanded sampling, and phylogeographic approaches, will be crucial to further test this hypothesis, and to elucidate the evolutionary dynamics within this fascinating group.

Conservation status of species of the *Magnolia pacifica* complex

Magnolia pacifica

Magnolia pacifica is restricted to montane cloud forests from the extreme west of the Trans-Mexican Volcanic Belt and Sierra Madre del Sur mountain ranges, in S Nayarit and W Jalisco. This species is threatened mainly by logging, forest fires, and forest conversion to pasturelands. Further threats include population growth in rural towns, increasing demand for water in both rural and urban areas, and pressures from mining, roads, tourism, and agriculture, such as crops of coffee, avocado, berries, guava, and citrus. Natural regeneration is hardly observed in most wild populations, such as La Morita, San Sebastián del Oeste, and Sierra San Juan. In a few populations, such as that of Talpa de Allende (Maple forest), the few seedlings and saplings encountered are under intense competition for light with many other associated tree species (Linsky and Muñiz-Castro 2022a). Even though some populations of *M. pacifica* are within protected natural areas (Reserva de la Biosfera Estatal Sierra de San Juan, Parque Estatal Bosque de Arce y Cuenca Alimentadora del Distrito Nacional de Riego 043), the low density of individuals and its low intra-population genetic diversity place this species in an endangered state (Muñiz-Castro et al. 2020). The estimated extent of occurrence (EOO) of *M. pacifica* is 4,581.3 km² and the area of occupancy (AOO) is 116 km². According to the IUCN Red List criteria (IUCN Standards and Petitions Committee 2024), *M. pacifica* must be categorized as Endangered: EN B1ab(ii,iii,v)+2ab(ii,iii,v), as assessed in the IUCN Red List by Khela and Gibbs (2014). The populations are severely fragmented (condition a), and there is a continuing decline (condition b) observed and projected in (ii) area of occupancy, (iii) extent and quality of its habitats, and (v) number of mature individuals.

Magnolia pugana

Magnolia pugana is endemic to the canyon region of central Jalisco and adjacent areas of south Zacatecas. Due to its small population and restricted distribution, this species is endangered. None of the *M. pugana* locations are in protected natural areas, except for a very small population in the La Primavera Forest Flora and Fauna Protection Area. The current threats for wild populations of *M. pugana* are high deforestation, fragmentation and isolation, low regeneration, changes in land use (mainly forest conversion to pasture lands and agriculture), forest fires, growth of urban and rural settlements, global climate change, causing less rain and more heat affecting the flow of the streams, and the intensive use of pesticides that deplete pollinators (Vázquez-García et al. 2021; Linsky and Muñiz-Castro 2022a; Janousek et al. 2023). The EOO of *M. pugana* is 2,188.2 km² and the AOO is 92 km². According the IUCN Red List criteria (IUCN Standards and Petitions Committee 2024), this species must be categorized as Endangered: EN

B1ab(ii,iii,v)+2ab(ii,iii,v), as assessed in the IUCN Red List by Gibbs and Khela (2014). Nevertheless, the IUCN criteria do not consider the levels of genetic diversity for assessing extinction risk, and *M. pugana* has very low genetic diversity (a total heterozygosity (H_T) of 0.158), even lower than other western and eastern endangered Mexican *Magnolia* species, such as *M. sharpii* Miranda and *M. schiedeana* (Newton et al. 2008; Muñiz-Castro et al. 2020). This low genetic diversity agrees with criterion C2 (intrinsic biological vulnerability with genetic heterozygosity < 20%) of the Mexican Standard NOM-059-SEMARNAT-2010, for being considered in the category of Endangered species (SEMARNAT 2010).

Magnolia tarahumara

This species is restricted to cool, moist canyons with perennial streams and nearly evergreen riparian forest of the Sierra Madre Occidental mountain range, in SE Sonora, SW Chihuahua, Sinaloa, E Durango, and N Nayarit (Vázquez-García 1994). Its habitats are isolated, fragmented, and surrounded by drier vegetation, such as pine-oak forest and tropical deciduous forest (Vázquez-García 1994, 1995). The threats for populations of *M. tarahumara* are the low number of individuals by population, increasing land surface temperature, loss of 34% of forest cover (from years 2000 to 2024) due to forest fires, logging and forest conversion to pasture lands and agriculture, increasing of droughts by global climate change (Sandoval and Escobar-Flores 2025), and the intensive use of pesticides that deplete pollinators (Janousek et al. 2023). None of the locations of *M. tarahumara* are in protected natural areas. The EOO of *M. tarahumara* is 19,601.7 km² and the AOO is 84 km². According to the IUCN Red List criteria (IUCN Standards and Petitions Committee 2024), this species must be categorized as Endangered: EN B2ab(ii,iii,v), as it was assessed by Vázquez-García et al. (2013) and in the IUCN Red List by Gibbs and Khela (2014).

Magnolia vallartensis

This species has a very restricted range, endemic to the windward slopes, ravines, and low mountains of the Sierra de Cuale and other small mountain ranges along the northern coast of Jalisco state, in the transition zone between the Pacific Lowlands and Sierra Madre del Sur Biogeographic Provinces (Morrone et al. 2017). It is only known from five small coastal watersheds of the rivers or streams El Nogalito, Palo María, Mismaloya (in Puerto Vallarta municipality), Las Lajitas, and Provincia (in Cabo Corrientes municipality). In Provincia, there is a population of *Magnolia pacifica* where some individuals with morphological characters and genetic groups or haplotypes of *Magnolia vallartensis* have been observed (Vázquez-García et al. 2012, 2013; Muñiz-Castro et al. 2020; Ordorica-Velarde et al. unpubl. data), which may represent a contact zone between these two species. None of the five locations of *M. vallartensis* is in a protected natural area. The EOO of *M. vallartensis* is only 72 km²

and the AOO is 36 km². According to the IUCN Red List criteria (IUCN Standards and Petitions Committee 2024), this species must be categorized as Critically Endangered: CR B1ab(ii,iii,v), as it was assessed by Vázquez-García et al. (2013) and in the IUCN Red List by Rivers et al. (2016). The forest habitat of *M. vallartensis* is declining due to forest fires, logging, and forest conversion to pasturelands, projected tourism developments, and road constructions (Vázquez-García et al. 2013; Linsky and Muñiz-Castro 2022b). Climate change could rapidly threaten this population because of its occurrence at unusually low elevations, increasing the risk of forest pest outbreaks and wildfires (Vázquez-García et al. 2013). In response to global warming, migration to higher elevations of the small mountains by tropical trees such as *Coussapoa purpusii* Standl., *Ficus* spp., *Aralia excelsa* (Griseb.) J.Wen, *Sideroxylon portoricense* Urb., *Dendropanax arboreus* (L.) Decne. & Planch., *Brosimum alicastrum* Sw., *Bursera simaruba* (L.) Sarg., *Nectandra* sp., *Attalea cohune* Mart., *Aphananthe monoica* (Hemsl.) J.-F.Leroy, *Hymenaea courbaril* L., *Lonchocarpus* spp., *Guarea glabra* Vahl, and others will confer greater pressure on *M. vallartensis* due to the high competitiveness for light and resources of these tall tropical tree species. Another threat for *M. vallartensis* is its low genetic diversity ($H_T = 0.171$, $I = 0.275$) (Muñiz-Castro et al. 2020), which is lower than the average values usually reported for plant genetic diversity ($H = 0.22$) (Nybom 2004).

Magnolia iltisiana

The distribution range of *Magnolia iltisiana* is restricted to montane cloud forests in the subhumid mountains of the Western Sierra Madre del Sur Biogeographic Subprovince, confined to the Jalisco-Manantlan District (Morrone 2017), in southern and southeastern Jalisco and eastern Colima. Previously, Rivers et al. (2016) categorized it as Vulnerable. The EOO of *M. iltisiana* is only 3,167.1 km², and the AOO is 128 km², known from ten locations. According to this and the IUCN Red List criteria (IUCN Standards and Petitions Committee 2024), this species must be categorized as Endangered: EN B1ab(ii,iii,v)+2ab(ii,iii,v). The main threats to the quality and extent of the habitat of *M. iltisiana* are deforestation and selective logging for its valued timber. These threats are likely to continue (Rivers et al. 2016). This species is protected in the Sierra de Manantlán Biosphere Reserve, but illegal logging by criminal gangs has devastated large populations even within protected natural areas. (García-Jiménez and Vargas-Rodríguez 2021; Torres-Rojo 2021). Furthermore, four biological factors appear to threaten this species: excessive seed predation by squirrels, rapid degradation of indehiscent fruits as they ripen on the ground, their apparent phased regeneration behaviour (intolerance to shade), and low efficiency in the dispersal of relatively large seeds (Vázquez-García 1994).

TAXONOMIC TREATMENT

Magnolia pacifica A.Vázquez (Vázquez-García 1994: 10)

Magnolia talpana A.Vázquez, Muñiz-Castro & A.S.Ortega (Vázquez-García et al. 2021: 7), **syn. nov.** – Type: MEXICO – **Jalisco** • Vicinity of Parque Estatal Bosque de Arce, Talpa de Allende municipality, 2.1 km SW from Los Sauces; 20°14'42"N, 104°47'41"W; 1340 m; 12 Apr. 2012; fr.; riparian cloud forest, besides a small tributary stream of the Talpa river; *M.Á. Muñiz-Castro* & *R. Murguía* 970; holotype: IBUG; isotype: IPN.

Type. MEXICO – **Jalisco** • Arroyo del Triángulo, San Sebastián; 1425 m; 20 Mar. 1927; *Y. Mexia* 1903; holotype: MICH; isotypes: A, CAS, F, GH, MO, NY, UCR, US.

Magnolia pugana (Iltis & A.Vázquez) A.Vázquez & Carvajal (Vázquez-García et al. 2002: 137)

Magnolia granbarrancae A.Vázquez, Muñiz-Castro & A.T.Nuño (Vázquez-García et al. 2021: 6), **syn. nov.** – Type: MEXICO – **Jalisco** • Zapotlanejo municipality, on a slope on the southern margin of the Río Verde river, beside a rocky spring, 80 m NE from Las Cruces ravine, 3–5 km NNW from Matatlán; 20°44'30.4"N, 103°09'56.8"W; 1073 m; 23 Jan. 2012; fl.; *M.Á. Muñiz-Castro*, *R. Murguía*, *J. Padilla-Lepe* & *M. Cházaro-Basañez* 918; holotype: IBUG; isotype: MEXU.

Type. MEXICO – **Jalisco** • Margin of forest along a small tributary of the San Lorenzo river, 35 km al NW de Guadalajara, 8 km NW de Tesistán, Zapopan; 20°50'N, 103°34'W; 1450 m; 18 Mar. 1987; *Iltis*, *Cházaro B.* & *R. López V* 29722; holotype: WIS; isotypes: IBUG, MEXU, MICH, MO, US.

CONCLUSION

In conclusion, our study reaffirms the monophyly of the *M. pacifica* complex within *M. sect. Magnolia*, consistent with previous morphological and molecular evidence. By applying a multispecies coalescent model to multi-locus data, we provide robust statistical support for recognizing five independently evolving lineages as the primary evolutionary entities within this complex: *Magnolia pacifica* (encompassing *M. pacifica* and *M. talpana*), *M. iltisiana*, *M. pugana* (encompassing *M. pugana* and *M. granbarrancae*), *M. tarahumara*, and *M. vallartensis*.

This delimitation resolves key taxonomic uncertainties and supports necessary synonymies among previously recognized species, providing a refined framework for the group's systematics and a clearer basis for management and conservation actions. Following the integrative spirit advocated by De Queiroz (2007), we emphasize that this hypothesis should be complemented by independent lines of evidence, such as quantitative morphometric and genomic data, along with population genetic processes within lineages (Maddison and Knowles 2006).

Based on our findings, we re-evaluated the conservation status of these species under the IUCN Red List criteria. The following changes are proposed: *M. tarahumara* from Vulnerable to Endangered; *M. pacifica* remains Endangered; *M. pugana* remains Endangered; *M. vallartensis* remains Critically Endangered; and *M. iltisiana* from Vulnerable to Endangered. These results highlight the urgent need to implement targeted management strategies to prevent further population declines and underscore the importance of integrating updated taxonomic frameworks into conservation planning efforts.

Ultimately, our work provides a solid and testable foundation for future studies on diversification, conservation, and the intriguing patterns of morphological divergence within the *M. pacifica* complex.

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SUPPLEMENTARY MATERIALS

Supplementary material 1

Primers and PCR conditions used for the six molecular markers in this study.

<https://doi.org/10.5091/plecevo.178054.suppl1>

Supplementary material 2

Newly generated sequences for individuals of the *Magnolia pacifica* species complex. The table lists the corresponding GenBank accession numbers and indicates with an X the phylogenetic divergence time estimation, and species delimitation analyses in which each sequence was included.

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Supplementary material 3

Sequences used in this study for the phylogenetic reconstruction of *Magnolia* sect. *Magnolia* and the *Magnolia pacifica* species complex, and for the divergence time estimation. This table lists the GenBank accession numbers for all downloaded sequences.

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