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Chapter

How to Save Endangered Magnolias? From Population Biology to Conservation Action: The Case of Allopatric Radiation in Western Mexico

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Abstract

Numerous Neotropical *Magnolia* species are endangered and red-listed by the IUCN. Here we highlight major results from over a decade of research on endangered magnolias in western Mexico. Particularly, we compare three species of *Magnolia* (*M. pugana*, *M. pacifica* and *M. vallartensis*) along a large-scale continentality and moisture gradients, in terms of a) their morphological adaptations, b) genetic structure, diversity, and differentiation, c) reproductive phenology, and d) floral scents and their floral visitors. Fieldwork along this gradient unveiled two new species of *Magnolia* sect. *Magnolia*; *M. granbarrancae* and *M. talpana*. We found that most continental populations have a higher extinction risk than those with greater maritime influence, due to their lower genetic diversity, and greater fragmentation, isolation, and water stress. Also, these populations are more vulnerable to the environmental conditions predicted with the global warming climate scenarios. We share fieldwork experience and advise on pre-germination treatments and seed dormancy. We propose an ex-situ and in-situ conservation strategy, identify new challenges, and suggest future directions of collaborative work as a global *Magnolia* conservation consortium.

Keywords: allopatric radiation, conservation, floral scents, genetic diversity and differentiation, new species, species distribution modeling, pre-germination treatments

1. Introduction

Magnolias have captivated mankind since ancient times, their lush evergreen foliage, varied floral scents and spectacular flowers make them highly attractive.

Distributed from temperate to tropical areas of the Americas and Asia, they have an extraordinary value in horticulture, they are harvested from natural forests, either as a beauty item for solemn ceremonial rites or as a source of wood for construction, food, and traditional medicine [1, 2].

The family Magnoliaceae Juss. [3] belongs to one of the basal clades in the angiosperms, it consists of 350 species, two genera, *Liriodendron* L. [4] and *Magnolia* L. [4] and 15 sections [5]. It is disjunct and bicontinental; in the America and SE Asia, from near sea level to 3400 m [6].

The fossil record suggests a North American origin for the family, migrating east through the Disko Island, Greenland, and Thulean Landbridge, with a range expansion into Europe and eventually to Asia. The cooling of the climate during the mid-Cenozoic resulted in a migration to lower latitudes and the extinction of Magnoliaceae in Europe and Siberia. Finally, the Ice-house conditions of the late-Cenozoic drove the group further south to highlands of Central and South America, as well as the mountains in South-east Asia [7].

In Mexico, as a result of allopatric speciation, there are 36 species in three sections of *Magnolia*: *M.* section *Macrophyllae* Figlar & Noot. [8] with five species of dorsal dehiscence [1, 9], *M.* sect. *Talauma* (Juss.) Baill. [3, 10] with 13 species mostly of ventral dehiscence [2] and *M.* sect. *Magnolia* with 18 species of dorsal dehiscence [11, 12].

In western Mexico, there are eight species of *Magnolia* [13], two belong to *M.* sect. *Talauma*: *M. jaliscana* A. Vázquez & R. Guzmán [14] and *M. ofeliae* A. Vázquez & Cuevas [15]— both confined to the lower slopes of the Pacific Lowlands province at the edge of the Sierra Madre del Sur province— and six belong to *M.* sect. *Magnolia*: *M. iltisiana* A. Vázquez [11], from the highlands of the Sierra Madre del Sur; *M. pacifica* A. Vázquez [11] from the western end of the Sierra Madre del Sur province to the western end of the Transmexican Volcanic Belt province; *M. pugana* (Iltis & A. Vázquez) A. Vázquez & Carvajal [16] from the canyons and valleys north of Guadalajara city, in the Transmexican Volcanic Belt; *M. vallartensis* A. Vázquez & Muñiz-Castro [14] in the Pacific Lowland province southeast of Puerto Vallarta; and the two species here described as new (**Figure 1**).

Here, we mostly focus in the species which constitute the *Magnolia pacifica* species complex [11], consisting of species with glabrous ellipsoid fruits distributed from tropical climate lowlands to temperate climate mountains in the confluence of four biogeographic provinces Sierra Madre Occidental, Sierra Madre del Sur, Pacific Lowlands and Transmexican Volcanic Belt [17] (**Figure 1**). Populations of the Barrancae Group, occurring in the Río Verde region in the Zapotlanejo e Ixtlahuacán del Río municipalities (north of Guadalajara city) [18] are here described as a new species (*M. granbarrancae*). Similarly, populations from the mountains of southern Talpa de Allende in W Jalisco are here described as a new species: *M. talpana*; while several populations from northern Nayarit, considered morphologically distinct from *M. pacifica* sensu stricto are here designated as the Huajicori group.

Magnolias in western Mexico, occur in four of its six biogeographic provinces, isolated in canyons (“barrancas”) or protected ravines of mountains, with minimum if any gene flow among their populations. They have undergone allopatric radiation, diverging in morphology, genetics, phenology, floral scents, and occupy different ecological niches (**Figure 1**) [11, 14, 18–20].

Here we aim to compare three species of *Magnolia* along a large-scale continentally and moisture gradients, in terms of (a) their morphological adaptations, (b) genetic structure, diversity, and differentiation, (c) reproductive phenology, and (d) floral scents and visitors; we also present an extinction risk assessment of each of the species and their vulnerability to predicted global warming climate scenarios.

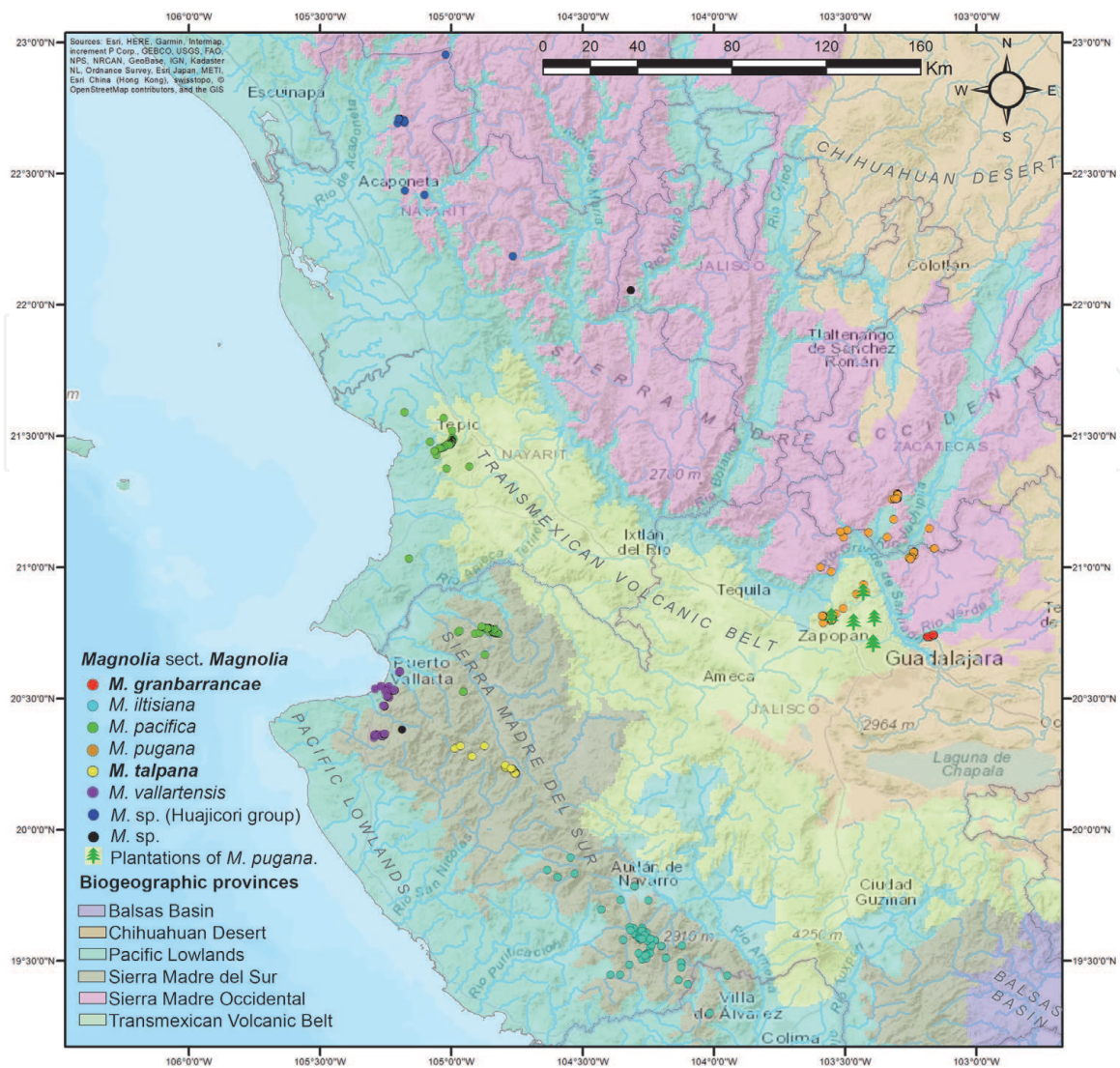


Figure 1. Current distribution of *Magnolia* sect. *Magnolia* in western México and their occurrence in biogeographic provinces [17]. Except for *M. iltisiana* all belong to the *Magnolia pacifica* complex. Undetermined *Magnolia* (*M. sp.*).

2. Population biology

2.1 Geographic distribution

Seven morphological distinct taxa of the *Magnolia pacifica* species complex occur in western Mexico (Figure 1), however most comparisons in this study include the three species growing along a continentality and moisture gradient, from west to east: *M. vallartensis*, *M. pacifica* and *M. pugana* and only few comparisons (morphological and genetical) also include discussion of the *M. granbarrancae* and *M. talpana* here described as new.

2.2 Morphological differentiation and adaptations

2.2.1 Habit and foliage

All species in the *Magnolia pacifica* complex are evergreen long leaved and usually sympodial and tortuous trees. *M. vallartensis* and the new *M. granbarrancae* are generally small trees (8.0–15.0(–23.0) m) with smaller trunk diameter at breast height (1.3 m) (0.2–0.5 m), while *M. pugana*, *M. pacifica* and the new *M. talpana*

are large trees (15.0–35.0 m) with thicker trunks, (0.3–1.6 m) in diameter at breast height.

The leaves in *Magnolia vallartensis* are distinctively broadly elliptic to elliptic or occasionally oblong, while the other four species are narrowly elliptical to elliptical to oblanceolate. The width to length ratio of mature leaves is larger in *M. vallartensis* (45–51%) than that of the other species *M. pacifica* (30–39%).

2.2.2 Flowers and floral visitors

Flowers of *Magnolias* in western Mexico differ in their pollination chamber, whether having a fully distinct chamber at female phase (day zero) involving all petals, like in *M. vallartensis*, *M. talpana* and *M. iltisiana*, or with an incipient or loose chamber involving mostly the inner petals. *M. vallartensis*, has a distinct subglobose pollination chamber, *M. iltisiana* has an oblongoid one and *M. talpana* has the narrowest oblongoid and tight chamber. *Magnolia granbarrancae* and *M. pugana* have the greater width to length ratio on petals and sepals while *M. talpana* has the smallest one.

Like other basal angiosperm lineages such as Araceae, Arecaceae, Cyclanthaceae, Nymphaeaceae and Annonaceae, the family Magnoliaceae exhibits floral traits that have been hypothesized as evolutionary adaptations to beetle pollination (cantharophily) [21]. In general, large, bisexual flowers, with petals, tepals or floral receptacles forming a bowl shape, have been considered as distinctive features of the beetle pollination syndrome [22]. Within the Magnoliaceae, specific traits include the development of female floral structures before the male ones (protogyny) to ensure cross pollination, floral odors, floral movements and the production of heat by reproductive structures as a result of biochemical reactions (thermogenesis) [23].

Coleoptera (Scarabaeidae) have been observed feeding and mating in flowers of *Magnolia pugana* and *M. pacifica* (*M. talpana*), causing damage to the petals and sepals (**Figure 2**). Despite cantharophily is the most common pollination syndrome in basal angiosperms, hymenopterans have been observed visiting flowers of *M. pacifica* s. s. (Huajicori group) and *M. vallartensis*. In the latter species we can confirm that hymenopterans play a role in pollination given that we were able to photograph some bees with significant amounts of pollen attached to the specialized hairs in their hind legs (**Figure 2**). The known floral visitors to each of the studied *Magnolia* species are listed in **Table 1**. Insect pollination represents a fundamental ecosystem service in natural habitats, particularly for obligate insect-pollinated plant species [26, 27], however, there is still little knowledge on insect floral visitors and the floral biology of most of *Magnolia* species, therefore, further studies including more field observations are much needed.

2.2.3 Fruits and seeds

The fruits of *Magnolia*, often called polyfollicles, consist of spirally arranged carpels, initially free and in some species becoming concrescent. Dehiscence in all three species of the *M. pacifica* complex is dorsal and bivalve. *Magnolia granbarrancae* and the Huajicori group have ovoid and smaller fruits (**Figure 3**). Basal carpels are larger in *M. pacifica* than in any other species, while the carpel apex are distinctively more pronounced in *M. vallartensis*.

Magnolia seeds are attached to the fruit by thin funicular threads that keep them suspended after release for one or two days before falling to the ground. They have a three-layer coat, the outer one called sarcotesta is bright, red to orange colored (that is attractive for their dispersers, the birds), fleshy and with persistent odor oils



Figure 2. Known floral visitors to the magnolias in the *Magnolia pacifica* species complex. A–C, *Strigoderma sulcipennis* on *M. pugana*; damage to the petals and sepals by the beetle is visible. D–F, *Cyclocephala* sp. sheltering inside flowers of *M. talpana*. G–I, representatives of *Frieseomelitta nigra* visiting stamens of *M. vallartensis*; pollen can be seen attached to their hind legs. Photographs A–C by G. Hernández-Vera, D–I by M. Muñoz-Castro.

Taxonomic rank of floral visitors					
	Family	Subfamily	Tribe	Genus	Species
<i>Magnolia pugana</i>	Scarabaeidae	Rutelinae	Anomalini	<i>Strigoderma</i>	<i>Strigoderma sulcipennis</i> Burmeister [24]
<i>Magnolia vallartensis</i>	Apidae	Apinae	Meliponini	<i>Frieseomelitta</i>	<i>F. nigra</i> Cresson [25]
<i>Magnolia talpana</i>	Scarabaeidae	Rutelinae	Anomalini	<i>Strigoderma</i>	x
	Scarabaeidae	Dynastinae	Cyclocephalini	<i>Cyclocephala</i>	x
<i>Magnolia pacifica</i> (Huajicori group)	Apidae	Apinae	x	x	x

Table 1. Known insect floral visitors to each of the studied *Magnolia* species. The “x” indicates unidentified taxonomic rank.

that make it impervious to water and gas exchange, containing inhibitors for germination and can protect it from some diseases and predators; the middle cover is called testa and provides mechanical protection as it is hard and bony, with a dark or light-colored depending on the species; the inner coat is a thin membrane that

surrounds the endosperm, within which a rudimentary embryo is present [28, 29]. For the magnolias of the pacific complex, the seeds are prismatic to triangular with scarlet red sarcotesta and light-colored testa, presenting more similarity between *M. pugana* and *M. vallartensis* with seeds of the same size (9–12 × 7–8 mm) and shape (sub-cylindrical or round-compressed) and small variations with *M. pacifica* seeds, that are slightly bigger (8–14 × 7–11 mm) and sub-cylindrical to cylindrical or rounded to compressed [13].

2.2.4 Two new species of *Magnolia* from Jalisco

Magnolia granbarrancae A. Vázquez, Muñiz-Castro & A. Nuño sp. nov. (Figure 3, sixth column).

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).

Diagnosis. *Magnolia granbarrancae* is similar to *M. pugana* in having broadly obovate sepals, but it differs from the latter in having flowers smaller, 7.0–8.0 vs. 11–14 cm; a tight pollination chamber vs. a loose one; fruit smaller 3.9–5.3 vs. 5.0–7.5 cm, subglobose to broadly obovoid vs. oblongoid to ellipsoid; carpels more numerous 24–32 vs. 16–22, seeds orange vs. scarlet red.

Trees of (8) 10.0–15.0 m tall, 60–70 cm dbh, *leaves* 11.2–16.6 × (3.5) 4.6–6.3 cm, oblanceolate to elliptical, obtuse at the apex, acute at the base, glabrous, with 20 pairs of lateral veins; *linear stipules* 0.95–1 × 0.15–0.2 cm; *petioles* glabrous 1.7–2.0 × 0.1–0.15 cm, *open flowers* 10.0–11.0 cm in diameter, white; glabrous



Figure 3. Morphological divergence in flowers and fruits of *Magnolia* sect. *Magnolia* in Jalisco, Mexico.

peduncles consisting of two internodes, 1.1–1.8 cm long, and 2.8–3.0 cm long; sepals 3, broadly obovate and strongly navicular, 5.8–6 × 3.1–3.5 cm, greenish beneath, glabrous on both sides; petals 6, at least, broadly obovate and strongly navicular, abruptly attenuated, the 3 outer ones 4.5–5 × 6–6.1 cm, white, glabrous in both faces, with a closed pollination chamber involving inner petals only 5.7–6.0 × 2.6–3.3 cm; stamens 40–46, *fruits* subglobose to broadly obovoid, glabrous, with 16–22 carpels, these, when mature and open, with almost round oval loci, 1.5–2.0 × 1.2–1.3 cm. *Seeds* broadly to narrowly ovoid or prismatic triangular or 0.9–1.1 × 0.6–0.9 cm, with orange sarcotesta.

Ethymology: the species was named after the great canyon in the vicinity of Guadalajara, as Henri Galeotii once name it: “la grande Barranca de Guadalaxara” (McVaugh 1952, *Asa Gray Bulletin*, Ann Arbor Michigan).

Distribution and habitat: Endemic to the Río Verde canyon, in the municipalities of Zapotlanejo and Ixtlahuacán del Río, Jalisco. Inhabits in steep slopes between 1073 and 1215 m asl, on rocky springs surrounded by tropical dry forest and some mesophytic plants as *Oreopanax peltatus*, *Trema micrantha*, *Ardisia* sp., *Aphananthe monoica*, *Piper* sp.

Additional specimens examined. JALISCO. Zapotlanejo municipality: Las Cruces ravine, 30 m down the stream pond and the water pump, 20°44'22.21" N, 103°9'48.01" W, 1215 m, 23 Jan 2012 (fl), *Muñiz-Castro, et al.* 919 (IBUG!). Ixtlahuacán del Río Municipality: North slope of the Barranca del Río Verde, next to a spring, in a mango orchard, 80 m above the river, 1.2 km ENE upriver of the Atengo canyon, 20°44'0.33" N, 103°11'23" W, 1204 m, 23 Feb 2013 (fr), *Muñiz-Castro, et al.* 1161 (IBUG!).

Magnolia talpana A. Vázquez, Muñiz-Castro & S. Ortega sp. nov. (**Figure 3**, third column).

Type: MEXICO. Jalisco: Vicinity of Parque Estatal Bosque de Arce. Municipio Talpa de Allende, 2.1 km SW from Los Sauces 20°14'42"N, 104°47'41"W, 1340 m, 12 Abr 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!).

Diagnosis. *Magnolia talpana* is similar to *M. pacifica* in sharing ellipsoid fruits with narrow locules during dehiscence, but it differs from the latter in having flowers smaller, 10.0–11.6 vs. 12–14 cm; a tight pollination chamber involving the outer and inner whorl of petals vs. a loose pollination chamber involving the inner whorl of petals only; open sepals narrowly oblongoid, reflexed and opening up to 170 degrees vs. oblongoid, not reflexed and opening less than 90 degrees; inner whorl of petals varying in size vs. subequal; peduncles pubescent vs. glabrous.

Trees of 15.0–25.0 m tall, *leaves* 8.1–14.9 × 5–7.5 cm, acute at the base, acute to obtuse at the apex, glabrous, with 14–15 pairs of lateral veins; stipules linear or conic when young, and abaxially golden pubescent, yellowish green; petioles glabrous, open *flowers* 10–11.6 cm in diameter, white; a tight pollination chamber involving the outer and inner of petals, 4.8 × 2.1–2.7 cm; peduncles of five internodes, the longest is the most distal one, half the length of sepals (ca. 2.5 cm long); sepals 3, narrowly oblongoid, navicular reflexed and opening up to 170 degrees; 4.3–5.5 × 1.4–2 cm, white, glabrous on both sides; petals 6, obovate, navicular, abruptly attenuated, the 3 outer ones 5.2–5.9 × 2.9–3.6 cm, white, glabrous in both faces, the 3 inner whorl of petals subequal, 5.3–6.1 × 2.5–2.8 cm; stamens 74–76; gynoecium 2.1 × 1.0 cm, *fruits* ellipsoid with narrow locules during dehiscence, immature fruit 2.0 × 1.1 cm. *Seeds* with red sarcotesta.

Distribution and habitat: Endemic to Talpa de Allende municipality, Jalisco, México, in the east and west branches of the high watershed of the Talpa river and in the Camacho and Desmoronado tributaries of the Tomatlán river, north and above Presa Cajón de Peñas. Inhabiting in cloud forest, riparian forests and ecotones with oak-pine forest.

Additional specimens examined: MEXICO. Jalisco: Municipio Talpa de Allende, 120 m SSW from Peña del Cuervo, 20°13'4.1"N, 104°44'11"W, 2077 m, 16 Jun 2012 (fl), *Muñiz-Castro and Murguía-Araiza 1094* (IBUG!); Cañada Ojo de Agua del Cuervo (Maple Forest), 18.2 km SSE from Talpa de Allende town, 1751 m, 01 Aug 2012 (fl) *Muñiz-Castro et al. 1106* (IBUG!); 17–19 km S from Talpa de Allende, margin W of Río de Talpa, 18–19 Oct 1960 (fr), *McVaugh 20375* (MICH!); 20–22 km S from Talpa de Allende, margin W of Río de Talpa, 28–30 Mar 1965 (fr), *McVaugh 23313* (MICH!); Cañada Ojo de Agua del Cuervo (Maple Forest), 200 m from the road, 1754 m, 24 Mar 2012 (fr), 20°12'46.5"N, 104°45'25.4"W, *Muñiz-Castro et al. 941* (IBUG!); path to Coamil de Méndez, tributary of Río Talpa, 2.5 km SW from Los Sauces, 1378 m, 31 Jul 2012 (fr), 20°14'19.1"N, 104°47'48.1"W, *Muñiz-Castro, et al. 1103* (IBUG!).

2.2.5 Key to the species of *Magnolia* in Jalisco.

1. Stipules adnate to petiole; fruits with connate carpels, circumcissil dehiscence and detachable mainly singly or in small irregular groups (sect. *Talauma*) ... 2.

- Free petiole stipules, free carpels, fruit with dorsal dehiscence (sect. *Magnolia*)

2. Leaf blades 24.0–25.0 × 11.9–12.6 cm; flowers 22.0 cm diameter, fruits 7.0–10.0 × 5.0–7.0 cm; carpels 47–58; basal carpels 4.3–4.5 × 1.2–1.4 cm and their decurrence from 0.4–0.6 cm long (S of Jalisco and Colima) ... *M. jaliscana*

- Leaf blades 35.0–45.0 × 23.0–29.0 cm; flowers 16.0 cm in diameter; fruits 14.5 × 8.5–9.0 cm; carpels 37–44; basal carpels 5–5.7 × 1.5–2 cm and their decurrence from 0.8–2.0 cm long (O of Jalisco) ... *M. ofeliae*

3. Bract, peduncular internodes and petioles densely pubescent ... *M. itisiana*

- Spataceae bract, peduncular internodes and petioles essentially glabrous or with pubescence limited to the nodes ... 4.

4. Widely obovate sepals (N of Jalisco and S of Zacatecas) ... 5.

- Narrowly oblong sepals ... 6.

5. Flowers 7.0–8.0 cm in diameter, with a tight pollination chamber; fruit 3.9–5.3 cm, subglobose to broadly obovoid; carpels 24–32, seeds orange ... *M. granbarrancae*

- Flowers 11–14 cm in diameter, with a loose pollination chamber, fruit 5.0–7.5, oblongoid to ellipsoid, carpels 16–22, with seeds scarlet red ... *M. pugana*

6. Pollination chamber subglobose to globose, leaves 13.5–27.8 × 6–14.8 cm, broadly elliptic to elliptic, often obtuse to rounded apex, petals 6–8, carpels 10–19 ... *M. vallartensis*

- Pollination chamber incipient or narrowly oblongoid, leaves 8.0–17.0 (18.0) × 3.0–6.0 (8.0) cm, elliptical-lanceolate, frequently acute apex, petals 6–7, carpels 17–25 ... 7.

7. Sepals oblongoid, not reflexed, opening less than 90 degrees, pollination chamber loose and incipient, inner whorl of petals of greatly varying in size ...

M. pacifica

- Sepals narrowly oblongoid, reflexed, opening up to 170 degrees, pollination chamber tight and narrowly oblongoid, inner whorl of petals subequal ...

M. talpana.

2.3 Genetic structure, diversity, and differentiation

In this section, we review what is known so far of genetic structure and diversity in three western Mexican *Magnolia* species: *Magnolia vallartensis*, *M. pacifica* s.l. (including the new species *M. talpana*), and *M. pugana* s.l. (including the new species *M. granbarrancae*), all belonging to the so-called *Magnolia pacifica* complex [11] (**Figure 4**). As they are distributed along a continentality and moisture gradient, the influences of their different distances to the Pacific Ocean in their genetic structure and diversity have been tested [18]. In this study, leaf tissues of 278 individuals from 10 localities representative of the *M. pacifica* complex were sampled. Three localities were sampled for *M. vallartensis*: Arroyo Palo María (APM), Las Lajitas (LL), and Provincia (PV); three for *M. pacifica* s.l.: Cerro San Juan (CSJ), San Sebastián del Oeste (SS), and Bosque de Arce (BA, *M. talpana*); and four for *M. pugana* s.l. Arroyo La Virgen (ALV), Arroyo San Lorenzo (ASL), Arroyo Palo

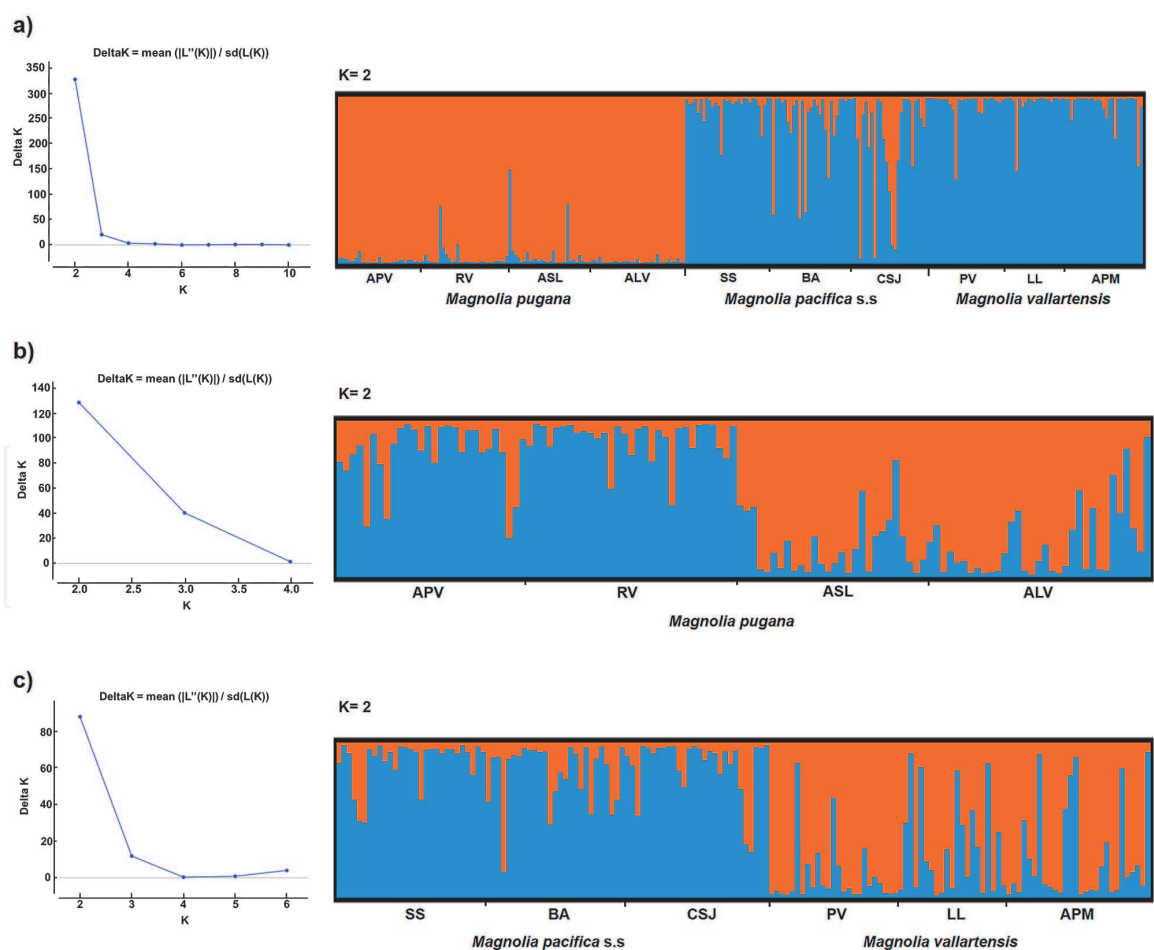


Figure 4. Results of Bayesian clustering based on STRUCTURE; analysis performed on a) the entire data set (278 individuals, 76 loci ISSR); b) the *M. pugana* s.l. group (120 individuals); c) the *M. pacifica* s.l.-*M. vallartensis* group (158 individuals). Relationships between K and Delta K values and structure bar plots are shown. Vertical bars represent each individual analyzed and bars are divided into distinct colors when there is evidence of admixture. From [18].

Verde (APV), and Río Verde (RV, *M. granbarrancae*) (**Figure 4**). A total of 76 reproducible DNA fragments of six Inter-Simple Sequence Repeats (ISSR) (primers UBC 810, 814, 834, 836, 855, and 857) were amplified and analyzed using Bayesian analysis with the program STRUCTURE, UPGMA clustering, Monmonier's algorithm with Barrier 2.2, AMOVA, and genetic diversity parameters. Also, a Mantel test was done to evaluate the relationship between genetic and geographic distances.

2.3.1 Genetic structure

Results of Bayesian analysis with STRUCTURE (**Figure 4**), UPGMA clustering (**Figure 5**), and the Exact Test for differentiation (**Table 2**) are in accordance that there are two main genetic clusters for the whole of three species, being *M. pugana* s.l. the eastern main group, and the cluster of *M. pacifica* s.l.-*M. vallartensis* the

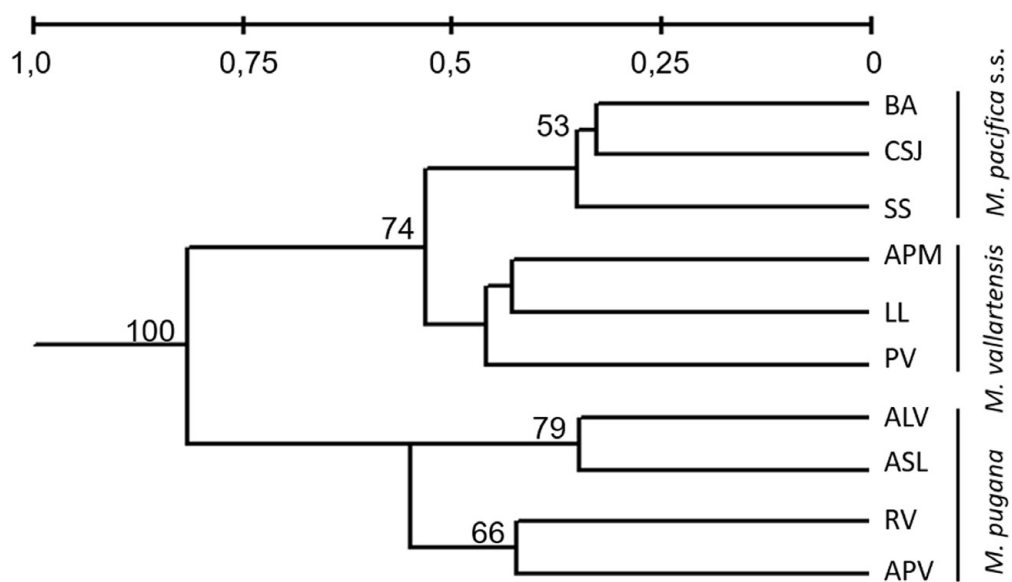


Figure 5. UPGMA dendrogram showing the genetic relationships of the *M. pacifica* species complex, the number at each node indicates the bootstrap percent having above 50% values. From [18].

	<i>M. pugana</i>				<i>M. pacifica</i> s.s.			<i>M. vallartensis</i>		
	APV	RV	ASL	ALV	SS	BA	CSJ	PV	LL	APM
APV	—	0.039	0.051	0.050	0.069	0.057	0.060	0.098	0.097	0.088
RV	0.079	—	0.054	0.052	0.074	0.070	0.073	0.110	0.112	0.088
ASL	0.006	0.001	—	0.032	0.060	0.065	0.050	0.077	0.079	0.072
ALV	0.013	0	0.144	—	0.070	0.069	0.066	0.099	0.097	0.080
SS	0	0	0.002	0	—	0.031	0.030	0.058	0.042	0.049
BA	0.002	0	0	0	0.520	—	0.032	0.065	0.042	0.045
CSJ	0	0	0	0	0.312	0.703	—	0.056	0.042	0.040
PV	0	0	0	0	0.060	0.052	0.050	—	0.039	0.043
LL	0	0	0	0	0.060	0.475	0.119	0.976	—	0.038
APM	0	0	0	0	0.360	0.210	0.162	0.550	0.360	—

Table 2.

Nei's unbiased genetic distance (above diagonal) and exact test differentiation probability values (below diagonal) among sampled localities of the *M. pacifica* species complex. From [18].

western grouping. But each one of these two main groups has its own structure, having two subgroups each (Figures 4 and 5). *M. pugana* s.l. group is segregated in the *M. pugana* s.s. subgroup (ALV-ASL localities), located SW of the Santiago river canyon, and the APV-*M. granbarrancae* (RV) group, located NE of that canyon. In the other main group, *M. pacifica* s.l. subgroup (BA-SS-CSJ) was separated from the *M. vallartensis* one (PV-LL-APM). Additionally, the 900 bp (primer 834) and 850 bp (primer 855) ISSR loci only amplified and were exclusive for *M. pacifica* s.l.-*M. vallartensis* group, these loci did not amplify *M. pugana* s.l. samples. The AMOVA based on the three species and the two Bayesian groups showed that only 8% of the genetic variation was explained by differences among species as well as among the Bayesian groups (Table 3), so AMOVA was less informative than the Bayesian analysis. Most of the variation ($\geq 82\%$) was explained by differences within localities, suggesting high levels of cross-pollination [30]. This predominance of outcrossing reproductive mode was also found by [31] in some Neotropical magnolias.

Other evidence for the structure represented by four groups was the results of the test with Monmonier's algorithm (Barrier 2.2), which detected three significant geographical barriers to gene flow, segregating the four groups (Figure 4). All geographical boundaries had 100% bootstrap support. One of these is the Trans-Mexican Volcanic Belt (TMVB), which is the main physiographic barrier between *M. pugana* s.l. and the group of *M. pacifica* s.l.-*M. vallartensis*. This mountain range has been identified as an important gene flow barrier for both plants [32–35] and animals [36, 37], even its complex orogenic processes have led to population isolation, speciation, and diversification [38]. The other two significant barriers detected correspond to the Santiago river canyon on the one hand (between the *M. pugana* s.s. and the APV-*M. granbarrancae* group), and the basins of the rivers Ameca, Mascota, Pitillal, and Cuale on the other hand (between *M. pacifica* s.l. and *M. vallartensis*). The effect of the deep Santiago river canyon (3–15 km wide and 500–700 m deep) and other river basins as significant gene flow barriers also have been documented for birds, reptiles, insects and other plant species [34, 39, 40].

The outcomes of this population genetics study reveal that *M. pugana* s.l., the easternmost and the most continental group, is the most geographically and genetically distinct group of all *M. pacifica* species complex, and clearly support its recognition at the species level [16]. The most likely process of speciation for *M. pugana* s.s. and *M. granbarrancae* is allopatric isolation, being the TMVB the main

Variation source	Percentage of variation				
	Groups		Taxa		
	Bayesian analysis (2)	Taxonomic (3)	<i>M. pugana</i> s.l.	<i>M. pacifica</i> s.l.	<i>M. vallartensis</i>
<i>Among</i>					
Taxa / Genetic groups	9 (0.001)	8 (0.001)			
Localities	9 (0.001)	8 (0.001)	12 (0.001)	7 (0.001)	7 (0.001)
<i>Within</i>					
Localities	82 (0.001)	84 (0.001)	88 (0.001)	93 (0.001)	93 (0.001)

(2) Two Bayesian groups, (3) three taxa, *p* value is given in parentheses.

Table 3. Molecular variance analysis (AMOVA) for Bayesian analysis and taxonomic groups of the *Magnolia pacifica* species complex. From [18].

gene flow barrier between them and their western close relatives. This is in congruence with Vázquez-García *et al.* [1], who suggest that allopatric speciation is a major driver of *Magnolia* diversification in Neotropical Magnoliaceae. The genetic divergence between *M. pacifica* s.l. and *M. vallartensis* is lesser than between this main group and *M. pugana* s.l., indicating a more recent process of segregation or current gene flow. There is no clear geomorphological barrier between some localities of *M. talpana* and *M. vallartensis* in their southern ranges (between BA and all the *M. vallartensis* localities). Therefore, genetic differences between these localities might be rather explained by a process of parapatric ecological differentiation [41, 42], as it is common that gene exchange among closely related taxa happens in at least 25% of plant species [43]. Nevertheless, the four identified genetic groups, independently of partially sharing genes, are distinctive evolutive entities and should be considered as separated conservation units.

2.3.2 Genetic differentiation

Differentiation indices were moderate in general, but higher in *M. pugana* s.l. ($GST = 0.120 \pm 0.021$, $D = 0.028 \pm 0.007$) than in *M. pacifica* s.l.–*M. vallartensis* ($GST = 0.106 \pm 0.016$, $D = 0.026 \pm 0.006$). The Exact Test for population differentiation showed significant genetic differences between localities of *M. pugana* s.s. and the *M. granbarrancae*, and between localities of *M. pugana* s.l. and *M. pacifica* s.l.–*M. vallartensis*, but not within each *M. pugana* subgroup and within the *M. pacifica* s.l.–*M. vallartensis* group (Table 2). The higher genetic differentiation of *M. pugana* s.l. is in accordance with its smaller, more fragmented, and more isolated populations. The highest distance to the Pacific Ocean of *M. pugana* s.l. causes a lower environmental humidity and more extreme cyclical temperature changes, that is, a greater continentality [44]. These drier and more extreme conditions result in declines, fragmentation, and isolation of *M. pugana* s.l. populations, which need a constant input of water to survive. In contrast, the more humid maritime environments of *M. pacifica* s.l.–*M. vallartensis* are more favorable to maintain populations of these cloud forest mesophytic species, which is reflected in less isolation and differentiation. Similarly, genetic differentiation has ranged from moderate to high in most Neotropical *Magnolia* species [31].

2.3.3 Genetic diversity:

M. pugana s.l. had lower genetic diversity than the *M. pacifica* s.l.–*M. vallartensis* group. *M. pugana* s.l. exhibited a Shannon Index (I) = 0.268, total heterozygosity (H_T) = 0.158 (0.023 SD), and intrapopulation heterozygosity (H_S) = 0.134 (0.020 SD). In contrast, *M. pacifica* had an I = 0.272, H_T = 0.175 (0.025), and H_S = 0.159 (0.023), and *M. vallartensis* an I = 0.275, H_T = 0.171 (0.024) and H_S = 0.153 (0.022). Genetic diversity also varied among localities; *M. pugana* s.l. localities had the lowest genetic diversity (H_E = 0.121–0.140, *M. granbarrancae* having the lowest I = 0.218 and H = 0.121), whereas the locality Bosque de Arce (BA) of *M. talpana* showed the highest genetic diversity (I = 0.280, H_E = 0.17) among the western *Magnolia* localities studied. The genetic diversity estimates for all the studied taxa here were lower than the average values reported for plant genetic diversity based on ISSR (H = 0.22) [45], and much lower than those reported for two threatened eastern Mexican *Magnolia* species: *M. sharpii* Miranda [46] (I = 0.56) and *M. schiedeana* Schlecht. [47] (I = 0.50) [48]. As with the higher genetic differentiation, the lowest genetic diversity of *M. pugana* s.s. and *M. granbarrancae* is consistent with their smaller populations, higher isolation, and fragmentation, all influenced by a drier and more extreme climate.

2.3.4 Isolation by distance pattern

The correlation between geographical and genetic distances among all localities of the *M. pacifica* species complex, revealed by Mantel tests, was high and significant ($r = 0.80$, $p < 0.001$), however, when the tests were applied to each of the two main genetic groups separately, no significant correlation was detected. Isolation by distance might explain the genetic structure and differentiation pattern within the *M. pacifica* species complex. Isolation by distance has been a strong pattern observed among plant studies, under this and in rapid environmental change, adaptive responses to environmental stress will be constrained by the natural dispersal mechanisms [49]. Pollination by beetles in small and isolated populations of *Magnolia* is not very efficient [50], and habitat fragmentation and other anthropogenic factors may also be troublesome for *Magnolia* seed dispersal by birds [50, 51]. Even if dispersal occurs, seeds may not germinate or seedlings may not survive in places without enough humidity [52].

In summary, based on ISSR genetic variation, the *M. pacifica* species complex exhibits a population genetic structure composed of two main groups, the eastern *M. pugana* s.l. more continental group, and the western *M. pacifica* s.l.-*M. vallartensis* group, with the more maritime climate. Both main groups are segregated by the physiographic barrier of the TMVB and isolation by distance, and are at the extremes of a maritime-continental climatic gradient. *M. pugana* s.s. and *M. granbarrancae* are subject to a drier and more extreme climate, therefore having more deforested, fragmented, and isolated habitats, which leads to lower genetic diversity and a higher genetic differentiation. This differentiation within *M. pugana* s.l. and the physiographic barrier of the Santiago river canyon have structured this taxa in two genetic subgroups, *M. pugana* s.s. and the *M. granbarrancae*. The *M. pacifica* s.l.-*M. vallartensis* group exhibited genetic segregation in two subgroups, having several canyon river barriers between both taxa, but maintaining a partial gene flow at their southern ranges. The three species of the *M. pacifica* complex have lower genetic diversity than eastern Mexican *Magnolia* species which are considered as endangered. Even more, the eastern and more continental *M. pugana* s.s. and *M. granbarrancae* undergo the lowest genetic diversity, which, together with their smaller and more isolated populations, makes these populations more vulnerable to gene drift and bottlenecks, therefore greater risk of extinction. All main genetic groups and subgroups defined in this study should be considered as separate conservation units, and concerted efforts are needed to protect them.

2.4 Phenological divergence

Phenology aims to characterize the behavior and the biological adaptation of the species in its natural habitats, which may help guide important management conservation strategies. We studied three different species of *Magnolia* (*M. vallartensis*, *M. pacifica* s.l., *M. pugana* s.l.) in western Mexico (**Figure 1**). Phenological observations were made at monthly intervals for one year and four months. The phenophases (flowering and fruiting) in an annual cycle and the intensity of each phenological event were estimated using the Fournier intensity index. The sum of all the intensity categories assigned to each individual was divided by the maximum amount that could be attributed to the population (relativizing the total number of selected individuals with the total sum sampled per month). Circular statistics were used to analyze and interpret phenological patterns. Using the statistical package Oriana version 4.0, the months were converted into angles, with intervals of 0.999° to 30° wide and it was calculated: 1) average angle (μ) or average date that refers to the time of the year around which the phenological activity of individuals is more

concentrated; 2) circular standard deviation; and 3) the vector r , which indicates the intensity of the concentration (0 to 1) around the average angle. The length of the vector r can be considered a measure of the degree of seasonality. In addition, the Rayleigh test was applied to indicate the significance ($P < 0.05$) of the mean angle.

The synchrony of the flowering phenophase between populations or species is essential to detect possibilities of gene flow between species and existence of temporary barriers in reproductive phenophases. Since the incipient asynchrony observed in the flowering of the three species was linked to their amplitude of flowering (which lasts up to six weeks with significant intersection) it was considered insufficient to prevent gene flow. The hypothesis of non-seasonality in the phenophases of the three species was rejected, which was high in *M. pugana*, intermediate in *M. pacifica* and low in *M. vallartensis*.

The flowering (female-flower) phenophase showed an incipient asynchrony among species, being earlier in *Magnolia vallartensis* (end of May), intermediate in *M. pacifica* (second week of June), and late in *M. pugana* (end of June). Seasonality was high in *M. pacifica* and *M. pugana* [20] (**Figure 6**). This incipient asynchronous female-flower phase differs from some Colombian *Magnolia* species, in which flowering is commonly markedly asynchronous among species and populations of each species [53].

The fruiting phase was asynchronous among species, and showed high seasonality only for *Magnolia pugana* (February) while in *M. vallartensis* the maximum fruiting was in August and for *M. pacifica* in December (**Figure 6**). This pattern is similar to that reported for *Leucaena* sp. and *Guazuma ulmifolia* Lam. [54, 55], a possible strategy to reduce competition among potential seed dispersers of *Magnolia* including birds, rodents and lizards. The intensity of fruiting is concentrated in rainy seasons [53]. Fruit dehiscence of *Magnolia vallartensis*, *M. pacifica* and *M. pugana* showed an asynchronous pattern between species. Similarly, eight

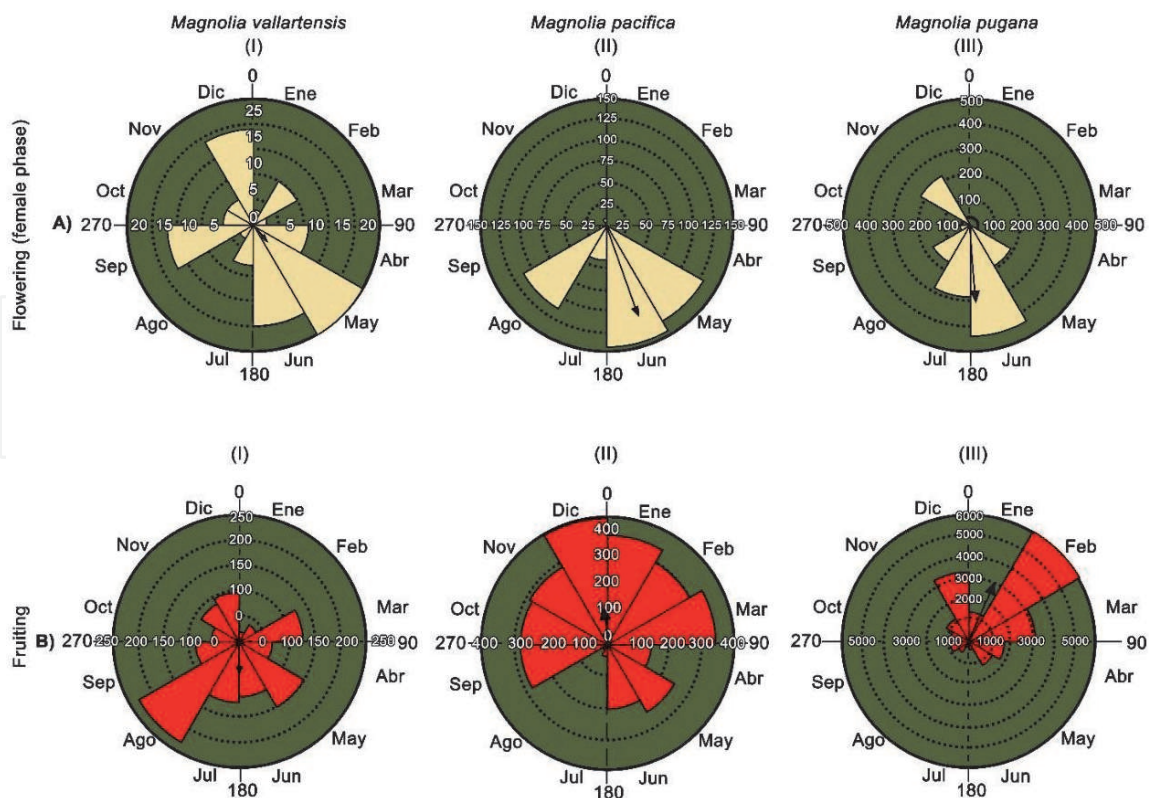


Figure 6.

Circular histograms of two phenophases for the three *Magnolia* species. A. Flowering (female-flower) phenophase. B. Fruiting phenophase. Based on monthly averages of frequencies from January to December 2017. *Magnolia vallartensis* (I), left column, *M. pacifica* (II), central column and *M. pugana* (III), right column. The arrows represent significant average angles (Rayleigh test $p < 0.001$), while the length of the vector r (0 to 1) indicates the concentration around the average angle [20].

species with an asynchronous pattern are reported for pioneer species in the central Amazon region of Brazil [56].

Differences in productivity were also evident, the higher productivity of reproductive phenophases (flowering and fruiting) of *Magnolia pugana* compared to that of *M. pacifica* and *M. vallartensis* makes it possible to reject the hypothesis of no significant differences. These differences may be due to their evident genetic differences [18], but also to their environmental differences; for instance, *M. pugana* populations thrive in permanent streams, surrounded by tropical deciduous forest and open oak-pine woodlands, a condition that provides more sunlight that can help to keep their reproductive phenophases more productive (flowers and fruits), whereas in the case of *M. vallartensis* and *M. pacifica*, their populations are usually surrounded by high and dense canopy cloud forest and tropical semi-evergreen forest trees that overshadow many *Magnolia* trees, which limits their productivity due to the scarcity of sunlight along the year.

2.5 Floral scents

More recently, several ecological studies have examined the roles of floral scent in the biology of the plant [57]. Scents, essential oils, or volatiles are secondary metabolites produced by plants to fulfill protection functions against herbivores, phytopathogens, and even other plants [58, 59]. These form a complex matrix with a lipophilic-volatile nature [60], from a chemical point of view, these volatile compounds belong to chemical classes such as terpene derivatives (oxygenated or hydrocarbons), phenylpropanoids, benzenoids, and nitrogen-containing compounds [61, 62]. The combinations of the constituents of this scent mixture give each flowering plant species a unique fragrance [57]. Through a compilation of data, the genus *Magnolia*, has been reported to be an interesting source of secondary metabolites found both in plant extracts and in essential oils, as shown by studies carried out mainly in species distributed in Asia and in which applications in traditional medicine are included [63–65]. In this sense, in many of the endemic species, studies related to describing the metabolic composition as a complement to determine potential uses, contribute to knowledge, and improve the conservation of their habitats. As an example, we extracted and characterized for the first time the essential oils present in flowers of *M. vallartensis*, *M. pacifica* and *M. pugana*, three endemic species of the genus *Magnolia* along a continentality and moisture gradient in western Mexico [19].

2.5.1 Methods

For details of flower collection techniques, essential oils extraction and determination of floral scents chemical composition see Mendeley Data repository [66].

2.5.2 Comparisons

The yield of essential oils from flowers of these three *Magnolia spp.* (**Table 4**) showed that in general these vegetal parts present a similar low yield between 0.2 to

Specie	Flower mass (g)	Essential oil (mL)	Yield	Odor
<i>M. pacifica</i>	100	0.22	0.21%	Sweet, citric
<i>M. vallartensis</i>	100	0.24	0.25%	Sweet, citric
<i>M. pugana</i>	100	0.30	0.30%	Sweet, woody

Table 4.
Essential oils of Magnolia flowers: Yield and organoleptic characteristics.

0.3%. On the other hand, the chemical composition of oils showed qualitative differences among individual components. The chemical profiles of these scents analyzed by GC/MS, observed in **Figure 7**, identified 97 compounds in total between species; 63 in *M. vallartensis*, 53 in *M. pacifica* and 39 in *M. pugana*.

In the chromatograms differences evidenced, in a specific manner, on the major components. Within the chemical composition of floral essential oils the major compounds greater than 3% of the total components obtained for each species from the most abundant were as follows. In the case of *M. pacifica*, (caryophyllene, bicyclo-dec-1-ene, 2-isopropyl-5-methyl-9-methylene, bicyclogermacrene, β -elemene and epi-cyclocolorenone); for *M. vallartensis* (caryophyllene, geranyl methyl ether, β -elemene and caryophyllene oxide); and in *M. pugana* (cyclocolorenone; 2Z,6E-farnesol; benzoic acid (5,5-dimethyl-4-oxo-2-cyclohexenyl ester, β -elemene, and caryophyllene oxide). Chemical composition data has an interesting application when essential oils occurrence is studied in the field of taxonomy. This assesses the possible chemotaxonomic relationship between chemical compounds and species, identifying compounds that could act as indicators. Based on this idea, the results obtained by a point-to-point analysis throughout the 90-minutes chromatograms and comparing the mass spectra including trace components, a matrix was constructed (**Figure 8**).

The presence of compounds could be associated to the closely related taxonomic affinity confirmed through molecular phylogenetic analysis; for example analyzing other three species closely related: *M. schiedeana*, *M. grandiflora* L. [67] and *M. tamaulipana* [11]; the pair *M. schiedeana*-*M. tamaulipana* (both endemic from Mexico) share the presence of geranyl methyl ether as the major compound up to 87%, while in *M. grandiflora* (widely distributed in the southern North America) shows the presence of its precursor: geraniol [68]. In this way, it was determined that the floral scents of the endemic species *M. pacifica*, *M. vallartensis* and *M. pugana* share qualitatively 14 components in their chemical profile, which is equivalent to 14.46% of total; these components suggested that could act as chemical markers for a determination at the gender level. Otherwise, floral oil compounds shared by pairs of species were also founded, the pair *M. pacifica*-*M. vallartensis* shared 27 more compounds of the total (42.47%); *M. vallartensis*-*M. pugana* 2 more compounds of the total (16.49%); and *M. pacifica*-*M. pugana* just one more additional compound to the total (15.46%). In the case of components particularly present in each species, *M. pugana* had 22 compounds, *M. vallartensis* 20 and

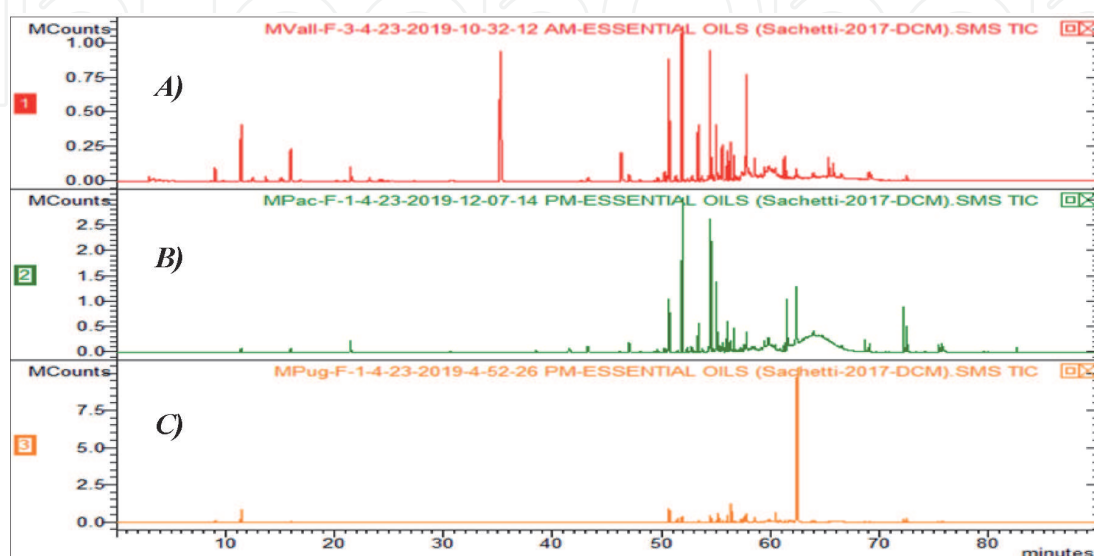


Figure 7. Floral scents chemical profiles by gas chromatography: A) *M. vallartensis*, B) *M. pacifica*, and C) *M. pugana*.

Matrix of chemical components and presence in *Magnolia* species floral scents

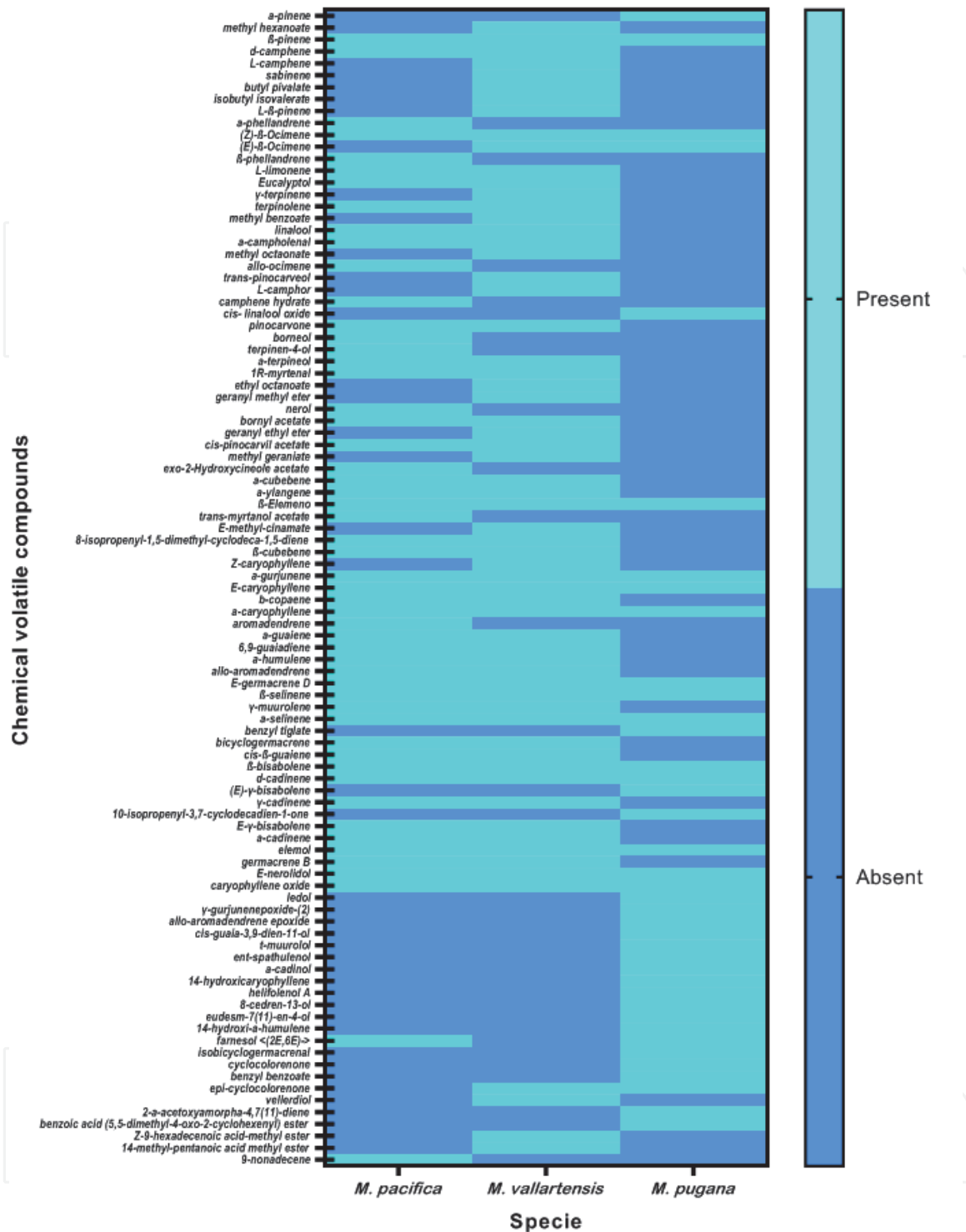


Figure 8. Matrix of chemical compounds present in *Magnolia* floral scents.

M. pacifica 11 components. The Jaccard similarity index allowed inferring the qualitative composition, the pair *M. pacifica* - *M. vallartensis* show the strongest similarity about 54.6%, while with *M. pugana* these species showed 19.5% and 18.6% of similarity respectively. The essential oils of flowers of *M. pacifica*, *M. vallartensis* and *M. pugana* showed a composition rich in sesquiterpenes with significant differences in their composition. Most species of *Magnolia* possess distinctive floral scent profiles, even though they may be taxonomically closely related, the chemical differences among these taxa may have arisen from interaction with pollinators or the environment [69]. There are factors that can affect the chemical composition and quality of an essential oil such as the age of the plant, altitude, climate, genetics, geography,

type of soil [70]. For this reason, it is necessary to continue with studies focused on establishing the relationships between the components and relating the chemotaxonomic field including more *Magnolia* species populations to conform a robust database that provides the necessary information to correlate chemical markers and to become a useful tool in species classification in addition to genetic analysis. These applications can help promote strategies in the conservation of this genus and its habitat.

2.6 Pre-germination treatments and seed dormancy

The most important and critical stage during the development of the plants is germination; therefore, it is also for the natural repopulation of the species and the dynamics of the populations [71]. The ability of a seed to germinate is known as viability, that is, the embryo is alive and can remain so for some time. A viable seed with dormancy is one that cannot germinate under any condition of physical environmental factors (humidity, temperature, light/dark, etc.), which are otherwise favorable for germination [72]. Therefore, the success of germination depends on whether or not the seeds have a dormancy mechanism, on their viability and on the time needed for them to germinate [73].

Magnolia seeds have viability percentages ranging from 50–100% [74, 75]. Different studies have shown a low percentage of germination because they can present different types of dormancy (including physical, chemical, mechanical, physiological and morphological) [76]. To eliminate dormancy and increase the percentage of germination and produce the greatest number of *Magnolia* seedlings, different pre-germination procedures have been applied [77–80]. This section will present the research carried out in *Magnolia iltisiana*, *M. pacifica*, *M. pugana* and *M. vallartensis*, to know the viability percentages and the different most successful pre-germination treatments, to break the lethargy and increase germination.

2.6.1 Seed management and germination

Seeds must be collected from 10 different plants of ripe fruit and extracted manually. To prevent fungal infection, a contact fungicide (Captan) was used for the seeds of *M. iltisiana* [81], while the other species were rinsed in a 3% sodium hypochlorite solution for 30 minutes. It is recommended that after being collected and disinfected, they are immediately stored in a well-ventilated and humid environment inside a refrigerator at 4–5°C, to avoid dehydration [76].

Were used 100 seeds per treatment with five replicates of 20 seeds per container for the four species. Once the treatments were concluded seeds of *M. pacifica*, *M. pugana* and *M. vallartensis* were sown inside a greenhouse at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) and were buried at 1.5 cm depth in 25 ml plastic containers. The substrate used for planting was “peat-moss” [29]. The study of *M. iltisiana* was carried out in the experimental greenhouse of Las Joyas Scientific Station (ECLJ), and they were planted in a mixture of sand and “germinaza” (1:1) as substrate. The number of germinated seeds was recorded daily for 60 days [75]. Seeds were considered to have germinated when the radicle emerged or when the hypocotyl was observed [82, 83].

2.6.2 Viability tests

The percentage of viability of all species was determined through tests in a 1% tetrazolium solution; two replicates of 50 seeds were used for *M. iltisiana*, while for

the other species it was in 30 seeds. The seeds were immersed without aril in this solution for 24 hours at 30°C, in darkness [72]. After this time, they were cut transversely and the tissues were observed in a stereoscope. What were stained in deep red is considered viable, while those not, are considered unviable (**Figure 9**) [84].

The viability is 80% for *M. iltisiana*, this result agrees with what was found in [77] where they obtained 80% of viable seeds in *M. schiedeana*. While the seeds of *M. pugana* registered 67% [76]. Reference [75] reported that in species of the same genus from southern North America it has an average of 50% viability, similar results are found in *M. vallartensis* and *M. pacifica* with 50% and 53% respectively (**Figure 10**). These percentages are lower than those recorded in other *Magnolia* species. For example, in [74] found 100% viability in seeds of *M. dealbata* Zucc. [85]. In Ref. [80] obtained 92% for *M. perezfarrerae* A. Vázquez & Gómez-Dominguez [86] and 87.5% in *M. sharpii*.

2.6.3 Pre-germination treatments, germination tests, and dormancy types

Four treatments were used for *M. iltisiana* [81]. In *M. pugana* six treatments were carried out [76]. Three treatments were performed for *M. pacifica* and *M. vallartensis* [87]. The germination of *M. iltisiana*, *M. pacifica*, *M. pugana* and *M. vallartensis*, is of the epigeal type, the embryo developed a pair of foliaceous cotyledons, a flaccid fatty endosperm, a hypocotyl and a radicle, confined to the micropile area [76, 81, 87], (**Figure 11**).

In general, the percentage of germination obtained for *M. iltisiana*, *M. pacifica*, *M. pugana* and *M. vallartensis* are low. The highest germination recorded are 60%, 21%, 52% and 12%, respectively. In these studies, it is found that for *M. pacifica*, cold stratification treatment promoted the highest number of germinated seeds. Similar results have been obtained in [77] for *M. schiedeana*, with this same treatment with 84% germination. Another important finding is that the manual aryl removal treatment for *M. iltisiana*, *M. pugana*, and *M. vallartensis* are the most successful. These results coincide with those of reference [80] reported for *M. perezfarrerae* (64%) and *M. sharpii* (73%) with the mechanical scarification treatment (i.e., the seeds are placed in purified water and then the aril is manually



Figure 9.
Staining of seed tissues with tetrazolium in the viability test in *Magnolia pugana*: a) viable seed, and b) non-viable seed.

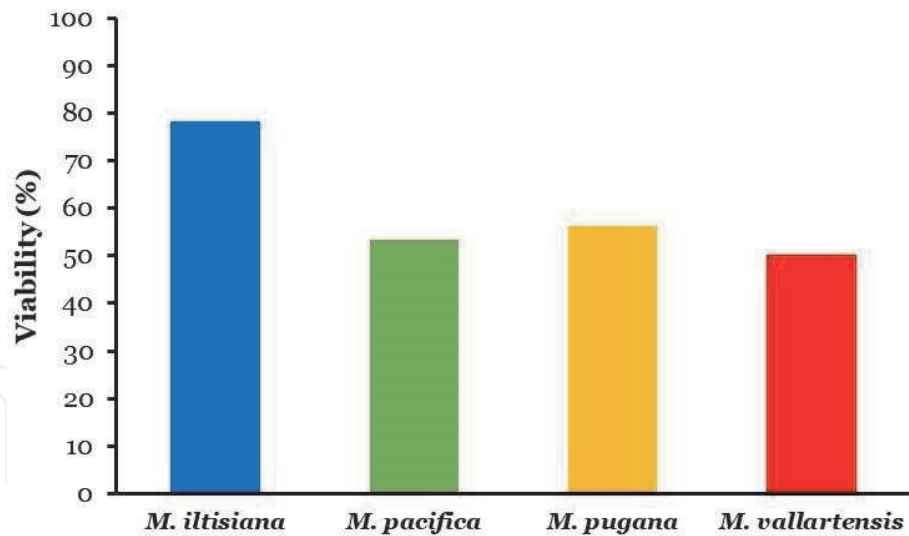


Figure 10. Viability percentages for: *Magnolia iltisiana*, *M. pacifica*, *M. pugana*, and *M. vallartensis*.

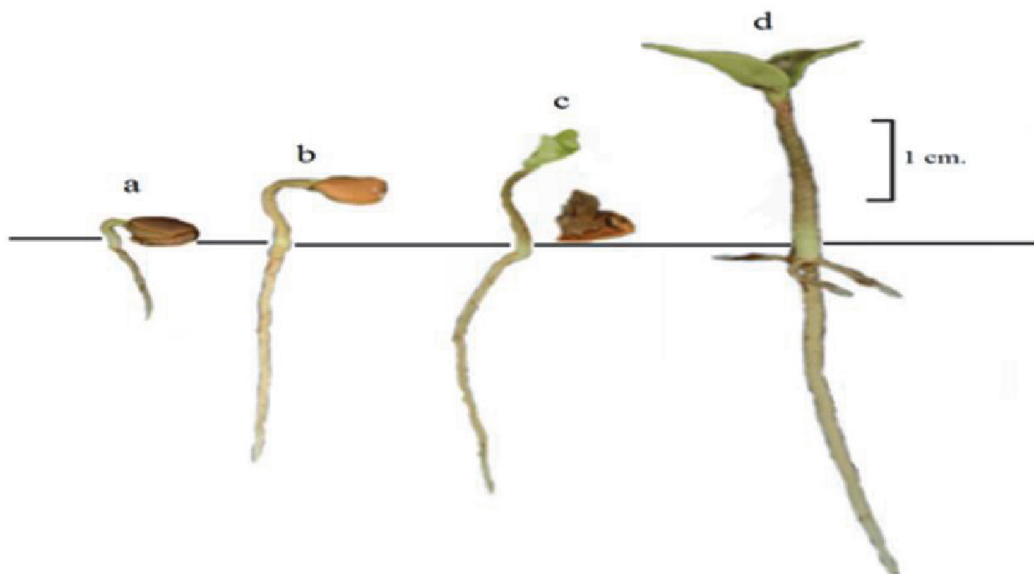


Figure 11. Germination process of *M. pugana*: a) exposure of the endosperm and hypocotyl, b) elongation of the hypocotyl and cotyledon, c) leaf cotyledons and testa flushing, d) seedling with leaf primordia exposed above ground.

removed). This treatment also proved to be effective with 90–100% germination in seeds of *M. dealbata* [74] and *M. champaca* (L.) Baill. ex Pierre [88] (73%) under the same treatment [89].

Low germination rates (< 70%) may indicate that the seeds are dormant and cannot be broken [90]. The results in these studies suggest that cold stratification treatments and manual aryl removal may indicate the presence of physiological and chemical dormancy, respectively [72]. Taken together, these results are consistent with reference [29] who recommends that *Magnolia* seeds undergo stratification periods so that immature embryos can develop, while in Ref. [91] reported that Magnoliaceae aryls contain inhibitors that delay germination.

On the other hand, it has been proven that the use of phytohormones is a promoter of germination with physiological dormancy [72]. Conversely, it was found that the phytohormone treatments used in the experiments on *M. iltisiana* and *M. pugana* did not increase the germination percentage, suggesting that the seeds of these species do not possess physiological dormancy. Although the seeds of

Species	Dormancy type	Reference
<i>M. iltisiana</i>	Chemical, physical and mechanical	[81]
<i>M. pacifica</i>	Chemical	[87]
<i>M. pugana</i>	Chemical	[76]
<i>M. vallartensis</i>	Chemical	[87]

Table 5.

Types of dormancy reported in *Magnolia iltisiana*, *M. pacifica*, *M. pugana* and *M. vallartensis*.

M. iltisiana have chemical dormancy, physical dormancy is also identified, that manifests itself through the development of a lignified testa that prevented the absorption of water, and the third type of dormancy is mechanical, which in this case the head exerted too much pressure on the embryo, delaying germination [81]. In the studies conducted for *M. pacifica*, *M. pugana* and *M. vallartensis* it was found that seeds do not have physical dormancy because they have the capacity to absorb water [76, 87] (Table 5).

3. Future scenarios

3.1 Vulnerability in climate change scenarios

Climate change is an important driver for future distributions of tree species, as it is expected to modify environmental conditions critical for plant populations maintenance. An analysis of 40 species of North American tree species from the temperate zone [92] revealed the higher vulnerability to climate change for the species growing in a colder climate, including the high-elevation mountain trees. In tropical mountain landscapes of Andes, the changes in habitat suitability are expected to be of a mixed character, while favorable for some tree species they may be prejudicial for others, with an overall general increase of species risk of extinction found in approximately 20% of tree species [93]. The increase of the risk of tree species extinction from climate change is related to the projected scale of the climate zones shift, which is highly variable across the globe [94]. In the situation of the terrain with complex irregular topography the extent of habitat suitable for species may be particularly sensitive to climate change [95], as the habitat tolerances of the mountain flora are generally narrow and the distribution shift upwards in the mountains frequently means the reduction of populations. At the same time, the complex topography may offer the high heterogeneity of habitat, which could provide opportunities for emerging local refugia, detectable at a fine spatial scale [96]. In the case of *Magnolia*, the narrow distribution ranges of species may constitute a risk factor, that could lead to the disappearance of the suitable habitat in some of the climatic projections for the end of the 21st century, as it was found in the case of the Andean species *M. mercedesiarum* D.A. Neill, A. Vázquez & F. Arroyo [97, 98].

An important step in the assessment of tree species vulnerability and extinction risk in the climate change scenarios is the analysis of habitat suitability dynamics. The habitat suitability belongs to the core concepts of the ecological niche theory, particularly of great importance in the field of plant ecology, as plants cannot evade adverse environmental conditions by sheltering or migrating within the single generation [99]. The assumption that healthy plant populations stay in the equilibrium with the contemporary or recent environmental conditions is central for accessing habitat suitability through niche modeling and projecting [100]. The climatic

envelope models are a particular class of habitat suitability and niche models that uses the climatic variables as an approximation to the maximal extent of the habitat suitable to support species [101]. This class of models is widely used for the analysis of species-climate relations in the current climate, and for the heterochronic studies using past and future climate strata, as well as for predicting species distributions.

After accepting the equilibrium assumption, we used the field observations of the presence of three *Magnolia* species to develop the corresponding climatic envelope models, which captured the range of the environmental conditions that are suitable for species persistence in time and space. Further projections of the habitat suitability in the climate change scenarios in the next 80 years allowed us to detect the probable habitat suitability changes for *Magnolia* species, and to evaluate species vulnerability to climate change.

3.1.1 Methods

The species presence dataset of three taxa of the *Magnolia pacifica* complex [*M. pacifica* s.l. (including *M. talpana*), *M. pugana* s.l. (including *M. granbarrancae*), and *M. vallartensis*] was compiled from herbarium specimens at IBUG, IEB, ZEA, WIS, MEXU, XAL, MO, MICH, field observations cited in taxonomic literature [11, 13, 16], virtual images at Naturalista [102]. Monthly averaged climatic variables from WorldClim 2.1 dataset [103] available for the recent past (1970–2000) were taken as an approximation to current conditions, while the future conditions under two CO₂ emission scenarios were taken from the down-scaled projections of the general circulation model CanESM5 [104]. The detailed description of the species distribution modeling procedure and predictions are available in Mendeley Data repository [66].

3.1.2 Results and discussion

As it was expected, the climatic envelope models recovered the suitable habitat extent larger than the known species distributions. The reasons of the overestimation are discussed in Shalisko *et al.* [66]. However, in the continental scale, the high suitability was predicted close to the occurrence records, the most distant grid cells identified as suitable were separated from known species occurrence sites in less than 150 km. The estimated suitable for the species presence zone in 1970–2000 varied from 9560 km² for *M. vallartensis* to 23940 km² for *M. pugana* s.l. (**Figure 12**). The suitable areas were well separated from another two species in the case of *M. pugana* s.l., overlapped with *M. pacifica* s.l. In the case of the *M. vallartensis* the estimated overlap of potentially suitable habitat with that of *M. pacifica* s.l. was of 4053 km², which equals to 42% of the entire high suitability zone for *M. vallartensis*.

Despite the systematic overestimation of the suitable area, the climatic envelope models are useful for the evaluation of the species vulnerability to climate change, as the same bias applies to the prediction of habitat suitability in current conditions and future projections. The changes in the area with suitable conditions may be proportional to the changes in true potential distribution.

The dynamics of suitable area in SSP2–4.5 scenario [105] (**Figure 13**) was favorable for *M. pugana* s.l., as the size of the potentially suitable area in 2080–2100 was about 13% larger than in current conditions, and almost all grid cells labeled as suitable in recent past persist at the end of 21st century. The models for both *M. pacifica* s.l. and *M. vallartensis* predicted suitable habitat reduction which is particularly fast in the second half of the century, with loss of about 45% of the suitable habitat in the case of the former species and 53% in the least. Interestingly, in the

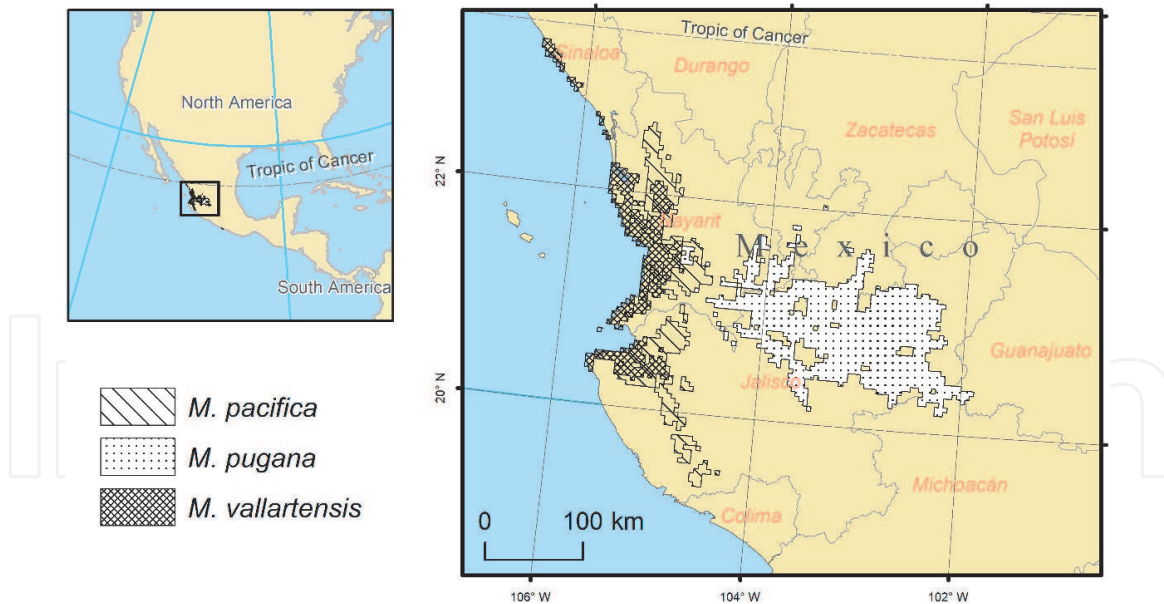


Figure 12. Areas of habitat suitability higher than ESS threshold in at least 50% of cross-validation runs of the climatic envelope model for recent (1970–2000) climatic conditions. The key map on the left part shows the location of the right frame within North America.

current climatic conditions, the habitat suitability for *M. vallartensis* was found to be high not only in the locations close to the known distribution, but also in the separate coastal zone northwards, and the persistence of the habitat suitability in future scenarios was higher in the northern area, where species observations are unknown. In the case of *M. pacifica* s.l. the future high habitat suitability in SSP2–4.5 scenario was predicted roughly in the same geographic zones as was identified for current conditions.

The baseline SSP3–7.0 scenario [105] produced habitat suitability projections that are concerning in terms of species survival (**Figure 13**). In the case of three species, the fast decline in habitat suitability was predicted from the middle of the century, resulting in a loss for the end of the century of 66% of the suitable area in the case of *M. pugana* s.l., dramatic 92% loss of habitat suitable for *M. pacifica* s.l. and complete disappearance of the habitat of *M. vallartensis*.

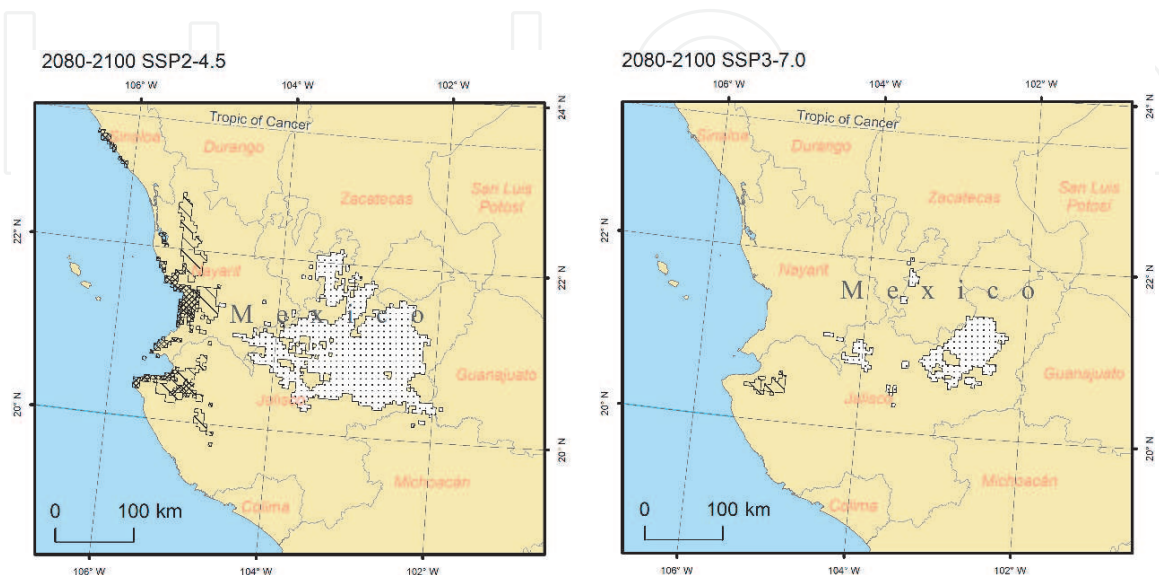


Figure 13. Areas of habitat suitability higher than ESS threshold in at least 50% of cross-validation runs of the climatic envelope model for projected future (2080–2100) climatic conditions under SSP2–4.5 and SSP3–7.0 scenarios. Symbology and extent is the same as in **Figure 12**.

The uncertainty associated with habitat loss projections remained high due to the limitations of climatic envelope modeling and the uncertainty from global circulation models and CO₂ emission scenarios. However, the general trend of probable habitat loss for *M. pacifica* s.l. and *M. vallartensis* detected in the analysis was not compromised by accounting for the uncertainty, as the similar trend of change between current conditions and projections for the end of the 21st century was found in the 95% confidence intervals. In the case of *M. pugana* s.l. the likely habitat suitability change cannot be interpreted as the sign of significant risk when taking into consideration the prediction uncertainties.

Similarly to reference [95] interpretation of the effect of habitat suitability reduction in the species survival, we consider that the risk of species extinction from habitat loss may be overestimated when the data was analyzed in coarse-scale, as the local small size refugia were excluded from consideration. The true vulnerability of species to climate change depends on several factors outside of the scope of the current analysis. Tree populations may have a lag in reaction to climate change as the long-living sessile organisms [106]. The result of this lag could be the absence of the immediate disappearance risk for adult tree individuals, that could successfully tolerate significant environmental stress, but the reduction of the reproductive success required for the populations maintenance. In many species of the North American trees, the observed distribution is not entirely concordant with the current climate, as the long-living organisms may present the 'extinction debts' and 'colonization credits' at some parts of their actual or potential ranges [106]. In the case of *Magnolia* species, the lag between the loss of the climatic suitability and the actual population disappearance may be at least several decades required for the eventual death of adult representatives of the populations. Another aspect that was not accounted for in the vulnerability analysis is the possibility of the wide environmental tolerance within the populations that were not manifested in the current biotic and abiotic conditions, but that could contribute to species survival in climate change scenarios.

4. Conservation strategy

4.1 Conservation status

More than 350 *Magnolia* species have been described worldwide, and of a total of 314 that have been evaluated in their conservation status, 48% (147 sp.) have been classified as threatened with extinction in the wild [107]. However, there are still 34% of *Magnolia* species that have not been evaluated mainly due to data deficiencies, despite that *Magnolia* conservation has become a major international task [108]. Therefore, in this section, we present an evaluation of the conservation status of the three western Mexican *Magnolia* species (section *Magnolia*) that have been evaluated so far [18], to propose and implement conservation measures.

4.1.1 Methods

Conservation status assessment. The threatened status of *M. pacifica* s.s., *M. talpana*, *M. pugana* s.s., *M. granbarrancae*, *M. vallartensis*, and *M. iltisiana* were assessed here using the IUCN Red List Criteria (criteria B1ab + B2ab, [109]) and the GeoCAT cloud software tool [110]. The Extent of Occurrence (minimum convex polygon, EOO) and the Area of Occupancy (grid cell area with occupancy, AOO) of each taxon were delimited from georeferenced records obtained from IBUG herbarium specimens, the GBIF, Tropicos.org, REMIB-CONABIO, REBIOMEX

databases, and field data. AOO was based on the IUCN default cell width of 2 km. The criterion C-2 (Genetics) of the Method of Extinction Risk Evaluation of Plants in Mexico (MER) from the Mexican Official Norm 059 [111] was used to contribute partially to the MER assessment. Criterion C-2 proposes that (1) if the population has heterozygosity (H_e) < 10–20% (depending on the molecular marker used) and (2) a genetic differentiation (G_{st} or F_{st}) > 20%, it has a higher threatened status or extinction risk.

4.1.2 Results and discussion

The estimated EOO (km^2) and AOO (km^2) were: for *M. pugana* s.s. = 1,259.3, 96, respectively; *M. granbarrancae* = 0.7, 12; *M. pacifica* s.s. = 1,216.2, 72; *M. talpana* = 91.8, 32; *M. vallartensis* = 124.0, 44; and *M. iltisiana* = 19,444.2, 196 (Table 6). These western Mexican *Magnolia* species are in an endangered status because of their higher fragmented populations, lower genetic diversity, and narrower extent of occurrence when compared with other threatened *Magnolia* species [48].

M. granbarrancae and *M. talpana* have extremely narrow geographical ranges (EOO < 100 km^2) that are in accordance with the IUCN criteria for the category of Critically Endangered species: B1ab (iii, v). Populations of these species are small and severely fragmented (criterion B1a), and present a continuing decline in the area, quality of habitat [criteria B1b (iii)], and number of mature individuals [criteria B1b (v)] [109]. Moreover *M. granbarrancae* fulfill with the IUCN criterion C1 for Critically Endangered species, having a known total number of 74 mature individuals for the species, and an estimated or projected continuing decline of at least 25% in the next three years because the flooding of its habitat by the construction of the El Zapotillo dam on the Río Verde.

The genetic diversity of a species is an important indicator of its conservation status due to its positive correlation with the capacity to adapt and overcome abiotic and biotic changes. The genetic diversity of the three species studied here is considered lower than the average ($H = 0.22$) [45], and it is even lower than that of *M. sharpii* ($I = 0.56$), a species categorized as endangered because of its narrow EOO (2,228 km^2), severely fragmented and degraded habitats, and the fact that it is known from only five locations [48, 107]. *M. pugana* together with *M. granbarrancae* had a total heterozygosity H_T of 0.158, *M. pacifica* together with *M. talpana*, of 0.175, and *M. vallartensis* of 0.171, this low genetic diversity of the three species is in accordance with criterion C-2 (intrinsic biological vulnerability with genetic heterozygosity < 20%) of the Mexican Standard NOM-059-SEMARNAT MER for being considered in the category of Endangered species [111].

The IUCN criteria do not consider the levels of genetic diversity and differentiation for assessing extinction risk, but *M. pugana* and *M. granbarrancae* suffer of very low genetic diversity [11] and very low number of individuals, so these two species should be categorized as Critically Endangered even when *M. pugana* s.s. has an area of occupancy of 96 km^2 . It is noteworthy that despite *M. pugana* s.s. EOO and AOO are in accordance with the IUCN Endangered category, their highly fragmented and isolated small populations suffering a seasonal dry and extreme climate, and its low genetic diversity make consider this species as Critically Endangered. *M. pugana* s.l. (including *M. granbarrancae*) has even lower genetic diversity and higher genetic differentiation than the other western Mexican *Magnolia* species studied. This fact, together with the fact of having more fragmented, more isolated, smaller populations, and being surrounded by a seasonally drier environment [103], make *M. pugana* s.s. and *M. granbarrancae* be proposed to be included in the category of Critically Endangered of extinction, as it had been previously cataloged by [112] for *M. pugana* s.l.

	<i>Magnolia iltisisana</i>	<i>Magnolia granbarrancae</i>	<i>Magnolia pacifica</i>	<i>Magnolia pugana</i>	<i>Magnolia talpana</i>	<i>Magnolia vallartensis</i>
IUCN Red List category [107]	Vulnerable	—	Endangered	Endangered	—	Critically Endangered
Current threats	Anthropogenic factors, seed predation by squirrels, rapid degradation of fallen indehisced fruits, shade intolerance, low seed dispersal, livestock, illegal logging	Agricultural expansion, construction of the El Zapotillo dam that will flood a large part of the species' population, climate warming	Illegal logging, livestock, mining, agricultural expansion, avocado and coffee plantations, fruit orchards (guava, citrus), forest fires	Isolated populations, fragmented habitats, high deforestation rate, low regeneration, changes in land use, forest fires	Agricultural expansion, illegal logging, avocado and coffee plantations, fruit orchards (guava, citrus), forest fires	Restricted distribution, highway construction for Puerto Vallarta bypass, forest fires, insect pests, global warming, agricultural expansion
No. of individuals	43 ind. in 20 0.1 ha plots, Villas de Cacoma	Only 74 known ind. From RV	48 ind. in six 0.1 ha plots, Cerro La Bufa +72 ind. in San Sebastián del Oeste region	735 known ind. From all known populations ASL, ALV, APV and others	40 ind. in 20 0.1 ha plots +54 outside of the plots, Talpa river watershed, maple forest	27 ind. in two 0.1 ha plots, from APM, 187 known ind. From APM, LL, PV and others
Ex-situ records	2	0	55	209	0	19
Genetic diversity	Unknown	Very low	Low	Very low	Low	Low
Seed productivity	High	Middle	Middle	High	Low	Low
Known populations in 1994	3	0	7	6	0	0
No. populations in 2020	23	3	14	14	5	6
Radius (km)	45	1.5	50	30	15	16
Biogeographic provinces	1	2	3	2	1	2
Altitude (m a.s.l.)	800–2400	1000–1240	750–2250 m	1300–1800	1050–1800	100–1100

	<i>Magnolia iltisisana</i>	<i>Magnolia granbarrancae</i>	<i>Magnolia pacifica</i>	<i>Magnolia pugana</i>	<i>Magnolia talpana</i>	<i>Magnolia vallartensis</i>
Latitudinal	19°10'–19°50'	20°45'–20°43'	20°30'–21°38'	20°46'–21°15'	20°12'–20°18'	20°20'–20°35'
EOO (km ²)	19,444.2	0.7	1,216.2	1259.3	91.8	124.0
AOO (km ²)	196	12	72	96	32	44
Updated category from IUCN criteria: B1ab (iii, v), B2ab (iii, v), or C1	Vulnerable B1b (iii,v) B2b (iii,v)	Critically Endangered B1ab(iii,v), C1 Low genetic diversity	Endangered B1ab (iii,v) B2ab (iii,v)	Critically Endangered B1ab (iii,v) B2ab (iii,v) Low genetic diversity	Critically Endangered B1ab(iii,v)	Critically Endangered B1ab(iii,v)

Table 6.

Relevant conservation data to guide establishing conservation priorities for *Magnolia* section *Magnolia* in western México. EOO: Extent of occurrence, AOO: Area of occupancy.

Magnolia vallartensis should also be considered as Critically Endangered, as it has been categorized by [107], due to having an EOO of only 124 km², low number of individuals, high deforestation rates, forest plagues and fires, fragmentation, climate change, cattle raising, roads and urban growth. Both, the EOO and the AOO of *M. pacifica* s.s. are in accordance with the IUCN category of Endangered (EOO < 5,000 km² and AOO < 500 km²). Furthermore, in the case of *M. pacifica* s.l. (including *M. talpana*) and *M. vallartensis* the extent of occurrence is projected to suffer a severe reduction in the next 80 years as a consequence of the shrinkage of the areas of high habitat suitability, estimated under the highly probable SSP3–7.0 and less probable SSP2–4.5 scenarios. The scope of the projected extent of occurrence reduction in the case of *M. vallartensis* may bear this species into the critically endangered status by the end of the century [criteria B1b (i, iii)]. In the case of *M. pacifica* s.l. the projected reduction of the area of suitable habitat for the end of the century is highly significant, however, may be not enough to move species to the critically endangered category by using only this criterion.

Despite *M. iltisiana* has an AOO < 500 km² (196), and that it has some populations with severe fragmentation, as those near Morelia city (in Michoacán), several populations of this species do not have severe fragmentation and inhabit in the protected area of Sierra de Manantlán Biosphere Reserve. Therefore *M. iltisiana* should maintain its category of Vulnerable, as it has been suggested by [107].

Magnolia granbarrancae which is located to the northeast side of the Santiago river canyon (composed by the RV locality), *M. pugana* s.s. (composed by ALV, ASL and APV), and the populations of *M. pacifica* s.s., *M. talpana* and *M. vallartensis* should be considered as five separate units of conservation. The implementation and enforcement of *in situ* and *ex situ* conservation actions should protect and preserve at least one locality of each population of *M. pugana* s.s., and *M. granbarrancae*, as well as the most divergent localities of *M. pacifica* s.s. (CSJ and SS), *M. talpana* (BA) and *M. vallartensis* (PV and APM). The BA locality of *M. talpana* (“Bosque de Arce”, maple forest) conserve the highest genetic diversity, which coincides with being one of the most important forests in terms of plant species richness, endemism, and floristic composition for western Mexico [113, 114]. Greater efforts must be made to preserve all of these taxa, and a higher focus is required to protect *M. pugana* s.s and *M. granbarrancae*, characterized by low levels of genetic variation and highly fragmented and small populations. All these *Magnolia* species must be fully evaluated with the MER method for inclusion in the list of endangered species of the official Mexican Standard NOM-059. Education, conservation, management, and ecological restoration plans are badly needed to decrease their threatened status and raise awareness of the fate of these important species in extinction risk.

4.2 Propagation and reintroduction

Magnolias, like other plant species, may be reproduced both sexually (by seeds) and asexually (through plant tissues).

4.2.1 Sexual reproduction

This kind of propagation involves genetic recombination, which provides a genetic variability that improves the plant’s ability to adapt to its environment [29, 115], ensuring that long-term survival by reducing the risk of suffering a bottleneck effect, which puts a species in danger of extinction [116]; Also, more vigorous seedlings are generated and the propagation is easier and cheaper than asexual reproduction, on the other hand, the plants take longer to reach maturity and bloom. In section “2.6 Pre-germination and seed dormancy treatments” of this

chapter, the aspect of sexual reproduction in magnolias of Western Mexico is addressed more extensively.

4.2.2 *Asexual propagation*

Asexual reproduction has been only reported for *M. tamaulipana* of sect. *Magnolia* and for species of sect. *Macrophylla*. Asexual propagation occurs through stems, shoots and roots and is more convenient for horticultural purposes, where it is sought to preserve certain characteristics through generation of clones and flower faster than those propagated by seeds [29], however, the methods used are more expensive and transportation is more complicated [117]. Although no studies have been conducted on vegetative propagation in the Mexican magnolias of the sect. *Magnolia*, we list the most used horticultural methods for the *Magnolia* genus [29, 117]:

4.2.2.1 *Cuttings*

This approach has the advantage of reducing costs in large-scale production and the plants obtained are more uniform, but to ensure survival it is necessary to have better-equipped facilities and develop specific protocols for Neotropical magnolias. Method for cuttings in *Magnolia* could be found in the following references: [29, 117, 118].

4.2.2.2 *Layering*

It is recommended when you want to get only a few plants. Produces larger plants in a shorter period of time, but requires more space and labor [29]. More information about this technique can be available in the following references [29, 117, 118].

4.2.2.3 *Harnpariphan's technique*.

Here named as such, is an intermediate cutting-layering technique. In the cutting a reed incision is made 5 cm above the basal cut, a small piece of plastic is inserted between the reed and the stem to ensure an acute angle separation. A paste with fungicide and rooting hormone (1:1) is applied to the exposed sides of the incision. Subsequently, the wound area is covered with a substrate, securing it as layering with a piece of plastic tied at the ends with ropes. A small segment is cut from the basal part of the cutting-layer in water, to ensure that the air does not interrupt the flow of water through the conductive vessels. The basal part must be in contact with the water and the covered region of the wound must remain in the air, taking care to always keep the layering moist, making sure that the plastic has small holes that allow the excess water to drain. Leaf areas with lesions or infections should be trimmed and the water always kept fresh by making replacements when necessary [117].

4.2.2.4 *Grafting*

It is an easy and cheap method but requires more space, labor and time. Recommended for species and cultivars difficult to root [29], the graft should be done between genetically closest species since there is better long-term compatibility [119]. It is a great alternative for threatened species with low fertility [117]. For major types of grafts of magnolias see the following references: chip-budding [29, 118], side-grafting [29], wedge or cleft grafting [117], crown grafting [117],

canutillo graft [117], shield budding [117], patch budding [117], and approach grafting [117].

4.2.2.5 Tissue culture

It involves the regeneration of a whole plant from a small portion taken from any part of a parent plant, producing enormous quantities of plants from a few cells. This method, in particular, represents a difficult and expensive task, coupled with the fact that some species may be more difficult to propagate by this means, a situation that is attributed to the high content of phenolic acid in magnolias, which can inhibit the growth of the crop, requiring frequently change the explant to a fresh medium, it has also been observed that magnolias tend to generate vitrified growth under tissue culture, which does not generate roots and rarely reverts to normal growth [29]. Phenolic acid content is lower in magnolias after dormancy is broken, so it is better to use dormant branches to obtain explants [120]. More information about this technique can be found in Refs. [29, 120, 121].

4.2.3 Reintroduction

The goal of reintroduction is to establish a viable population of any species in the wild and is essential to increase its long-term survival and to reestablish key species in an ecosystem and restore its natural biodiversity, so it must be carried out within the area of distribution and primitive natural habitat of the target species [122]. For a reintroduction to be successful, it should be considered the awareness of the population and community participation, the planting time and composition of the individuals in quantity and quality (**Figures 14 and 15**). It must be ensured that the site has the appropriate biotic and abiotic requirements for the species in all its life stages, considering seasonal and post-establishment needs, continuous monitoring and management is required to provide feedback [123]. In the case of magnolias, it has been found that they belong to an intermediate and late-successional state, so reintroduction and reforestation projects must consider planting individuals under a pre-existing plant cover [124]. In tropical magnolias, no tolerance to prolonged dry seasons has been found, so to plant them, humid regions, well-drained sites with slightly acidic soils (pH close to 6) and little compacted should be chosen [117]. For transplantation to the ground, reference [117] recommends loosening the soil first, taking care not to injure any roots when removing them from the bag, always keeping the soil moist, adding mulch around the stem to keep moisture and avoid weeds, and, in case of if necessary, apply dilute phosphoric acid to lower the soil pH.



Figure 14. Course-workshop on conservation challenges for *Magnolia* conservation in the subdeciduous forest of the Nahuan community of Ayotitlán, in the sierra de Manantlán, western México. Photos by A. Vázquez-García.



Figure 15. Left: Reintroduction of *Magnolia pugana* by Dra. Rosa Romo and her assistants near arroyo san Nicolas. Central: Plantation of *Magnolia ofeliae* at the campus of UDG-CUCBA, Zapopan, by the kids: Alberto Aguilar and Quetzalcóatl Vázquez. Right: Urban plantation of *Magnolia mexicana* at Jardines Alcalde, Guadalajara by Jesús Cortés and Paula Cortés. Photos by M. Muñiz-Castro and A. Vázquez-García.

4.3 In-situ and ex-situ conservation

4.3.1 In-situ conservation proposals

Of the six species of *Magnolia* sect. *Magnolia* five of them have populations within Protected Natural Areas; *M. iltisiana* is located within the Sierra de Manantlán Biosphere Reserve, a federally protected area, while *M. talpana* is located within the Bosque de Arce State Park and *M. granbarrancae* in the Natural Formation of State Interest Barrancas de los Ríos Santiago y Verde, *M. pacifica* has one of its populations in the Sierra de San Juan Ecological Reserve in Nayarit and in the Cuenca Alimentadora del Distrito Nacional de Riego 043 (APRN-CADNR-043), and *M. pugana* has populations within the La Primavera Flora and Fauna Protection Area and in the Natural Resources Protection Area of Cuenca Alimentadora del Distrito Nacional de Riego 043 (APRN-CADNR-043, southern Zacatecas polygons). However, for some of these species it is necessary to establish additional measures for their protection [13].

In the case of *M. pacifica*, it is important to create a protection zone in its type locality, in San Sebastián del Oeste, Jalisco, where it is currently threatened and its population has been reduced by deforestation, agricultural expansion and mining. For *M. pugana*, it is proposed to create a protection zone in the perimeter of Arroyo La Virgen, which is located near Rancho San Nicolás in Zapopan, Jalisco. This stream has relict elements of cloud forest, so declaring it a Protection Area would benefit the conservation of other species such as *Populus luziarum* A. Vázquez, Muñiz-Castro & Padilla-Lepe [125] (CR), an endemic species found only in two Zapopan ravines.

With *M. vallartensis*, it is recommended to decree a protection zone in the Palo María stream basin, since it is one of its main distribution sites in addition to having other endemic species such as *Pinus vallartensis* Pérez de la Rosa & Gernandt [126] and *Miconia vallartensis* Zabalgoitia, Figueroa & Muñiz-Castro [127], which would contribute significantly to the conservation of Puerto Vallarta's biodiversity. In this particular case, the importance of establishing a protection zone and rescuing this species also rests on the fact that this species was declared an emblematic tree of the Municipality from which it bears its name, Puerto Vallarta.

Although all these species, except for the new ones described here, are under some risk category of the IUCN Red List, at the national level, only one of them, *M. iltisiana*, is found in the Official Mexican Norm NOM-059 on Environmental Protection for native species of flora and fauna; low genetic diversity of

M. granbarrancae (Rio Verde locality), *M. pacifica* s.s., *M. talpana*, *M. pugana* s.s., and *M. vallartensis*, agrees with the C-2 criterion of NOM-059 MER [18], therefore that it would be appropriate to integrate them into this Norm as Endangered species.

It is vitally important to create integrated management and conservation strategies according to each species, which include reaching stakeholders of the communities where these magnolias are distributed since the first step is to increase conservation awareness and foster appropriation of their natural resources. An example is a Workshop held by some of the authors of this chapter in the Nahua community of Ayotitlán, where *M. jaliscana* is located, in which they taught about the importance of conserving magnolias and of its propagation, as well as the importance of ecosystem services (**Figure 14**).

Alternatively, Wildlife Conservation Management Units (UMA for its acronym in Spanish) can be created in which, in addition to conserving the site, the owner obtains benefits through the sustainable use of its natural resources and can be beneficiaries of subsidies for the conservation and sustainable use of wildlife native at UMA [128].

Property owners can also be creditors of the Payment for Environmental Services (PES), which is a program whose purpose is to promote the recognition of the value of the services provided by ecosystems by creating a market for them [129].

4.3.2 Ex-situ conservation

The ex-situ conservation centers aim to reduce the risk of extinction of threatened species and act as a complement to in-situ conservation by supporting wild populations with the reintroduction of specimens and restoration of habitats, acting as gene banks, promoting research and continuing to raise social awareness as elements of diffusion and environmental education [130]. For *Magnolia*, there are 9,918 ex-situ records of 152 Magnoliaceae species, however less than half of the most threatened taxa are represented [107], Latin America and the Caribbean region are of particular concern because many of their endemic Magnoliaceae are

Species	University of Guadalajara, Zapopan	Vallarta Botanic Garden	National Center of Genetic Resources, Tepatitlán
<i>M. dealbata</i>	0	0	1
<i>M. grandiflora</i>	11	1	1
<i>M. iltisiana</i>	0	2	0
<i>M. jaliscana</i>	1	0	0
<i>M. mexicana</i>	0	0	1
<i>M. oaxacensis</i>	0	3	0
<i>M. ofeliae</i>	2	4	0
<i>M. pacifica</i>	17	38	0
<i>M. pugana</i>	180	29	0
<i>M. rzedowskiana</i>	0	1	0
<i>M. tarahumara</i>	80	35	0
<i>M. vallartensis</i>	14	5	0

Table 7.

Magnolia ex-situ collections from Jalisco, Mexico. *M. mexicana* DC. [132], *M. oaxacensis* A. Vázquez [14], *M. rzedowskiana* A. Vázquez, Domínguez-Yescas & Pedraza-Ruiz [133], *M. tarahumara* (A. Vázquez) A. Vázquez [15].

not represented in ex-situ collection [131]. In Jalisco, some of the *Magnolia* ex-situ collections for 2020 are here presented (**Table 7**), and particularly the species of sect. *Magnolia* discussed in this chapter are cultivated.

In March 2020, a Magnolia Conservation and Propagation Workshop was held in the Nahua community of Ayotitlán, in which a small greenhouse was installed so that the community could reproduce *M. jaliscana*, currently, they have 20 seedlings (**Figure 14**).

4.3.2.1 Plantations of *M. pugana*

Dr. Rosa de Lourdes Romo Campos has dedicated herself for several years to the conservation of *M. pugana*, which has led to a series of reintroduction plantations for this species, whose origin is from San Lorenzo, Zapopan. These plantations can be located in the map (**Figure 1**) with a tree symbol. In 2013 two plantations were carried out, one in the “Los Colomos” urban forest and another near the “Balneario El Encanto” in Zapopan, Jalisco, both with 50 plants; in 2016 one plantation with 30 individuals were made near highway San Cristobal de la Barranca-Tesistán; in 2018 one plantation with 25 trees was carried out in “Los Colomos” urban forest and another with 200 individuals in San Esteban, Zapopan, Jalisco was executed; in 2020 only one plantation was made in Parques de Tesistán, Zapopan, with 18 trees.

5. Conclusions

1. The *Magnolia pacifica* species complex shows high morphological divergence and displays a remarkable pattern of allopatric radiation, where the species are distributed among five biogeographic provinces and or separated by canyons, along an elevational, latitudinal and continentality or moisture gradient. This is demonstrated by the two newly described taxa, including: *M. granbarrancae*, confined to the 500 m deep and narrow Rio Verde canyon, in the vicinity of Guadalajara city and *M. talpana* confined to the southern portion of the Talpa de Allende municipality, Jalisco, in the high watersheds of the ríos Camacho, Desmoronado and Talpa. Further fieldwork is needed in northern Nayarit, Sinaloa, Durango, Sonora and Chihuahua to enhance our understanding of the morphological divergence of this species complex.
2. The genetic structure of the *Magnolia pacifica* species complex exhibits two main groups separated along a moisture gradient by the Transmexican Volcanic Belt province and by distance. The western more maritime group differentiated into two genetic subgroups *M. pacifica* s.s. and *M. vallartensis*, and the eastern most continental group differentiated into two genetic subgroups *M. pugana* s.s. and *M. granbarrancae*. *M. pugana* s.l. is subject to a drier and more extreme climate, therefore having more deforested, fragmented, and isolated habitats, which leads to lower genetic diversity and a higher genetic differentiation. This differentiation and the physiographic barrier of the Santiago river canyon have structured *M. pugana* s.l. in the two subgroups, *M. pugana* s.s. and *M. granbarrancae*. The *M. pacifica* s.s.-*M. vallartensis* group exhibits genetic segregation in two subgroups, *M. vallartensis* and *M. pacifica*, separated by several canyon river barriers, but maintaining a partial gene flow at their southern ranges. The three species of the *M. pacifica* complex have lower genetic diversity than eastern Mexican *Magnolia* species which are considered as endangered. All main genetic groups and subgroups defined in this study should be considered as separate conservation units, and concerted efforts are needed to protect them.

3. Similar to other basal angiosperm lineages, beetle pollination has been associated with the Magnoliaceae, however, we have documented that hymenopterans could also play a significant role as pollinators in some species as demonstrated in *Magnolia vallartensis*, being pollinated by meliponid bees. The pollination efficiency between Hymenoptera and Coleoptera needs to be assessed.
4. Flower showed an incipient asynchrony, insufficient to prevent gene flow, while fruiting had a marked asynchrony among the three species, suggesting specialization by reducing competitors and enhancing dispersal. Flowering seasonality was significant for *M. pacifica* and *M. pugana* while fruiting seasonality was significant only for *M. pugana*. In terms of productivity *M. pugana* had a significantly higher productivity than *M. vallartensis* or *M. pacifica*, despite this large productivity, there is little or no recruitment of juveniles.
5. Floral scents in the three studied species of *Magnolia pacifica* complex display distinctive floral scent profiles, the chemical differences may have arisen from interaction with pollinators or the environment. The Jaccard similarity index between *M. pacifica* - *M. vallartensis* was 54.6%, while with *M. pugana* these showed 19.5% and 18.6% of similarity respectively. It is necessary to continue with studies focused on establishing the relationships between the components and the chemotaxonomic field including more *Magnolia* species populations to conform a robust database.
6. The conservation status of the western Mexican *Magnolia* species are currently as endangered status because of their highly fragmented populations, low genetic diversity, and narrower extent of occurrence compared with other threatened *Magnolia* species. But it is noteworthy that *M. pugana* s.l. group has even lower genetic diversity and higher genetic differentiation than the other western Mexican *Magnolia* species. This fact, together with the fact of having more fragmented, more isolated, smaller populations, and being surrounded by a seasonally drier environment, make *M. pugana* a candidate species to be included in the category of Critically Endangered, as it had been previously cataloged.
7. Climate change scenarios projected in the next 80 years suggest high vulnerability for two species (*M. pacifica* and *M. vallartensis*). The area of high habitat suitability is expected to abruptly reduce for both species, which may result in persistence of populations only within the small local refugia, which are located within the extent of the current high suitability area. The species in-situ preservation effort may be particularly important in the areas that were found to have high habitat suitability in the climate change scenarios.
8. Given that there is still little knowledge on insect floral visitors and the floral biology of most *Magnolia* species, further studies including more field observations are needed. Studies of seed predation and dispersal are also highly recommended to understand the apparent lack of recruitment of juveniles.
9. The results of the percentages of viability differ from those of germination in *M. iltisiana*, *M. pacifica*, *M. pugana* and *M. vallartensis*. Therefore, it is necessary to continue with the studies of seed ecology, to determine whether there are other types of dormancy as morphological or morpho-physiological, as has been detected in other species of *Magnolia*. Considering that the highest percentage of germination is achieved with the manual removal of the aril, for its low cost and in greenhouse conditions, it can be a successful practice for the

propagation of seedlings of these species, which help reforestation and restoration of their populations in their natural environment.

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