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Seed germination and population structure of two endangered tree species: *Magnolia perezfarrerae* and *Magnolia sharpii*

Germinación de semillas y estructura poblacional de dos especies de árboles en peligro: *Magnolia perezfarrerae* y *Magnolia sharpii*

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Abstract

Background: Due to habitat fragmentation many *Magnolia* species are at risk of extinction in Mexico.

Question: What type of population structure is exhibited by *Magnolia perezfarrerae* and *M. sharpii,* endemic species of Chiapas, Mexico? Does the extreme reduction in the population of both *Magnolia* affect their reproductive capacity? What is the propagation potential in both species of *Magnolia*? Is it necessary to use pregerminative treatments for *Magnolia* species propagation?

Studied species: *Magnolia perezfarrerae* A. Vázquez & Gómez-Domínguez and *Magnolia sharpii* Miranda.

Study sites and years of study: Highlands and Central Depression of Chiapas, Mexico, from 2015 to 2017.

Methods: In this study, the density and distribution of diametric sizes of *M. perezfarrerae* and *M. sharpii* were assessed in natural populations. Their reproductive capacity and the *ex-situ* germination of both species was analyzed.

Results: The population density in *M. perezfarrerae* was 40-53 ind. ha^{-1}, and 24-83 ind. ha^{-1} in *M. sharpii*. Size structure varied between populations of each species: one apparently without problems of regeneration, and another with discontinuous distribution in several size categories. The seeds presented physical, chemical and mechanical dormancy. The pre-germination treatments applied proved mostly effective for *M. sharpii* by 73 % and by 64 % for *M. perezfarrerae*.

Conclusions: The current condition of *Magnolia* species is critical and their persistence is compromised. Any viable conservation option would need to identify potential sites for the reintroduction of new populations.

Keywords: endangered species, endemic, *ex situ* propagation, pre-germination treatment.

Resumen

Antecedentes: Las especies de *Magnolia* en México se encuentran en riesgo de extinción debido a la fragmentación de su hábitat.

Preguntas: ¿Qué tipos de estructura poblacional exhiben *Magnolia perezfarrerae* y *M. sharpii*, dos especies endémicas de Chiapas, México? ¿La extrema reducción de las poblaciones presentan alguna afectación en la capacidad reproductiva de las dos especies de *Magnolia*? ¿Cuál es el potencial de propagación en ambas especies de *Magnolia*? ¿Es necesario usar tratamientos pregerminativos para incrementar la propagación de ambas especies de *Magnolia*?

Especies estudiadas: *Magnolia perezfarrerae* A. Vázquez & Gómez-Domínguez y *Magnolia sharpii* Miranda.

Sitios y años de estudio: Los Altos y la Depresión Central de Chiapas, México, de 2015 a 2017.

Métodos: En este estudio evaluamos la densidad y distribución de tamaños diamétricos de *M. perezfarrerae* y *M. sharpii* en dos poblaciones naturales. Se evaluó su capacidad reproductiva y la germinación *ex situ*.

Resultados: La densidad poblacional de *M. perezfarrerae* fue de 40-53 ind. ha^{-1} y 24-83 ind. ha^{-1} en *M. sharpii*. La estructura de tamaños varía entre las poblaciones de cada especie: una aparentemente sin problemas de regeneración y otra con distribución discontinua en varias categorías de tamaños. Las semillas presentaron latencia física, química y mecánica. Los tratamientos pregerminativos aplicados fueron efectivos para *M. sharpii* con 73 % y 64 % para *M. perezfarrerae*.

Conclusiones: La condición actual de las poblaciones de *Magnolia* es crítica, por lo que su persistencia está comprometida. Las opciones viables de conservación requieren de identificar sitios potenciales para la reintroducción de nuevas poblaciones.

Palabras clave: endémica, especies en peligro, propagación *ex situ*, tratamientos pre-germinativos.

There are 314 species of *Magnolia* (Magnoliaceae) worldwide, and about of 46 % of them are classified under any threatened category. In Mexico 30 species of *Magnolia* are distributed, six are categorized as Critically Endangered (CR), 13 as endangered (EN), four as vulnerable (VU), one as almost threatened (NT), and six under defficient data (DD); Rivers *et al*. 2016). However, the Norma Oficial Mexicana (SEMARNAT-2010) considers only *M. dealbata* Zucc., in danger of extinction, and *M. iltisiana* A. Vázquez, *M. mexicana* DC., and *M. schiedeana* Schltdl., as threatened (SEMARNAT 2010). The main threats are felling and wood extraction (Kundu 2009, Vásquez-Morales *et al*. 2017), land-use changes, illegal extraction (Cicuzza *et al*. 2007, He *et al*. 2009), and population reduction due to the negative effects of global climate change (McKenney *et al*. 2007, Vásquez-Morales *et al*. 2014). Seven species of *Magnolia* have been recognized in Chiapas. Three species are common in the tropical montane cloud forest (*M. faustinomirandae* A. Vázquez, *M. montebelloensis* A. Vázquez, Pérez-Farr., and *M. sharpii* Miranda), and the rest are present in lower montane semi-evergreen rainforest and dry forest (*M. lacandonica* A. Vázquez, Pérez-Farr. & Mart.-Camilo, *M. mayae* A. Vázquez & Pérez-Farr., *M. perezfarrerae* A. Vázquez & Gómez-Domínguez and *M. zamudioi* A. Vázquez; Miranda 1955, Vázquez-García *et al*. 2013, 2017, Rivers *et al*. 2016).

The Magnoliaceae are known as living fossils because of their long history on our planet, dating from 100 to 120 Mya (Kim *et al*. 2004). They belong to the basal angiosperms that are characterized by having ancestral flowers pollinated by beetles (Dieringer *et al*. 1999, Kim *et al*. 2005, Wang *et al*. 2014). Commonly, the population structure of *Magnolia* is aggregated with geographic isolation and allopatric speciation, which contributes to diversification and endemism (Jiménez-Ramírez *et al*. 2007, Kundu 2009). Currently, *Magnolia* populations live in forest relicts and rainforest fragments. In Chiapas, *M. sharpii* is restricted to the pine-oak forest and montane rain forests in the Central Highlands and part of the Northern region of Chiapas. Over the last 25 years, tropical montane cloud forest cover has decrease of 50 %, with an annual deforestation rate of 4.8 % (Ochoa-Gaona & González-Espinosa 2000, Cayuela *et al*. 2006). Therefore, its necessary to establish conservation and restoration programs for threatened native species, this of course can only be established with the proper knowledge on handling such species (Ramírez-Marcial *et al*. 2001, Toledo-Aceves 2017).

Germination is one of the most important and critical stages in the life cycle of trees (Baskin & Baskin 2001, Toledo-Aceves 2017). Some studies of *Magnolia* have shown low germination percentage due to different types of dormancy (including physical, chemical, mechanical, physiological and morphological; Jacobo-Pereira *et al*. 2016). To eliminate dormancy, several pre-germination treatments have been successfully applied: mechanical scarification on *M. iltisiana* and *M. vovidesii* A. Vázquez, Domínguez-Yescas & L. Carvajal, (Saldaña-Acosta *et al*. 2001, Toledo-Aceves 2017), stratification at different temperatures in *M. punduana* (Hook.f. & Thomson) Figlar, and *M. schiedeana* (Vásquez-Morales & Sánchez-Velásquez 2011, Iralu & Upadhaya 2016), and chemical compounds imbibition on *M. champaca* (L.) Figlar (Fernando *et al*. 2013). In general, pre-germination treatments increase the germination potential of *Magnolia* seeds, producing highest number of seedlings which can be useful for conservation and restoration programs.

We study two *Magnolia* species with a restricted distribution in the state of Chiapas by assessing the structure of diametric sizes in two natural populations for each of them, their reproductive capacity in terms of size and vigor (number and size of polyfollicles and number of seeds), and their germination capacity. The main objective was to determine and compare the population structure of *M. perezfarrerae* and *M. sharpii* in natural populations, and to assess the *ex-situ* germination of both species, as to obtain the greatest number of seedlings for *circa-situ* conservation programs. We address four primary questions: (1) What type of population structure is exhibited by *Magnolia perezfarrerae* and *M. sharpii,* endemic species of Chiapas, Mexico? (2) Does the extreme reduction in the population of both *Magnolia* affect their reproductive capacity? (3) What is the propagation potential in both species of *Magnolia*? (4) Is it necessary to use pregerminative treatments for *Magnolia* species propagation?

Materials and methods

Species under study. Magnolia perezfarrerae is a perennial tree up to 25 m tall with glabrous and elliptical leaves; a fissured grayish bark covered with lichens; and creamy white flowers with tepals, sepals and petals in series of three (Figure 1A-D). Flowering occurs between the months of June and August. The fruit is a dehiscent ellipsoid polyfollicle, containing between 41 to 115 seeds. The seeds have a red sarcotesta (Figure 1E,F). Fruiting occurs during the months of December to February. Similarly, *M. sharpii* is a perennial tree up to 25 m tall, bark with gray to greenish-white lenticels with abundant lichens and mosses and concave coriaceous leaves. It presents glabrous white flowers between the months of July and August (Figure 2A-D). The fruit is a conical ovoid polyfollicle with about 85 to 120 seeds (Figure 2E,F). *Magnolia sharpii* have a wide phenological spectrum and is possible to obtain mature fruits in different time, the fruiting occurs in altitude low to 1,600 m during winter, to high of 2,400 m snm during spring (Miranda 1955).

Study area. Two populations of *M. perezfarrerae* from the Central Depression region of Chiapas were chosen. The first one located at the Ejido El Divisadero, in the municipality of Berriozábal, and the second one in the community of Ocuilapa, municipality of Ocozocoautla de Espinosa, Chiapas. *Magnolia perezfarrerae* in this study, was determinated in voucher No. 23948 of herbarium CH – El Colegio de la Frontera Sur.

Three populations of *M. sharpii* were located in the Highland region of Chiapas: the first one in the community of Bazom, municipality of Huixtán (Martínez-Icó *et al*. 2015), and

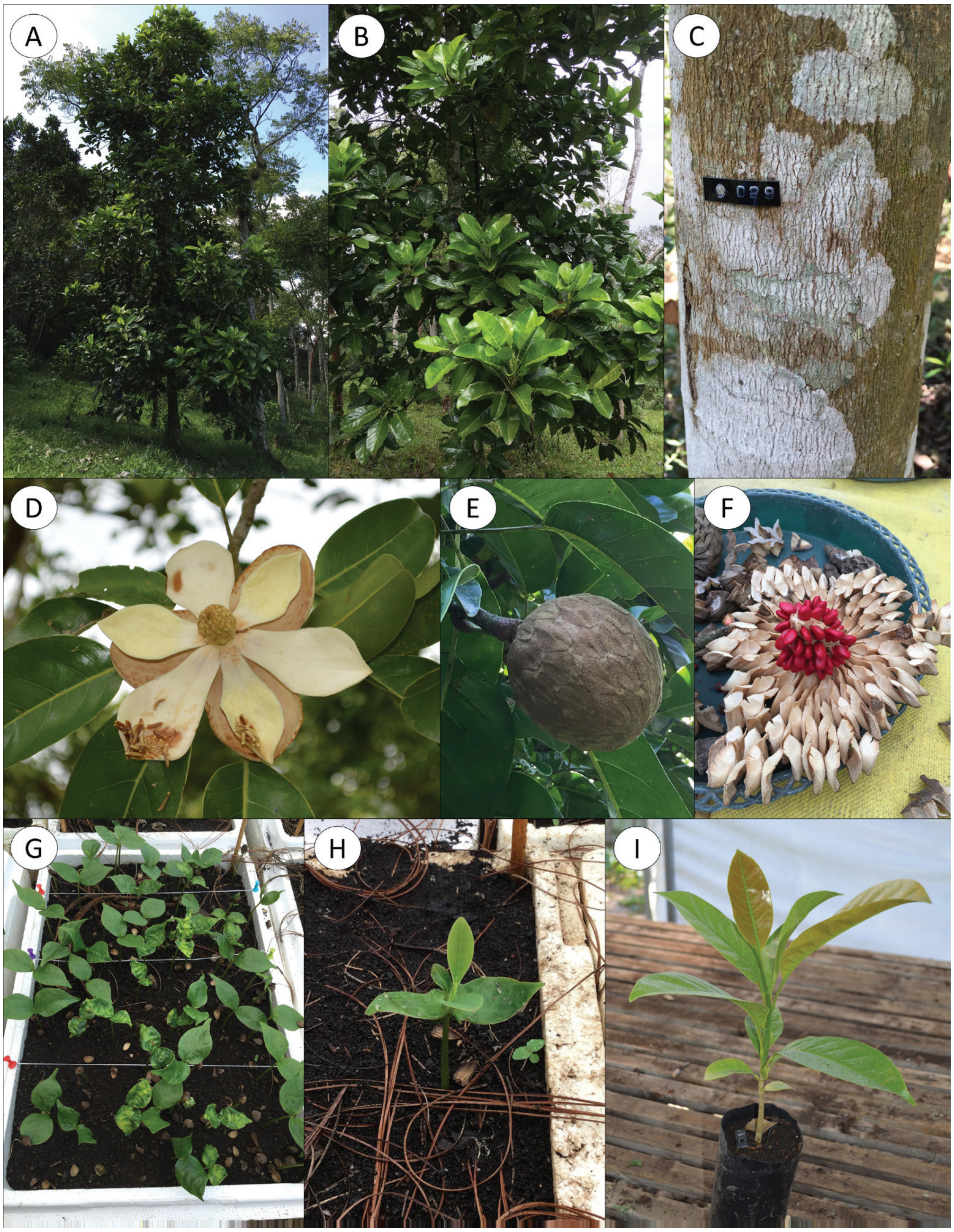

Figure 1. Morphological characteristics of *Magnolia perezfarrerae*. A) mature tree, B) foliage, C) bark, D) flower, E) polyfollicle on tree, F) mature polyfollicle exposing its seeds, G) seedlings, H) seedling with true leaves, I) 1 year-old plant. Photo credit: SGVM.

compared voucher No. 608 of herbarium CH, the second one was identified in the community of El Retiro, and the third one in Tzajalchen, the municipality of Tenejapa (Newton *et al*. 2008). The physical, climatic and cultural characteristics of the sites are detailed in the Table 1.

Population structure. To obtain the population structure of *M. perezfarrerae,* all individuals in the population of Ejido El Divisadero were included, and 70 % of the individuals in Ocuilapa population. In the case of *M. sharpii*, we included about 60 % of the individuals from Bazom population, and 80 % of the El Retiro population. At Tzajalchen, we recorded only two adult reproductive individuals of this species (19.9 and 22.4 cm in diameter at breast height), so the structural analysis was not carried out.

On each population, diameter at breast height (DBH) for all individuals of *Magnolia* whose stems taller than 1.3 m were included. To individuals < 1.3 m in height the basal

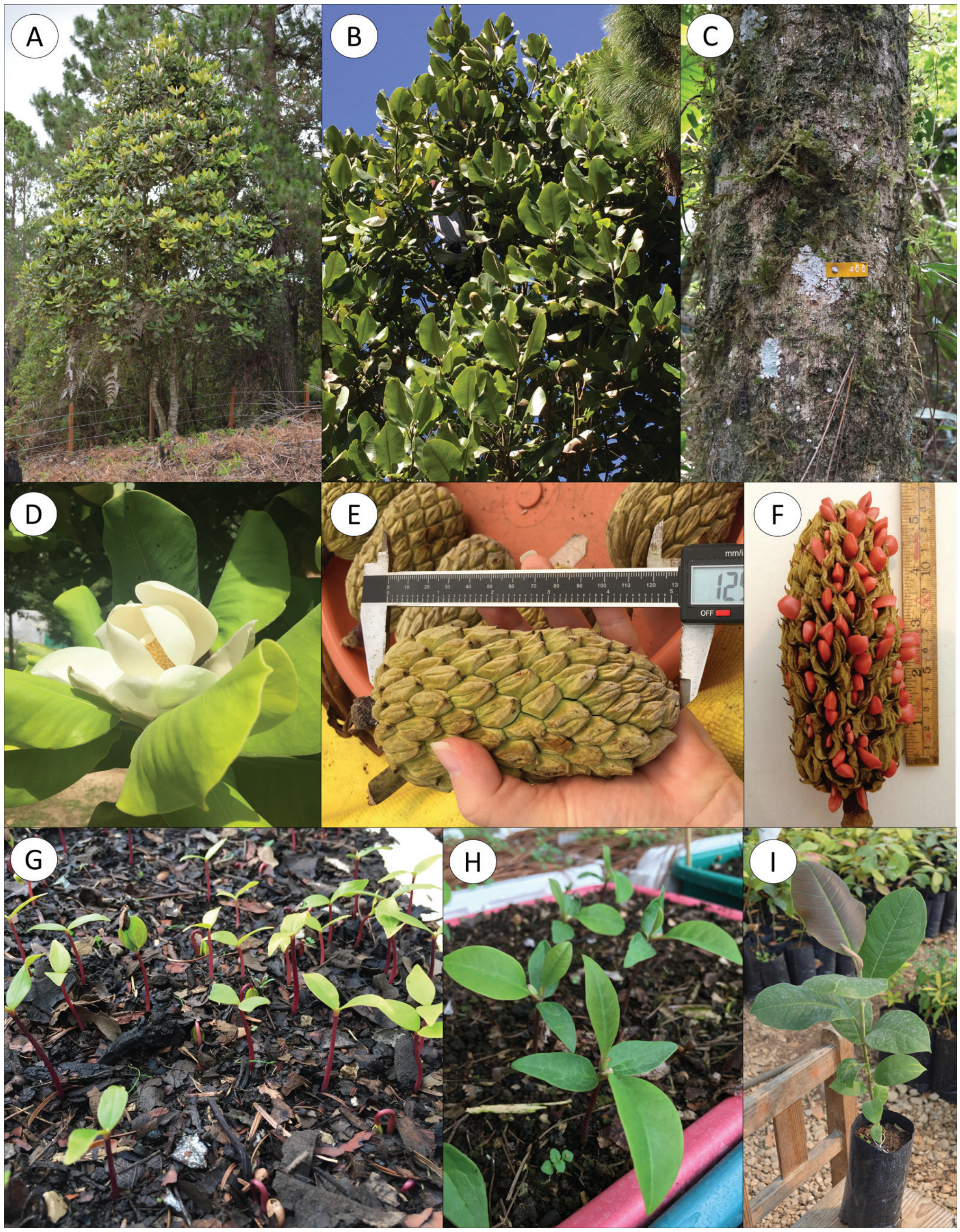

Figure 2. Morphological characteristics of *Magnolia sharpii*. A) mature tree, B) foliage, C) bark, D) flower, E) polyfollicle, F) mature polyfollicle exposing its seeds, G) seedlings, H) seedling with true leaves, I) 1 year-old plant. Photo credit: SGVM.

diameter of the stem was recorded. Each individual was grouped into one of the following four size categories: a) seedlings or recent-regeneration individuals, *i.e*., those less than 1.3 m in height; b) juveniles, *i.e*., individuals taller than 1.3 m but with a diameter smaller than 5 cm; c) pre-adults, *i.e*., individuals with a diameter between 5 and 10 cm, d) adults, *i.e*., individuals with a diameter at breast height larger than 10 cm. Frequency of the diametric categories and population structure of each species and population were analyzed by means of a generalized linear model with Poisson distribution, using R package (R Core Team 2013).

Polyfollicles and seeds. Due to the little availability of reproductive individuals, 10 adult reproductive individuals with no apparent damage were chosen for each population of both species. In January 2016, the mature polyfollicles of *M. perezfarrerae* were collected at each population, nine polyfollicles from El Divisadero and 55 from Ocuilapa. The mature polyfollicles of *M. sharpii* were collected at Bazom in March 2016, and in October 2015 at Tzajalchen (75 and 13 polyfollicles, respectively). No polyfollicles were obtained from the population of El Retiro, due to the absence of reproductive individuals. The polyfollicles were then transferred to a nursery located in ECOSUR-San Cristóbal de Las Casas, Chiapas, México, and placed in an illuminated and ventilated area at room temperature (Mean annual temperature = 16.7 °C).

To characterize the reproduction of both species in each population, nine to 15 polyfollicles were chosen from each of them to carry out the following measurements: size of polyfollicles (length and width in cm), number of seeds per polyfollicle, time required for the release of the seeds, and seed size (length and width in cm). Each variable were subjected to an one-way analysis of variance, using R package (R Core Team 2013).

Viability test. From each set of seeds by species and population, a random sample of 50 seeds with two replicates were taken. The seeds were placed in glass Petri dishes (150 × 25 mm) and the sarcotesta was removed by manual scarification, washed in running water, split with a scalpel and immediately treated with a 2,3,5 Triphenyltetrazolium chloride solution (1 % in a phosphate buffer pH 7), and placed in a drying oven at a temperature of 30 ºC during 24 h in complete darkness (Baskin & Baskin 2001).

The seeds from each replicate were scrutinized by way of a dissection microscope and classified according to the coloration of the embryo. Those that presented the embryo completely red were recorded as live seeds; on the contrary, a seed was declared dead when the embryo was partially red or colorless.

Germination treatments. In order to determine the most effective pre-germination treatment for the seeds of two *Magnolia* species, all available seeds in good condition were used. In view of the scarcity and inequality of the number of seeds obtained per population of each species, pre-germination treatments were carried out with different seed quantities and replicates (Table 2).

The treatments used in this research were: (a) SWS = seeds with sarcotesta or control; (b) MSS = mechanically scarified seeds, *i.e.*, the seeds are placed in purified water for 48 h and subsequently the sarcotesta is removed manually; (c) CSS = mechanically scarified seeds are immersed in a hydrogen peroxide solution (H_2O_2) and purified water (1:3) for 30 min, then rinsed with purified water; (d) IS = mechanically scarified seeds are incubated at 4-10 °C for 15 days in glass Petri dishes with moist blotting paper, then soaked in purified water at room temperature for 24 h; (e) WWS = mechanically scarified seeds are incubated in water purified at 30 °C for 15 min, then soaked in purified water at room temperature for 24 h.

After applying pre-germination treatments, the *M. perezfarrerae* seeds from each treatment and replicate were placed in polystyrene foam trays of 30 cm wide, 40 cm long and 15 cm high, with forest soil substrate for germination. The seeds of *M. sharpii* were placed in plastic trays of 30 cm wide, 35 cm long and 15 cm high, with forest soil substrate for germination. The germination experiments were carried out at the institutional nurseries of ECOSUR-San Cristóbal, located at 2,125 m of altitude, mean temperature of driest period 13.04 ºC and mean temperature of wettest period 15.69 ºC, with a interval de 40-60 % of humidity. Once the radicle emerged, seeds were considered as germinated

Table 1. Characteristics of study sites in Chiapas, México.

	Magnolia perezfarrerae		***Magnolia sharpii***		
Populations	**El Divisadero**	**Ocuilapa**	**Bazom**	**El Retiro**	**Tzajalchen**
Polygon area (ha)	0.8	3.4	4.8	0.5	< 0.5
Latitude (N)	16º 56’ 36”	16º 50’ 57”	16º 44’ 30”	16º 49’ 11”	16º 50’ 29”
Longitude (W)	93º 22’ 57”	93º 24’ 35”	92º 29’ 30”	92º 29’ 11”	92º 27’ 42”
Elevation (m)	820	959	2,450	2,070	1,571
Climate	Warm sub humid	Warm sub humid	Temperate Sub humid	Warm wet	Warm wet
Mean annual temperature (ºC)	23	22	14.5	15	15
Mean annual rainfall (mm)	1,000	1,000	1,350	1,500	1,500
Soil type	Acrisol, vertisol and litosol	Litosol, rendzinas, luviosol and regosol	Nitosol and acrisol	Nitosol and acrisol	Nitosol and acrisol
Vegetation type	Lower mountain tropical rainforest, secondary vegetation and grassland	Dry tropical forest, oak forest, riparian forest and grassland	Pine forest, pine-oak forest and grassland	Pine-oak forest	Pine-oak forest
Ethnic group	Zoque	Zoque	Tzoltzil	Tzeltal	Tzeltal

Table 2. Pregerminative treatments applied to *Magnolia* spp. Number of replicates to each treatments and number of seeds (between parentheses) in each population. SWS = seeds with sarcotesta (control); MSS = mechanically scarified seeds, *i.e.*, the seeds are placed in purified water for 48 h and subsequently the sarcotesta is removed manually; CSS = mechanically scarified seeds are immersed in a hydrogen peroxide solution (H_2O_2) and purified water (1:3) for 30 min, then rinsed with purified water; IS = mechanically scarified seeds are incubated at 4-10 °C for 15 days in glass Petri dishes with moist blotting paper, then soaked in purified water at room temperature for 24 h; WWS = mechanically scarified seeds are incubated in water purified at 30 °C for 15 min, then soaked in purified water at room temperature for 24 h.

		Pregerminative treatments*				
Species	**Populations**	**SWS**	**MSS**	**CSS**	**IS**	**WWS**
M. perezfarrerae	El Divisadero	2(50)	2 (50)	2 (50)	2 (50)	2 (50)
	Ocuilapa	5(100)	5(100)	5(100)	5(100)	5(100)
M. sharpii	Bazom	5(100)	5(100)	5(100)	5(100)	5(100)
	Tzajalchen	5(25)	-	5(25)	5(25)	5(25)

(Baskin & Baskin 2001). In each experiment, germinated seeds were quantified for 60 days, counting starting with the germination of the first seed of each treatment. For each species, the percentage of seeds viability and the germination time between populations and treatments was compared by means of a generalized linear model with Poisson distribution, using R package (R Core Team 2013).

Results

Population structure. The size structure of *M. perezfarrerae* populations presented an inverted *J* form, that is, skewed to the left, which indicates a high frequency of small individuals with few adult reproductive individuals. No significant differences were found among populations ($Z = -3.50$, $p > 0.05$, Figure 3A). The population density in El Divisadero amounted to 53 individuals ha^{-1} and for Ocuilapa reached 40 individuals ha^{-1}, with no significant difference between them ($Z = -0.69$, $p = 0.48$). As for *M. sharpii*, the population in Bazom also presented an inverted *J* form, but in the population of El Retiro a discontinuous curve was observed in the frequency of the diametric sizes, with no new individuals being recruited, and without the presence of individuals less than 1.3 m tall or reproductive adult individuals with DBH greater than 40 cm. The structures of these two populations of *M. sharpii* present significant differences ($Z = -5.37$, $p < 0.05$, Fig. 3B). While the population of Bazom amounted to 83 individuals ha^{-1}, the population of El Retiro reached only 24 individuals per ha ($Z = -5.35$, $p < 0.05$).

Polyfollicles and seeds. Except for a greater seed size and produccion of polyfollicles in the population of Ocuilapa, measurement values of the polyfollicles and seeds of the two populations of *M. perezfarrerae* did not present significant differences between them. As for *M. sharpii*, the population of Bazom presented a high productivity of larger-size polyfollicles and, consequently, a higher seed production (Table 3).

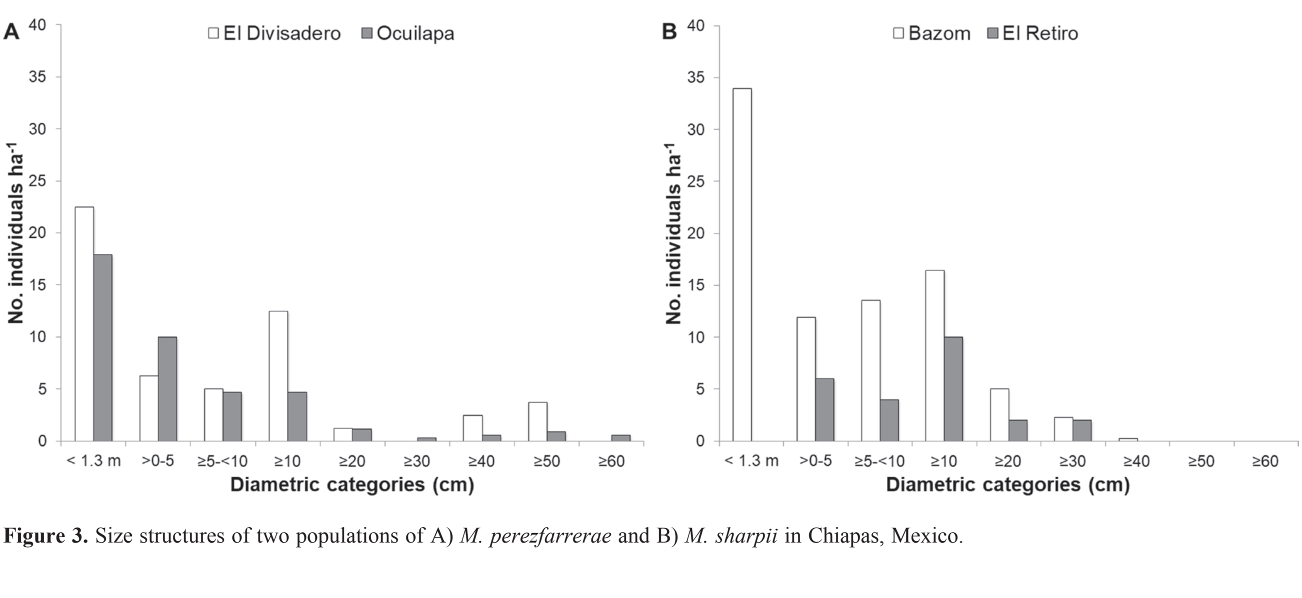

Figure 3. Size structures of two populations of A) *M. perezfarrerae* and B) *M. sharpii* in Chiapas, Mexico.

Table 3. Measurements of polyfollicles and seeds of two endangered *Magnolia* species. Mean ± standard deviation. Different letters in each row mean significant differences between populations (ANOVA test, $p < 0.05$).

		Magnolia perezfarrerae		***Magnolia sharpii***	
Populations		**El Divisadero**	**Ocuilapa**	**Bazom**	**Tzajalchen**
Polyfollicles	Long (cm)	11.23 ± 1.67 a	10.10 ± 0.79 a	12.76 ± 0.45 a	10.83 ± 0.33 b
	Width (cm)	7.96 ± 0.83 a	8.58 ± 0.46 a	5.50 ± 0.66 a	4.87 ± 0.24 b
	Opening (day)	4 a	4 a	14 a	14 a
Seed	Number per polyfollicles	78 ± 37 a	89 ± 26 a	104 ± 23 a	33 ± 13 b
	Long (cm)	0.94 ± 0.11 b	1.17 ± 0.15 a	1.04 ± 0.06 a	1.03 ± 0.08 a
	Width (cm)	0.88 ± 0.18 a	0.90 ± 0.07 a	0.65 ± 0.09 a	0.64 ± 0.08 a

Viability test. The viability of *M. perezfarrerae* seeds was 92 ± 0.8 %, and that of *M. sharpii* 87.5 ± 4.1 %. No significant variations were found between populations of *M. perezfarrerae* ($Z = 0.10$, $p = 0.91$) and *M. sharpii* ($Z = 0.53$, $p = 0.59$).

Germination treatments. The seeds of both species showed epigeous germination, the cotyledons emerging from the soil as the hypocotyl develops, until they become photosynthetic organs (Figure 1G,H y 2G,H). After two months, the reddish hue of the stem of *M. sharpii* seedlings changes to green.

With MSS and WWS treatments, the seeds of *M. perezfarrerae* from Ocuilapa started germination 70 days after sowing, and those from El Divisadero after 75 days with MSS treatment, without significant differences between populations or treatments ($F = 3.53$, df = 4, $p = 0.12$; Figure 4). As for *M. sharpii*, the seeds from Tzajalchen started to germinate 43 days after planting with the CSS and WWS treatment, and the seeds from Bazom after 46 days with the CSS, MSS and WWS treatments, with no significant differences between populations or treatments ($F = 1.32$, df = 4, $p = 0.40$; Figure 5).

Germination of *M. perezfarrerae* seeds varies according to their origin ($Z = -3.22$, $p < 0.05$), the seeds from Ocuilapa being the ones that achieved greater germination. In both populations of *M. perezfarrerae*, the most effective pre-germination treatment was MSS, with 64 ± 15.8 % on seeds from Ocuilapa, and 63 ± 3.5 % on seeds from El Divisadero (Figure 6A). MSS and WWS pre-germination treatments presented significant differences with respect to the other treatments ($F = 6.21$, df = 4, $p < 0.05$).

The same is true for *M. sharpii* seeds, those from Bazom obtaining greater germination than seeds from other populations ($Z = -21.60$, $p < 0.05$). The most effective germination treatment with *M. sharpii* seeds was WWS, with 73 ± 7.5 % on the seeds from Bazom, whereas with the CSS treatment 64 ± 2.1% was obtained on seeds from Tzajalchen (Figure 6B). The CSS, IS and WWS pre-germination treatments presented significant differences in both populations of *M. sharpii* ($F = 69.42$, df = 4, $p < 0.05$). Finally, with the SWS treatment, used as control, 10 ± 12 % of germination was

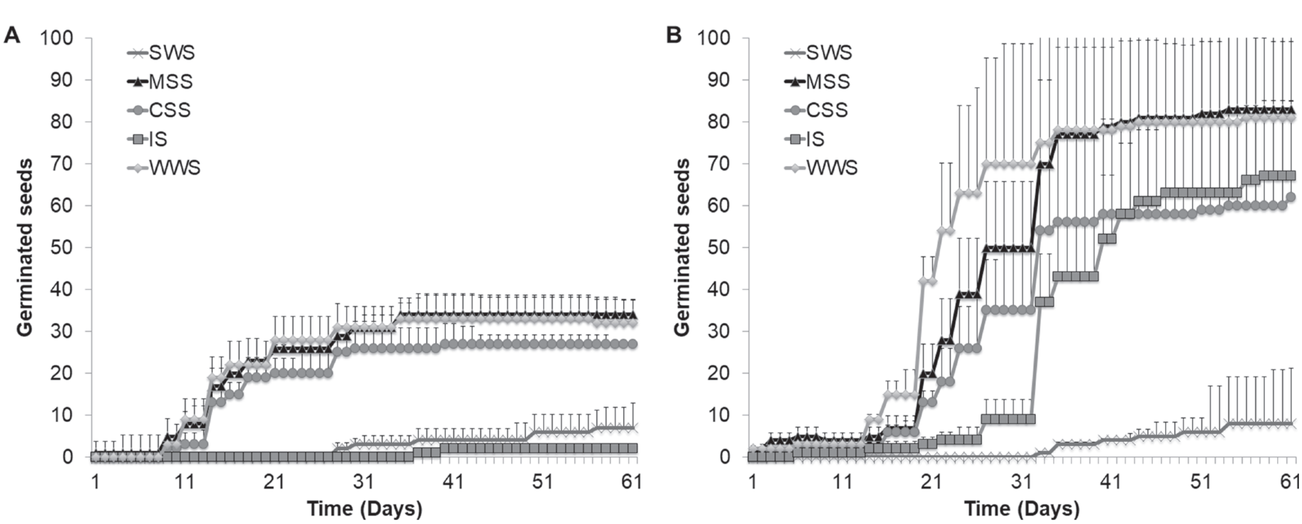

Figure 4. Number of germinated seeds of *M. perezfarrerae* by pre-germination treatment in two populations: A) El Divisadero and B) Ocuilapa. The bars indicate average ± standard deviation

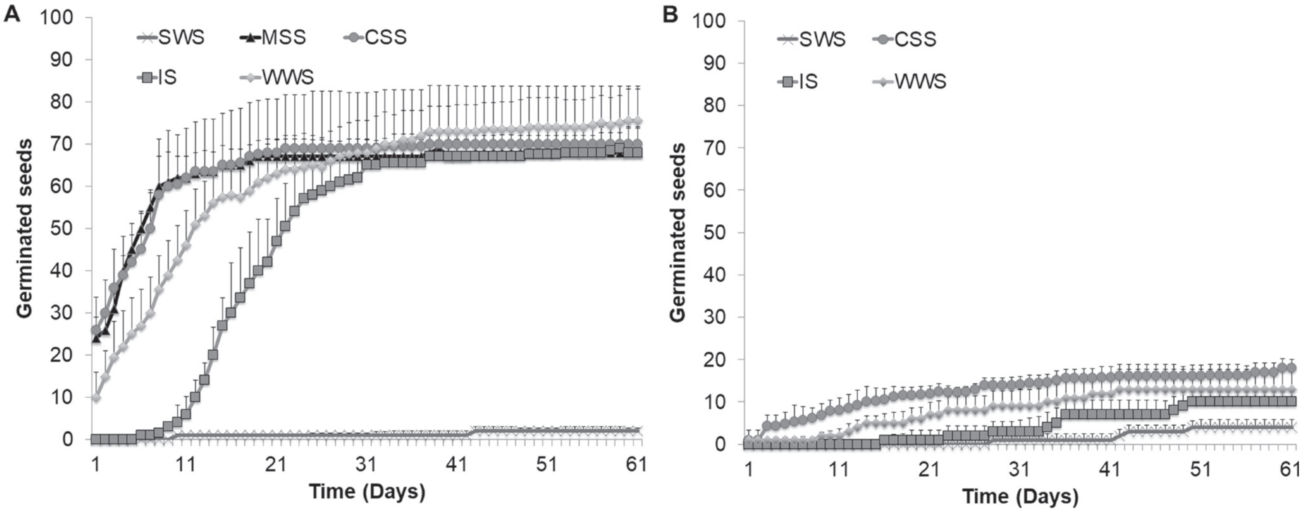

Figure 5. Number of germinated seeds of *M. sharpii* by pre-germination treatment in two populations: A) Bazom and B) Tzajalchen. The bars indicate average ± standard deviation.

obtained with *M. perezfarrerae* seeds, and 5 ± 1.3 % with *M. sharpii* seeds.

Discussion

The populations under study of *Magnolia perezfarrerae* and *M. sharpii* present a good population density (53 individuals ha^{-1} y 83 individuals ha^{-1} respectively), as individuals tend to concentrate on a relatively small area. Lower densities have been observed in other *Magnolia* species. For example, *M. schiedeana* populations present 12 and 14 individuals ha^{-1} (Vásquez-Morales *et al*. 2017); *M. officinalis* Rehder & E.H. Wilson subsp. *biloba* (Rehder & E.H. Wilson) Y.W. Law presents 0.15 to 5 individuals ha^{-1} (He *et al*. 2009), while *M. longipedunculata* (Q.W. Zeng & Y.W. Law) V.S. Kumar and *M. zenii* W.C. Cheng present 11 and 18 individuals ha^{-1}, respectively, also *M. crassifolia* presents a relict population of six individuals (Rivers *et al*. 2016).

Size structure is a useful indicator of both population composition in terms of size, sex, age and reproduction, and the presence or absence of recruitment under natural conditions (Caswell 2001, Sánchez-Velásquez *et al*. 2016). The size structure of the *M. perezfarrerae* and *M. sharpii* populations in this study comprise large number of small and non-reproductive individuals and a small number of reproductive adults. Although this structure has been documented

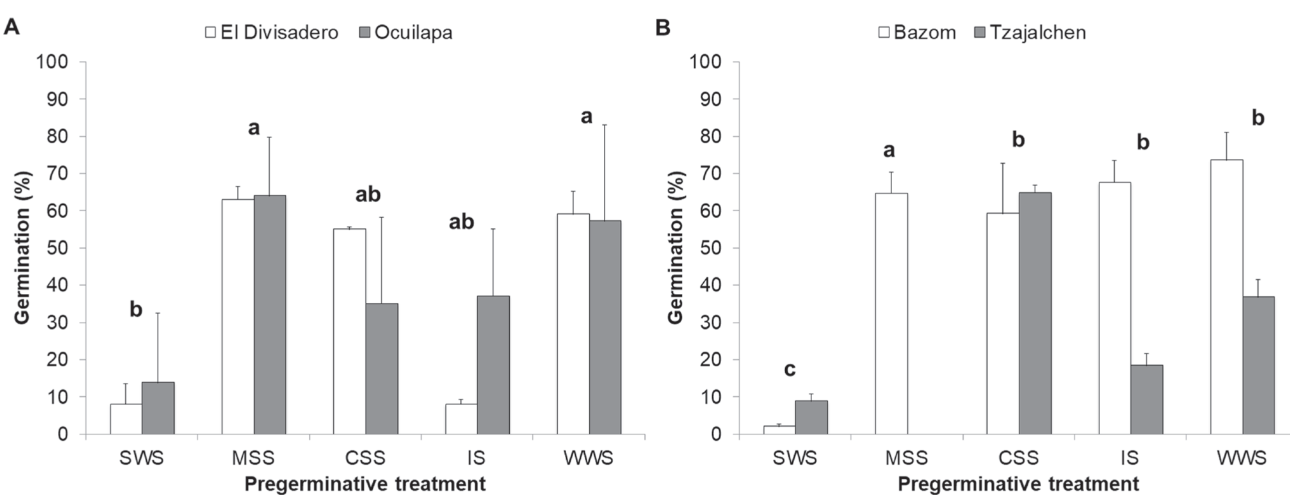

Figure 6. Germination percentage of A) *M. perezfarrerae* and B) *M. sharpii* seeds from two populations in response to pre-germination treatments. The treatments are SWS (seeds with sarcotesta), MSS (seeds with mechanical scarification), CSS (seeds with mechanical and chemical scarification), IS (seeds with mechanical scarification and cold stratification) and WWS (seeds with mechanical scarification and incubation). Different letters indicate significant differences ($p < 0.05$).

for *M. sharpii* (Ramírez-Marcial *et al*. 2001), *M. vovidesii* (Sánchez-Velásquez & Pineda-López 2010) and *M. obovata* Thunb., it is not a constant in all populations (Hoshino *et al*. 2002). In the community of El Retiro, the *M. sharpii* population showed a discontinuous curve, indicating pulses of regeneration by the establishment of seedling banks, as has been observed in *M. schiedeana* (Vásquez-Morales *et al*. 2017). Low densities are attributed to the effect of habitat deforestation and felling of individuals of large diametric size (Ramírez-Marcial *et al*. 2001, Sánchez-Velásquez *et al*. 2016). Conservation programs for both *Magnolia* species (including the reintroduction of seedlings in the remaining forest relicts; Ramírez-Bamonde *et al*. 2005) are therefore necessary, especially in natural populations, where low densities and few adult reproductive individuals prevail.

Like other *Magnolia* species, *M. perezfarrerae* and *M. sharpii* seeds present a high percentage of viability. *M. vovidesii* seeds have 100 % viability (Corral-Aguirre & Sánchez-Velásquez 2006), and *M. schiedeana* and *M. iltisiana* 80 % (Saldaña-Acosta *et al*. 2001, Vásquez-Morales & Sánchez-Velásquez 2011). In this study, the germplasm with the highest germination potential was collected at the Ocuilapa and Bazom populations.

Mechanical scarification (MSS) of the two studied *Magnolia* showed high germination rates being the most effective treatment for *M. perezfarrerae*. However, germination rates are lower than those obtained with *M. vovidesii* (previously named *M. dealbata*, 90-100 %; Corral-Aguirre & Sánchez-Velásquez 2006, Toledo-Aceves 2017) and *M. champaca* (73 %) under the same treatment (Candiani *et al*. 2004), indicating that sarcotesta must be eliminated in order to break chemical and physical dormancy, as it inhibits germination and impedes water absorption.

Mechanical scarification and incubation in warm water (WWS) was the most conducive treatment for the germination of *M. sharpii* seeds. This treatment proved also to be effective (84 %) with seeds of *M. schiedeana*, an endemic species from the tropical montane cloud forest of the state of Veracruz, Mexico (Vásquez-Morales & Sánchez-Velásquez 2011). In this study, both species, experienced mechanical dormancy as a result of the pressure exerted by the sclerotesta on the seedling cotyledons as observed in *M. iltisiana* (Saldaña-Acosta *et al*. 2001).

Although the seeds of *M. perezfarrerae* and *M. sharpii* have a high percentage of viability, the pre-germination treatment applied made the germination percentages differ. It is therefore necessary to continue seed ecology studies, so as to determine whether other types of dormancy the seeds present, such as morphological or physiological dormancy, as has been detected in other *Magnolia* species (Jacobo-Pereira *et al*. 2016).

In this study, a feasible route for conservation and possible repopulation of *M. perezfarrerae* and *M. sharpii* through seed management for plant production has been proposed. It has been confirmed that the populations of both species in the area of study present a small number of individuals with high regeneration potential but few parental trees, due to immoderate felling and high deforestation. The seeds of both species have a high percentage of viability, but without the application of pre-germination treatments, physical, chemical and mechanical dormancy result in low germination rates. In spite of this, the size structure of natural populations seems to be unaffected. It is essential to implement restoration and conservation programs for threatened *Magnolia* species in Mexico.

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