

## Unravelling the Mexican *Magnolia dealbata* (Magnoliaceae) species complex

FABIÁN AUGUSTO ALDABA NÚÑEZ<sup>1,5,\*</sup>, SALVADOR GUZMÁN-DÍAZ<sup>1,6</sup>, SUHYEON PARK<sup>2,7</sup>, SANGTAE KIM<sup>2,8</sup>, ESTEBAN MANUEL MARTÍNEZ SALAS<sup>3,9</sup> & MARIE-STÉPHANIE SAMAIN<sup>1,4,10</sup>

<sup>1</sup>Red de Diversidad Biológica del Occidente Mexicano, Instituto de Ecología, A.C., Avenida Lázaro Cárdenas 253, 61600, Pátzcuaro, Michoacán, Mexico



<sup>2</sup>Department of Biology, Sungshin Women's University, 76 Ga-gil 55, Gangbuk-gu, Seoul, 01133, Republic of Korea



<sup>3</sup>Herbario Nacional de México, Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Zona Deportiva, 4510, Mexico City, Mexico


<sup>4</sup>Botanical Garden, Ghent University, K.L. Ledeganckstraat 35, 9000, Gent, Belgium



<sup>5</sup> [fabian.aldaba@outlook.com](mailto:fabian.aldaba@outlook.com);  <https://orcid.org/0000-0002-0271-4467>

<sup>6</sup> [guzmandsalvador@gmail.com](mailto:guzmandsalvador@gmail.com);  <https://orcid.org/0000-0002-5123-1197>

<sup>7</sup> [shpark817@naver.com](mailto:shpark817@naver.com);  <https://orcid.org/0000-0002-3321-1950>

<sup>8</sup> [amborella@sungshin.ac.kr](mailto:amborella@sungshin.ac.kr);  <https://orcid.org/0000-0002-1821-4707>

<sup>9</sup> [ems@ib.unam.mx](mailto:ems@ib.unam.mx);  <https://orcid.org/0000-0003-3882-899X>

<sup>10</sup> [mariestephanie.samain@gmail.com](mailto:mariestephanie.samain@gmail.com);  <https://orcid.org/0000-0002-7530-9024>

\*Corresponding author:  [fabian.aldaba@outlook.com](mailto:fabian.aldaba@outlook.com)

### Abstract

In the last two decades, approximately 80 new *Magnolia* species have been described from the Neotropics; thus this region now hosts almost half of the world's known *Magnolia* diversity. Many of these likely are not segregate taxa but rather separate populations or groups of populations of the previously broadly circumscribed, widespread species. Such is possibly the case of the *Magnolia dealbata* species complex (belonging to *Magnolia* sect. *Macrophylla*), distributed throughout the Sierra Madre Oriental mountain range in Eastern Mexico. This species complex has been divided into six morphospecies based on morphological criteria only. However, recent microsatellite markers have suggested that these may be a single entity. Considering geographical data and the isolation of populations, we hypothesised that the different morphospecies could form two entities, corresponding to the north and centre of the Sierra Madre Oriental. This hypothesis was tested by morphological observations, chloroplast comparisons and phylogenetic analyses of plastomes, angiosperm DNA plastid barcodes and *Magnolia*-specific plastid DNA barcodes from hypervariable regions. Phylogenetic results from plastomes and angiosperm DNA plastid barcodes refute the multispecies hypothesis and show that the six morphospecies of this complex inhabiting the Sierra Madre Oriental form a single entity. Evidence is also provided that the morphological characters used to delimit the morphospecies of the complex, mainly numbers of carpels and the absence-presence and colour of a spot in the petals, are, in fact, phenotypic variation and have no taxonomic significance. Therefore, the taxa *M. alejandrae*, *M. nuevoleonensis*, *M. rzedowskiana*, *M. vovidesii* and *M. zoticitla* are synonymised here under *M. dealbata*. However, the possibility remains of including the latter as a variety of *M. macrophylla*, based on the results of the *Magnolia*-specific plastid DNA barcodes. Furthermore, this study proposes an updated conservation status for *M. dealbata*, highlighting the urgent need for effective conservation measures. The taxonomic clarification presented here is essential to properly target such efforts, especially in the face of threats such as indiscriminate collection and vulnerability to environmental disturbance.

**Key words:** Barcodes, Conservation status, *Macrophylla*, Plastome, Sierra Madre Oriental

### Introduction

The infrageneric classification of *Magnolia* Linnaeus (1753: 535) subdivides the genus into 15 sections (Wang *et al.* 2020; Aldaba *et al.*, in prep.) comprising a total of ca. 400 species distributed mainly in temperate and tropical mountainous areas of Asia and the Americas (Meyer 1993; POWO 2023; TROPICOS 2023; Xia *et al.* 2008). One of these sections is the North American endemic clade *Magnolia* sect. *Macrophylla* Figlar & Nootboom (2004: 92), the species of which are morphologically distinguished by being deciduous trees, the presence of false leaf whorls on new growth branches, generally large (>20 cm) and auriculiform leaves with auriculate or cordate base, pulverulent abaxial

surface, stipules semi-attached to the petiole and leaving a scar on it, and rather large flowers (>25 cm), usually with a purple or orange spot at the base of the adaxial side of the petals. The morphologically most similar clade is the *Magnolia* sect. *Tuliparia* Spach (1839: 477; *Magnolia* sect. *Auriculata* Figlar & Nooteboom (2004: 92) *sensu* Figlar & Nooteboom 2004), with only two known varieties of a single species: *M. fraseri* Walter (1788: 159) var. *fraseri* and *M. fraseri* var. *pyramidata* (W.Bartram) Pampanini (1915: 230), inhabiting the Appalachian region (Meyer 1993; Wang *et al.* 2020).

The main distinction between the two sections is the pubescence on the abaxial leaf surface of the taxa in *Magnolia* sect. *Macrophylla*, which is absent in *Magnolia* sect. *Tuliparia* (Meyer 1993). Phenologically, differences in floral odours and anthesis have also been observed, with the *Magnolia* sect. *Tuliparia* flowers closing completely at night (Figlar 2019; Thien 1974). However, molecular evidence provides varying insights into the relationships of *Magnolia* sect. *Macrophylla*: Plastid data confirm *Magnolia* sect. *Tuliparia* as its sister group, while nuclear data suggest that its closest relative is *Magnolia* sect. *Magnolia*; the latter group also inhabits the SMOr and other mountain ranges in Mexico (Aldaba Núñez *et al.* 2024).

*Magnolia* sect. *Macrophylla* includes nine currently accepted species. Of these, seven are endemic to Mexico (García-Morales *et al.* 2017; Sánchez-González *et al.* 2021; Vázquez-García *et al.* 2013, 2015, 2016, 2021; Zuccarini 1837). Six species are distributed along the Sierra Madre Oriental (SMOr): *M. alejandrae* García-Morales & Iamónico (2017: 239) occurs in the northern SMOr (central-western Tamaulipas state); *M. dealbata* Zuccarini (1837: 373) in the southern SMOr (central Oaxaca state); *M. nuevoleonensis* A.Vázquez & Domínguez-Yescas (2016: 49) in the extreme north of the SMOr (central Nuevo León state); *M. rzedowskiana* (A.Vázquez, Domínguez-Yescas & R.Pedraza 2015: 23) in the north-central SMOr (Hidalgo, Querétaro, and San Luis Potosí states); *M. vovidesii* (A.Vázquez, Domínguez-Yescas & L.Carvajal 2013: 478) in the south-central SMOr (Veracruz state); and *M. zotictla* A.Sánchez-González, Gutiérrez-Lozano & A.Vázquez (2021: 272) in the central SMOr (Hidalgo and Puebla states). One species, *M. mixteca* A. Vázquez & Domínguez-Yescas (2021: 202), is found in the Sierra Madre del Sur (SMS). The remaining two species are found in the Appalachians, in the southeastern United States of America (Meyer 1993): *M. ashei* Weatherby (1926: 35) and *M. macrophylla* Michaux (1803: 327).

The taxa from *Magnolia* sect. *Macrophylla* are known for their diverse insecticidal and medicinal applications, such as an analgesic, antidiarrheal, gastrointestinal aid, respiratory aid, and toothache remedy, some of which have been documented since pre-Hispanic times (Alonso-Castro *et al.* 2011, 2014; Chen *et al.* 2021; Domínguez *et al.* 2009, 2010, 2016; Domínguez-Yescas & Tapia 2013; Flores-Estévez *et al.* 2013; González-Trujano *et al.* 2015; Guzmán Gutiérrez *et al.* 2014; Guzmán-Trampe *et al.* 2015; Jacobo-Salcedo *et al.* 2011; Martínez *et al.* 2006; Moerman 1991; Ramírez-Reyes *et al.* 2015; Rauf *et al.* 2021; Rodríguez-Ramírez *et al.* 2021).

Among the Mexican species of *Magnolia* sect. *Macrophylla*, only *M. dealbata* was originally known to be distributed throughout the SMOr, especially montane cloud forests, one of the most threatened ecosystems in Mexico (Gual-Díaz & Rendón-Correa 2017; Miranda & Hernández-X. 2014; Rzedowski 2006; Williams-Linera *et al.* 2016b). This species was long believed to be extinct (Gutiérrez Carvajal 1993; Vovides 1981); however, since 2013, different populations (or localities disjointed by at least 70 km) throughout the central part of the SMOr have been segregated as new species: *M. rzedowskiana*, *M. vovidesii*, and *M. zotictla* (García-Morales *et al.* 2017; Sánchez-González *et al.* 2021; Vázquez-García *et al.* 2013, 2015, 2016, 2021).

Those new species have been described based on a few specimens (1 to 13 vouchers), and the morphological characters used to define them mostly considered colours (fruits, flowers), sizes (stamens, gynoecium, leaves, petals, size) and numbers of floral structures (carpels, stamens), which are highly variable traits in angiosperms (Basnett *et al.* 2024; Dai *et al.* 2016; Delgado *et al.* 2021; Zu *et al.* 2020) and in *Magnolia* (Aldaba Núñez 2020). Moreover, their delimitation does not integrate data on genetics, geography, biogeography, or geology, only a few differences in vegetation. Hence, the delimitation of these new species needs further investigation, especially in light of the development of conservation actions, but also because of their enormous potential in medicinal applications.

The existence of a species complex comprising these three taxa from the central SMOr segregated from *M. dealbata* and two others from the northern SMOr (*M. alejandrae* and *M. nuevoleonensis*) has been suggested based on genetic data of a population genetics study for the conservation of these species. In this work, Chávez-Cortázar *et al.* (2021) showed, using microsatellite markers, that all the species of the *Magnolia dealbata* complex were not reflected in the analyses of genetic structure, while the greatest genetic variability was found between groups of populations instead of groups of species. It was therefore concluded that gene pools may instead represent well-differentiated populations of a single widely distributed species: *M. dealbata*. This statement is also supported by preliminary morphological observations, where the observed differences in colour, size, and number of parts may be intraspecific population variation along a north-south latitudinal gradient.

In addition to the genetic data, observations of phenotypic plasticity, both inter- and intraspecific, such as those realized by Gutiérrez-Lozano *et al.* (2020) and Rodríguez-Ramírez *et al.* (2021) confirm the existence of this species complex. The first study focused on the morphology (using 26 traits) of four populations of *M. rzedowskiana* and found a significant variation in leaves and flowers. The second study analysed the leaf vein morphological variation in four species (*M. alejandrae*, *M. nuevoleonensis*, *M. vovidesii*, and *M. rzedowskiana*), using four traits, and found no significant differences between them. Subsequently, (Aldaba Núñez *et al.* 2024) conducted a study of Neotropical magnolias using morphological, nuclear genomic and plastome data. Following these analyses, the taxa from *Magnolia* sect. *Macrophylla* did not exhibit a consistent pattern in the phylogenetic trees; although they conform a monophyletic group, the samples appeared scattered across various branches of the trees, indicating a lack of clear lineage grouping. In addition, several polytomies were observed, complicating the understanding of the phylogenetic relationships among the taxa. Moreover, many of the clades recovered had low support values (< 0.5), showing the absence of strong evidence for these groupings.

A similar pattern has previously been found in *Magnolia* sect. *Talauma* (Juss.) Baillon (1868: 142), where the microsatellite markers together with morphological observations of *M. lopezobradorii* A.Vázquez (2012: 110) and *M. zoquepopolucae* A.Vázquez (2012: 52 ; two species with doubtful taxonomic delimitation segregated from *M. mexicana* Candolle (1818: 451) showed that both conform to a single taxon (Aldaba Núñez *et al.* 2021). Similarly, in *Magnolia* sect. *Magnolia*, chloroplast and nuclear DNA sequences, as well as microsatellite markers, did not support the genetic differentiation of *M. pedrazae* A.Vázquez (2013: 475) which had been segregated from *M. schiedeana* Schlechtendal (1864: 144; Rico & Becerril 2019).

Accurate species delimitation within the *Magnolia dealbata* complex is crucial for conservation efforts. Although there are already several conservation studies on some of the taxa, if their taxonomic delimitation is not clear or without a clear understanding of the species diversity within the complex, the conservation strategies may be ineffective and would hinder their management (Corral-Aguirre & Sánchez-Velásquez 2006; Galván-Hernández *et al.* 2020; García-Hernández & Toledo-Aceves 2020; Gutiérrez Carvajal 1993; Gutiérrez & Vovides 1997; Martínez *et al.* 2006; Ramírez-Bamonde *et al.* 2005; Sánchez-Velásquez *et al.* 2016; Sánchez-Velásquez & Pineda-López 2010; Sánchez-Velásquez & Pineda-López 2006; Smit 2013; Velazco-Macías *et al.* 2008; Vovides & Iglesias 1996). Furthermore, the taxa within the complex inhabit highly threatened cloud forests (Domínguez-Yescas *et al.* 2020).

Therefore, the work aimed to resolve the species complex present in the Mexican species of the *Magnolia* sect. *Macrophylla* by morphological observations and molecular analyses. The main questions addressed in this study were: 1) How many species of *Magnolia* sect. *Macrophylla* are found in the Sierra Madre Oriental in Mexico?, 2) What are the species' taxonomic limits based on morphological and molecular data?, 3) To what extent are the characters (reproductive and vegetative) used for species delimitation within the *Magnolia dealbata* complex taxonomically reliable? and 4) How do they relate to each other phylogenetically? Our hypothesis, based on preliminary taxonomic analyses as well as previous molecular and morphological work, is that the six-species complex of *M. dealbata* from the SMOr constitute at least two entities: 1) The northern SMOr species: *M. alejandrae* and *M. nuevoleonensis* and 2) The central-south SMOr species: *M. dealbata*, *M. rzedowskiana*, *M. vovidesii*, and *M. zoticlla*.

## Materials and methods

### Field sampling

Field sampling was conducted in the SMOr, focusing on regions known to harbour taxa from the *M. dealbata* complex. The sampling process took place over two years, from 2014 to 2015. During this period, approximately 2.5 cm portions of young leaves were collected and preserved in silica gel for subsequent laboratory analyses. They were labelled with field information such as the identity, date of collection, geographical location, and observed phenotypic characteristics (flowering or fruiting).

Furthermore, four molecular samples of the study taxa, collected and provided by Sangtae Kim (BioProject ID: PRJNA994423), as part of a project to study evolutionary relationships in the Magnoliaceae Jussieu (1789), were used to reinforce the sampling. We included at least one sample per taxon, comprising in total 14 molecular samples from nine taxa (all six morphospecies in the *Magnolia dealbata* complex, the two US taxa from *Magnolia* sect. *Macrophylla* and *M. fraseri* from *Magnolia* sect. *Tuliparia*), with each sample representing one different population covering the full distribution of the species complex. Detailed sampling information can be found in Table 1.

**TABLE 1.** Sampling list of the 14 North American *Magnolia* taxa studied. Note: \*: Transplanted or seedlings provided by Wisley Garden.

Taxa	Lab ID	Section	Field origin	Voucher (herbarium)	GenBank accession number
<i>M. alejandrae</i> García-Mor. & Iamónico	MA1160A	<i>Macrophylla</i>	Mexico, Tamaulipas	Mata 1160 (XAL)	OR730681
<i>M. alejandrae</i>	MA1188B	<i>Macrophylla</i>	Mexico, Tamaulipas	Mata 1188 (XAL)	OM455404
<i>M. ashei</i> Weath.	MA0016	<i>Macrophylla</i>	*South Korea, Chungcheongnam-do (Chollipo arboretum)	Kim 1016 (NPRI)	PQ842611
<i>M. cf. dealbata</i>	MA0008	<i>Macrophylla</i>	*South Korea, Chungcheongnam-do (Chollipo arboretum)	Kim 1008 (NPRI)	OR730743
<i>M. dealbata</i> Zucc.	MA3190	<i>Macrophylla</i>	Mexico, Oaxaca	Mata 807 (XAL)	OR730679
<i>M. fraseri</i> Walter	MA0291	<i>Tuliparia</i>	*South Korea, Chungcheongnam-do (Chollipo arboretum)	Kim 1111 (NPRI)	OR730746
<i>M. macrophylla</i> Michx.	MA0015	<i>Macrophylla</i>	*South Korea, Chungcheongnam-do (Chollipo arboretum)	Kim 1015 (NPRI)	PQ842612
<i>M. nuevoleonensis</i> A.Vázquez & Domínguez-Yescas	MA1138A	<i>Macrophylla</i>	Mexico, Nuevo León	Mata 1138 (XAL)	OR730704
<i>M. rzedowskiana</i> A.Vázquez, Domínguez-Yescas & R.Pedraza	MA1004A	<i>Macrophylla</i>	Mexico, Querétaro	Mata 1004 (XAL)	OR730716
<i>M. rzedowskiana</i>	MA3188B	<i>Macrophylla</i>	Mexico, San Luis Potosí	Mata 1118 (XAL)	OR730717
<i>M. vovidesii</i> A.Vázquez, Domínguez-Yescas & L.Carvajal	MA0092B	<i>Macrophylla</i>	Mexico, Veracruz	Chávez-Cortázar & Hernández-Sánchez 92 (XAL)	OR730729
<i>M. vovidesii</i>	MA0624B	<i>Macrophylla</i>	Mexico, Veracruz	Chávez-Cortázar & Hernández-Sánchez 624 (XAL)	OR730730
<i>M. vovidesii</i>	MA0877A	<i>Macrophylla</i>	Mexico, Veracruz	Mata 877 (XAL)	OR730680
<i>M. zoticitla</i> A.Sánchez-Gonz., Gut.-Lozano & A.Vázquez	MA0866A	<i>Macrophylla</i>	Mexico, Puebla	Mata 866 (XAL)	OM455410

### Morphological observations

Morphological analyses were conducted at population and morphospecies level to gain insights into the variations within the *M. dealbata* complex. A total of 168 vouchers (including the types) were consulted from 26 herbaria, both physically and through their digital collections: BR, BRIT, F, HUAP, IBUG, IEB, ITCV, K, LL, LSU, M, MA, MEXU, MO, NCU, NCSC, NY, P, PH, TEX, US, USCH, URV, VT, WAG, XAL (Herbarium names according to Thiers continuously updated). Field pictures from iNaturalist.org (whose taxonomic identity was verified) were also examined. The protologues of each taxon were also considered and revised (García-Morales *et al.* 2017; Sánchez-González *et al.* 2021; Vázquez-García *et al.* 2013, 2015, 2016, 2021; Zuccarini 1837).

All specimens and pictures underwent detailed morphological observations. From the visual evaluation of the phenotypic characteristics of the specimens, a list of 13 characters was compiled (table 2). The botanical terms of the character states were standardized following the proposal of Moreno (1984).

### Preliminary conservation status assessment

We conducted a preliminary assessment of the conservation status of the taxa based on the morphological observations and the molecular and phylogenetic results. Our methodology involved field surveys to collect data on species distribution, population size, and habitat conditions. We also reviewed historical records and consulted with local experts to gather additional insights. The criteria set forth by the International Union for Conservation of Nature (IUCN) Red List (IUCN 2013, 2021; IUCN Standards and Petitions Committee 2024) were applied to evaluate the risk of extinction faced. This included analysing factors such as geographic range, population trends, and threats to habitat integrity. The online tool GeoCAT (Bachman *et al.* 2011) was used to calculate the Extent of Occurrence (EOO) and the Area of Occupancy (AOO), using the data from vouchers and carefully curated GBIF samples.



**TABLE 2.** Traits for the *Magnolia* sect. *Macrophylla* taxa occurring in the Sierra Madre Oriental in Mexico.

Taxa	Indumentum of young branches	Indumentum of young petioles	Indumentum of stipule	Leaf shape	Leaf base shape	Leaf apex shape	Under surface veins indumentum	Peduncle indumentum	Bract indumentum	Indumentum of gynoecium	Fruit shape	Fruit indumentum	Beaked mature follicles
<i>Magnolia alejandrae</i> García-Mor. & Iamónico	Pulverulent	Glabrous	Pubescent	Auriculiform	Auriculate, Cordate	Acute	Pulverulent	Pulverulent	Pulverulent	Glabrous	Ovoid	Glabrous	Absent
<i>M. dealbata</i> Zucc.	Pulverulent	Pulverulent	Pubescent	Auriculiform	Auriculate, Cordate	Acute	Pulverulent	Pulverulent	Pulverulent	Glabrous	Ovoid	Glabrous	Absent
<i>M. nuevoleonensis</i> A. Vázquez & Domínguez-Yescas	Pulverulent	Glabrous	Glabrous	Auriculiform	Auriculate, Cordate	Acute	Pulverulent	Pulverulent	Pulverulent	Tomentose	Ovoid	Glabrous	Absent
<i>M. rzedowskiana</i> A. Vázquez, Domínguez-Yescas & R. Pedraza	Sericeous	Pulverulent	Pubescent	Auriculiform	Auriculate, Cordate	Acute	Pulverulent	Pulverulent	Pulverulent	Tomentose	Ovoid	Glabrous	Absent
<i>M. vovidesii</i> A. Vázquez, Domínguez-Yescas & L. Carvajal	Pulverulent	Glabrous	Pubescent	Auriculiform	Auriculate, Cordate	Acute	Pulverulent, Sericeous	Pulverulent	Pulverulent	Glabrous	Ovoid	Glabrous	Absent
<i>M. zotitclla</i> A. Sánchez-Gonz., Gut.-Lozano & A. Vázquez	Sericeous	Pulverulent, Sericeous	Pubescent	Auriculiform	Auriculate, Cordate	Acute	Pulverulent	Glabrous, Pulverulent	Pulverulent	Glabrous	Ovoid	Glabrous, Sericeous	Absent, Present

## DNA extraction and sequencing

A modified CTAB method (Larridon *et al.* 2015) was utilized for the DNA extraction process. The quality of the extracted DNA was then evaluated using a Nanodrop 2000 UV-Vis spectrophotometer. Subsequently, the samples were dispatched to RAPiD Genomics located in Gainesville, Florida, US, for sequencing. The sequencing was carried out using the Genome Skimming technique to compile complete chloroplast genomes.

## Plastome assembly and annotation

A first quality check of the demultiplexed samples was performed using the software FastQC v. 0.11.7 (Andrews 2019). Trimmomatic v. 0.38 (Bolger *et al.* 2014) was used to filter low-quality reads and perform the adapter trimming, applying a sliding window of 5:20 and removing all the reads shorter than 30 bases.

GetOrganelle v. 1.7.0 (Jin *et al.* 2020) was used to assemble the plastome; this is a complete Python software that uses Illumina reads to perform *de novo* plastome assemblies. This software makes use of Bowtie2 (Langmead & Salzberg 2012), SPAdes (Bankevich *et al.* 2012), BLAST (Camacho *et al.* 2009) and Biopython (Cock *et al.* 2009). The program “Get\_organelle\_from\_reads.py” was used with the “embplant\_pt” option, as well as 15 extension rounds and kmer values between 21 and 105. The results were visualized with Bandage v. 0.8.1 (Wick *et al.* 2015) to ensure that a correct assembly graph was produced.

The plastome annotation was performed using the online software GeSeq v. 1.55 (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>) (Tillich *et al.* 2017). Chloë v. 0.1.0 (<https://chloe.plantenergy.edu.au/unpublished>) was used as a support annotator, and ARAGORN v. 1.2.38 (Laslett & Canback 2004) and tRNAscan-SE v. 2.0.7 (Chan & Lowe 2019) were used as tRNA annotators, keeping the best annotation only. As a reference for the annotation, we utilized the base MPI-MP reference set, as well as the Magnoliaceae plastomes available at the NCBI RefSeq (O’Leary *et al.* 2016).

## Plastome comparative analysis

All assembled plastomes and their annotations were submitted to mVista (Frazer *et al.* 2004). We used the Shuffle-LAGAN mode (Brudno *et al.* 2003) to align the sequences and perform pairwise comparisons. Next, the plastomes were aligned using Mauve v. 2.4.0 (Darling *et al.* 2004), with the progressive alignment option, and the gene orders were compared.

DNAsp v. 6.12.03 (Librado & Rozas 2009) was used to calculate the nucleotide diversity among the aligned genomes using a sliding-window approach with a window length of 600 bp and a step size of 200 bp. All the complete annotated plastomes were submitted to IRscope (Amiryousefi *et al.* 2018) to analyse the expansion and contraction of the IR regions.

## Phylogenetic analyses

Three different plastid datasets were used independently to elucidate the *Magnolia dealbata* complex: 1) The complete plastomes of each sample; 2) The complete sequences of the following genes: *matK*, *rbcL*, *trnH*, *psbA* and *trnL-F*, which have been suggested as plastid barcodes to infer phylogenetic relationships in closely related taxa in angiosperms (Hu & Wang 2023; Li *et al.* 2015); and 3) The group-specific DNA barcodes selected from hypervariable regions in Neotropical *Magnolia* plastomes, *ccsA*, *ndhD*, *petL*, and *rpl32*, have been identified in previous studies (Guzmán-Díaz *et al.* 2022).

Each dataset was aligned using MAFFT with the default parameters. The MAFFT alignment was used to build species trees with maximum likelihood (ML) and Bayesian inference (BI). The ML approach was performed in IQ-Tree v. 2.0.3 (Minh *et al.* 2020) with an ultrafast bootstrap to estimate the branch support values. The software MrBayes v. 3.2.7 was used for the BI analysis, applying a GTR + I + gamma model with 10 million generations and a burn-in of 25%. *Magnolia fraseri* from the -2016633688 *Magnolia sect. Auriculata* was used to root the trees.

## Results

### Morphological observations

According to the list of 13 characters obtained from the observations of the specimens collected, the 168 herbarium samples and the photographs taken in the field (Table 2), it has been observed that the taxa of the *Magnolia dealbata* complex have a few traits that may allow them to be distinguished. These are mainly the indumentum on the veins of the abaxial surface of the leaves, as well as differences in the pubescence of the stipules, bracts, and fruits.

However, it is important to emphasize that while certain morphological characteristics were found to be relatively conserved, they are not sufficient for distinguishing between taxa, as these features varied among individuals from the same locality (e.g., abaxial leaf surface indumentum in *M. vovidesii*). Similarly, traits initially identified as unique to *M. zoticla* (e.g., sericeous indumentum on young petioles and fruits, glabrous indumentum on peduncles, and beaked mature follicles) were found to vary among individuals within its populations.

The observed lamina sizes were quite variable and did not show any pattern among the taxa, rather the maximum leaf lengths overlapped between the taxa. The same was true for the size of the floral parts. However, no length or width measurements nor counting of parts (number of carpels or stamens) have been carried out as most material was seen digitally due to the COVID-19 pandemic and because we wanted to test different characters based on shapes and textures. Nevertheless, we do not consider this to be a problem for our study since we are focusing on variations in organ shapes, which are proving to be more informative and of greater taxonomic importance, as has already been confirmed in species from the *Magnolia* sect. *Talauma* (Aldaba Núñez 2020).

The most stable or conserved traits among the species complex were those related to leaves shapes (all with acute apex, auriculate or cordate base and auriculiform lamina), bract indumentum (pulverulent) and fruit shape (ovoid); while the most variable traits were related to the indumentum of: young branches (from pulverulent to sericeous), young petioles (from glabrous to sericeous), stipules (glabrous or pubescent), gynoecium (glabrous or tomentose) and fruits (glabrous or sericeous).



*Magnolia alejandrae*  
García-Mor. & Iamónico



*Magnolia dealbata* Zucc.



*Magnolia nuevoleonensis*  
A. Vázquez & Domínguez-Yescas



*Magnolia rzedowskiana* A. Vázquez,  
Domínguez-Yescas & R. Pedraza



*Magnolia vovidesii* A. Vázquez,  
Domínguez-Yescas & L. Carvajal

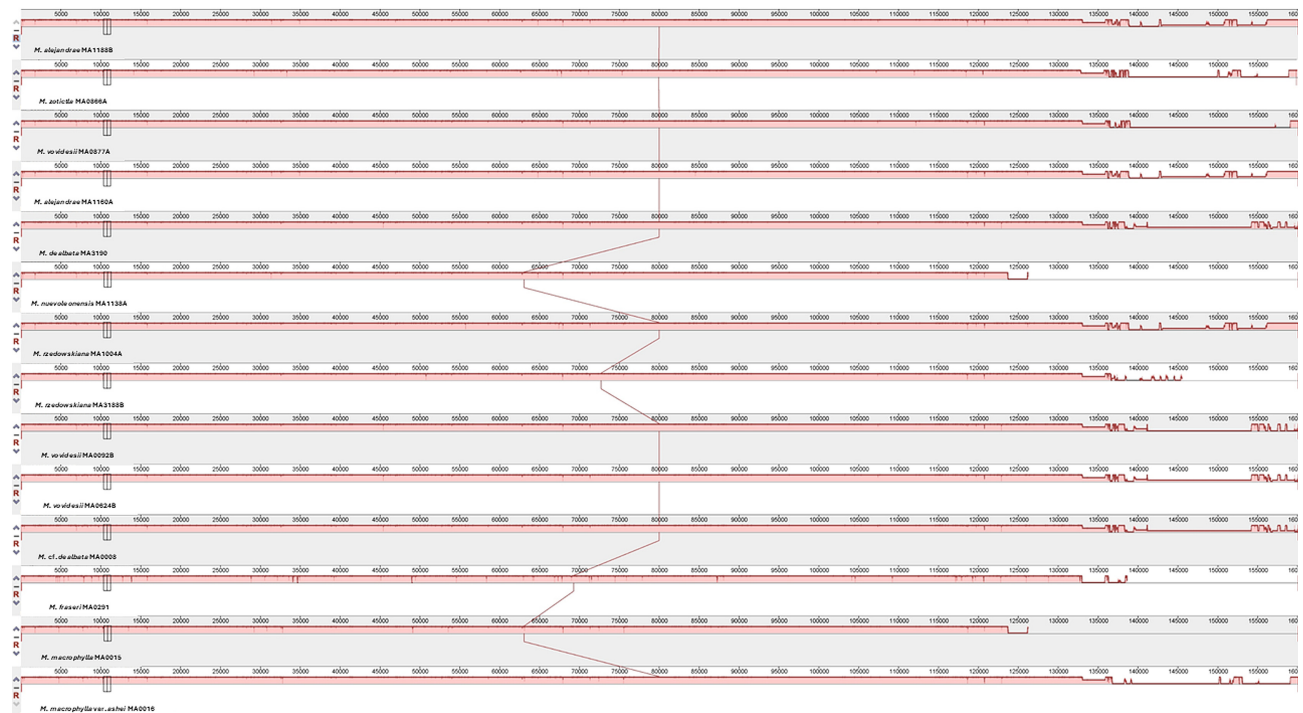


*Magnolia zoticla* A. Sanch. Gonz.,  
Gut.-Lozano & A. Vázquez

**FIGURE 1.** Taxa comprising the *Magnolia dealbata* Zucc. (Magnoliaceae) species complex in the Sierra Madre Oriental in eastern Mexico.

## Plastome features and comparative analysis

The *Magnolia* plastomes ranged in size length from 159 879 bp in *M. zotictla* (sample MA0866A) to 160 087 bp in *M. macrophylla* (sample MA0015) and exhibited a quadripartite structure, including a large single-copy region (LSC, ranged from 87 967 to 88 175 bp), a small single-copy region (SSC, ranged from 18 735 to 18 787 bp), and two inverted repeated regions (IRs, ranged from 26 585 to 26 597 bp). The total GC content was 39% for all studied samples. The Shuffle-LAGAN alignment in mVista resulted in the similarity plot shown in Supplementary file 1; overall, the *Magnolia* plastomes presented high similarity values across the whole genome, with small regions of lower similarity. The Mauve alignment results are presented in Figure 2. One colinear block was identified, while all regions of the 14 *Magnolia* plastomes were in the same order and orientation.



**FIGURE 2.** Mauve progressive alignment, including all 14 North American *Magnolia* taxa plastomes. Blocks of the same colour linked by a line represent colinear regions. Blocks below the graphs represent coding regions.

From the sliding window analysis performed in DNAsp, we obtained the nucleotide diversity values ( $P_i$ ) from all samples (Fig. 3). These ranged from 0 to 0.0095 and presented a mean of 0.0007. The most diverse sites corresponded to genes, such as *psbJ*, *petL*, *rpl22*—*rps18*, *ccsA*, *ndhD*, and *ycf1*; as well as intergenic regions in the LSC and SSC regions, while the IR regions presented the lowest diversity.

The plastomes of all 14 samples (corresponding to nine taxa) were compared to visualize the overall sequence divergence (Fig. 4). Comparing the IR/LSC and IR/SSC boundaries in the 14 plastomes uncovered stable IRs with little expansion or contraction. The LSC-IRb borders were found to be located within the *rps19* gene, and the SSC/IRa was located in the coding region of the *ycf1* gene. The IR regions of all taxa were well-conserved, ranging from 25 585 bp in *M. macrophylla* and *M. ashei* to 26 597 bp in *M. fraseri*. The IR regions of almost all Mexican taxa recovered 26 587 bp, except for the samples *M. vovidesii* MA0877A and *M. zotictla*, which recovered 26588 bp. The gene distribution at the four boundaries was the same in all plastomes.

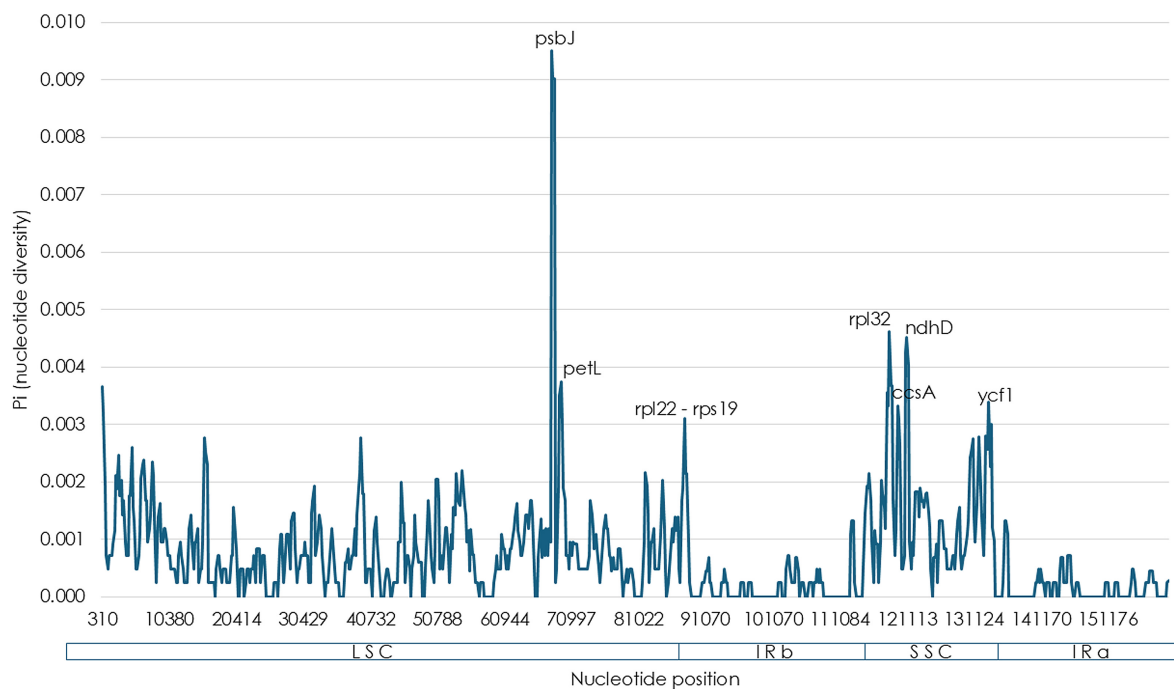
## Phylogenetic analyses

Overall, both the Bayesian Inference (BI) and Maximum-likelihood (ML) analyses produced fairly similar topologies in each of the three molecular datasets analysed (angiosperm DNA barcodes, *Magnolia* DNA barcodes and *Magnolia* plastomes).

In the BI phylogenetic hypothesis based on the complete chloroplasts, the samples of *Magnolia* sect. *Macrophylla* formed two differentiated clades (Fig. 5, Fig. S1). The first consisted of the two US taxa (posterior probability, PP=1),



while the second grouped all Mexican taxa (PP=1). Within the Mexican clade, *M. nuevoleonensis* was placed as the sister group to this Mexican clade, which was composed of *M. zotictla* and all the remaining taxa (PP=0.71), hereafter referred to as the core group (PP=1). Within this core group, the two samples of *M. alejandrae* formed a clade separated from the rest, maintaining high support values between them (PP=1). Within the core group, a three-branch polytomy was formed consisting of a sample of *M. vovidesii* (MA0877A), the remaining samples of this taxon together with those of *M. dealbata*, and the two samples of *M. rzedowskiana*, which formed their own clade but with low support values between them (PP=0.66); while the samples of *M. dealbata* and *M. vovidesii* formed a single clade with low support (PP=0.58) intersecting among them. The same pattern was maintained in the ML-based trees, with the only difference being that no polytomy was recorded in the core group, but all *M. dealbata* and *M. vovidesii* samples were grouped into a single clade (bootstrap support percentage, BP=47). The BP was also similar to the PP in all clades.



**FIGURE 3.** Nucleotide diversity values (Pi) resulting from the sliding window analysis of the 14 North America *Magnolia* plastomes.

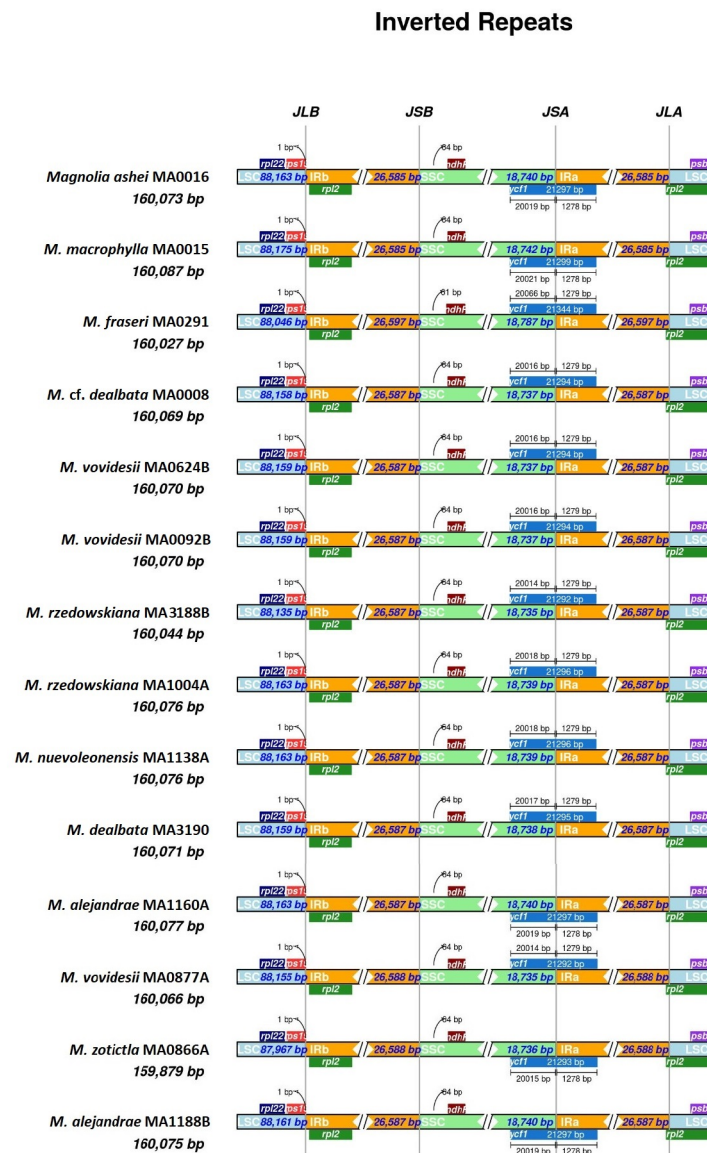
The BI phylogenetic hypothesis derived from the DNA barcodes also recovered the two main clades in the tree based on chloroplast data: the Mexican taxa on the one hand (PP=0.64) and the US taxa on the other (PP=1; Fig. 6, Fig. S2). However, in this case, there was no resolution, and all Mexican taxa were grouped into a large polytomy. In the ML tree, a sample of *M. vovidesii* (MA0877A) behaved as a sister to all other Mexican taxa, including the remaining samples of *M. vovidesii* (BP=63). Furthermore, the relationships between the *M. alejandrae* and *M. rzedowskiana* samples were no longer recovered, but the taxa appeared within a large polytomy.

Finally, the topologies obtained from the hypervariable regions were completely different from the two previously described (Fig. 7, Fig. S3). In the BI tree, two clades were also recovered: on the one hand, taxa from the US (PP=1) and the northern SMO ( *M. alejandrae* and *M. nuevoleonensis*, the latter as sister to the others) and, on the other hand, the remaining taxa from the central and southern SMO (PP=0.98), both clades forming polytomies. In the ML tree, this north-south pattern was maintained, the only difference being that in the northern clade, *M. nuevoleonensis* was the sister of all other taxa, while in the southern clade, the sister group was *M. zotictla* (BP=63).

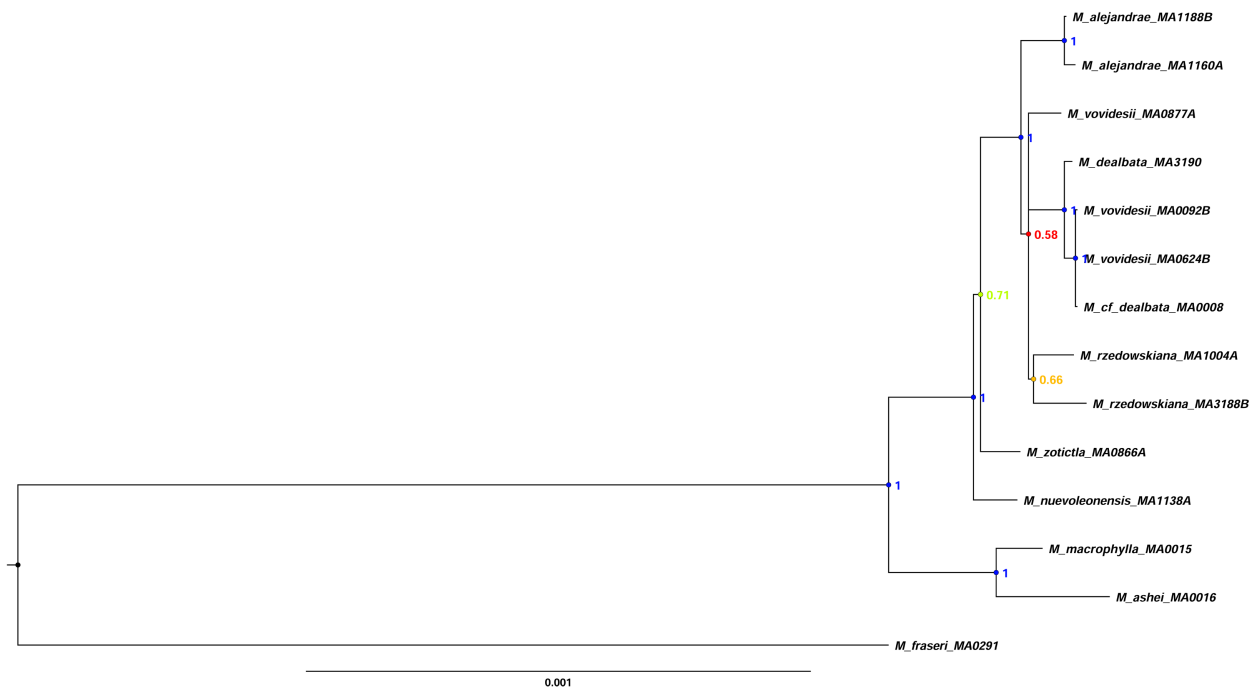
## Taxonomic treatment

*Magnolia dealbata* Zucc. Abh. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. 2: 373. 1837.  $\equiv$  *Magnolia macrophylla* var. *dealbata* (Zucc.) D.L.Johnson, Baileya 23: 56. 1989. Type:—MEXICO. Oaxaca, prope Rincou [Rincon, Villa Alta], 600–900 m, *Karwinski s.n.* [epitype, designated by García-Morales *et al.* (2017: 242): M239898!; lectotype, designated by García-Morales *et al.* (2017: 242): [icon]: “*Magnolia dealbata*” in Zuccarini, Abh. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. 2. Icon. 373: t. 3. 1836 ].

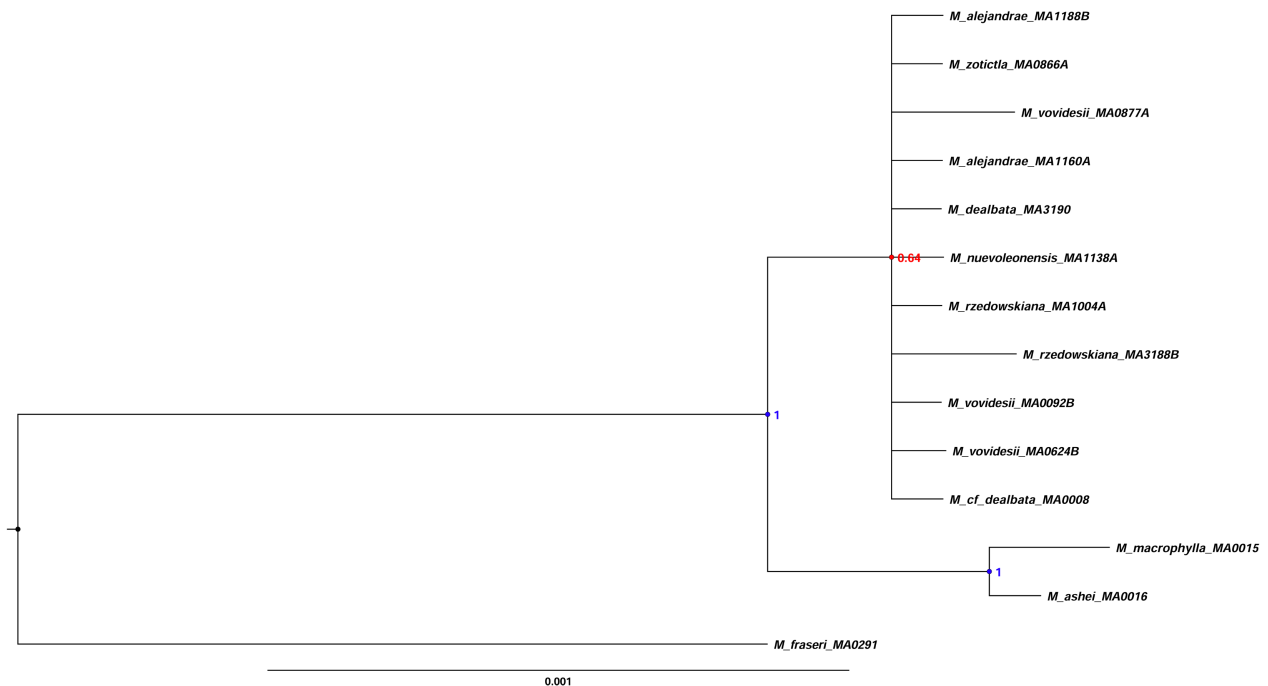
- = *Magnolia alejandrae* García-Mor. & Iamónico, Phytotaxa 309(3): 239. 2017. Type:—MEXICO. Tamaulipas: Municipio de Victoria, Rancho El Molino, bosque mesófilo de montaña, 23°45'56.02"N 99°19'31.27"W, 1500 m, 1 May 2016, *García-Morales 5435* (holotype: ITCV!; isotypes: GBH, HFLA, HUAP!, ITCV!, MEXU!, SLP, UAT).
- = *Magnolia nuevoleonensis* A. Vázquez & Domínguez-Yescas, Nordic J. Bot. 34: 49. 2015. Type:—MEXICO. Nuevo León: Municipio de Montemorelos, Ejido La Trinidad, 25°11'45"N 100°06'59"W, 1600 m, 21 Mar 2006, *C. G. Velasco-Macias s. n.* (holotype: UNL; isotype: IBUG).
- = *Magnolia rzedowskiana* A. Vázquez, Domínguez-Yescas & R. Pedraza, Acta Bot. Mex. 112: 23. 2015. Type:—MEXICO. Querétaro, municipio de Landa de Matamoros, Sierra Gorda, laderas calizas con bosque mesófilo de montaña, 17 Aug de 1996, *S. Zamudio & E. Pérez-Calix 9921* (holotype: IEB!; isotype: IEB!, MEXU).



**FIGURE 4.** Comparison and visualization of the inverted repeat (IR), small single copy (SSC) and large single copy (LSC) sequences boundary positions in the 14 North America *Magnolia* plastomes.

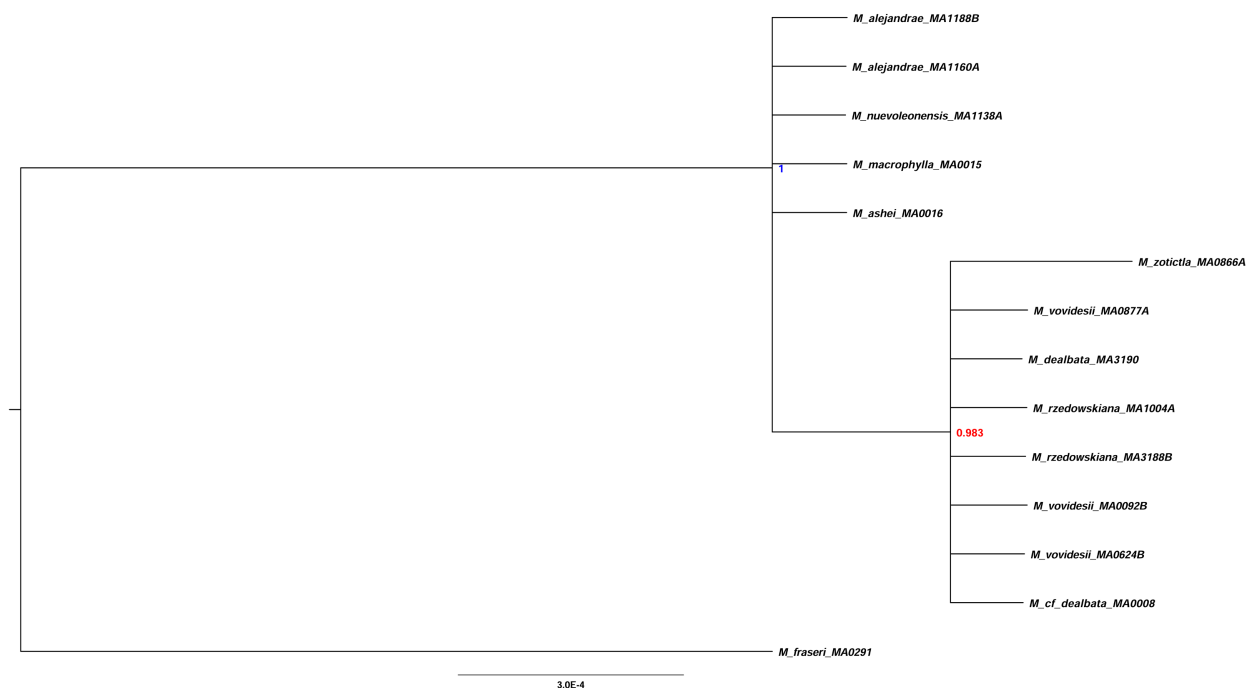


**FIGURE 5.** Phylogenetic relationships of 14 North America *Magnolia* accessions inferred from complete plastomes generated by Bayesian inference approach; values on nodes represent posterior probabilities (PP).



**FIGURE 6.** Phylogenetic relationships of 14 North America *Magnolia* accessions inferred from plastid DNA barcodes (*matK*, *rbcL*, *trnH*, *psbA* and *trnL-F*) generated by Bayesian inference approach; values on nodes represent posterior probabilities (PP).

- = *Magnolia vovidesii* A.Vázquez, Domínguez-Yescas & L.Carvajal, Recursos Forest. Occid. México 4: 478. 2013. Type:—MEXICO. Veracruz. Ixhuacán de los Reyes municipality: 5 km de Coyopolan camino a Ixhuacán, Jul 1988, *Vázquez-García 4644* (holotype: IBUG!; isotypes: F!, MO, WIS).
- = *Magnolia zotictla* A.Sánchez-Gonz., Gut.-Lozano & A.Vázquez Phytotaxa 513: 272. 2021. Type:—MEXICO. Hidalgo: Acaxochitlán municipality, Zotictla, 0.3 km al SE de San Miguel del Resgate, bosque mesófilo de montaña, 1743 m, 20°13'32.5"N 98°09'48"W, 5 May 2021. *Gutiérrez-Lozano et al. 10186* (holotype: HGOM; isotypes: ENCB, IBUG, OAX).



**FIGURE 7.** Phylogenetic relationships of 14 North America *Magnolia* accessions inferred from a group-specific DNA barcode combination selected from hypervariable regions in Neotropical *Magnolia* plastomes (*ccsA*, *ndhD*, *petL*, and *rpl32*) generated by Bayesian inference approach; values on nodes represent posterior probabilities (PP).

**Description:**—Trees, 4–35 m high, deciduous. **Branches** short in pyramidal arrangement. **Bark** smooth, light brown with white and dark brown spots. **Young branches** light green to dark brown, with whitish spots, puberulent with whitish or yellowish trichomes. **Leaves:** simple, up to 50 cm long and 30 cm wide, adaxially dark green, glabrous, abaxially light green to whitish, veins sericeous, base cordate to auriculate, apex acute, margin entire, petioles cylindrical, sericeous. **Stipules** deciduous, adnate to the petiole covering up to 60% of it, lanceolate, tomentose. **Pedicels** cylindrical, pulverulent to tomentose. **Bracts** deciduous, ovate, pulverulent at the base. **Flowers** terminal, solitary, sepals 3, whitish green, lanceolate, up to 20 cm long and 5 cm wide, adaxially glabrous, abaxially pulverulent, base truncate, apex acute; petals 6, white, the youngest with an orange or purple spot at base adaxially, elliptic, lanceolate or ovate, glabrous, coriaceous, up to 25 cm long and 10 cm wide, base obtuse, apex acute. **Stamens** spirally inserted, laminar, cymbiform, yellowish, base truncate, apex obtuse, thecae 2, introrse, longitudinal dehiscence. **Gynoecium** ovoid, yellowish-white, recurved styles. **Peduncles** cylindrical, pulverulent. **Fruits** polyfollicles, ovoid or botuliform, glabrous, rarely puberulent with yellowish trichomes, 4–15 cm long and up to 10 cm wide, brown, green, orange or reddish when young, longitudinal dehiscence, follicles beaked. **Seeds** 1–2 per follicle, obloid to ellipsoid, with funicle, sarcotesta red.

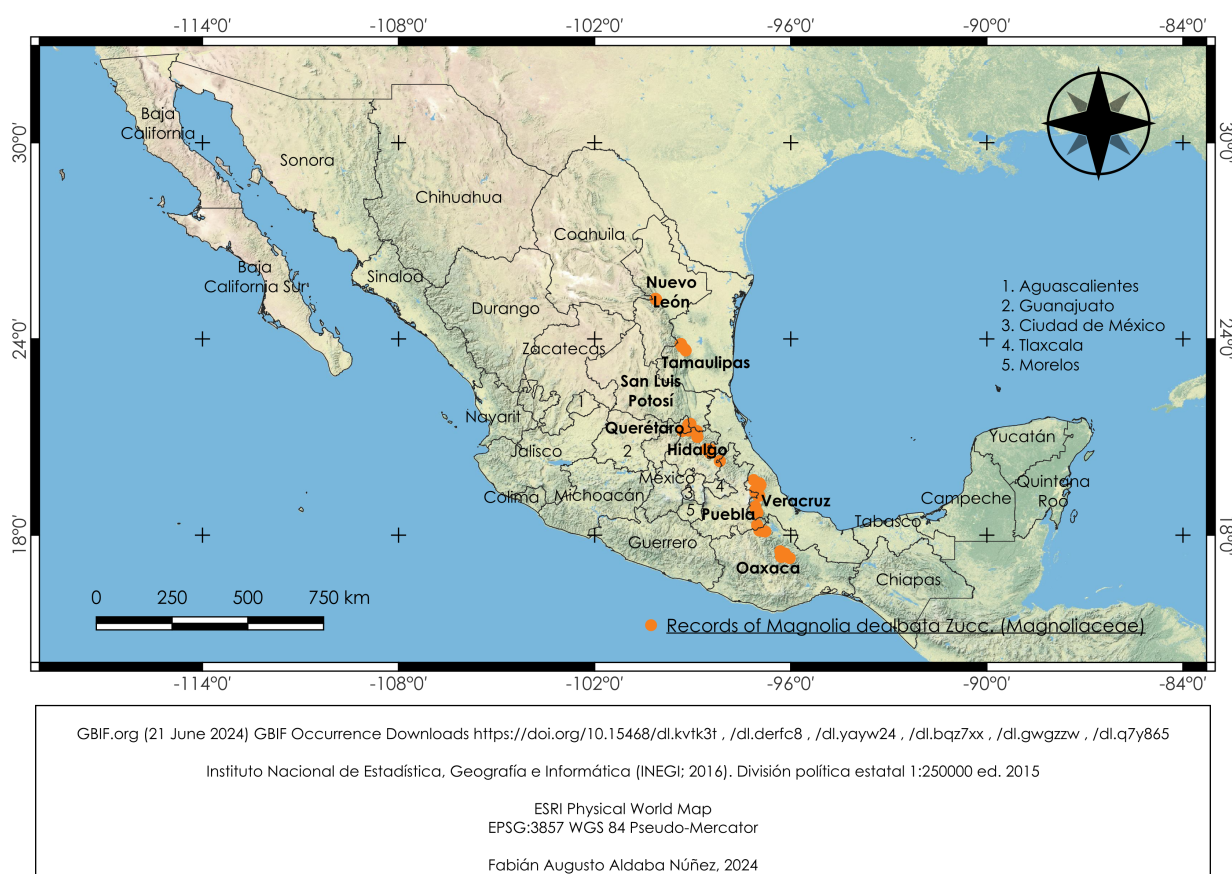
**Distribution and habitat:**—*Magnolia dealbata* is endemic to eastern Mexico, where it is distributed along the Sierra Madre Oriental mountain range in the states (from north to south) of Nuevo León, Tamaulipas, San Luis Potosí, Querétaro, Hidalgo, Veracruz, Puebla, and Oaxaca (Fig. 8). It grows in temperate forests: cloud forest, pine forest and pine-oak forest at elevations of 1000–2300 m.

**Common names:**—Chirimoya, elosúchil, eloxóchitl, magnolia, ya-nacho yote, yagsa, yolosóchil.

**Phenology:**—Shedding leaves from October to March, flowering from April to June, and fruiting from June to September.

**Preliminary conservation status:**—Although new populations have recently been discovered along the Sierra Madre Oriental, they consist of only a few individuals. The Extent of Occurrence (EOO) is 77 874.487 km<sup>2</sup> and the Area of Occupancy (AOO) is 268 km<sup>2</sup>. Hence, following the IUCN criteria (IUCN 2013; IUCN Standards and Petitions Committee 2024), the proposed assessment category under criterion B is Least Concern (LC). The primary threats to the species are habitat fragmentation, deforestation and climate change, the most threatened populations are located in the central part of the SMOr (Hidalgo, Puebla and Veracruz).





**FIGURE 8.** Map of the known geographic distribution of *Magnolia dealbata* Zucc. (Magnoliaceae).

**Specimens examined:—MEXICO. HIDALGO STATE: Tepehuacán de Guerrero municipality:** Chilijapa, 21°0'19.44"N 98°51'41.23"W, 1400 m, 29 Aug. 2015, *A. Chávez Cortazar 1015* (XAL); *A. Chávez Cortazar 1013* (XAL). **Tlanchinol municipality:** 3 km al NNE de Chapulhuacán, 1400m, May 1960, *F. Sanchez s.n.* (MEXU), **Zacualtipán municipality:** Vereda entre El Reparo y Zacualtipán, 3 km de la Mojonera, 29 Jun. 1988, *A. Vázquez 4631*, *M. Cházaro & M. Rosales* (MEXU). **NUEVO LEÓN STATE: Montemorelos municipality:** Ejido La Trinidad, 25°12'24.28"N 100°7'13.39"W, 1526 m, 5 May 2015, *A. Chávez Cortazar 990* (XAL). **OAXACA STATE: Huautla de Jiménez municipality:** La Providencia, 1500, 19 Apr. 1975, *Rzedowski 32842* (MEXU). **Ixtlán de Juárez municipality:** Tiltepec, 17°30'48"N 96°19'29"W, 1380 m, 25 Apr. 1998, *J. García R. 226* (MEXU); camino a las pueblas de San Juan Yaeé and Tanetze, 17°21'52"N 96°15'37"W, 1841 m, 22 Mar. 2016, *M. Sundue et al. 4065* (BRIT, MEXU, VT). **San Juan Juquila Vijanos municipality:** 5 Km al NE de la desviación a Juquila Vijanos, hacia Talea, 17°21'28"N 96°16'19"W, 1650 m, 11 Jul. 1996, *R. Aguilar Santelises 620* (IEB, XAL); La Cumbre, 17°21'38.39"N 96°16'27.45"W, 1924 m, 15 Apr. 2015, *A. Chávez Cortazar 715* (XAL). **San Juan Yaeé municipality:** San Juan Yaeé, 17°24'28.53"N 96°16'50.43"W, 1715 m, 16 Apr. 2015, *A. Chávez Cortazar 768*. **Santa María Teopoxco municipality:** Los Duraznos, 18°8'41.83" N 96°57'44.31" W, 2187 m, 13 Apr. 2015, *A. Chávez Cortazar 922* (XAL); 39 km de Teotitlan de Flores Magón, por la carretera a Huautla de Jiménez (1-2 km antes de llegar al Plan de Guadalupe), 16 Apr. 2002, *X. Munn-Estrada 2231* y *F. Mendoza* (MEXU, XAL). **Santiago Camotlán municipality:** 500 m al este de Santiago Camotlán, rumbo a Yadoó 17°26'33"N 96°10'42"W, 1500 m, 14 Mar. 2013, *M.L. Pérez-Nicolás 109*, *J. Galicia & O. Quishehualt* (IBUG). **Talea de Castro municipality:** El Faisán, 17°23'8.62"N 96°15'54.33"W, 1895 m, 16 Apr. 2015, *A. Chávez Cortazar 674* (XAL); Distr. Villa Alta, Talea de Castro a 1.5 km al N, 25 Mar. 2016, *R. Torres Colin 18088*, *A. Vasco y E. Vasquez Pérez* (MEXU). **Teotitlán del Camino municipality:** Loma Chapultepec, a 4 km. al S de Huautla de Jiménez, 1750 m, 29 Apr. 1978, *M. Sousa S. et al. 9357* (MEXU). **Totontepec Villa De Morelos municipality:** Tepitongo, 17°18'00"N 96°02'00"W, 1700 m, 10 Mar. 1990, *E. Velasco López 406* (MEXU); Totontepec, 17°15'N 96°00'W, 1900 m, 9 Mar. 1986, *J. Rivera Reyes 0191 & G.J. Martin* (MEXU). **Yetzelalag municipality:** 1300 m, 5 Mar. 1919, *B.P. Riko 4139* (US). **Without municipality data:** Rincon (al NE de Totontepec, probablemente San Ildefonso Villa Alta): 1833, *Karwinski* nd (BR 31083553); Sierra de Ixtlán:

21 Apr. 1906, *C. Conzatti* 1370 (US); 1846, *H. Galeotti* 4588 (BR 31083577, BR 31083581, BR 31083607, P 1963173, P 1963172); *H. Galeotti* 4991 (P 1963175); *Karwinski s.n.* (M 239896, M 239898, M 239897, M 239899). PUEBLA STATE: **Pahuatlán municipality**: Ahila, 20°15'52.83" N, 98°10'24.28" W, 1785 m, 6 Apr. 2015, *A. Chávez Cortazar* 857 (XAL). **Zoquitlán municipality**: San Francisco Xitlana, 18°18'32.99" N 97°1'59.4" W, 2274 m, 6 Apr 2015, *M. Castañeda-Zárate MCZ-1042* (MEXU). QUERÉTARO STATE: **Landa de Matamoros municipality**: Joya del Cedro, 21°14'31.42" N, 99°9'55.58" W, 1167 m, 31 May 2015, *A. Chávez Cortazar* 997 (XAL); Joya del Hielo, 17 km al N de Acatitlán de Zaragoza, 11 Apr. 1989, *S. Zamudio & E. Carranza* 7197 (MEXU); Joya del Hielo, 21°12'18" N 99°11'24" W, 1800 m, 17 Aug. 1996, *S. Zamudio y E. Pérez C.* 9921 (MEXU, XAL); La Lima, 3 km al Noroeste de La Florida, 21°13'38" N 99°66'12" W, 1680 m, 5 May 1989, *E. González* 540 (MEXU, XAL); Sótano Colorado, 21°14'26.68" N 99°9'22.44" W, 1673 m, 4 Oct. 2015, *A. Chávez Cortazar* 976 (XAL). SAN LUIS POTOSÍ STATE: **Xilitla municipality**: José Coronel Castillo, 21°24'21.06" N 99°4'24.89" W, 1958 m, 4 May 2016, *A. Chávez Cortazar* 1211 (XAL); *A. Chávez Cortazar* 1212 (XAL); *A. Chávez Cortazar* 1132 (XAL); *A. Chávez Cortazar* 1209 (XAL). TAMAULIPAS STATE: **Güémez municipality**: 1 km al Noreste de Los San Pedro, 1430 m, 8 Jun. 1990, *F. González Medrano* 17562, *V. Juárez & P. Tenorio* (MEXU). **Victoria municipality**: Rancho El Molino, 1500 m, 1 May 2016, *Leccinum J. Garcia-Morales* 5435 (ICTV, MEXU). VERACRUZ STATE: **Coatepec municipality**: Plan de La Cruz, carretera antigua Xalapa-Coatepec, 19°30'16" N 96°56'42" W, 1358 m, 10 Oct. 2018, *F.G. Lorea H. & L.R. Tlaxcalteco T.* 6754 (XAL); Puerto Rico, 19°30'16" N 96°56'42" W, 1374 m, 14 May 2018, *F.G. Lorea H. & L.R. Tlaxcalteco T.* 6706-B (XAL); Plan de la Cruz, 19°30'16" N 96°56'42" W, 1374 m, 14 May. 2018, *F.G. Lorea H. et al.* 6706 (XAL). **Huayacocotla municipality**: Helechales, 20°37' N 98°26' W, 1800 m, 5 May 1980, *F. Ramírez R. & J. Palma G.* 1003 (XAL); Helechales, 12 Aug. 1980, *R. Ortega O.* 1489 (XAL); El Salto por Agua de la Calabaza, 26 Apr. 1981, *L. Ballesteros & F. Ballesteros* 438 (MEXU, XAL); Agua de la Calabaza, 1820 m, 17 May 1980, *L. Ballesteros & H. Morales* 214 (XAL); Agua de la Calabaza, 8 Apr. 1980, *L. Ballesteros & J. I. Calzada* 141 (XAL); Agua de la Calabaza, 23 Mar. 1989, *A. Vázquez* 4889 (TEX); Agua de la Calabaza, 1820 m, 4 Jun. 1980, *L. Ballesteros & H. Morales* 260 (MEXU, XAL). **Ixhuacan de Los Reyes municipality**: Ejido Coyopola, 2 Jun. 1986, *L. Gutiérrez Carvajal* 13 (XAL); Ejido Coyopola, 2 Jun. 1986, *L. Gutiérrez Carvajal* 2 (XAL); 5 km delante de Coyopola en ruta de a pie a Ixhuacán, 1650 m, 28 Aug. 1985, *M. Chazaro y P. Padilla* 293725 (MEXU, XAL); Coyopolan, 19°22'0.33" N 97°4'4.40" W, 1567 m, 11 Apr. 2015, *A. Chávez Cortazar* 473 (XAL); Coyopola, 19°23' N 97°2' W, 1450 m, 6 Jun. 1984, *R. Ortega O & G. Pattison* 2638 (XAL); Coyopolan, 19°22'0.33" N, 97°4'4.40" W, 1567 m, 11 Apr. 2015, *A. Chávez Cortazar* 992 (XAL); Coyopolan, 19°21'58" N, 97°3'11" W, 1600 m, 18 Jul. 2016, *J.A. Guerrero Analco* 3 (XAL); Ejido Coyopola, 1500 m, 2 Jun. 1986, *L. Gutiérrez Carvajal* 12 (XAL); Coyopolan, 19°22'0.33" N 97°4'4.40" W, 1567 m, 11 Apr. 2015, *A. Chávez Cortazar* 994 (XAL); 5 km de Coyopola camino a Ixhuacan, Jul. 1988, *A. Vázquez, M. Cházaro & M. Rosales* 4644 (F, IBUG, MEXU); en una cañadita que está entre Coyopola y Tlalchi, 1700 m, 9 Jul. 1984, *M. Chazaro B. s.n.* (MEXU); brecha a Coyopola, entrando por la carretera Ixhuacán-Coatepec, 19°21'5.9" N 97°3'30.8" W, 1585 m, 3 Sep. 2009, *A. Campos V.* 6469, *S.M. Guzmán T. & A. Troyo B.* (MEXU); Coyopolan, 19°21'59" N, 97°4'5" W, 1570 m, 17 Oct. 2022, *D.A. Infante* 13 (XAL); Coyopolan, 19°22'0.33" N, 97°4'4.40" W, 1567 m, 11 Apr 2015, *A. Chávez Cortazar* 479 (XAL). **Los Reyes municipality**: Colonia Bugambilia-Congocotepec (terrenos particulares), carretera Orizaba-Zongolica (km 32), 18°41'20" N, 97°1'18" W, 1700 m, 9 Apr. 2000, *A. Rincón G.* 1413 & *C. Durán E.* (MEXU, XAL); Cuacaballo, 18°41'2.77" N, 97°1'59.82" W, 1736 m, 9 Apr. 2015, *A. Chávez Cortazar* 874 (XAL). **Tequila municipality**: Moxala, 18°42'55.42" N, 97°2'15.40" W, 1725 m, 10 Apr. 2015, *A. Chávez Cortazar* 881 (XAL). **Tlatetela municipality**: Axocuapan, 19°12'18.55" N 96°59'34.56" W, 1501 m, 12 Apr. 2015, *A. Chávez Cortazar* 915 (XAL). **Xalapa municipality**: 22 May 1899, *J.N. Rose* 4316 & *W. Hough* (US); Along rt. 140, on the way from Los Pinos to Xalapa, ca. 10 km W of Xalapa, 19°35'28.106" N 96°57'27.2" W, 1524 m, *J. Wen* 17812 (US). **Without municipality data**: 23 Mar. 1989, *A. Vázquez* 4889 (TEX). **Without state/locality data**: 1975, *Paulsen s.n.* (L 1741325).

**Notes and taxonomic discussion.**—*Magnolia alejandrae*, *M. nuevoleonensis*, *M. rzedowskiana*, *M. vovidesii*, and *M. zotictla* are synonymized here under *Magnolia dealbata* Zucc., following the principle of priority according to the ICN-International Code of Nomenclature (Turland *et al.* 2018). The different molecular analyses performed showed that the different taxa form a single entity; no differences were observed in the chloroplast comparisons of the taxa, and the different phylogenetic analyses of both plastomes and angiosperm plastid DNA barcodes (Figures 5, 6) showed high levels of support that the taxa form a single entity, and morphospecies are not disjunct in the branches of the phylogenetic trees but intermingle with each other. Furthermore, the main morphological characters used to delimit them have no taxonomic weight: the number of carpels is light-dependent and the absence/presence and colour of the blotch at the base of the petals represents normal development of *Magnolia* flowers and based on extensive field sampling of several populations no differences in this colouring are observed; see below for further discussion. Traits

initially identified as unique to some taxa (*M. vovidesii*, *M. zotictla*) are better interpreted as polymorphic rather than diagnostic autapomorphies. This variability supports the hypothesis that they do not represent a distinct taxon, but rather fall within the variation observed in *M. dealbata* s.l.

*Magnolia dealbata*, as a broadly circumscribed species is also supported by geological and climate data. Its distribution area is nearly entirely restricted to the morphotectonic province of the Sierra Madre Oriental, with a small portion in the province of the Sierra Madre del Sur *sensu* Ferrusquía-Villafranca (1993), both areas being separated by a small area of the Trans-Mexican Volcanic Belt. However, until the 1960s its distribution area in southeastern Puebla and adjacent regions of Veracruz and Oaxaca was even considered to be part of the Sierra Madre Oriental (De Cserna 1961, 1989; Murray 1961). The Sierra Madre Oriental is a nearly continuous system of folded mountain ranges (De Cserna 1989; Ferrusquía-Villafranca, 1993). Moreover, the species is restricted to the temperate humid and subhumid climate areas following García (2004), with the northernmost localities of its occurrence area restricted to humid ravines. Thus, the geological substrate is not a limiting factor for the species but rather characterizes their distribution according to a particular climate.

There remains the possibility that *M. dealbata* is a variety of *M. macrophylla*, as suggested by the specific DNA barcodes of hypervariable regions. To confirm this hypothesis, it is proposed to include more samples from different populations of *M. ashei* and *M. macrophylla* and compare them phylogenetically with *M. dealbata* and to make morphological observations.

## Discussion

### *Magnolia* sect. *Macrophylla* plastome features and variations

Variations of plastomes between different taxa of the *Magnolia* sect. *Macrophylla* were minimal (Figures 2, 4), underscoring the overall conservation of the *Magnolia* sect. *Macrophylla* and *Magnolia* plastome (Guzmán-Díaz *et al.* 2022; Palmer 1985). Moreover, there were important and noticeable differences between the taxa from the *Magnolia* sect. *Macrophylla* and *M. fraseri* (*Magnolia* sect. *Auriculata*), confirming, in a molecular way, that they are indeed completely distinct lineages and clades.

In particular, among the taxa of the *Magnolia dealbata* complex, the two samples that differed most from the rest were *M. vovidesii* MA0877A and the one corresponding to *M. zotictla*. For the former, the variations recorded in comparison with the other two samples of *M. vovidesii* could be explained by the fact that this sample comes from a different population than the rest of *M. vovidesii*, or that it inhabits a different ecosystem, so these variations are a response to the habitat or simply related to the individual itself. However, variations in plastomes can be the result of many other causes, including random mutations or genetic drift (Cao *et al.* 2024; Chen *et al.* 2021; Dong *et al.* 2023; Du *et al.* 2022).

### Phylogenetic relationships within *Magnolia dealbata* complex

Phylogenetic hypotheses derived from plastomes and angiosperm plastid DNA barcodes suggest that all taxa in the *Magnolia dealbata* complex belong to a single entity (Figures 5, 6). This refutes the initial hypothesis that the different taxa of the complex would be grouped into two entities in the SMOr (one in the north and one in the south). However, the topology recovered from the *Magnolia*-specific plastid DNA barcodes from hypervariable regions suggests a north-south geographical association (Figure 7). In similar studies, phylogenetic trees based on chloroplast or plastome data have also resolved relationships more satisfactorily than other sources of molecular evidence (Hu *et al.* 2023; Li *et al.* 2022).

In most angiosperms, phylogenetic inconsistencies are commonly recorded between different genes or genomes as well as among phylogenetic and phylogenomic data (Duan *et al.* 2023; Jiao *et al.* 2023; Oliver 2013; Wanke & Wicke 2023; Zhou *et al.* 2023; Zuntini *et al.* 2024), especially between plastome and other molecular datasets (Giaretta *et al.* 2022; Hu *et al.* 2016, 2023; Rokas & Chatzimanolis 2008; Su *et al.* 2021; Villar *et al.* 2019). There are several factors (mainly biological and methodological) that cause conflicts in phylogenies; these include chloroplast capture, incomplete lineage sorting, hybridisation, introgression in species undergoing rapid radiation and convergent molecular evolution, sampling error, rate signal, model selection, heterotaxy, and low genetic variability (Cai *et al.* 2021; Doyle 2022; Hu *et al.* 2023; Steenwyk *et al.* 2023; Zhang *et al.* 2020). The latter has been widely reported for



the genus *Magnolia* (Aldaba Núñez *et al.* 2021; Budd *et al.* 2015; Chávez-Cortázar *et al.* 2021; Guzmán-Díaz *et al.* 2022; Hernández *et al.* 2020; Rico & Becerril 2019; Veltjen *et al.* 2019).

DNA barcodes have been widely used to delimit species complexes and/or problematic angiosperm groups, with varying results. In some cases, sequences are useful in clarifying the relationships of certain groups (Binh *et al.* 2018; Gu *et al.* 2011; Hu *et al.* 2023; Su *et al.* 2021; Zhou *et al.* 2023), while in others they are of little or no use (Ding *et al.* 2022; Du *et al.* 2011; Starr *et al.* 2009; Terrones *et al.* 2022; Zhang *et al.* 2012). Rather, the current trend is to use multiple sources of molecular evidence, taking advantage of the fact that next-generation sequencing techniques make it possible to work with much larger amounts of data at an increasingly lower cost.

## Unravelling reproductive organ variations

Phenotypic variations in the reproductive organs of the taxa comprising the *Magnolia dealbata* species complex used to distinguish species, are either variations in flowers or in fruits. The former comprises the number of stamens, petal sizes, and the presence or absence and colour of a purple or yellow spot at the base of the petals, while fruit variations focus on size, the number of carpels, and whether the latter have a beaked apex (García-Morales *et al.* 2017; Gutiérrez-Lozano *et al.* 2020; Rodríguez-Ramírez *et al.* 2021; Sánchez-González *et al.* 2021; Vázquez-García *et al.* 2012b, 2013, 2015, 2016). Each of these characteristics will be discussed below.

## Decoding the colour spectrum: a look at flower variations

The presence and colour of the spot at the base of the petals (either purple or yellowish) have been used as characters of taxonomic importance to delimit the recently described Mexican taxa of *Magnolia* sect. *Macrophylla* (García-Morales *et al.* 2017; Sánchez-González *et al.* 2021; Vázquez-García *et al.* 2013, 2015, 2016, 2021). However, in the field, it has been observed that this characteristic is more of a facultative type and may vary. Individuals in bloom have been recorded with flowers that may or may not have the blotch, i.e., they have flowers with the blotch, others with a less marked, barely perceptible blotch, and even other flowers that are completely white, all occurring on the same tree (pers. obs.).

In addition, a similar phenological phenomenon has been observed in the US taxa of the section: *M. ashei* and *M. macrophylla* (Chafin 2000; Gilman & Watson 1994; Meyer 1993; Pattison 1985), as well as in other *Magnolia* taxa from *Magnolia* sect. *Talauma* (Aldaba Núñez 2020), which are also distributed in the SMO (specifically *M. mexicana* DC.). On the one hand, both *M. ashei* and *M. macrophylla* are described with only the innermost petals as purple, while the outer petals are greenish, but it has been observed that the outermost petals also exhibit the purple stain and are the last to lose it so that in the most mature flowers both the inner and outer petals are completely white (pers. obs.). There are also literature sources reporting entire populations of *M. macrophylla* in Alabama and Mississippi with all-white flowers (Treseder & Blamey 1981). On the other hand, in *M. mexicana*, the youngest flowers have a completely purple colouring, which fades as they open and continue to mature so that the purple hue is reduced until it becomes a spot at the base of the petals that finally disappears completely so that the most mature flowers are white and devoid of the purple spot (pers. obs.).

Based on the above, it could be deduced that the presence of an orange stain instead of a purple blotch in the taxa of the *Magnolia* sect. *Macrophylla* is more likely to be a consequence of such a fading of the colouring from purple to white; this pattern has also been seen in other angiosperms (Bar-Akiva *et al.* 2010; Rezende *et al.* 2020; Vaknin *et al.* 2005). Furthermore, no ecological correlation (such as differences in vegetation types, soil, or climate) or geographical correlation (e.g. distribution in altitudinal ranges or north-south longitude) has been found that could explain the presence of such a patch. That is, one would expect that taxa from the North would present such a spot, while those from the South would not or vice versa (and perhaps those from the centre would be the ones to present the orange colouration, thus exhibiting a spectrum of gradation in petal shades). However, this is not the case, it is present in an interspersed manner in the taxa regardless of their distribution. The same is true when the altitudinal range of the taxa is taken into account, which does not influence the presence or absence of the spot at the base of the petals.

However, to understand this phenomenon in depth, phenological and phytochemical studies are needed to elucidate the development and function of these purple shades and their changes during the process of flower maturation. Colour change in angiosperm flowers can be due to various factors, such as soil pH, senescence, sun exposure, or even in response to pollinators (Casper & La Pine 1984; Cruzan *et al.* 1988; Del Valle *et al.* 2019; Delph & Lively 1989; Gori 1989; Ida & Kudo 2003; Jones & Cruzan 1999; Kudo *et al.* 2007; Li *et al.* 2019; Luo *et al.* 2017; Martínez-Harms *et al.* 2022; Narbona *et al.* 2021; Oberrath & Böhning-Gaese 1999; Odell *et al.* 1999; Ram & Mathur 1984; Ruxton &



Schaefer 2016; Teppabut *et al.* 2018; Weiss 1991; Weiss & Lamont 1997; Zhang *et al.* 2023). In the particular case of magnolias, the colour change may be associated more with senescence, as has been documented in other families (Brito *et al.* 2015; Teppabut *et al.* 2018; Weiss 1995).

### Fruitful insights: exploring fruit variations

Characters related to fruit shape and size have also been used to delimit the different taxa that make up the species complex studied. However, based on the detailed observations of herbarium specimens, it was noted that there is a marked intraspecific morphological variation in both shape and size; therefore, these characteristics are not of taxonomic importance. Particularly noteworthy is the case of *M. alejandrae*, a taxon which, according to the description, has the smallest fruits of all the taxa in both the complex and the section (4–7 cm × 3–4.5 cm; García-Morales *et al.* 2017), but specimens with larger fruits were observed, whose measurements overlap with the other taxa of the complex, thus making taxonomic delimitation difficult. It is precisely this situation, which also occurs in the other synonymised taxa, complemented by molecular data, both from previously studied microsatellites (Chávez-Cortázar *et al.* 2021) and from the phylogenomic data generated in the present study, that has led to the taxonomic decisions presented.

Another of the main morphological characteristics traditionally used to delimit *Magnolia* species, especially in the Neotropical region, is the number of carpels (Cruz-Durán *et al.* 2014; García-Morales *et al.* 2017; Lozano-Contreras 1994; Vázquez-García 1994; Vázquez-García *et al.* 2013, 2015, 2021). However, when statistical analyses based on a large sample of fruits have been carried out, it has been found that carpel numbers overlap among taxa (Aldaba Núñez 2020). Furthermore, it has been shown in model organisms that this trait is genetically related and regulated by light (Reymond *et al.* 2012). In addition, some recent studies on the evolutionary processes of carpels in angiosperms have concluded that these processes are quite complex due to their diversity in shape and arrangement; their ontogeny is subject to a large number of developmental processes, which are well documented for *Arabidopsis thaliana* (L.) Heynhold (1842: 538), but not for non-model organisms (Liu *et al.* 2000, 2022; Pfannebecker *et al.* 2016; Reyes-Olalde *et al.* 2023; Rivarola 2020; Sattler 2024). In this species complex, it has been observed that fruits from the same tree that were more exposed to sunlight had a higher number of carpels than those that were more shaded (pers. obs.).

### Shedding light on sterile organ variations

Once the morphological variations of the reproductive organs have been analysed, the variations of the sterile organs remain to be dealt with. The main characteristic of this type, that has been proposed as taxonomically important for delimiting taxa within the *Magnolia dealbata* complex, is leaf size (García-Morales *et al.* 2017; Gutiérrez-Lozano *et al.* 2020; Sánchez-González *et al.* 2021; Vázquez-García *et al.* 2013, 2015, 2016).

*Magnolia* leaves exhibit considerable variation in size and shape (Gutiérrez-Lozano *et al.* 2020; Rodríguez-Ramírez *et al.* 2021). Morphological observations did not reveal any pattern that would allow the different morphospecies of the complex to be separated by leaf size or shape. It was also observed that other traits, such as leaf pubescence, showed a wide phenotypic variation, especially in individuals that had been identified as *M. vovidesii*, where specimens from the same locality can be either glabrous or pubescent.

Other deciduous plant organs, such as stipules and bracts, are less conspicuous and thus underrepresented in collections, often remaining overlooked (Aldaba Núñez 2020). These organs exhibit relatively stable and conserved morphology, which could be significant for species delimitation, particularly concerning the type of pubescence. However, it was also observed that these structures did not prove useful in delineating species within the complex.

Finally, some discrepancies were observed in the morphology of the studied taxa. For instance, in *M. mixteca*, although molecular samples were not included in this study, morphological observations were made. The taxon's description mentions that the stipule has sericeous pubescence (Vázquez-García *et al.* 2021). However, specimens from the type locality were observed with a completely glabrous stipule. In principle, this suggests that it indeed may be a distinct taxon. However, additional field expeditions are required to gather more specimens and make a determination.

Overall, the traits that exhibit the most intraspecific variation are the ones used to define the new taxa of the *Magnolia dealbata* complex (number of carpels, number of stamens, length of leaves (Gutiérrez-Lozano *et al.* 2020). It may be tempting to consider these variations as a result of significant phenotypic plasticity, but further study is required. *Magnolia* morphology is more intricate than it appears, and this phenomenon has only recently been explored

in depth (Aldaba Núñez *et al.* 2024; Gutiérrez-Lozano *et al.* 2020; Lozano-Contreras 1994; Rodríguez-Ramírez *et al.* 2021). Additionally, the morphological characteristics used to define certain sections may not apply to other sections.

As part of the morphological observations, characters previously proposed as diagnostic autapomorphies for certain taxa—such as the indumentum on peduncles and petioles in *M. zotictla*—were re-evaluated. Field and herbarium studies revealed considerable variability in these characters, even between individuals within the same population. For example, peduncle pubescence ranged from pulverulent to sericeous, while petiole indumentum varied from glabrous to sericeous. This evidence suggests that these characters are better interpreted as polymorphic rather than autapomorphic, highlighting their inadequacy for diagnosing *M. zotictla* as a distinct species. Interestingly, *M. zotictla* stands out within the *Magnolia dealbata* complex as the morphospecies with the most restricted distribution and the smallest known population size (Sánchez-González *et al.* 2021). These findings underscore the phenotypic plasticity and morphological variation present within each morphospecies of the *Magnolia dealbata* complex

### Taxonomic limits within the *Magnolia dealbata* complex

In the last two decades, around 80 new *Magnolia* species have been described in the Neotropical region; consequently, nearly half of the world's *Magnolia* species are now found in the Neotropics. These newly segregated taxa often have narrow distributions and are sometimes microendemic. (i.e., taxa segregated from *M. schiedeana* and *M. mexicana* (Vázquez-García *et al.* 2012b, 2013). However, the case of *M. dealbata sensu lato* may be an exception to this rule. Although the distribution of the closest taxon (*M. macrophylla*) is quite wide compared to other species in other sections (García-Morales *et al.* 2017; Gilman & Watson 1994; Meyer 1993), it is important to note that this is not always the case. In general, *Magnolia* species from temperate clades tend to have larger distributions than those from tropical habitat sections (pers. obs.), which could also be since there are more studies in temperate zones than in tropical ones, which are more difficult to access; it could also be due to the fact that temperate climates are wider and more stable. In the tropics, climatic conditions can change dramatically within a few kilometres, so the plant has a limited range (Sentinella *et al.* 2020). However, this statement needs to be investigated, as several factors explain the distribution and diversification of angiosperms (Gehrke 2018; Pennington *et al.* 2009; Tietje *et al.* 2022). The deciduous leaves of *Magnolia* sect. *Macrophylla* taxa may contribute to their broader geographical distribution, suggesting adaptability to diverse habitats. However, the distribution of *M. dealbata* s.l. across various forest types in the SMO indicates that factors beyond leaf type influence their occurrence. Interestingly, despite their wider distribution, deciduous groups of *Magnolia* in both continents have lower species richness compared to evergreen groups, hinting at complex underlying factors that govern species diversity.

Furthermore, the minimal variation in plastome structure and arrangement suggests a single identity with multiple populations comprising *Magnolia dealbata* complex taxa and potentially the two US varieties of *M. macrophylla*. However, from a broader perspective, both US and Mexican taxa could belong to the same entity: *M. macrophylla*, with Mexican taxa forming the variety *M. macrophylla* var. *dealbata* (Zucc.) D.L. Johnson (1989: 55), as previously proposed (Johnson 1989). Additional samples of *M. macrophylla* from the USA are needed to confirm this hypothesis. When comparing plastomes of different taxa within a species complex, differences and variations in plastomes are usually evident and consistent with the results of phylogenetic hypotheses (Cao *et al.* 2024; Hu *et al.* 2023; Li *et al.* 2022). Although the various studies of morphological and molecular variation conclude that the different taxa that make up the *Magnolia dealbata* complex are indeed differentiated species, the results obtained here suggest otherwise. On the one hand, morphological variation is based on very few characters from a larger matrix, so most morphological characters support the hypothesis that the taxa form a single entity with specific morphological variations (Gutiérrez-Lozano *et al.* 2020; Rodríguez-Ramírez *et al.* 2021). On the other hand, the most recent molecular results from microsatellites do indeed show that individuals of the different taxa intermingle, and no real differentiation is observed (López-Ramírez *et al.* 2024), as previously reported (Chávez-Cortázar *et al.* 2021). This could be a matter of scale: if sampling is extended to include most of the taxa in the complex, little variation is observed, but if it is focused on a smaller number of taxa and populations, more differentiation is observed, and genetic groups become more apparent.

The species complex has a discontinuous distribution along the SMO, the gaps can be explained conclusively, as it is highly probable that *M. dealbata* can be found in these areas, as several of them have a similar climate and vegetation type suitable for *M. dealbata*. From north to south, the gap between the populations of '*M. nuevoleonensis*' in Nuevo León and those of '*M. alejandrae*' in Tamaulipas is explained by the fact that this area is difficult to access and has been little studied (Martínez Salas, pers. comm.). Next, the gap between the populations of '*M. alejandrae*' and those of '*M. rzedowskiana*' in San Luis Potosí and '*M. zotictla*' in Hidalgo and Puebla is due to the lower foothills and drier climate, which limits their distribution to the Huasteca, a more humid area that represents the northern limit

of the high forests; however, around Ciudad del Maiz, San Luis Potosí in the middle of the gap there are reports of montane cloud forest (Martínez Salas, pers. comm.). Although generic differentiation between both morphospecies has been reported, it was not significant (Chávez-Cortázar *et al.* 2021). Subsequently, the gap between the populations of ‘*M. rzedowskiana*’–‘*M. zoticlla*’ and ‘*M. vovidesii*’ in Veracruz is explained by the steep slopes in the Misantla area, (Martínez-Salas, pers. comm.). Finally, the gap between the populations of ‘*M. vovidesii*’ and *M. dealbata* s.s. is since there are several natural communal zones in the area, to which the local people do not allow access, which is another little explored area, but this time due to social factors rather than natural factors (Martínez Salas, pers. comm.).

Despite these gaps, it must be considered that magnolias are pollinated by beetles and dispersed by birds, organisms that can easily evade the steep slopes of the SMOr (Gottsberger *et al.* 2012; Gutiérrez-Zúñiga 2018; Sun *et al.* 2023; Thien 1974; Wang *et al.* 2014; Werle 2002). Furthermore, magnolias from other sections (i.e., *M. tamaulipana* from sect. *Magnolia* in Reserva de la Biósfera El Cielo, Tamaulipas and *M. mexicana* from *Magnolia* sect. *Talauma* in the Sierra Norte de Puebla, Puebla) can be found in these areas, which reinforces the fact that these gaps are due to limited collection efforts and that *M. dealbata* could inhabit these areas and maintained historical gene flow throughout the SMOr, as showed in a previous study (Chávez-Cortázar *et al.* 2021).

*Magnolia mixteca* is another species of the *Magnolia* sect. *Macrophylla* but it occurs in a different mountain range, the SMS in Oaxaca state, so it was not included as the study focused on the SMOr species. This species was described “*de novo*”, rather than being segregated populations from *M. dealbata* as happened with the SMOr taxa (Vázquez-García *et al.* 2021).

### Implications for conservation

During our field expeditions, we observed that magnolias are integral components of the primary vegetation (particularly in cloud forests) and are particularly vulnerable to environmental disturbances. We especially found individuals away from forest margins, where the species composition indicated less disturbance, suggesting that these species may prefer more secluded, undisturbed habitats, as also reported in the literature (Chávez-Cortázar *et al.* 2021; López-Ramírez *et al.* 2024) and observed in other sections (Aldaba Núñez *et al.* 2021; Budd *et al.* 2015; Hernández *et al.* 2020; Sánchez-Velásquez *et al.* 2016). Furthermore, due to their medicinal properties, these plants are often indiscriminately collected for commercial purposes, especially their flowers, preventing fruiting and reproduction. Their distinctive growth habit makes them easy to identify in the field, but unfortunately, this also makes them an easy target for collectors. Historical and current literature note that their flowers are highly aromatic (Argueta-Villamar 2009; Hernández 1959; Hernández-Cerda 1988; Meyer 1993; Mociño & Sessé 2010), which not only aids in their location but also increases their collection appeal. Despite this, there are places, such as Xalapa, Veracruz, where people are more protective of the species and use it for ornamental purposes, and where the trade is more local, without intensive exploitation.

According to the IUCN Red List (IUCN 2013, 2021; IUCN Standards and Petitions Committee 2024), five of the taxa that comprise the complex are classified as ‘Endangered’ (Akanke & Yobal 2020; Rivers 2015b, 2016a; b; Vasquez-Garcia *et al.* 2023), with *M. zoticlla* being the most threatened with a ‘CR’ category (Critically Endangered; (Caeaeun Her & Sanchez Gonzalez 2023), while only *M. dealbata* s.s. is classified as ‘NT’ (Near Threatened; (Rivers 2015a). However, we propose a new provisional conservation status for *M. dealbata* as Least Concern (LC). *Magnolia dealbata* occupies an extensive EOO covering the entire range of the SMOr, although the AOO value falls into the EN category. It is mainly found in cloud forests, the most threatened habitat in Mexico, and the one that has suffered the greatest area loss (Castillo-Hernández & Flores-Olvera 2017; Domínguez-Yescas *et al.* 2020; Gual-Díaz & Rendón-Correa 2017; Ramírez-Bamonde *et al.* 2005; Williams-Linera *et al.* 2016a). Although new small populations have been discovered, in the central part of the SMOr, in the southern SMOr larger populations can be found, sometimes forming forests of up to 250 individuals, especially in Oaxaca (Galindo 2010; García Padilla *et al.* 2022; Rodríguez-Robayo & Merino-Pérez 2018). The species is found in several federal, state and communal natural areas in Mexico, which reduces conservation concerns. This is particularly the case in Oaxaca, the state with the highest number of communal natural areas and where, due to the uses and customs of its inhabitants, it is one of the few places in Mexico and the world where forest cover has increased in the last 50 years (Martínez Salas, pers. comm.).

In addition, further exploration and work on population genetics is suggested, especially in the northern and southern parts of the SMOr, considering that all populations belong to the same species, as exposed here, to develop appropriate conservation strategies and propose conservation units. As well as including *M. mixteca* in conservation genetics studies, this taxon is under the Endangered (EN) category according to the Red List (IUCN 2013, 2021; Vasquez-Garcia *et al.* 2023), so more considerable conservation efforts are needed.

## Conclusion

In this study, comparative analyses of the complete chloroplast of a species complex of *Magnolia* were performed. They can be a source of information on species relationships and taxonomic changes in taxa.

Several sources of molecular evidence were used: plastomes, angiosperm plastid DNA barcodes, and *Magnolia*-specific plastid DNA barcodes obtained from hypervariable plastid regions. The first two sources of evidence suggest that the Mexican taxa of the *Magnolia dealbata* complex studied form a single entity.

Traditional morphological traits used to distinguish *Magnolia* species are no longer sufficient due to the increased description of new species and the discovery of new populations. Specifically, the number of carpels and the presence or absence of a colour spot at the base of the petals, traditionally used to delimit species, have been found to be less reliable. This underscores the need for a reassessment of the morphological characters used in *Magnolia* taxonomy and the proposal of new morphological characteristics. The development of more reliable morphological markers for species delimitation will have important implications for studies on the medicinal uses and conservation efforts of these taxa. Accurate species identification is essential for understanding their medicinal properties. It is also suggested that historical, geological, and ecological factors be taken into account when considering separating populations and elevating them to species rank. Although there may be gaps in the distribution of populations of a species, these can be explained when considering the vegetation, geology, geomorphology, climate, and previous collections in the region.

Further research on this species complex is suggested. The present study represents a preliminary advance in the delimitation of Mexican *Magnolia* sect. *Macrophylla* species based on molecular and morphological data. However, further morphometric and statistical studies can be carried out to provide some quantitative support to the taxonomic analysis, which is more qualitative in nature.

Finally, based on the results, an update of the conservation status of *M. dealbata* as Least Concern (LC) is proposed so that it would no longer be in any risk category according to the IUCN Red List guidelines.

## Acknowledgements

The authors thank the authorities in Mexico for the collection permits (SGPA/DGGFS/712/1643/13 and SGPA/DGGFS/712/1063/18). We are grateful to the following colleagues who supported us with lab or fieldwork, providing samples, logistics and/or obtaining the necessary permits: Pieter Asselman, Angélica Chávez Cortázar, Isabel Larridon, Martín Mata Rosas, and Emily Veltjen. This work was supported by the Fondation Franklinia 2019-003; the Arboretum Wespelaar; the King Leopold III Fund for Nature Exploration and Conservation; the Instituto de Ecología, A.C. and the Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT scholarships 916022 and 848616 to FA and SG).

## Author contributions

Conceptualization: FAAN. Writing—original draft: FAAN. Writing—review and editing: EMMS, FAAN, MSS, SK. Data curation: EMMS, FAAN, SGD, SK, SP. Formal analysis: FAAN, SGD. Investigation: FAAN. Methodology: FAAN, SGD. Project administration: FAAN, MSS. Visualization: FAAN. Resources: SGD, SK, SP. Software: SGD. Validation: EMMS, MSS. SGD. Supervision: EMMS, MSS. Funding acquisition: MSS.

## References

- Akande, C. & Yobal, S. (2020) *Magnolia alejandrae*. IUCN Red List of Threatened Species 2021: e.T182247087A196811648. <https://doi.org/10.2305/IUCN.UK.2021-2.RLTS.T182247087A196811648.en>
- Aldaba Núñez, F.A. (2020) *Sistématica, diversidad genética y conservación de Magnolia en Veracruz, México*. Master thesis. Instituto de Ecología, A.C.
- Aldaba Núñez, F.A., Guzmán-Díaz, S., Veltjen, E., Asselman, P., Jiménez, J.E., Sánchez, J.V., Testé, E., Infante, G.P., Sierra, D.S., Posada, R.C., Najarro, F.H., Vázquez-García, J.A., Larridon, I., Park, S., Kim, S., Salas, E.M.M. & Samain, M.-S. (2024) Phylogenomic



- insights into Neotropical *Magnolia* relationships. *Heliyon* 10: e39430.  
<https://doi.org/10.1016/j.heliyon.2024.e39430>
- Aldaba Núñez, F.A., Veltjen, E., Martínez Salas, E.M. & Samain, M.-S. (2021) Disentangling Species Delineation and Guiding Conservation of Endangered Magnolias in Veracruz, Mexico. *Plants* 10: 673–700.  
<https://doi.org/10.3390/plants10040673>
- Alonso-Castro, A.J., Domínguez, F., García-Regalado, A., González-Sánchez, I., Cerbón, M.A. & García-Carrancá, A. (2014) *Magnolia dealbata* seeds extract exert cytotoxic and chemopreventive effects on MDA-MB231 breast cancer cells. *Pharmaceutical Biology* 52: 621–627.  
<https://doi.org/10.3109/13880209.2013.859160>
- Alonso-Castro, A.J., Zapata-Bustos, R., Domínguez, F., García-Carrancá, A. & Salazar-Olivo, L.A. (2011) *Magnolia dealbata* Zucc and its active principles honokiol and magnolol stimulate glucose uptake in murine and human adipocytes using the insulin-signaling pathway. *Phytomedicine* 18: 926–933.  
<https://doi.org/10.1016/j.phymed.2011.02.015>
- Amiryousefi, A., Hyvönen, J. & Poczaí, P. (2018) IRscope: an online program to visualize the junction sites of chloroplast genomes. J. Hancock (Ed.) *Bioinformatics* 34: 3030–3031.  
<https://doi.org/10.1093/bioinformatics/bty220>
- Andrews, S. (2019) FastQC. A quality control tool for high throughput sequence data.
- Argueta-Villamar, A. (2009) Atlas de las Plantas de la Medicina Tradicional Mexicana. Available from: <http://www.medicinatradicionalmexicana.unam.mx/> (accessed 18 December 2018)
- Bachman, S., Moat, J., Hill, A.W., laTorre, J. de & Scott, B. (2011) Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126.  
<https://doi.org/10.3897/zookeys.150.2109>
- Baillon, H. (1868) *Histoire des plantes*. Librairie Hachette & Co., Paris, France. 502 pp.
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A. & Pevzner, P.A. (2012) SPAdes: A new genome assembly algorithm and Its applications to single-cell sequencing. *Journal of Computational Biology* 19: 455–477.  
<https://doi.org/10.1089/cmb.2012.0021>
- Bar-Akiva, A., Ovadia, R., Rogachev, I., Bar-Or, C., Bar, E., Freiman, Z., Nissim-Levi, A., Gollop, N., Lewinsohn, E., Aharoni, A., Weiss, D., Koltai, H. & Oren-Shamir, M. (2010) Metabolic networking in *Brunfelsia calycina* petals after flower opening. *Journal of Experimental Botany* 61: 1393–1403.  
<https://doi.org/10.1093/jxb/erq008>
- Basnett, S., Krpan, J. & Espíndola, A. (2024) Floral traits and their connection with pollinators and climate. *Annals of Botany* 2024: mcae046.  
<https://doi.org/10.1093/aob/mcae046>
- Binh, H.T., Ngoc, N.V., Tagane, S., Toyama, H., Mase, K., Mitsuyuki, C., Strijk, J.S., Suyama, Y. & Yahara, T. (2018) A taxonomic study of *Quercus langbianensis* complex based on morphology and DNA barcodes of classic and next generation sequences. *PhytoKeys* 95: 37–70.  
<https://doi.org/10.3897/phytokeys.95.21126>
- Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.  
<https://doi.org/10.1093/bioinformatics/btu170>
- Brito, V.L.G., Weynans, K., Sazima, M. & Lunau, K. (2015) Trees as huge flowers and flowers as oversized floral guides: the role of floral color change and retention of old flowers in *Tibouchina pulchra*. *Frontiers in Plant Science* 6.  
<https://doi.org/10.3389/fpls.2015.00362>
- Brudno, M., Malde, S., Poliakov, A., Do, C.B., Couronne, O., Dubchak, I. & Batzoglou, S. (2003) Glocal alignment: Finding rearrangements during alignment. *Bioinformatics* 19: i54–i62.  
<https://doi.org/10.1093/bioinformatics/btg1005>
- Budd, C., Zimmer, E. & Freeland, J.R. (2015) Conservation genetics of *Magnolia acuminata*, an endangered species in Canada: Can genetic diversity be maintained in fragmented, peripheral populations? *Conservation Genetics* 16: 1359–1373.  
<https://doi.org/10.1007/s10592-015-0746-9>
- Caeacun Her, E. & Sanchez Gonzalez, A. (2023) *Magnolia zoticlla*. *IUCN Red List of Threatened Species* 2023: e.T216693205A216693467.  
<https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T216693205A216693467.en>
- Cai, L., Xi, Z., Lemmon, E.M., Lemmon, A.R., Mast, A., Buddenhagen, C.E., Liu, L. & Davis, C.C. (2021) The perfect storm: Gene

- tree estimation error, incomplete lineage sorting, and ancient gene flow explain the most recalcitrant ancient angiosperm clade, Malpighiales. *Systematic Biology* 70: 491–507.  
<https://doi.org/10.1093/sysbio/syaa083>
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. & Madden, T.L. (2009) BLAST+: architecture and applications. *BMC Bioinformatics* 10: 421.  
<https://doi.org/10.1186/1471-2105-10-421>
- Candolle, A.P. de (1818) *Regni vegetabilis systema naturale, sive Ordines, genera et species plantarum secundum methodi naturalis normas digestarum et descriptorum*. Treuttel et Würtz, Paris, France. 580 pp.  
<https://doi.org/10.5962/bhl.title.59874>
- Cao, Z., Qu, Y., Song, Y. & Xin, P. (2024) Comparative genomics and phylogenetic analysis of chloroplast genomes of Asian *Caryodaphnopsis* taxa (Lauraceae). *Gene* 907: 148259.  
<https://doi.org/10.1016/j.gene.2024.148259>
- Casper, B.B. & La Pine, T.R. (1984) Changes in corolla color and other floral characteristics in *Cryptantha humilis* (Boraginaceae): Cues to discourage pollinators? *Evolution* 38: 128–141.  
<https://doi.org/10.2307/2408552>
- Castillo-Hernández, L.A. & Flores-Olvera, H. (2017) Floristic composition of the cloud forest of the Bicentenario Reserve, Zongolica, Veracruz, México. *Botanical Sciences* 95: 1–25.  
<https://doi.org/10.17129/botsci.1223>
- Chafin, L.G. (2000) *Field Guide to the Rare Plants of Florida*. Florida Natural Areas Inventory, Tallahassee, Florida.
- Chan, P.P. & Lowe, T.M. (2019) tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. *Methods in Molecular Biology (Clifton, N.J.)* 1962: 1–14.  
[https://doi.org/10.1007/978-1-4939-9173-0\\_1](https://doi.org/10.1007/978-1-4939-9173-0_1)
- Chávez-Cortázar, A., Oyama, K., Ochoa-Zavala, M., Mata-Rosas, M., Veltjen, E., Samain, M.-S. & Quesada, M. (2021) Conservation genetics of relict tropical species of *Magnolia* (section *Macrophylla*). *Conservation Genetics* 22: 259–273.  
<https://doi.org/10.1007/s10592-021-01334-5>
- Chen, C., Zhang, Q.-W., Ye, Y. & Lin, L.-G. (2021) Honokiol: A naturally occurring lignan with pleiotropic bioactivities. *Chinese Journal of Natural Medicines* 19: 481–490.  
[https://doi.org/10.1016/S1875-5364\(21\)60047-X](https://doi.org/10.1016/S1875-5364(21)60047-X)
- Cock, P.J.A., Antao, T., Chang, J.T., Chapman, B.A., Cox, C.J., Dalke, A., Friedberg, I., Hamelryck, T., Kauff, F., Wilczynski, B. & de Hoon, M.J.L. (2009) Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* 25: 1422–1423.  
<https://doi.org/10.1093/bioinformatics/btp163>
- Corral-Aguirre, J. & Sánchez-Velásquez, L. (2006) Seed ecology and germination treatments in *Magnolia dealbata*: An endangered species. *Flora—Morphology, Distribution, Functional Ecology of Plants* 201: 227–232.  
<https://doi.org/10.1016/j.flora.2005.07.004>
- Cruzan, M.B., Neal, P.R. & Willson, M.F. (1988) Floral Display in Phyla Incisa: Consequences for Male and Female Reproductive Success. *Evolution* 42: 505–515.  
<https://doi.org/10.2307/2409035>
- Cruz-Durán, R., Jiménez-Ramírez, J. & Olivera-Martínez, M. de L. (2014) Magnoliaceae, Olacaceae. In: Diego-Pérez, N. & Fonseca, R.M. (eds.) *Flora de Guerrero*. Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, Mexico, pp. 1–16.
- Dai, C., Liang, X., Ren, J., Liao, M., Li, J. & Galloway, L.F. (2016) The mean and variability of a floral trait have opposing effects on fitness traits. *Annals of Botany* 117: 421–429.  
<https://doi.org/10.1093/aob/mcv189>
- Darling, A.C.E., Mau, B., Blattner, F.R. & Perna, N.T. (2004) Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Research* 14: 1394–1403.  
<https://doi.org/10.1101/gr.2289704>
- De Cserna, Z. (1961) *Tectonic Map of Mexico*.
- De Cserna, Z. (1989) An outline of the geology of Mexico. In: Bally, A.W. & Palmer, A.R. (eds.) *The Geology of North America—An Overview*. Geological Society of America, Boulder, Colorado, pp. 233–364.  
<https://doi.org/10.1130/DNAG-GNA-A.233>
- Del Valle, J.C., Alcalde-Eon, C., Escribano-Bailón, M.T., Buide, M.L., Whittall, J.B. & Narbona, E. (2019) Stability of petal color polymorphism: the significance of anthocyanin accumulation in photosynthetic tissues. *BMC Plant Biology* 19: 496.  
<https://doi.org/10.1186/s12870-019-2082-6>
- Delgado, A., Quinet, M. & Dapena, E. (2021) Analysis of the variability of floral and pollen traits in apple cultivars—selecting suitable

- pollen donors for cider apple orchards. *Agronomy* 11: 1717.  
<https://doi.org/10.3390/agronomy11091717>
- Delph, L.F. & Lively, C.M. (1989) The evolution of floral color change: Pollinator attraction versus physiological constraints in *Fuchsia excorticata*. *Evolution* 43: 1252–1262.  
<https://doi.org/10.2307/2409360>
- Ding, H., Han, S., Ye, Y., Bi, D., Zhang, S., Yi, R., Gao, J., Yang, J., Wu, L. & Kan, X. (2022) Ten plastomes of *Crassula* (Crassulaceae) and phylogenetic implications. *Biology* 11: 1779.  
<https://doi.org/10.3390/biology11121779>
- Domínguez, F., Chávez, M., Garduño-Ramírez, M.L., Chávez-ávila, V.M., Mata, M. & Cruz-Sosa, F. (2009) Production of honokiol and magnolol in suspension cultures of *Magnolia dealbata* Zucc. *Natural Product Communications* 4: 939–943.  
<https://doi.org/10.1177/1934578X0900400713>
- Domínguez, F., Chávez, M., Garduño-Ramírez, M.L., Chávez-Avila, V.M., Mata, M. & Cruz-Sosa, F. (2010) Honokiol and magnolol production by in vitro micropropagated plants of *Magnolia dealbata*, an endangered endemic Mexican species. *Natural Product Communications* 5: 235–240.  
<https://doi.org/10.1177/1934578X1000500213>
- Domínguez, F., González-Trujano, E., Gallardo, J.M. & Orozco-Suárez, S. (2016) Antidepressant medicinal plants and compounds used in traditional medicines in North America. In: Grosso, C. (ed.) *Herbal medicine in depression*. Springer, Cham, pp. 381–431.  
[https://doi.org/10.1007/978-3-319-14021-6\\_8](https://doi.org/10.1007/978-3-319-14021-6_8)
- Domínguez-Yescas, R. & Tapia, R. (2013) *Estudio etnobiológico de Magnolia dealbata Zucc., en San Juan Juquila Vijanos, Oaxaca*.
- Domínguez-Yescas, R., Vázquez-García, J.A., Muñoz-Castro, M.Á., Hernández-Vera, G., Salcedo-Pérez, E., Rodríguez-Pérez, C. & Gallardo-Yobal, S.I. (2020) Small-scale environmental drivers of plant community structure and diversity in Neotropical montane cloud forests harboring threatened *Magnolia dealbata* in southern Mexico. *Diversity* 12: 444.  
<https://doi.org/10.3390/d12120444>
- Dong, W., Gao, L., Xu, C., Song, Y. & Poczar, P. (2023) Editorial: Rise to the challenges in plastome phylogenomics. *Frontiers in Plant Science* 14.  
<https://doi.org/10.3389/fpls.2023.1200302>
- Doyle, J.J. (2022) Defining coalescent genes: Theory meets practice in organelle phylogenomics. *Systematic Biology* 71: 476–489.  
<https://doi.org/10.1093/sysbio/syab053>
- Du, X.-Y., Kuo, L.-Y., Zuo, Z.-Y., Li, D.-Z. & Lu, J.-M. (2022) Structural variation of plastomes provides key insight into the deep phylogeny of ferns. *Frontiers in Plant Science* 13.  
<https://doi.org/10.3389/fpls.2022.862772>
- Du, Z.-Y., Qimike, A., Yang, C.-F., Chen, J.-M. & Wang, Q.-F. (2011) Testing four barcoding markers for species identification of Potamogetonaceae. *Journal of Systematics and Evolution* 49: 246–251.  
<https://doi.org/10.1111/j.1759-6831.2011.00131.x>
- Duan, L., Fu, L. & Chen, H.-F. (2023) Phylogenomic cytonuclear discordance and evolutionary histories of plants and animals. *Science China. Life sciences* 66.  
<https://doi.org/10.1007/s11427-023-2456-6>
- Ferrusquía-Villafranca, I. (1993) Geology of Mexico: A Synopsis. In: Ramamoorthy, T.P., Bye, R., Lot, A. & Fa, J. (eds.) *Biological Diversity of Mexico: Origins and Distribution*. Oxford University Press, New York, USA, pp. 3–107.
- Figlar, R.B. (2019) *Ex-situ cultivation of magnolias in South Carolina facilitates observations of tepal movements during their 24-hour protogynous flowering cycles*. Jalisco, Mexico.
- Figlar, R.B. & Nootboom, H.P. (2004) Notes on Magnoliaceae IV. *Blumea: Journal of Plant Taxonomy and Plant Geography* 49: 87–100.  
<https://doi.org/10.3767/000651904X486214>
- Flores-Estévez, N., Vasquez-Morales, S.G., Cano-Medina, T., Sánchez-Velásquez, L.R., Noa-Carrazana, J.C. & Díaz-Fleischer, F. (2013) Insecticidal activity of raw ethanolic extracts from *Magnolia dealbata* Zucc. on a tephritid pest. *Journal of Environmental Science and Health, Part B* 48: 582–586.  
<https://doi.org/10.1080/03601234.2013.774933>
- Frazer, K.A., Pachter, L., Poliakov, A., Rubin, E.M. & Dubchak, I. (2004) VISTA: Computational tools for comparative genomics. *Nucleic Acids Research* 32: 273–279.  
<https://doi.org/10.1093/nar/gkh458>
- Galindo, C. (2010) Áreas comunitarias protegidas en Oaxaca. In: Carabias, J., Sarukhán, J., De La Maza, J. & Galindo-Leal, C. (eds.) *Patrimonio Natural de México, Cien Casos de Éxito*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, D.F., pp. 240.

- Galván-Hernández, D.M., Octavio-Aguilar, P., Bartolo-Hernández, C. de J., García-Montes, M.A., Sánchez-González, A., Ramírez-Bautista, A. & Vovides, A. (2020) Current status of *Magnolia vovidesii* (Magnoliaceae, Magnoniales): New data on population trends, spatial structure, and disturbance threats. *Tropical Conservation Science* 13: 1940082920923894.  
<https://doi.org/10.1177/1940082920923894>
- García, E. (2004) *Modificaciones al Sistema de Clasificación Climática de Köppen*. Universidad Nacional Autónoma de México, Instituto de Geografía, Mexico City, Mexico. 90 pp.
- García Padilla, E., DeSantis, D.L., Rocha, A., Fucsko, L.A., Johnson, J.D., Lazcano, D. & Wilson, L.D. (2022) Biological and cultural diversity in the state of Oaxaca, Mexico: Strategies for conservation among indigenous communities. *Biología y Sociedad* 5: 48–72.  
<https://doi.org/10.29105/bys5.9-5>
- García-Hernández, M. de los Á. & Toledo-Aceves, T. (2020) Is there potential in elevational assisted migration for the endangered *Magnolia vovidesii*? *Journal for Nature Conservation* 53: 125782.  
<https://doi.org/10.1016/j.jnc.2019.125782>
- García-Morales, L.J., Iamónico, D. & Jiménez, J.G. (2017) Nomenclatural remarks on *Magnolia* sect. *Macrophylla* (Magnoliaceae), with description of a new species from North America (Tamaulipas, Mexico). *Phytotaxa* 309: 238–244.  
<https://doi.org/10.11646/phytotaxa.309.3.4>
- Gehrke, B. (2018) Staying cool: preadaptation to temperate climates required for colonising tropical alpine-like environments. *PhytoKeys* 96: 111–125.  
<https://doi.org/10.3897/phytokeys.96.13353>
- Giarretta, A., Murphy, B., Maurin, O., Mazine, F.F., Sano, P. & Lucas, E. (2022) Phylogenetic relationships within the hyper-diverse genus *Eugenia* (Myrtaceae: Myrteae) based on target enrichment sequencing. *Frontiers in Plant Science* 12.  
<https://doi.org/10.3389/fpls.2021.759460>
- Gilman, E.F. & Watson, D.G. (1994) *Magnolia macrophylla* bigleaf magnolia. In: *Southern trees fact sheets*. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, pp. 3.
- González-Trujano, M., Lopez-Meraz, M.-L., Navarrete, A. & Martinez, A. (2015) Preclinical studies of three mexican plants used in folk medicine to treat epilepsy. A short review. *Revista Latinoamericana de Química* 43: 31–40.
- Gori, D.F. (1989) Floral color change in *Lupinus argenteus* (Fabaceae): Why should plants advertise the location of unrewarding flowers to pollinators? *Evolution* 43: 870–881.  
<https://doi.org/10.2307/2409314>
- Gottsberger, G., Silberbauer-Gottsberger, I., Seymour, R.S. & Dötterl, S. (2012) Pollination ecology of *Magnolia ovata* may explain the overall large flower size of the genus. *Flora—Morphology, Distribution, Functional Ecology of Plants* 207: 107–118.  
<https://doi.org/10.1016/j.flora.2011.11.003>
- Gu, J., Su, J.-X., Lin, R.-Z., Li, R.-Q. & Xiao, P.-G. (2011) Testing four proposed barcoding markers for the identification of species within *Ligustrum* L. (Oleaceae). *Journal of Systematics and Evolution* 49: 213–224.  
<https://doi.org/10.1111/j.1759-6831.2011.00136.x>
- Gual-Díaz, M. & Rendón-Correa, A. (2017) Los bosques mesófilos de montaña de México. *Agroproductividad* 10: 3–9.
- Gutiérrez Carvajal, L. (1993) *Estudio biológico de una especie forestal endémica (Magnolia dealbata Zucc.)*. Master thesis. Universidad Autónoma de Nuevo León.
- Gutiérrez, L. & Vovides, A. (1997) An in situ study of *Magnolia dealbata* Zucc in Veracruz State: An endangered endemic tree of Mexico. *Biodiversity and Conservation* 6: 89–97.  
<https://doi.org/10.1023/A:1018327700030>
- Gutiérrez-Lozano, M., Sánchez-González, A., Vázquez-García, J.A., López-Mata, L. & Octavio-Aguilar, P. (2020) Diferenciación morfológica poblacional de *Magnolia rzedowskiana* (Magnoliaceae): especie endémica en peligro de extinción de la Sierra Madre Oriental, México. *Revista Mexicana de Biodiversidad* 91: 913101e.  
<https://doi.org/10.22201/ib.20078706e.2020.91.3101>
- Gutiérrez-Zúniga, J. (2018) *Dispersores de semilla de yoloxóchitl (Magnolia mexicana DC) en dos localidades de Zongolica, Ver.* Instituto Tecnológico Superior de Zongolica.
- Guzmán Gutiérrez, S.L., Reyes Chilpa, R. & Bonilla Jaime, H. (2014) Medicinal plants for the treatment of “nervios”, anxiety, and depression in Mexican Traditional Medicine. *Revista Brasileira de Farmacognosia* 24: 591–608.  
<https://doi.org/10.1016/j.bjp.2014.10.007>
- Guzmán-Díaz, S., Núñez, F.A.A., Veltjen, E., Asselman, P., Larridon, I. & Samain, M.-S. (2022) Comparison of Magnoliaceae plastomes: Adding Neotropical *Magnolia* to the discussion. *Plants* 11: 448.  
<https://doi.org/10.3390/plants11030448>
- Guzmán-Trampe, S., Lemus, D., Jiménez, O., Ruiz-Villafán, B., García-Carrancá, A., Hernández-Fernández, R., García-Zepeda, E.,



- Rodríguez-Sanoja, R., Macías-Rubalcava, M.L. & Sánchez, S. (2015) Evaluation of the potential bioactivity of an endophytic bacteria isolated from *Magnolia dealbata* Zucc. *International Journal of Current Microbiology and Applied Sciences* 4: 515–525.
- Hernández, F. (1959) *Historia Natural de Nueva España*. Universidad Nacional de México, Mexico City, Mexico. 476 pp.
- Hernández, M., Bejerano, A., Veltjen, E., Asselman, P., Testé, E., Larridon, I., Samain, M.-S. & Luis Roberto, G.-T. (2020) Population structure and genetic diversity of *Magnolia cubensis* subsp. *acunae* (Magnoliaceae): effects of habitat fragmentation and implications for conservation. *Oryx* 54: 1–9.  
<https://doi.org/10.1017/S003060531900053X>
- Hernández-Cerda, M.E. (1988) Magnoliaceae. In: Gómez-Pompa, A. (ed.) *Flora de Veracruz*. Instituto Nacional de Investigaciones Sobre Recursos Bióticos, Xalapa, Mexico, pp. 1–38.
- Heynhold, G. (1842) *Arabidopsis thaliana*. *Flora von Sachsen* 1: 538.
- Hu, H., Hu, Q., Al-Shehbaz, I.A., Luo, X., Zeng, T., Guo, X. & Liu, J. (2016) Species delimitation and interspecific relationships of the genus *Orychophragmus* (Brassicaceae) inferred from whole chloroplast genomes. *Frontiers in Plant Science* 7.  
<https://doi.org/10.3389/fpls.2016.01826>
- Hu, H., Wang, Q., Hao, G., Zhou, R., Luo, D., Cao, K., Yan, Z. & Wang, X. (2023) Insights into the phylogenetic relationships and species boundaries of the *Myricaria squamosa* complex (Tamaricaceae) based on the complete chloroplast genome. *PeerJ* 11: e16642.  
<https://doi.org/10.7717/peerj.16642>
- Hu, H. & Wang, X. (2023) Insights into the phylogenetic relationships and species boundaries of the *Myricaria squamosa* complex (Tamaricaceae) based on the complete chloroplast genome. *PeerJ* 11: e16642.  
<https://doi.org/10.7717/peerj.16642>
- Ida, T.Y. & Kudo, G. (2003) Floral color change in *Weigela middendorffiana* (Caprifoliaceae): Reduction of geitonogamous pollination by bumble bees. *American Journal of Botany* 90: 1751–1757.  
<https://doi.org/10.3732/ajb.90.12.1751>
- IUCN (2013) *Documentation standards and consistency checks for IUCN Red List assessments and species accounts. Version 2*. IUCN, Cambridge, UK. 68 pp.
- IUCN (2021) Red List of Threatened Species. Version 2020-3. Available from: <https://www.iucnredlist.org/> (accessed 8 February 2021)
- IUCN Standards and Petitions Committee (2024) *Guidelines for Using the IUCN Red List Categories and Criteria. Version 16*. Prepared by the Standards and Petitions Committee.
- Jacobo-Salcedo, M. del R., Gonzalez-Espindola, L.A., Alonso-Castro, A.J., Gonzalez-Martinez, M. del R., Domínguez, F. & Garcia-Carranca, A. (2011) Antimicrobial activity and cytotoxic effects of *Magnolia dealbata* and its active compounds. *Natural Product Communications* 6: 1934578X1100600818.  
<https://doi.org/10.1177/1934578X1100600818>
- Jiao, B., Chen, C., Wei, M., Niu, G., Zheng, J., Zhang, G., Shen, J., Vitales, D., Vallès, J., Verloove, F., Erst, A.S., Soejima, A., Mehregan, I., Kokubugata, G., Chung, G.-Y., Ge, X., Gao, L., Yuan, Y., Joly, C., Jabbour, F., Wang, W., Shultz, L.M. & Gao, T. (2023) Phylogenomics and morphological evolution of the mega-diverse genus *Artemisia* (Asteraceae: Anthemideae): Implications for its circumscription and infrageneric taxonomy. *Annals of Botany* 131: 867–883.  
<https://doi.org/10.1093/aob/mcad051>
- Jin, J.-J., Yu, W.-B., Yang, J.-B., Song, Y., de Pamphilis, C.W., Yi, T.-S. & Li, D.-Z. (2020) GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology* 21: 241.  
<https://doi.org/10.1186/s13059-020-02154-5>
- Johnson, D.L. (1989) Nomenclatural changes in *Magnolia*. *Baileya* 23: 55–56.
- Jones, C.E. & Cruzan, M.B. (1999) Floral morphological changes and reproductive success in deer weed (*Lotus scoparius*, Fabaceae). *American Journal of Botany* 86: 273–277.  
<https://doi.org/10.2307/2656943>
- Jussieu, A.L. (1789) *Genera plantarum: secundum ordines naturales disposita, juxta methodum in Horto regio parisiensi exarata*. Herrisant, Paris. 498 pp.  
<https://doi.org/10.5962/bhl.title.284>
- Kudo, G., Ishii, H.S., Hirabayashi, Y. & Ida, T.Y. (2007) A test of the effect of floral color change on pollination effectiveness using artificial inflorescences visited by bumblebees. *Oecologia* 154: 119–128.  
<https://doi.org/10.1007/s00442-007-0820-1>
- Langmead, B. & Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9: 357–359.  
<https://doi.org/10.1038/nmeth.1923>
- Larridon, I., Walter, H.E., Guerrero, P.C., Duarte, M., Cisternas, M.A., Hernández, C.P., Bauters, K., Asselman, P., Goetghebeur, P. & Samain, M.-S. (2015) An integrative approach to understanding the evolution and diversity of *Copiapoa* (Cactaceae), a threatened endemic Chilean genus from the Atacama Desert. *American Journal of Botany* 102: 1506–1520.

<https://doi.org/10.3732/ajb.1500168>

- Laslett, D. & Canback, B. (2004) ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Research* 32: 11–16.  
<https://doi.org/10.1093/nar/gkh152>
- Li, M., Sun, Y., Lu, X., Debnath, B., Mitra, S. & Qiu, D. (2019) Proteomics reveal the profiles of color change in *Brunfelsia acuminata* flowers. *International Journal of Molecular Sciences* 20: 2000.  
<https://doi.org/10.3390/ijms20082000>
- Li, Q., Liu, Y., Wang, A., Chen, Q., Wang, J., Peng, L. & Yang, Y. (2022) Plastome comparison and phylogenomics of *Fagopyrum* (Polygonaceae): Insights into sequence differences between *Fagopyrum* and its related taxa. *BMC Plant Biology* 22: 339.  
<https://doi.org/10.1186/s12870-022-03715-5>
- Li, X., Yang, Y., Henry, R.J., Rossetto, M., Wang, Y. & Chen, S. (2015) Plant DNA barcoding: from gene to genome. *Biological Reviews of the Cambridge Philosophical Society* 90: 157–166.  
<https://doi.org/10.1111/brv.12104>
- Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.  
<https://doi.org/10.1093/bioinformatics/btp187>
- Linnaeus, C. (1753) *Species plantarum, exhibentes plantas rite cognitatas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus secundum systema sexuale digestas*. Impensis Laurentii Salvii, Holmiae [Stockholm]. 560 pp.  
<https://doi.org/10.5962/bhl.title.59734>
- Liu, H., Li, J., Gong, P. & He, C. (2022) The origin and evolution of carpels and fruits from an evo-devo perspective. *Journal of Integrative Plant Biology* 65: 1–16.  
<https://doi.org/10.1111/jipb.13351>
- Liu, Z., Franks, B. & Klink, V. (2000) Regulation of gynoeceium marginal tissue formation by LEUNIG and AINTEGUMENTA. *The Plant cell* 12: 1879–92.  
<https://doi.org/10.2307/3871199>
- López-Ramírez, L., Hernández-Vera, G., Galván-Hernández, D.M., Aguilar, P.O., Carranza-Aranda, A.S., Vázquez-García, J.A. & Sánchez-González, A. (2024) Structure, diversity, and genetic delimitation of two species endemic to eastern Mexico. *Silvae Genetica* 73: 35–47.  
<https://doi.org/10.2478/sg-2024-0004>
- Lozano-Contreras, G. (1994) *Dugandiodendron y Talauma (Magnoliaceae) en el Neotrópico*. Academia Colombiana de Ciencias Exactas, Bogotá, Colombia. 147 pp.
- Luo, H., Deng, S., Fu, W., Zhang, X., Zhang, X., Zhang, Z. & Pang, X. (2017) Characterization of active anthocyanin degradation in the petals of *Rosa chinensis* and *Brunfelsia calycina* reveals the effect of gallated catechins on pigment maintenance. *International Journal of Molecular Sciences* 18: 699.  
<https://doi.org/10.3390/ijms18040699>
- Martínez, A.L., Domínguez, F., Orozco, S., Chávez, M., Salgado, H., González, M. & González-Trujano, M.E. (2006) Neuropharmacological effects of an ethanol extract of the *Magnolia dealbata* Zucc. leaves in mice. *Journal of Ethnopharmacology* 106: 250–255.  
<https://doi.org/10.1016/j.jep.2006.01.003>
- Martínez-Harms, J., Guerrero, P., Martínez-Harms, M., Poblete, N., González, K., Stavenga, D. & Vorobyev, M. (2022) Mechanisms of flower coloring and eco-evolutionary implications of massive blooming events in the Atacama Desert. *Frontiers in Ecology and Evolution* 10.  
<https://doi.org/10.3389/fevo.2022.957318>
- Meyer, G.F. (1993) Magnoliaceae. *Flora of North America North of Mexico [Online]*. Available from: <http://floranorthamerica.org/Magnoliaceaeceae> (accessed 31 January 2023)
- Michaux, A. (1803) *Flora Boreali-Americana, sistens characteres plantarum quas in America septentrionali collegit et detexit*. Levrault, Paris & Strasbourg, France. 371 pp.  
<https://doi.org/10.5962/bhl.title.330>
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear, R. (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 37: 1530–1534.  
<https://doi.org/10.1093/molbev/msaa015>
- Miranda, F. & Hernández-X., E. (2014) *Los tipos de vegetación de México y su clasificación: edición conmemorativa 1963-2013*. Fondo de Cultura Económica, México, D.F. 216 pp.  
<https://doi.org/10.17129/botsci.1084>

- Mociño, J.M. & Sessé, M. (2010) *La Real Expedición Botánica a Nueva España: De familia Lamiaceae a familia Malvaceae. Volumen VII. Siglo XXI*. 424 pp.
- Moerman, D.E. (1991) The medicinal flora of native North America: An analysis. *Journal of Ethnopharmacology* 31: 1–42.  
[https://doi.org/10.1016/0378-8741\(91\)90141-Y](https://doi.org/10.1016/0378-8741(91)90141-Y)
- Moreno, N.P. (1984) *Glosario botánico ilustrado*. Instituto Nacional de Investigaciones Sobre Recursos Bióticos, Editorial Continental, S.A. de C.V., Xalapa, Mexico. 300 pp.
- Murray, G.E. (1961) *Geology of the Atlantic and Gulf Coastal Province of North America*. Harper & Row, New York, USA
- Narbona, E., del Valle, J.C. & Whittall, J.B. (2021) Painting the green canvas: how pigments produce flower colours. *The Biochemist* 43: 6–12.  
[https://doi.org/10.1042/bio\\_2021\\_137](https://doi.org/10.1042/bio_2021_137)
- Oberrath, R. & Böhning-Gaese, K. (1999) Floral color change and the attraction of insect pollinators in lungwort ( *Pulmonaria collina* ). *Oecologia* 121: 383–391.  
<https://doi.org/10.1007/s004420050943>
- Odell, E., Raguso, R.A. & Jones, K.N. (1999) Bumblebee foraging responses to variation in floral scent and color in snapdragons ( *Antirrhinum* : Scrophulariaceae). *The American Midland Naturalist* 142: 257–265.  
[https://doi.org/10.1674/0003-0031\(1999\)142\[0257:BFRTVI\]2.0.CO;2](https://doi.org/10.1674/0003-0031(1999)142[0257:BFRTVI]2.0.CO;2)
- O’Leary, N.A., Wright, M.W., Brister, J.R., Ciufu, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C.M., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V.S., Kodali, V.K., Li, W., Maglott, D., Masterson, P., McGarvey, K.M., Murphy, M.R., O’Neill, K., Pujar, S., Rangwala, S.H., Rausch, D., Riddick, L.D., Schoch, C., Shkeda, A., Storz, S.S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R.E., Vatsan, A.R., Wallin, C., Webb, D., Wu, W., Landrum, M.J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T.D. & Pruitt, K.D. (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research* 44: D733–745.  
<https://doi.org/10.1093/nar/gkv1189>
- Oliver, J.C. (2013) Microevolutionary processes generate phylogenomic discordance at ancient divergences. *Evolution* 67: 1823–1830.  
<https://doi.org/10.1111/evo.12047>
- Palmer, J.D. (1985) Comparative organization of chloroplast genomes. *Annual Review of Genetics* 19: 325–354.  
<https://doi.org/10.1146/annurev.ge.19.120185.001545>
- Pampanini, R. (1915) *Magnolia fraseri* var. *pyramidata*. *Bullettino della R[eale] Società Toscana d’Orticoltura. Florence* 20: 230.
- Pattison, G. (1985) *Magnolia dealbata*. *Magnolia Journal of the Magnolia Society* 21: 17–18.
- Pennington, R., Lavin, M. & Oliveira-Filho, A. (2009) Woody plant diversity, evolution, and ecology in the tropics: Perspectives from seasonally dry tropical forests. *Annual Review of Ecology, Evolution, and Systematics* 40: 437–457.  
<https://doi.org/10.1146/annurev.ecolsys.110308.120327>
- Pfannebecker, K., Lange, M., Rupp, O. & Becker, A. (2016) An evolutionary framework for carpel developmental control genes. *Molecular Biology and Evolution* 34: 330–348.  
<https://doi.org/10.1093/molbev/msw229>
- POWO (2023) Plants of the World Online. Royal Botanic Gardens, Kew. Available from: <http://www.plantsoftheworldonline.org/> (accessed 25 May 2021)
- Ram, H.Y.M. & Mathur, G. (1984) Flower colour changes in *Lantana camara*. *Journal of Experimental Botany* 35: 1656–1662.  
<https://doi.org/10.1093/jxb/35.11.1656>
- Ramírez-Bamonde, E.S., Sánchez-Velásquez, L.R. & Andrade-Torres, A. (2005) Seedling survival and growth of three species of mountain cloud forest in Mexico, under different canopy treatments. *New Forests* 30: 95–101.  
<https://doi.org/10.1007/s11056-004-5397-5>
- Ramírez-Reyes, T., Luna-Rodríguez, M., Noa-Carrazana, J.C., Díaz-Fleischer, F., Sánchez-Velásquez, L.R. & Flores-Estévez, N. (2015) Influence of season and organ on antibacterial activity of *Magnolia dealbata* Zucc. against two phytopathogenic bacteria. *Chemistry and Ecology* 31: 47–52.  
<https://doi.org/10.1080/02757540.2014.932779>
- Rauf, A., Olatunde, A., Imran, M., Alhumaydhi, F.A., Aljohani, A.S.M., Khan, S.A., Uddin, Md.S., Mitra, S., Emran, T.B., Khayrullin, M., Rebezov, M., Kamal, M.A. & Shariati, M.A. (2021) Honokiol: A review of its pharmacological potential and therapeutic insights. *Phytomedicine* 90: 153647.  
<https://doi.org/10.1016/j.phymed.2021.153647>
- Reyes-Olalde, J., Aida, M. & de Folter, S. (2023) An Evo-Devo view of the gynoecium. *Journal of Experimental Botany* 74: 3933–3950.  
<https://doi.org/10.1093/jxb/erad135>
- Reymond, M.C., Brunoud, G., Chauvet, A., Martínez-García, J.F., Martin-Magniette, M.-L., Monéger, F. & Scutt, C.P. (2012) A light-

- regulated genetic module was recruited to carpel development in *Arabidopsis* following a structural change to SPATULA. *The Plant Cell* 24: 2812–2825.  
<https://doi.org/10.1105/tpc.112.097915>
- Rezende, F.M., Clausen, M.H., Rossi, M. & Furlan, C.M. (2020) The regulation of floral colour change in *Pleroma raddianum* (DC.) Gardner. *Molecules* 25: 4664.  
<https://doi.org/10.3390/molecules25204664>
- Rico, Y. & Becerril, B.A.G. (2019) Species delimitation and genetic structure of two endemic *Magnolia* species (section *Magnolia* ; Magnoliaceae) in Mexico. *Genetica* 147: 57–68.  
<https://doi.org/10.1007/s10709-019-00052-8>
- Rivarola, A.C. (2020) *The molecular basis of carpel evolution*. PhD Thesis. Université de Lyon.
- Rivers, M.C. (2015a) *Magnolia dealbata*. *The IUCN Red List of Threatened Species* 2016: e.T88558975A2796189.  
<https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T88558975A2796189.en>
- Rivers, M.C. (2015b) *Magnolia vovidesii*. *IUCN Red List of Threatened Species* 2016: e.T67513624A67513853.  
<https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67513624A67513853.en>
- Rivers, M.C. (2016a) *Magnolia nuevoleonensis*. *IUCN Red List of Threatened Species* 2016: e.T84786942A84786994.  
<https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T84786942A84786994.en>
- Rivers, M.C. (2016b) *Magnolia rzedowskiana*. *IUCN Red List of Threatened Species* 2016: e.T82782448A82783726.  
<https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T82782448A82783726.en>
- Rodríguez-Ramírez, E.C., García-Morales, L.J., Alcántara-Ayala, O., Vázquez-García, J.A. & Luna-Vega, I. (2021) Leaf vein morphological variation in four endangered Neotropical *Magnolia* species along an elevation gradient in the Mexican tropical montane cloud forests. *Plants* 10: 2595.  
<https://doi.org/10.3390/plants10122595>
- Rodríguez-Robayo, K.J. & Merino-Pérez, L. (2018) Preserve and produce: experience in implementing payments for environmental services in two indigenous communities in the Northern and Southern ranges of Oaxaca, Mexico. *Journal of Sustainable Forestry* 37: 504–524.  
<https://doi.org/10.1080/10549811.2018.1432363>
- Rokas, A. & Chatzimanolis, S. (2008) From gene-scale to genome-scale phylogenetics: The data flood in, but the challenges remain. In : Murphy, W.J. (ed.) *Phylogenomics*. Methods in Molecular Biology™. Humana Press, Totowa, NJ, pp. 1–12.  
[https://doi.org/10.1007/978-1-59745-581-7\\_1](https://doi.org/10.1007/978-1-59745-581-7_1)
- Ruxton, G.D. & Schaefer, H.M. (2016) Floral colour change as a potential signal to pollinators. *Current Opinion in Plant Biology* 32: 96–100.  
<https://doi.org/10.1016/j.pbi.2016.06.021>
- Rzedowski, J. (2006) *Vegetación de México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico. 504 pp.
- Sánchez-González, A., Gutiérrez-Lozano, M., Yescas, R.D., Hernández-Álvarez, A.G., Ortega-Peña, A.S. & Vázquez-García, J.A. (2021) *Magnolia zoticlla* (*Magnolia* sect. *Macrophylla*, Magnoliaceae): A new species from the southern Sierra Madre Oriental, México. *Phytotaxa* 513: 271–281.  
<https://doi.org/10.11646/phytotaxa.513.4.1>
- Sánchez-Velásquez, L., María del Rosario, P.-L., Vázquez-Morales, S. & Avendaño-Yáñez, M. (2016) Ecology and conservation of endangered species: The case of magnolias. *Endangered Species: Threats, Conservation and Future Research*. Nova Science Publishers, Inc., pp. 63–84.
- Sánchez-Velásquez, L.R. & Pineda-López, M. del R. (2006) Species diversity, structure and dynamics of two populations of an endangered species, *Magnolia dealbata* (Magnoliaceae). *Revista de Biología Tropical* 54: 997–1002.  
<https://doi.org/10.15517/rbt.v54i3.13974>
- Sánchez-Velásquez, L.R. & Pineda-López, M.D.R. (2010) Comparative demographic analysis in contrasting environments of *Magnolia dealbata* : An endangered species from Mexico. *Population Ecology* 52: 203–210.  
<https://doi.org/10.1007/s10144-009-0161-5>
- Sattler, R. (2024) Morpho evo-devo of the gynoecium: Heterotopy, redefinition of the carpel, and a topographic approach. *Plants* 13: 599.  
<https://doi.org/10.3390/plants13050599>
- Schlechtendal, D.F.L. von (1864) Ueber *Magnolia mexicana* DC. *Botanische Zeitung* 22: 144.
- Sentinella, A.T., Warton, D.I., Sherwin, W.B., Offord, C.A. & Moles, A.T. (2020) Tropical plants do not have narrower temperature tolerances, but are more at risk from warming because they are close to their upper thermal limits. *Global Ecology and Biogeography* 29: 1387–1398.  
<https://doi.org/10.1111/geb.13117>



- Smit, M. (2013) *Assessing botanic gardens' support for integrated plant conservation—with focus on specific threatened Magnolia taxa (Magnoliaceae)*. University of Delaware.
- Spach, E. (1839) *Histoire naturelle des végétaux. Phanérogames*. Librairie encyclopédique de Roret, Paris, France. 562 pp.
- Starr, J.R., Naczi, R.F.C. & Chouinard, B.N. (2009) Plant DNA barcodes and species resolution in sedges ( *Carex* , Cyperaceae). *Molecular Ecology Resources* 9: 151–163.  
<https://doi.org/10.1111/j.1755-0998.2009.02640.x>
- Steenwyk, J.L., Li, Y., Zhou, X., Shen, X.-X. & Rokas, A. (2023) Incongruence in the phylogenomics era. *Nature Reviews Genetics* 24: 834–850.  
<https://doi.org/10.1038/s41576-023-00620-x>
- Su, N., Zhao, L., Potter, D. & Wen, J. (2021) On the species delimitation of the *Maddenia* Group of *Prunus* (Rosaceae): Evidence from plastome and nuclear sequences and morphology. *Frontiers in Plant Science* 12.  
<https://doi.org/10.3389/fpls.2021.743643>
- Sun, L.-Y., Wan, X.-X., Nie, T.-J., Chen, Y. & Yin, Z.-F. (2023) Floral ontogeny of *Magnolia* (Magnoliaceae) species provides insights into floral evolution but does not possess taxonomic value at the genus level. *Phytotaxa* 583: 153–162.  
<https://doi.org/10.11646/phytotaxa.583.2.3>
- Teppabut, Y., Oyama, K., Kondo, T. & Yoshida, K. (2018) Change of petals' color and chemical components in *Oenothera* flowers during senescence. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry* 23: 1698.  
<https://doi.org/10.3390/molecules23071698>
- Terrones, A., van der Bank, M., Moreno, J. & Juan, A. (2022) DNA barcodes and microsatellites: How they complement for species identification in the complex genus *Tamarix* (Tamaricaceae). *Journal of Systematics and Evolution* 60: 1140–1157.  
<https://doi.org/10.1111/jse.12830>
- Thien, L.B. (1974) Floral biology of *Magnolia*. *American Journal of Botany* 61: 1037–1045.  
<https://doi.org/10.1002/j.1537-2197.1974.tb12321.x>
- Thiers, B. (2018 [continuously updated]) Index Herbariorum: A global directory of public herbaria and associated staff. Available from: <http://sweetgum.nybg.org/science/ih/> (accessed 18 December 2018)
- Tietje, M., Antonelli, A., Baker, W.J., Govaerts, R., Smith, S.A. & Eiserhardt, W.L. (2022) Global variation in diversification rate and species richness are unlinked in plants. *Proceedings of the National Academy of Sciences of the United States of America* 119: e2120662119.  
<https://doi.org/10.1073/pnas.2120662119>
- Tillich, M., Lehwark, P., Pellizzer, T., Ulbricht-Jones, E.S., Fischer, A., Bock, R. & Greiner, S. (2017) GeSeq—versatile and accurate annotation of organelle genomes. *Nucleic Acids Research* 45: W6–W11.  
<https://doi.org/10.1093/nar/gkx391>
- Treseder, N.G. & Blamey, M. (1981) *The book of Magnolias*. Faber & Faber, London, UK
- TROPICOS (2023) Tropicos.org. Missouri Botanical Garden. Available from: <https://tropicos.org/> (accessed 25 May 2021)
- Turland, N., Wiersema, J., Barrie, F., Greuter, W., Hawksworth, D., Herendeen, P., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T., McNeill, J., Monro, A., Prado, J., Price, M. & Smith, G. (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Glashütten: Koeltz Botanical Books.  
<https://doi.org/10.12705/Code.2018>
- Vaknin, H., Bar-Akiva, A., Ovadia, R., Nissim-Levi, A., Forer, I., Weiss, D. & Oren-Shamir, M. (2005) Active anthocyanin degradation in *Brunfelsia calycina* (yesterday–today–tomorrow) flowers. *Planta* 222: 19–26.  
<https://doi.org/10.1007/s00425-005-1509-5>
- Vasquez-Garcia, A., Luna Vega, I., Domínguez-Yescas, R., Smith, C., Rodríguez-Ramírez, E. & Rodríguez-Pérez, C. (2023) *Magnolia mixteca*. *IUCN Red List of Threatened Species* 2023: e.T216693067A216693437.  
<https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T216693067A216693437.en>
- Vázquez-García, J.A. (1994) *Magnolia* (Magnoliaceae) in Mexico and Central America: A synopsis. *Brittonia* 46: 1–23.  
<https://doi.org/10.2307/2807454>
- Vázquez-García, J.A., Castro-Arce, E.D., Múñiz-Castro, M.Á. & Cházaro-Basáñez, M. de J. (2012a) *Magnolia zoquepopolucae* (subsection *Talauma*, Magnoliaceae), a new species from Sierra de Santa Marta, Veracruz, Mexico. *Phytotaxa* 57: 51–55.  
<https://doi.org/10.11646/phytotaxa.57.1.7>
- Vázquez-García, J.A., Domínguez Yescas, R., Luna-Vega, I., Rodríguez-Ramírez, E.C. & Rodríguez-Pérez, C. (2021) Corncob flower, *Magnolia mixteca* (M. sect. *Macrophylla* , Magnoliaceae) a new species endemic to the Alto Balsas Basin (Baja Mixteca), in the Pacific slopes of Oaxaca, Mexico. *Phytotaxa* 522: 200–210.  
<https://doi.org/10.11646/phytotaxa.522.3.3>

- Vázquez-García, J.A., Domínguez-Yescas, R., Pedraza-Ruiz, R., Sánchez-González, A. & Muñoz-Castro, M.Á. (2015) *Magnolia rzedowskiana* (Magnoliaceae), una especie nueva de la sección *Macrophylla* de la parte central de la Sierra Madre Oriental, México. *Acta Botanica Mexicana* 112: 19–36.  
<https://doi.org/10.21829/abm112.2015.1086>
- Vázquez-García, J.A., Domínguez-Yescas, R., Velazco-Macias, C., Shalisko, V. & Merino-Santi, R.E. (2016) *Magnolia nuevoleonensis* sp. nov. (Magnoliaceae) from Northeastern Mexico and a key to species of section *Macrophylla*. *Nordic Journal of Botany* 34: 48–53.  
<https://doi.org/10.1111/njb.00800>
- Vázquez-García, J.A., Muñoz-Castro, M.Á., Arroyo, F., Pérez-Castañeda, Á.J., Serna, M., Cuevas Guzmán, R., Domínguez-Yescas, R., De Castro-Arce, E. & Gurrola-Díaz, C.M. (2013) Novelties in Neotropical *Magnolia* and an addendum proposal to the IUCN Red List of Magnoliaceae. In: Salcedo-Pérez, E., Hernández-Álvarez, E., Vázquez-García, J.A., Escoto-García, T. & Díaz-Echavarría, N. (eds.) *Recursos forestales en el Occidente de México: Diversidad, manejo, producción, aprovechamiento y conservación*. Fronteras de Biodiversidad. Amaya Ediciones, Guadalajara, México, pp. 458–496.
- Vázquez-García, J.A., Muñoz-Castro, M.Á., De Castro-Arce, E., Murguía Araiza, R., Nuño Rubio, A.T. & Cházaro-Basáñez, M. de J. (2012b) Twenty new neotropical tree species of *Magnolia* (Magnoliaceae). In: Salcedo-Pérez, E., Hernández-Álvarez, E., Vázquez-García, J.A., Escoto-García, T. & Díaz-Echavarría, N. (eds.) *Recursos forestales en el Occidente de México: Diversidad, manejo, producción, aprovechamiento y conservación*. Fronteras de Biodiversidad. Amaya ediciones, Guadalajara, México, pp. 91–130.
- Velazco-Macias, C.G., Foroughbakhch-Pournavab, R., Alanís-Flores, G.J. & Alvarado-Vázquez, M.A. (2008) *Magnolia dealbata* en Nuevo León, México. *Revista Mexicana de Biodiversidad* 79: 459–463.  
<https://doi.org/10.22201/ib.20078706e.2008.002.554>
- Veltjen, E., Asselman, P., Hernández Rodríguez, M., Palmarola Bejerano, A., Testé Lozano, E., González Torres, L.R., Goetghebeur, P., Larridon, I. & Samain, M.-S. (2019) Genetic patterns in Neotropical magnolias (Magnoliaceae) using *de novo* developed microsatellite markers. *Heredity* 122: 485–500.  
<https://doi.org/10.1038/s41437-018-0151-5>
- Villar, J.L., Alonso, M.Á., Juan, A., Gaskin, J.F. & Crespo, M.B. (2019) Out of the Middle East: New phylogenetic insights in the genus *Tamarix* (Tamaricaceae). *Journal of Systematics and Evolution* 57: 488–507.  
<https://doi.org/10.1111/jse.12478>
- Vovides, A. & Iglesias, C. (1996) Seed germination of *Magnolia dealbata* Zucc. (Magnoliaceae), an endangered species from Mexico. *HortScience* 31: 877.  
<https://doi.org/10.21273/HORTSCI.31.5.877>
- Vovides, A.P. (1981) Lista preliminar de plantas mexicanas raras o en peligro de extinción. *Biotica* 6: 219–228.
- Walter, T. (1788) *Flora Caroliniana, secundum systema vegetabilium perillustris Linnaei digesta; characteres essentielles naturalesve et differentias veras exhibens; cum emendationibus numerosis: Descriptionum antea evulgatarum: Adumbrationes stirpium plus mille continens: Necnon, generibus novis non paucis, speciebus plurimis novisq. ornata*. J. Fraser, Londini. 270 pp.  
<https://doi.org/10.5962/bhl.title.9458>
- Wang, R., Xu, S., Liu, X., Zhang, Y., Wang, J. & Zhang, Z. (2014) Thermogenesis, flowering and the association with variation in floral odour attractants in *Magnolia sprengeri* (Magnoliaceae). M. Renou (Ed.) *PLoS ONE* 9: e99356.  
<https://doi.org/10.1371/journal.pone.0099356>
- Wang, Y.-B., Liu, B.-B., Nie, Z.-L., Chen, H.-F., Chen, F.-J., Figlar, R.B. & Wen, J. (2020) Major clades and a revised classification of *Magnolia* and Magnoliaceae based on whole plastid genome sequences via genome skimming. *Journal of Systematics and Evolution* 58: 673–695.  
<https://doi.org/10.1111/jse.12588>
- Wanke, S. & Wicke, S. (2023) Editorial: Phylogenomic discordance in plant systematics. *Frontiers in Plant Science* 14.  
<https://doi.org/10.3389/fpls.2023.1308126>
- Weatherby, C.A. (1926) A new magnolia from West Florida. *Rhodora* 28: 35.
- Weiss, M.R. (1991) Floral colour changes as cues for pollinators. *Nature* 354: 227–229.  
<https://doi.org/10.1038/354227a0>
- Weiss, M.R. (1995) Floral color change: A widespread functional convergence. *American Journal of Botany* 82: 167–185.  
<https://doi.org/10.2307/2445525>
- Weiss, M.R. & Lamont, B.B. (1997) Floral color change and insect pollination: a dynamic relationship. *Israel Journal of Plant Sciences* 45: 185–199.  
<https://doi.org/10.1080/07929978.1997.10676683>
- Werle, C.T. (2002) *Insects associated with Southern magnolia (Magnolia grandiflora L.) in east Tennessee*. Master thesis. University of Tennessee, Knoxville University of Tennessee, Knoxville.
- Wick, R.R., Schultz, M.B., Zobel, J. & Holt, K.E. (2015) Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics*

- Williams-Linera, G., Manson, R.H. & Isunza Vera, E. (2016a) La fragmentación del bosque mesófilo de montaña y patrones de uso del suelo en la región oeste de Xalapa, Veracruz, México. *Madera y Bosques* 8: 73–89.  
<https://doi.org/10.21829/myb.2002.811307>
- Williams-Linera, G., Manson, R.H. & Vera, E.I. (2016b) La fragmentación del bosque mesófilo de montaña y patrones de uso del suelo en la región oeste de Xalapa, Veracruz, México. *Madera y Bosques* 8: 73–89.  
<https://doi.org/10.21829/myb.2002.811307>
- Xia, N., Liu, Y. & Nooteboom, H.P. (2008) Magnoliaceae. In: Wu, Z., Raven, P.H. & Hong, D. (eds.) *Flora of China*. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press, pp. 48–91.
- Zhang, C.-Y., Wang, F.-Y., Yan, H.-F., Hao, G., Hu, C.-M. & Ge, X.-J. (2012) Testing DNA barcoding in closely related groups of *Lysimachia* L. (Myrsinaceae). *Molecular Ecology Resources* 12: 98–108.  
<https://doi.org/10.1111/j.1755-0998.2011.03076.x>
- Zhang, Q.-Y., Chen, Z., Sun, H. & Niu, Y. (2023) Intraspecific floral colour variation in three *Pedicularis* species. *Plant Diversity*.  
<https://doi.org/10.1016/j.pld.2023.03.011>
- Zhang, X., Sun, Y., Landis, J.B., Lv, Z., Shen, J., Zhang, H., Lin, N., Li, L., Sun, J., Deng, T., Sun, H. & Wang, H. (2020) Plastome phylogenomic study of Gentianeae (Gentianaceae): widespread gene tree discordance and its association with evolutionary rate heterogeneity of plastid genes. *BMC Plant Biology* 20: 340.  
<https://doi.org/10.1186/s12870-020-02518-w>
- Zhou, S.-M., Wang, F., Yan, S.-Y., Zhu, Z.-M., Gao, X.-F. & Zhao, X.-L. (2023) Phylogenomics and plastome evolution of *Indigofera* (Fabaceae). *Frontiers in Plant Science* 14.  
<https://doi.org/10.3389/fpls.2023.1186598>
- Zu, P., Schiestl, F.P., Gervasi, D., Li, X., Runcie, D. & Guillaume, F. (2020) Floral signals evolve in a predictable way under artificial and pollinator selection in *Brassica rapa*. *BMC Evolutionary Biology* 20: 127.  
<https://doi.org/10.1186/s12862-020-01692-7>
- Zuccarini, J.G. (1837) Plantarum novarum vel minus cognitarum, quae in horto botanico herbarioque regio monacensi servantur. Fasciculus secundus. In: *Abhandlungen der Mathematisch-Physikalischen Classe der Königlich Bayerischen Akademie der Wissenschaften*. Mich. Lindauer'schen Hofbuchdruckerei, Munich, Germany, pp. 309–380.
- Zuntini, A.R., Carruthers, T., Maurin, O., Bailey, P.C., Leempoel, K., Brewer, G.E., Epitawalage, N., Françoso, E., Gallego-Paramo, B., McGinnie, C., Negrão, R., Roy, S.R., Simpson, L., Toledo Romero, E., Barber, V.M.A., Botigué, L., Clarkson, J.J., Cowan, R.S., Dodsworth, S., Johnson, M.G., Kim, J.T., Pokorny, L., Wickett, N.J., Antar, G.M., DeBolt, L., Gutierrez, K., Hendriks, K.P., Hoewener, A., Hu, A.-Q., Joyce, E.M., Kikuchi, I.A.B.S., Larridon, I., Larson, D.A., de Lirio, E.J., Liu, J.-X., Malakasi, P., Przelomska, N.A.S., Shah, T., Viruel, J., Allnutt, T.R., Ameka, G.K., Andrew, R.L., Appelhans, M.S., Arista, M., Ariza, M.J., Arroyo, J., Arthan, W., Bachelier, J.B., Bailey, C.D., Barnes, H.F., Barrett, M.D., Barrett, R.L., Bayer, R.J., Bayly, M.J., Biffin, E., Biggs, N., Birch, J.L., Bogarin, D., Borosova, R., Bowles, A.M.C., Boyce, P.C., Bramley, G.L.C., Briggs, M., Broadhurst, L., Brown, G.K., Bruhl, J.J., Bruneau, A., Buerki, S., Burns, E., Byrne, M., Cable, S., Calladine, A., Callmander, M.W., Cano, Á., Cantrill, D.J., Cardinal-McTeague, W.M., Carlsen, M.M., Carruthers, A.J.A., de Castro Mateo, A., Chase, M.W., Chatrou, L.W., Cheek, M., Chen, S., Christenhusz, M.J.M., Christin, P.-A., Clements, M.A., Coffey, S.C., Conran, J.G., Cornejo, X., Couvreur, T.L.P., Cowie, I.D., Csiba, L., Darbyshire, I., Davidse, G., Davies, N.M.J., Davis, A.P., van Dijk, K.-J., Downie, S.R., Duretto, M.F., Duvall, M.R., Edwards, S.L., Egli, U., Erkens, R.H.J., Escudero, M., de la Estrella, M., Fabiani, F., Fay, M.F., Ferreira, P. de L., Ficinski, S.Z., Fowler, R.M., Frisby, S., Fu, L., Fulcher, T., Galbany-Casals, M., Gardner, E.M., German, D.A., Giarretta, A., Gibernau, M., Gillespie, L.J., González, C.C., Goyder, D.J., Graham, S.W., Grall, A., Green, L., Gunn, B.F., Gutiérrez, D.G., Hackel, J., Haevermans, T., Haigh, A., Hall, J.C., Hall, T., Harrison, M.J., Hatt, S.A., Hidalgo, O., Hodgkinson, T.R., Holmes, G.D., Hopkins, H.C.F., Jackson, C.J., James, S.A., Jobson, R.W., Kadereit, G., Kahandawala, I.M., Kainulainen, K., Kato, M., Kellogg, E.A., King, G.J., Klejevska, B., Klitgaard, B.B., Klopfer, R.R., Knapp, S., Koch, M.A., Leebens-Mack, J.H., Lens, F., Leon, C.J., Lévillé-Bourret, É., Lewis, G.P., Li, D.-Z., Li, L., Liede-Schumann, S., Livshultz, T., Lorence, D., Lu, M., Lu-Irving, P., Luber, J., Lucas, E.J., Luján, M., Lum, M., Macfarlane, T.D., Magdalena, C., Mansano, V.F., Masters, L.E., Mayo, S.J., McColl, K., McDonnell, A.J., McDougall, A.E., McLay, T.G.B., McPherson, H., Meneses, R.I., Merckx, V.S.F.T., Michelangeli, F.A., Mitchell, J.D., Monro, A.K., Moore, M.J., Mueller, T.L., Mummenhoff, K., Munzinger, J., Muriel, P., Murphy, D.J., Nargar, K., Nauheimer, L., Nge, F.J., Nyffeler, R., Orejuela, A., Ortiz, E.M., Palazzesi, L., Peixoto, A.L., Pell, S.K., Pellicer, J., Penneys, D.S., Perez-Escobar, O.A., Persson, C., Pignal, M., Pillon, Y., Pirani, J.R., Plunkett, G.M., Powell, R.F., Prance, G.T., Puglisi, C., Qin, M., Rabeler, R.K., Rees, P.E.J., Renner, M., Roalson, E.H., Rodda, M., Rogers, Z.S., Rokni, S., Rutishauser, R., de Salas, M.F., Schaefer, H., Schley, R.J., Schmidt-Lebuhn, A., Shapcott, A., Al-Shehbaz, I., Shepherd, K.A., Simmons, M.P., Simões, A.O., Simões, A.R.G., Siros, M., Smidt, E.C., Smith, J.F., Snow, N., Soltis, D.E., Soltis, P.S., Soreng, R.J., Sothers, C.A., Starr, J.R., Stevens, P.F., Straub, S.C.K., Struwe, L., Taylor, J.M., Telford,

I.R.H., Thornhill, A.H., Tooth, I., Trias-Blasi, A., Udovicic, F., Utteridge, T.M.A., Del Valle, J.C., Verboom, G.A., Vonow, H.P., Vorontsova, M.S., de Vos, J.M., Al-Wattar, N., Waycott, M., Welker, C.A.D., White, A.J., Wieringa, J.J., Williamson, L.T., Wilson, T.C., Wong, S.Y., Woods, L.A., Woods, R., Worboys, S., Xanthos, M., Yang, Y., Zhang, Y.-X., Zhou, M.-Y., Zmarzty, S., Zuloaga, F.O., Antonelli, A., Bellot, S., Crayn, D.M., Grace, O.M., Kersey, P.J., Leitch, I.J., Sauquet, H., Smith, S.A., Eiserhardt, W.L., Forest, F. & Baker, W.J. (2024) Phylogenomics and the rise of the angiosperms. *Nature* 629: 843–850.  
<https://doi.org/10.1038/s41586-024-07324-0>

**Supplementary Materials.** The following supporting information can be downloaded at the DOI landing page of this paper:

**Supplementary file 1.** Sequence identity plot produced by Shuffle-LAGAN alignment in mVista comparing 13 North American *Magnolia* taxa using *Magnolia fraseri* Walter as the reference. The x-axis represents the base sequence of the alignment, and the y-axis represents per cent identity (50–100%). Grey arrows represent genes with their orientation. Pink areas represent conserved non-coding sequences (CNS). Blue areas represent exons.

**Supplementary figure 1.** Phylogenetic relationships of 14 North America *Magnolia* accessions inferred from complete plastomes generated by maximum Likelihood approach; values on nodes represent bootstrap support percentages (BP).

**Supplementary figure 2.** Phylogenetic relationships of 14 North America *Magnolia* accessions inferred from plastid DNA barcodes (*matK*, *rbcL*, *trnH*, *psbA* and *trnL-F*) generated by maximum Likelihood approach; values on nodes represent bootstrap support percentages (BP).

**Supplementary figure 3.** Phylogenetic relationships of 14 North America *Magnolia* accessions inferred from a group-specific DNA barcode combination selected from hypervariable regions in Neotropical *Magnolia* plastomes (*ccsA*, *ndhD*, *petL*, and *rpl32*) generated by maximum Likelihood approach; values on nodes represent bootstrap support percentages (BP).