



Research article

Phylogenomic insights into Neotropical *Magnolia* relationships

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ABSTRACT

Despite extensive research into the phylogenetic relationships of the genus *Magnolia*, Neotropical taxa have been neglected. This is partly because their numbers have recently doubled and now account for almost half of the global richness. Therefore, by sampling one-third of all Neotropical taxa their relationships were studied using morphological, nuclear, and plastome data. Two major clades were identified: Clade I, comprising *Magnolia* sect. *Talauma*, *Magnolia* sect. *Splendentes* from the Neotropics, and the Asian *Magnolia* sect. *Gwillimia*; while Clade II included the Neotropical clades *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Magnolia*, along with the remaining non-Neotropical sections. Within Clade I, *Magnolia* sect. *Talauma* was geographically

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divided into a northern subclade grouping Mexican and Central American taxa, and a southern subclade comprising South American and Caribbean taxa. *Magnolia* sect. *Splendentes* was also dichotomously divided, corresponding to the former *Magnolia* sect. *Talauma* subsect. *Cubenses* and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*. In Clade II, the relationships within *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Magnolia* were unclear, suggesting a species complex in all *Magnolia* sect. *Macrophylla* taxa. In total, 25 morphological traits were assessed, and ancestral state reconstructions were carried out. Only the joined clustering of mature follicles was a synapomorphy for the southern subclade of *Magnolia* sect. *Talauma*. In conclusion, this highlights the need to re-assess the taxonomic delimitation of certain groups, to update the infrageneric classification of Neotropical clades and to explore morphological traits to support them.

Abbreviations

ASR	Ancestral character-state reconstructions
BI	Bayesian inference
BP	Bootstrap percentages
HTS	High-throughput sequencing
MA	Million years ago
ML	Maximum likelihood
MSC	Multispecies coalescent model
PP	Posterior probabilities
SL	Single locus

Table 1

Comparison among the most widely accepted *Magnolia* infrageneric classification (Figlar, 2012) and the most recent proposal (Wang et al., 2020).

Figlar, 2012	Wang et al., 2020
Subg. <i>Magnolia</i>	Sect. <i>Magnolia</i>
Sect. <i>Magnolia</i>	
Sect. <i>Gwillimia</i>	Sect. <i>Gwillimia</i>
Subsect. <i>Gwillimia</i>	
Subsect. <i>Blumiana</i>	
Sect. <i>Auriculata</i>	Sect. <i>Tuliparia</i>
Sect. <i>Macrophylla</i>	Sect. <i>Macrophylla</i>
Sect. <i>Rytidospermum</i>	Sect. <i>Rytidospermum</i>
Subsect. <i>Rytidospermum</i>	
Subsect. <i>Oyama</i>	Sect. <i>Oyama</i>
Sect. <i>Talauma</i>	Sect. <i>Talauma</i>
Subsect. <i>Talauma</i>	
Subsect. <i>Chocotalauma</i>^a	
Subsect. <i>Cubenses</i>	Sect. <i>Splendentes</i>
Subsect. <i>Dugandiodendron</i>	
Sect. <i>Kmeria</i>	Sect. <i>Kmeria</i>
Sect. <i>Manglietia</i>	Sect. <i>Manglietia</i>
Subg. <i>Gynopodium</i>	Sect. <i>Gynopodium</i>
Sect. <i>Gynopodium</i>	
Sect. <i>Manglietiastrum</i>	
Subg. <i>Yulania</i>	Sect. <i>Yulania</i>
Sect. <i>Yulania</i>	
Subsect. <i>Yulania</i>	
Subsect. <i>Tulipastrum</i>	Sect. <i>Tulipastrum</i>
Sect. <i>Michelia</i>	Sect. <i>Michelia</i>
Subsect. <i>Michelia</i>	
Subsect. <i>Elmerrillia</i>	
Subsect. <i>Aromadendron</i>	Sect. <i>Maingola</i>
Subsect. <i>Maingola</i>	

Notes: Bold identifies Neotropical clades.

^a Subsection *Chocotalauma* is proposed by Pérez et al., 2016 following Figlar's system.

1. Introduction

The genus *Magnolia* L. represents almost the entire richness of the extant Magnoliaceae, comprising about 390 tree or shrub species [1–5]. They are mainly distributed in temperate and tropical mountainous areas of the Nearctic, Neotropical, Oriental, and Palearctic biogeographic regions [6–11]. Approximately 170 recognised taxa (including four subspecies) occur in the Neotropical region, with the highest number of species being found in Colombia (38 species), Mexico (37 species), Ecuador (24 species) and Guatemala (20 species) [3–5,12–17].

Despite its diversity, the phylogenetic relationships within the genus have only partially been elucidated. Early molecular studies included the plastid-based study of Azuma [6,18], who found that the genus *Magnolia* sensu stricto was polyphyletic. Subsequently, Kim et al. [10] used evidence from the plastid *ndhF* gene to delimit the main lineages within the family and suggested an intrafamilial rearrangement. Subsequent studies using various molecular markers and morphological data have continued to improve our understanding of the relationships within the genus clarifying the relationships of some nodes and clades [6,8,19,20]. However, relationships within the *Magnolia* subg. *Magnolia* remain poorly resolved to date, mainly due to limited sampling and sequence variation (almost all studies conducted before 2020 were based on Sanger sequencing of a few amplified regions). Newer techniques are therefore needed to address its phylogenetic history [21].

Phylogenomic studies based on high-throughput sequencing (HTS) techniques based on target-enrichment approaches such as Hyb-Seq [22–24] are becoming increasingly common. Hyb-Seq combines target enrichment of nuclear genes [25] and genome skimming to recover organellar data [26,27], allowing the simultaneous collection of single to low-copy nuclear gene data and recovery of plastid genomic data [23,24]. These genome-scale datasets can be used to answer questions at different taxonomic levels based on phylogenomic or population genomic analyses [22,24,26,28,29]. The application of HTS techniques in conjunction with genome-scale data in taxonomy and systematics has clarified the phylogenetic relationships of groups that were difficult to interpret in an evolutionary framework [30–33]. However, these approaches are only beginning to be applied to Magnoliaceae phylogenomics [21,34]. Two phylogenetic studies based on plastome data found that a reorganisation of the infrageneric classification of *Magnolia* is needed and made proposals for this [21,35]. In addition, Neotropical *Magnolia* taxa have been underrepresented in most previous studies; a recent preliminary study highlighted the need to investigate these lineages through more extensive sampling [34].

The most widely accepted infrageneric classification proposal is that of Figlar [2], which includes 3 subgenera, 12 sections and 14 subsections based on morphological data, chloroplast and nuclear DNA sequences. However, the most recent proposal using morphological and plastome data is that of Wang et al. [21], which proposes 15 sections and confirms with previous studies that the three subgenera are not monophyletic, especially the *Magnolia* subg. *Magnolia* (the richest subgenus), which occurs in two out of three different clades. Both schemes, with emphasis on the Neotropical clades, are shown in Table 1.

The main aim of the present work was therefore to use a nuclear- and plastid-based phylogenomic approach to resolve the delimitation and phylogenetic relationships of *Magnolia* in the Neotropics employing a representative sampling of Neotropical taxa (Fig. 1). We addressed the following questions: 1. What are the overall phylogenetic relationships among Neotropical *Magnolia*

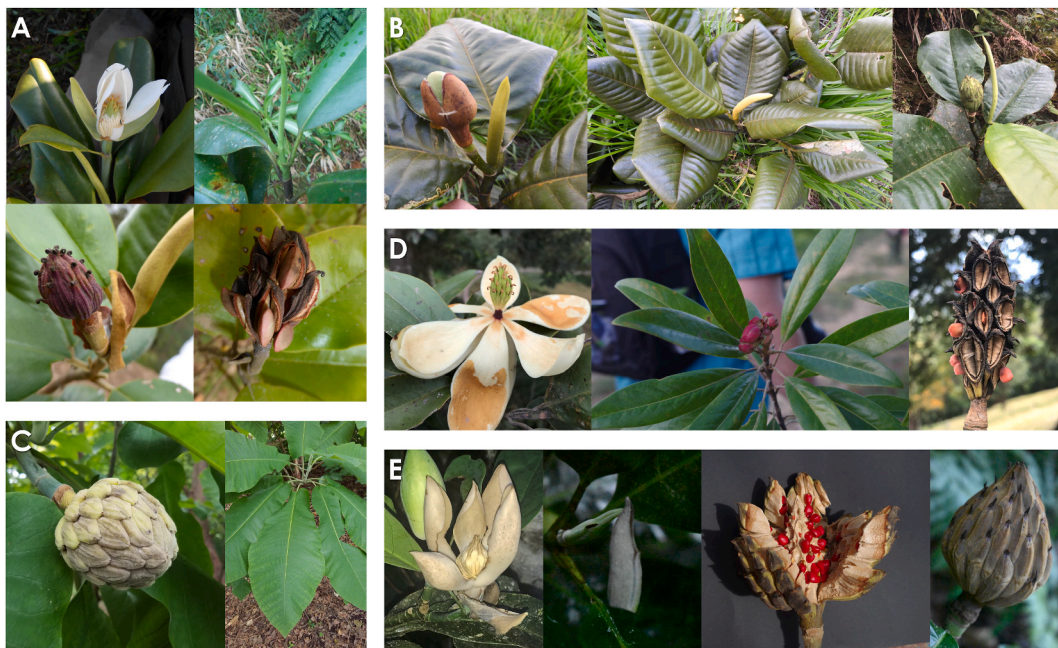


Fig. 1. *Magnolia* clades of Neotropical distribution. A: *Magnolia* sect. *Talauma* subject. *Cubenses*. B: *Magnolia* sect. *Talauma* subject. *Dugandiodendron*. C: *Magnolia* sect. *Macrophylla*. D: *Magnolia* sect. *Magnolia*. E: *Magnolia* sect. *Talauma* subject. *Talauma*.

species? 2. Which morphological characters can adequately differentiate *Magnolia* species and clades in the Neotropics? 3. How has the morphology of Neotropical *Magnolia* evolved? and 4. How useful are these traits for updating the infrageneric classification in combination with phylogenomic evidence?

2. Results

2.1. Bioinformatic analyses

We successfully obtained complete plastid sequences for 104 samples from 70 taxa (49 accepted Neotropical taxa, 7 Neotropical morphospecies and 13 non-Neotropical taxa). The length of the assembled chloroplast sequences ranged from 159 244 bp in *Magnolia ptaritepuiana* Steyerem. to 160 218 bp in *M. sieboldii* K.Koch. The mean depth coverage ranged from 16.05x in *M. ofeliae* A.Vázquez & Cuevas (ID sample MA3236) to 6302.9x in *M. globosa* Hook.f. & Thomson (ID sample MA068) with a mean of 605.67 x.

Regarding the nuclear target assembly, a set of 4 597 baits was designed in collaboration with RAPID Genomics (Gainesville, Florida, USA) to capture the identified low- to single-copy nuclear genes (LSCN). We successfully recovered between 386 and 488 loci for each species; the coverage ranged from 43x in *M. chiriquiensis* A.Vázquez (ID sample MA3140B) to 1174.77x in *M. aff. silvii* (Lozano) Govaerts (ID sample MA3129B) with a mean coverage of 309 x. The mean sequence length for each target locus ranged from 7 to 2 721 bp (assembled sequences with a length shorter than 50% of the original target were excluded from further analysis) with an overall mean of 79 bp per gene. On average, we recovered approximately 387 557 bp for each sample; those with fewer than 250 genes less than 50% of the mean target (GenesAt50pct) were discarded.

2.2. Phylogenetic analyses

As the main phylogenetic results, we presented both the single locus (SL) plastid and nuclear trees derived from Bayesian inference (BI) and their comparison in tanglegram (Fig. 2), as well as the multispecies coalescent model (MSC) tree (Fig. 3); as they had higher support values (posterior probabilities; PP) compared to maximum likelihood (ML) trees (bootstrap percentages; BP; Supplementary Figs. S1 and S2), although the topologies were the same.

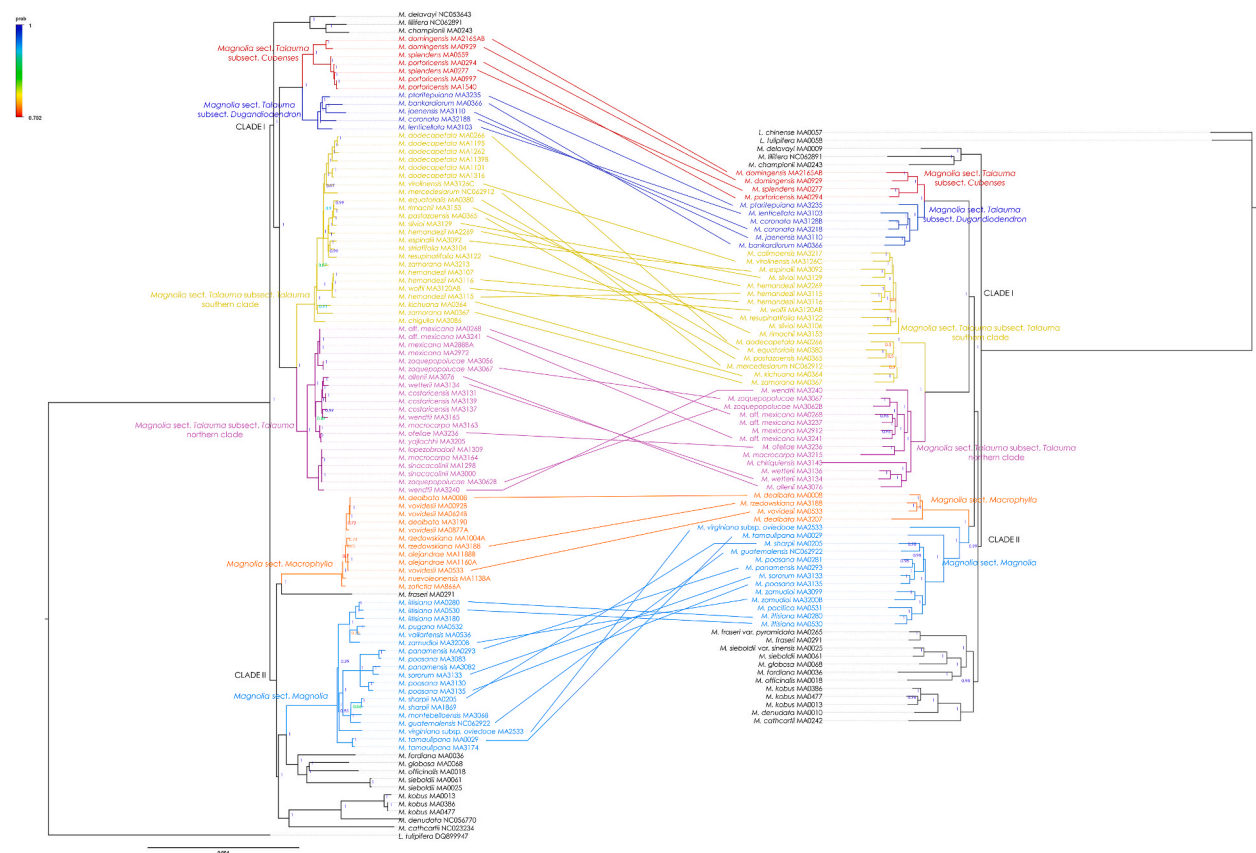


Fig. 2. Plastid and nuclear data phylogenetic topologies obtained from Bayesian Inference and comparison among them in tanglegram. Values on nodes represent Posterior Probabilities (PP).

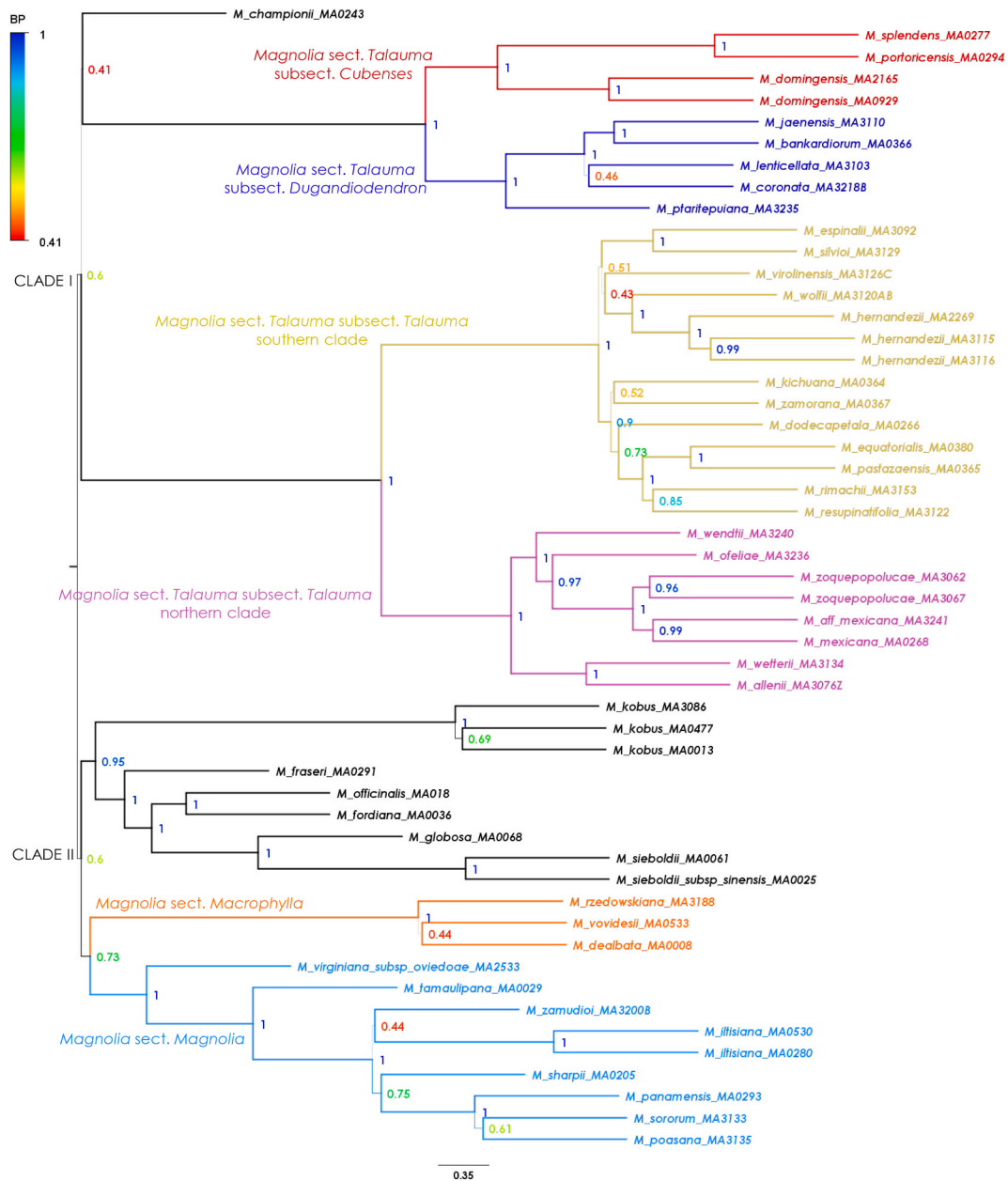


Fig. 3. Multispecies coalescent tree based on maximum likelihood from 74 nuclear sequences of 53 *Magnolia* species. Values on nodes represent Bootstrap Percentages (BP).

2.3. Phylogenetic relationships in *Magnolia*

In all SL and MSC trees, we recovered two distinct main clades: the first (which we refer to as Clade I) grouped *Magnolia* sect. *Gwillimia* DC. and *Magnolia* sect. *Talauma* sensu lato (referring to *Magnolia* sect. *Talauma* (Juss.) Baill. and *Magnolia* sect. *Splendentes* Dandy ex J.A.Vázquez together; PP = 1, Fig. 2; BP = 0.6, Fig. 3; Supplementary Fig. S1), while the second (Clade II) comprised all remaining sections studied here: *Magnolia* sect. *Macrophylla* Figlar & Noot., *Magnolia* sect. *Magnolia*, *Magnolia* sect. *Maingola* Dandy, *Magnolia* sect. *Manglietia* (Blume) Baill., *Magnolia* sect. *Oyama* Nakai, *Magnolia* sect. *Rytidospermum* Spach, *Magnolia* sect. *Tuliparia* Spach (formerly *Magnolia* sect. *Auriculata* Figlar & Noot.) and *Magnolia* sect. *Yulania* (Spach) Dandy (PP = 1, Fig. 2; BP = 0.6, Fig. 3; Supplementary Fig. S1). However, in the trees based on SL nuclear data, *Magnolia* sect. *Gwillimia* was instead recovered as a sister group to *Magnolia* sect. *Talauma* s.l. and Clade II (PP = 1, Fig. 2; Supplementary Fig. S2).

Within Clade I, in plastid trees, *Magnolia* sect. *Gwillimia* was found to be the sister group of *Magnolia* sect. *Talauma* s.l. (PP = 0.6, Fig. 2), within which two clades are apparent: the first grouped *Magnolia* sect. *Talauma* subsect. *Cubenses* and *Magnolia* sect. *Talauma*

subject. *Dugandiodendron* (both referred to as *Magnolia* sect. *Splendentes* by Wang et al., 2020), and the second comprised all the other species of *Magnolia* sect. *Talauma* subject. *Talauma* (Juss.) Figlar & Noot (here referred to as *Magnolia* sect. *Talauma* sensu stricto). However, in the MSC phylogeny, *M. championii* Benth. from *Magnolia* sect. *Gwillimia* was recovered as a sister group to *Magnolia* sect. *Splendentes*, but with a low support value (PP = 0.4, Fig. 3).

In Clade II, different analyses showed different topologies, although all yielded two major subclades. In the MSC and SL nuclear trees, the first subclade consisted of the Neotropical clades *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Magnolia* (PP = 1, Fig. 2; BP = 0.7, Fig. 3; Supplementary Fig. S2), whereas the second subclade grouped all Asian and Nearctic sections (PP = 1, Fig. 2; BP = 0.9, Fig. 3; Supplementary Fig. S2), showing a geographic pattern. However, in the SL plastid data trees, one subclade contained *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Tuliparia* (PP = 1, Fig. 2; Supplementary Fig. S1), whereas the second one consisted of all remaining groups (PP = 1, Fig. 2; Supplementary Fig. S1). In this case, a morphological affinity was observed.

2.4. Phylogenetic relationships in *Magnolia* sect. *Macrophylla*

Within *Magnolia* sect. *Macrophylla*, the taxa studied formed a species complex, where no consistent pattern was observed in the phylogenetic trees; instead of forming a clear lineage grouping, samples were found scattered across different branches. Furthermore, the presence of several polytomies added complexity to the understanding of the phylogenetic relationships between the taxa, making it difficult to interpret evolutionary relationships. In addition, many of the recovered clades had low support values (BP or PP < 0.5). These patterns were more apparent in the SL plastid phylogenies (Fig. 2; Supplementary Fig. S1), where more samples from this group were included.

2.5. Phylogenetic relationships in *Magnolia* sect. *Magnolia*

In the *Magnolia* sect. *Magnolia* clade, we observed the following four patterns, all of which are new: 1) Support for a close relationship between four species from western Mexico (*M. iltisiana* A.Vázquez, *M. pacifica* A.Vázquez, and *M. pugana* (Iltis & A.Vázquez) A.Vázquez & Carvajal and *M. vallartensis* A.Vázquez & Muñiz-Castro) and one from southeastern Mexico (*M. zamudioi* A.Vázquez) in the SL nuclear and plastid phylogenetic hypotheses (Fig. 2; Supplementary Fig. S2); 2) Support for a close relationship between *M. panamensis* Iltis & A.Vázquez (from Panama), *M. poasana* (Pittier) Dandy and *M. sororum* Seibert (both from Costa Rica) based on the SL nuclear and plastid phylogenetic hypotheses (Fig. 2; Supplementary Figs. S1 and S2), most likely in the form of a species complex, as the duplicate samples never clustered together (Fig. 2; Supplementary Fig. S1); 3) A close relationship between *M. guatemalensis* Donn. Sm., *M. montebelloensis* A.Vázquez & Pérez-Farr. (from southern Mexico), *M. sharpii* Miranda (from southern Mexico) in all SL phylogenetic hypotheses; Fig. 2; Supplementary Fig. S1); 4) Strongly genetically distinct taxa: *M. virginiana* subsp. *oviedoae* Palmarola, M.S.Romanov & A.V.Bobrov (from Cuba) in SL and MSC trees (Figs. 2 and 3; Supplementary Fig. S2) and *M. tamaulipana* (from northern Mexico) in plastid trees (Fig. 2; Supplementary Fig. S1).

2.6. Phylogenetic relationships in *Magnolia* sect. *Splendentes*

In all phylogenetic hypotheses obtained, the taxa of *Magnolia* sect. *Splendentes* formed two distinct clades, the first consisting of the taxa belonging to the *Magnolia* sect. *Talauma* subject. *Cubenses* and the second with the taxa of *Magnolia* sect. *Talauma* subject. *Dugandiodendron*, both with high support values (BP = 1; PP = 100). In both SL plastid trees (Fig. 2; Supplementary Fig. 2), the samples of *M. portoricensis* Bello (from western Puerto Rico) and *M. splendens* Urb. (from western Puerto Rico) intermingled among them within the clade of *Magnolia* sect. *Talauma* subject. *Cubenses*.

2.7. Phylogenetic relationships in *Magnolia* sect. *Talauma*

Within Clade I, *Magnolia* sect. *Talauma* s.s. was divided into two clades with a geographical pattern (Figs. 2 and 3). The first included the species from Mexico and Central America (hereafter referred to as the “northern clade”; PP = 1, Fig. 2; BP = 1, Fig. 3; Supplementary Figs. S1 and S2), and the second comprised the species from South America and the Caribbean (hereafter referred to as the “southern clade”; PP = 1, Fig. 2; BP = 1, Fig. 3; Supplementary Figs. S1 and S2). Furthermore, in the SL nuclear data topologies, the species from Mexico also formed a clade distinct from those from Central America (PP = 1, Fig. 2; Supplementary Fig. S2), but in the plastid data trees, species from both regions grouped together regardless of their geographic distribution (Fig. 2; Supplementary Fig. S1).

In the northern clade, the Central American taxa *M. allenii* Standl., *M. chiriquiensis* (both from Panama) and *M. wetterii* A.Vázquez (from Costa Rica) formed a well-supported (PP = 1) sister clade to all the Mexican *Magnolia* sect. *Talauma* species in the SL nuclear data and MSC phylogenies tree (Figs. 2 and 3; Supplementary Fig. S2). However, in the SL plastid phylogenies (Fig. 2; Supplementary Fig. S1), *M. allenii*, *M. costaricensis* A.Vázquez, *M. wetterii* and a sample of *M. wendtii* (from southern Mexico) were nested within the Mexican *Magnolia* sect. *Talauma* taxa.

With regard to the southern clade, the three taxa of the *Magnolia* sect. *Talauma* subject. *Chocotalauma* J.A.Vázquez, Á.J.Pérez & F. Arroyo included were nested within this clade in all trees (Figs. 2 and 3; Supplementary Figs. S1 and S2). *Magnolia calimaensis* was included in the SL nuclear phylogenetic hypotheses (Fig. 2; Supplementary Fig. S2), resulting as a sister species to *M. virolinensis* (Lozano) Govaerts with high support values: (PP = 1; Fig. 2; Supplementary Fig. S2). *Magnolia chiguila* was included in the plastid phylogenetic hypotheses (Fig. 2; Supplementary Fig. S1), although its position varied depending on the approach used: in BI (Fig. 2) it

appeared as the sister species to all other South American taxa with a high support value (PP = 1), while in ML (Figure [Supplementary S1](#)) it formed a clade together with *M. kichuana* and *M. zamorana* F.Arroyo (both from Ecuador), albeit with a medium support value (BP = 75). Finally, *M. striatifolia* was included in the plastid phylogenetic hypotheses, in both cases as a sister species to *M. espinalii* (Lozano) Govaerts (from the Cordillera Central of the Colombian Andes) with high support values: BI (PP = 1; [Fig. 2](#)) and ML (BP = 100; [Supplementary Fig. S1](#)). There was no pattern for the remaining taxa of the *Magnolia* sect. *Talauma* s.s.

2.8. Morphological studies and ancestral-state reconstructions

Of the 25 morphological characters assessed ([Table 2](#)), the joined clustering of mature follicles represented a synapomorphy of the southern clade of *Magnolia* sect. *Talauma* s.s., as all other branches of the tree have follicles that fall individually ([Fig. 4](#)). In the case of species where the carpels split longitudinally and remain attached to the receptacle, the code was set as 'individual'. According to the ASR, the clustered mature follicles represented a derived state, whereas the individually falling follicles represented the ancestral state.

In addition, in the plastid trees, auriculate ([Fig. 5A and B](#)) and cordate leaf base shapes ([Fig. 5C and D](#)) were unique to *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Tuliparia*. This was also a derived state for *Magnolia*, as it was absent in the rest of the nodes. The rest of the characters studied have appeared independently in *Magnolia* in different clades, without representing homologies.

According to the BI plastid tree in the ASRs, some traits represented possible autapomorphies for certain species, all of which are derived states: glabrescent indumentum on the peduncle and 10 petals for *M. coronata* M.Serna, C.Velásquez & Cogollo (from northwestern Colombia); 12 petals and obovoid fruits for *M. dodecapetala* (Lam.) Govaerts (from Martinique); obovate-shaped leaves for *M. kichuana* A.Vázquez, F.Arroyo & Á.J.Pérez (from Ecuador) and glabrescent indumentum on the abaxial surface for *M. lenticellata* (Lozano) Govaerts (from northwestern Colombia). For the Asian and Nearctic groups, autapomorphies were not considered because of the small number of species included, which would not be representative.

Following the ASR on the BI plastid tree, the ancestral states for our *Magnolia* taxa matrix are characterised as follows ([Supplementary Fig. S3](#)): Evergreen trees, with no false leaf whorls on young branches, young branches glabrous, young petioles glabrous, absence of pulvinus, with full scars left by the stipules (covering more than 75% of total petiole length), stipules glabrous, leaves elliptic, glabrous, apex acute, base cuneate, one glabrous bract, six petals, gynoeceum glabrous, style branches straight, peduncle glabrous, fruit shape ovoid, follicles glabrous, not recurved, with longitudinal dehiscence, and carpel apex acuminate.

Derived states have appeared independently at different times across the evolutionary history of *Magnolia*, such as deciduous foliage in the *Magnolia* sect. *Macrophylla*, *Magnolia* sect. *Tuliparia*, and *Magnolia* sect. *Yulania*; follicles with circumscissile dehiscence in *Magnolia* sect. *Talauma* and *Magnolia* sect. *Splendentes* (subclade *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*) or pubescence on different organs (young branches, stipules, leaves, gynoeceum, peduncles or follicles) in many taxa of the different clades.

Regarding the Neotropical groups, most of them also have a conserved morphology, since most of the traits present the ancestral state. Some exceptions of derived states unique to Neotropical lineages are the circumscissile dehiscent follicles in the *Magnolia* sect. *Talauma* and *Magnolia* sect. *Splendentes* (especially in the subclade *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*), and the curved style branches in the *Magnolia* sect. *Macrophylla*, *Magnolia* sect. *Magnolia* and *Magnolia* sect. *Splendentes*. In the case of multi-state characters, the derived states (fruit or leaf shapes or type of indumentum) are present in most of the clades studied.

Table 2

List of the 25 morphological traits and their character states used to distinguish the species and infrageneric clades of *Magnolia*.

	Morphological character	Character states and codification
1	Maximum tree height	0. 4–8.9 m; 1. 9–16.9 m; 2. 17–24.9 m; 3. 25–32.9 m; 4. 33–40.9 m; 5. >41 m
2	Leaf persistence	0. Evergreen; 1. Deciduous
3	Indumentum of young branches	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Glabrescent; 4. Pubescent; 5. Lanate
4	False leaf whorls on young branches	0. Absent; 1. Present
5	Presence of pulvinus	0. Absent; 1. Present
6	Indumentum of young petioles	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Glabrescent; 4. Pubescent; 5. Lanuginose; 6. Lanate
7	Presence of stipular scar	0. Absent; 1. Present
8	Length of stipular scar	0. Absent; 1. Partial (>1% – <74.9%); 2. Full (>75%)
9	Indumentum of stipule	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Pubescent; 4. Lanuginose; 5. Lanate; 6. Auriculiform
10	Leaf shape	0. Elliptic; 1. Ovate; 2. Obovate; 3. Oblanceolate; 4. Lanceolate; 5. Orbicular; 6. Oblate
11	Leaf base shape	0. Cuneate; 1. Obtuse; 2. Rounded; 3. Attenuate; 4. Truncate; 5. Auriculate; 6. Cordate
12	Leaf apex shape	0. Acute; 1. Apiculate; 2. Obtuse; 3. Rounded; 4. Truncate
13	Indumentum of abaxial surface	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Glabrescent; 4. Pubescent; 5. Lanate
14	Indumentum of peduncle	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Glabrescent; 4. Pubescent; 5. Lanuginose; 6. Lanate
15	Number of bracts	0. 1 bract; 1. 2 bracts; 2. 3 bracts; 3. 4 bracts; 4. 5 bracts
16	Indumentum of bract	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Glabrescent; 4. Pubescent; 5. Lanuginose; 6. Lanate
17	Number of petals	0. 6 petals; 1. 7 petals; 2. 8 petals; 3. 9 petals; 4. 10 petals; 5. 11 petals; 6. 12 petals
18	Indumentum of gynoeceum	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Pubescent; 4. Lanate
19	Shape of style branches	0. Curved; 1. Straight
20	Fruit shape	0. Spheroid; 1. Globose; 2. Ovoid; 3. Clavate; 4. Obovoid; 5. Botuliform; 6. Fusiform
21	Indumentum of fruit	0. Glabrous; 1. Pulverulent; 2. Puberulent; 3. Pubescent; 4. Lanate
22	Recurved mature follicles	0. Absent; 1. Present
23	Type of follicle dehiscence	0. Longitudinally; 1. Circumscissile
24	Clustering of mature follicles	0. Individual; 1. Clustered
25	Acuminate carpel apices	0. Absent; 1. Present

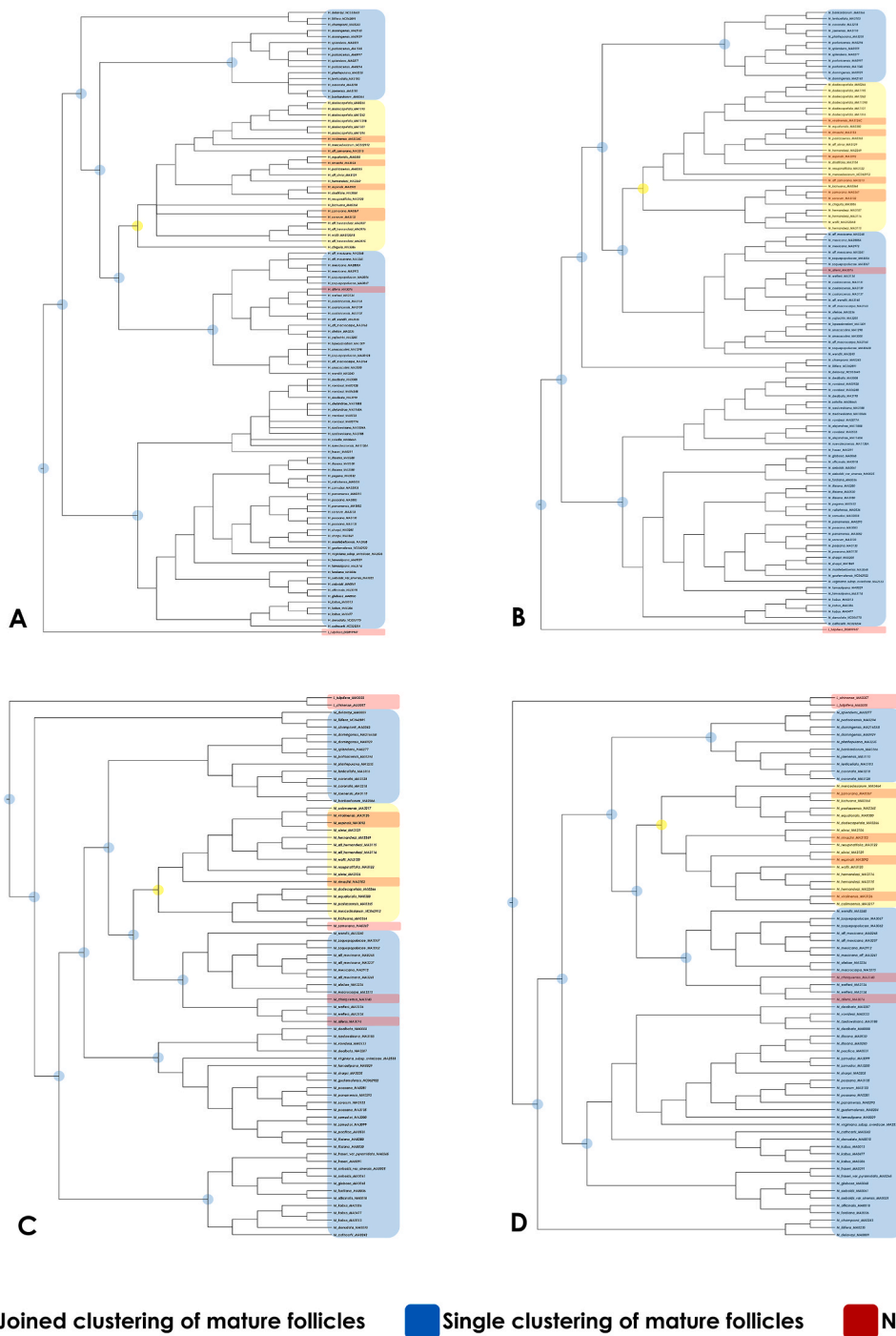
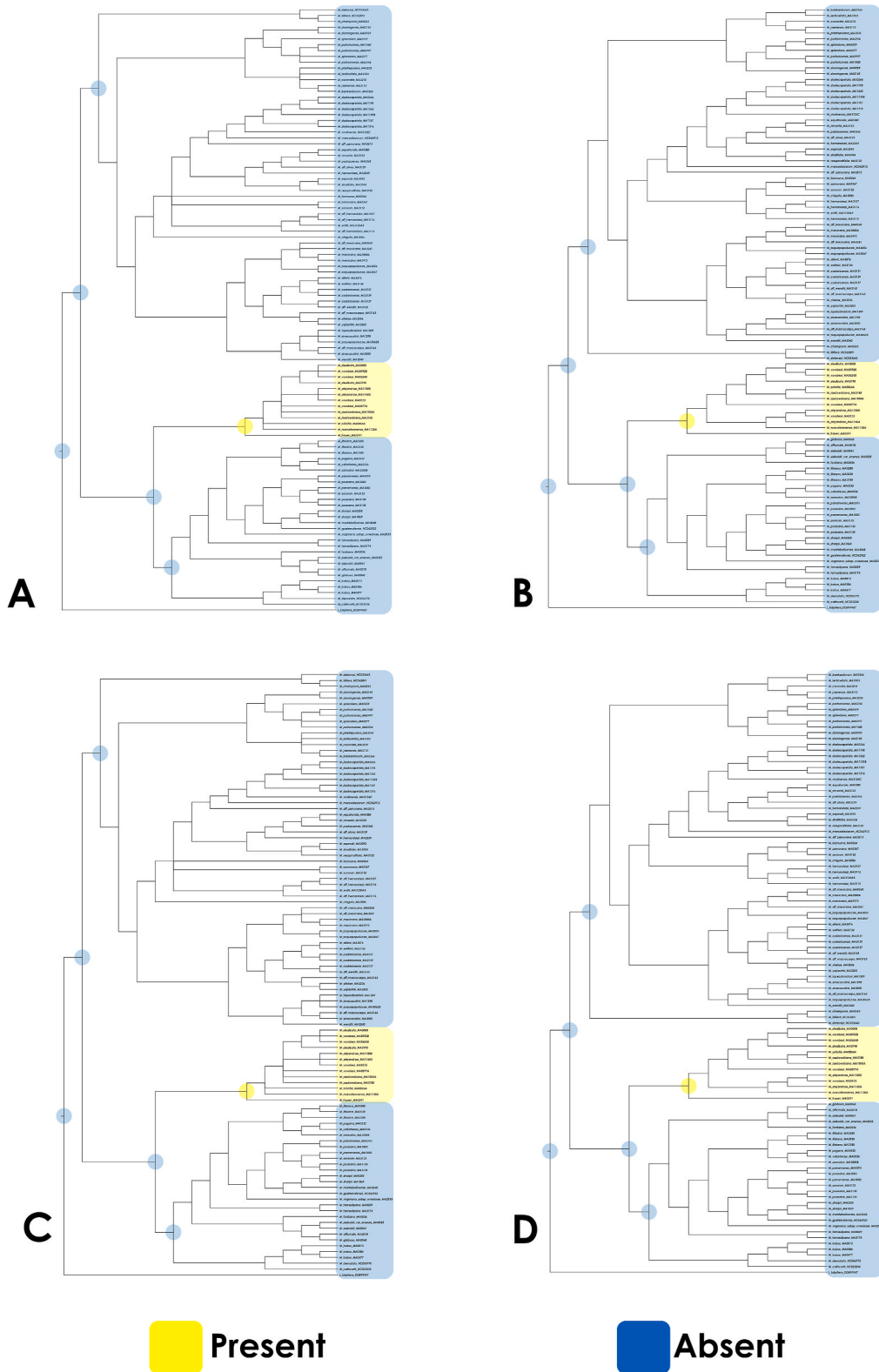


Fig. 4. Ancestral-state reconstructions for the clustering type of mature follicles based on Bayesian Inference (BI) and maximum likelihood (ML) approaches A. Plastid tree based on BI from 104 sequences of 68 Magnoliaceae taxa. B. Plastid tree based on ML from 104 sequences of 68 Magnoliaceae taxa. C. Nuclear tree based on BI from 74 sequences of 58 Magnoliaceae taxa. D. Nuclear tree based on ML from 74 sequences of 58 Magnoliaceae taxa.

3. Discussion

3.1. Evolutionary relationships in Magnolia

Phylogenomic data from both plastomes and nuclei were newly generated and applied here, revealing novel patterns and



(caption on next page)

Fig. 5. Ancestral-state reconstructions for the auriculate and cordate leaf base shapes from 104 plastid sequences of 68 Magnoliaceae taxa based on Bayesian inference (BI) and maximum likelihood (ML) approaches: A. Auriculate leaf base shape ancestral estimation tree based on BI; B. Auriculate leaf base shape ancestral estimation tree based on ML; C. Cordate leaf base shape ancestral estimation tree based on BI; D. Cordate leaf base shape ancestral estimation tree based on ML.

confirming previous patterns in *Magnolia* phylogenetic relationships known from limited nuclear markers [6,8,10,19,20]. On the one hand, all nuclear trees data showed similar topologies (both SL and MSC); on the other hand, the SL plastid phylogenies showed different relationships. However, they all showed a well-supported (PP > 1 or BP > 90) dichotomous pattern separating the *Magnolia* species into two major clades (Figs. 2 and 3; Supplementary Figs. S1 and 2). These corresponded to those reported by Wang et al. [21], also based on plastid data which did not correspond to the three known subgenera [21,34].

Neotropical *Magnolia* species have been poorly represented in previous phylogenetic analyses of the genus worldwide [6,8,10,18,20,21]. Therefore, the present study is the first to focus on the phylogenetic relationships of Neotropical species. This emphasis on the study of underrepresented Neotropical taxa is one of the main differences between this study (which includes just over a third of the described Neotropical species) and previous studies. This is also one of the strengths of our study, as it does not compromise the global representativeness of the genus, nor does it introduce geographical biases, as species from related Asian and Nearctic clades were included.

Although the current Neotropical *Magnolia* species belong to *Magnolia* sect. *Talauma* s.l., *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Magnolia* do not belong to the same major clade. Therefore, we hypothesise that the current Neotropical magnolias arose from two major colonisation events (Guzmán-Díaz et al., in review). In the first event, the ancestral species of *Magnolia* sect. *Talauma* s.l. likely arrived in the Neotropical region about 36 million years ago (MA), initially colonising areas of what are now Colombia and Venezuela. However, this hypothesis is challenged by the relatively recent geological emergence of Colombia compared to Mesoamerican regions [36–38]; Vázquez-García, comm. pers.); it is therefore likely that Colombia was then populated by *Magnolia* species more recently. In the second colonisation event, the ancestor of both *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Magnolia*, which have temperate affinities, would have started to colonise the Neotropics later, around 20 MA, primarily via the Trans-Mexican volcanic belt.

In clade I, the position of the Asian *Magnolia* sect. *Gwillimia* was also interesting, as it has not been stable in previous studies and has changed depending on the type of molecular data used [6,8,10,18,20,21]. When considering plastid data, it is grouped with *Magnolia* sect. *Talauma* s.s. and *Magnolia* sect. *Splendentes*. However, based on nuclear data, *Magnolia* sect. *Gwillimia* is either sister to *Magnolia* sect. *Splendentes* or placed in an early divergent lineage. This incongruence between plastid and nuclear phylogenomic data may be attributed to several factors. One potential explanation for this discrepancy is incomplete lineage sorting, whereby ancestral genetic variation is randomly sorted into descendant lineages, leading to discrepancies in phylogenetic relationships. Another possible explanation is hybridisation or ancient introgression events, wherein genetic material from different lineages is mixed through interbreeding, resulting in a reticulated evolutionary pattern [39–43]. Further investigation of these possible explanations is suggested to better understand the observed patterns.

Within Clade II, *Magnolia* sect. *Macrophylla* (distributed throughout eastern and southern Mexico [13,44–52]) and *Magnolia* sect. *Magnolia* (occurring in eastern and western Mexico, Costa Rica and Panama [13,53–56]) are sister clades that are grouped against all other sections (Figs. 2 and 3). However, this dichotomy is not well supported in the MSC phylogeny (BP = 0.6); this may be due to inconsistent placement of the plastid data. Nevertheless, our phylogenetic hypotheses based on SL nuclear data show the well-supported sister relationship of *Magnolia* sect. *Magnolia* and *Magnolia* sect. *Macrophylla* (PP = 1, Fig. 2; Supplementary Fig. S2). Finally, our plastid phylogenies support with high values the following sister relationships: 1) *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Tuliparia* (PP = 1, Fig. 2; Supplementary Fig. S1), similar to the results of Wang et al. [21] and, 2) *Magnolia* sect. *Magnolia* with a clade containing representatives of *Magnolia* sect. *Rytidospermum*, *Magnolia* sect. *Oyama* and *Magnolia* sect. *Manglietia* (PP = 1, Fig. 2; Supplementary Fig. S1), similar to the results of Kim and Suh [20] and Wang et al. [21].

3.2. Evolutionary relationships in *Magnolia* sect. *Macrophylla*

Morphologically, the most similar group to *Magnolia* sect. *Macrophylla* is *Magnolia* sect. *Tuliparia*; this relationship was recovered only in the plastid trees (Fig. 2; Supplementary Fig. S1) and is consistent with the results of Wang et al. [21], whereas in the MSC nuclear topologies, the Neotropical vs. Nearctic-Asian geographic pattern was maintained (Figs. 2 and 3; Supplementary Fig. S2). Such discrepancies between plastome and nuclear data are common in angiosperms [57–60], and various approaches have been proposed to resolve them (simulations, gene tree dating, network analyses), but there is still no consensus [61–63]. The species complex present in *Magnolia* sect. *Macrophylla* was not featured in previous phylogenetic and phylogenomic studies, but its existence was suspected by population genetic studies [46] and preliminary morphological observations (Samain, comm. pers.). In both cases, it was observed that the ~6 taxa studied could represent fewer entities, as there was no significant genetic differentiation in the populations [46] and morphological variation would correspond to ecotypes rather than species (Samain, comm. pers.).

A comparison with the phylogenomic results obtained confirms this observation, as no geographical or morphological correspondence was found between the taxa. In fact, they all inhabit the Sierra Madre Oriental mountain range in eastern Mexico along a narrow north-south distribution in cloud forests [44,45,47,52,64].

3.3. Evolutionary relationships in *Magnolia* sect. *Magnolia*

The morphologically closest groups to *Magnolia* sect. *Magnolia* are *Magnolia* sect. *Gynopodium* Dandy and *Magnolia* sect. *Manglietia* from Asia and *Magnolia* sect. *Rytidospermum* from Asia and the Nearctic [2,21]. The relationships of the *Magnolia* sect. *Magnolia* have varied in previous works depending on the type of molecular data used. When nuclear sequences have been used, *Magnolia* sect. *Magnolia* is grouped with *Magnolia* sect. *Gynopodium* and *Magnolia* sect. *Gwillimia* [8]. However, in phylogenomic studies using plastid data, it has been placed as a sister group to *Magnolia* sect. *Manglietia* and *Magnolia* sect. *Rytidospermum* [20,21], a pattern that was also recovered in our plastid phylogenies (Fig. 2; Supplementary Fig. S1).

Although the arrangement of the species within *Magnolia* sect. *Magnolia* varied according to the type of data used, a general pattern emerged in which *M. tamaulipana* A.Vázquez (from northeastern Mexico) was the sister species to the rest of the clade in all SL trees (Fig. 2). Furthermore, the close relationship between *M. panamensis*, *M. poasana*, and *M. sororum* could be due to different genetic factors (such as ancient hybridisation or introgression [65–67]), as they are quite dissimilar morphologically.

3.4. Evolutionary relationships in *Magnolia* sect. *Talauma* subsect. *Chocotalauma*

Magnolia sect. *Talauma* subsect. *Chocotalauma* is the most recently described infrageneric category within the genus *Magnolia* segregated from *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* [68]. It is also the least species-rich subsection, with only six taxa endemic to the Chocó biogeographic region (Colombia and Ecuador [69]). Three species were included in the present study: *M. calimaensis* (Lozano) Govaerts, *M. chiguila* F.Arroyo, Á.J.Pérez & A.Vázquez and *M. striatifolia* Little, representing 50 % of the total number of species in the subsection. It is noteworthy that in all our phylogenetic hypotheses they were nested within the southern clade of the *Magnolia* sect. *Talauma* s.s. Moreover, a recent related analysis using complete chloroplast genomes [34] corroborated our results, confirming the same placement within the southern clade of *Magnolia* sect. *Talauma* s.s. for *M. chiguila* and a fourth *Chocotalauma* species, *M. neomagnifolia* I.M.Turner.

3.5. Evolutionary relationships in *Magnolia* sect. *Splendentes* (*magnolia* sect. *Talauma* subsect. *Cubenses* and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*)

Wang et al. [21] proposed the merging of *Magnolia* sect. *Talauma* subsect. *Cubenses* Imkhanitskaja and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* (Lozano) Figlar & Noot. due to the close genetic relationship and common morphological features, they, therefore, reinstated the name section *Splendentes* for the combined clade consisting of the former subsections *Cubenses* and *Dugandiodendron*. However, this change was made based on only four taxa. This relationship is confirmed in this study, as well as in a previous preliminary study with a smaller sample size [34]. Although *Magnolia* sect. *Talauma* subsect. *Cubenses* and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* are closely related, a clear dichotomy was recovered in all the phylogenies obtained (Figs. 2 and 3; Supplementary Figs. S1 and S2), so that each of them could still represent a distinct taxonomic entity.

Magnolia sect. *Splendentes* was proposed by Vázquez-García [13]; the distinguishing features were stamens with the connective extended into a long setiform curved and variously hooked appendage, embedded in the gynoeceum and supporting the stamens. Afterwards, Figlar and Nootboom proposed the subsections *Splendentes* and *Dugandiodendron* based on their difference in carpel dehiscence: circumscissile in *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* and longitudinal in *Magnolia* sect. *Splendentes*. The *Magnolia* sect. *Splendentes* should be correctly referred to as the *Magnolia* sect. *Talauma* subsect. *Cubenses* based on the work of Imkhanitzkaja [70,71]. Based on our evidence we propose to maintain the two subsections separate based on the genetic synapomorphies that support their existence in all phylogenetic hypotheses, as well as the existing geographical separation of the two: Caribbean islands for the *Magnolia* sect. *Talauma* subsect. *Cubenses* versus South America for *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*.

The fact that samples of *M. portoricensis* and *M. splendens* were mixed in the plastome trees could be due to the presence of hybrids or remnants of gene flow between the two taxa, both of which are easily distinguishable (golden pubescence on *M. splendens* leaves) and would not constitute a species complex. Although both occur on the same island, *M. portoricensis* populations extend from the west to the centre [72], while *M. splendens* is endemic to the east in the Sierra de Luquillo [73], so hypothetical hybrids or gene flow would occur in the Carité forest, where *M. portoricensis* occurs and is close to the *M. splendens* area.

3.6. Evolutionary relationships in *Magnolia* sect. *Talauma* subsect. *Talauma*

The separation of the *Magnolia* sect. *Talauma* subsect. *Talauma* into two distinct clades based on geographical distribution highlights the potential for unrecognised complexity within this group and underscores the need for further research focusing on these underrepresented Neotropical taxa. This pattern could be attributed to the possibility that *Magnolia* sect. *Talauma* s.l. species populated the Caribbean islands first from Mesoamerica and later from South America in at least three distinct events: 1) A colonisation inferred from the sister relationship between the islands (Cuba and Hispaniola) and Mexican species of *Magnolia* sect. *Talauma* subsect. *Talauma*; 2) A colonisation of the Lesser Antilles by *M. dodecapetala* from South America; 3) A colonisation of the Caribbean islands inferred from the sister relationship between species of *Magnolia* sect. *Talauma* subsect. *Cubenses* and South American species of *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*. Although the geographical distribution supports the clades, the morphological characters studied (Table 2) provide only one characteristic: the joined clustering of mature follicles (*Syncarpae* group of Vázquez-García et al., p. 4 [14]), a synapomorphy of the southern clade (Fig. 4), since all other branches showed follicles falling individually

(*Apocarpae* and *Follicetae* groups of Vázquez-García et al., p.4 [14]).

In the southern clade of *Magnolia* sect. *Talauma* s.s., no pattern was found in the topologies and the species were intermingled. However, we discuss the cases of *M. dodecapetala* and *M. hernandezii* (Lozano) Govaerts (from southwestern Colombia). On the one hand, *M. dodecapetala* is distributed on five islands in the Lesser Antilles and its sequence data formed a well-supported single clade in the plastid phylogenetic hypotheses (PP = 1; Fig. 2; Fig. S1), where subclades of *M. dodecapetala* could be distinguished according to Veltjen et al. [74]. Our data confirm previous patterns that the Lesser Antilles were most likely colonised by an ancestor related to the extant South American *Magnolia* sect. *Talauma* species [74–76]. Therefore, the samples from the five islands need to be studied in detail morphologically, because genetically there are differences, there is no sign of recent exchange between the islands (in evolutionary terms) and the fruits show different sizes and number of carpels. In fact, it is suggested that they should be recognised and managed as different species, as they have not exchanged genetic material for a long time and will only continue to diverge [77].

On the other hand, all samples of *M. hernandezii* clustered together with high support values (PP = 1) in the MSC tree (Fig. 3). This species has a remarkably wide distribution in Colombia (compared to most other species of *Magnolia* sect. *Talauma*) and is morphologically easily recognised by its large fruits with many carpels and large ovate, leathery, glabrous leaves. Therefore, the unusual pattern in Fig. 3 can be explained by the plastid data; here the type specimen (sample MA2269 from Valle del Cauca) does not cluster with the other *M. hernandezii* individuals. The other samples came from juvenile specimens grown in a botanical garden, from which identifications of other species have already been corrected. In the case of the *M. hernandezii* samples, these could also be other species. In the nuclear topology; however, all the *M. hernandezii* samples clustered together.

3.7. Evolution of morphological traits in *Magnolia*

In general, the morphological traits studied emerged at different times in the evolutionary history of *Magnolia*. This is evidenced by the independent occurrence of these traits in different clades within the phylogenies. In addition, there were few macromorphological differences between Neotropical and other clades, suggesting that despite its disjunct distribution, *Magnolia* morphology is conservative (traits are conserved and present in most extant species worldwide [6,9,78,79]). In particular, deciduous foliage characterises clades with Holarctic distributions (*Magnolia* sect. *Tuliparia*, *Magnolia* sect. *Macrophylla*, *Magnolia* sect. *Oyama*, *Magnolia* sect. *Rytidospermum*, and *Magnolia* sect. *Yulania*), in contrast to the evergreen foliage of the tropical clades (*Magnolia* sect. *Gwillimia*, *Magnolia* sect. *Magnolia*, *Magnolia* sect. *Maingola*, *Magnolia* sect. *Manglietia*, *Magnolia* sect. *Talauma* and *Magnolia* sect. *Splendentes*). Regarding the ASR based on the BI plastid tree, the most likely ancestral foliage deciduousness of our *Magnolia* taxa data set was evergreen. However, this character has changed among the different Neotropical clades: deciduous in *Magnolia* sect. *Macrophylla* and evergreen in *Magnolia* sect. *Magnolia*, *Magnolia* sect. *Splendentes* and *Magnolia* sect. *Talauma*.

In general, traits that are present in only a few clades are derived states, whereas those that are more widely distributed throughout the phylogeny are ancestral states (Supplementary Fig. S3). For example, traits traditionally used to delimit clades, such as the absence of stipular scars on the petioles (absent in *Magnolia* sect. *Magnolia*, *Magnolia* sect. *Maingola*, and *Magnolia* sect. *Splendentes*, or at least not recognisable). This supports the hypothesis that the *Magnolia* morphology is widely conserved with few innovations.

Historically, several morphological characters have been used to delimit infrageneric categories of *Magnolia*, including leaf deciduousness, stipular scars along the petiole, style branch shape, and fruit dehiscence type [1,2,21,80,81]. However, this study included previously overlooked characters, such as bracts, which are often difficult to collect due to their deciduous nature and are rarely found in herbarium specimens [82], similar to stipules [83]. Glabrous bracts were the most likely ancestral state for all our *Magnolia* datasets based on the BI plastid tree. The number of carpels has traditionally been used to distinguish *Magnolia* species [13, 16,17,54,54,68,84,85], but this may not be accurate [86]. Observations and preliminary studies on the fruits of four *Magnolia* sect. *Talauma* species in Veracruz revealed too many outliers and overlapping carpel numbers between species, with variations influenced by light exposure [87]. Some works focused on the morphological characters of *Magnolia* sect. *Macrophylla* and *Magnolia* sect. *Talauma* in Mexico mainly considers the size, number, and colour of structures, while other characters such as hairiness or shape are rarely considered and often involve small sample sizes [64,86,88–90]. Therefore, distinguishing *Magnolia* species requires additional characters and larger sample sizes.

Regarding the separation of *Magnolia* sect. *Talauma* s.s. from *Magnolia* sect. *Talauma* subsect. *Cubenses* and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron*, as mentioned above, the latter has in common the absence of a stipular scar on the petiole, unlike the *Magnolia* sect. *Talauma* s.s., which has it. Although this character has traditionally been used to separate these two groups from the *Magnolia* sect. *Talauma* [1,2,21], when it is analysed in a wider sample of taxa, it is found that it is not unique to them, as they also share it with other groups, so it has appeared at different times in the history of *Magnolia* (i.e., the absence of a stipular scar on the petiole is also present in some -to many-species of *Magnolia* sect. *Gynopodium*, *Magnolia* sect. *Magnolia*, *Magnolia* sect. *Maingola* and *Magnolia* sect. *Michelia*); being a derived state. In addition, *Magnolia* sect. *Talauma* and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* share the circumscissile dehiscence of the carpels (a derived state in *Magnolia*), unlike the *Magnolia* sect. *Talauma* subsect. *Cubenses* which has longitudinal dehiscence (the ancestral form). However, longitudinal dehiscence may not be a true synapomorphy, as it has appeared at different times in other clades from Asia, such as the *Magnolia* sect. *Gwillimia* (sister of *Magnolia* sect. *Talauma* and *Magnolia* sect. *Splendentes*), *Magnolia* sect. *Gynopodium* and *Magnolia* sect. *Maingola*. It is therefore necessary to continue the search for morphological characters to support this arrangement.

The segregation of the *Magnolia* sect. *Talauma* subsect. *Chocotalauma* from the *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* was based on both micromorphology (i.e., the absence of long staminal connective appendages) and macromorphology, (i.e., the presence of globose fruits [68]). However, this micromorphological feature has rarely been studied in *Magnolia*, so it requires more attention and studies to know if it can be a character of taxonomic importance in the genus [17,91]. Furthermore, all six species of

Magnolia sect. *Talauma* subsect. *Chocotalauma* were described from less than 10 specimens each, so these differences in fruit shapes may be masked by the small number of collections.

4. Implications for *Magnolia* infrageneric classification

All phylogenies confirmed that *Magnolia* is divided into two major clades which do not correspond to the three traditionally recognised subgenera. *Magnolia* subg. *Magnolia* is largely polyphyletic and includes species from both major clades according to Wang et al. [21]. Thus, the inclusion of genomic molecular data changes the traditional view and highlights the need to update the infrageneric classification of *Magnolia*.

A key difference between the two classifications concerning Neotropical clades is the separation of the *Magnolia* sect. *Talauma* subsect. *Cubenses* and *Magnolia* sect. *Talauma* subsect. *Dugandiodendron* from *Magnolia* sect. *Talauma* to form the *Magnolia* sect. *Splendentes*. However, our results suggest that *Magnolia* sect. *Splendentes* and *Magnolia* sect. *Talauma* can be further subdivided into two subsections.

The consistent placement of species from the *Magnolia* sect. *Talauma* subsect. *Chocotalauma* within the southern clade of *Magnolia* sect. *Talauma* s.s. in our study, together with the corroborating results of Guzmán-Díaz et al. [34], emphasises the need to re-evaluate the current infrageneric classification of Neotropical *Magnolia*. The observation of this pattern in half of the species from the *Magnolia* sect. *Talauma* subsect. *Chocotalauma* underlines its potential importance. In the light of these results, we propose to reconsider the current classification of this subsection within *Magnolia*.

Based on our character matrix, we did not find any synapomorphy that defines each of the Neotropical lineages, except for the common clustering of mature follicles, which is a synapomorphy of the southern clade of the *Magnolia* sect. *Talauma* s.s. It is therefore proposed to investigate whether there are combinations of characters that can distinguish them from and morphologically support an updated infrageneric classification proposal. We were not able to assess floral parts or micromorphological traits (such as trichomes, stomata numbers or anatomy), so these types of characters may be useful for lineage delimitation together with the character matrix provided here.

5. Conclusions

Our study provides new insights into the phylogenetic relationships within Neotropical *Magnolia*, particularly within the species-rich *Magnolia* sect. *Talauma*. We identified major inconsistencies in the current classification and showed that the three subgenera are not monophyletic. Our results highlight the need for an updated classification of Neotropical clades. In addition, our research highlights the importance of a closer examination of flowers and fruits, particularly through micromorphological characters, to better understand evolutionary patterns within the genus. We also identified complex relationships within the *Magnolia* sect. *Magnolia* and *Magnolia* sect. *Macrophylla*, indicating the need for a more detailed study of these clades. Finally, to gain a more comprehensive understanding of phylogenetic relationships within the genus, future studies should include more samples of Neotropical *Magnolia* and further morphological analysis.

Materials and methods

Taxonomic sampling

Field sampling was carried out between 2016 and 2019 in the following Neotropical countries: Colombia, Costa Rica, Dominica, the Dominican Republic, Ecuador, France (Guadeloupe and Martinique), Mexico, Panama, Peru, Puerto Rico, Saint Lucia, and Saint Vincent, considering information contained in the herbarium vouchers, protologues and other morphological descriptions of the Neotropical *Magnolia* species [13,15–17,47,68,92–95]. The aim was to obtain a representative sampling of Neotropical taxa, and a great effort was made to visit as many localities as possible where populations were known to occur or where, based on previous field observations of vegetation types suitable for the genus, magnolias could be found.

Portions of young leaves (~2 × 2 cm) were sampled and placed in tea bags, which in turn were preserved in plastic bags containing silica gel. Voucher specimens were also collected and deposited in local herbaria in each country and in GENT, IEB and MEXU herbaria (Thiers, continuously updated). When a fresh sample could not be obtained, we extracted from herbarium material (HUA, K, MEDEL, MEXU, PMA, USM, XAL [96]; [Supplementary Tables S1 and S2](#)).

We also included samples representing other major clades of the genus from the Nearctic and Asia, including at least one sample from each of the clades considered to be most closely related to the Neotropical groups, following Wang et al. [21]. These include representatives of *Magnolia* sect. *Gwillimia*, *Magnolia* sect. *Maingola*, *Magnolia* sect. *Manglietia*, *Magnolia* sect. *Oyama*, *Magnolia* sect. *Rytidospermum*, *Magnolia* sect. *Tuliparia* (formerly *Magnolia* sect. *Auriculata*) and *Magnolia* sect. *Yulania* ([Supplementary Tables S1 and S2](#)).

The sampling included a total of 11 sections: all four Neotropical clades, and the remainder from Asia and the Nearctic region ([Supplementary Tables S1 and S2](#)). The final plastid dataset consisted of 104 samples from 70 taxa and the nuclear dataset consisted of 74 samples from 59 taxa; therefore, the plastid and nuclear datasets do not contain exactly the same taxa. Most of the discarded samples were due to poor DNA quality and they corresponded to ~50 species or morphospecies already included in our sampling. Our final sampling covered almost a third of the ~170 recognised Neotropical species and ~20 % of the global *Magnolia* richness.

DNA extraction and sequencing

DNA was extracted using a modified CTAB technique [97] and quality was assessed using a spectrophotometer (Nanodrop 2000 UV–Vis); where possible, 1 000 ng of total DNA was prepared. Samples were stored briefly at -20°C and sent to RAPiD Genomics, Gainesville, Florida, US (www.rapid-genomics.com) for quantification, normalisation, bead-based cleanup, library preparation, and multiplex sequencing using an Illumina HiSeq 3000 (Illumina, San Diego, California, USA) with paired-end 150-bp reads. Two different approaches were used: target capture (TC; to recover nuclear genes) using a taxon-specific bait kit (see details below) and genome skimming (GS; to assemble complete plastid genomes) with an estimated median of 2M PE150 reads per sample and a minimum of 1.8M reads. Standard Illumina library processing was performed by RAPiD Genomics with the modification that depending on fragment size, DNA was mechanically sheared to an average size of 300 bp or not sheared (e.g. for samples with fragmented DNA extracted from herbarium specimens). Standard hybridisation protocols were performed by RAPiD Genomics using the taxon-specific bait kit. Sequence reads were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA; BioProject PRJNA1035522).

For TC, we used a taxon-specific bait set developed for Magnoliaceae (Kim, BioProject ID: PRJNA994423). This bait set was designed using MarkerMiner [98] to identify LSCN genes based on a preliminary assembly of the nuclear genome of *Magnolia kobus* DC. (1.91 Gbp; Kim, unpublished data), transcriptome data from *M. kobus* (Kim, unpublished data), the genome assembly of *Amborella trichopoda* Baill. (Amborellaceae [99]), and the reference genomes of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), *Populus trichocarpa* Torr. & A.Gray ex Hook. (Salicaceae), *Vitis vinifera* L. (Vitaceae) and *Oryza sativa* L. (Poaceae). A total of 504 LSCN were identified, representing a total combined length of 443 Kbp.

Bioinformatic analyses

Demultiplexing of the TC and GS datasets was performed using BCLtofastq, provided by RAPiD Genomics (www.rapid-genomics.com). We performed an initial quality control of the demultiplexed samples using FastQC v. 0.11.7 [100] and multiQC [101]. We then used Trimmomatic v. 0.38 [102] to filter out low-quality reads and perform adapter trimming with a 5:20 sliding window, removing all reads below 30 bp. A second quality check was then performed using FastQC and multiQC to ensure correct adapter removal. We obtained complete plastid genomes (GS data) using the GetOrganelle pipeline [103], which uses Bowtie2 [104], SPAdes [105] and BLAST [106] for the assembly.

We used the HybPiper pipeline v. 1.3 [27] to assemble the TC data, using the BLAST [106] and BWA [107] packages to map the reads to each target locus. Reads were then distributed into folders for each target using Biopython [108], and each target was assembled from the selected reads using SPAdes [105]. Finally, for the assembled contigs for each gene, HybPiper scripts were used to extract the sequences for exons, introns, or both (supercontig) of each target. The BWA approach was chosen for the assembly of the nuclear bait set developed by Kim (in progress) for Magnoliaceae.

Phylogenetic analyses

We used the genus *Liriodendron* L. (Magnoliaceae) as an outgroup, as it is the sister group to the remaining taxa [78,79]. We downloaded 10 additional suitable sequences from GenBank [109] to reinforce the sampling (Supplementary Tables S1 and S2). We treated the entire plastid sequences as a single partition, but aligned each of the nuclear targets separately and then set them as individual partitions in the subsequent analysis. Both maximum likelihood (ML) and Bayesian inference (BI) methods were used. For ML, we employed IQ-Tree v. 2.0.3 [110] with an ultra-fast bootstrap approach to estimate branch support values. For BI analysis, we used MrBayes v. 3.2.7 [111], with the GTR invgamma model, running the analysis for 10 000 000 generations with a burn-in of 25 %.

We performed phylogenetic analyses on both the plastid and nuclear datasets, based on a total of 178 samples (104 plastid samples and 74 nuclear samples); both datasets were aligned using MAFFT v. 7.475 [112]. We then analysed each dataset using BI and/or ML with the following three general approaches: 1. Single-locus (SL) analysis using only the plastid sequences with BI and ML, 2. SL analysis employing only the nuclear targets with BI and ML, and 3. Multi-locus analysis using a multispecies coalescent (MSC) model analysis in ASTRAL-III [113] with nuclear data only. For this analysis, we inferred ML gene trees from the aligned nuclear data using IQTREE v.2.0.3 [110] with 1000 ultrafast bootstraps using the -bb option. These gene trees were then used as input for ASTRAL-III v. 5.7.3 to infer a species tree. The output trees from approaches 1 and 2 are shown in tanglegram for comparison.

These different phylogenomic approaches were used to avoid bias in the analyses and allowed us to explore the phylogenetic relationships within *Magnolia* more thoroughly. In addition, by using both plastid and nuclear data, we aimed to obtain a more comprehensive picture of the evolutionary relationships between the taxa.

Morphological studies and ancestral-state reconstructions

A total of about 800 vouchers of Neotropical *Magnolia* species were carefully examined in detail, including ~250 type specimens examined in JSTOR Global Plants [114] or in digital herbaria (F, MEXU, MO, P [96]). We used several databases such as the International Plant Names Index [3], Plants of the World Online [4] and Tropicos [5] to consult the names and protologues of Neotropical *Magnolia*; the oldest protologues were downloaded from the Biodiversity Heritage Library (BHL; biodiversitylibrary.org). In addition, we analysed photographs taken in the field and consulted morphological descriptions of Neotropical *Magnolia* from several literature sources to complement the study [13,15–17,70,71,91–94,115–117].

Most of the resources were digitally examined due to limitations imposed by COVID-19 at the time of the study, so characters based on textures, type of trichomes and measurements (diameter, length and width of organs) were excluded. Only 25% of the *Magnolia* collections had flowers, and these were often poorly preserved on vouchers, making them difficult to characterise, so traits based on them could not be considered here.

We compiled a list of 25 macromorphological characters that were expected to distinguish clades and morphospecies (Table 2). The definitions of the discrete traits and their qualitative states were based on Moreno [118], while a codification of the single continuous character and its quantitative state (maximum tree height) was made following Thiele's approach [119], as follows:

$$C = \left(\frac{H_i - H_{\min}}{H_{\max} - H_{\min}} \right) S$$

C: character codification; H_i : height; H_{\min} : minimum height (4 m); H_{\max} : maximum height (45 m); S: arbitrary number of states of character (5).

Ancestral character-state reconstructions (ASRs) were performed on the 25 macromorphological traits. These were carried out in ape [120] using the *ace* (Ancestral State Estimation) function, with the type parameter set to discrete and the method set to ML. Trees were then plotted using the *plotTree* function from the phytools [121] package. Both packages were used in the R programming language v. 4.3.2 (R Core Development Team, 2008). We repeated these analyses for the five phylogenetic trees resulting from the approaches described above (SL and MSC) using the same taxa dataset.

CRedit authorship contribution statement

Fabián A. Aldaba Núñez: Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Salvador Guzmán-Díaz:** Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Emily Veltjen:** Visualization, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Pieter Asselman:** Resources, Investigation. **José Esteban Jiménez:** Resources, Investigation. **Jorge Valdés Sánchez:** Resources, Investigation. **Ernesto Testé:** Writing – review & editing, Resources, Investigation, Resources, Investigation. **Guillermo Pino Infante:** Resources, Investigation. **Daniel Silva Sierra:** Resources, Investigation. **Ricardo Callejas Posada:** Resources, Investigation. **Francisco Hernández Najarro:** Resources, Investigation. **Isabel Larridon:** Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Suhyeon Park:** Resources, Methodology, Investigation. **Sangtae Kim:** Resources, Methodology, Investigation. **Esteban M. Martínez Salas:** Supervision, Resources, Investigation. **Marie-Stéphanie Samain:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Data availability statement

The nuclear dataset (target capture) is available in the Sequence Read Archive (SRA) under the BioProject: PRJNA1035522, which can be accessed at the following link: <https://www.ncbi.nlm.nih.gov/sra/>

The plastid dataset (genome skimming) is available on GenBank with the accession numbers: OR730675 – OR730764 and can be accessed via the following link: <https://www.ncbi.nlm.nih.gov/genbank/>

The 4597 baits designed in collaboration with RapidGenomics to capture the identified low- to single-copy nuclear genes are hosted on Zenodo: <https://zenodo.org/doi/10.5281/zenodo.10428043>.

The sequence assembly lengths of the target used reported by HybPiper are also available on Zenodo: <https://zenodo.org/doi/10.5281/zenodo.10428090>.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e39430>.

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