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Ernesto Testé Lozano

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# Integrative taxonomic analysis of *Magnolia* subsect. *Talauma* (*Magnoliaceae*) in Cuba

Analyse taxonomique intégrative des espèces cubaines de *Magnolia*  
subsect. *Talauma* (*Magnoliaceae*)

**Thèse de doctorat de l'université Paris-Saclay et de l'université de  
La Havane**

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(Université de La Havane), sous la direction de **Thierry ROBERT**, maître de  
conférences, et la co-direction d'**Eldis R. BÉCQUER GRANADOS**, chercheur principal.

**Thèse soutenue à La Havane, le 14 juillet 2023, par**

**Ernesto TESTÉ LOZANO**

## Composition du Jury

Membres du jury avec voix délibérative

<b>Roberto ALONSO BOSCH</b> Chargé de recherche, Université de La Havane	Président
<b>Ángel MOTITO</b> Chercheur Principal (eq. HDR), BIOECO	Rapporteur & Examineur
<b>Nathalie MACHON</b> Professeure, Muséum National d'Histoire Naturelle	Rapporteuse
<b>Leneidy PÉREZ PELEA</b> Professeur, Université de La Havane	Examinatrice
<b>Juan FERNÁNDEZ-MANJARRES</b> Directeur de recherche, Université Paris-Saclay	Examineur
<b>René Pablo CAPOTE LÓPEZ</b> Directeur de recherche, IES	Examineur

**Titre :** Analyse taxonomique intégrative des espèces cubaines de *Magnolia* subsect. *Talauma* (*Magnoliaceae*)

**Mots clés :** concept d'espèce, délimitation des espèces, structure génétique, phylogénie, flux génétique, hybridation, marqueurs génétiques, microsatellites, modèles de niche écologique, morphométrie géométrique

**Résumé :** Une délimitation taxonomique précise, basée sur une compréhension complète des processus évolutifs impliqués dans la différenciation des taxons, peut être obtenue à partir d'une combinaison d'approches écologiques, morphologiques et moléculaires. La taxonomie de *Magnolia* subsect. *Talauma* à Cuba a longtemps été débattue et exclusivement basée sur une étude morphologique traditionnelle d'un nombre limité d'individus. L'objectif de la présente étude est de délimiter les taxons cubains de *Magnolia* subsect. *Talauma* afin de mettre à jour leur traitement taxonomique par l'intégration de critères écologiques, morphologiques, génétiques et phylogénétiques.

Des échantillons pour les analyses écologiques (858), morphologiques (243), génétiques (461) et phylogénétiques (7) ont été collectés sur l'ensemble de l'aire de répartition. Pour toutes les analyses, les résultats et les représentations graphiques ont été construits en utilisant le système de classification à quatre taxons. Ce système a divisé la variabilité morphologique en le plus grand nombre d'unités et a été utilisé sur le terrain pour identifier chaque taxon. Afin d'éviter toute confusion avec les noms scientifiques des espèces et les différents traitements taxonomiques effectués sur le groupe, les taxons ont été traités comme des groupes morphologiques (*Orbiculata*, *Minor*, *Oblongifolia*, *Ophiticola*).

La variabilité de chaque groupe a été analysée à travers des modèles de niche écologique, des morphométries multivariées et géométriques, 21 marqueurs génétiques et un plastome entièrement séquencé. *Orbiculata* avait les plus petites zones de convenance de niche, et les groupes de la région nord-est ont montré un niveau élevé de similarité écologique. La variabilité morphologique observée des feuilles était différente entre les groupes, et de celle décrite par les études précédentes. Les résultats génétiques ont montré trois comme le nombre le plus probable de groupes génétiques, avec un niveau élevé d'admixture génétique entre les groupes du nord-est de Cuba. L'existence d'individus purs, de rétrocroisements vers *Minor* et de F2 dans les groupes du nord-est de Cuba a été détectée.

La phylogénie du plastome valide la monophylie de *Magnolia* subsect. *Talauma* à Cuba, mais ne résout pas la délimitation taxonomique des groupes du nord-est de Cuba. *Orbiculata* a été clairement distingué des autres groupes de la sous-section sur la base des différentes sources de preuves qui soutiennent fortement leur statut d'espèce valide. Les principaux problèmes taxonomiques concernent les populations du nord-est de *Magnolia* subsect. *Talauma* à Cuba. Les données morphologiques et génétiques ont soutenu l'existence de deux groupes : correspondant principalement à *Minor-Oblongifolia* et *Ophiticola*.

**Title :** Integrative taxonomic analysis of *Magnolia* subsect. *Talauma* (*Magnoliaceae*) in Cuba

**Keywords :** species concept, species delimitation, genetic structure, phylogeny, gene flow, hybridization, genetic markers, microsatellites, ecological niche models, geometric morphometrics

**Abstract :** An accurate taxa delimitation, based on a full understanding of evolutionary processes involved in taxon differentiation, can be gained from a combination of ecological, morphological, and molecular approaches. The taxonomy of *Magnolia* subsect. *Talauma* in Cuba has long been debated and exclusively based on a traditional morphological study of a limited number of individuals. The goal of the present study is to delimit the Cuban taxa of *Magnolia* subsect. *Talauma* in order to update their taxonomic treatment through the integration of ecological, morphological, genetic, and phylogenetic criteria.

Samples for the ecological (858), morphological (243), genetic (461), and phylogenetic (7) analyses were collected throughout the entire distribution range. For all the analyses, the results and graphical representations were built using the four taxa classification system. This system divided the morphological variability into the highest number of units and was used in the field to identify each taxon. In order to avoid confusion with the scientific names of the species and the different taxonomic treatments carried out on the group, the taxa were treated as morphological groups (Orbiculata, Minor, Oblongifolia, Ophiticola).

The variability of each group was analysed through ecological niche models, multivariate and geometric morphometrics, 21 genetic markers, and a fully-sequenced plastome. Orbiculata had the smallest areas of niche suitability, and the groups from northeastern region showed a high level of ecological similarity. The observed leaf morphological variability was different between groups, and from that described by previous studies. The genetic results showed three as the most probable number of genetic groups, with a high level of genetic admixture between the groups from northeastern Cuba. The existence of pure individuals, backcrosses to Minor, and F2 in the groups from northeastern Cuba were detected.

The plastome phylogeny validates the monophyly of *Magnolia* subsect. *Talauma* in Cuba, but does not solve the taxonomic delimitation of the groups from northeastern Cuba. Orbiculata was clearly distinguished from the other groups of the subsection based on the different sources of evidence that strongly supported their status as a valid species. The main taxonomic issues concern northeastern populations of *Magnolia* subsect. *Talauma* in Cuba. The morphological and genetic data supported the existence of two groups: corresponding mainly to Minor-Oblongifolia and Ophiticola.



**Título :** Análisis taxonómico integrativo de *Magnolia* subsect. *Talauma* (*Magnoliaceae*) en Cuba

**Palabras clave :** concepto de especie, delimitación de especies, estructura genética, filogenia, flujo genético, hibridación, marcadores genéticos, microsatélites, modelos de nicho ecológico, morfometría geométrica

**Resumen :** Una delimitación de taxones más precisa, basada en una comprensión completa de los procesos evolutivos involucrados en la diferenciación de los taxones, se puede obtener a partir de una combinación de enfoques ecológicos, morfológicos y moleculares. La taxonomía de *Magnolia* subsect. *Talauma* en Cuba ha sido ampliamente debatida, y se ha basado exclusivamente en estudios morfológicos tradicionales de un número limitado de individuos. El objetivo del presente estudio es delimitar los taxones cubanos de *Magnolia* subsect. *Talauma* para actualizar su tratamiento taxonómico mediante la integración de criterios ecológicos, morfológicos, genéticos y filogenéticos.

Se colectaron muestras para los análisis ecológicos (858), morfológicos (243), genéticos (461) y filogenéticos (7) en todo el rango de distribución del grupo. Para todos los análisis, los resultados y las representaciones gráficas se construyeron utilizando el sistema de clasificación de cuatro taxones. Este sistema divide la variabilidad morfológica en el mayor número de unidades y se utilizó en campo para identificar cada taxón. Para evitar confusiones con los nombres científicos de las especies y los diferentes tratamientos taxonómicos realizados sobre el grupo, los taxones fueron tratados como grupos morfológicos (Orbiculata, Minor, Oblongifolia, Ophiticola).

La variabilidad de cada taxón se analizó a través de modelos de nicho ecológico, morfometría multivariada y geométrica, 21 marcadores genéticos y la secuencia completa del cloroplasto. Orbiculata presentó las áreas más pequeñas de idoneidad climática, mientras que los taxones del norte de oriente mostraron altos niveles de similitud ecológica. La variabilidad morfológica de la hoja fue diferente entre grupos y con respecto a la descrita por estudios previos. Los resultados genéticos mostraron a tres como el número más probable de grupos genéticos, con un alto nivel de mezcla genética entre los taxones del norte de oriente. Se detectó la existencia de individuos puros, retrocruzamientos con Minor y F2 en los taxones del norte de Cuba oriental.

La filogenia del cloroplasto valida la monofilia de *Magnolia* subsect. *Talauma*, pero no resuelve la delimitación taxonómica de los grupos del norte de oriente. Orbiculata se distinguió claramente de los otros taxones de la subsección en función de las diferentes fuentes de evidencia, lo que respaldó su condición de especie válida. Las principales cuestiones taxonómicas se refieren a las poblaciones de *Magnolia* subsect. *Talauma* del norte de oriente. Los datos morfológicos y genéticos apoyaron la existencia de dos grupos correspondientes principalmente a Minor-Oblongifolia y Ophiticola.

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## RÉSUMÉ DÉTAILLÉ DE LA THÈSE

Définir ce qu'est une espèce a fait l'objet de longs débats dans l'histoire de la biologie, débats qui ont produit de multiples concepts d'espèces au fil du temps. Au cours des deux dernières décennies, l'idée selon laquelle les espèces peuvent être définies comme des lignées de métapopulations évoluant séparément (SC unifié) s'est de plus en plus répandue au sein de la communauté scientifique. Délimiter les espèces nécessite ainsi d'accumuler des preuves que les taxons considérés évoluent actuellement de manière indépendante. Il est maintenant largement reconnu qu'une délimitation précise des taxons, basée sur une compréhension complète des processus évolutifs impliqués dans la différenciation des taxons, peut être obtenue à partir d'une combinaison d'approches écologiques, morphologiques et moléculaires pour évaluer la diversité des taxons et leur différenciation sur l'ensemble de leur aire de répartition géographique. Cette approche multi-niveaux est connue sous le nom de taxonomie intégrative.

Le genre *Magnolia* est un bon modèle pour explorer l'efficacité d'une approche intégrative dans des problèmes taxonomiques complexes. Les données moléculaires récentes remettent en question la délimitation des espèces basée sur la morphologie. La taxonomie de *Magnolia* subsect. *Talauma* à Cuba a longtemps fait débat avec de nombreux changements dans le nombre, et les limites des espèces au cours du siècle dernier. Toutes les révisions taxonomiques étaient exclusivement basées sur les descripteurs morphologiques traditionnels des feuilles, et sur seulement quelques individus, limitant leur capacité à élucider les limites des taxons. La présente étude propose l'objectif suivant: délimiter les taxons cubains de *Magnolia* subsect. *Talauma* afin de mettre à jour leur traitement taxonomique par l'intégration de critères écologiques, morphologiques, génétiques et phylogénétiques.

Les échantillons pour les analyses écologiques, morphologiques et génétiques ont été collectés (2015-2020) sur des individus représentant tous les taxons cubains de *Magnolia* subsect. *Talauma*, dans toute leur aire de répartition dans les montagnes de Nipe-Sagua-Baracoa et Sierra Maestra à l'Est de Cuba. Sur le terrain, les individus ont été identifiés en fonction de la forme des arbres et des feuilles selon les critères morphologiques qui a défini le plus grand nombre d'unités taxonomiques. Pour toutes les analyses, les résultats et les représentations graphiques ont été construits en utilisant le système de classification de quatre espèces. Afin d'éviter toute confusion avec les noms scientifiques des espèces et les différents traitements taxonomiques effectués sur le groupe, les taxons ont été considérés comme des groupes morphologiques (*Orbiculata*, *Minor*, *Oblongifolia*, *Ophiticola*), et ils ont été traités comme tels dans toutes les analyses suivantes.

Pour l'analyse écologique, les coordonnées GPS ont été enregistrées pour chaque individu. Un ensemble des variables bioclimatiques a été choisi comme prédicteur de niche écologique. Après un processus de calibration du modèle, les modèles finaux ont été exécutés dans Maxent v. 3.4.1. Un test d'identité a été utilisé afin d'examiner la divergence de niche entre les groupes. Les valeurs de pixel pour chaque couche environnementale ont été extraites à chaque point d'occurrence du *Magnolia* subsect. *Talauma*, et utilisées pour évaluer les différences de niche entre les groupes à l'aide d'une analyse en composantes principales (ACP). Pour les analyses morphologiques, 243 individus ont été analysés. Deux types d'analyses morphologiques ont été effectuées sur trois ensembles de variables indépendantes, permettant d'appréhender la forme de la feuille. La signification statistique des différences entre les groupes pour chaque variable mesurée a été évaluée. La variabilité de l'ensemble de l'échantillon a été décrite à l'aide d'une ACP. Une approche de classification bayésienne basée sur des modèles de mélanges de distributions a été réalisée en utilisant chacun des trois ensembles de données de variables morphologiques.

Pour les analyses génétiques, l'ADN des échantillons de jeunes feuilles saines d'un total de 461 individus a été extrait. Les individus ont été génotypés à l'aide de 21 marqueurs microsatellites. Les valeurs de diversité génétique et la différenciation entre les groupes ont été estimées. L'identification des clusters génétiques et l'attribution des individus ont été réalisées à l'aide de STRUCTURE et de l'analyse DAPC. La présence ou l'absence d'individus hybrides a été estimée avec NEWHYBRIDS. La reconstruction phylogénétique a été réalisée avec un plastome entièrement séquencé (deux individus pour: *Minor*, *Oblongifolia* et *Ophiticola*; un individu pour *Orbiculata*). En outre, un échantillon de chaque taxon des différentes régions des Caraïbes et des sous-sections *Magnolia* ont été inclus dans l'analyse.

Presque tous les modèles de niche écologique réalisés pour chaque groupe ont montré des régions potentiellement appropriées dans de vastes zones des montagnes de Nipe-Sagua-Baracoa et de la Sierra Maestra. *Orbiculata* était le groupe avec la plus petite zone d'aptitude potentielle, ce qui pourrait s'expliquer par le faible nombre d'individus connus et leur répartition géographique limitée. L'analyse du test d'identité de niche a indiqué des niveaux élevés de chevauchement de niche entre les différents groupes. L'ACP a montré que le groupe *Orbiculata* possède la niche environnementale distincte la plus petite sans chevauchement avec celle des autres groupes. Les groupes du nord-est de Cuba vivent dans des conditions environnementales similaires.

Pour la plupart des douze variables, des différences significatives entre chaque paire de groupes ont été trouvées. Sur la base de l'ACP pour les trois ensembles de variables, les différents groupes taxonomiques ont montré peu de chevauchement dans l'espace d'ordination, ainsi que des moyennes statistiquement différentes les unes des autres. La variabilité morphologique des feuilles observée pour les magnolias de Cuba était plus forte que celle décrite par les études précédentes, probablement en raison de la plus grande taille de l'échantillon utilisé dans le présent travail et de sa plus large représentativité géographique. Les variables linéaires et angulaires ont permis une discrimination claire entre *Orbiculata*, *Ophitcola* et *Minor*, ce dernier groupe étant divisé en deux sous-groupes. L'un d'eux était commun avec la majorité des individus d'*Oblongifolia*. Les contours de la feuille ont permis d'obtenir une distinction très claire entre *Orbiculata* et les trois autres groupes. L'analyse effectuée sur la matrice de Landmark a montré un schéma similaire à celui obtenu avec les variables linéaires et angulaires pour *Minor*, *Ophitcola* et *Orbiculata*. Le niveau élevé de différenciation morphologique entre les taxons dans cette étude renforce la valeur des caractéristiques foliaires dans les études taxonomiques des magnolias cubains.

Les groupes ont montré des valeurs de diversité génétique similaires, avec les valeurs les plus basses chez *Orbiculata*. Son aire de distribution plus réduite que celle des autres groupes, ainsi que son relatif isolement géographique permet de supposer que la variabilité génétique plus faible chez *Orbiculata* pourrait traduire un effectif efficace plus faible que ceux des autres groupes. La différenciation génétique entre les taxons était relativement élevée ( $F_{ST}$  global = 0,10,  $D_{JOST}$  = 0,23), *Orbiculata* contribuant principalement à ce résultat, tandis que *Minor* et *Oblongifolia* étaient les deux taxons les moins différenciés l'un de l'autre. L'analyse de classification bayésienne a permis d'identifier sans ambiguïté trois groupes génétiques, correspondant majoritairement à *Orbiculata*, *Minor* (comprend *Oblongifolia*) et *Ophitcola*, respectivement. Elle montre aussi l'existence d'admixture génétique entre ces groupes, essentiellement au sein des groupes du nord-est de Cuba. Ces résultats ont été confirmés dans une analyse de classification multivariée (méthode DAPC). L'analyse NEWHYBRIDS a permis de préciser que les individus montrant des profils génétiques admixés au sein des taxons du nord-est Cuba sont vraisemblablement des individus issus de rétrocroisements sur *Minor*, à partir d'hybrides (*Minor* x *Ophitcola*) et des individus de type F2. Par ailleurs, certains individus d'*Ophitcola* avaient une assignation génétique purement *Minor*, ce qui appuie l'existence d'introgessions génétiques entre ces espèces. Les données génétiques démontrent ainsi l'existence de flux de gènes dans les zones hybrides entre *Minor* et *Ophitcola*.

La phylogénie a montré que les taxons cubains de *Magnolia* subsect. *Talauma* forment un groupe monophylétique. Les talaumas sont plus proches des espèces de *Talauma* de Mésoamérique que des espèces cubaines de *Magnolia* subsect. *Talauma*. Les espèces cubaines de *Magnolia* subsect. *Cubenses*, sont plus proches des espèces de la République dominicaine et de Porto Rico. Le groupe *Orbiculata* forme un clade très bien soutenu. Le groupe du nord-est de Cuba forme lui aussi un clade bien soutenu au sein duquel il n'est pas possible d'établir des limites claires entre les différents groupes (*Minor*, *Oblongifolia*, *Ophiticola*).

Dans la présente étude, *Orbiculata* a été clairement distingué des autres groupes de *Magnolia* subsect. *Talauma* à Cuba sur la base des données écologiques, morphologiques, génétiques et des reconstructions phylogénétiques. Ces résultats soutiennent fortement la conclusion selon laquelle *Orbiculata* peut être considérée comme une espèce bien délimitée: *Magnolia orbiculata* (Britton & P. Wilson) Palmarola. Sans aucun doute, nos données ont confirmé les problèmes taxonomiques épineux que posent les populations du nord-est de Cuba de *Magnolia* subsect. *Talauma*. Les résultats de la thèse me conduisent à rejeter les systèmes de classification précédents proposés pour le groupe d'espèces à Cuba. En ce sens, les individus considérés comme *Minor* et *Oblongifolia* sont génétiquement très proches. En revanche, malgré l'existence notable d'introgressions génétiques entre *Minor* et *Ophiticola*, ce dernier groupe constitue clairement une entité génétique propre. Bien que différenciation ne soit pas complète entre les deux groupes, les différences morphologiques et génétiques soutiennent l'existence de deux lignées évolutives différentes.

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## ABBREVIATIONS

**B:** Herbarium “Botanischer Garten, Freie Universität Berlin” (Berlin, Germany)

**B<sub>ci</sub>:** Calculated Index of Bisse

**BC-Oph:** Backcross to *Ophitcola*

**Bio12:** Annual precipitation

**Bio15:** Precipitation seasonality (Coefficient of variation)

**Bio5:** Maximum temperature of warmest month

**Bio7:** Temperature annual range

**BM:** Herbarium “The Natural History Museum” (London, United Kingdom)

**BR:** Herbarium “Meise Botanic Garden” (Meise, Belgium)

**DAPC:** Discriminant Analysis of Principal Components

**DEM:** Digital Elevation Model

**DNA:** Deoxyribonucleic Acid

**EDMA:** Euclidean Distance Matrix Analysis

**ENMs:** Ecological Niche Models

**ESC:** Ecological Species Concept

**F1:** First Generation Hybrid

**F2:** Second Generation Hybrid

**G:** Herbarium Conservatoire et Jardin botaniques de la Ville de Genève (Genève, Switzerland)

**GH:** Herbarium “Harvard University” (Massachusetts, U.S.A.)

**GM:** Geometric Morphometrics

**GPS:** Global Positioning System

**GSC:** Genetic Species Concept

**HAC:** Herbarium “Instituto de Ecología y Sistemática” (Havana, Cuba)

**HAJB:** Herbarium “Jardín Botánico Nacional, Universidad de La Habana” (Havana, Cuba)

**HFC:** Acronyms of the serial numbers of collections of Herbario de la Flora de Cuba

**JE:** Herbarium “Friedrich Schiller University Jena” (Jena, Germany)

**K:** Herbarium “Royal Botanic Gardens” (Kew, United Kingdom)

**LE:** Herbarium “Komarov Botanical Institute of RAS” (Saint Petersburg, Russia)

**LS:** Herbarium “Colegio de La Salle” (Havana, Cuba) transferred to HAC

**m.a.s.l.:** meter above sea level

**MANOVA:** Multivariate Analysis of Variance

**MCMC:** Markov Chain Monte Carlo

**ML:** Maximum Likelihood

**MM:** Multivariate Morphometrics

**MSC:** Morphological Species Concept

**mya:** millions of years

**NPMANOVA:** not parametric Multivariate Analysis of Variance

**NY:** Herbarium "The New York Botanical Garden" (New York, U.S.A.)

**P:** Herbarium "Muséum National d'Histoire Naturelle" (Paris, France)

**PCA:** Principal Component Analysis

**PCR:** Polymerase Chain Reaction

**PCs:** Principal Components

**pers. obs.:** personal observation

**PSC:** Phylogenetic Species Concept

**S:** Herbarium "Swedish Museum of Natural History" (Stockholm, Sweden)

**SC:** Species Concepts

**SSR:** Simple Sequence Repeats (microsatellite markers)

**SV:** Herbarium "Antigua Estación Experimental Agronómica" (Havana, Cuba), transferred to HAC

**US:** Herbarium "Smithsonian Institution" (Washington DC, U.S.A.)

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## INTRODUCTION

Defining what a species is has been the subject of long debates in the history of biology, debates that have produced multiple species concepts (SC) over time (e.g., Genetic SC, Morphological SC, Phylogenetic SC, Ecological SC, Biological SC, among others (Mayr 1996; de Queiroz 2007). However, as Hey (2006) pointed out, this theoretical dilemma should not hinder the fact that biologists agree about general and straightforward ideas such as species are fundamental units in biology and that individuals belonging to the same species share a higher co-ancestry than individuals from other species. As this author states, the problem arises when one defines criteria for defining a species because these criteria are linked to methodologies used to delineate species, therefore leading to different ways to define species. In the last two decades, there has been an ever-growing shared idea that species can be defined as separately evolving metapopulation lineages (Unified SC) (de Queiroz 1998, 2007). Therefore, delimiting species boundaries calls for accumulating evidence that the considered taxa are currently evolving independently. This task is especially challenging for taxa that have recently diverged due to several evolutionary and genetic factors previously described (see Naciri & Linder 2015, for a review).

It is now largely recognized that an accurate taxon delimitation, based on a full understanding of the evolutionary processes involved in taxon differentiation, can be gained from, and even should rely on, a combination of ecological, morphological, and molecular approaches to assess within-taxon diversity and among-taxon differentiation, across their whole geographical range (de Queiroz 2007; Padial *et al.* 2010). The combination of several species concepts to broadly support species limits is known as integrative taxonomy (Padial *et al.* 2010). Dayrat (2005) and Will *et al.* (2005) recommended that species should only be named when their limits are supported by multiple lines of evidence. Integrative taxonomy does not replace traditional taxonomy but uses complementarity among disciplines to improve accuracy (Schlick-Steiner *et al.* 2010; Yeates *et al.* 2011). The potential for such integrative taxonomic approaches has not yet been fully

embraced in botany, particularly in the tropics (Damasco *et al.* 2019). The works of Zheng *et al.* (2017), Alvarado-Sizzo *et al.* (2018), Damasco *et al.* (2019), Denham *et al.* (2019), Moein *et al.* (2019), Yang *et al.* (2019) are good examples of the use of an integrative approach carried out to solve the taxonomic problems in different plant families.

The genus *Magnolia* L. is a good model for applying an integrative taxonomic approach. It is the largest genus of the family *Magnoliaceae* Juss. It includes three subgenera, 13 sections, and an equal number of subsections (Figlar & Nootboom 2004; Veltjen *et al.* 2022). *Magnolia* section *Talauma* Baill., with a Neotropical distribution, includes around 120 species distributed in four subsections: *Dugandiodendron* Lozano, *Chocotalauma* A. Vázquez, Á.J. Pérez and F. Arroyo, *Cubenses* Imkhan., and *Talauma* Juss. (Figlar & Nootboom 2004; Vázquez-García *et al.* 2017). The 85 taxa of the subsection *Talauma*, the most species-rich of all *Magnolia* subsections, occur in lowlands and mountainous areas (0 - 3 300 m.a.s.l.) of Central and South America and the Caribbean Islands (Vázquez-García *et al.* 2017). The genus *Magnolia* includes species that could be distinguished morphologically and genetically conservative (Treseder 1978; Callaway 1994; Lee & Chappell 2008; Li *et al.* 2013; Shen *et al.* 2018; Sun *et al.* 2020). Recent molecular research is challenging species delimitation based on morphology (Azuma *et al.* 2011; Rico & Gutiérrez-Becerril 2019; Aldaba-Núñez *et al.* 2021). The majority of these studies conclude that more evidence from ecology and morphology is needed to understand the discrepancies with molecular data.

Cuba has the highest diversity of magnolias among the Caribbean islands, with seven endemic taxa (Veltjen *et al.* 2019). The Cuban taxa of *Magnolia* belong to two sections, *Magnolia* and *Talauma*. The section *Talauma*, the most diverse with six taxa, is represented by two subsections in Cuba: *Cubenses* and *Talauma*. The taxonomy of *Magnolia* subsect. *Talauma* in Cuba has long been debated, although based until now only on leaf morphology. The first taxon described was *Talauma minor* Urb. followed by *T. orbiculata* Britton & P. Wilson, in 1912

and 1923, respectively (Urban 1912; Britton 1923). Howard (1948) recognized two species of *Talauma*, *T. minor* Urb., and *T. truncata* (Moldenke) R.A. Howard, previously described by Moldenke (1946) as *Svenhedinia truncata* Moldenke. Two years later, León & Alain (1950) described a variety of *Talauma minor* with extremely oblong leaves that they named *T. minor* var. *oblongifolia* León. In the taxonomic treatment for the "Flora of Cuba", León & Alain (1951) mentioned four taxa of *Talauma*: *T. minor* var. *minor*, *T. minor* var. *oblongifolia*, *T. orbiculata*, and *T. truncata*. These authors distinguished *T. orbiculata* and *T. truncata* based on the largely truncate leaves of the latter, which inhabits only areas around Pico Turquino (León & Alain 1951). However, due to the large variation of leaf-base shape observed in these two taxa, they were not recognized as separated entities in the subsequent taxonomic reviews of the group (e.g., Bisse 1988; Imkhanitzkaja 1993; Palmarola *et al.* 2016).

In the Supplement for the "Flora of Cuba" published by Alain (1969), he suggested that all previously described taxa of *Talauma* from Cuba could be considered as one single taxon. Later, Borhidi & Muñiz (1971) considered *T. minor* as the only species of *Talauma* in Cuba and defined two subspecies: *T. minor* subsp. *oblongifolia* (León) Borhidi and *T. minor* subsp. *orbiculata* (Britton & P. Wilson) Borhidi. Afterward, Bisse (1974) described *T. ophitcola* Bisse and recognized *T. oblongifolia* (León) Bisse. Years later, Bisse (1988) referred four species: *T. orbiculata*, *T. minor*, *T. oblongifolia*, and *T. ophitcola*. The delimitation of each taxon, made by Bisse (1974, 1988), was mainly supported, as in previous works, by leaf morphological characters. Imchanickaja (1993) recognized *T. minor* and considered their variability as subspecies: *T. minor* Urb. subsp. *minor* and *T. minor* subsp. *oblongifolia* (León) Borhidi. *Talauma orbiculata* was still considered by Imchanickaja (1993) as a different valid species.

Based on anatomical and morphological (vegetative and reproductive) traits described by Nootboom (1993), Frodin & Govaerts (1996) made the combination of *Talauma minor* to *Magnolia minor* (Urb.) Govaerts, and considered all the other names of the Cuban *Talauma*

species as synonyms of *M. minor* (Acevedo-Rodríguez & Strong 2012; Rivers *et al.* 2016). The latest taxonomic review of *Magnolia* subsect. *Talauma* in Cuba was published by Palmarola *et al.* (2016), who made the new combinations from *Talauma* to *Magnolia*. Palmarola *et al.* (2016) recognize three species: *Magnolia orbiculata* (Britton & P. Wilson) Palmarola, *Magnolia minor*, and *Magnolia oblongifolia* (León) Palmarola (Palmarola *et al.* 2016). In the absence of additional evidence, these authors considered *T. ophiticola* (without name under *Magnolia*) a synonym of *M. oblongifolia* due to the existence of one specimen (Bisse & Kohler HFC 5358 HAJB, JE) that has leaves with the characteristics used by Bisse (1974, 1988) to define both taxa.

All above-mentioned taxonomic revisions (e.g., León & Alain 1950, 1951; Borhidi & Muñiz 1971; Bisse 1974, 1988; Imkhanitzkaja 1993; Palmarola *et al.* 2016) were exclusively based on traditional leaf morphological descriptors and only a few individuals, limiting their ability to elucidate taxon boundaries. Therefore, our research questions are:

- It is the current delimitation of the Cuban taxa of *Magnolia* subsect. *Talauma*, based on morphological criteria, consistent with other criteria to delimit the species?
- What is the ecological, morphological, and genetic variability of the Cuban taxa of *Magnolia* subsect. *Talauma*?

The present work is based on the following hypothesis: **For the Cuban members of *Magnolia* subsect. *Talauma*, the number and limits of species defined by the genetic, ecological, and phylogenetic criteria are the same as that defined by the morphological species criteria.**

In order to test the research hypothesis, the present study proposes the **goal**: to delimit the Cuban taxa of *Magnolia* subsect. *Talauma* in order to update their taxonomic treatment through the integration of ecological, morphological, genetic, and phylogenetic criteria.



## Tasks

1. To analyze the ecological limits of the Cuban taxa of *Magnolia* subsect. *Talauma* in Cuba throughout their ecological niche.
2. To evaluate the morphological limits of the Cuban taxa of *Magnolia* subsect. *Talauma* using multivariate and geometric morphometrics of the leaves.
3. To describe the genetic structure of the Cuban taxa of *Magnolia* subsect. *Talauma* using microsatellite markers.
4. To analyze the phylogenetic relationships of the Cuban taxa of *Magnolia* subsect. *Talauma*, using the complete plastome sequence.
5. To integrate the ecological, morphological, genetic, and phylogenetic criteria in the taxonomic delimitation of *Magnolia* subsect. *Talauma* in Cuba.
6. To update the taxonomy and nomenclature of *Magnolia* subsect. *Talauma* in Cuba.

## SCIENTIFIC NOVELTIES OF THE THESIS

1. The delimitation of the taxa of *Magnolia* subsect. *Talauma* through the integration of the ecological, morphological, genetic, and phylogenetic criteria. This is the first research of this type in *Magnoliaceae* family and in Cuban species.
2. The analysis of hybridization in *Magnolia* subsect. *Talauma*. The first research on population genetics applied to natural hybridization in Cuban plants.
3. The application of the ecological and genetic species concept in *Magnolia* subsect. *Talauma*.
4. The evaluation of ecological differences using two niche identity tests in Cuban plants.
5. The morphological characterization of *Magnolia* subsect. *Talauma* in Cuba, using multivariate and geometric morphometrics.
6. The description of specific microsatellite markers for the Cuban taxa of *Magnolia* subsect. *Talauma*.

7. The genetic characterization of *Magnolia* subsect. *Talauma* in Cuba.
8. The complete plastome phylogeny of *Magnolia* sect. *Talauma* in Cuba.
9. The definition of Significant Evolutionary and Conservation units of *Magnolia* subsect *Talauma* in Cuba.

The **theoretical importance** is the delimitation of the different taxa of *Magnolia* subsect. *Talauma* in Cuba using four different approaches (ecological, morphological, population genetics, and phylogenetic). The genetic characterization of the Cuban taxa of *Magnolia* subsect. *Talauma*, using microsatellite markers and chloroplast genome, will allow the analyses of the evolutionary history of this group and their conservation. The ecological and morphological data will help to understand the variability of the group and the possible effect of the environment on morphology and genetics.

The **practical importance** is reflected in the methodological value of the thesis for future taxonomic studies in Cuban species and magnolias since it is the first work in Cuba that analyses the limits of species through an integrative approach. In addition, it serves from the methodological point of view in the analysis of ecological niche, leaf morphology, population genetics, and phylogeny of other species of Cuban flora.

The thesis document consists of 102 pages of content, 11 tables, 23 figures, and 9 supplementary materials. It includes the chapters Introduction, Bibliographic Revision, Materials and Methods, Results, Discussion, Conclusions, and Recommendations. A total of 268 bibliographic references were used, of which 81.7 % are after the year 2000, and 44 % correspond to works from the last decade.

Three articles related to the thesis have been published in indexed journals (Revista del Jardín Botánico Nacional-Universidad de La Habana, Botanical Sciences, and PhytoKeys), and three other manuscripts are in process (see page 120). The results of this thesis have been presented in five scientific event.

## BIBLIOGRAPHIC REVISION

### 2.1 Species concept

The species concept (SC), also called the species problem, is one of the most controversial and debated issues within biology (Mayden 1997; Zachos 2016; Aldhebiani 2018). One of the reasons for this is that the term species has been poorly defined since its beginning more than three centuries ago (Roberts 2016; Aldhebiani 2018). A species is one of the fundamental units of biology, comparable in importance to genes, cells, and organisms (de Queiroz 2007). Defining species is central in biodiversity sciences because changes in the number of species can directly affect fields such as taxonomy, biogeography, ecology, agriculture, and macroevolution (Agapow *et al.* 2004; Denham *et al.* 2019; Yang *et al.* 2019). Species constitute the basic unit of conservation (Prebble 2016), and are the basis for the creation and implementation of policies (Agapow *et al.* 2004; Zachos 2016). The term species has two basic functions: (1) the species category as a rank in the Linnaean hierarchy created by taxonomists for grouping organisms and (2) the species as a taxon with a location in space and time and referring to objective, observable entities, to living objects perceptible by touch (Mayr 1996).

Species are an artificial category created to organize information (Mayden 1997). There are different arguments on how a species should be defined, which has resulted in more than 30 concepts (Mayden 1997; Zachos 2015; Haider 2018). Most species concepts have marked incompatibilities, which leads to different interpretations of species boundaries. The incompatibility between the different species concepts is given by the different biological properties on which each of the concepts is based (de Queiroz 1998, 2007), which do not escape criticism due to their limitations and counterexamples. The problem of the definition of species cannot be solved easily, given that the problem is philosophical and not a simple question of science (Rusmana *et al.* 2019). Natural populations constitute a continuum of variability (morphological, genetic, reproductive, ecological, etc.), composed of phenomena such as speciation, introgression, and hybridization that contribute to unclear boundaries

between groups of individuals (Nyléhn & Ødegaard 2018). This complexity becomes more relevant in groups like trees, where factors such as generation length and effective population sizes can delay lineage differentiation (Zheng *et al.* 2017).

The ecological SC, the morphological SC, the genetic SC, and the phylogenetic SC have been widely used by scientists. All these concepts have advantages and limitations (explained below) in establishing limits between species.

### **2.1.1 Ecological Species Concept (ESC)**

The ecological niche is considered a central concept in modern biology (Hutchinson 1957; Qiao *et al.* 2016). The ecological niche is the set of environmental conditions and requirements that allow the species to maintain stable populations without the occurrence of immigration (Peterson 2003; Pearman *et al.* 2007; Soberón & Nakamura 2009). It can also be defined as an N-dimensional hyper-volume where each dimension represents the range of environmental conditions and resources necessary for the survival, stability, and reproduction of a population (Hutchinson 1957). There are two approaches to the niche concept: the Eltonian and the Grinnellian. The Eltonian niche is spatially fine, focuses on the functional role of the species and its place in the biotic environment, and takes into account biological interactions between species (Peterson 2003). The niche from the Grinnellian approach is understood as the range of conditions defined in terms of variables that are not consumed or affected by the presence of the species (Soberón & Nakamura 2009). This approach, unlike the previous one, does not take biological interactions into account and works on a large scale (Jiménez-Valverde *et al.* 2011). The concept of ecological niche is vital for understanding patterns of distribution and biological diversity, the identification of critical habitats to mitigate the effect of climate change, and the reconstruction of evolutionary trajectories (Smith *et al.* 2019).

The ESC, proposed by Van Valen (1976), emphasizes the importance of natural selection based on ecology for the long-term survival of species. A species is a lineage (or a group of related

lineages) that occupies a minimally different adaptive zone from other lineages in its range and that has evolved separately from all lineages outside its range. The ESC is based on the differential occupation of the ecological niche or an adaptive zone (de Queiroz 1998; Aldhebiani 2018; Haider 2018). In this sense, each species has a specific ecological niche that is different from other species, including related and sympatric species (Soberón & Peterson 2005; Kurnaz & Hosseinian-Yousefkhani 2019). The existence of two or more groups of related individuals occupying different niches can be seen as evidence of genetic differences between them.

#### **2.1.1.1 Ecological Niche Models (ENMs)**

The ENMs are a set of analytical tools that allows identifying, in a quantitative way, the ideal environmental conditions for the maintenance of species or natural populations (Peterson *et al.* 2011; Smith *et al.* 2019). From this analysis, the Grinnellian ecological niche of the species is characterized and projected into geographic space, generating probability maps of favorable conditions for the presence and development of populations (Mateo *et al.* 2011; Peterson & Soberón 2012). One of the main assumptions of the ENMs is that environmental adaptation and stress tolerance are the primary determinants of the current distribution of species (Rödger *et al.* 2008) and that the ecological niche is quite conserved (stable in time and space) (Thuiller *et al.* 2005; Rödder *et al.* 2008; Mateo *et al.* 2011). ENMs are a complex tool that manages to integrate elements of biology, statistics, cartography, databases, computing, remote sensing, and climatology (Mateo 2008). This has meant that ENMs have been integrated into a wide variety of fields within the natural sciences, such as geography, species conservation, invasive species, evolution, and systematics (Guisan & Thuiller 2005; Peterson *et al.* 2011; Moein *et al.* 2019).

There are several statistical modeling algorithms (Phillips *et al.* 2006; Wolmarans *et al.* 2010; Escobar *et al.* 2018); among the most used are GARP (Stockwell & Peters 1999), BIOCLIM (Busby 1991), BIOMOD (Thuiller *et al.* 2009), and Maxent (Phillips *et al.* 2006). Maxent, which implements a learning machine-based algorithm, is based on the principle of maximum entropy

and assumes that the best approximation to an unknown distribution is the one that will maximize the entropy of the probability distribution (Phillips *et al.* 2006; Mateo 2008; Elith *et al.* 2011). A certain probability of maximum entropy is subject to restrictions that represent our incomplete information; this is what allows us to justify a distribution as an inference (Phillips *et al.* 2006). Maxent is a robust method which has a high performance with few data and generates stable results, despite not being easy to interpret (Mateo 2008; Escobar *et al.* 2018). Furthermore, it only requires records documenting the presence or the absence of the studied species (Phillips *et al.* 2006; Elith *et al.* 2011). Thus, information from databases, herbaria, and other collections can be used (Mateo *et al.* 2011).

Ecological niche modeling can be used as a taxonomic framework that helps define or delimit species based on the differences in their ecological niches (Wiens & Graham 2005; Raxworthy *et al.* 2007; Kurnaz & Hosseinian-Yousefkhani 2019; Hosseinian-Yousefkhani *et al.* 2019). Niche models can also provide evidence of geographic isolation between populations (based on divergence or conservation of the ecological niche). In addition, they provide elements that support that these populations are separate lineages when gene flow is unlikely due to unsuitable regions (Raxworthy *et al.* 2007).

### **2.1.2 Morphological Species Concept (MSC)**

The comparison of the morphological characteristics of organisms has been a central element in biology for centuries (Remagnino *et al.* 2017). The taxonomic classification of organisms and the understanding of biological diversity have historically been based on morphological forms (Adams *et al.* 2004). The MSC defines species as the smallest group that is consistently and persistently different and distinguishable by ordinary measures (Mayden 1997; Nyléhn & Ødegaard 2018; Haider 2018). Species can also be defined as a group or related groups that have different morphological characters. In the opinion of the taxonomist, these characters are enough to delimit them (Mayden 1997). Species are categorized based on observable physical characteristics, such

as structures, sexual organs, sizes, and shapes (Rusmana *et al.* 2019).

The MSC, also known as Linnean, Phenetic, Classic, Typological, Essentialist (Haider 2018), is one of the most used species concepts (Soltis & Soltis 2009; Padial *et al.* 2010; Karbstein *et al.* 2020). The delimitation of species based on the MSC has been the basis of taxonomy since its origin and has been used in almost all classification systems (Jensen 2003). The MSC can be applied in sexual and asexual organisms and is very useful for fossil records. However, morphological characteristics are sometimes quite subjective and depend on specialized opinions for some key features. MSC has shortcomings when working with natural hybrids and does not consider the evolutionary origin of organisms (Haider 2018).

As previously mentioned, MSC is based on morphological differences between groups of individuals. It is at this point that morphometrics (from the Greek *morphé* - shape; *metrics* - measurement) becomes relevant, which focuses on the statistical analysis of quantitative morphological variations within and between species (Remagnino *et al.* 2017). Comparing the morphological characteristics of organisms and understanding how these characteristics vary in association with other traits are of great interest to ecologists, taxonomists, and evolutionists (Adams & Otárola-Castillo 2013). At present, morphometrics remains an invaluable tool for the delimitation of species. However, it is necessary to reexamine the classical concept of morpho-species and their limits in light of new molecular evidence (Roberts 2016). There are two morphometrics methods to assess the morphological limits between species: multivariate morphometrics (MM) and geometric morphometrics (GM).

#### **2.1.2.1 Multivariate Morphometrics (MM)**

Morphological measurements have been used for a long time in the description and characterization of morphological groups. However, it was not until 1960 that the use of traditional or multivariate morphometrics began in quantitative description and analysis of shape variation (Marcus 1990; Adams *et al.* 2004). Multivariate morphometrics is based on linear and angular measurements

and their relationships (Rohlf & Marcus 1993; Adams *et al.* 2004; Slice 2007; Portillo *et al.* 2019), which can be compared independently or together using multivariate analysis (Remagnino *et al.* 2017). Multivariate morphometrics plays a fundamental role in establishing the limits of taxa and in evaluating the relationships between them (Jensen 2003). Although there has been significant development of new morphometrics techniques, traditional measurements (linear and angular) continue to be present in descriptions, comparisons, and exploration of patterns of variation at different taxonomic levels (van der Niet *et al.* 2010; Remagnino *et al.* 2017).

Unfortunately, the use of MM in the analysis of structures does not guarantee a complete understanding of the shape of objects (Dryden & Mardia 1998; Karbstein *et al.* 2020). Multivariate morphometrics does not have a high statistical value for defining shapes because different shapes can be obtained for the same set of distance measurements, and in general, it is not possible to generate graphic representations of the shape based on linear distances (Adams *et al.* 2004; Slice 2007). According to Jensen (2003), if a researcher is really interested in focusing on shape rather than only on size, traditional approaches are not the most appropriate.

#### **2.1.2.2 Geometric Morphometrics (GM)**

The development of GM techniques at the beginning of the 1980s, based on the spatial relationship between homologous points, has revolutionized the studies of the variation of shape in objects and biological structures (Bookstein 1991; Rohlf & Marcus 1993; Jensen 2003; Adams *et al.* 2004; Chen *et al.* 2018). MG is defined as the set of methods and procedures with a geometric basis for the analysis of shape patterns, where the measurements are usually derived from contours and landmarks (Adams *et al.* 2004; Viscosi & Fortini 2011; Karbstein *et al.* 2020). Bookstein (1984), who established the theoretical basis of the GM, proposed the description of the shape using Cartesian coordinates that allowed the visualization of differences between individuals and the possibility of applying statistical tests. Kendall (1984) theorized the existence of a space of the form (or Kendall's space) by demonstrating how the work of Bookstein (1984) was interpreted



in this space. MG allows obtaining a more useful and consistent definition of shape and size (Bookstein 1991; Rohlf 2000), which makes it possible to analyse their variations separately. The first step in GM is to remove the effect of size through uniform transformations, which includes isotropic underlining (equal size), rotations, and translations of the analysed structure (Kuhly & Giardina 1982; Klingenberg & McIntyre 1998; Remagnino *et al.* 2017).

Contour methods were the first to be used in MG analyses. These consist of digitizing points that quantify the periphery of an object through the use of sequences of coordinates (x, y) or (x, y, z) (Adams *et al.* 2004). Contours can be opened (start and end at different points) or closed (start and end at the same point). Open contours are studied using polynomial equations, cubic splines, and Bezeir curves (Evans *et al.* 1985; Engel 1986), while closed contours usually use elliptical Fourier descriptors, elliptical Fourier analysis, and eigenshapes (Adams *et al.* 2004).

Elliptical Fourier descriptors (EFDs) were proposed by Kuhl & Giardina (1982) and allow any closed and bi-dimensional contour to be described (Iwata & Ukai 2002), starting from a sequence of coordinates (X, Y). The set of coordinates allows the formation of mathematical functions (sins and cosines) known as Fourier harmonics, from which the coefficients are calculated, which will form the EFDs. These coefficients present similar values for similar shapes (Viscosi & Fortini 2011). The higher the number of harmonics, the greater the precision in the descriptors (Cope *et al.* 2012), but there is a number beyond which the description is not more efficient. Chuanromanee *et al.* (2019) suggest that between 15 and 20 Fourier harmonics is sufficient to find differences in leaf shape in various plant species.

The landmark methods allow characterizing the shape through coordinates of a set of points that can be unequivocally located in each of the specimens under study (Klingenberg *et al.* 2002; Slice 2007). Landmarks are points in space that correspond to the position of a particular feature on an object of interest, which allow the shape of each specimen to be quantified (Adams *et al.* 2004). There are three types of key points: i) type I or morphological landmarks, which

are points whose presumed homology between taxa is supported by biological significance; ii) type II or mathematical landmarks are points whose presumed homology between individuals is supported only by geometry and not by anatomical evidence, and iii) type III or semi landmarks which are extreme points and allow the form to be analysed to be fully covered (Bookstein 1986). The landmark methods are superior to the contour methods, especially when the landmarks represent biologically defined and homologous points (Jensen 2003).

In a general sense, GM has proven to be a powerful tool to capture the shape of biological structures (Rohlf & Marcus 1993) and helps to increase the rigor of scientific descriptions (Viscosi & Fortini 2011). The GM techniques have been used in the delimitation of plants species in many studies: Jensen *et al.* (2002) in *Acer* (*Sapindaceae*), Viscosi *et al.* (2009) in *Quercus* (*Fagaceae*), Chen *et al.* (2018) in *Delphinieae* (*Ranunculaceae*), Renner *et al.* (2018) in *Plagiochila arbuscula* (Brid. ex Lehm. & Lindenb.) Lindenb. complex (*Plagiochilaceae*), Menini-Neto *et al.* (2019) in *Pseudolaelia vellozicola* (Hoehne) Porto & Brade complex (*Orchidaceae*), and Nery & Fiaschi (2019) in *Hydrocotyle quinqueloba* Ruiz & Pav. (*Araliaceae*). Almost of these works are based on leaves. Without question, leaf morphology is central to plant taxonomy, and it has mainly been studied using traditional morphometrics. However, in recent years has been an increasing interest in using geometric morphometrics to study the form of leaves because this structure is particularly suitable for outline and landmark based description.

### 2.1.3 Genetic Species Concept (GSC)

Like many other fields in the life sciences, systematics has advanced significantly with genomics and evolutionary modeling. The development of coalescent modeling, Bayesian approaches, and capacities to generate large datasets, thanks to progress in sequencing technologies, allow us to assess patterns of genetic diversity and infer evolutionary processes that underlie them (Funk *et al.* 2012). Genetic data provide a great power to identify differentiation and divergence within and between species, requiring further consideration of how this affects the delineation of

taxonomic units (Coates *et al.* 2018). Genetic data can contribute to the delimitation of taxonomic groups, whose identity can be masked by other types of information such as morphology or environment (Postaire *et al.* 2016; Rico & Gutiérrez-Becerril 2019); these groups can be identified as independent evolutionary lineages.

From the genetic point of view, a species can be defined as the largest and most inclusive reproductive community of individuals who share a common gene pool (Mayr 1969; Mayden 1997). The GSC is quite similar to the MSC, except that the method used to delimit species is based on genetic distances (Mayden 1997). The main problem with this concept is that there is no genetic information for most of the diversity, and obtaining it is not a simple or cheap process. Furthermore, most species are not genetically uniform, as processes such as divergence (genetic drift and geographic isolation) and gene flow can shape geographic and genomic patterns of variation within species (Coates *et al.* 2018).

Despite all these limitations, GSC is very useful when working with cryptic species and natural hybrids with unclear morphological limits. Furthermore, it provides a good level of resolution in the study of the number of species and their limits (Groves *et al.* 2017; Ramírez-Barahona *et al.* 2017; Rico & Gutiérrez-Becerril 2019; Muñoz-Castro *et al.* 2020).

### **2.1.3.1 Population genetics**

Population genetics studies the origin, quantity, and distribution of the variation of genes present in populations, as well as the fate of this variation through space and time (Templeton 2006). Population genetics is based on the measurement and characterization of genetic variation within and between populations. This analysis allows making inferences about processes like genetic drift, mutation rates, gene flow, and natural selection, among others (Hartl & Clark 1997). Within-population, genetic variability and the population genetic structure of a species is determined by habitat, reproductive system, pollen and seed dispersal range, and evolutionary history, as well as by the spatial and temporal stability of the environment used by the species to complete its

life cycle (selection) (Templeton 2006; Hirsch *et al.* 2018). There is a wide variety of methods and statistics to describe the genetic structure and diversity of populations and to summarize the information brought by dozens to billions of genetic markers in manageable terms.

Population genetic structure describes the composition and distribution of genetic variability (genes and genotypes) among populations of a given species. The methods for evaluating population genetic structure aim at identifying the number of different genetic groups (species/populations) in a sample and classify individuals or fractions of individual genomes into the inferred groups (Godoy 2009; Grünwald *et al.* 2017). Prior geographical or ecological information, when available, can be included in the clustering methods. Genetic diversity is the variation in the DNA sequence (in terms of the number and frequency of alleles and genotypes) between different individuals of a population or species (Futuyma & Kirkpatrick 2018).

Genetic diversity contributes to the ability of species to respond to environmental changes (Frankham *et al.* 2002), which has implications for the development and maintenance of natural populations in the long term. The levels of genetic diversity in populations are tightly linked to the population genetic effective size ( $N_e$ ), a parameter that is strongly influenced by different so-called neutral mechanisms such as demography, genetic structure, migration, and their adaptation to the environment (selection) (Kim *et al.* 2017). Because each species has its own evolutionary history, the genetic diversity and structure vary considerably between species (Ellegren & Galtier 2016).

#### **2.1.3.2 Molecular markers: microsatellites**

A molecular marker (also known as genetic marker) is any molecular phenotype without any contribution to the environment that can be used to infer or obtain genetic information about the organism or population to which it belongs (Schlötterer 2004). Molecular markers provide information about allelic variation and allow the description of genetic patterns in natural populations at scales ranging from individuals to species (Schlötterer 2004; Godoy 2009). Molecular markers can be classified into three general classes: protein variants (allozymes,

no longer used by now), polymorphisms at the non-repetitive DNA sequence level (RFLPs: Restriction Fragment Length Polymorphisms -no longer used by now-, AFLPs: Amplified Fragment Length Polymorphisms, SNPs: Single Nucleotide Polymorphism), and DNA variation with repetitive motifs (minisatellites and microsatellites) (Schlötterer 2004; Arif & Khan 2009). Molecular markers have been applied to various biological issues, from gene mapping, phylogenies, population genetics, paternity testing, forensic analysis, and conservation genetics, among others (Schlötterer 2004; Godoy 2009).

Microsatellites or simple sequence repeat (SSR) are short motifs of DNA sequences (1-10 base pairs) tandemly repeated (Allendorf & Luikart 2007). These markers are generally highly polymorphic (mostly due to the variation in the number of tandem repeats), abundant, and widely distributed within the genomes of most eukaryotic species. They are found in gene sequences (exons, introns, regulatory regions) and in non-functional DNA sequences as well (Schlötterer 2004). Apart from their level of polymorphism, another advantage of microsatellites is that they are easily amplified by PCR, which makes them highly useful markers for evaluating genetic diversity (Arif & Khan 2009). Microsatellite markers have proven to be efficient and widely used in the evaluation of the population genetic structure and diversity in several species of the *Magnoliaceae* family (Hirayama *et al.* 2007; Setsuko *et al.* 2007; Tamaki *et al.* 2008; He *et al.* 2009; Yu *et al.* 2011; Rico & Gutiérrez-Becerril 2019; Aldaba-Nuñez *et al.* 2021; Hernández *et al.* 2021). In all these works, between 10-20 microsatellite loci were used.

#### **2.1.4 Phylogenetic Species Concept (PSC)**

According to the PSC, a species is the smallest aggregation of populations (sexual) or lineages (asexual) with a specific common ancestry (monophyly) diagnosable by unique combinations of character states (Mayden 1997; Wheeler & Platnick 2000; Agapow *et al.* 2004; Soltis & Soltis 2009; Haider 2018). Wheeler (1999) stated that the phylogenetic concept is simple, broadly applicable, and testable. The PSC is more widely applicable in practice than other

species concepts because it can be applied to asexual organisms and allopatric populations. Phylogenetic analysis is a powerful approach that provides new lines of taxonomic evidence for species identifications and biodiversity assessments (Roberts 2016).

It has been argued that the PSC is more objective than other definitions of species and may reveal morphologically unremarkable but significant populations (Agapow *et al.* 2004). Within the PSC, there is no privileged phylogenetic level that corresponds to a species other than “the smallest aggregate”. Where the PSC is used to delimit species, sufficient individuals and characters should be sampled to ensure an adequate resolution of the species status across clades (Agapow *et al.* 2004). It is important to remark that all species phylogenies are, in reality, genes genealogies. The evolutionary history of a species and its genes may not match because identifying truly orthologous genes is not always obvious, the existence of long branches issues, and incomplete lineage sorting (Mindell & Meyer 2001). Thus, it is widely recognized that the PSC will often split a species determined under non-phylogenetic criteria with two or more “new” species (Agapow *et al.* 2004; Sukumaran & Knowles 2017).

The chloroplast, an important plant organelle, is essential for photosynthesis and several metabolic pathways (Zoschke & Bock 2018; Liu *et al.* 2020). The chloroplast genome has a highly conserved circular DNA arrangement ranging from 75 to 250 kb (kilo base pair) (Song *et al.* 2017). This molecule has four parts: a large single-copy (LSC) region, a small single-copy (SSC) region and two inverted repeat (IRa and IRb) regions (Liu *et al.* 2020; Dong *et al.* 2022). Chloroplast genomes have often been used for phylogenetic studies and species identification, especially in difficult complexes of morphologically homogeneous species (Kim *et al.* 2006; Sun *et al.* 2020; Dong *et al.* 2022). Genetic information from the chloroplast genome is usually maternal inheritance in angiosperms, making the chloroplast genome a good indicator of maternal ancestry (Song *et al.* 2017; Li *et al.* 2019).

The complete chloroplast genome in *Magnoliaceae* family has been found to have the typical structure with approximately 160 000 bp (base pair) in length (Liu *et al.* 2020). Comparative analysis of full plastome provides greater insight into the evolutionary history and interspecies relationships in the family, with many examples in the last years (Kuang *et al.* 2011; Li *et al.* 2019; Liu *et al.* 2020; Sun *et al.* 2020; Guzmán-Díaz *et al.* 2022; Wang *et al.* 2020; Dong *et al.* 2022).

## 2.2 Integrative taxonomy

Integrative taxonomy is a relatively young discipline, which was introduced independently into the literature by Dayrat (2005) and Will *et al.* (2005). Will *et al.* (2005) used the term integrative taxonomy to refer to a taxonomic process that was inclusive of all available data sources to delimit, discover, and identify species at all levels. Dayrat (2005) used the term integrative taxonomy in a set of guidelines that taxonomists should follow when describing new species. Guideline 5: 'species should only be named when their limits are supported by multiple lines of evidence'. Species are always hypothetical, even when they are 'broadly supported', but our level of confidence is much higher for entities supported by different kinds of data than for entities supported by only one kind (Dayrat 2005). Both papers focused on a taxonomy that integrated all available sources of data to develop hypotheses of species-level taxa.

Ancient and more divergent species can usually be easily recognized by considering any of the different species concepts (Prebble 2016; Denham *et al.* 2019). However, difficulties arise during the early stages of lineage divergence, when lineages may have diverged and formed new species but may not have acquired all of the different properties on which different species concepts are based (de Queiroz 2007; Denham *et al.* 2019). In those cases, the use of a single taxonomic data source (morphological, ecological, genetic, phylogenetic, reproductive, etc.) to delimitate species would fail to resolve the species limits. In complex cases, the integrative taxonomy approach that integrates lines of evidence from morphology, genetics, phylogeny, and ecological traits is often required to reliably delineate, identify, and help reveal evolutionary



relationships among taxa (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010; Yeates *et al.* 2011). The integrative approach is especially useful when using a single line of evidence fails, which is common in polymorphic, cryptic groups, and species complexes (Masonick & Weirauch 2019). Evaluating multiple criteria not only increases our ability to detect recently separated lineages but also can provide more robust support for lineage separation (Prebble 2016). Integrative taxonomy does not replace traditional taxonomy but uses complementarity among disciplines to improve rigor (Schlick-Steiner *et al.* 2010; Yeates *et al.* 2011).

Since taxonomy has moved towards being an integrative science, Damasco *et al.* (2019) believe that formal species should represent evolutionarily diverged populations that: (1) form highly supported monophyletic clades according to molecular evidence, (2) have not (natural) gene flow among closely related lineages or sister groups, and (3) exhibit conserved features that enable their recognition. A pragmatic way to approach the question of species delimitation is to start with the null hypothesis that two or more individuals belong to the same species and then aim to falsify this null hypothesis by one or more sources of evidence (the more, the better). Therefore if one piece of evidence does not falsify the null hypothesis, it is not proof that there is only one species present; it simply indicates that that particular piece of evidence is not informative, yet others might be (Prebble 2016).

There is probably no magic solution for species discovery and delimitation, but an integrative and evolutionary framework provides taxonomists with a larger arsenal to face the underestimated biodiversity (Schlick-Steiner *et al.* 2010); with important implications for conservation, biosecurity, trade and agriculture, and ecological research (Prebble 2016). The potential for such integrative taxonomic approaches has not yet been fully embraced in botany, particularly in the tropics, where biodiversity studies are especially needed (Damasco *et al.* 2019). However, the publications of Duminil *et al.* (2011) in *Meliaceae*, Prebble (2016) in *Boraginaceae*, Ranarivelo (2017) in *Hypericaceae*, Zheng *et al.* (2017) in *Salicaceae*, Alvarado-Sizzo *et al.* (2018) in *Cactaceae*,



Damasco *et al.* (2019) in *Burseraceae*, Denham *et al.* (2019) in *Calyceraceae*, Moein *et al.* (2019) in *Lamiaceae*, Yang *et al.* (2019) in *Gesneriaceae*, Karbstein *et al.* (2020) in *Ranunculaceae*, De Giorgi *et al.* (2022) in *Asteraceae*, Meng *et al.* (2022) in *Crassulaceae*, showed a rise in the number of works that applied the integrative taxonomy approach to plants. These works have contributed to a better understanding species limits, evolution, and diversity.

### **2.3 Inter-specific hybridization and genetic introgression in plants**

Hybridization is the crossing between two individuals, whatever they belong to the same variety, population, or species (Allendorf & Luikart 2007; Soltis & Soltis 2009). Many interspecific hybrids are sterile so that introgression (gene flow between populations whose individuals hybridize followed by backcrossing) does not occur. But in many cases, hybrids are fertile and may produce offspring with one or both parental taxa (Allendorf & Luikart 2007; Soltis & Soltis 2009). Hybridization and genetic introgression are an important and natural part of the evolutionary process in plants and represent a valuable source of genetic variation (Allendorf & Luikart 2007; Soltis & Soltis 2009; An *et al.* 2017). Many plant taxa have hybrid origins, and hybridization is a key mechanism for the production of new species and novel adaptations.

The levels of hybridization and introgression have increased dramatically on a global scale, caused mainly by the introduction of exotic species, fragmentation, and habitat modifications (Grabenstein & Taylor 2018; Ottenburghs 2021). According to Allendorf & Luikart (2007), hybridization is a serious problem for conservation, whose impact has been underestimated by conservation biologists and, in the future, will become even more problematic. Hybridization and genetic introgression can contribute to the decline and eventual extinction of species. In the case of outbreeding depression, the hybrid progeny has lower performance or fitness than either parent (Allendorf & Luikart 2007; Becker *et al.* 2013). Also, the sterile or partially sterile hybrids could result in a loss of reproductive potential and may reduce the population growth rate. In the case of fertile hybrids, genetically distinct populations may be lost through

genetic mixing (Allendorf *et al.* 2001; Allendorf & Luikart 2007; An *et al.* 2017). On the other hand, hybridization and introgression may have a wide variety of effects on fitness. In the case of heterosis, or hybrid vigor, hybrids have enhanced performance or fitness relative to either parental taxa. Hybrids may also have a fitness advantage because they possess advantageous traits from both parental populations.

The assessment of the inter-population or inter-specific gene flow has therefore been the focus of interest of a huge number of evolutionary studies. Interspecific hybridization and introgression seem to be a common and widespread phenomenon in plants and even more in angiosperms and ferns (Soltis & Soltis 2009; Burgarella *et al.* 2009). Estimates of several well-studied floras suggest that about 11% of plant species hybridize, and about a quarter of them are rare or endangered (Allendorf & Luikart 2007). Species with strong pre-mating barriers, for example, or that have coexisted naturally for thousands of generations, are unlikely to be threatened by hybridization. However, plants from islands are particularly vulnerable to hybridization due to the pre-mating barriers often being weak and their smallest geographic ranges. In the specific cases of *Magnoliaceae*, hybridization is likely to occur and may have played an important role in speciation (Nie *et al.* 2008; Wang *et al.* 2020). Studies of natural hybridization in *Magnoliaceae* are not common (Muranishi *et al.* 2011; Muranishi *et al.* 2013). Yet, many interspecific hybrids have been created by artificial crosses for ornamental use (Treseder 1978; Callaway 1994; Muranishi *et al.* 2013; Xu *et al.* 2017); suggesting that the involved species have diverged only recently.

#### **2.4 Genus *Magnolia***

The family *Magnoliaceae* Juss. is included in the order *Magnoliales* and belongs to the clade of Magnoliids (APG IV 206). The family is divided into two genera: *Magnolia* L. and *Liriodendron* L. (Figlar & Nootboom 2004). The genus *Magnolia*, the largest within the family, are trees and shrubs that could be evergreen or deciduous (Rivers *et al.* 2016). Its leaves are simple and

alternate, with stipules surrounding the terminal bud. The flowers are solitary and large with numerous tepals (sometimes sepals and petals), bisexual with an elongated receptacle, and numerous stamens and carpels (Treseder 1978; Judd *et al.* 2016). The fruit is an aggregate of follicles, and the seeds have a fleshy aril. Pollination in the family is mainly mediated by beetles and dispersal by birds and small mammals (Callaway 1994; Judd *et al.* 2016). The genus *Magnolia* includes approximately 350 species (Vázquez-García *et al.* 2021), distributed in the temperate and tropical regions of East and Southeast Asia and America (Azuma *et al.* 2001; Cires *et al.* 2013), although they are known from fossil records in Europe (Callaway 1994).

Magnolias have attracted the interest of many botanists due to their ancestral evolutionary history and the retention of primitive characters (Treseder 1978; Callaway 1994; Rivers *et al.* 2016). This group has also been relevant from a commercial point of view since most of its species are globally appreciated as ornamentals due to their attractive flowers and foliage (Vázquez-García 1994). These plants are also valued for their medicinal properties and the quality of their wood (Cires *et al.* 2013; Rivers *et al.* 2016; Wang *et al.* 2020). Due to the high economic value of magnolias, their conservation status has been recognized as a priority by international organizations (Rivers *et al.* 2016). Approximately 48 % of the species in the family are threatened. Among the main threats faced by the species of the family are logging, agriculture and livestock, anthropization, and climate change, among others (Rivers *et al.* 2016).

Seven taxa of *Magnoliaceae* occur in Cuba, all of which belong to the genus *Magnolia*. *Magnolia virginiana* subsp. *oviedoae* Palmarola, M.S. Romanov & A.V. Bobrov is the only species within the section *Magnolia* L. The remaining six taxa are part of the section *Talauma* Baill., which has three taxa in the subsection *Cubenses* Imkhan.: *Magnolia cristalensis* Bisse, *Magnolia cubensis* subsp. *acunae* Imkhan., and *Magnolia cubensis* Urb. subsp. *cubensis*. The other three taxa belong to the subsection *Talauma* Juss.: *Magnolia minor* (Urb.) Govaerts, *Magnolia oblongifolia* (León) Palmarola, and *Magnolia orbiculata* (Britton & P. Wilson) Palmarola (Palmarola *et al.*

2016; Greuter & Rankin 2022; Veltjen *et al.* 2022).

#### 2.4.1 *Magnolia* sect. *Talauma*

The members of *Magnolia* section *Talauma* are evergreen trees with a stamen connective with a long appendix and embedded in the gynoeceium (except in the subsection *Talauma*). The carpels present circumscissile dehiscence (except in the subsection *Cubenses*) and two ovules per carpel (Figlar & Nootboom 2004). The section *Talauma* occurs in Central America, South America, and the Caribbean Islands (Pérez *et al.* 2016). This section is divided into four subsections (following Figlar & Nootboom 2004; Veltjen *et al.* 2022): 1) *Magnolia* subsect. *Cubenses* Imkhan.: from the Antilles, 2) *Magnolia* subsect. *Dugandiodendron* Lozano: from the middle elevations in the Andes and Guyana; 3) *Magnolia* subsect. *Chocotalauma* A. Vázquez, Á.J. Pérez & F. Arroyo: from the Chocó biogeographical region, and 4) *Magnolia* subsect. *Talauma*: from the lowland of the Caribbean and Continental Neotropical zone to low montane areas (Vázquez-García *et al.* 2018; Domínguez-Yescas & Vázquez-García 2019). The subsection *Talauma* is the group with the largest number of species, characterized by the presence of early deciduous stipules adnate to the petioles, leaving a scar of variable length depending on the species on both edges of the adaxial side of petioles and converging apically (Figlar & Nootboom 2004; Pérez *et al.* 2016; Vázquez-García *et al.* 2017).

#### 2.4.2 *Magnolia* subsect. *Talauma* in Cuba: taxonomic history

In 1912 Ignatius Urban published the first species of *Magnolia* subsect. *Talauma* from Cuba, as *Talauma minor* Urb. (Urban 1912), based on a recollection of Charles Wright from 1860. In 1923, Nathaniel Britton and Percy Wilson described *Talauma orbiculata* Britton & P. Wilson (Britton 1923), based on the collection of Brother León from 1922. Urban (1927) transferred *Talauma minor* Urb. to *Svenhedinia minor* (Urb.) Urb., and considered *T. orbiculata* as a synonym. The supposition of *T. orbiculata* as a synonym of *Svenhedinia minor* was a misunderstanding in the analysis of the locus classicus since Urban assumed it was the same for both taxa (León & Alain

1950). In 1946, Harold N. Moldenke described *Svenhedinia truncata* Moldenke (Moldenke 1946). Howard (1948) studied the Cuban taxa of *Magnolia* subsect. *Talauma*, and only recognizes two Cuban taxa: *T. minor* Urb. (including *T. orbiculata* as a synonym) and *T. truncata* (Moldenke) R.A. Howard. The main criterion used by Howard (1948) to differentiate both taxa was the truncated base of the leaves present in *T. truncata*, against rounded or acute base leaves in *T. minor*. However, he pointed out that the material used to describe *T. truncata* may have been a vegetative branch of *T. minor*.

In 1950 Brother León and Brother Alain, described *Talauma minor* var. *oblongifolia* León (León & Alain 1950). They recognized that the shape of the leaves, the shorter and more widely petiole, as well as the carpels and smaller seeds, seem to indicate a new species; however, in the absence of flowers, only the range of variation was given. These authors state that type specimens have been collected on the coast of Moa, as well as intermediate forms with oblong leaves mixed with elliptical leaves. The shared distribution area was the reason to consider it as a variety of *T. minor*. Also, León & Alain (1950) revalidated the name *Talauma orbiculata* Britton & P. Wilson, considered by Urban (1927) and Howard (1948) as synonymous of *Talauma minor* Urb. León & Alain (1951), in the treatment of *Magnoliaceae* for the "Flora de Cuba", recognized four taxa of *Magnolia* subsect. *Talauma*: (1) *T. minor* Urb. var. *minor*, (2) *T. minor* var. *oblongifolia* León, (3) *T. orbiculata* Britton & P. Wilson, and (4) *T. truncata* (Moldenke) R.A. Howard. León & Alain (1951) report that *T. truncata* is exclusive to the Pico Turquino region, while *T. orbiculata* is found throughout the Sierra Maestra.

In the Supplement for the "Flora of Cuba" published by Alain (1969), he suggested that all previously described taxa of *Talauma* from Cuba could be considered as one single taxon. Later, Borhidi & Muñiz (1971) considered *T. minor* as the only species of *Talauma* in Cuba and defined two subspecies: *T. minor* subsp. *oblongifolia* (León) Borhidi and *T. minor* subsp. *orbiculata* (Britton & P. Wilson) Borhidi. Bisse (1974, 1988) split all the morphological variability, previously reflected

by León & Alain (1950, 1951) and Borhidi & Muñiz (1971), into four species. For *T. minor* Urb. and *T. orbiculata* Britton & P. Wilson considered the criteria of Urban (1912) and Britton (1923), respectively. Bisse (1974) accepted *T. oblongifolia* (León) Bisse as a species and described *Talauma ophiticola* Bisse as a new species. Imchanickaja (1993) recognized *T. orbiculata* Britton & P. Wilson as a different valid species and considered the other taxa as subspecies of *T. minor*: *T. minor* Urb. subsp. *minor* and *T. minor* subsp. *oblongifolia* (León) Borhidi.

Based on anatomical and morphological (vegetative and reproductive) traits described by Nootboom (1993), Frodin & Govaerts (1996) made the combination of *T. minor* to *Magnolia minor* (Urb.) Govaerts and considered all the other names of the Cuban *Talauma* synonyms of *M. minor* (Acevedo-Rodríguez & Strong 2012; Rivers *et al.* 2016). The last taxonomic treatment of *Magnolia* subsect. *Talauma* in Cuba was published by Palmarola *et al.* (2016), who made the new combinations from *Talauma* to *Magnolia*. Palmarola *et al.* (2016) recognize *Magnolia minor* (Urb.) Govaerts and *Magnolia orbiculata* (Britton & P. Wilson) Palmarola in the same sense as Urban (1912) and Britton (1923), respectively. Palmarola *et al.* (2016) considered *Magnolia oblongifolia* (León) Palmarola, including *Talauma ophiticola* Bisse (without name under *Magnolia*), as a synonym, based on the existence of the collection *Bisse & Kohler HFC 5358* in the herbaria HAJB and JE, that combines oblong and elliptical leaves in a presumably unique individual. León & Alain (1950) had already mentioned this situation when they described *T. minor* var. *oblongifolia*: “on the coast of Moa, typical specimens have been collected as well as intermediate forms with oblong leaves mixed with elliptical leaves”. The different taxonomic classification criteria of *Magnolia* subsect. *Talauma* in Cuba are shown in Table 1.

**Table 1.** Historical classification of the Cuban taxa of *Magnolia* subsect. *Talauma*.

ID	Urban (1912)	Britton (1923)	Urban (1927)	Moldenke (1946)	Howard (1948)	León & Alain (1950)
1	<i>Talauma minor</i> Urb.		<i>Svenhedinia minor</i> (Urb.) Urb.		<i>Talauma minor</i> Urb.	<i>Talauma minor</i> Urb. var. <i>minor</i>
2						<i>Talauma minor</i> var. <i>oblongifolia</i> León
3						
4		<i>Talauma orbiculata</i> Britton & P. Wilson				<i>Talauma orbiculata</i> Britton & P. Wilson
5				<i>Svenhedinia truncata</i> Moldenke	<i>Talauma truncata</i> (Moldenke) R.A. Howard	

ID	León & Alain (1951)	Alain (1969)	Borhidi & Muñiz (1971)	Bisse (1974, 1988)	Imchanickaja (1993)	Palmarola <i>et al.</i> (2016)
1	<i>Talauma minor</i> Urb. var. <i>minor</i>	<i>Talauma minor</i> Urb.	<i>Talauma minor</i> Urb. subsp. <i>minor</i>	<i>Talauma minor</i> Urb.	<i>Talauma minor</i> Urb. subsp. <i>minor</i>	<i>Magnolia minor</i> (Urb.) Govaerts
2	<i>Talauma minor</i> var. <i>oblongifolia</i> León		<i>Talauma minor</i> subsp. <i>oblongifolia</i> (León) Borhidi	<i>Talauma oblongifolia</i> (León) Bisse	<i>Talauma minor</i> subsp. <i>oblongifolia</i> (León) Borhidi	<i>Magnolia oblongifolia</i> (León) Palmarola
3				<i>Talauma ophiticola</i> Bisse		
4	<i>Talauma orbiculata</i> Britton & P. Wilson		<i>Talauma minor</i> subsp. <i>orbiculata</i> (Britton & P. Wilson) Borhidi	<i>Talauma orbiculata</i> Britton & P. Wilson	<i>Talauma orbiculata</i> Britton & P. Wilson	<i>Magnolia orbiculata</i> (Britton & P. Wilson) Palmarola
5	<i>Talauma truncata</i> (Moldenke) R.A. Howard					



## MATERIALS AND METHODS

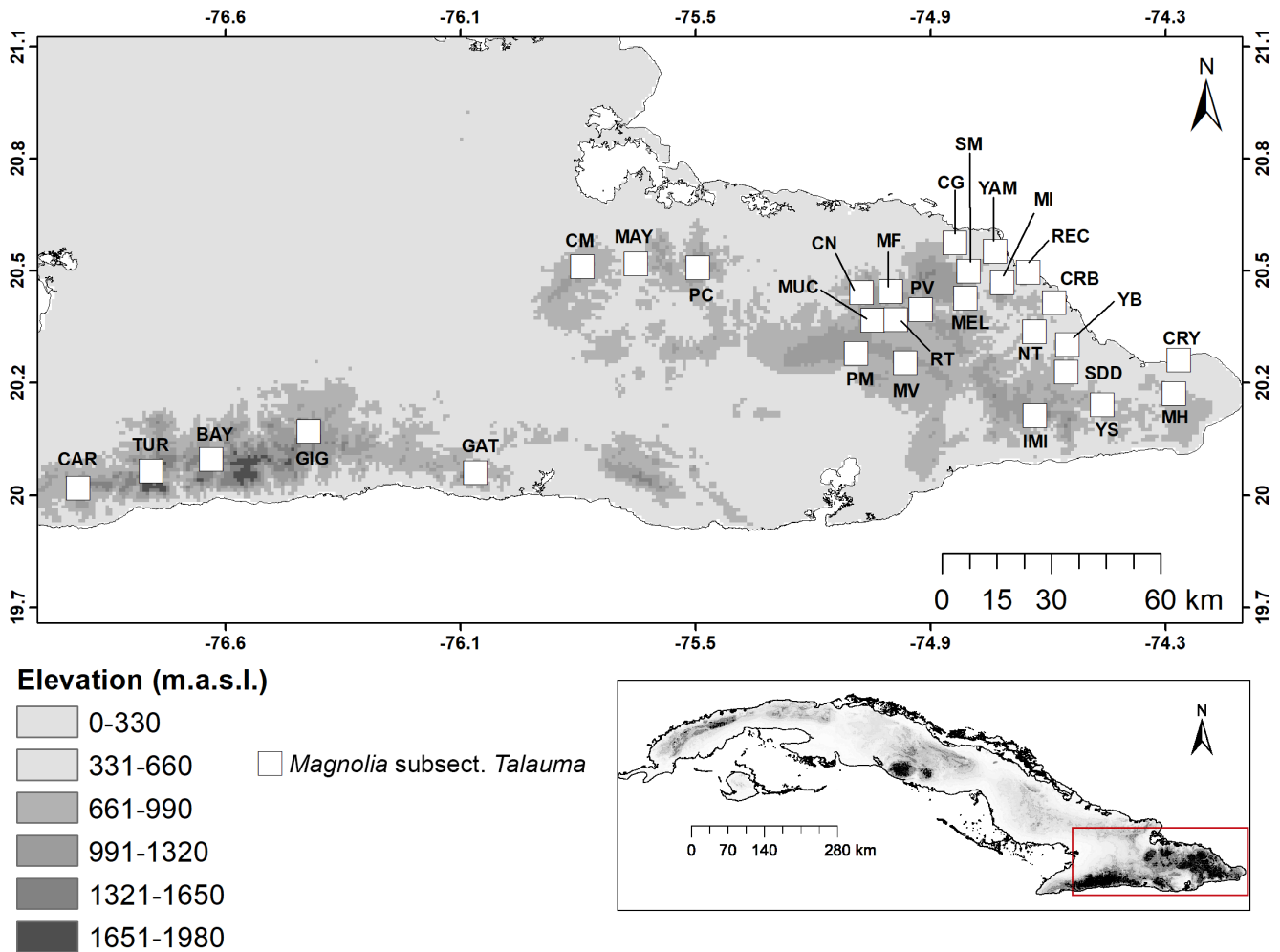
### 3.1 Data sampling: occurrence, leaf and DNA records

The samples for the ecological, morphological, and genetic analyses were collected (2015-2020) from individuals representing all the Cuban taxa of *Magnolia* subsect. *Talauma* throughout their entire distribution range in the mountains of Nipe-Sagua-Baracoa and Sierra Maestra in eastern Cuba (Fig. 1). In the field, individuals were identified based on tree and leaf shape according to the morphological criteria outlined by Bisse (1974, 1988) because this author defined the highest number of species units (see Table 1). To confirm species identity, 37 herbarium vouchers were collected or consulted (Table 2). All herbarium vouchers were deposited in the Herbarium Johannes Bisse (HAJB, herbarium codes follow Thiers 2023+) at the National Botanic Garden, University of Havana. The number of samples per species and localities is shown in Table 2. For the ecological and morphological analyses, data from herbarium specimens (deposited in HAC, HAJB, NY, and B) were included to cover not visited localities. Herbarium specimens reported before 1950 were not considered for niche analysis.

For ecological analysis, the GPS coordinates and the type of soil (serpentine derivative, limestone derivative, volcanic, and limestone derivate) were recorded for each individual. For the morphological analyses, 4-8 adult healthy leaves from 200 individuals were randomly collected and herborized. A leaf was considered healthy if the full outline of the leaf was undamaged. Leaves were photographed with a Nikon camera on a white background with a fixed ruler. The petiole of the leaf was removed before taking pictures, and the camera was mounted on a tripod to standardize the angle and distance of the photographs. To expand the geographic scope of the study, leaf samples from 43 herbarium specimens were included (deposited in HAC, HAJB, NY, and B). Hence, 243 individuals of *Magnolia* sect. *Talauma* subsect. *Talauma* in Cuba were morphologically analysed. For the genetic analyses, healthy young leaf samples of a total of 461 individuals belonging to 26 of 31 known localities were stored in self-sealed bags with silica gel for DNA to be extracted from. The resulting number of DNA samples represented 52 % of



the known individuals of *Magnolia* subsect. *Talauma* in Cuba (close to 900 individuals, data unpublished).



**Figure 1.** Geographic distribution of the sampled individuals of *Magnolia* subsect. *Talauma* in Cuba. Calizas de Mucaral (MUC), Camarones-Río Báez (CRB), Cañón del Río Yumurí (CRY), Cayo Guam (CG), Cayo Mujeres (CM), Cupeyal del Norte (CN), El Recreo (REC), El Gigante (GIG), Loma del Gato (GAT), Pico Caracas (CAR), La Melba (MEL), Mina Iberia (MI), Mina la Hoya (MH), Monte Fresco (MF), Monte Verde (MV), Naranjo del Toa (NT), Pico Cristal (PC), Pico La Bayamesa (BAY), Piedra La Vela (PV), Pinares de Montecristo (PM), Presa de Mayarí (MAY), Región del Toa (RT), Río Yamanigüey (YAM), Sierra de Imías (IMI), Subida a La Melba (SM), Sur de las Delicias del Duaba (SDD), Turquino (TUR), Yumurí del Sur (YS), Yunque de Baracoa (YB).

**Table 2.** Recorded localities, demographic information, DNA samples and herbarium vouchers of *Magnolia* subsect. *Talauma* in Cuba. Serie Herbario de la Flora de Cuba (HFC), Herbarium “Instituto de Ecología y Sistemática” (HAC), Herbarium “Jardín Botánico Nacional, Universidad de La Habana” (HAJB), Herbarium “The New York Botanical Garden” (NY), Herbarium “Botanischer Garten, Freie Universität Berlin” (B), National Park (NP), Ecological Reserve (ER), Natural Outstanding Element (NOE), Protected Area of Management Resources (PAMR), Extinct in the locality (\*), No field data available (?), no voucher (NV), Abreviature (Abrev.), Number of individuals (Indiv.).

Group	Localities (Protected Area)	Abrev.	Indiv.	Leaves	DNA	Vouchers (Herbarium)
Orbiculata	ER El Gigante	GIG	4	4	1	<i>Testé E. HFC 90667</i> (HAJB)
Orbiculata	ER Loma del Gato	GAT	?	4	0	<i>Hno. León 23366</i> (HAC)
Orbiculata	ER Pico Caracas	CAR	26	1	14	<i>Palmarola A. et al. HFC 89194</i> (HAJB)
Orbiculata	NP Pico La Bayamesa	BAY	6	0	1	<i>Molina Y. HFC 89590</i> (HAJB)
Orbiculata	NP Turquino	TUR	43	26	20	<i>Palmarola A. &amp; González L.R. HFC 89394</i> (HAJB)
Minor	Calizas de Mucará (NP Alejandro de Humboldt)	MUC	35	9	16	<i>Bécquer E.R. et al. HFC 90656</i> (HAJB)
Minor	Camarones-Río Báez (PAMR Cuchillas del Toa)	CRB	16	5	15	<i>Bécquer E.R. et al. HFC 89579</i> (HAJB)
Minor	Cañón del Río Yumurí (NOE Cañón del Río Yumurí)	CRY	5	5	4	<i>Bécquer E.R. et al. HFC 89829</i> (HAJB)
Minor	Cayo Guam	CG	43	3	22	<i>Palmarola A. et al. HFC 89243</i> (HAJB)
Minor	Cayo Mujeres	CM	2	0	1	<i>Palmarola A. et al. HFC 89213</i> (HAJB)
Minor	Cupeyal del Norte (NP Alejandro de Humboldt)	CN	34	4	19	<i>Falcón B. et al. HFC 88955</i> (HAJB)
Minor	El Recreo (NP Alejandro de Humboldt)	REC	4	2	4	<i>Bécquer E.R. et al. HFC 89467</i> (HAJB)
Minor	La Melba (NP Alejandro de Humboldt)	MEL	5	1	5	<i>Palmarola A. et al. HFC 89584</i> (HAJB)
Minor	Mina la Hoya (NOE Cañón del Río Yumurí)	MH	29	9	12	NV
Minor	Monte Fresco (NP Alejandro de Humboldt)	MF	18	0	12	<i>García A. et al. HFC 90715</i> (HAJB)
Minor	Monte Verde	MV	*	1	0	<i>Wright 1100</i> (B)
Minor	Naranjo del Toa (NP Alejandro de Humboldt)	NT	15	7	13	NV
Minor	NOE Pinares de Montecristo	PM	33	8	16	<i>Bécquer E.R. et al. HFC 90421</i> (HAJB)
Minor	NOE Yunque de Baracoa	YB	3	2	3	<i>Bisse J. HFC 5321</i> (HAJB)
Minor	Pico Cristal (NP Pico Cristal)	PC	16	13	15	<i>Bécquer E.R. et al. HFC 89921</i> (HAJB)
Minor	Piedra La Vela (NP Alejandro de Humboldt)	PV	13	3	11	<i>Bécquer E.R. et al. HFC 90519</i> (HAJB)
Minor	Presa de Mayarí	MAY	*	1	0	<i>Shafer 3691</i> (NY)
Minor	Región del Toa (NP Alejandro de Humboldt)	RT	29	7	15	<i>Bécquer E.R. et al. HFC 90660</i> (HAJB)

**Table 2.** Recorded localities, demographic information, DNA samples and herbarium vouchers of *Magnolia* subsect. *Talauma* in Cuba. Serie Herbario de la Flora de Cuba (HFC), Herbarium “Instituto de Ecología y Sistemática” (HAC), Herbarium “Jardín Botánico Nacional, Universidad de La Habana” (HAJB), Herbarium “The New York Botanical Garden” (NY), Herbarium “Botanischer Garten, Freie Universität Berlin” (B), National Park (NP), Ecological Reserve (ER), Natural Outstanding Element (NOE), Protected Area of Management Resources (PAMR), Extinct in the locality (\*), No field data available (?), no voucher (NV), Abreviature (Abrev.), Number of individuals (Indiv.).

Group	Localities (Protected Area)	Abrev.	Indiv.	Leaves	DNA	Vouchers (Herbarium)
Minor	Río Yamanigüey (NP Alejandro de Humboldt)	YAM	72	5	28	<i>Bécquer E.R. et al. HFC 89449</i> (HAJB)
Minor	Sierra de Imías (PAMR Cuchillas del Toa)	IMI	?	2	0	<i>Álvarez A. et al. HFC 27534</i> (HAJB)
Minor	Sur de las Delicias del Duaba (PAMR Cuchillas del Toa)	SDD	2	1	2	<i>Díaz J. et al. HFC 89435</i> (HAJB)
Minor	Yumurí del Sur	YS	8	5	5	<i>Bécquer E.R. et al. HFC 89510</i> (HAJB)
Oblongifolia	Calizas de Mucaral (NP Alejandro de Humboldt)	MUC	1	1	1	<i>Bécquer E.R. et al. HFC 90655</i> (HAJB)
Oblongifolia	Cayo Guam	CG	11	11	3	<i>Palmarola A. et al. HFC 89249</i> (HAJB)
Oblongifolia	Cupeyal del Norte (NP Alejandro de Humboldt)	CN	31	12	15	<i>Falcón B. et al. HFC 88959</i> (HAJB)
Oblongifolia	La Melba (NP Alejandro de Humboldt)	MEL	5	0	2	<i>Palmarola A. et al. HFC 89589</i> (HAJB)
Oblongifolia	NOE Yunque de Baracoa	YB	2	2	1	<i>Bécquer E.R. et al. HFC 89531</i> (HAJB)
Oblongifolia	Pico Cristal (NP Pico Cristal)	PC	5	4	3	<i>Bécquer E.R. et al. HFC 89933</i> (HAJB)
Oblongifolia	Piedra La Vela (NP Alejandro de Humboldt)	PV	4	3	3	<i>Bécquer E.R. et al. HFC 90543</i> (HAJB)
Oblongifolia	Río Yamanigüey (NP Alejandro de Humboldt)	YAM	6	6	6	<i>Bécquer E.R. et al. HFC 89452</i> (HAJB)
Oblongifolia	Sur de las Delicias del Duaba (PAMR Cuchillas del Toa)	SDD	1	1	1	<i>Díaz J. et al. HFC 89435</i> (HAJB)
Ophitcola	Cayo Guam	CG	130	15	33	<i>Bécquer E.R. &amp; Testé E. HFC 89439</i> (HAJB)
Ophitcola	Cupeyal del Norte (NP Alejandro de Humboldt)	CN	82	23	39	<i>Falcón B. et al. HFC 88950</i> (HAJB)
Ophitcola	La Melba (NP Alejandro de Humboldt)	MEL	12	5	12	<i>Palmarola A. et al. HFC 89587</i> (HAJB)
Ophitcola	Mina Iberia (NP Alejandro de Humboldt)	MI	77	16	45	<i>Palmarola A. et al. HFC 89261</i> (HAJB)
Ophitcola	Monte Fresco (NP Alejandro de Humboldt)	MF	11	0	8	NV
Ophitcola	NOE Yunque de Baracoa	YB	19	2	8	<i>Bécquer E.R. et al. HFC 89529</i> (HAJB)
Ophitcola	Pico Cristal (NP Pico Cristal)	PC	8	6	7	<i>Bécquer E.R. et al. HFC 89917</i> (HAJB)
Ophitcola	Piedra La Vela (NP Alejandro de Humboldt)	PV	4	0	3	<i>Bécquer E.R. et al. HFC 90531</i> (HAJB)
Ophitcola	Sierra de Imías (PAMR Cuchillas del Toa)	IMI	?	0	0	<i>Bisse J. et al. HFC 53300</i> (HAJB)
Ophitcola	Subida a la Melba (km 10)	SM	7	0	7	<i>Álvarez A. et al. HFC 42531</i> (HAJB)
Ophitcola	Sur de las Delicias del Duaba (PAMR Cuchillas del Toa)	SDD	12	8	10	<i>Bécquer E.R. et al. HFC 89556</i> (HAJB)

For all the following analyses (ecological, morphological, genetic, and phylogenetic), the results and graphical representations were built using the classification system proposed by Bisse (1974, 1988). This system divided the morphological variability into the highest number of units (*M. minor*, *M. orbiculata*, *M. oblongifolia*, and *T. ophiticola*), and was used in the field to identify each taxon. For the three taxa CS: *M. oblongifolia* = *M. oblongifolia* + *Talauma ophiticola*; and for the Two taxa CS: *M. minor* = *M. minor* + *M. oblongifolia* + *T. ophiticola*. However, in order to avoid confusion with the scientific names of the species and the different taxonomic treatments carried out on the group (see table 1), the taxa were considered morphological groups (Orbiculata, Minor, Oblongifolia, Ophiticola), and they will be treated as such in the following analyses.

## 3.2 Ecological analysis

### 3.2.1 Ecological Niche Models (ENMs)

In order to avoid autocorrelation and model overfitting (Anderson 2012), a spatial thinning of the occurrences of *Magnolia* subsect. *Talauma* was performed, leaving gaps of 3 km between records. This “gap distance” was based on the spatial resolution of the environmental variables (~1 km) and the environmental heterogeneity of Cuban mountain habitats. The final occurrence data used for the ENMs are in Table 3. As niche predictors, a set of 15 bioclimatic variables (spatial resolution: 0.86 km<sup>2</sup>) and the digital elevation model (DEM) were chosen from the WorldClim database (<http://www.worldclim.com>; Hijmans *et al.* 2005). Four of the bioclimatic variables (Bio8: Mean Temperature of Wettest Quarter, Bio9: Mean Temperature of Driest Quarter, Bio18: Precipitation of Warmest Quarter, Bio19: Precipitation of Coldest Quarter) were excluded from all analyses because they show odd spatial anomalies in the form of discontinuities between neighboring pixels in the absence of environmental gradients on the ground (Escobar *et al.* 2014). The variables were trimmed to the eastern Cuba region (extent of accessible area - M) using ArcGis v. 10.2 (ESRI 2014). A pre-model using Maxent v. 3.4.1 (Phillips *et al.* 2006) was built. For model calibration, the *Magnolia* subsect. *Talauma* individuals' thinned occurrence data and the bioclimatic variables were used, excluding the ones with the lower contribution of any

set of correlated variables ( $r \geq 0.85$ ). To improve the model calibration process, we designed three sets of variables (Table 4) as Cobos *et al.* (2019) recommended.

Considering the distribution in sympatry of the population from northeastern Cuba (see results), we treat these as only one ecological group for the calibration process. We built 1116 candidate models for the northeastern group and Orbiculata. These models integrated 12 values of regularization multiplier (0.5 to 6, at intervals of 0.5), all combinations of the five features classes (linear = l; quadratic = q; product = p; threshold = t; hinge = h), and the three set of environmental variables. The background extent selected for the models was based on a minimum convex polygon with a buffer distance of 0.5 degrees, and the models were limited to the calibration area.

**Table 3.** Number of original records and thinning records (after 3 km spatial thinning) used to create the ecological niche model of *Magnolia* subsect. *Talauma* in Cuba.

Taxa	Initial number of records	Number of records after 3 km spatial thinning
Minor	371	28
Orbiculata	78	6
Oblongifolia	58	10
Ophiticola	351	15

**Table 4.** Sets of environmental variables used for model calibration of *Magnolia* subsect. *Talauma* in Cuba. Source of the variables: WorldClim (Hijmans *et al.* 2005). Sets 2 and 3 did not include variable(s) with the lowest contribution to the model.

Climatic variables	Code	Set 1	Set 2	Set 3
Annual precipitation	Bio12	x	x	x
Precipitation seasonality (Coefficient of variation)	Bio15	x	x	x
Maximum temperature of warmest month	Bio5	x	x	x
Digital elevation model	DEM	x	x	x
Temperature annual range (BIO5-BIO6)	Bio7	x	x	x
Temperature seasonality (standard deviation *100)	Bio4	x	x	-
Isothermality (BIO2/BIO7) (*100)	Bio3	x	-	-

The performance of the candidate models was evaluated by considering the significance (partial ROC), omission rates, and model complexity (AICc). The selected best models were the significant models with omission rates  $\leq 5\%$  and  $\Delta AICc \leq 2$ . The model calibration process was done using *kuenm* (Cobos *et al.* 2019), an R v. 4.2.0 (R Core Team 2022) package for detailed development of ENMs using Maxent v. 3.4.1.

The parameter combination of the best model was used for the niche modeling. The final models were created with the full set of thinned records (Table 3) and the selected sets of variables (Table 4). Modeling was performed in Maxent v. 3.4.1 with 50 bootstrap replicates, and the best configurations obtained during the evaluation process. The median models of the logistic output were converted into a binary map in ArcGis v. 10.2 (ESRI 2014). The minimum training presence (MTP) was used as a threshold. It considers as suitable habitats all the pixels with higher values than the smallest value of probability corresponding to a real presence point. The contribution of each variable to the final model was assessed with a jackknife analysis in Maxent v. 3.4.1, and the total suitable area was calculated for each model.

### 3.2.2 Niche Identity Test

A Niche Identity Test was used in order to examine the niche divergence within *Magnolia* subsect. *Talauma*. This test is used to ask whether ENMs generated from two or more species are more different than expected if they are drawn from the same underlying distribution (Warren *et al.* 2008). Two indices for niche identity (Schoener's D and Hellinger's-based I) were computed with ENMTools package with 1 000 replicates in R v. 4.2.0 (R Core Team 2022). Schoener's D calculates the suitable range for a given species based on the probability distributions for inhabiting particular regions and assumes that the suitability scores produced by Maxent are proportional to the species abundance. Hellinger's-based also estimates habitat suitability from probability distributions but without the assumption of Schoener's D (Warren *et al.* 2010). Both indices range from zero (no overlap) to one (complete overlap) (Warren *et al.* 2008).

### 3.2.3 Point-based and cluster analyses

Using the best suitable dataset of variables (from ENMs analysis), pixel values for each environmental layer were extracted at each point of occurrence of *Magnolia* subsect. *Talauma*, and used to assess niche differences between groups. A Principal Component Analysis (PCA) was performed in R v. 4.2.0 (R Core Team 2022) to explore the relation across the climate variables. To test the differences in elevation over sea level between groups, a MonteCarlo analysis in add-ins PopTools v. 3.23 MSEXcel (Hood 2010) with 10 000 random permutations was made. We used the total number of geographical records (before spatial thinning) for *Magnolia* subsect. *Talauma* (858 records).

A Bayesian clustering approach based on Gaussian finite mixture models was carried out using the best suitable dataset of variables using the “mclust” R package (Fraley *et al.* 2012; Scrucca *et al.* 2016). The method tests the number of clusters and different mixture models that best fit the data according to the number of clusters (CI) chosen a priori. The method allows comparing the quality of the discrimination among clusters based on the Bayesian Information Criteria (BIC), allowing one to choose the best value(s) of CI without any information about individual assignment to the different clusters. The default “mclust” setting was used to assess the 14 types of models, which all differ in the covariance matrix landscape (see Scrucca *et al.* 2016; Zhang & Di 2020, for further details about the models). We varied CI values between 1 and 12. Because the analysis sometimes provided clusters having only one individual, those clusters were therefore considered “ghost” clusters and hence not considered true clusters.

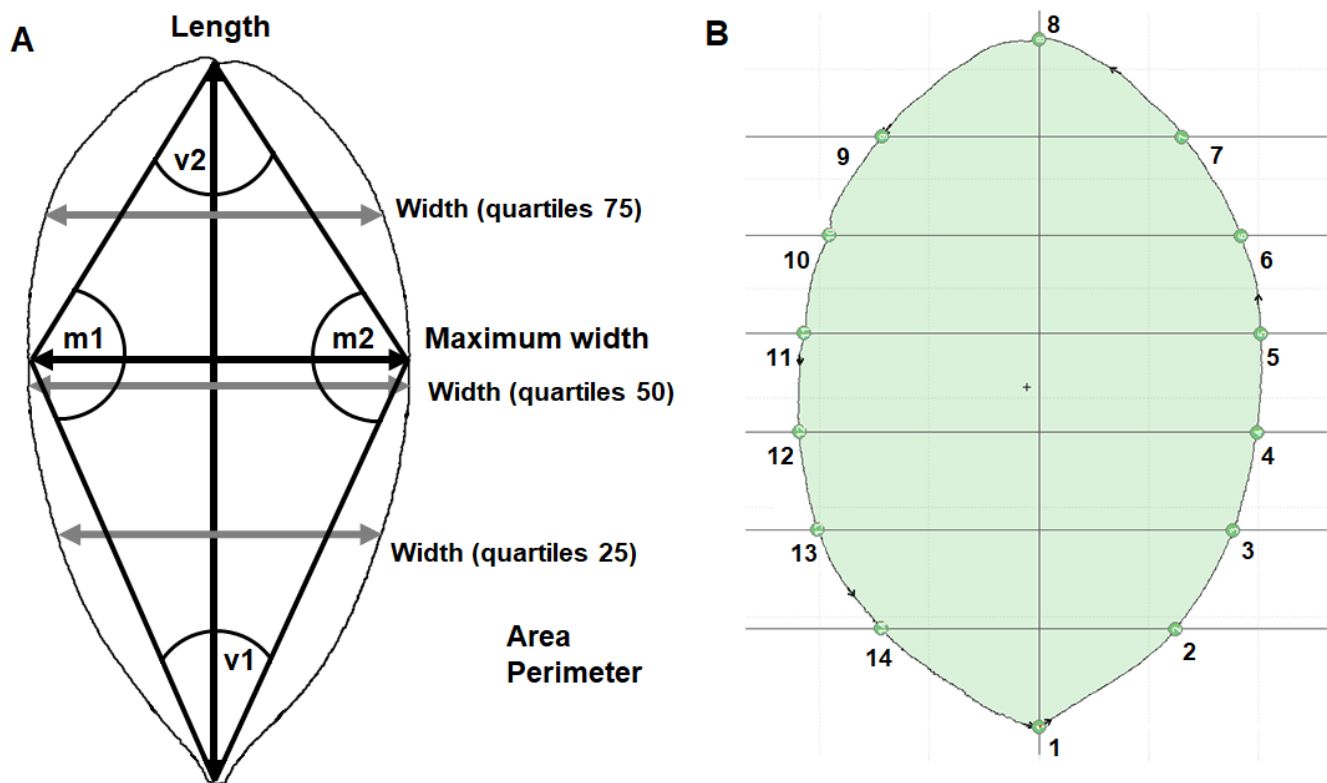
## 3.3 Foliar morphological analyses

### 3.3.1 Multivariate and Geometric morphometrics

Two types of morphological analyses were carried out on three independent datasets: 1) multivariate morphometrics analyses: a linear and angular measures dataset, 2) geometric morphometrics analyses: an outline dataset, and 3) a landmark coordinates dataset.



In the multivariate morphometrics analyses, linear and angular measures of leaf characters were automatically taken from the digital photographs using the R v. 4.2.0 (R Core Team 2022) package FoliometriK v. 0.2.2 (Ramírez-Arrieta & Denis 2020). Eleven leaf variables were measured for each leaf: central axis length (Length), maximum width, width at the three main quartiles (25, 50, and 75 quartiles), the perimeter of the contour (Perimeter), surface area (Area), and internal angles ( $v1$  = angle of the base,  $v2$  = angle of the apex;  $m1$  and  $m2$  = lateral angles at the maximum width) (Fig. 2A). Additional to the eleven measured variables, the maximum width/length ratio, named Calculated Index of Bisse ( $B_{CI}$ ), was calculated for each leaf. The twelve variables were averaged per individual for the 4-8 leaves available per individual. The averages of the twelve variables were used for all the subsequent statistical analyses.



**Figure 2.** A: The 11 morphological variables measured on leaves of *Magnolia* subsect. *Talauma* in Cuba;  $v1$  = angle of the base,  $v2$  = angle of the apex;  $m1$  and  $m2$  = lateral angles in the maximum wide. B: Quadratic grid with six lines and the position of the 14 landmarks (type 1: points 1 and 8; type 2: the other 12 points), placed on the leaves of *Magnolia* subsect. *Talauma* in Cuba.



In the geometric morphometrics analyses, the outline dataset was obtained through a semi-automated shape analysis performed in FoliometriK v. 0.2.2 (Ramírez-Arrieta & Denis 2020). We set the program outputs to the elliptic Fourier descriptors (EFDs) (Jensen 2003), to obtain the first 25 harmonics (Chuanromanee *et al.* 2019). The harmonics were normalized to eliminate the differences in size, position, rotation, and starting point. This allowed removing the undesired experimental source of random variation and analysing true differences of leaf shape between individual measurements (Jensen 2003). The landmark coordinates dataset was obtained as follows. The positions of landmarks were determined by placing a quadratic grid with six lines on each leaf. In between the intersections of the grid and the border of the leaf, we set 14 landmarks, two of them anatomical (type 1, i.e. apex and base) and the other 12 mathematically defined (type 2) (Fig. 2B). The landmarks X and Y coordinates were standardized using a Generalized Procrustes Analysis in Past v. 2.14 (Hammer *et al.* 2001). Next, two variables: the Sum EDMA (Euclidean Distance Matrix Analysis) and centroid size, were calculated.

The statistical significance of the differences among groups (Orbiculata, Minor, Oblongifolia, and Ophiticola) for each measured variable (linear and angular variables, Sum EDMA, and centroid size) was assessed by a MonteCarlo analysis in PopTools v. 3.23 (Hood 2010) with 10 000 random permutations. The variability of the whole sample was described by using a normalized Principal Component Analysis (PCA). Differences among groups were tested according to a one-way nonparametric MANOVA, using Euclidian distance, with 10 000 random permutations. Correction of p-values for multiple testing was done using the Bonferroni method. The multivariate comparisons were made independently for each dataset (linear and angular measures dataset, outline datasets, and landmark coordinates dataset). All statistical analyses were conducted in R v. 4.2.0 (R Core Team 2022) and Past v. 2.14 (Hammer *et al.* 2001), and the threshold used to decide for statistical significance was a p-value of 0.001.

### 3.3.2 Clustering Analysis based on morphological variability

A Bayesian clustering approach based on Gaussian finite mixture models was carried out using each of the three datasets of morphological variables using the “mclust” R package (Scrucca *et al.* 2016). The default “mclust” setting was used to assess the 14 types of models and varied CI (number of clusters) between 1 and 9 (default option). Three independent analyses were performed using the three datasets; to compare the power of those three sets of variables to discriminate among groups. The clusters with only one individual were considered “ghost” clusters and hence not considered true clusters.

## 3.4 Genetic analyses

### 3.4.1 DNA extraction and PCR

DNA was extracted from dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle & Doyle 1990) with MagAttract Suspension G solution-mediated cleaning (Xin & Chen 2012). For each sample, between 50 and 70 mg ( $\approx 1\text{cm}^2$ ) of plant tissue was used and placed in 2 ml Eppendorf tubes. To the fragmented tissue, 700  $\mu\text{l}$  of CTAB lysis buffer, 5  $\mu\text{l}$  of B-mercaptoethanol, 5  $\mu\text{l}$  of RNAase, and 5  $\mu\text{l}$  of proteinase k were added and mixed by vigorous vortexing and left to incubate at 60°C for at least one hour or for all night. Subsequently, 500  $\mu\text{l}$  of chloroform-isoamylalcohol was added. The mix was centrifuged for 8 minutes at maximum speed (15 000 rpm) and 4°C, and the supernatant was later transferred to a new 1.5 mL tube to which the precipitation buffer was added (7.5 M ammonium acetate) and isopropanol. The tubes were kept at room temperature (RT) for 5 minutes, and after centrifuging again at 15 000 rpm and 4°C for 30 minutes, the supernatant was removed by decantation.

The DNA was washed with 1 ml of 70 % ethanol (inverting the tube 2-3 times to clean the inner surface), and after centrifuging for 5 min at maximum speed (15 000 rpm), the fluid was removed with a pipette. The tubes were allowed to dry at 37°C for 7 min, and the DNA was subsequently resuspended in 100  $\mu\text{l}$  of high-salt TBE buffer (10mM Tris pH 8.0, 2mM EDTA, 1M NaCl). The

samples were incubated at 60°C for 30 minutes and subsequently left at RT overnight. Finally, 5 µl of MagAttract Suspension G solution was added to the bottom of each tube.

A total of 120 µl of 100 % ethanol was added, mixed by pipetting, and incubated at RT for 5 minutes. The tubes were placed in the Dynamag, which attracts the MagAttract particles and allows for easier extraction of the ethanol solution. Samples were washed twice with 200 µl of 70 % ethanol and allowed to dry at 37°C for 7 minutes. To resuspend the DNA, 100 µl of TBE buffer (10 mM Tris, 1 mM EDTA, pH 8) was added to each tube and mixed by light vortexing. The samples were incubated at 60°C for 5 minutes and placed back in the Dynamag to transfer the TE-DNA solution to a new tube. DNA quality was assessed by reading the Optical Density values at the wavelengths of 230 nm, 260 nm, and 280 nm using a spectrophotometer NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A).

A total of 174 microsatellite markers (SSR) developed on four Neotropical *Magnolia* species: *M. lacandonica* A. Vázquez, Pérez-Farr. & Mart.-Camilo (MA39), *M. mayae* Vázquez & Pérez-Farrera (MA40), *M. dealbata* Zucc. (MA41) and *M. cubensis* subsp. *acunae* Imkhan. (MA42) (Veltjen *et al.* 2019), were tested. The individuals of *Magnolia* subsect. *Talauma* were finally genotyped using 21 microsatellite markers (the only ones which amplified for all the groups) (Table 5). Simplex-PCRs were performed with the 21 selected microsatellite markers. Samples with high DNA purity and quality were chosen, with ratios 260/230 and 260/280 Optical Density close to 2. These samples were diluted in distilled water in a ratio of 1 DNA: 10 H<sub>2</sub>O. Two samples per group were selected.

PCR reactions were performed with 2 µl of microsatellite marker (in ratio 1 forward primer: 4 reverse primer: 4 fluorescent probe), 1 µl of DNA, and 2 µl of master mix for a final volume of 5 µl. The master mix consisted of 1×TrueStart Taq Buffer (Thermo Fisher Scientific), 1.5 µM MgCl<sub>2</sub> (Thermo Fisher Scientific), 0.125 µM dNTP, 5U of True Start Hot Start DNA polymerase (Thermo Fisher Scientific), and 0.4 mg /ml BSA (bovine serum albumin) per reaction. The

amplification program used was as follows: 95°C for 4 minutes, 35 cycles of 95°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute, and a final extension step at 72°C for 15 minutes. To the products of the PCR reactions were added 20 µl distilled water for a final volume of 25 µl and plated in 96-well plates. Identification by sequencing of amplified allelic variants was performed by MacroGen Inc. (Seoul, South Korea). The results were analyzed in the Geneious Prime program (<http://www.geneious.com>, Kearse *et al.* 2012).

Multiplex-PCRs were designed with microsatellite markers that showed clear peaks, amplification quality, and more than one allele in the Multiplex Manager program (Holleley & Geerts 2009). This program considers the range of each marker, the fluorescent probe attached to it, the base sequence, and the possibility of hybridization between markers and shows possible multiplex-PCR options. The variants obtained in the Multiplex Manager were modified and refined until the desired marker combinations were achieved.

For the multiplex-PCRs execution, the forward primers were attached to a universal tail at the 5' position (Vartia *et al.* 2014). The labels of the tails were the following: T3: 5' AATTAACCCTCACTAAAGGG 3', Hill: 5' TGACCGGCAGCAAATTG 3', Neomycin inverse: 5' AGGTGAGATGACAGGAGATC 3' and M13(-20): 5' GTAAAACGACGGCCAGT 3' (Tozaki *et al.* 2001). The forward oligonucleotide with the universal tail was used in combination with a primer with the same 5' sequence labeled with a fluorescent probe. FAM (blue), NED (yellow), PET (red), and VIC (green) fluorescent probes were ligated to the T3, Hill, Neo, and M13 tails, respectively. The combination and parameters of the four multiplex reactions are given in Table 5.

The lengths of the DNA fragments were detected using an ABI 3130XL fragment analyser, quantified with a GeneScan™ 500 LIZ™ size standard (Thermo Fisher Scientific), and analysed in Geneious v. 8.0.5 (Kearse *et al.* 2012) with the microsatellite plugin.

**Table 5.** Multiplex designed with the 21 microsatellites marker used for the genetic characterization of *Magnolia* subsect. *Talauma* in Cuba. The following universal tags were used: T3: 5' AATTAACCTCACTAAAGGG 3', M13 (-20): 5' GTAAAACGACGGCCAGT 3', Hill: 5' TGACCGGCAGCAAATTG 3' (Tozaki *et al.* 2001) and Neomycin reverse: 5' AGGTGAGATGACAGGAGATC 3'. The reverse primers had a PIG-tail (Brownstein 1996). Tm: Primer Melting Temperature (temperature at which one-half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability).

Marker (Multiplex)	Conc. (uM)	Forward primer Reverse primer	Tm	Amplicon size (pb)	Motif (No. repeats)	GenBank References
MA39_120_M13 (1)	1	F: GTGAGAACCGGTGGACAGTT R: ATGCATGTACAAAGGTGCGC	F: 59.894 R: 59.829	96	AC (15)	Unpublished
MA41_120_M13 (1)	1	F: ATTGTACACAGGTGTGGAGGC R: AAGTCTGGCTCCCAATGGTG	F: 60.251 R: 59.961	143	AG (17)	Unpublished
MA42_421_M13 (1)	3	F: GACAGCAGACCTGACCGATT R: GACCAGTGCATCCCATCAAA	F: 59.752 R: 58.162	280	AG (10)	MH923428
MA42_072_Hill (1)	2	F: CCCACCTAGGTTTCCAGTGC R: TGC GTTCGAAAGGCACAATG	F: 60.323 R: 60.041	245	AC (5)	MH923401
MA39_280_Neo (1)	2	F: AGCCGAACAGTCACTCCATC R: AGTGGCATTTCACCGCAATG	F: 59.752 R: 59.756	144	AG (14)	Unpublished
MA42_495_Neo (1)	2	F: TGCATCTCCTCATCCTCCCA R: ACGCCATTCAATTACCTACGG	F: 60.031 R: 58.443	97	AG (26)	MH923433
MA39_182_T3 (1)	1	F: CTACACGGGTGAAGCCTACC R: GGCCGTAATCAGAGTCCACC	F: 59.825 R: 60.179	129	AG (12)	MH923376
MA40_045_M13 (2)	1	F: TTGTGGGCCAAGCTCGATAG R: ATTGTGGCATGTACCTCGCA	F: 60.108 R: 60.036	232	AG (13)	MH923387
MA42_130_Hill (2)	1	F: CAGTCGACCGACCTCTCAAC R: CACGTCTGATGCCTCCTCAC	F: 60.11 R: 60.459	96	AG (22)	Unpublished
MA42_150_Neo (2)	2	F: TGCTCAGTGCTCACATGAGG R: CCGGTGTTACCTGAGCATT	F: 60.037 R: 60.606	94	AC (15)	Unpublished
MA39_259_T3 (2)	1	F: TGATAGAGTGGGATGGCGGA R: TTGGTTCATGCATCGGTCT	F: 60.106 R: 59.672	96	AG (11)	MH923380
MA42_255_M13 (3)	1	F: ACGTGGGTCGAGGATCAAGT R: GGACCCACCTCCAACAGATC	F: 60.899 R: 59.747	137	AG (14)	MH923417
MA42_231_Hill (3)	0.5	F: GGGTGC GAAATGTGCATCAA R: GGGCCAGTGAGCATTAGAGC	F: 59.757 R: 60.817	131	AG (14)	MH923413
MA42_028_Neo (3)	1	F: GGATCGTCTTCCGCCATTCT R: TTCCGTACGATGCTCCCATG	F: 59.895 R: 59.896	151	AG (33)	MH923398
MA39_191_Neo (3)	1	F: TCCAACGAGTACTTGGGCAG R: GATGCGTCCTTGAGTCCCAA	F: 59.68 R: 60.036	171	AG (22)	Unpublished
MA42_441_M13 (4)	1	F: TTGACTGCATCTCCCTTCT R: CTCATCTCCGCTTCAGCAGG	F: 58.339 R: 60.53	305	AG (5)	Unpublished
MA42_471_M13 (4)	1	F: TGATGAAGAGCCCAGATCGTC R: TGGCCTTGTTCTCCATACGT	F: 59.589 R: 59.019	153	AG (16)	MH923429
MA41_373_Hill (4)	1	F: GCGCCCAATCAGAACAACAAC R: GGGGAAGAGCTTCTTCGCCA	F: 60.387 R: 60.322	165	AG (16)	MH923396
MA39_442_Neo (4)	1	F: AGTCGATCCTCTTGCTGCAC R: GAGGGAGCATCGGCCATTAC	F: 60.108 R: 60.604	109	AAG (8)	MH923386
MA39_185_Neo (4)	1	F: CGGGTGTGTAGATGACGCT R: AAGACACGGAATGGGACGAG	F: 60.109 R: 59.753	209	AG (15)	MH923377
MA39_342_T3 (4)	1	F: TCCCTTCAGTCTTCACACGC R: AAAGGAGCGTTGAGTGGTGG	F: 59.966 R: 60.535	146	AG (14)	MH923384

### 3.4.2 Population Genetic structure at subsection level

Genetic diversity values per group (Orbiculata, Minor, Oblongifolia, and Ophiticola) were calculated using GeneAlex v. 6.5 (Peakall & Smouse 2012), Genepop v. 4.7.5 (Rousset 2008), and FSTAT v. 2.9.4 (Goudet 2001). Genetic differentiation between groups was estimated through pairwise comparisons of  $F_{ST}$  (Weir & Cockerham 1984) and  $D_{JOST}$  values (Jost 2008) using the fastDivPart function of the R package diveRsity (Keenan *et al.* 2013). The identification of genetic clusters and the assignment of individuals was performed in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000), which uses a Bayesian clustering approach using MCMC (Markov Chain Monte Carlo) for posterior distribution sampling. STRUCTURE analyses were conducted by selecting a model that assumes admixture, correlated allele frequencies, and without prior population information. First, 10 replicates were run for each number of genetic clusters considered (K), with K varying between 1 to 20 and a burn-in period of 50 000 iterations followed by a run-length of 150 000 iterations of the Markov Chain. The model (value of K) choice was made according to the method of Evanno *et al.* (2005) as implemented in STRUCTURE HARVESTER v. 0.6.94 (<http://taylor0.biology.ucla.edu/structureHarvester>) (Earl & von Holdt 2012). Then, 100 new repetitions of the MCMC method were run for the best K value. CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) was used to estimate similarities between runs and to average the membership probabilities. Final bar plots displaying individual admixture coefficients were obtained thanks to STRUCTURE PLOT v. 2.0 (Ramasamy *et al.* 2014). An individual was considered as a member of a genetic cluster when its probability of belonging to that group was higher than or equal to 0.9. A second STRUCTURE analysis was executed (using the same configuration) without considering the individuals of Oblongifolia (see the result section for rationale).

Because the MCMC method implemented in STRUCTURE is based on a population genetic model (assuming Hardy-Weinberg genotypic proportions and no linkage disequilibrium within each inferred genetic cluster), the results of genetic clusters and assignment of individuals may be affected by the potential low model fit to data. Thus, a non-model-based multivariate

clustering analysis was also performed. A DAPC analysis (Discriminant Analysis of Principal Components) was executed in R v. 4.2.0 (R Core Team 2022) using the *adegenet* R package (Jombart *et al.* 2010). Firstly, a PCA was run on the whole dataset for which the first 200 Principal Components (PCs) were retained. Secondly, a discriminant analysis was executed using the number of genetic clusters defined in the previous step. Parallel to the STRUCTURE analysis, a second DAPC analysis was done without *Oblongifolia*. An individual was considered a member of a genetic cluster when its probability of belonging to that group was higher than or equal to 0.9.

### 3.4.3 Testing hybrids

The presence or absence of hybrid individuals was estimated with NEWHYBRIDS (Anderson & Thompson 2002; Anderson 2008), using the 21 microsatellite markers. This analysis computes the posterior probability that an individual in the sample belongs to each of the different hybrid categories. The program was run to identify F1 (First Generation), F2 (Second Generation), and backcrossing hybrids from the whole genotypic data. The posterior probability reflects the level of certainty that an individual belongs to a hybrid category. The analysis was performed based on ten iterations carried out using Jeffreys's prior (see Anderson & Thompson 2002; Anderson 2008) and setting the burn-in period to 150 000, with a MCMC length of 500 000 replicates. A first performance was made without previous information on populations or taxonomic identity. A second analysis was made using prior information about the groups identity based on the individuals perfectly classified based on morphological (*mclust*) and genetic (STRUCTURE) probabilities. The 75 % of the best-classified individuals were used for calibrating the analysis, and the other 25 % for tested (Minor: 38 individuals for calibration and 13 for a test; *Ophiticola*: 12 individuals for calibration and 5 for a test). A probability threshold of 0.9 and 0.7 was used to classify the individuals in pure, F1, F2, and backcrosses.



### 3.5 Phylogenetic analysis

The complete chloroplast sequence with the annotations for *Magnolia* subsect. *Talauma* in Cuba and the outgroup was obtained from the research group of Marie-Stéphanie Samain PhD. (Instituto de Ecología, Red de Diversidad Biológica del Occidente Mexicano, Pátzcuaro, Mexico). The phylogenetic reconstruction was performed with a fully-sequenced plastome (two samples: Minor, Oblongifolia, Ophiticola; one sample: Orbiculata). Based on the result of Veltjen *et al.* (2022), as outgroups, one sample of each taxon from different Caribbean regions (Mexico, Puerto Rico, Dominican Republic, and Cuba) and subsections of *Magnolia* were selected. *Magnolia* sect. *Talauma* subsect. *Cubenses*: *M. cubensis* Urb. subsp. *cubensis* (Cuba), *M. cubensis* subsp. *acunae* Imkhan. (Cuba), *M. cristalensis* Bisse (Cuba), *M. domingensis* Urb. (Dominican Republic), *M. portoricensis* Bello (Puerto Rico); *Magnolia* sect. *Talauma* subsect. *Talauma*: *M. lacandonica* A. Vázquez, Pérez-Farr. & Mart.-Camilo (Mexico), and *M. sinacacolinii* A. Vázquez (Mexico); *Magnolia* sect. *Macrophylla*: *M. dealbata* Zucc.

These plastomes were trimmed to include only one copy of the IR (inverted repeat region), as proposed by Dong *et al.* (2022). The aligned was made using MAFFT v. 7.272 (Kato & Standley 2013) in its default settings. The resulting alignment was visually inspected in Geneious v. 8.0.5 (Kearse *et al.* 2012). The length of the plastome sequence after the alignment was 133 450 pb. ML (Maximum Likelihood) analyses were run under the GTR + G substitution model using the software RAxML-HPC v. 8 (Stamatakis 2014). The analysis was run on XSEDE ([www.sxede.org](http://www.sxede.org)) in the CIPRES Science Gateway v. 3.1 (Miller *et al.* 2010). The options “searching the best ML tree” and “performing a rapid bootstrapping (1 000 replicates)” were selected. Branches were considered statistically supported for bootstrap values > 70 % (Hillis & Bull 1993). The model GTRGAMMA was selected for bootstrapping. MrBayes v. 3.2.7a (Ronquist & Huelsenbeck 2003) was used to estimate Bayesian trees, which were conducted on XSEDE in the CIPRES Science Gateway v. 3.1 (Miller *et al.* 2010), under the following settings: starting from random trees, Markov Chain Monte Carlo (MCMC) simulations for 10 million generations, sampling every 1 000



generations. The first 10 % of the trees were discarded as burn-in. Output trees were visualized and exported in TreeGraph v. 2.15.0-887 beta (Stöver & Müller 2010).

For the ancestral range reconstruction, the plastome sequence alignment was used to infer a calibrated phylogeny using BEAST 2.6.7 (Bouckaert *et al.* 2019). Substitution model was estimated using the bModelTest 1.2.1 package (Bouckaert & Drummond 2017). A relaxed clock Log Normal and a Yule prior were used. Due to the lack of fossil data for the species included in our analysis, we calibrated our tree using the dates presented by Veltjen *et al.* (2022) for *Magnolia* sect. *Talauma*. A uniform prior was set with a minimum bound of 24 and a maximum of 36 mya for the section. BEAST analysis was set to run for 100 million generations with 10 % as burn-in. We checked the convergence values using Tracer 1.7 (Rambaut *et al.* 2018) to ensure an effective sample size (ESS) > 200. Treeannotator 2.6.7 (Bouckaert *et al.* 2019) was used to create a maximum clade credibility tree with node height representing the mean heights.

The ancestral range reconstruction was estimated using R 4.2.2 (R Core Team 2022) and the BioGeoBEARS package (Matzke 2014, 2013). Six regions were defined: Mesoamerica, Puerto Rico, Hispaniola, and the three Cuban regions of Guamuhaya, Nipe-Sagua-Baracoa, and Sierra Maestra. The calibrated tree was used to test a total of six models that included three different base models (DEC, DIVALIKE, and BAYAREALIKE) along with their jump dispersal (+J) variants. The models were compared using the AICc criterion (Burnham & Anderson 2002).

### **3.6 Integrative taxonomy**

The values of probabilities of cluster assignment (mclust and STRUCTURE) based on morphological, ecological, and genetic data were related. For this analysis, a filter was made to ensure that the same individual had the three types of data (ecological, morphological, and genetic), resulting in 138 individuals. A Chi<sup>2</sup> Contingency Table was computed, in Past v. 2.14 (Hammer *et al.* 2001), to assess the agreement between ecological, morphological, and genetic classification. A heatmap was made to analyse the variation in the cluster assignment inside

each group; this analysis was only done for the morphological and genetic classification.

Three independent Mantel paired tests, with 10 000 random permutations, were made in order to prove the existence of a correlation between the matrix of ecological, genetic, and geographic distances. The test ecological vs. morphological variables was made based on 222 individuals, ecological vs. genetic variables: 461 individuals, and morphological vs. genetic: 138 individuals. A partial Mantel test was made to correlate, at the same time, the three sets of data: ecological, morphological, and genetic. In this case, the analysis was made based on the 138 individuals, which are the only individuals with values for the three sets of data. For the Mantel tests, a statistical significance probability value of 0.05 was chosen, and in the partial Mantel test, a Bonferroni correction was made, and the significance probability was 0.02. The group's limits based on ecological, morphological, genetic, and phylogenetic data were combined to analyse their similarities.

The genetic distances between individuals were estimated from the Codom-Genotypic distances and the linear genetic distances, calculated according to the method proposed by Smouse & Peakall (1999) with interpolation of the missing data. When data from a locus have been lost in an individual, this procedure allows interpolating the average of the genetic distances (calculated across all pairwise comparisons of the individual distances from the non-missing data).

### 3.7 Taxonomic treatment

Protologues of all Cuban names of *Magnolia* subsect. *Talauma* as well as all relevant taxonomic literature were consulted (Urban 1912; Britton 1923; Urban 1927; Moldenke 1946; Howard 1948; León & Alain 1950, 1951; Borhidi & Muñiz 1971; Bisse 1974, 1988; Imchanickaja 1993; Frodin & Govaerts 1996; Palmarola *et al.* 2016). The present revision was based on the study of nomenclatural types and other specimens. High-resolution images of 134 specimens from the herbaria: B, BM, BR, G, GH, HAC, HAJB, JE, K, LE, NY, P, S, US, were included in the analysis. Herbarium acronyms were cited according to Thiers (2023+), while the series of specimens

were cited according to the recommendations of Regalado *et al.* (2010). The digital images were consulted online on the websites of the respective herbaria or via <http://plants.jstor.org>. Lectotypes were designated in accordance with the relevant articles of the Shenzhen Code (International Code of Nomenclature; Turland *et al.* 2018). For the types of names based on collections by Charles Wright, the considerations proposed by Howard (1988) were followed. Due to the uncertainty that “duplicates” of a Charles Wright numbers correspond to the same collection (since most do not correspond to the same location and date), such specimens were designated as possible duplicates of the type and indicated with the legend “isolectotypes?”. The growth type of each group was registered during the field work. The size of the petioles was measured on the images of the herbarium materials with the TPSdig program of the TPS series (Rohlf 2015).

## RESULTS

### 4.1 Ecological limits

#### 4.1.1 ENMs and identity test

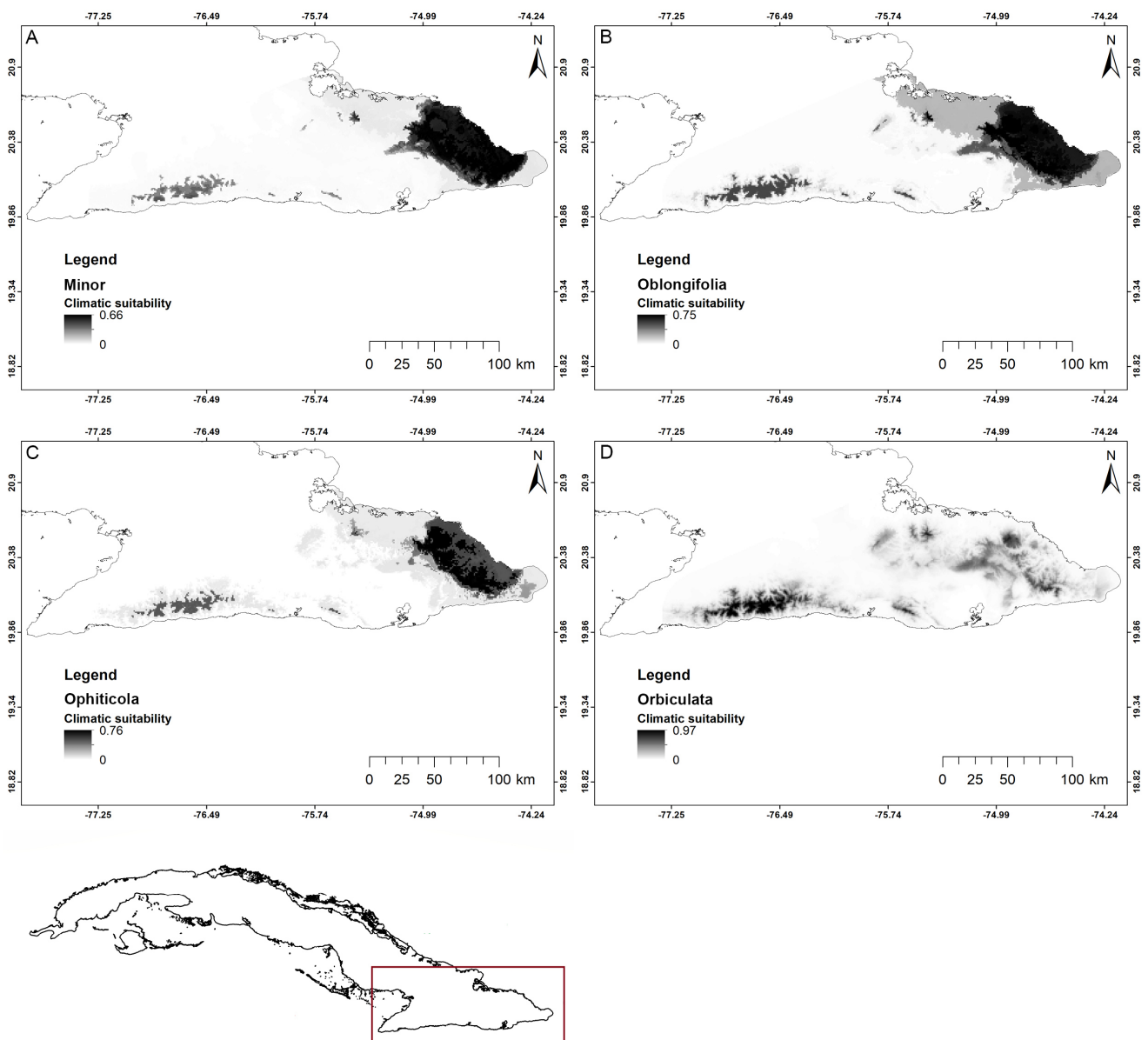
For the northeastern groups, the best model resulted from the combination of 1.5 regularization multipliers, feature class threshold, and set 3 of variables (Bio5, Bio7, Bio12, Bio15, and DEM). For Orbiculata group, the best model resulted from the combination of 3.5 regularization multipliers, feature class quadratic, and set 3 of variables. Annual precipitation (Bio12) and precipitation seasonality (Bio15) were the variables with the highest contribution to the models for all the groups, except for Orbiculata, in which maximum temperature (Bio5) and elevation (DEM) contributed the most to the model. Almost all ENMs performed for each group showed potentially suitable regions in large areas of the mountains of Nipe-Sagua-Baracoa and the Sierra Maestra (Fig. 3). Orbiculata was the group with the smallest potential area of suitability (Fig. 3D), which was restricted to the Sierra Maestra and a many places in northeastern Cuba. Based on the MTP (Minimum training presence) suitability area, Oblongifolia and Minor were the groups with the largest predicted area, and Orbiculata had the lowest value of predicted area (Table 6). Niche identity test analysis indicated high levels of niche overlap between the different groups (Table 7). Orbiculata had the lowest values for both indexes. Based on the comparison with the replicates (Supplementary material 1), it is clear that all groups are more similar than expected by chance.

**Table 6.** Values of MTP (minimum training presence) and area of the habitat suitability of the groups of *Magnolia* subsect. *Talauma* in Cuba.

Groups	MTP	Suitability area (km <sup>2</sup> )
Minor	0.097	5 206
Oblongifolia	0.298	7 765
Ophiticola	0.225	4 058
Orbiculata	0.548	685

**Table 7.** Niche identity test between the groups of *Magnolia* subsect. *Talauma* in Cuba. Numbers below diagonal refer to Schoener's D and numbers above diagonal refer to Hellinger's-based I values.

Groups	Minor	Oblongifolia	Ophiticola	Orbiculata
Minor	0	0.959	0.986	0.904
Oblongifolia	0.994	0	0.945	0.941
Ophiticola	0.998	0.976	0	0.945
Orbiculata	0.966	0.970	0.976	0



**Figure 3.** Areas of climatic suitability for *Magnolia* subsect. *Talauma* in Cuba.

#### 4.1.2 Point based and cluster analysis

Principal Component Analysis showed that *Orbiculata* group has the smallest and the clearest distinct environmental niche with no overlap ( $p < 0.001$ ) with the other group (Fig. 4A). The groups from northeastern Cuba, which showed the highest space of occupied environmental conditions, live under similar environmental conditions. In the PCA, the DEM was the variable with the higher contribution to the analysis. Figure 4B shows the clear disjointed geographic distribution of *Orbiculata* compared with the groups from the northeastern, with a distance of 83 km between the closest localities. The other groups of *Magnolia* subsect. *Talauma* seem to live in sympatry.

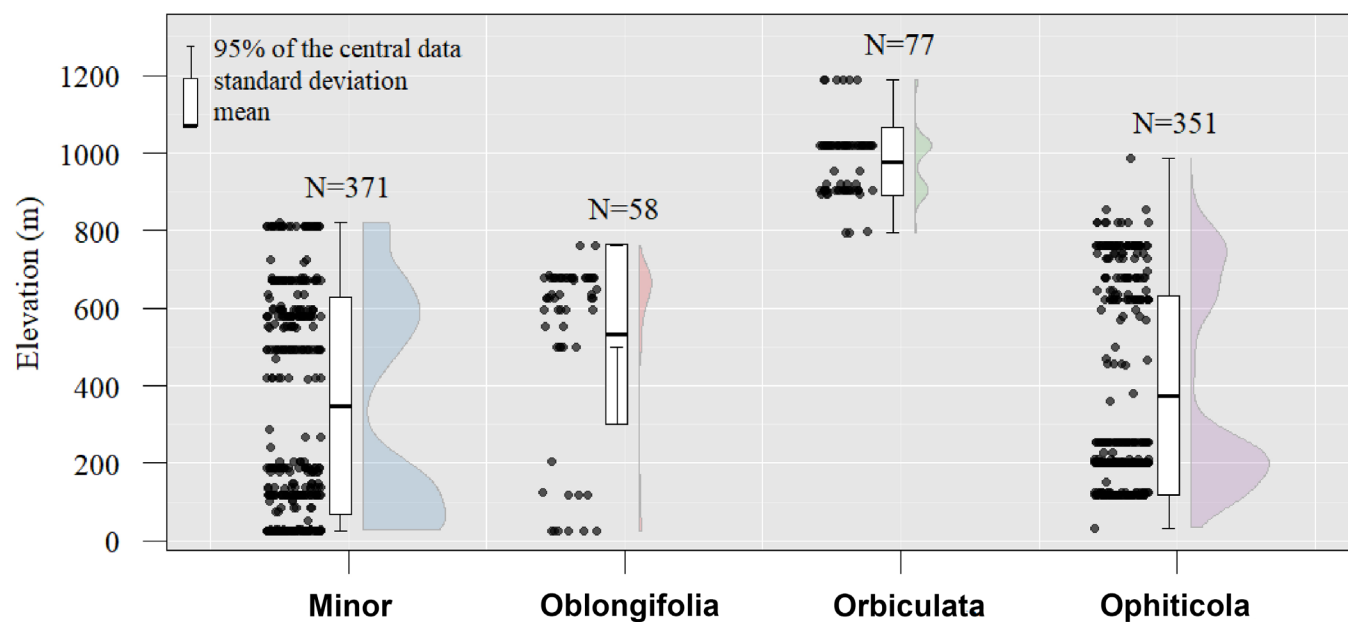
*Orbiculata* was the only group distributed at higher elevations (795-1 200 m.a.s.l.), while *Ophiticola* was the group with the highest variability in its elevation range (34-987 m.a.s.l.) (Fig. 5). Individuals of the groups *Minor* and *Oblongifolia* were found to be present at the same altitudes ( $p = 0.033$ ), and similar results were found between the groups *Minor* and *Ophiticola* ( $p = 0.109$ ). All the individuals of *Ophiticola* and *Orbiculata* were found on serpentine derivate and volcanic-limestone derivate soils, respectively. In the case of *Minor*, 43 % of individuals were found on limestone derivative, and the rest on serpentine. In *Oblongifolia*, only one individual was found on limestone derivative.

The clustering analysis based on ecological data showed  $CI = 9$  as the number of groups inferred by the best models (Table 8; Supplementary material 2). The individuals of *Oblongifolia* were included in all cases with the individuals of *Minor*; in this last group, almost all individuals were organized in seven clusters. 57.26 % of the individuals of *Ophiticola* were included in the same groups of *Minor* and *Oblongifolia*, but 37.75 % of the individuals of this group were organized in an independent cluster, only shared with one individual of *Minor*. In the case of *Orbiculata*, 95 % of its individuals were grouped in a unique cluster ( $CI = 7$  on Table 8), not shared with another group. An apparent correspondence between the assigned clusters and the geographic distribution was found due to the existence of many individuals of the same

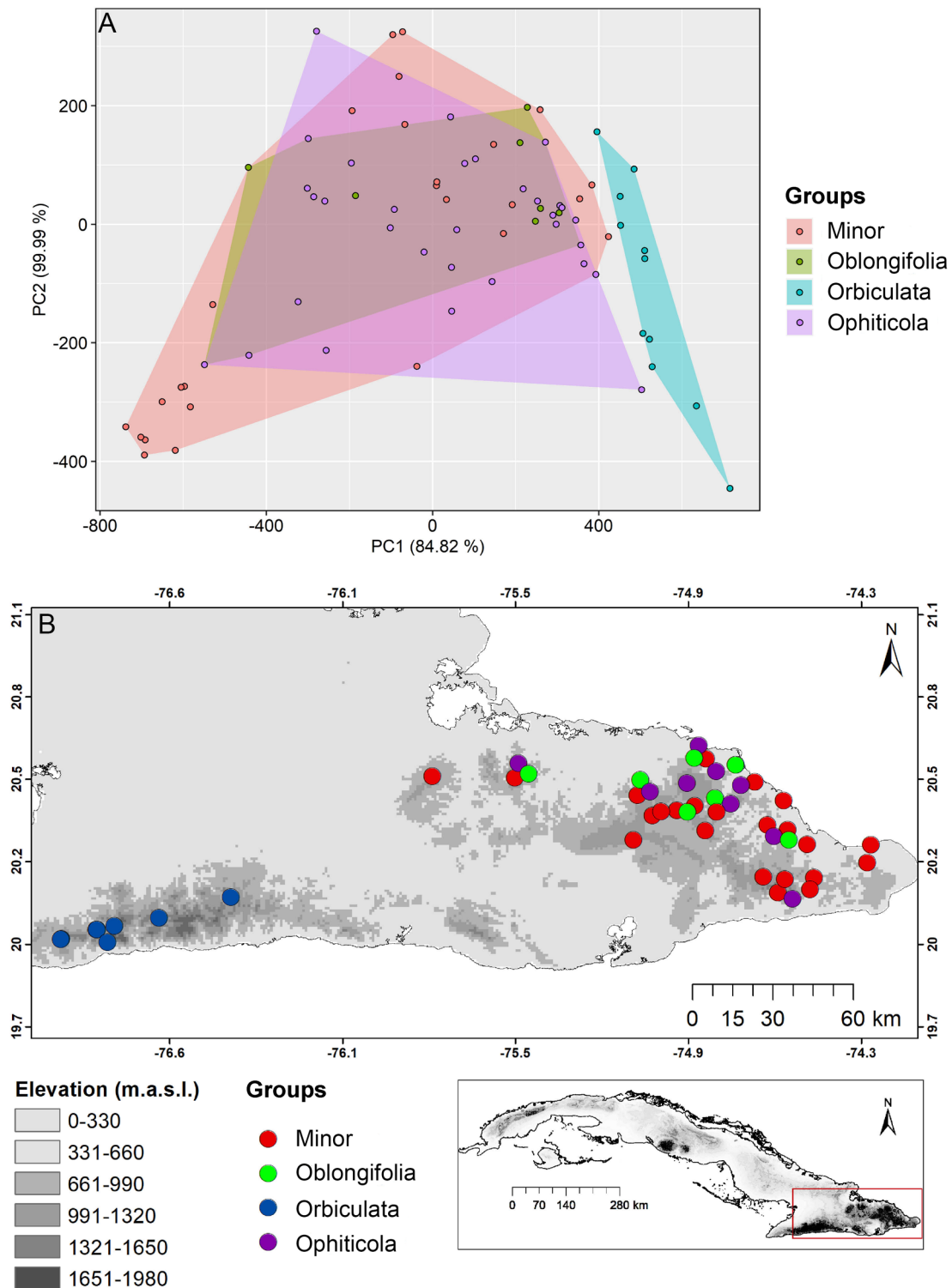
group/locality who were assigned to the same clusters. However, more analyses are needed to prove this association. The probabilities of assignment of each individual were higher than 0.9 in all cases.

**Table 8.** Classification matrix obtained after the cluster (CI) analysis using the environmental data of *Magnolia* subsect. *Talauma* in Cuba.

Groups	CI-1	CI-2	CI-3	CI-4	CI-5	CI-6	CI-7	CI-8	CI-9
Minor	98	31	78	38	49	82	0	1	10
Oblongifolia	13	2	3	23	9	6	0	0	1
Ophiticola	8	53	44	16	84	0	0	128	25
Orbiculata	4	0	0	0	0	0	74	0	0



**Figure 5.** Graphical representation of digital elevation model values extracted from the geographic position of *Magnolia* subsect. *Talauma* in Cuba.



**Figure 4.** A: Results of the Principal Component Analysis (PCA) performed with five bioclimatic variables (Bio5, Bio7, Bio12, Bio15, and DEM) used in the description of the ecological niche of *Magnolia* subsect. *Talauma* in Cuba. B: Current geographic distribution of *Magnolia* subsect. *Talauma* in Cuba.



## 4.2 Foliar morphological limits

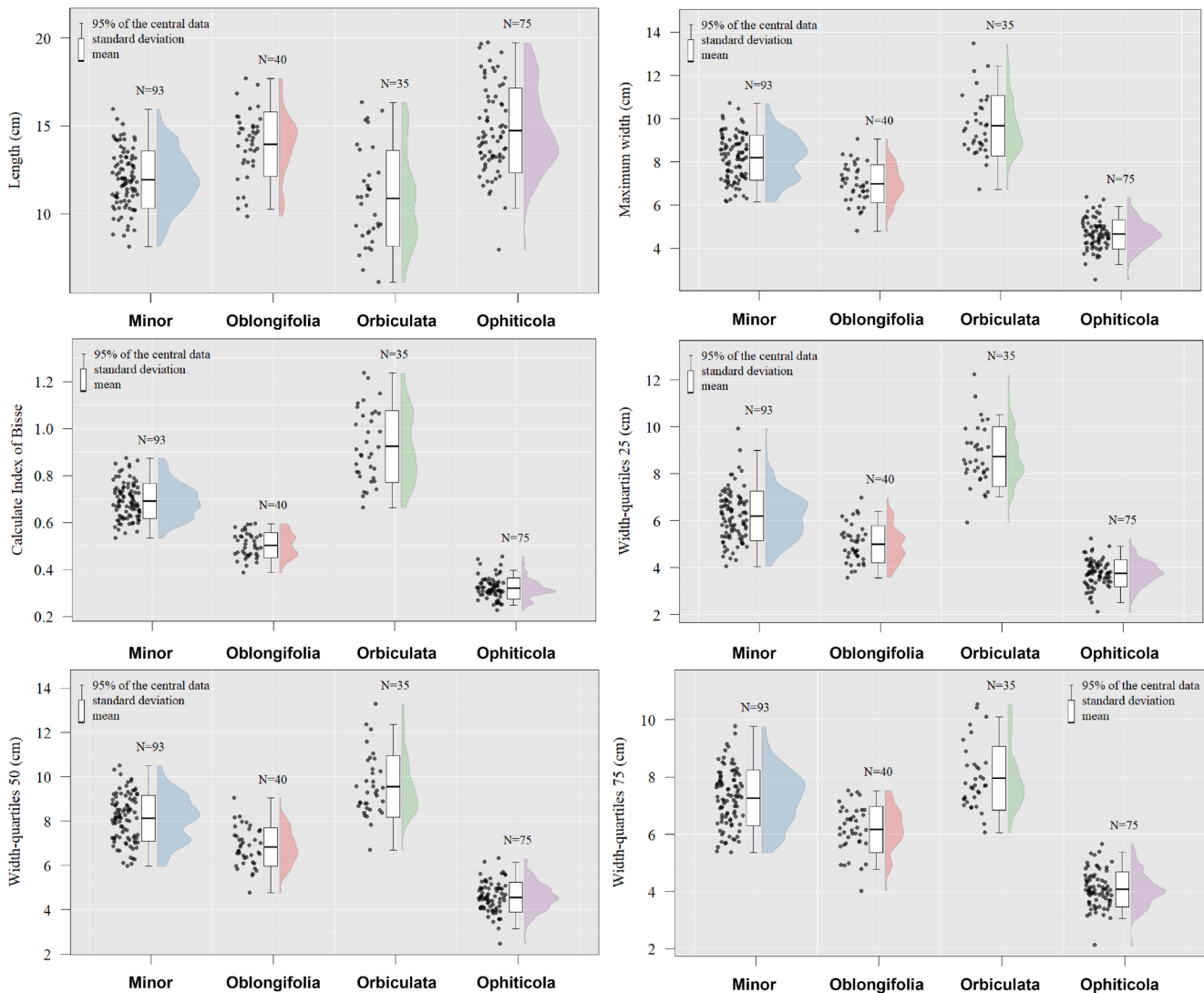
### 4.2.1 Multivariate and Geometric morphometrics

For most of the twelve variables, significant differences between each pair of groups were found (Fig. 6). There were three exceptions: the leaf perimeter did not show significant differences between Minor and Ophiticola ( $p=0.211$ ), and between Oblongifolia and Ophiticola ( $p=0.132$ ). Ophiticola showed higher values for the length and a lower one for the width. On the other hand, Orbiculata showed higher values for the width and lower for the length of the leaf. The relation width-length was close to 1 in Orbiculata; the minimum value for this relation was found in Ophiticola (Supplementary material 3).

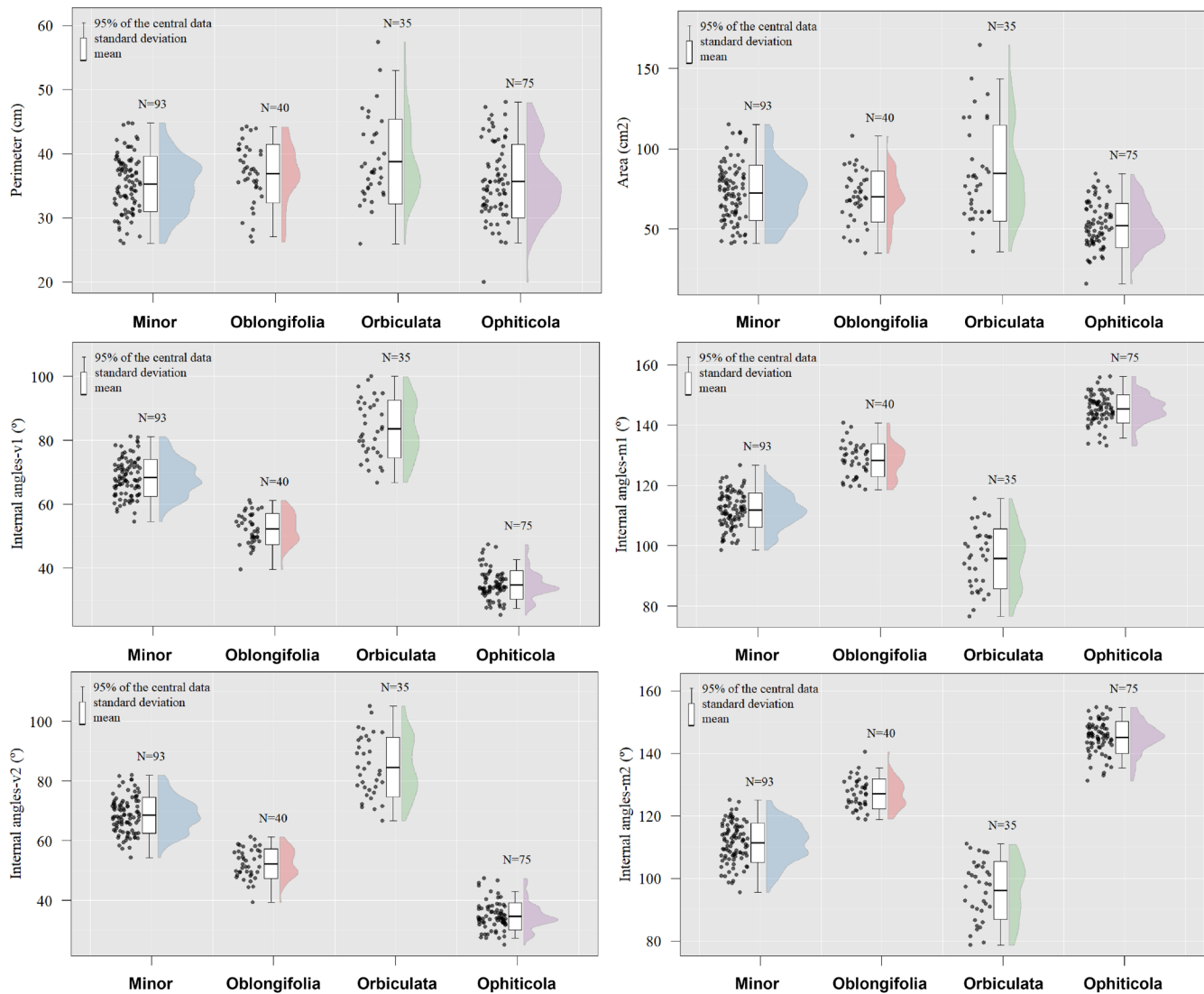
Despite the clear morphological differentiation between groups, overlap in the multivariate distributions of leaf morphology variables was observed (Fig. 7). The internal angle of the base (-0.324) and the leaf perimeter (0.5627) displayed the highest weight in the first two principal components, respectively (Supplementary material 4). It is possible to observe a clear differentiation between Minor and Ophiticola (Fig. 7). The variability of the individuals considered as Oblongifolia is distributed between Minor and Ophiticola. The morphological variation of Orbiculata and Minor (Fig. 7) showed some levels of superposition. The individuals of Orbiculata showed the highest morphological variation. The NPMANOVA showed significant statistical differences ( $p<0.0001$ ) between groups.

The comparison between groups, based on Sum EDMA and centroid size, showed significant differences for most comparisons (Fig. 8). The exceptions were: the Sum EDMA between Minor and Oblongifolia ( $p=0.316$ ) and between Orbiculata and Ophiticola ( $p=0.406$ ). Based on PCA for elliptic Fourier descriptors (Fig. 9A) and matrix of Landmark (Fig. 9B), the different groups had little overlap in the ordination space (Fig. 9). However, a clearer distinction among groups was obtained with landmark positions than with other quantitative variables. This was especially obvious with Orbiculata, which was strongly differentiated from other groups when using landmark

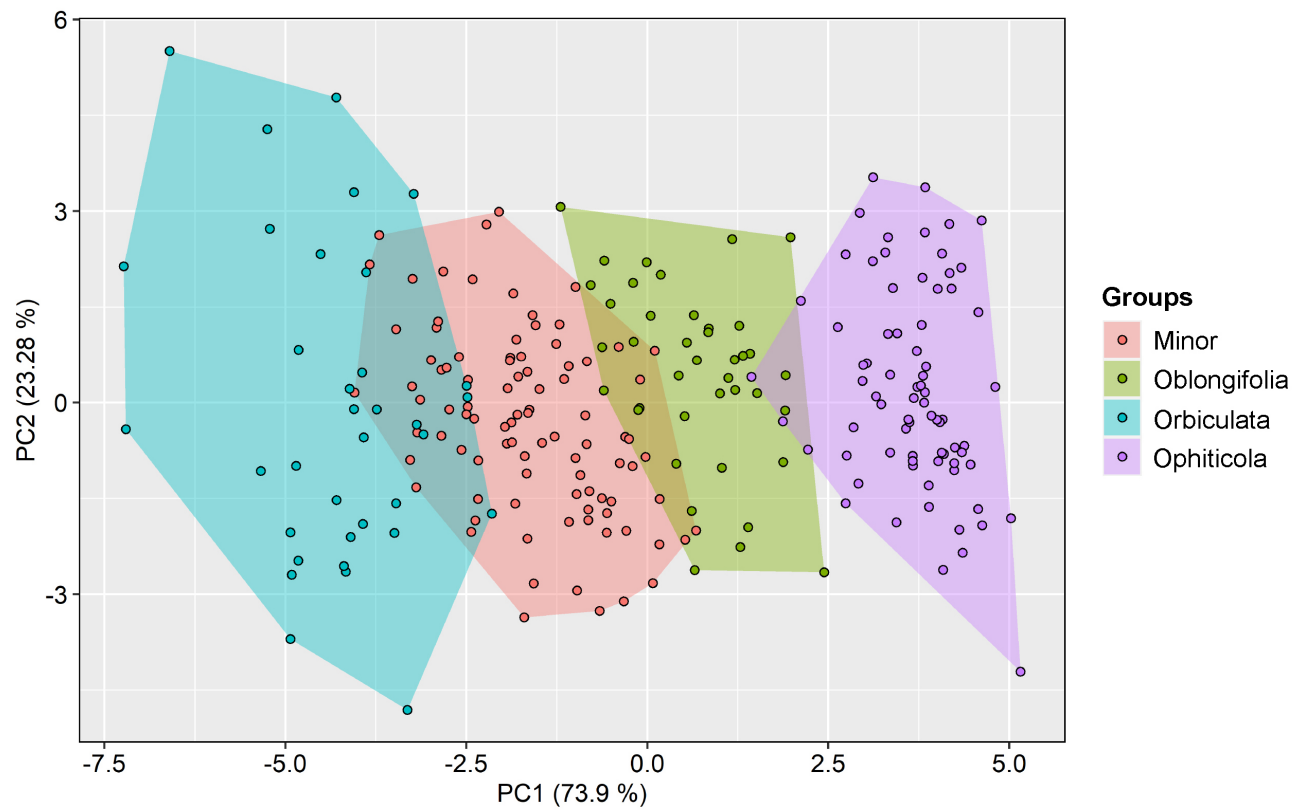
positions. In all cases, the NPMANOVA showed significant statistical differences ( $p < 0.001$ ) between the groups. The shape reconstruction based on the elliptic Fourier descriptors and the matrix of Landmark is shown in Figures 10 and 11.



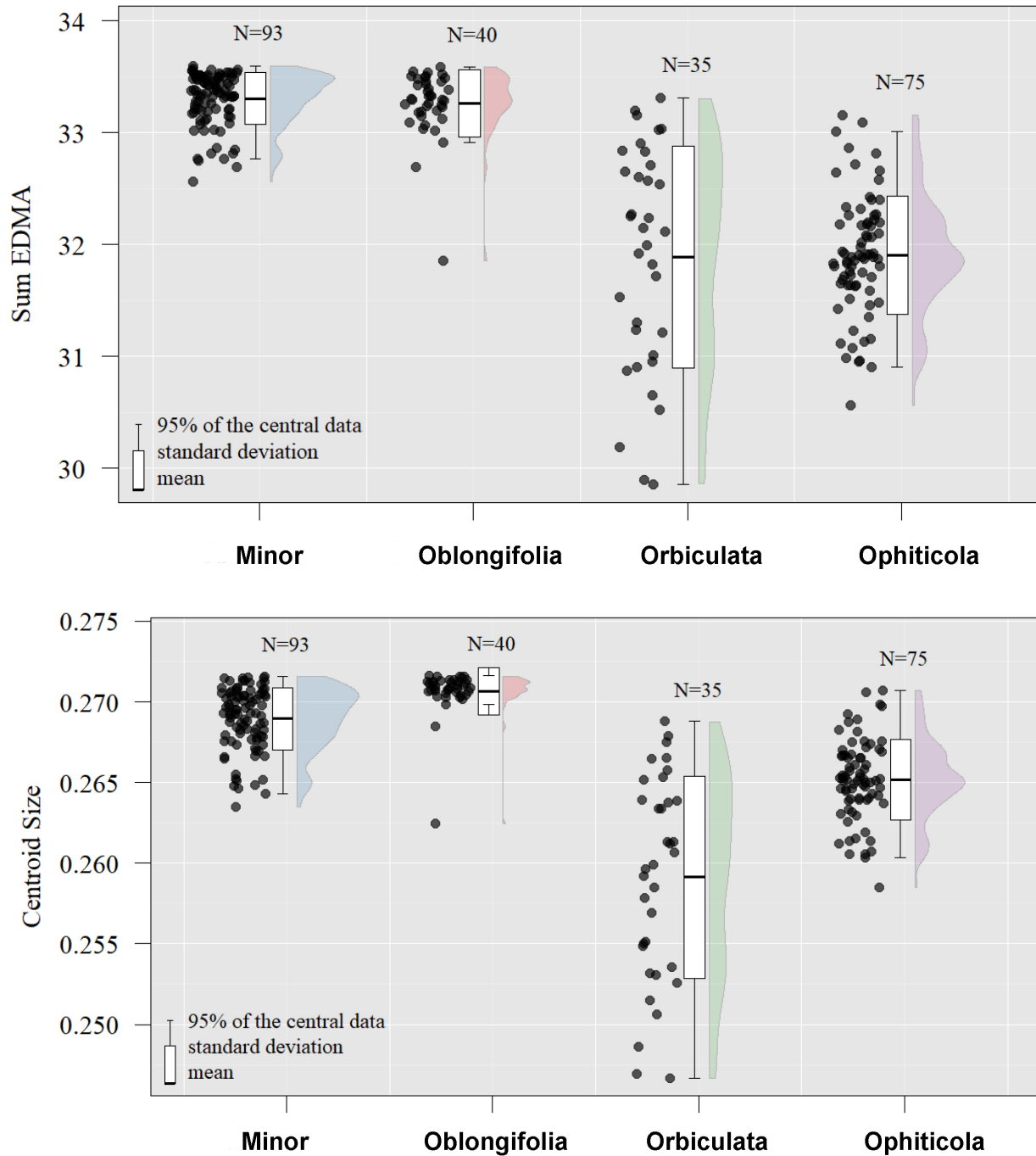
**Figure 6.** Graphic representation of the leaf's morphological variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba. The MonteCarlo probabilities were less than 0.001; except in the perimeter for Minor-Ophiticola ( $p=0.211$ ) and Oblongifolia-Ophiticola ( $p=0.132$ ).



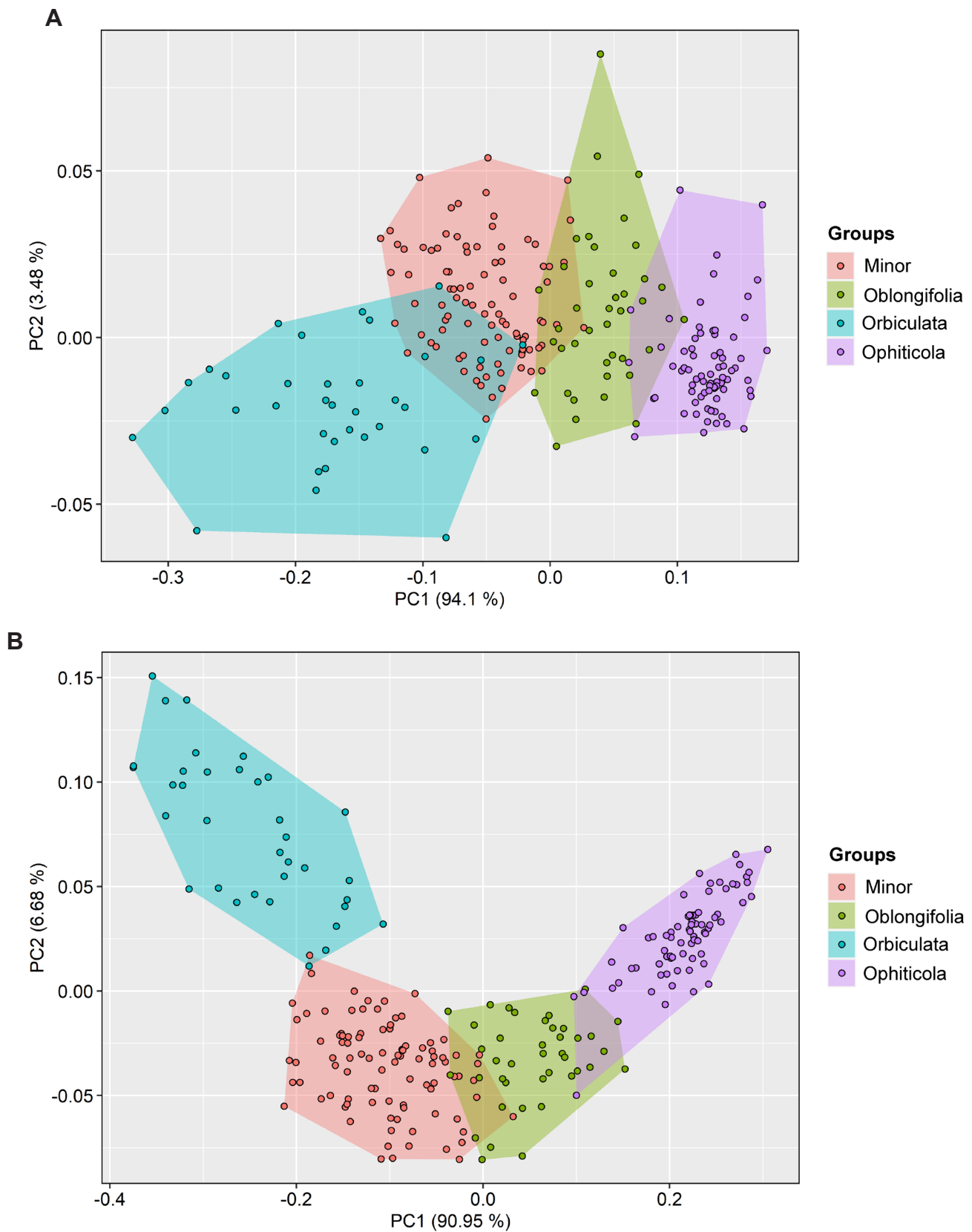
**Figure 6 (cont.).** Graphic representation of the leaf's morphological variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba. The MonteCarlo probabilities were less than 0.001; except in the perimeter for Minor-Ophiticola ( $p=0.211$ ) and Oblongifolia-Ophiticola ( $p=0.132$ ).



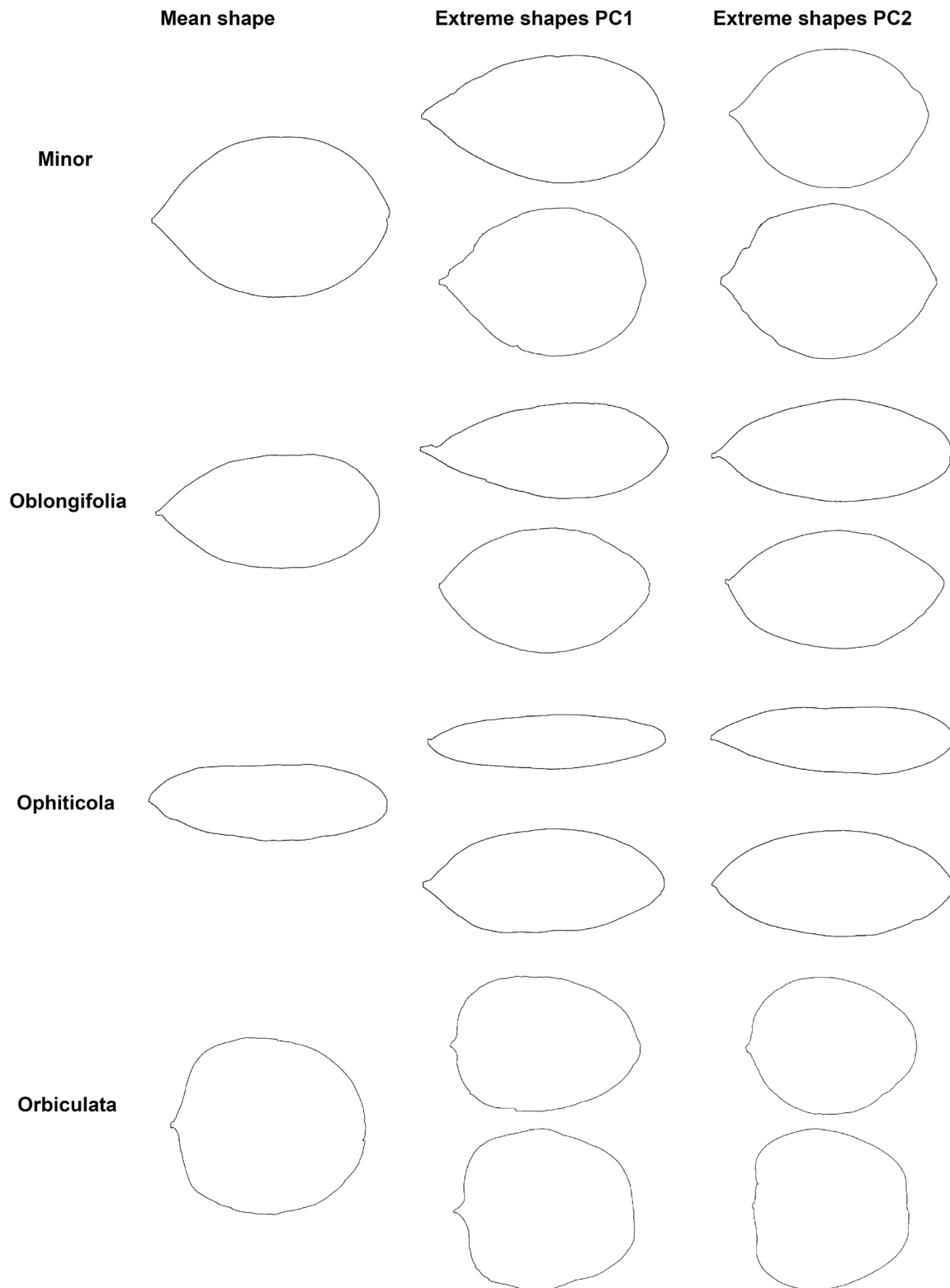
**Figure 7.** Principal Component Analysis for the multivariate morphometrics variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba.



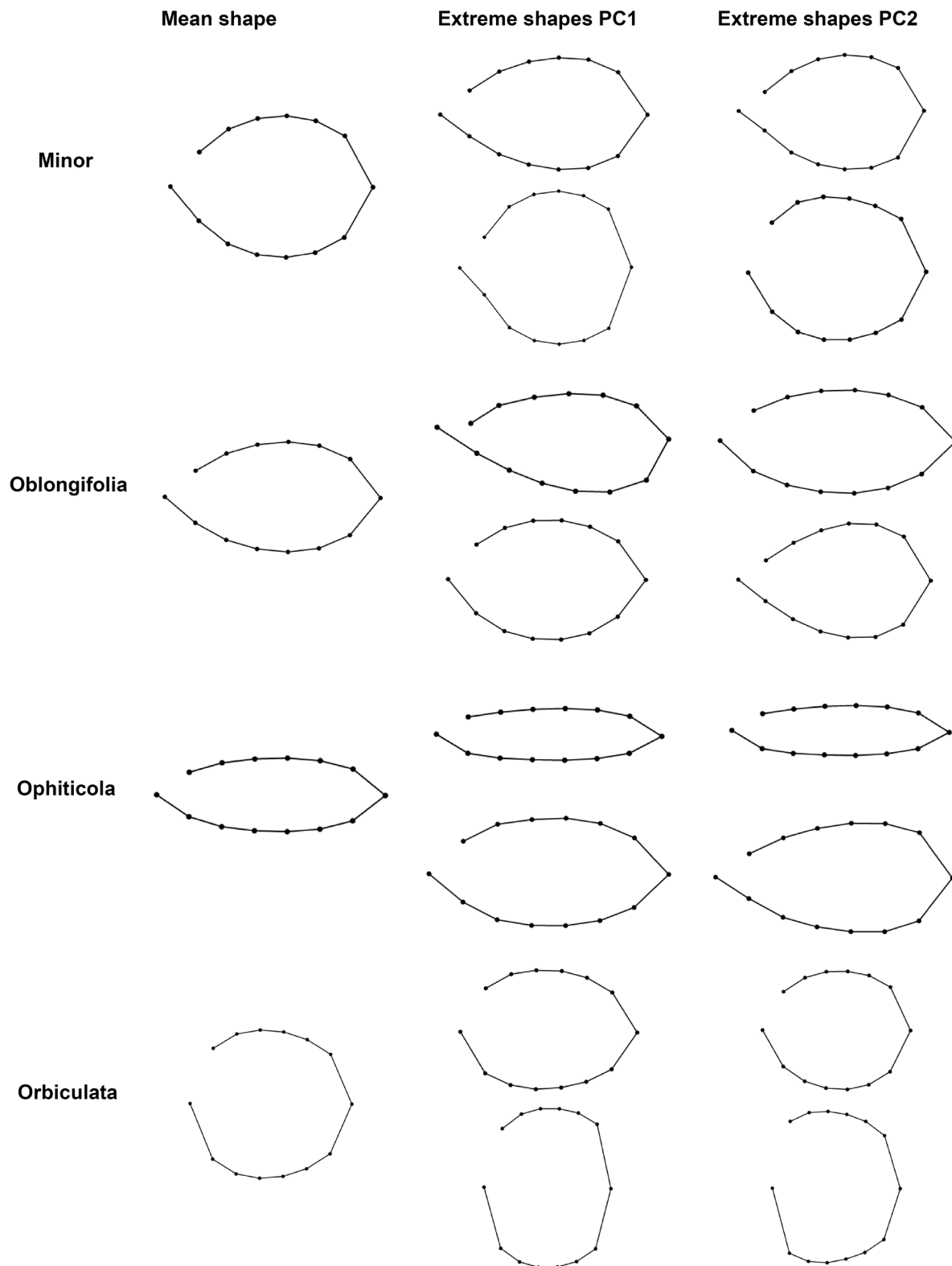
**Figure 8.** Graphic representation of the Sum of EDMA (Euclidean Distance Matrix Analysis) and Centroid Size calculated in the individuals of *Magnolia* subsect. *Talauma* in Cuba. The pair Minor-Oblongifolia ( $p=0.316$ ) and Orbiculata-Ophiticola ( $p=0.406$ ) were the only comparisons with MonteCarlo probabilities over 0.001.



**Figure 9.** Principal Component Analysis for the elliptic Fourier descriptors (A) and matrix of Landmark (B) which characterized the leaves of *Magnolia* subsect. *Talauma* in Cuba.



**Figure 10.** Shape reconstruction of the leaves of *Magnolia* subsect. *Talauma* in Cuba based on the elliptic Fourier descriptors. The extremes shapes are based on the Principal Components, from the Principal Component Analysis.



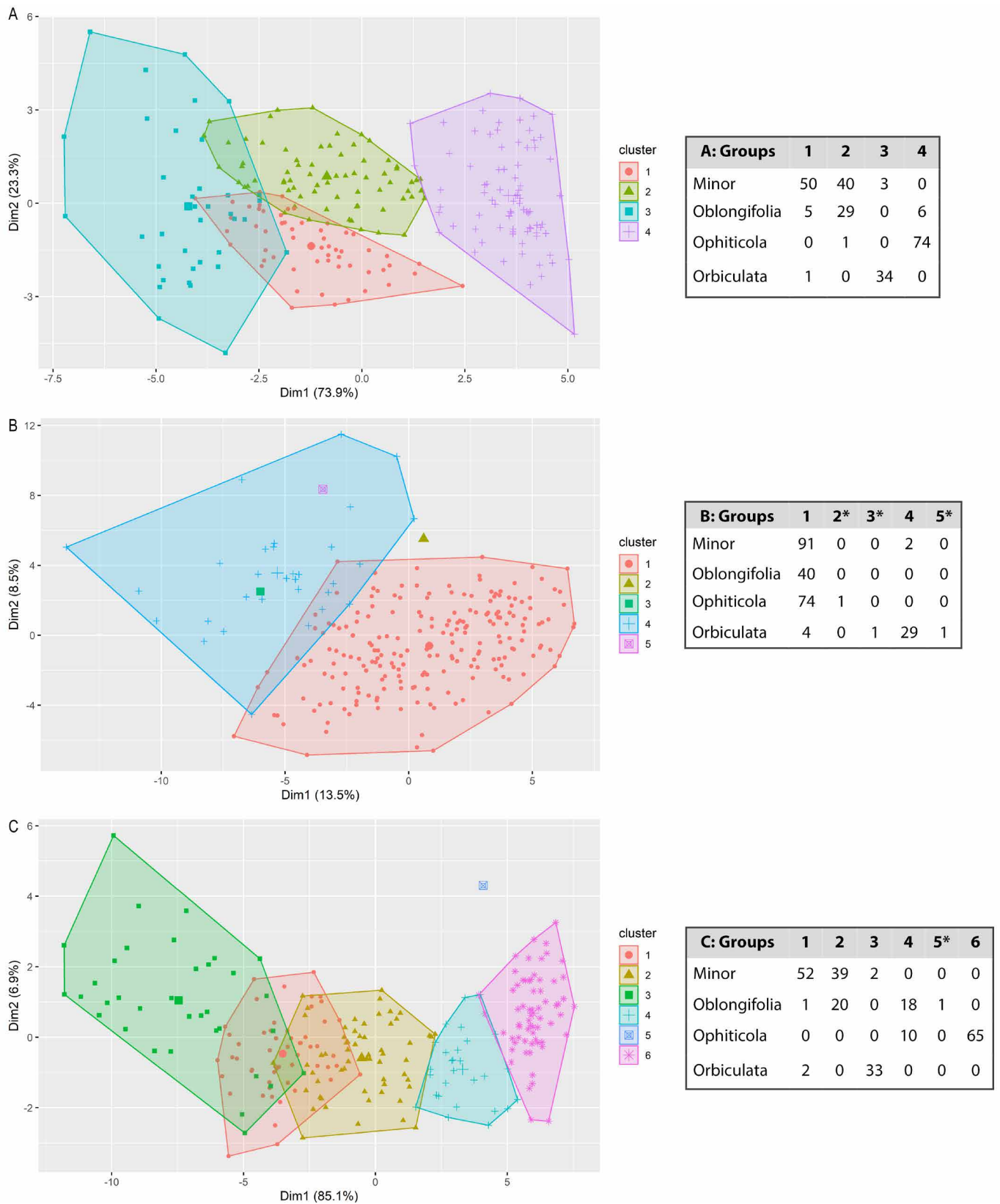
**Figure 11.** Shape reconstruction of the leaves of *Magnolia* subsect. *Talauma* in Cuba based on the matrix of Landmark. The extremes shapes are based on the Principal Components, from the Principal Component Analysis.



### 4.2.2 Clustering analysis

The clustering analysis based on morphological variability showed differences in the number of groups inferred by the best models according to the different datasets (Fig. 12; Supplementary material 5). The highest BIC scores were retrieved for  $Cl=4$  for the linear and angular dataset,  $Cl=2$  for the EDFs dataset (with other three ghost clusters), and  $Cl=6$  for the matrix of Landmark (with another ghost clusters) (Fig. 12; Supplementary material 5). The probabilities of assignment of each individual were higher than 0.9 based on the EDFs. In the case of the linear and angular variables and the matrix of Landmark, only 5 and 22 individuals showed probabilities of an assignment less than 0.9, respectively (data not shown). The linear and angular variables allowed a clear discrimination between *Orbiculata*, *Ophiticola*, and *Minor*, the latter group being split into two clusters. One of these two clusters was shared only with the majority of *Oblongifolia* individuals.

The clustering analysis based on EFDs provided only two clusters (Fig. 12). The assignment of individuals was, therefore, different from that obtained with linear and angular variables. Indeed, all individuals of *Oblongifolia*, and most individuals of *Ophiticola* and *Minor*, were assigned to the same cluster (cluster 1), while most individuals of *Orbiculata* were assigned to a different cluster (cluster 4). Therefore, EFDs were efficient in discriminating between *Orbiculata* on the one hand and the three other groups on the other hand. Finally, the analysis carried out on the matrix of Landmark showed a similar pattern to that obtained with the linear and angular variables for *Minor*, *Ophiticola*, and *Orbiculata*. The main difference between these two analyses (matrix of Landmark and linear and angular dataset) was that in the first one, *Oblongifolia* was split into two clusters, one of which is shared with *Minor* and the other one with *Ophiticola* (Fig. 12). Thus, despite a continuous variation of leaf morphology across groups, a clear delimitation of *Orbiculata* is shown by our analysis whatever the dataset used. In cases where individuals of the same group were assigned to different clusters, no obvious correspondence between the assigned clusters and the geographic origin of those individuals was found. Indeed, many individuals of the same group/locality were assigned to different clusters (data not shown).



**Figure 12.** Graphic representation and classification matrix obtained after the cluster analysis using the morphological data of *Magnolia* subsect. *Talauma* in Cuba. A: Linear and angular variables, B: elliptic Fourier descriptors, C: matrix of Landmark. \* Ghost Cluster.

### 4.3 Genetic limits

#### 4.3.1 Genetic Structure in *Magnolia* subsect. *Talauma*

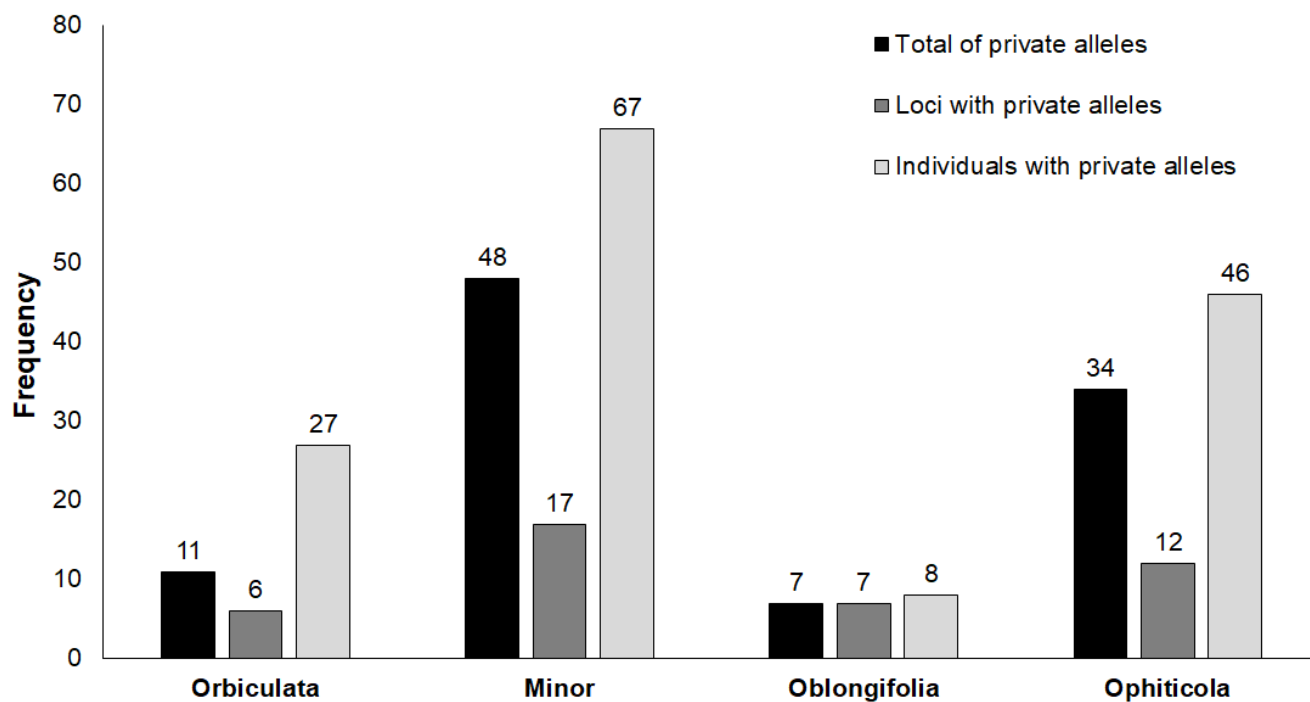
The groups showed similar genetic diversity values, with the lowest values in Orbiculata (Table 9). The genetic differentiation among groups was relatively high (global  $F_{ST}=0.10$ ,  $D_{JOST}=0.23$ ). Orbiculata contributed mainly to this result since it was highly differentiated from the three other groups, while Minor and Oblongifolia were the less differentiated groups (Table 10). The number of alleles and the diversity values per locus are resumed in supplementary material 6-7. Minor showed the highest private allele values, followed by Ophiticola (Fig. 13). In both groups, the total number of individuals with private alleles represents 30.7 % and 26.7 % of the total number of known individuals, respectively. In Minor, 81 % of the markers showed private alleles.

**Table 9.** Average values and standard error of the measures of genetic diversity by groups of *Magnolia* subsect. *Talauma* in Cuba. N: sample size, Np: number of private alleles, Na: number of mean alleles,  $A_R$ : allele richness, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity.

Groups	N	Np	Na	$A_R$	Ne	Ho	He
Orbiculata	36	11	6.81 ±1.18	6.652 ±1.143	3.618 ±0.543	0.584 0.078	0.564 ±0.064
Minor	218	48	13.619 ±2.043	9.091 ±1.412	5.732 ±1.169	0.518 ±0.072	0.588 ±0.072
Oblongifolia	35	7	9.810 ±1.360	9.674 ±1.339	5.420 ±0.929	0.579 ±0.066	0.630 ±0.065
Ophiticola	172	34	12.524 ±1.896	9.163 ±1.306	5.658 ±1.023	0.574 ±0.060	0.650 ±0.057

**Table 10.** Pairwise genetic differentiation and distances measures: fixation indices ( $F_{ST}$ ) (below diagonal) and allelic differentiation index ( $D_{JOST}$ ) (above diagonal) calculated for the groups of *Magnolia* subsect. *Talauma* in Cuba. In all case significant differences were found ( $p < 0.001$ ).

Groups	Orbiculata	Minor	Oblongifolia	Ophiticola
Orbiculata	-	0.3127	0.2937	0.3921
Minor	0.1721	-	0.0056	0.0999
Oblongifolia	0.1613	0.0092	-	0.0705
Ophiticola	0.1982	0.0859	0.045	-



**Figure 13.** Privates alleles by taxa, locus, and individuals in *Magnolia* subsect. *Talauma* in Cuba.

The Bayesian clustering analysis clearly provided three genetic clusters as the unambiguously best solution in the two analyses (with and without *Oblongifolia*) (Fig. 14). In the following, an individual was considered to be correctly assigned to a unique genetic cluster if the ancestry coefficient of this individual to this cluster was higher than or equal to 0.9. One of those clusters corresponded obviously to *Orbiculata* (red cluster in Figure 15A). The 88.8 % (32/36) of individuals from *Orbiculata* were assigned to this cluster, while only 4/36 *Orbiculata* individuals displayed genome admixture (see hereafter). The second genetic cluster (green cluster in Figure 15A) consisted mainly of the majority of *Minor* (171) and *Oblongifolia* (16) individuals but also included some individuals (14) of *Ophiticola* (Fig. 15A). The third genetic cluster (blue cluster on Figure 15A) was predominantly composed of *Ophiticola* with only one individual of *Minor*. We will therefore refer hereafter to the “orbiculata”, “minor-oblongifolia”, and “ophiticola” genetic clusters, keeping in mind that ancestry coefficients within each group to these genetic clusters still varied. Indeed, despite a clear delimitation between the three genetic clusters, a significant proportion of individuals (130/461) displayed genetic admixture. Based on these individuals, the level of genetic admixture varied according to groups; the mean value of probability to belong to each cluster was 0.624 ( $\pm 0.104$ ) for *Orbiculata*, 0.678 ( $\pm 0.172$ ) for *Minor* (including *Oblongifolia*), and 0.477 ( $\pm 0.262$ ) for *Ophiticola*.

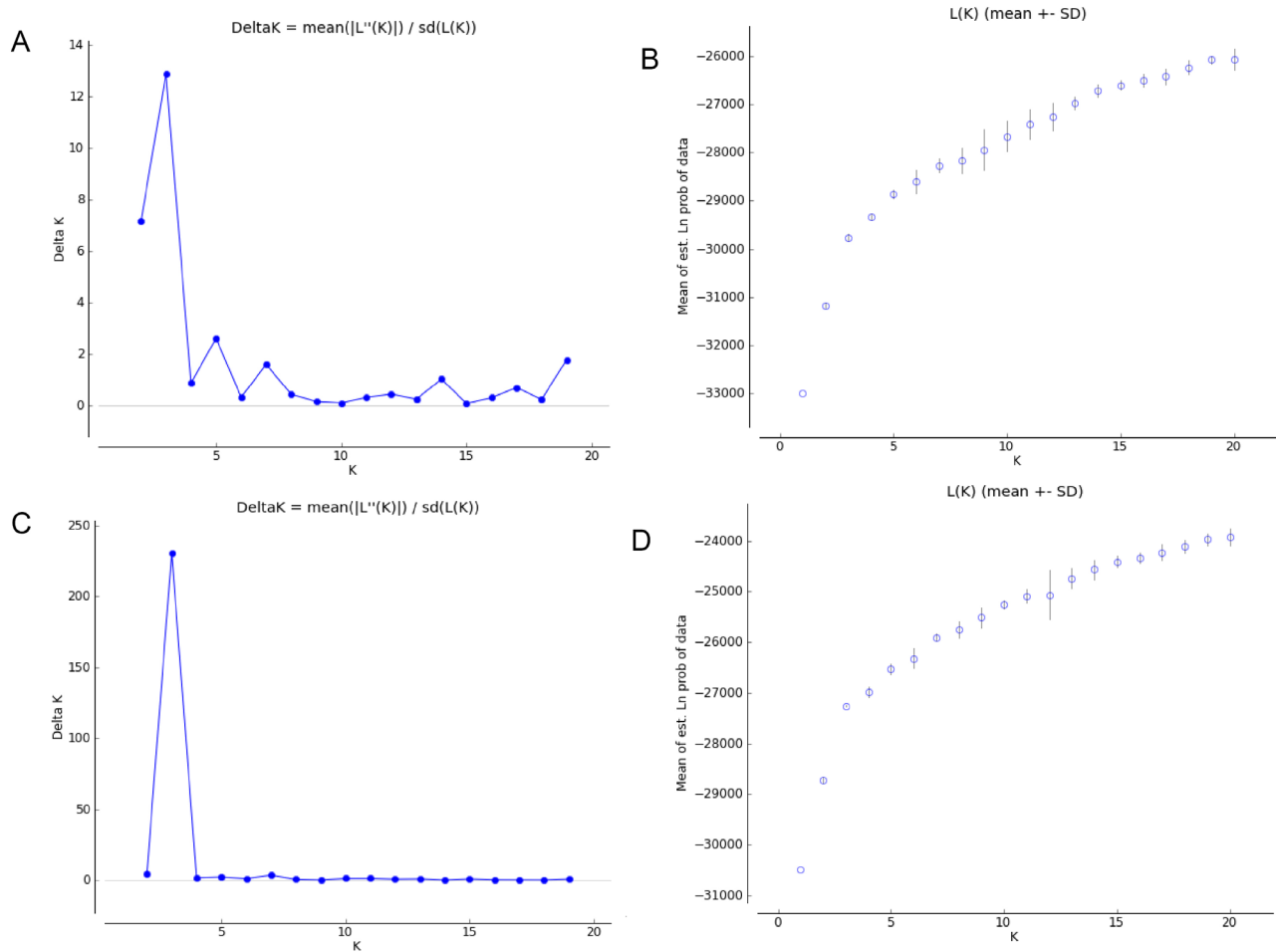
*Orbiculata* showed strongly homogeneous ancestry coefficient values, with only four individuals displaying genome admixture with the “minor-oblongifolia” cluster (Fig. 15A). *Minor* and *Oblongifolia* displayed a high level of genome admixture with the “ophiticola” cluster. Thirty-five individuals (16.1 %) of *Minor* showed genome admixture with the “ophiticola” cluster. In *Oblongifolia*, 51 % of the individuals exhibited an ancestry coefficient over 0.9 to belong to the “minor-oblongifolia” cluster; the rest showed high admixture levels. Moreover, it is noticeable that one individual of *Oblongifolia* displayed a very high ancestry to *Orbiculata*. It was noticeable that the level of admixture within the “minor-oblongifolia” cluster was not evenly distributed among sites. The localities of Cupeyal del Norte (CN), Monte Fresco (MF), Piedra la Vela (PV), and

Cayo Guam (CG) show the highest levels of “misclassification” in the “minor-oblongifolia” cluster.

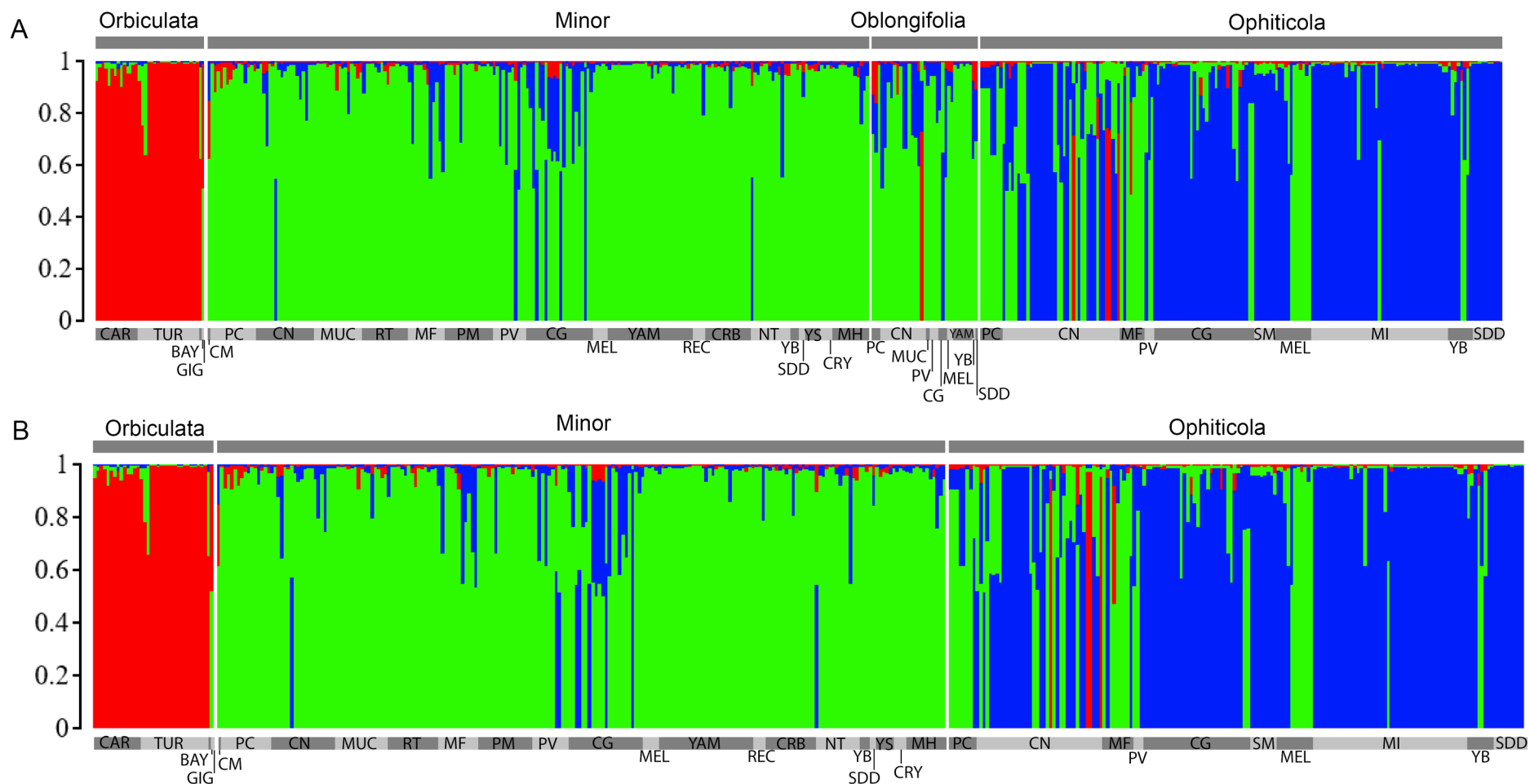
For *Ophiticola*, 56.4 % (97/172) of individuals could be assigned to the third (blue in Figure 15A) genetic cluster, while 8.14 % (14/172) could be assigned to the “minor-oblongifolia” genetic cluster (referred to as “misclassified” individuals hereafter). Similar to *Minor* and *Oblongifolia*, many individuals of *Ophiticola* (61/172) also displayed signals of genetic admixture, mainly with the “minor-oblongifolia” cluster, but also, for a few of them, with the “orbiculata” cluster. The localities of Subida a la Melba (SM), Mina Iberia (MI), and Sur de las Delicias del Duaba (SDD) showed the lowest levels of misclassification. Most individuals from La Melba (MEL), Pico Cristal (PC), and Monte Fresco (MF) showed an ancestry coefficient similar to the “minor-oblongifolia cluster”. The clustering analysis without individuals of *Oblongifolia* also provided  $K=3$  as the best solution (Fig. 14, 15B), reinforcing the conclusion of the analysis carried out on the whole sample. Moreover, it was very striking that this analysis provided an ancestry pattern very similar (Fig. 15B) to the analysis including this taxon (Fig. 15A).

The PCA analysis on the whole SSR dataset showed that the 200 first principal components explained 99.3 % of the variation, which were therefore kept for the discriminant analyses. Based on the number of groups that have been defined across the history of Cuban talaumas taxonomy, but also on the STRUCTURE results, two solutions for the number of genetic clusters were considered in the following:  $K=3$  and  $K=4$ . When  $K=3$ , individual assignment displayed a pattern very similar to that found with the Bayesian clustering approach; many individuals “misclassified” in the DAPC analysis were also “misclassified” based on the STRUCTURE analysis. The DAPC result confirmed the correspondence of *Orbiculata* to a unique genetic cluster and also showed that *Orbiculata* is more genetically differentiated from the three other defined groups (Fig. 16). For  $K=3$  only one individual of *Ophiticola* showed an assignment probability value less than 0.9 to that genetic cluster.  $K=4$  (Fig. 16) seems to be a less meaningful solution. In this case three clusters were predominantly composed of *Minor*,

Ophitcola, Orbiculata, respectively, confirming the main pattern found with  $K=3$ , with the difference that a higher proportion of Minor and Ophitcola, but also a majority of Oblongifolia, were not assigned to unique clusters. When  $K=4$ , seven and three individuals of Minor and Oblongifolia, respectively, showed probabilities values under 0.9. As for STRUCTURE, the analysis without considering Oblongifolia with  $K=3$  displayed very similar results to the analysis including this taxon (Fig. 16); in this case, only one individual of Ophitcola showed probabilities values under 0.9. The genetic characterization of *Magnolia* subsect. *Talauma* represents the first work made in the group in Cuba.

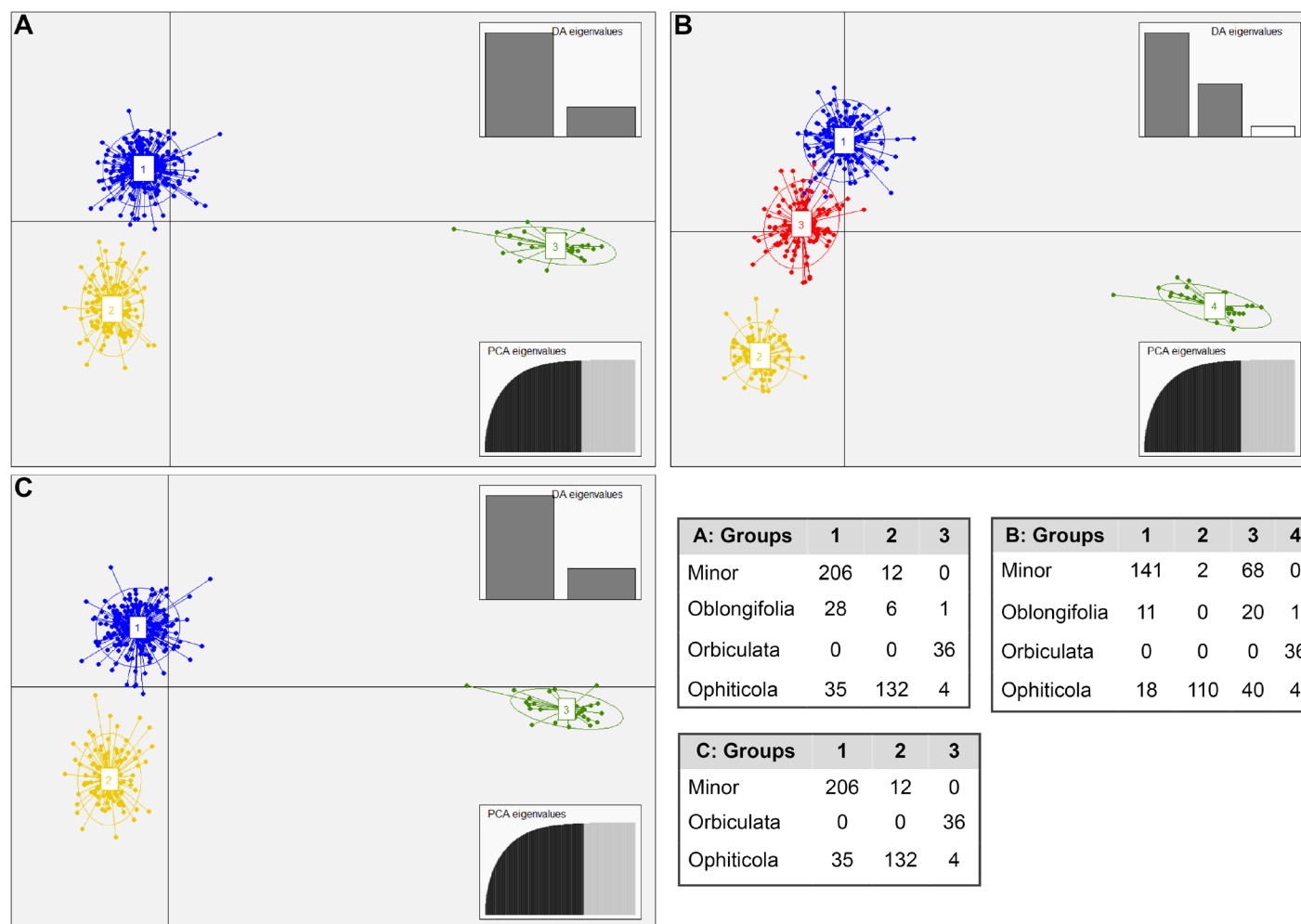


**Figure 14.** Delta K plot (A) and mean Ln(K) plot (B) for the complete dataset of *Magnolia* subsect. *Talauma* in Cuba. Delta K plot (C) and mean Ln(K) plot (D) for the dataset without the individuals of Oblongifolia.



**Figure 15.** STRUCTURE results of *Magnolia* subsect. *Talauma* in Cuba. A: Representative bar plot (out of 100 en replicates) for K=3 based on the complete dataset; B: Representative bar plot (out of 100 en replicates) for K=3, without Oblongifolia. Calizas de Mucaral (MUC), Camarones-Río Báez (CRB), Cañón del Río Yumurí (CRY), Cayo Guam (CG), Cayo Mujeres (CM), Cupeyal del Norte (CN), El Gigante (GIG), El Recreo (REC), La Bayamesa (BAY), La Melba (MEL), Mina Iberia (MI), Mina la Hoya (MH), Monte Fresco (MF), Naranjo del Toa (NT), Pico Caracas (CAR), Pico Cristal (PC), Piedra La Vela (PV), Pinares de Montecristo (PM), Región del Toa (RT), Río Yamanigüey (YAM), Subida a la Melba (SM), Sur de las Delicias del Duaba (SDD), Turquino (TUR), Yumurí del Sur (YS), Yunque de Baracoa (YB).





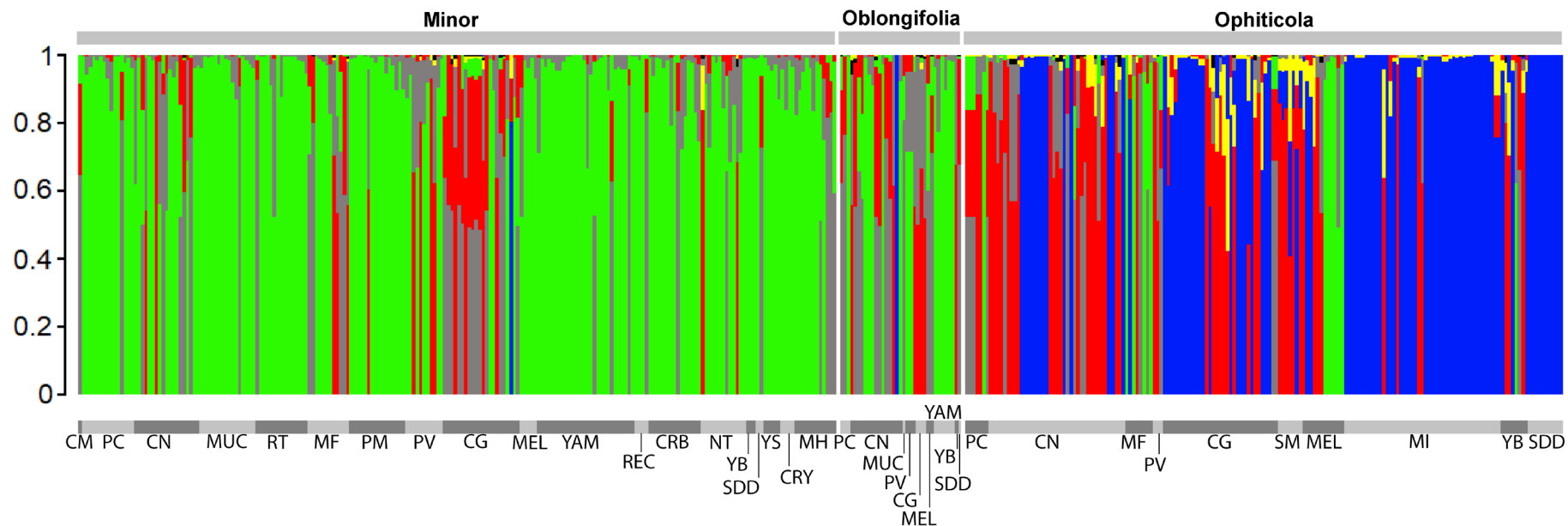
**Figure 16.** Discriminant Analysis of Principal Components (DAPC) of *Magnolia* subsect. *Talauma* in Cuba. The axes represent the first two linear discriminant functions. The upper left graph (Principal Component Analysis -PCA- eigenvalues) inset displays the variance explained by the principal component axes used for DAPC and the bottom-right inset (DA eigenvalues) displays in relative magnitude the variance explained by the two discriminant axes plotted. DAPC graph of the all the taxa, 200 principal components (PCs) retained. A: K=3, B: K=4, C: K=3 without *Oblongifolia*.

### 4.3.2 Testing hybrids

The NEWHYBRIDS analysis with and without prior information showed very similar results; the following results are based only on the data with prior information. 100 % of the individuals used to validate the analysis were correctly classified as pure Minor and pure Ophiticola. The pure individuals of Minor and Ophiticola were the most abundant genetic groups (Fig. 17; Table 11). The confusion matrix, based on a probability threshold of 0.9, showed a high number of individuals as non-classified; because this 0.7 was also used as a second threshold. Based on 0.7, no individuals were classified as F1 or backcrossed with Ophiticola. Among Oblongifolia individuals, 14 were classified as pure Minor and only as pure Ophiticola. Ophiticola was the group with the higher level of confusion (Table 11) since a high proportion of individuals were classified either as pure Minor, F2, or backcrosses with Minor. 23 % of individuals of Ophiticola were considered by the analysis as being F2 individuals, the second more frequent group inside Ophiticola, after pure Ophiticola with 51 %. Also, 87 (20.5 %) individuals were considered non-classified. The hybridization analysis represents the first research on population genetics applied to natural hybridization in Cuban plants.

**Table 11.** Confusion matrix of the individuals of *Magnolia* subsect. *Talauma* from northeastern Cuba, based on the NEWHYBRIDS result with a probability threshold of 0.9 and 0.7. Mm: Minor; Oph: Ophiticola; F1: first generation hybrid; F2: second generation hybrid; BC-Mm: Backcross to Minor; BC-Oph: Backcross to Ophiticola; NR: non-classified (individuals with probabilities under the threshold value).

Groups – 0.9	N	Mm pure	F1	F2	BC-Mm	BC-Oph	Oph pure	NC
Minor	218	127	0	2	3	0	0	86
Oblongifolia	35	10	0	0	0	0	1	24
Ophiticola	172	10	0	12	1	0	80	69
<b>Total</b>	425	147	0	14	4	0	81	179
Groups – 0.7	N	Mm pure	F1	F2	BC-Mm	BC-Oph	Oph pure	NC
Minor	218	155	0	6	15	0	1	41
Oblongifolia	35	14	0	0	4	0	1	16
Ophiticola	172	10	0	40	3	0	89	30
<b>Total</b>	425	179	0	46	22	0	91	87



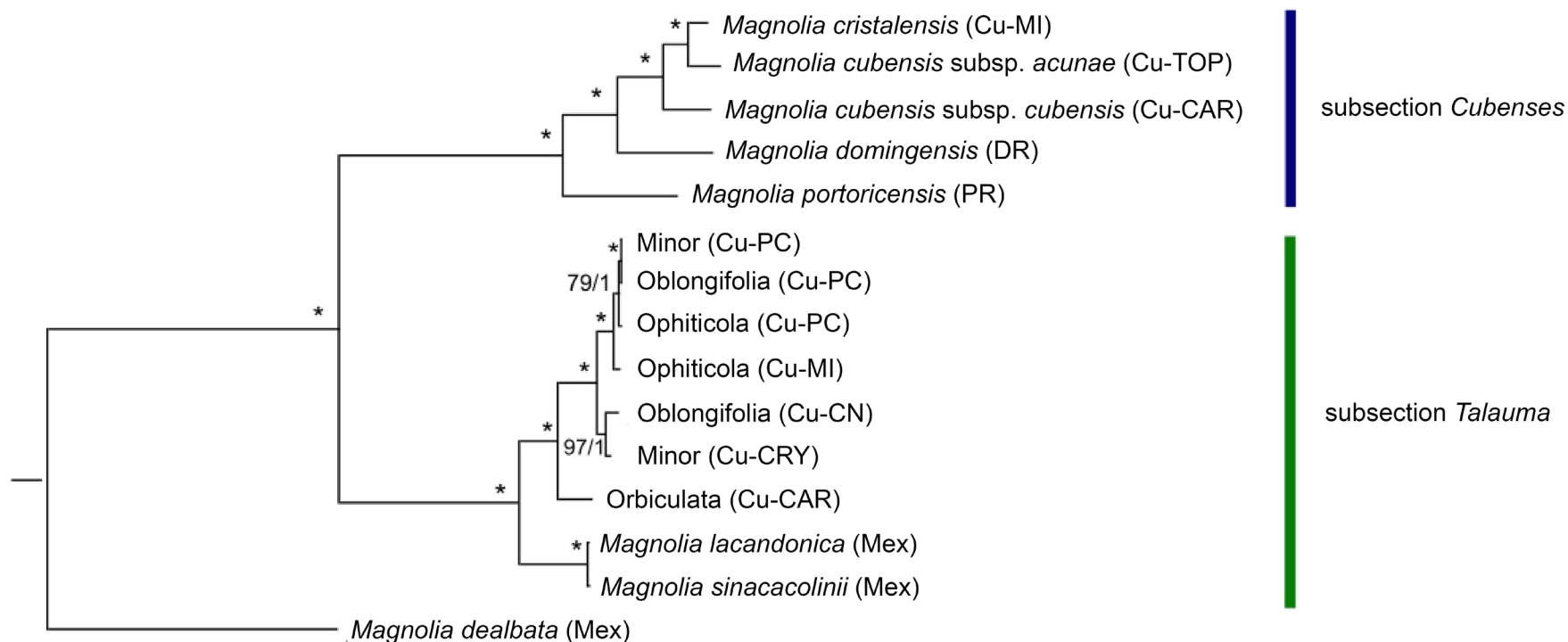
**Figure 17.** Genotype assignment of the individuals of *Magnolia* subsect. *Talauma* from northeastern Cuba based on the program NEWHYBRIDS using microsatellite data and location prior information: Minor pure (green); Ophiticola pure (blue); F1 (black); F2 (red); Backcross to Minor (gray); Backcross to Ophiticola (yellow). Calizas de Mucaral (MUC), Camarones-Río Báez (CRB), Cañón del Río Yumurí (CRY), Cayo Guam (CG), Cayo Mujeres (CM), Cupeyal del Norte (CN), El Recreo (REC), La Melba (MEL), Mina Iberia (MI), Mina la Hoya (MH), Monte Fresco (MF), Naranjo del Toa (NT), Pico Cristal (PC), Piedra La Vela (PV), Pinares de Montecristo (PM), Región del Toa (RT), Río Yamanigüey (YAM), Subida a la Melba (SM), Sur de las Delicias del Duaba (SDD), Yumurí del Sur (YS), Yunque de Baracoa (YB).

#### 4.4 Phylogenetic limits

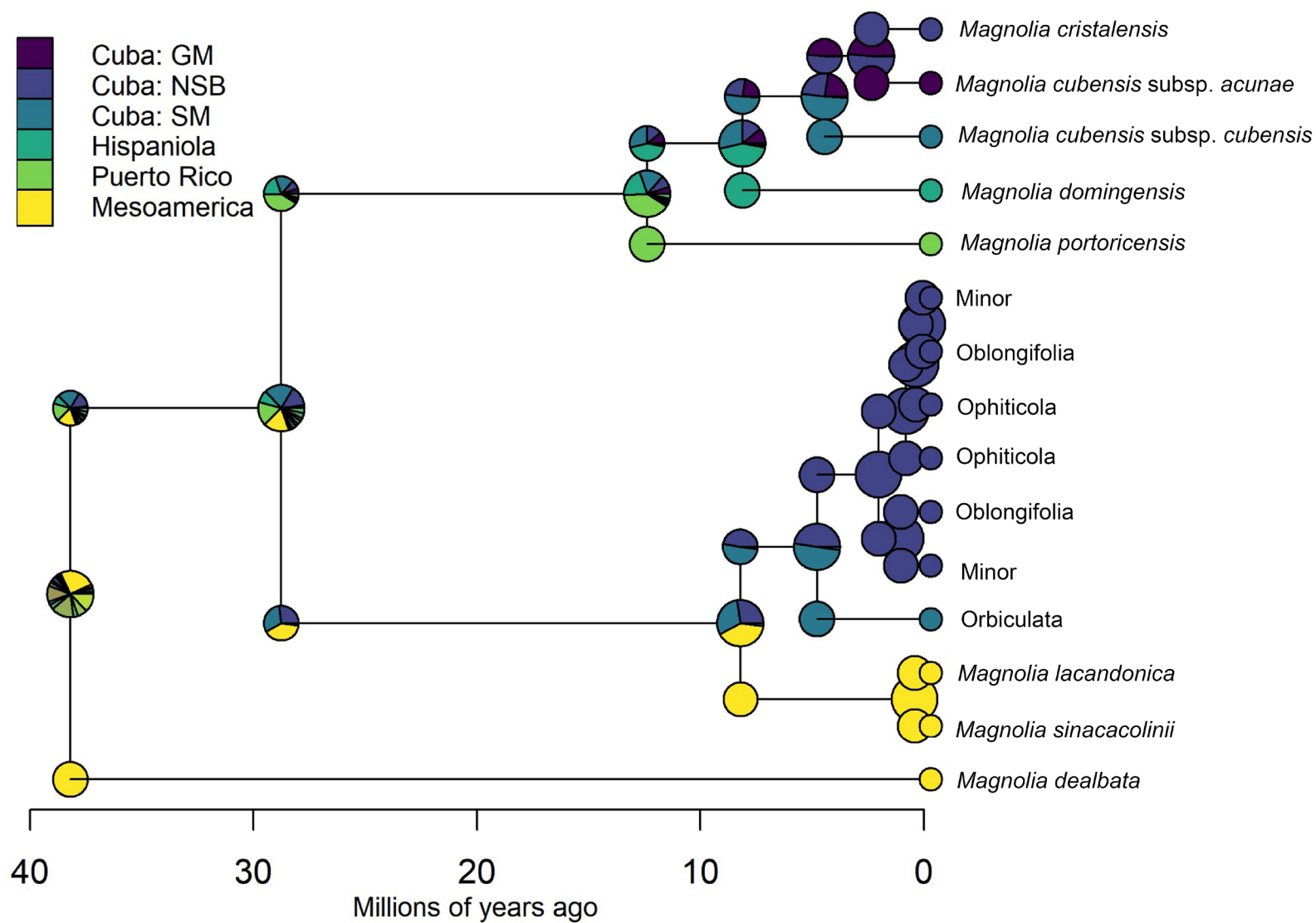
A total of 104 nucleotide sites were considered parsimony-informative; among them, only 21 sites were informative within the groups of *Magnolia* subsect. *Talauma* in Cuba. Both the ML and the BI analyses resulted in the same phylogenetic tree. The phylogeny showed *Magnolia* subsect. *Talauma* in Cuba as a monophyletic group. The Cuban groups of *Magnolia* subsect. *Talauma* are closer to the talaumas species from Mesoamerica compared with the Cuban species of *Magnolia* subsect. *Cubenses*, which are closer to those from Dominican Republic and Puerto Rico. Orbiculata group looks like a lineage with solid support (Fig. 18, 19). The group of northeastern Cuba is a well-supported clade in which it is not possible to establish the limits between the different groups (Minor, Oblongifolia, Ophiticola). The first branch of this group included one individual of Minor and Oblongifolia, and the second branch included the three groups. The ancestral range reconstruction showed that the DIVALIKE+J model was the best according to the AICc values, while the BAYAREALIKE+J and DIVALIKE models achieved the second and third-best scores. The six models included in the BioGeoBEARS analysis are shown in supplementary material 8. Ancestral range estimation reconstructed by the DIVALIKE+J model is presented in Figure 19. This phylogeny represents the first one made using the complete plastome sequence of all the species of *Magnolia* sect. *Talauma* in Cuba.

#### 4.5 Integrative taxonomy

An obvious association between genetic vs. morphological classification (using the three sets of data), genetic vs. niche classification, and niche vs. morphological classification were found based on Chi<sup>2</sup> test (values probabilities less than 0.0001). In addition, the heatmap analysis showed a high concordance between morphological and genetic classification for Orbiculata and Minor; and to a lesser extent for Oblongifolia and Ophiticola (Fig. 20). In this last group, several individuals with different genetic characteristics whose leaf morphology was the same were found. In Minor (Fig. 20), only a few individuals showed different morphology having a similar genetic pattern. A relation between the three datasets of variables (ecological, morphological, and genetic) was



**Figure 18.** Phylogenetic relationships inferred for *Magnolia* subsect. *Talauma* from Cuba based on full plastome sequences. Tree constructed by the best tree based on maximum likelihood (ML). Support values marked above the branches follow the order bootstrap support/bayesian posterior probability. Cuba (Cu), Mexico (Mex), Puerto Rico (PR), Dominican Republic (DR); Cañón del Río Yumurí (CRY), Cupeyal del Norte (CN), Mina Iberia (MI), Pico Caracas (CAR), Pico Cristal (PC).

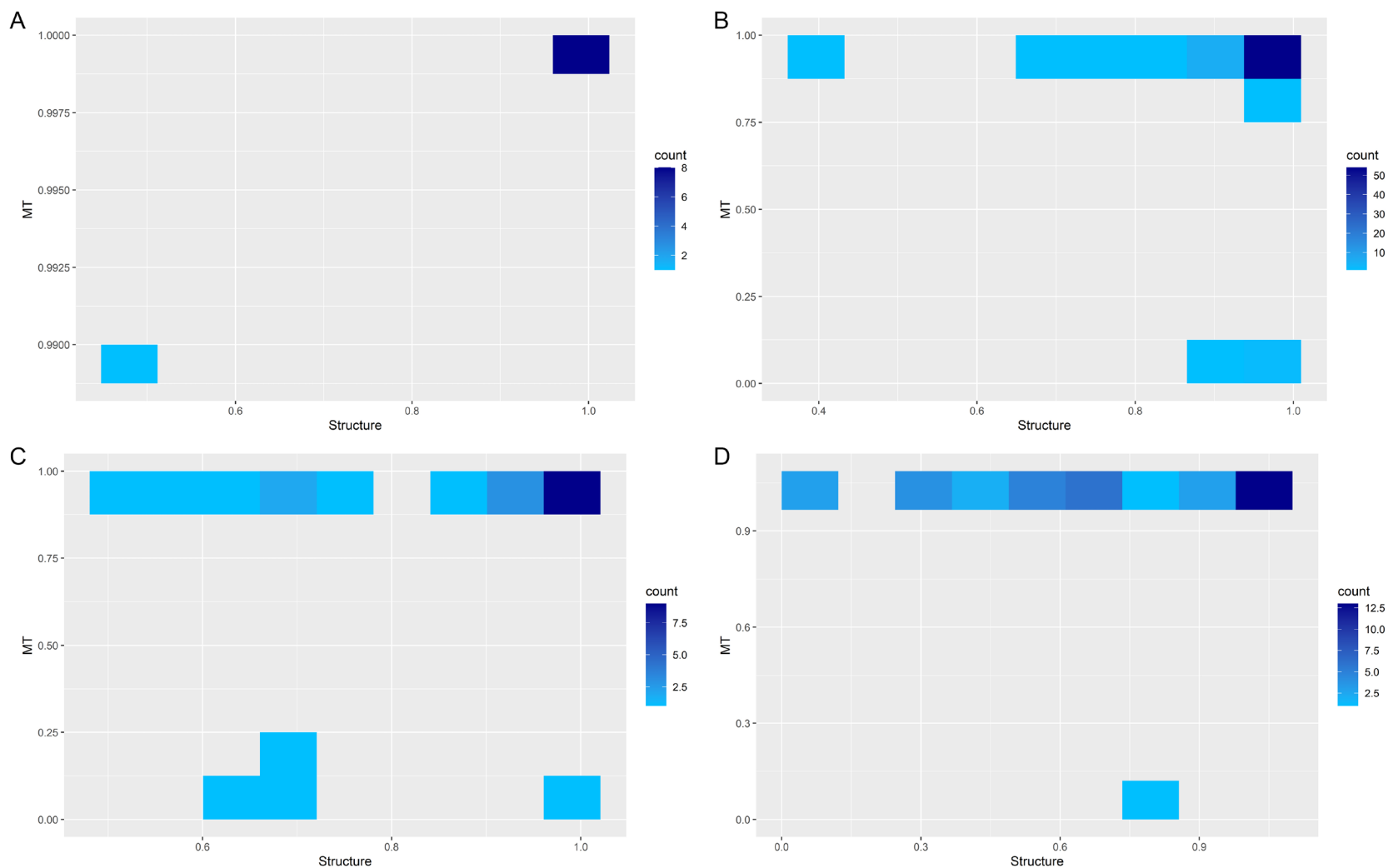


**Figure 19.** Ancestral range reconstruction resulting from the BioGeoBEARS analysis of the Cuban groups of *Magnolia* subsect. *Talauma* using the DIVALIKE+J model. Colors represent geographic regions. GM: Guamuhaya, NSB: Nipe-Sagua-Baracoa, SM: Sierra Maestra.

found based on the bi-dataset and multiple comparisons (ecological vs. morphological vs. genetic). The mantel correlation values were moderate in the multiple comparisons ( $r=0.402$ ) and between morphological vs. genetic distances ( $r=0.41$ ); and low between ecological vs. morphological ( $r=0.115$ ) and ecological vs. genetic distances ( $r=0.118$ ). In all cases, the probability values were less than 0.0001.

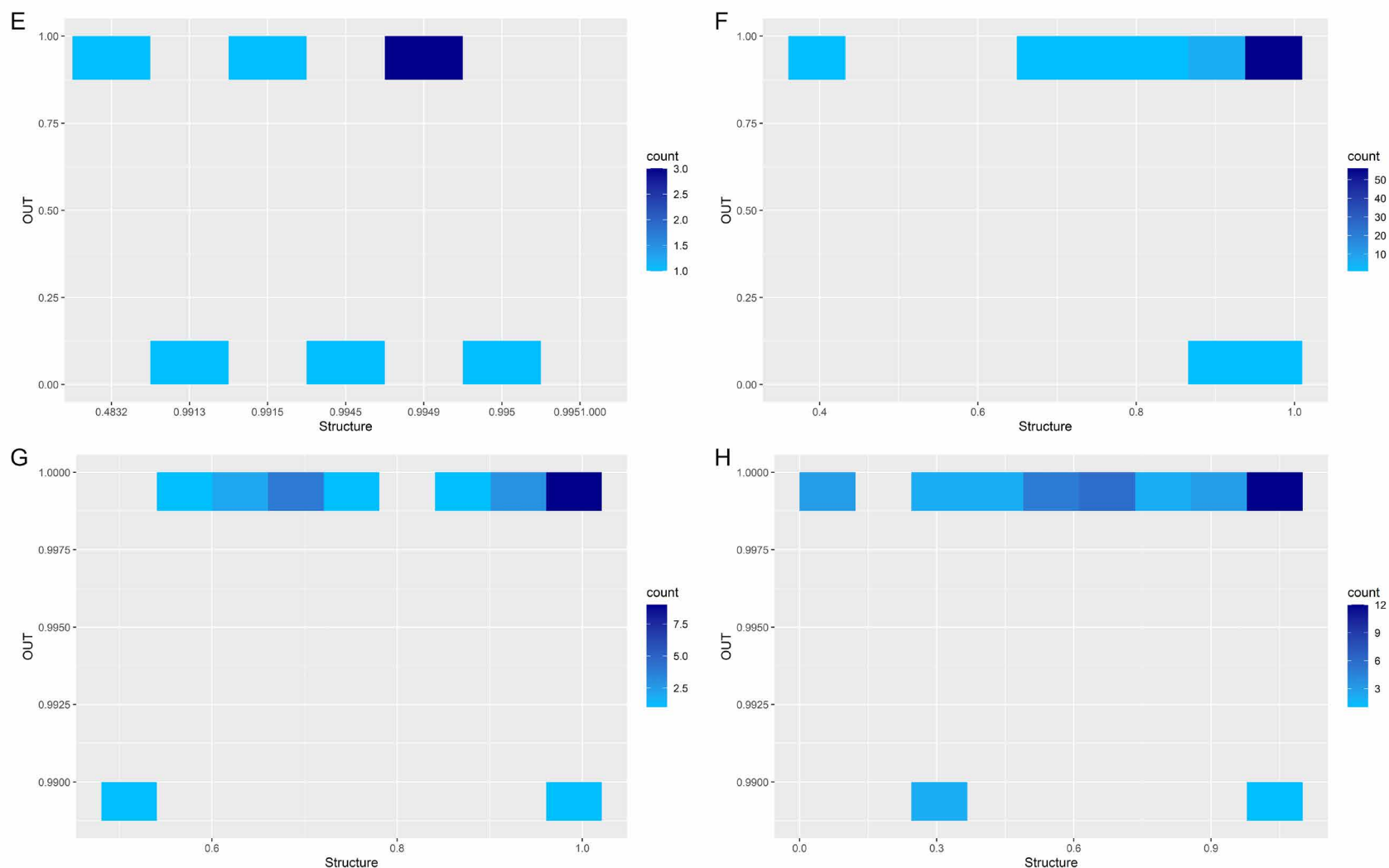
Figure 21 shows the group's limits based on the results obtained in this thesis. Orbiculata was the only group with consistent limits based on morphology, ecology, genetics, and phylogenetics data. Concerning the group of northeastern Cuba, the different sets of data showed different limits. Based on the ecological and EFDs dataset (morphology), everything could be considered as only one species. The linear and angular dataset (morphology) considered Minor and Oblongifolia as the same species, different from Ophiticola. These same limits were shown by the matrix of Landmark and the genetic data. In these last two datasets, the boundaries between both groups (Minor-Oblongifolia and Ophiticola) were more confused.

Even when the limits in the groups from northeastern Cuba were not totally clear, based on the result of the thesis, it is possible to define three groups of species of *Magnolia* subsect. *Talauma* in Cuba: (1) Orbiculata, an endemic tree from Sierra Maestra range with leaves as long as wide; (2) Minor (including the individuals considered as Oblongifolia), an endemic tree from Nipe-Sagua-Baracoa range, with leaves elliptic-rounded (equal or twice larger than wide); and (3) Ophiticola, a endemic small tree or decumbent shrub from Nipe-Sagua-Baracoa range, with leaves oblong-elliptic (two to four time larger than wider).

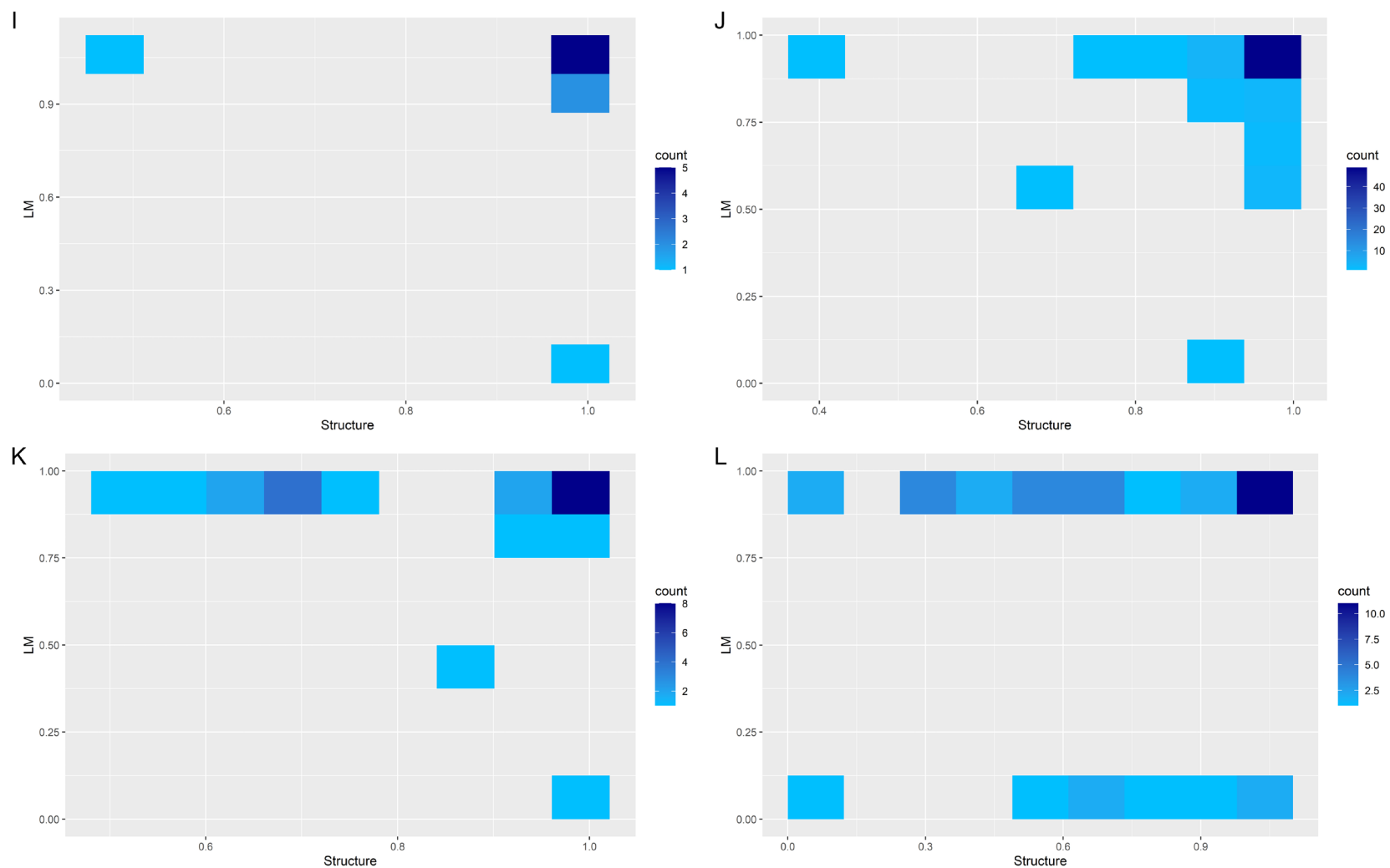


**Figure 20.** Heatmap with the congruence between morphological (MT: Linear and angular variables, OUT: elliptic Fourier descriptors, LM: matrix of Landmark) and genetic (STRUCTURE) cluster probabilities, inside each group of *Magnolia* subsect. *Talauma* in Cuba. Orbiculata (A-E-I); Minor (B-F-J); Oblongifolia (C-G-K); Ophiticola (D-H-L); The blue color represents the number of individuals (less individuals: light blue; more individuals: dark blue).

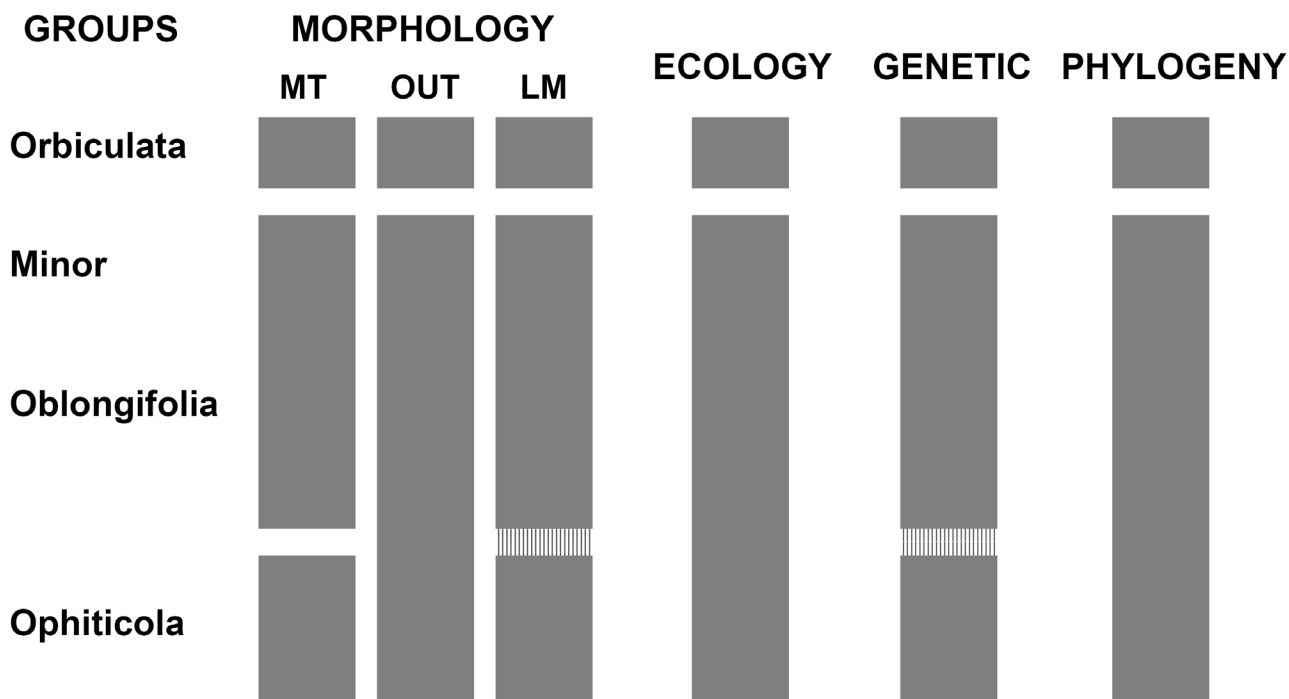




**Figure 20.** Heatmap with the congruence between morphological (MT: Linear and angular variables, OUT: elliptic Fourier descriptors, LM: matrix of Landmark) and genetic (STRUCTURE) cluster probabilities, inside each group of *Magnolia* subsect. *Talauma* in Cuba. Orbiculata (A-E-I); Minor (B-F-J); Oblongifolia (C-G-K); Ophiticola (D-H-L); The blue color represents the number of individuals (less individuals: light blue; more individuals: dark blue).



**Figure 20.** Heatmap with the congruence between morphological (MT: Linear and angular variables, OUT: elliptic Fourier descriptors, LM: matrix of Landmark) and genetic (STRUCTURE) cluster probabilities, inside each group of *Magnolia* subsect. *Talauma* in Cuba. Orbiculata (A-E-I); Minor (B-F-J); Oblongifolia (C-G-K); Ophiticola (D-H-L); The blue color represents the number of individuals (less individuals: light blue; more individuals: dark blue).



**Figure 21.** Integration of the morphological, ecological, genetic, and phylogeny limits of *Magnolia* subsect. *Talauma* in Cuba. Linear and angular variables (MT); elliptic Fourier descriptors (OUT); matrix of Landmark (LM). Discontinuous lines represent a not clear differentiation between the groups.

#### 4.6 Taxonomic treatment

***Magnoliaceae*** Juss., Gen. Pl.: 280. 1789. Type: *Magnolia* L.

***Magnolia*** L., Sp. Pl. : 1: 535. 1753. Type: *Magnolia virginiana* L.

***Magnolia*** sect. ***Talauma*** (Juss.) Baill., Adansonia 7: 3, 66. 1866. Type: *Magnolia plumieri* Sw.

***Magnolia*** subsect. ***Talauma*** (Juss.) Figlar & Noot., Blumea 49(1): 90. 2004.

Trees or little trees, evergreen; stipules adnate to the petioles and soon deciduous, leaving a scar on both edges of the adaxial side of petiole and converging apically; leaf blade generally coriaceous to subcoriaceous, entire margin; leaf orbicular, widely elliptic, ovate, reniform, oblong-elliptical, obovate; apex rounded, retuse, truncate; apex rounded and obtuse, retuse, truncate, subtruncated, emarginate and cuneiform; base from truncate to rounded, base rounded, obtuse;

rarely subtruncated; surface glabrous and prominently reticulate-veined in both sides, the midrib flat above, prominent beneath; adaxial surface dark green, abaxial surface light green.

***Magnolia orbiculata* (Britton & P. Wilson) Palmarola**, Revista Jard. Bot. Nac. Univ. Habana 37: 3. 2016.  $\equiv$  *Talauma orbiculata* Britton & P. Wilson, Bull. Torrey Bot. Club 50: 37. 1923.  $\equiv$  *T. minor* subsp. *orbiculata* (Britton & P. Wilson) Borhidi, Acta Bot. Acad. Sci. Hung. 17: 7. 1971. Lectotype (designated here): “Cuba. Prov. Granma, “tree, 6-8 m. tall; Loma de Quintín-Nagua”, VII-1922, León LS 10955 [NY #73699!]. Isolectotypes: GH [#39075!], HAC ex LS [#SV 10703!].

= *Svenhedinia truncata* Mold., Phytologia 2: 142. 1946.  $\equiv$  *Talauma truncata* (Mold.) Howard, Bull. Torrey Bot. Club 75: 357. 1948. Holotype: Cuba. Prov. Santiago de Cuba, “Alto Babiney, Sur del Turquino” *Acuña* SV 14069 [HAC ex SV!].

Trees, up to 25 m tall and 62 cm in trunk diameter, leaves with petiole of 2 – 7 cm long, leaf blade of 6 – 16 × 6 – 13 cm, width-length relation between 0.66 to 1.23 (sometime wider than large), orbiculate, widely elliptic, ovate and reniform; apex rounded, retuse, truncate; base from truncate to rounded, adaxial surface dark green and the abaxial light green; glabrous and prominently reticulate-veined in both sides, the midrib flat above, prominent beneath.

***Magnolia minor* (Urb.) Govaerts**, Word Checkl. Bibliogr. *Magnoliaceae* 71. 1996.  $\equiv$  *Talauma minor* Urb., Symb. Antill. 7: 222. 1912.  $\equiv$  *Svenhedinia minor* Urb., Repert. Spec. Nov. Regni Veg. 24: 3. 1927.

Lectotype (designed by Palmarola *et al.* 2016: 4): Cuba. Prov. Holguín, “Prope villam Monte Verde dictam Cuba Orientali”, I-VII.1859, *Wright 1100 B* [#100248228!]. Isolectotypes?: BM [#574752!].

- *Talauma oblongifolia* sensu Bisse (1974,1988) pro parte (excludes the type, LS 22588 HAC ex LS!).

- *Magnolia oblongifolia* sensu Palmarola *et al.* (2016) pro parte (excludes the type, LS 22588 HAC ex LS!)

Trees, up to 30 m tall and 76.8 cm in trunk diameter, leaves with petiole of 1.5 – 5 cm long, leaf

blade de 8 – 17.7 × 4.8 – 10.7 cm, elliptic, widely elliptic, width-length relation between 0.38 to 0.87; apex rounded and obtuse, sometime retuse or truncate; base rounded and obtuse; adaxial surface dark green, abaxial surface light green; glabrous and prominently reticulate-veined in both sides, the midrib flat above, prominent beneath.

***Magnolia oblongifolia* (León) Palmarola**, Revista Jard. Bot. Nac. Univ. Habana 37: 3. 2016. ≡ *T. minor* var. *oblongifolia* León, Contr. Ocas. Mus. Hist. Nat. Colegio “De La Salle” 9: 5. 1950. ≡ *T. minor* subsp. *oblongifolia* (León) Borhidi, Acta Bot. Acad. Sci. Hung. 17: 6. 1971. ≡ *Talauma oblongifolia* (León) Bisse, Feddes Repert. 85: 589. 1974. Holotype: Cuba. Prov. Holguín, “Monte de la Breña, Sierra de Moa, Moa, Oriente”, 1-VII-1945, *León, Alain, Clemente, Crisógono LS 22588* (HAC ex LS!).

= *Talauma ophiticola* Bisse, Feddes Repert. 85: 9-10: 589-590. 1974. Lectotype (designated here): Cuba, Prov. Holguín, “Baracoa, Mina Iberia, en las orillas del arroyo Iberia”, IV-1968, *Bisse & Köhler HFC 6519* [HAJB 634!]; isotypes: JE [#1224!, #1225!], HAJB [#635!].

Small trees or decumbent shrubs, up to 14 m tall and 23 cm in trunk diameter, leaves with petiole of 0.8 – 3.4 cm long, leaf blade de 7.9 – 19.7 × 2.5 – 6.4 cm, oblong-elliptic or oblanceolate, width-length relation between 0.22 to 0.46; apex rounded, retuse-emarginate or acute; base cuneate or narrowly cuneate; adaxial surface dark green, abaxial surface light green; glabrous and reticulate-veined in both sides, the midrib flat above, prominent beneath.

*Talauma ophiticola* was described based on the gathering *Bisse & Köhler HFC 6519* (Fig. 22A), which is strongly similar to the holotype of *Talauma minor* var. *oblongifolia* (*León, Alain, Clemente, Crisógono LS 22588*) (Fig. 22B). The types of *Talauma minor* var. *oblongifolia* León, and *Talauma ophiticola* Bisse have the same morphological variability (Fig. 22). Based on this, the correct name for the individuals considered as *Ophiticola* group, is *Magnolia oblongifolia* (León) Palmarola, which was the first used name.



Figure 22. Lectotype of *Talauma ophiticola* Bisse (A) and *Talauma minor* var. *oblongifolia* León (B).



## DISCUSSION

### 5.1 Ecological Niche

Two precipitation variables, annual rainfall and precipitation seasonality, presented the highest contribution to the ENMs of *Magnolia* subsect. *Talauma* in Cuba, except for *Orbiculata*, which was more influenced by extreme temperature. Rainfall and temperature are among the factors that influence the most the distribution of plants because both climate parameters are the primary control of biomes and are closely correlated with terrestrial net productivity (Hull 2008; Zhao *et al.* 2019). In the specific case of magnolias, some authors (Song *et al.* 2019; Song & Liu 2019; Rodríguez-Ramírez *et al.* 2020) have already report the importance of these bioclimatic variables in the distribution of this group of plants. The differences observed between *Orbiculata* and the remaining groups in the contributions of the variables used for the niche model reflect the differences that exist between the Sierra Maestra and the Nipe-Sagua-Baracoa range. The latter is the region with the highest annual rainfall values in Cuba, above 3 000 mm per year (Borhidi 1996), and the Sierra Maestra is the one with the highest elevation in Cuba, 1 974 m.a.s.l. According to Borhidi (1996), in the highest mountains, the vertical gradients of temperature are more abrupt when the mountains are closest to the south coast. This author reports in the Sierra Maestra a change of 0.99°C every 100 m, and for Nipe-Sagua-Baracoa 0.66°C. Song & Liu (2019) report a high correlation between elevation and maximum temperature of the warmest month, the most influencing variables for *Orbiculata*. This correlation was not detected in our dataset, but a possible association between both variables could explain the strong influence on the model of *Orbiculata*.

The significant contribution of annual precipitation, precipitation seasonality, and maximum temperature of the warmest month to the distribution of *Magnolia* subsect. *Talauma* in Cuba is indicative of the sensitivity of this taxon to Climate Change. Global warming is likely to reach between 1-5°C in 2100, depending on the model, and the precipitation could likely decrease (Stocker *et al.* 2013). Climate change could exceed the adaptive capacity of these trees, causing

geographic displacement and local extinctions. The individuals of *Orbiculata*, with a constrained distribution to the higher elevations in Sierra Maestra, will have a higher risk of extinction. However, other groups of the subsection may also be affected by climate change, as proposed by Fuentes-Marrero *et al.* (2019). These authors report that by 2050, under different climatic scenarios, the suitable areas of distribution of *Magnolia minor* (sensu Bisse 1988 and Palmarola *et al.* 2016) will be considerably reduced.

The small areas found to be suitable for *Orbiculata* could be explained by the low numbers of known individuals and their limited geographic distribution (see Figure 3). In the case of the magnolias from northeastern Cuba, their ecological niche has large extensions, which is in concordance with their higher values of extent of occurrence and area of occupancy. According to the results of this thesis, all the groups exclusive to this area (northeastern Cuba) showed large areas of environmental suitability in the region of the Sierra Maestra. The absence of individuals of *Minor*, *Oblongifolia*, and *Ophiticola* in this last area could be due to large unsuitable areas between both mountain systems. Another possible explanation may be that the common ancestor of *Magnolia* subsect. *Talauma* in Cuba lived in the Sierra Maestra, and the extant taxa that diverged from it maintain similar niche characteristics. According to Wiens & Graham (2005), the species tends to retain ancestral ecological aspects of their fundamental niche over time. Another result was the absence of *Magnolia* subsect. *Talauma* in the region of Gran Piedra, in the Sierra Maestra. This locality showed climatic conditions for the presence of individuals of the subsection. Also in Gran Piedra lives *Magnolia cubensis* subsp. *cubensis*, a Cuban endemic magnolia from subsection *Cubenses* (Hernández 2022).

The individuals of *Magnolia* subsect. *Talauma* from northeastern Cuba can live on serpentine and limestone soils, *Ophiticola*'s individuals being exclusive of serpentine. According to Borhidi (1996), the serpentine areas in Cuba have been proven to be extreme and successful places for plant speciation and diversification. In this sense, the extreme conditions in this area could have



influenced the diversification of this botanical group. It is true that many individuals of *Minor* occur on serpentine too, but in this case, all of them were found closer to rivers and streams (pers. obs.). More analyses about the influence of the type of soil over the morphology and the genetic differentiation due to the effect of local adaptation (soil type) are needed.

## 5.2 Morphological variability

The observed leaf morphological variability for Cuban magnolias was different from that described by previous studies. The values of leaf length and width were higher than those reported by León & Alain (1950, 1951) for *Magnolia minor* and *M. orbiculata*. Likewise, these values were higher than what was previously reported by Bisse (1974, 1988), except for *M. oblongifolia*. These differences with previous studies are due to the larger sample size used in the present work and its wider geographic representativeness. Palmarola *et al.* (2016) report similar values of length and width for *M. minor* and lower values for *M. oblongifolia* and *M. orbiculata*. According to Padial *et al.* (2010), one of the disadvantages of the MSC is that demonstrating the fixation of a state requires large sample sizes. Based on the Calculated Index of Bisse ( $B_{Ci}$ ), the average values were similar to those of Bisse (1974, 1988) for *M. orbiculata* and *M. oblongifolia*. For the individuals considered in this research as *Minor* and *Ophiticola*, the average values of  $B_{Ci}$  are slightly lower and slightly higher, respectively, than those reported by Bisse (1974, 1988) for *M. minor* and *T. ophiticola*. The other nine linear and angular variables are not possible to compare with previously published papers because this work represents their first use in *Magnoliaceae* family.

The use of Geometric Morphometrics methodology is not frequent in magnolias, even when Jensen (2003) recommends their uses in the family in which all the species have simple leaves. Only the works from Hernández (2014), Tamaki *et al.* (2018), and Tamaki *et al.* (2019) use this approach in order to clarify the delimitation among taxa. The landmark methods allow a better visualization and characterization of the differences in the leaves in *Magnolia* subsect. *Talauma*

in Cuba, compared with the method based on EFDs (see Figure 9). Also, the landmark methods are better for discriminating the individuals based on blind analysis, just like clustering methods (see Figure 12). Hernández (2014) recommends the use of both methods in the delimitation of *Magnolia cubensis*, a Cuban sister species from *Magnolia* subsect. *Cubenses*. The high level of morphological differentiation between taxa in this study reinforces the value of leaf characteristics in taxonomic studies of Cuban magnolias (León & Alain 1950, 1951; Alain 1969; Bisse 1974, 1988; Imkhanitzkaja 1991, 1993; Hernández 2014; Palmarola *et al.* 2016). Leaf morphology is a key to species delimitation (Jensen *et al.* 2002; Jensen 2003), and this study confirms that they are highly relevant in groups like *Magnolia*.

It is widely recognized that the variation in the leaf shape is used in taxonomy since it varies between species, subspecies, and populations in plants; it is also a highly sensitive trait to the influence of biotic and abiotic factors. Numerous investigations have shown that the shape of angiosperm leaves is strongly affected by the availability of water (Picotte *et al.* 2009; Reichgelt & Lee 2021), temperature (Royer *et al.* 2009; Walter *et al.* 2009; Reichgelt & Lee 2021) or lighting (Xu *et al.* 2009; Fan *et al.* 2013). According to Reichgelt & Lee (2021), environmental changes can frequently produce alterations in leaf morphology, even for the same species growing in different habitats. The analysis of the influence of the environment on the leaf morphology should be carried on in order to understand its effect on the variation among populations.

Not including flowers and fruits in the morphological analysis of *Magnolia* subsect. *Talauma* in Cuba was given by the fact that finding both structures is very rare. However, field observations and literature revision (León & Alain 1950, 1951; Bisse 1974; Palmarola *et al.* 2016) confirm the differences in the fruit shape and size between the groups of the subsection. *Orbiculata* and *Minor* display spherical fruits but with differences in size, the fruit of *Orbiculata* being larger. In the case of *Ophiticola*, fruits have a rhombic shape, with a size more similar to those of *Orbiculata*.

### 5.3 Genetic Structure

Some of the genetic markers used in the present work are used by Hernández (2022) in the genetic characterization of *Magnolia cubensis* subsp. *acunae* (five markers in common), *Magnolia cubensis* subsp. *cubensis* and *Magnolia cristalensis* (both with six markers in common); and by Veltjen (2020) in *Magnolia dodecapetala* (Lam.) Govaerts (eleven markers in common). The first three taxa are from Cuba and belong to *Magnolia* subsect. *Cubenses*, while the other taxon belongs to the subsection *Talauma* (Veltjen *et al.* 2022). According to Veltjen *et al.* (2022), in the phylogeny of the Caribbean magnolias, and supported by our own phylogeny (see Figures 18, 19), *Magnolia* subsect. *Talauma* in Cuba is more closely related to *M. dodecapetala* than to the other taxa of *Magnoliaceae* in Cuba.

The genetic differentiation and distance measures ( $F_{ST}$  and  $D_{JOST}$ ) between Orbiculata and the other groups of the subsection were the highest (see Table 10). In the case of the groups from northeastern Cuba, both measures showed very low values, less than 0.01. When compared with other studies of Neotropical *Magnolia*, the  $F_{ST}$  values among northeastern groups of *Magnolia* subsect. *Talauma* were lower than values usually found at the interspecific level. For example, Rico & Gutiérrez-Becerril (2019) consider *M. pedrazae* A. Vázquez and *M. schiedeana* Schltldl. as being a single species with pairwise  $F_{ST}$  values ranging between 0.053 and 0.283; Chávez-Cortázar *et al.* (2021) propose the synonymization of *M. nuevoleonensis* A. Vázquez & Domínguez-Yescas and *M. alejandrae* García-Mor. & Iamónico with *M. dealbata* based on  $F_{ST}$  values equal to 0.21 and 0.43, respectively. Aldaba-Nuñez *et al.* (2021) recommend a formal taxonomic revision of *M. decastroi* A. Vázquez & Muñoz-Castro and *M. mexicana* DC. ( $F_{ST}=0.12$ ,  $D_{JOST}=0.19$ ) and *M. lopezobradorii* A. Vázquez and *M. zoquepopolucae* A. Vázquez ( $F_{ST}=0.11$ ,  $D_{JOST}=0.28$ ) species complexes. The values reported by Hernández (2022) between the populations of *Magnolia cubensis* subsp. *cubensis* and *Magnolia cristalensis*, and by Veltjen (2020) in *Magnolia dodecapetala* between the different islands of the Less Antilles, are higher or equal to the values found between the groups of *Magnolia* subsect. *Talauma* in Cuba in the present work.

The STRUCTURE results (see Figure 15), with and without *Oblongifolia* individuals, showed the same genetic structure and admixture pattern. These results showed the excellent stability of inferences made on individual assignment and genetic admixture between clusters, probably because of the strong genetic differentiation between the genetic clusters. Also, this is an indicator that *Oblongifolia* does not represent an independent genetic group, as proposed by Bisse (1974, 1988). The algorithm implemented in STRUCTURE is very useful for the delimitation in Neotropical *Magnolia*, with good results as demonstrated by Rico & Gutiérrez-Becerril (2019), Muñiz-Castro *et al.* (2020), Veltjen (2020), Aldaba-Nuñez *et al.* (2021), Chávez-Cortázar *et al.* (2021), and Hernández *et al.* (2021).

The STRUCTURE results showed that some individuals of each group displayed genetic admixture with the other groups, being more evident in the groups of northeastern Cuba. According to Hey & Pinho (2012), the levels of genetic differentiation among species are influenced by the time of separation and the amount of gene exchange. According to Zhou *et al.* (2017), genetic variation shared between closely related species may be due to the retention of ancestral polymorphisms because of incomplete lineage sorting (ILS) and/or introgression following secondary contact. Distinguishing between those two causes from observed patterns is challenging, although coalescence modeling can help (e.g. Zhou *et al.* 2017; Meleshko *et al.* 2021).

It seems more likely that the admixture signal between *Orbiculata* and the other groups could be explained by the common origin and recent diversification of the subsection in Cuba, which is less than five mya according to Veltjen *et al.* (2022), and the recent separation of *Orbiculata* from the other groups. However, the possibility of rare events of inter-taxa hybridization involving *Orbiculata*'s individuals as one parent cannot be totally ruled out, especially because individuals that have displayed admixed genomes involving this group have intermediate ancestry coefficients, which is compatible with a hypothetical first- or early-generation hybrid status. The ecological differentiation between *Orbiculata* and the other groups of the subsection may suggest that

selection against first- or early-generation hybrids due to local adaptation could also contribute to preventing genetic exchanges with the other groups. In the groups from northeastern Cuba, the level of genetic admixture could be explained by a recent diversification that led to numerous genetic loci with incomplete lineage sorting and overlaps in the distribution of morphological traits. In trees, factors such as long generation time, and large effective population sizes, increase the opportunity of sharing ancestral polymorphisms through incomplete lineage sorting, which makes species identification based on neutral markers even more problematic (Zhou *et al.* 2017).

The individual assignment in the solution with three genetic clusters in the DAPC analysis (see Figure 16) was strongly similar to the previously obtained using STRUCTURE. This result strongly supported these three genetic groups corresponding to: *Orbiculata*, *Minor* (includes *Oblongifolia*), and *Ophiticola*. However, a second solution with four genetic clusters was also likely. DAPC analysis is not based on a population genetic model, which is the case of STRUCTURE (Jombart *et al.* 2010); this makes that the results of genetic clusters and assignment of individuals obtained using this analysis (DAPC) are not affected by the potential low model fit to data. The fact that two different analyses get the same results validates the inference made in the number of genetic groups and individuals assignment of *Magnolia* subsect. *Talauma* in Cuba.

In the future, a complete analysis of the population genetic structure of each species would be needed in order to obtain a better insight into the link between spatial distance, ecological differentiation, and genetic diversity. Such a landscape genetic approach would be necessary to deeply understand environmental parameters that influence the genetic connectivity between populations and gene flow between species in *Magnolia* subsect. *Talauma* in Cuba. Such knowledge is necessary to define conservation units and propose management measures accordingly. However, this analysis goes beyond the objectives of the present work.

#### 5.4 Testing hybrids

The foliar phenotype observed in the individuals of *Oblongifolia* appears to be intermediate between *Minor* and *Ophiticola*. A feasible explanation is a recent diversification process of these two groups, opening the possibility that reproductive isolation would not be completed. Natural hybridization might explain the intermediate characteristics of *Oblongifolia*, as has been observed for *Quercus* species (Burgarella *et al.* 2009; An *et al.* 2017) and for the genus *Rhizophora* (Francisco *et al.* 2018). According to Lihová *et al.* (2007), An *et al.* (2017), and Teixeira *et al.* (2019), morphological data have been widely used in the detection of hybrid individuals. In *Magnolia* subsect. *Talauma*, genetic data support rather the fact the two groups (*Minor* and *Ophiticola*) are totally genetically compatible and showed that they could be considered as a single genetic group. However, not all morphological variation has a genetic basis. Also, the assumption that hybrid individuals are phenotypically intermediate to parents is not necessarily true because of dominance effects or because of recombination from first-generation hybrids, leading to interspecific hybrids displaying a mosaic of parental phenotypes (Allendorf *et al.* 2001). In the specific case of *Oblongifolia* individuals, even when their foliar phenotype looks intermediate (between *Minor* and *Ophiticola*), the genetic data showed unambiguously that this group is closer to *Minor*.

Yet, backcrosses between first-generation hybrids and parental species and crosses between hybrids may occur. Morphological characters do not allow us to determine whether an individual is a first-generation hybrid (F1), a backcross, or a later generation hybrid, which reduces our capacity to assess the extent of interspecific gene flow (Allendorf & Luikart 2007; Soltis & Soltis 2009). The previous elements raise the importance of using molecular markers as an indicator for hybrid origins and subsequently comparing them with morphology. According to Lihová *et al.* (2007), in plants, hybridization processes can cause discordance between morphological and molecular data.

The NEWHYBRIDS analysis (see Figure 17 and Table 11) detected the existence of pure individuals, backcrosses to Minor, and F2 in the groups from northeastern Cuba. Some individuals of *Ophiticola* were identified as pure Minor, which supports the existence of genetic introgression between those species. Detection of backcross hybrids and evidence of historic introgression suggest hybrid zones between Minor and *Ophiticola* displayed a complex genetic structure, which is not limited to the first generation of hybrids. The occurrence of F2 hybrids indicates that F1 hybrids can mate with each other and are fertile.

In any case, F1 and backcross to *Ophiticola* were found, even when a 0.5 threshold was used (data not shown). The absence of BC-Oph and the existence of *Ophiticola*'s individuals considered pure Minor seem to indicate asymmetric introgression from *Ophiticola* to Minor. According to some authors (Arnold *et al.* 2010; Hu *et al.* 2011; Muranishi *et al.* 2013), asymmetric introgression has been reported in several species (including magnolias), and relevant explaining mechanisms have been explored from the perspective of prezygotic (gamete competition, spatial, temporal, mechanical and gametic isolation, and pollination behavior) and postzygotic (hybrid zygote abnormality, hybrid infertility, and low hybrid viability) barriers. Based on the results, the BC-Oph individuals could have low fitness, or crosses between F1/F2 with pure *Ophiticola* may have some prezygotic or postzygotic barriers, which does not allow the viability of BC-Oph individuals.

The absence of F1 individuals in hybrid populations could be due, in some degree, to fairly strong pre-zygotic reproductive isolation (Arnold *et al.* 2010), minimizing initial F1 formation events. Once an F1 is formed, all of its descendants will be hybrid derivatives, and these can hence rapidly multiply in number, producing later-generation hybrids, from which the transfer of novel alleles between hybridizing lineages via repeated backcrossing (introgression) becomes possible (Yan *et al.* 2017). Further studies are required to understand the causes of this observed asymmetric introgression, and it will be helpful for conservation to determine the contribution of each hybrid class on the population diversity.



Without analysing young individuals in these groups, it is impossible to confirm that the hybridization process still occurs. However, the high admixture level observed using SSR markers, as well as the few cases of reciprocal “miss-assignment”, suggest that gene flow between the groups of northeastern Cuba has occurred recently and must still be occurring. The latter hypothesis is reinforced by the observation that reciprocal genetic admixture between the two genetic clusters mainly corresponds to Minor and to *Ophiticola*, respectively, as it is more frequent in the localities where both groups occur (see Figures 15, 16, 17). This hypothesis is well-supported because these groups live in the same habitats and in similar ecological conditions (see Figure 4), a situation that is not favorable for the emergence of reproductive barriers. The phylogenetic closeness between those three groups (Minor, *Oblongifolia*, and *Ophiticola*) and the high overlap detected in the ecological conditions are parameters that may favor hybridization between them. According to Callaway (1994), hybridization is a common process in magnolias and is obviously more common when the distribution ranges of two or more highly related taxa overlap (Soltis & Soltis 2009).

According to Allendorf & Luikart (2007) and Becker *et al.* (2013), hybridization may have a wide variety of effects on fitness, whether positive (heterosis or adaptive introgression) or negative (outbreeding depression). Also, hybridization could lead the introgressed species to become extinct due to the dilution of the genetic pool of one species involved. Hybridization is an important evolutionary phenomenon, and therefore a detailed understanding of the processes involved in hybridization is of widespread interest. Many plant species have hybrid origins, and hybridization is a key mechanism for the production of new species and novel adaptations. The loss of a species by hybridization is not necessarily a conservation problem as long as its origin is natural and never provoked by human intervention. In the present case, it is hard to say if hybridization is natural or human induced because the mountains of Nipe-Sagua-Baracoa have been under the influence of human activity for many years (Carabia 1945; González-Torres *et al.* 2016). This problem remains in our days but to a lesser extent. Knowing if hybridization has



been promoted by human impact is very complicated. Researches focusing on incompatibility, phenology, pollination, dispersion, seed production and viability, local adaptability, and response to the environment and climatic change are necessary in order to understand the possible effect (negative or positive) of hybridization on the fitness of the Cuban species of *Magnolia* subsect. *Talauma*.

### 5.5 Plastome phylogeny

The previous molecular phylogeny of the group in Cuba (Veltjen *et al.* 2022) is based on eleven genome regions (five partial nuclear genes, three partial chloroplast genes, and three chloroplast intergenic spacers). Unfortunately, the work of Veltjen *et al.* (2022) does not solve, just like in the present case, the taxonomic delimitation of the species from northeastern Cuba. According to Wang *et al.* (2020) and Dong *et al.* (2022), using a few plastids or nuclear loci in the reconstruction of the phylogenetic relations inside *Magnoliaceae* family has recovered some well-defined clades which are in concordance with relevant morphological circumscriptions. However, when using only a few loci, some deep nodes are still unresolved, possibly due to the slow nucleotide substitution rates, incomplete lineage sorting, hybridization, or a combination of these factors (Dong *et al.* 2022). Our results showed that the Cuban species of *Magnolia* sect. *Talauma* did not constitute a monophyletic group, similar to Veltjen *et al.* (2022) and Guzmán-Díaz *et al.* (2022).

The plastome phylogeny in *Magnolia* subsect. *Cubenses* showed the proximity of the Cuban taxa with those from Hispaniola and Puerto Rico. The present research is in concordance with Veltjen *et al.* (2022), in the state that the migration of *Magnolia* species across the Caribbean could have occurred from South America to Puerto Rico, from there to Hispaniola, and finally to Cuba (around 5 mya). The time of arrival in Cuba corresponds to the Pliocene and Pleistocene, according to Iturralde-Vinent (2006).

The plastome phylogeny validates the statement of Veltjen *et al.* (2022) that *Magnolia* subsect. *Talauma* from Cuba represents a monophyletic group, more related to the Mexican species from the same subsection than to the other magnolias of Cuba (see Figures 18, 19). The phylogeny showed the proximity between *Magnolia* subsect. *Talauma* from Cuba and Central America. According to our result, and the one found by Veltjen *et al.* (2022), the ancestor of *Magnolia* subsect. *Talauma* in Cuba came from Mesoamerica, with a colonization time of Cuba less than 10 mya ago. According to Iturralde-Vinent (2006), during this period of time, the mountains of Sierra Maestra and Nipe-Sagua-Baracoa had emerged for at least 16 mya. During the Upper Miocene (9-7 mya) and Miocene-Pliocene (7-3.7 mya), there were shallow marine areas (Nicaragua Rise, Pedro Bank, and Western Jamaica) between Central America and the Sierra Maestra that could have served as migration pathway for the ancestor, probably mediated by birds.

Taking into account the results, Sierra Maestra could be the first population founded by the ancestor of the subsection (assuming a unique colonization event); and, from them, another migration event to northeastern Cuba. This hypothesis is supported by the statement that *Orbiculata* is genetically the most different group of the subsection, probably reflecting more time of isolation and divergence. Concerning the group from northeastern Cuba, probably the plastome genome divergence is still low because of the recent differentiation of this group. According to Song *et al.* (2017), the chloroplast genome is highly conserved, which makes it, in some cases, very hard to find differences, especially in poorly evolved groups.

The retention of ancient features characterizes the *Magnoliaceae* family (Treseder 1978; Callaway 1994; Rivers *et al.* 2016). Therefore, the ancestor may have displayed the current red-orange fleshy aril characteristic of *Magnoliaceae* seeds (Callaway 1994; Judd *et al.* 2016), which is easily visible and ingested by birds. As a result, the seeds can be widely dispersed through long-distance dispersal, and as a consequence these characteristics may have facilitated the colonization of new areas by *Magnolia* species.

### 5.6 An integrative classification of *Magnolia* subsect. *Talauma* in Cuba

In the present study, *Orbiculata* was clearly distinguished from the other groups of *Magnolia* subsect. *Talauma* in Cuba based on ecological, morphological, genetic markers, and phylogenetic data. The previously observed large variation of leaf morphology across *Magnolia* subsect. *Talauma* in Cuba, although based on the observation of only a few specimens, has been the basis for several authors to consider a unique species in this subsection, therefore including *Orbiculata* within *Minor* (Howard 1948; Alain 1969; Borhidi & Muñiz 1971; Lozano-Contreras 1994). In contrast, the present study and the line of evidence already brought by the molecular phylogeny of *Magnolia* subsect. *Talauma* (Veltjen *et al.* 2022) strongly support that *Orbiculata* may be considered a well-delineated species (*Magnolia orbiculata* (Britton & P. Wilson) Palmarola, *nom. nov.*). However, in our study, a few cases of confusion with *Minor* on the basis of leaf morphology traits were observed. This confusion could be explained by the similar rounded shape and relation width-length present in both taxa. Different specialists have already erroneously identified some herbarium specimens of *M. orbiculata* as *Minor* in the past (personal observation on herbarium records).

Regular gene flow seems unlikely between *M. orbiculata* individuals with respect to other groups. The clear morphological and genetic differentiation of *M. orbiculata* (see Figures 12, 15, 16) from other groups of *Magnolia* subsect. *Talauma* in Cuba strongly suggested that, at least, the lowland between the Sierra Maestra (habitat of *M. orbiculata*) and Nipe-Sagua-Baracoa (habitat of the other groups) may have acted and still acts as a barrier to gene flow by limiting pollination and seed dispersal. This is strongly supported by the differentiation in the ecological niche of *M. orbiculata* (see Figure 4), respecting the other groups of the subsection in Cuba. However, the genetic data of our study do not allow us to reject the possibility of rare events of gene flow. It is possible that post-zygotic reproductive barriers also contribute to the genetic isolation between the two groups (*M. orbiculata* and northeastern populations) by counter-selection of hybrids. Hernández (2022) reports high levels of genetic differentiation between *Magnolia cubensis* subsp. *cubensis* (from

the Sierra Maestra) and *Magnolia cristalensis* (from Nipe-Sagua-Baracoa), both from *Magnolia* subsect. *Cubenses*. Vázquez-García *et al.* (2016) state that allopatric speciation seems to be a major driver of *Magnolia* diversification in the Neotropics.

Undoubtedly, our data confirmed the main taxonomic issues concerning the northeastern Cuban populations of *Magnolia* subsect. *Talauma*. León & Alain (1950, 1951) considered all the variations of this group in a single species: *Magnolia minor*, with two varieties (*Magnolia minor* var. *minor* and *Magnolia minor* var. *oblongifolia*). León & Alain (1950) point out that individuals with more oblong leaves, considered by them as *Talauma minor* var. *oblongifolia*, may belong to a different species. However, these authors do not assign the species rank to this group because of the absence of reproductive structures in the available specimens. The absence of flowers and fruits is still a major concern in this group because it is tough to find these structures in the field. The existence of a single species (*Magnolia minor*), including the three groups considered in the present thesis (Minor, Oblongifolia, and Ophiticola) was supported neither by our morphological results (based on linear and angular variables and the matrix of Landmark) nor by genetic markers, which both showed differentiation between the individuals of Minor and Ophiticola.

On the other hand, Bisse (1974, 1988) proposes to divide *Magnolia minor* (sensu León & Alain 1950, 1951) into three separate species (*M. minor*, *M. oblongifolia*, and *Talauma ophiticola*). The morphological and genetic data did not support this proposal. Considering *M. minor* (Minor group) and *M. oblongifolia* (Oblongifolia group) as separate species is supported neither by ecological (see Figures 4, Table 8), morphological (see Figures 7, 8, 9, 12) nor by genetic data (see Figures 15, 16). Indeed, concerning the individual of *M. oblongifolia*, the foliar phenotype observed in this species appears to be intermediate between *M. minor* and *T. ophiticola* (Ophiticola group); but their similarity, based on genetic and morphological data, with *M. minor* discharged this idea.

The results of the thesis do not support either the combination of *Talauma ophiticola* and *Magnolia oblongifolia* (sensu Bisse 1974, 1988) in a single taxon, as recently proposed by Palmarola *et al.* (2016) based on the gathering *Bisse & Kohler HFC 5358* (HAJB, JE) from the coast of Moa, which shows both oblong and elliptical leaves. In fact, the individuals considered as *M. oblongifolia* (Oblongifolia group) are, morphologically and genetically, more similar to *M. minor* (Minor group) than to *T. ophiticola* (Ophiticola group). Nevertheless, the delimitation of the individuals of Ophiticola group is still challenging. In the present study, a significant proportion of individuals that were assigned to that group based on leaf morphology were unambiguously assigned to the “minor-oblongifolia” genetic cluster, while only one individual from Minor group was assigned to the “ophiticola” genetic cluster. This could be explained by a recent and/or continued asymmetrical gene flow among both species. This statement could be possible taking into consideration the sympatric distribution and their ecological similarities (not favorable for the emergence of reproductive barriers).

After analysing the results and taking into account, the differences found for the evaluated taxa, the hypothesis of this thesis ("For the Cuban members of *Magnolia* subsect. *Talauma*, the number and limits of species defined by the genetic, ecological, and phylogenetic criteria are the same as that defined by the morphological species criteria") is partially refuted. According to the results, not all taxa of *Magnolia* subsect. *Talauma* in Cuba are consistent in their morphological, ecological, genetic, and phylogenetic limits. Only for *Magnolia orbiculata* (Orbiculata group in the thesis), the different sources of evidence support this group as independent lineages and, consequently, a valid species. In the case of northeastern groups, each approach used showed different species limits.

Based on the absence of solid evidence of species delimitation, two possible taxonomic solutions could be considered 1) the population of northeastern Cuba could be considered as one single and highly variable species: *M. minor* (Urb.) Govaerts, the oldest name, which includes all the

variability of the groups from northeastern Cuba (Minor, Oblongifolia, and Ophiticola); or 2) two species: *M. minor* (Urb.) Govaerts (include the individuals of the groups Minor and Oblongifolia) and *M. oblongifolia* (León) Palmarola (include the individuals considered in the present as Ophiticola) without very strong limits may be recognized. The decision to treat this group as a complex of two different species that can still be hybridized is validated by the consensus protocol for integrative taxonomy proposed by Padial *et al.* (2010). The start of this protocol is two groups of specimens with differences in a taxonomic character (e.g. morphology), followed by the search for the existence of congruent differences in another taxonomic character (e.g. population genetics). In the present thesis, differences between both species were found based on morphological characters (see Figure 12) and genetic data (see Figures 15, 16).

Furthermore, most evolutionary biologists agree that species are separately evolving lineages of populations or meta-populations (Unified SC) (de Queiroz 1998, 2007). According to de Queiroz (2007) and Naciri & Linder (2015) this implies that the populations share a common history (largely) and have a common future. Contrary to the biological species concept of Mayr (1942), this concept allows for some gene flow between species. There is no doubt about the common evolutionary history of *Magnolia* subsect. *Talauma* from northeastern Cuba, which is supported by the phylogeny (monophyletic group, see Figure 18) and their biogeographic history (see Figure 19). Also, there is strong evidence of a current or recent gene flow between both taxa (see Figures 15, 16, 17).

Evolution is a continuous process in which different lineages can separate and diverge (de Queiroz 2007). During the speciation process, the daughter lineages become more and more different from one another through time and acquire different properties relative to each other (morphological, ecological, genetic, reproductive incompatibility, etc.). Definitely, it is not a simple matter to propose an unbiased approach for species delimitation given the nature of the speciation process; in which no order is expected in the appearance of divergences among

sister species, such as morphological differentiation, ecological adaptation to new niches and reproductive isolation among other criteria (de Queiroz 2007; Prata *et al.* 2018).

It is possible that *Magnolia minor* (includes Minor and Oblongifolia groups) and *Magnolia oblongifolia* (includes Ophiticola group) are still in a speciation process. According to de Queiroz (2007), both taxa could be in a grey zone of speciation, in which they have not yet acquired all the necessary properties to be two completely differentiated species. This could explain why the different species concepts evaluated here come into conflict. Even when there is not a complete differentiation, the found differences between both taxa concerning morphology and genetics seem to support two different evolutionary lineages. Based on the Unified SC and the Integrative Approach, both taxa could be considered as two independent species.

### 5.7 Taxonomic treatment

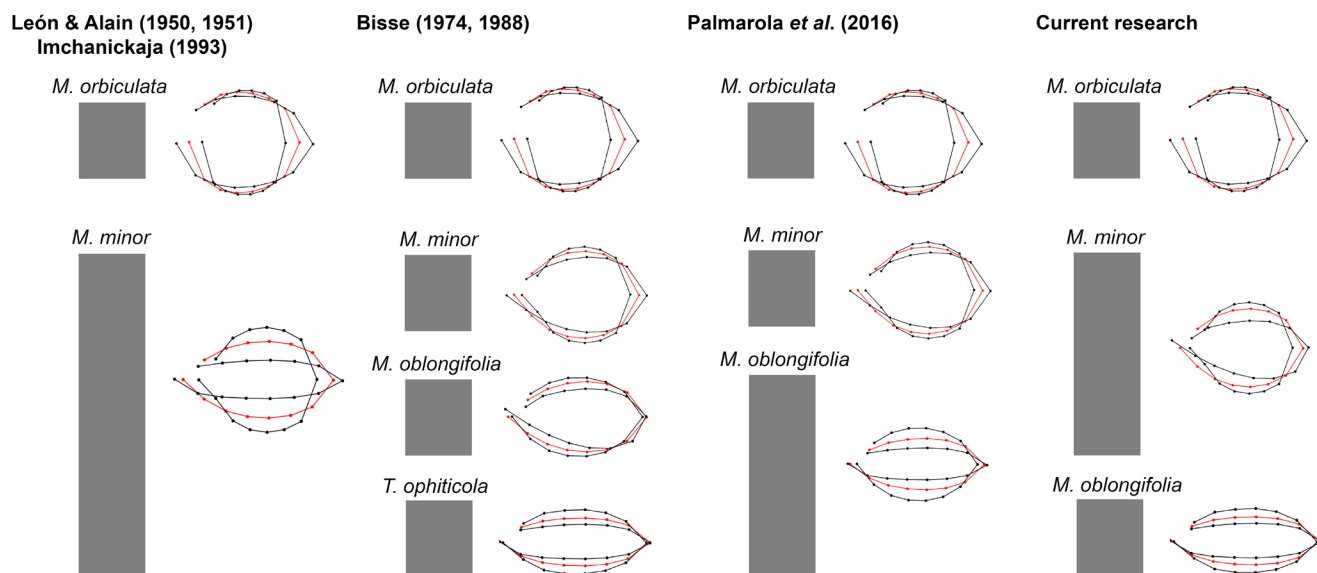
The taxonomic circumscription for *Magnolia orbiculata* (Britton & P. Wilson) Palmarola remains similar to the one proposed by Bisse (1974, 1988), Imchanickaja (1993), and Palmarola *et al.* (2016). However, Britton (1923) only mentions the material *León LS 10955* in the description of the species without specifying the herbarium where it was deposited. Because of this, all duplicates of such a collection in all herbaria are syntypes (Turland *et al.* 2018: Art. 40 Note 1). However, Palmarola *et al.* (2016) recognize the NY specimen as a holotype, but such designation cannot be considered effective lectotypification (inadvertent lectotypification) since, as of January 1<sup>st</sup>, 2001, the phrase “designated here” or an equivalent is required (Turland *et al.* 2018: Art. 7.11). For this reason, *Talauma orbiculata* Britton & P. Wilson is lectotypified here with the material (*León LS 10955*, NY #73699) suggested by Palmarola *et al.* (2016).

In the case of *Magnolia minor* (Urb.) Govaerts and *Magnolia oblongifolia* (León) Palmarola, the results of this research change the limits of these species. *Magnolia minor* extends its species limits from the proposed by Bisse (1974, 1988) and Palmarola *et al.* (2016) to include the individuals considered by Bisse (1974, 1988) as *Talauma oblongifolia* (except the type).



This last taxon is considered by Palmarola *et al.* (2016) together with *Talauma ophiticola*. The individuals considered by Bisse (1974, 1988) as *Talauma ophiticola* (considered as *M. oblongifolia* in the present taxonomic treatment) are an independent species (Fig. 23). In this part, it is important to clarify the confusion in the names and types that Bisse (1974) makes for *Talauma oblongifolia* (León) Bisse and *Talauma ophiticola* Bisse. This confusion is not cleared up by Palmarola *et al.* (2016) in the revision of *Magnolia* subsect. *Talauma* of Cuba.

In the species description of *Talauma ophiticola* Bisse (Bisse 1974), the type material (Bisse & Köhler HFC 6519) is referred in HAJB, without mention of which of the two specimens (HAJB #634, #635) is the holotype. Palmarola *et al.* (2016) recognize the specimen HAJB #634 as the holotype, but such designation cannot be considered effective lectotypification (inadvertent lectotypification) since, as of January 1<sup>st</sup>, 2001, the phrase “designated here” or an equivalent is required (Turland *et al.* 2018: Art. 7.11). For this reason, *Talauma ophiticola* Bisse is lectotypified here with the material suggested by Palmarola *et al.* (2016).



**Figure 23.** The main classification systems of *Magnolia* subsect. *Talauma* in Cuba, with the taxonomic results of this research. The lines and points on the right of the blocks represents the leaf shape variability obtained from the morphological analyses.



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**CONCLUSIONS**

1. *Magnolia orbiculata* has the most restricted potential suitability area and is the only taxon with a different ecological niche, supporting its species status. The taxa from northeastern Cuba are sympatric and show high ecological similarity, making it impossible to determine their ecological limits.
2. Three morphological groups are possible to delimited in *Magnolia* subsect. *Talauma* in Cuba, being *Magnolia orbiculata* the most differentiated one. Also, these taxa showed a high inter-specific leaf morphological variability, which reinforces the value of leaf characteristics in taxonomic studies of Cuban magnolias.
3. In *Magnolia* subsect. *Talauma* in Cuba it is possible to differentiate three genetic groups, with *Magnolia orbiculata* the clearest one. The taxa from northeastern Cuba showed high levels of genetic admixture given by a recent or still going asymmetric hybridization process.
4. *Magnolia* subsect. *Talauma* in Cuba is a monophyletic group, closer to the species from Central America than to the other species of *Magnolia* in Cuba. *Magnolia orbiculata* represent an independent lineage and the group from northeastern Cuba is a well-supported clade with no resolution between their different taxa.
5. *Magnolia orbiculata* appeared to be an evolutionary lineage separated from other Cuban taxa of *Magnolia* subsect. *Talauma* , in which the morphological limits are consistent with the ecological, genetic, and phylogenetic limits. Therefore, this taxon can thus be considered a valid species. The morphological limits of *Magnolia minor* and *Magnolia oblongifolia*, are not consistent with the ecological, genetic, and phylogenetic limits. Each approach used showed different species limits.
6. *Magnolia* subsect. *Talauma* in Cuba is integrated by three species: *Magnolia orbiculata* (Britton & P. Wilson) Palmarola, *Magnolia minor* (Urb.) Govaerts, and *Magnolia oblongifolia* (León) Palmarola. Only the species limits of *Magnolia orbiculata* remain similar to the proposed by the last taxonomic revision of the group in Cuba.

**RECOMMENDATIONS**

1. To analyse the venation patterns of the leaves in *Magnolia* subsect. *Talauma* in Cuba.
2. To study the reproductive structures (flowers and fruits) of the Cuban taxa of *Magnolia* subsect. *Talauma*.
3. To analyse the phenology patterns of *Magnolia* subsect. *Talauma* in Cuba.
4. To increase the number of plastome sequences and include nuclear genes of the Cuban taxa of *Magnolia* subsect. *Talauma* and the outgroups taxa, in order to have a better understanding of the phylogenetic relationships and the biogeography of the group.
5. The taxonomic treatment must be published to make the typifications valid.

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Jhonny Quintana-Delgado, Majela Hernández-Rodríguez, **Ernesto Testé**, Alejandro Palmarola, Emily Veltjen, Pieter Asselman, Marlyn Valdés de la Cruz, Isabel Larridon, Marie-Stéphanie Samain & Luis R. González-Torres. 2022. Estructura y diversidad genética de *Magnolia cubensis* subsp. *cubensis* (*Magnoliaceae*). *Revista del Jardín Botánico Nacional - Universidad de La Habana* 43: 33-46.

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### **Thesis supervision related to *Magnoliaceae* family**

Yaniela Infante Columbié. 2022. Comparación de la diversidad y estructura genética entre generaciones de *Magnolia* subsect. *Cubenses*. Bachelor Thesis, University of Havana, Cuba.

Gabriel Martínez Alvarez. 2021. Evaluación de la estructura y la diversidad genética de *Magnolia cristalensis* (*Magnoliaceae*) en Cuba. Bachelor Thesis, University of Havana, Cuba.

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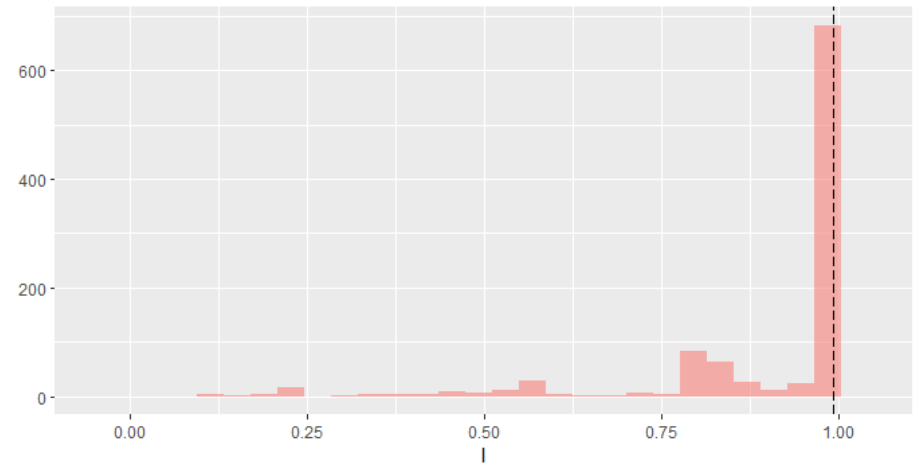
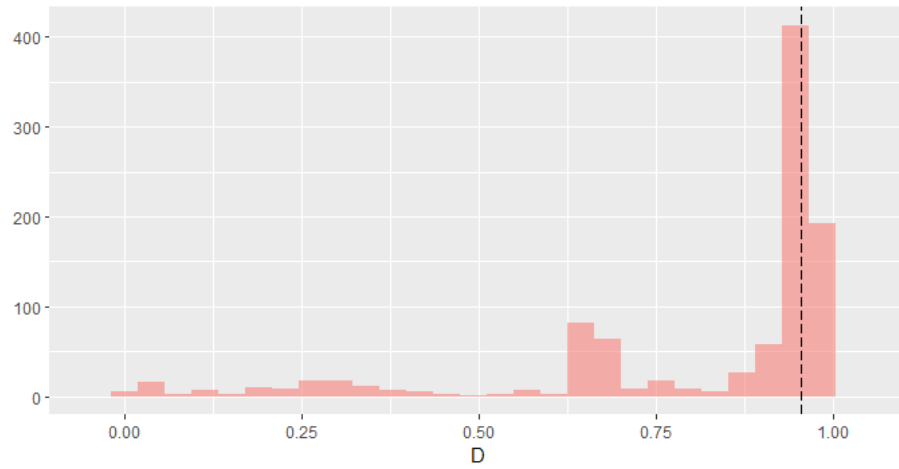
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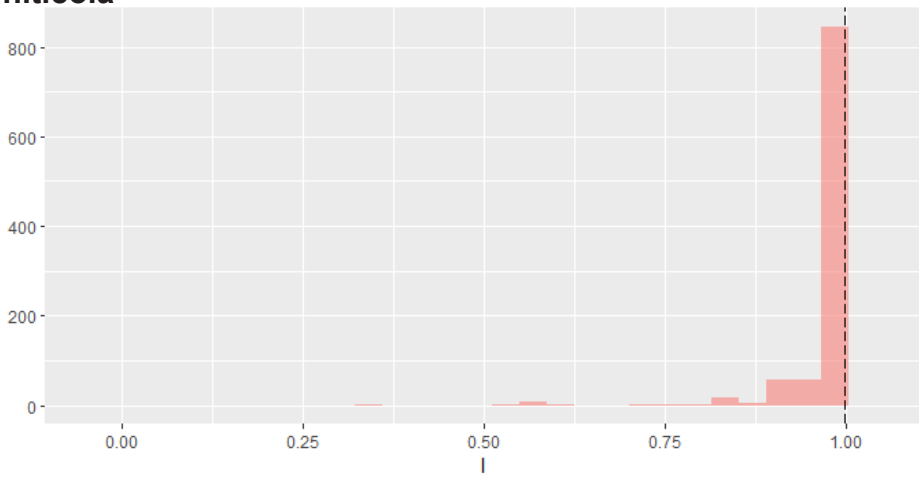
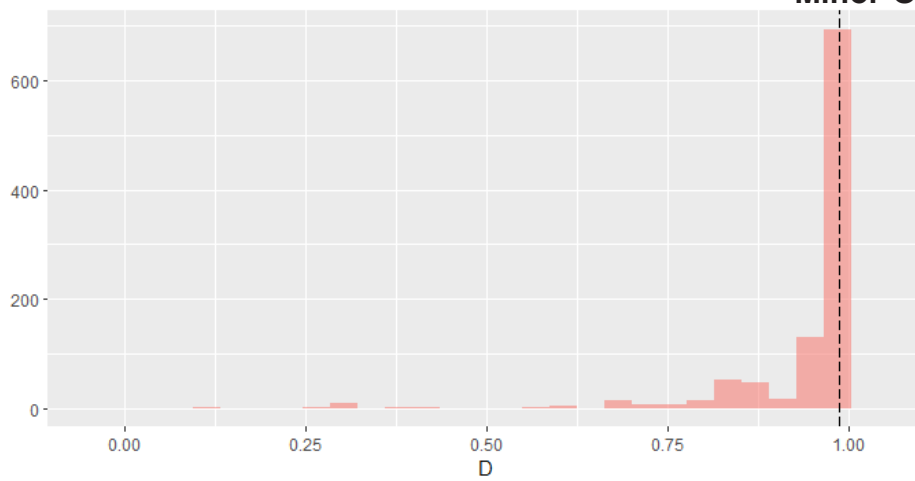


**Supplementary material 1.** Results of the niche identity test in *Magnolia* subsect. *Talauma* in Cuba. Black dashed arrows refer to the actual niche overlap as calculated by ENMTools (D and I). The color bars are calculated by replicates with identity test mode.

**Minor-Oblongifolia**

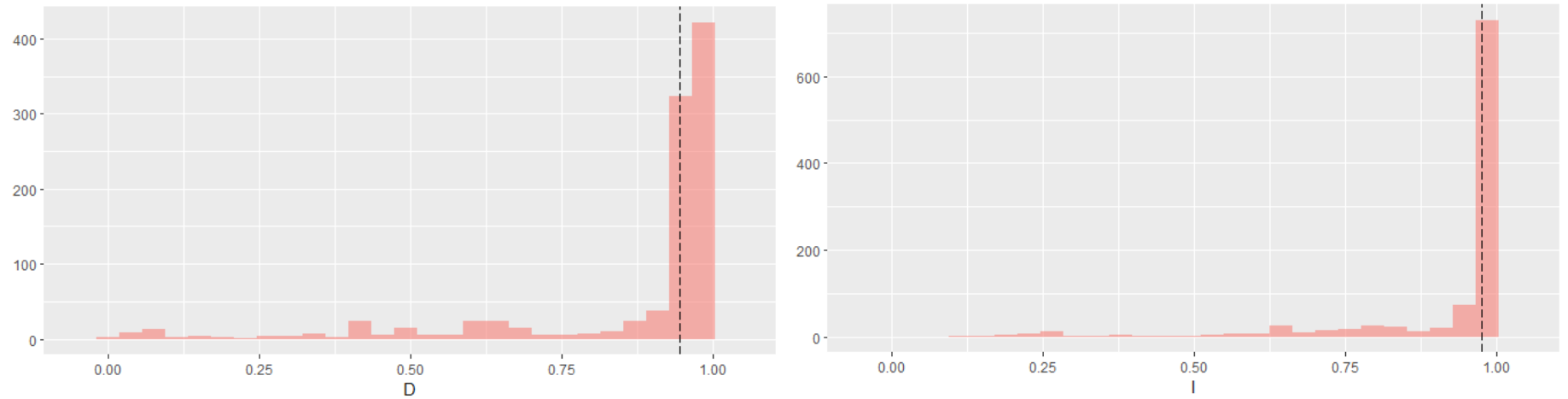


**Minor-Ophiticola**

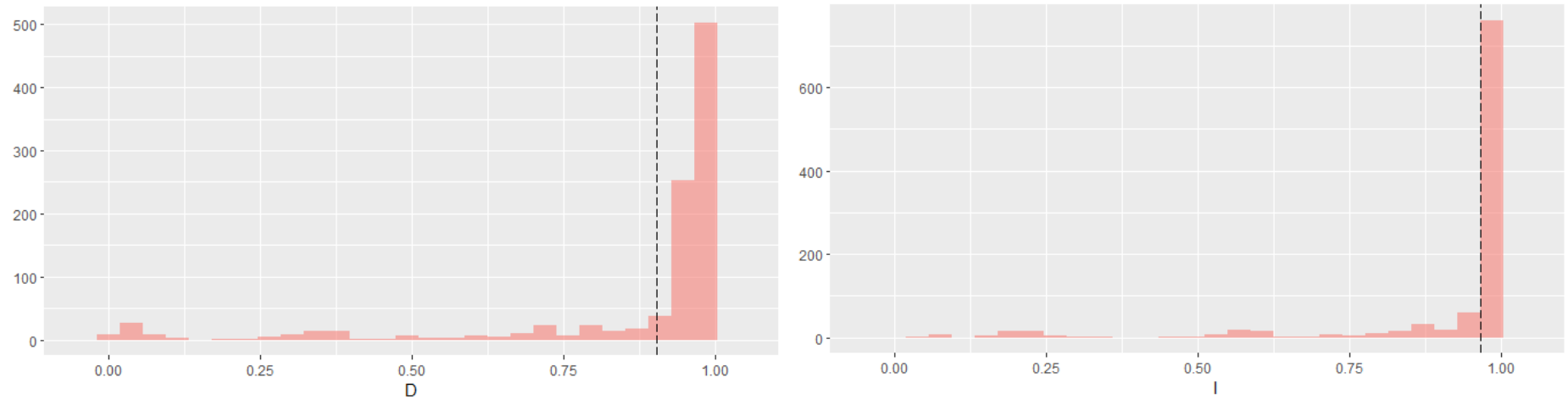


**Supplementary material 1.** Results of the niche identity test in *Magnolia* subsect. *Talauma* in Cuba. Black dashed arrows refer to the actual niche overlap as calculated by ENMTools (D and I). The color bars are calculated by replicates with identity test mode.

**Oblongifolia-Ophiticola**



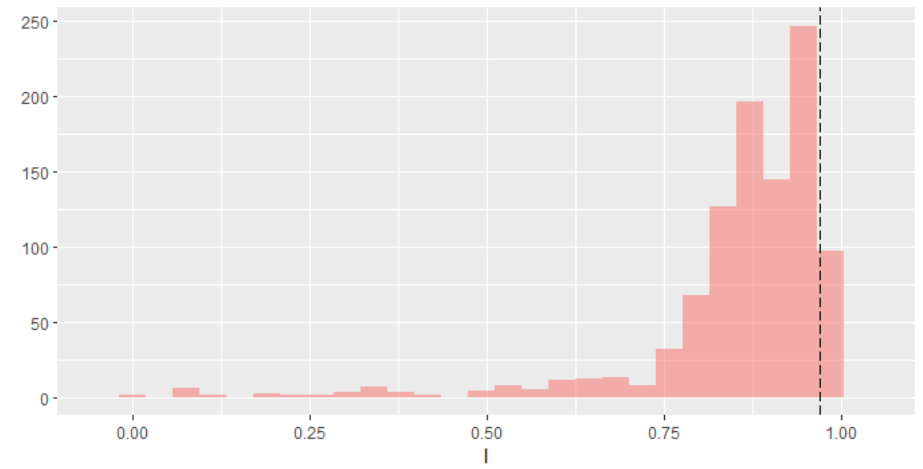
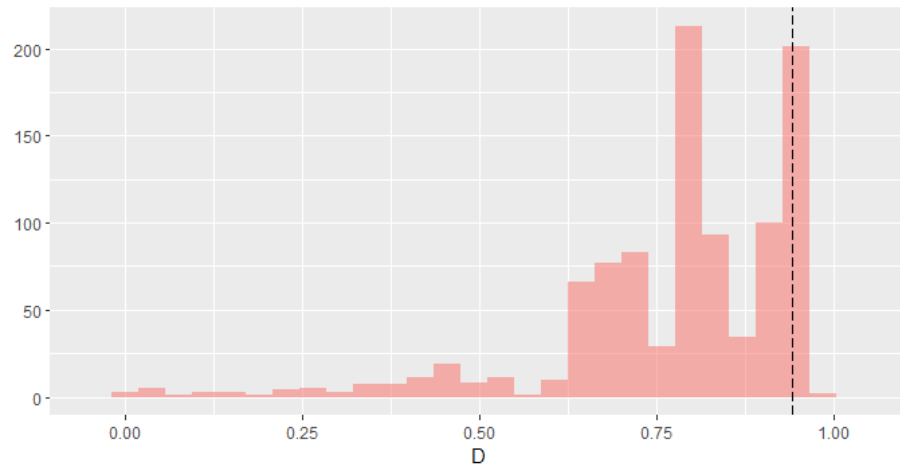
**Minor-Orbiculata**



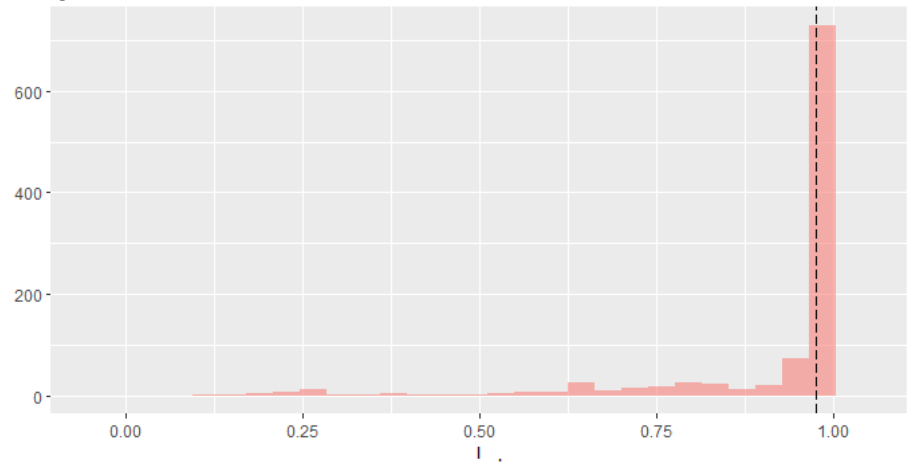
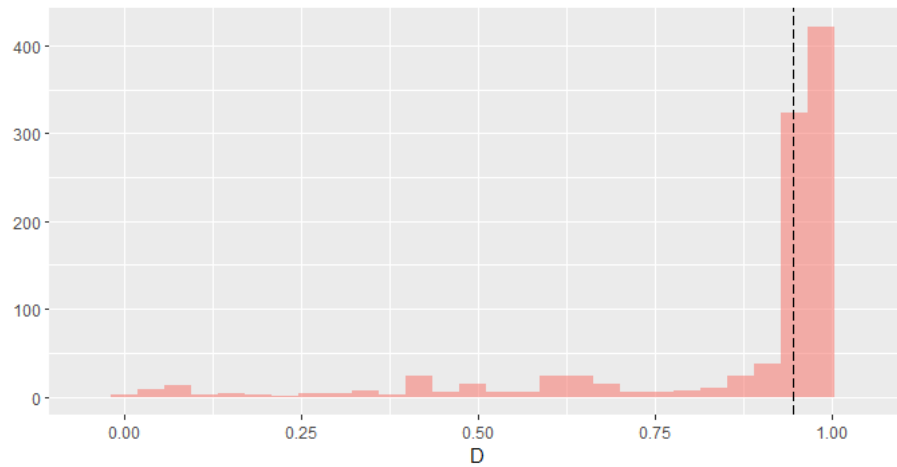


**Supplementary material 1.** Results of the niche identity test in *Magnolia* subsect. *Talauma* in Cuba. Black dashed arrows refer to the actual niche overlap as calculated by ENMTools (D and I). The color bars are calculated by replicates with identity test mode.

**Orbiculata-Oblongifolia**



**Orbiculata-Ophiticola**



**Supplementary material 2.** Number of most probable (highest BIC score) groups (CI) resulting for the ecological niche data of the taxa of *Magnolia* subsect. *Talauma* in Cuba. EEI (diagonal, equal volume and shape), EEV (ellipsoidal, equal volume and shape), BIC (Bayesian information criterion), ICL (integrated complete-data likelihood criterion).

CI	Model	Log-likelihood	BIC	ICL
2	EEV	-13 381.82	-27 007.71	-27 008.17
3	EEV	-13 382.11	-27 116.77	-27 118.55
4	EEV	-13 359.55	-27 180.13	-27 201.76
5	EEV	-13 693.38	-27 956.28	-28 169.34
6	EEI	-13 837.49	-27 946.18	-27 962.64
7	EEV	-13 291.58	-27 369.64	-27 441.23
8	EEI	-13 837.49	-27 946.18	-27 962.64
9	EEV	-11 134.12	-23 271.68	-23 280.05
10	EEV	-11 384.64	-23 881.19	-23 921.43

**Supplementary material 3.** Descriptive statistics of the 12 linear and angular variables measures in the leaves of *Magnolia* subsect. *Talauma* in Cuba.

Variables	Minor (N=93)	Oblongifolia (N=40)	Ophiticola (N=75)	Orbiculata (N=35)
Length (cm)	11.957 ±1.630 (8.127-15.951)	13.970 ±1.841 (9.856-17.684)	14.739 ±2.417 (7.961-19.732)	10.891 ±2.720 (6.105-16.335)
Maximum width (cm)	8.199 ±1.046 (6.143-10.708)	6.979 ±0.879 (4.791-9.058)	4.649 ±0.679 (2.534-6.359)	9.669 ±1.399 (6.718-13.471)
Calculate Index of Bisse	0.691 ±0.075 (0.533-0.873)	0.503 ±0.054 (0.386-0.595)	0.320 ±0.045 (0.225-0.455)	0.924 ±0.153 (0.663-1.237)
Width-quartiles 25 (cm)	6.200 ±1.054 (4.061-9.923)	5.004 ±0.791 (3.579-6.977)	3.765 ±0.563 (2.111-5.241)	8.739 ±1.279 (5.928-12.225)
Width-quartiles 50 (cm)	8.123 ±1.045 (5.969-10.509)	6.824 ±0.869 (4.759-9.032)	4.562 ±0.671 (2.452-6.303)	9.560 ±1.388 (6.697-13.268)
Width-quartiles 75 (cm)	7.272 ±0.981 (5.361-9.769)	6.168 ±0.806 (4.017-7.528)	4.073 ±0.614 (2.117-5.652)	7.958 ±1.116 (6.060-10.537)
Perimeter (cm)	35.261 ±4.321 (26.015-44.796)	36.866 ±4.563 (26.217-44.192)	35.7 ±5.721 (19.967-48.012)	38.781 ±6.568 (25.911-57.353)
Area (cm <sup>2</sup> )	72.438 ±17.344 (40.922-115.108)	70.180 ±15.787 (34.894-107.921)	52.115 13.785 (15.644-84.336)	84.858 ±30.022 (35.987-164.647)
Internal angles-v1 (°)	68.246 ±5.827 (54.455-81.171)	52.291 ±4.879 (39.476-61.186)	34.726 ±4.510 (25.166-47.409)	83.540 ±9.001 (66.751-99.994)
Internal angles-m1 (°)	111.848 ±5.761 (98.537-126.789)	128.357 ±5.452 (118.615-140.726)	145.473 ±4.704 (133.176-156.135)	95.689 ±9.987 (76.489-115.627)
Internal angles-v2 (°)	68.514 ±5.993 (54.352-82.046)	52.281 ±4.945 (39.320-61.327)	34.717 ±4.530 (25.040-47.437)	84.630 ±10.037 (66.611-105.084)
Internal angles-m2 (°)	111.393 ±6.319 (95.479-125.017)	127.071 ±4.741 (118.872-140.478)	145.085 ±5.071 (131.250-154.648)	96.142 ±9.306 (78.586-111.011)

**Supplementary material 4.** The Eigenvalue and percent of the total variance of the three first principal components (PC); and the relative weight of each variable to the Principal Component Analysis for the Cuban taxa of *Magnolia* subsect. *Talauma*. \* The variable with the highest weight per component.

	Eigenvector		
	PC-1	PC-2	PC-3
Eigenvalue	8.87	2.79	0.18
Variance (%)	73.90	23.28	1.52
<b>Variables</b>			
Length	0.1651	0.5140	-0.0435
Maximum width	-0.3208	0.1607	-0.2513
Calculated Index of Bisse	-0.3200	-0.1605	0.2134
Width-quartile 25	-0.3100	0.1407	0.5557*
Width-quartile 50	-0.3220	0.1549	-0.1740
Width-quartile 75	-0.3145	0.1527	-0.5180
Perimeter	-0.0763	0.5627*	0.4639
Area	-0.2101	0.4570	-0.2318
Internal angles-v1	-0.3245*	-0.1493	0.0386
Internal angles-m1	0.3233	0.1485	-0.0869
Internal angles-v2	-0.3237	-0.1537	0.0622
Internal angles-m2	0.3227	0.1536	-0.0134

**Supplementary material 5.** Number of most probable (highest BIC score) groups (CI) resulting for the morphological data of the taxa of *Magnolia* subsect. *Talauma* in Cuba. Abbreviations: VEV (ellipsoidal, equal shape), EEI (diagonal, equal volume and shape), VVV (ellipsoidal, varying volume, shape, and orientation), EEE (ellipsoidal, equal volume, shape and orientation), BIC (Bayesian information criterion), ICL (integrated complete-data likelihood criterion).

CI	Model	Log-likelihood	BIC	ICL
<b>Linear and angular dataset</b>				
G=2	VEV	1834.648	2735.475	2727.775
G=3	VEV	2368	3362.735	3361.879
G=4	VEV	2617.274	3421.837	3418.993
G=5	VEV	2783.715	3315.276	3314.166
G=6	VEV	2840.613	2989.626	2987.493
G=7	VEV	2951.995	2772.946	2771.089
G=8	VEV	3124.385	2678.279	2676.927
G=9	VEV	3250.297	2490.66	2488.738
<b>EDFs dataset</b>				
G=2	EEI	151 956.5	302 259.6	302 259.6
G=3	EEI	152 188.4	302 168.6	302 168.6
G=4	EEI	152 358.6	301 954.1	301 954.1
G=5	EEI	153 380.4	303 443	303 442.9
G=6	EEI	153 610.6	303 348.6	303 346
G=7	EEI	153 649.5	302 871.7	302 871.3
G=8	EEI	153 812.5	302 642.9	302 642.9
G=9	EEI	153 953.5	302 370	302 370
<b>Matrix of Landmark</b>				
G=2	VVV	44 329.82	83 886.17	83 886.07
G=3	EEE	43 324.66	83 946.72	83 945.98
G=4	EEE	43 375.32	83 888.76	83 882.96
G=5	EEE	43 410.8	83 800.42	83 781.46
G=6	EEE	44 014.69	84 848.89	84, 828.99
G=7	EEE	44 037.63	84 735.48	84 716.24
G=8	EEE	44 101.98	84 704.88	84 688.31
G=9	EEE	44 197.58	84 736.77	84, 715.57

**Supplementary material 6.** Size of the alleles identified in the microsatellite loci used in the characterization of the taxa of *Magnolia* subsect. *Talauma* in Cuba.

Loci	Orbiculata
MA39_120	131, 135
MA39_182	144, 146
MA39_185	223, 225, 227, 229, 231, 233
MA39_191	192, 194, 196, 198, 200, 202, 204, 206, 208, 210
MA39_259	121, 123, 125
MA39_280	160, 162, 164, 166, 172, 180, 184, 188, 198, 200, 204, 210, 218, 220
MA39_342	168, 170, 172, 174, 176, 178, 180, 182, 186, 188
MA39_442	140
MA40_045	255, 259, 261, 265, 267, 269, 271, 275, 277, 285, 287, 289, 301, 311, 323
MA41_120	163, 169, 171, 173, 177, 183, 185, 189, 191, 193, 195, 197, 199, 201, 203, 205, 211, 213
MA41_373	176
MA42_028	127, 129, 131
MA42_072	268, 269, 270
MA42_130	123, 127
MA42_150	144, 146, 148, 150, 152, 154, 156
MA42_231	145, 147, 153, 159, 163
MA42_255	154, 156, 158, 160, 162, 164, 166, 170, 172
MA42_421	307, 347, 349, 351, 353
MA42_441	327
MA42_471	142, 168, 170, 172, 174, 176, 178, 180, 186, 188
MA42_495	101, 103, 107, 109, 111, 115, 117, 119, 123, 125, 129, 131, 133, 135, 139, 145

**Supplementary material 6.** Size of the alleles identified in the microsatellite loci used in the characterization of the taxa of *Magnolia* subsect. *Talauma* in Cuba.

Loci	Minor
MA39_120	115, 119, 123, 125, 131, 135, 137, 141, 143
MA39_182	144, 146, 148, 150
MA39_185	221, 223, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 257
MA39_191	180, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236
MA39_259	119, 121, 123, 125, 127, 131, 133, 137, 185
MA39_280	164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 214, 220, 224, 226, 228, 230, 232, 234
MA39_342	154, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200
MA39_442	130, 140, 142
MA40_045	249, 251, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 295, 297
MA41_120	149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 213, 219, 223, 225
MA41_373	154, 162, 164, 166, 174, 176
MA42_028	125, 127, 129, 131, 133, 135, 137
MA42_072	268, 269, 270
MA42_130	121, 123, 127, 163, 173,
MA42_150	142, 144, 146, 148, 150, 152, 154
MA42_231	145, 147, 153, 155, 157, 165, 171
MA42_255	144, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 180
MA42_421	343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 393, 395, 401
MA42_441	317, 325, 327, 333, 337
MA42_471	154, 156, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190
MA42_495	95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139



**Supplementary material 6.** Size of the alleles identified in the microsatellite loci used in the characterization of the taxa of *Magnolia* subsect. *Talauma* in Cuba.

Loci	Oblongifolia
MA39_120	115, 119, 123, 125, 131, 137, 141
MA39_182	146, 148, 152
MA39_185	221, 229, 233, 235, 237, 239, 241, 243, 245, 247, 249
MA39_191	150, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 224, 228, 232
MA39_259	119, 121, 123, 125, 131, 187,
MA39_280	166, 168, 170, 172, 178, 180, 184, 186, 188, 192, 216, 218, 256
MA39_342	166, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 200
MA39_442	136, 140, 142
MA40_045	249, 255, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287
MA41_120	149, 151, 153, 159, 161, 163, 165, 167, 171, 173, 175, 177, 179, 181, 185, 187, 189, 191, 195, 197, 201, 203
MA41_373	162, 174, 176, 190,
MA42_028	125, 127, 129, 131, 133, 135
MA42_072	268, 269, 270
MA42_130	121, 123, 127
MA42_150	122, 124, 142, 144, 146, 148, 150, 152, 154
MA42_231	145, 147, 165, 167
MA42_255	152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 182
MA42_421	319, 343, 347, 351, 353, 355, 357, 359, 361, 363, 365, 369, 371, 395
MA42_441	325, 327, 335
MA42_471	156, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 186
MA42_495	105, 107, 109, 111, 113, 115, 117, 121, 123, 125, 127, 129, 131, 133, 135, 137, 149

**Supplementary material 6.** Size of the alleles identified in the microsatellite loci used in the characterization of the taxa of *Magnolia* subsect. *Talauma* in Cuba.

Loci	Ophiticola
MA39_120	119, 123, 125, 131, 135, 137, 141
MA39_182	142, 146, 148, 150
MA39_185	223, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 259
MA39_191	156, 160, 176, 180, 182, 186, 188, 190, 192, 194, 196, 198, 200, 201, 202, 204, 205, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226
MA39_259	119, 121, 123, 125, 133, 145, 185, 187
MA39_280	164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 256
MA39_342	158, 160, 164, 166, 168, 170, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 200, 202
MA39_442	136, 140, 142
MA40_045	251, 253, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 293, 299, 301
MA41_120	151, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 211
MA41_373	172, 174, 176, 196, 198
MA42_028	123, 125, 127, 129, 131, 133, 135
MA42_072	268, 269, 270
MA42_130	121, 123, 127
MA42_150	120, 122, 124, 134, 142, 144, 146, 148, 150, 152
MA42_231	145, 147, 149, 151, 153, 163, 165, 167
MA42_255	150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174
MA42_421	307, 309, 311, 313, 315, 319, 321, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 373, 375, 393, 395, 397, 399, 401,
MA42_441	317, 325, 327
MA42_471	156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 188
MA42_495	101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 145, 151

**Supplementary material 7.** Average values of the measures of genetic diversity by loci of *Magnolia* subsect. *Talauma* in Cuba. Np: number of private alleles, Nr: number of rare alleles Na: number of mean alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity.

Loci	Orbiculata					
	Np	Nr	Na	Ne	Ho	He
MA39_120	0	0	2	1.946	0.611	0.486
MA39_182	0	0	2	1.927	0.472	0.481
MA39_185	2	2	6	2.188	0.486	0.543
MA39_191	0	5	10	5.851	0.917	0.829
MA39_259	0	0	3	2.008	0.278	0.502
MA39_280	4	7	14	8.229	0.750	0.878
MA39_342	0	6	10	3.735	0.889	0.732
MA39_442	0	0	1	1.000	0.000	0.000
MA40_045	2	7	15	9.324	0.833	0.893
MA41_120	0	14	18	6.715	0.972	0.851
MA41_373	0	0	1	1.000	0.000	0.000
MA42_028	0	1	3	1.285	0.250	0.222
MA42_072	0	0	3	2.976	0.194	0.664
MA42_130	0	0	2	1.994	0.944	0.498
MA42_150	1	2	7	4.194	0.972	0.762
MA42_231	1	3	5	1.876	0.314	0.467
MA42_255	0	4	9	4.431	0.972	0.774
MA42_421	0	2	5	2.589	0.667	0.614
MA42_441	0	0	1	1.000	0.000	0.000
MA42_471	1	3	10	5.143	0.833	0.806
MA42_495	0	11	16	6.562	0.917	0.848

**Supplementary material 7.** Average values of the measures of genetic diversity by loci of *Magnolia* subsect. *Talauma* in Cuba. Np: number of private alleles, Nr: number of rare alleles Na: number of mean alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity.

Loci	Minor					
	Np	Nr	Na	Ne	Ho	He
MA39_120	1	6	9	1.700	0.404	0.412
MA39_182	0	2	4	1.207	0.170	0.172
MA39_185	1	8	14	6.034	0.802	0.834
MA39_191	3	21	27	14.366	0.876	0.930
MA39_259	2	6	9	2.515	0.573	0.602
MA39_280	10	24	28	3.773	0.408	0.735
MA39_342	3	12	19	8.569	0.857	0.883
MA39_442	1	1	3	1.285	0.198	0.222
MA40_045	3	15	22	9.983	0.890	0.900
MA41_120	5	27	34	19.264	0.881	0.948
MA41_373	3	4	6	1.193	0.171	0.162
MA42_028	1	4	7	3.134	0.619	0.681
MA42_072	0	1	3	1.595	0.137	0.373
MA42_130	2	3	5	1.243	0.106	0.196
MA42_150	0	2	7	2.933	0.664	0.659
MA42_231	3	6	7	1.028	0.028	0.027
MA42_255	3	7	16	8.950	0.862	0.888
MA42_421	0	10	20	11.996	0.849	0.917
MA42_441	2	4	5	1.083	0.042	0.077
MA42_471	2	11	18	5.457	0.440	0.817
MA42_495	3	14	23	13.067	0.899	0.923

**Supplementary material 7.** Average values of the measures of genetic diversity by loci of *Magnolia* subsect. *Talauma* in Cuba. Np: number of private alleles, Nr: number of rare alleles Na: number of mean alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity.

Loci	Oblongifolia					
	Np	Nr	Na	Ne	Ho	He
MA39_120	0	3	7	2.552	0.686	0.608
MA39_182	1	2	4	1.597	0.400	0.374
MA39_185	0	6	11	5.947	0.829	0.832
MA39_191	1	17	22	12.895	0.971	0.922
MA39_259	0	2	6	2.549	0.629	0.608
MA39_280	1	10	13	3.436	0.371	0.709
MA39_342	0	7	14	6.964	0.765	0.856
MA39_442	0	1	3	1.772	0.429	0.436
MA40_045	0	8	16	9.041	0.829	0.889
MA41_120	0	15	22	14.000	0.914	0.929
MA41_373	1	2	4	1.228	0.143	0.186
MA42_028	0	2	6	3.793	0.657	0.736
MA42_072	0	1	3	1.782	0.303	0.439
MA42_130	0	1	3	1.270	0.176	0.213
MA42_150	0	5	9	3.810	0.800	0.738
MA42_231	0	3	4	1.091	0.057	0.083
MA42_255	1	2	11	8.688	0.857	0.885
MA42_421	0	6	14	9.245	0.886	0.892
MA42_441	1	2	3	1.090	0.029	0.083
MA42_471	0	4	14	9.879	0.571	0.899
MA42_495	1	9	17	11.187	0.857	0.911

**Supplementary material 7.** Average values of the measures of genetic diversity by loci of *Magnolia* subsect. *Talauma* in Cuba. Np: number of private alleles, Nr: number of rare alleles Na: number of mean alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity.

Loci	Ophiticola					
	Np	Nr	Na	Ne	Ho	He
MA39_120	0	4	7	2.306	0.384	0.566
MA39_182	0	2	4	2.080	0.407	0.519
MA39_185	2	6	12	6.257	0.808	0.840
MA39_191	6	20	28	12.343	0.928	0.919
MA39_259	1	5	8	1.658	0.390	0.397
MA39_280	0	13	16	3.159	0.473	0.683
MA39_342	4	12	19	7.865	0.812	0.873
MA39_442	0	1	3	2.093	0.413	0.522
MA40_045	3	11	21	11.957	0.882	0.916
MA41_120	0	20	26	13.481	0.895	0.926
MA41_373	3	3	5	1.858	0.380	0.462
MA42_028	1	2	7	3.785	0.512	0.736
MA42_072	0	1	3	1.810	0.287	0.448
MA42_130	0	1	3	1.611	0.470	0.379
MA42_150	2	6	10	2.986	0.622	0.665
MA42_231	2	6	8	1.273	0.058	0.214
MA42_255	0	7	13	4.825	0.686	0.793
MA42_421	7	19	28	13.281	0.843	0.925
MA42_441	0	2	3	1.048	0.047	0.046
MA42_471	1	8	16	10.139	0.801	0.901
MA42_495	2	14	23	12.999	0.953	0.923

**Supplementary material 8.** Results of the six models included in the BioGeoBEARS analysis of *Magnolia* subsect. *Talauma* in Cuba.

Model	LnL	numparams	d	e	j	AICc	AICc_wt
DIVALIKE+J	-20.65	3	1.00E-12	1.00E-12	0.1	49.48	0.73
BAYAREALIKE+J	-21.78	3	1.00E-07	1.00E-07	0.099	51.74	0.23
DIVALIKE	-25.21	2	0.016	0.0078	0	55.43	0.037
DEC	-29.44	2	0.024	0.033	0	63.87	0.0005
DEC+J	-29.44	2	0.024	0.033	0	63.87	0.0005
BAYAREALIKE	-33.63	2	0.039	0.08	0	72.26	8.20E-06



**Supplementary material 9.** List of revised herbarium specimens of the Cuban species of *Magnolia*. subsect. *Talauma*. HAC: Instituto de Ecología y Sistemática, HAJB: Jardín Botánico Nacional, Universidad de La Habana, HFC: Serie Herbario de la Flora de Cuba, JE: Friedrich Schiller University Jena, LS: Colegio de La Salle (en HAC), NSC: Colegio Ntra. Sra. de la Caridad, Santiago de Cuba (en HAC). NY: The New York Botanical Garden, S: Swedish Museum of Natural History, SV: Antigua Estación Experimental Agronómica (en HAC y HAJB).

***Magnolia orbiculata* (Britton & P. Wilson) Palmarola**

**Cuba: Granma: Bartolomé Masó:** Firme de la Sierra Maestra entre Lagunitas y Aguada de Joaquín, 19.IV.1979, *Bisse J. et al. HFC 40417* (HAJB); Campamento Aguada de Joaquín (PN Pico Turquino), 10.XII.2014, *Palmarola A. & González-Torres L.R. HFC 89393* (HAJB); Campamento Aguada de Joaquín (PN Pico Turquino), 10.XII.2014, *Palmarola A. & González-Torres L.R. HFC 89394* (HAJB); Campamento Aguada de Joaquín (PN Pico Turquino), 10.XII.2014, *Palmarola A. & González-Torres L.R. HFC 89395* (HAJB); Campamento Aguada de Joaquín (PN Pico Turquino), 10.XII.2014, *Palmarola A. & González-Torres L.R. HFC 89396* (HAJB); Loma Cala, 21.V.1948, *J. Acuña 15177* (HAC ex SV); Loma de Quintín-Nagua, 5.VII.1922, *E.L. Ekman 14178* (S); Subida al pico Caracas desde la Estación Biológica, 19.II.2016, *Palmarola A. et al. HFC 89194* (HAJB); **Buey Arriba:** El Maguito, 30.II.2016, *Molina Y. HFC 89590* (HAJB); Subida al Pico Caracas desde la Estación biológica 19-20.II.2016 *Palmarola A. et al. HFC 89195* (HAJB); Yao Arriba, 14.III.1952, *Smith 384* (HAC ex SV, HAC ex LS). **Santiago de Cuba: Guamá:** Entre Loma del Sabicú y Palma Mocha-Región del Turquino, I.1948, *Ambrosio 23367* (HAC ex LS); Pluvilsilva Alto de la Francia, 05.II.1972, *Bisse J. HFC 21369* (HAJB, JE); Sierra Maestra: Alto de la Siberia, falda norte, IV.1969, *Bisse J. HFC 13589* (HAJB); Sierra Maestra: El uvero, monte semicaducifolio cerca de la Francia entre 300-600 m, 13.IV.1969, *Bisse J. & Lippold H. HFC 14289* (JE); Sierra Maestra: El uvero, monte semicaducifolio cerca de la Francia entre 300-600 m, 13.IV.1969, *Bisse J. & Lippold H. HFC 14294* (JE); Sierra Maestra: Ocuja, camino de la Cantimplora, monte seco entre 100-600 m, 24.V.1971, *Bisse J. & Lippold H. HFC 19473* (JE); Santiago de Cuba: Cerca de Loma del Gato-Maestra-Oriente, VIII.1944, *Hnos. Alain & Crisógono LS 255* (LS); Santiago de Cuba: Loma del Gato-Victoria, XII.1947, *Hnos. Crisógono & Néstor NSC 5794* (HAC ex NSC); Loma San Juan-Gato (Oriente), XII.1947, *Hno. León 23366* (HAC ex SV, HAC ex LS).

***Magnolia minor* (Urb.) Govaerts**

**Cuba: Guantánamo: Baracoa:** Cuchillas del Toa, Cayo Fortuna, en lomas de roca caliza 2.IV.1972, *Bisse J. & Berazaín R. HFC 22312* (HAJB, JE); Cuchillas del Toa, pluvilsilva en las orillas del río Toa, cerca de Cayo Fortuna 26.III.1972, *Bisse J. et al. HFC 22580* (HAJB, JE); Cuchillas del Toa; orillas del arroyo Cayo Fortuna 22.III.1972, *Bisse J. et al. HFC 22812* (HAJB, JE); Falda suroeste Yunque de Baracoa II.1968 *Bisse J. HFC 5321* (HAJB, JE); In the foothills 11.I.1915,

*E.L. Ekman 4198* (S); Las Cuevas de Cayo Guin, orillas del arroyo suelo calizo 12.IV.1985, *Álvarez A. et al. HFC 55740* (HAJB, JE); Las Delicias del Duaba 11.VIII.2017, *Díaz J. et al. HFC 89435* (HAJB); Palenque: Cuchillas del Toa; Cayo Fortuna X.1970, *Bisse J. HFC 16890* (HAJB, JE); Río Báez, en el camarones, a 4 km de la carretera de Moa-Baracoa 22.VII.2017, *Bécquer E. et al. HFC 89579* (HAJB); Río Báez, en el puente de hierro a 1 km de la carretera Moa-Baracoa 17.VII.2017, *Bécquer E.R. et al. HFC 89472* (HAJB); Río Báez, en el puente de hierro a 1 km de la carretera Moa-Baracoa 17.VII.2017, *Bécquer E.R. et al. HFC 89473* (HAJB); Sendero El Recreo, a 1 km de Taco Bay en dirección a Moa 17.VII.2017, *Bécquer E.R. et al. HFC 89467* (HAJB); Sierra de Frijol, cayo Fortuna, resto de pluviosilva, en la orilla del río Toa 11.V.1983, *Bisse J. et al. HFC 49206* (HAJB); Sierra de Nipe, in charrascales at Río Piloto 14.III.1915, *E.L. Ekman 5023* (S); **Imías:** Comunidad Yumurí del Sur, a 4 km desde el mirador de Alto de Cotilla en dirección a Baracoa 18.VII.2017, *Bécquer E.R. et al. HFC 89510* (HAJB); Comunidad Yumurí del Sur, a 4 km desde el mirador de Alto de Cotilla en dirección a Baracoa 18.VII.2017, *Bécquer E.R. et al. HFC 89511* (HAJB); Cotilla: orillas del arroyo Cotilla matorral xeromorfo 29.IV.1986, *Genes E. et al. HFC 59405* (HAJB); En un valle al sureste del Alto de Cotilla 29.V.1982, *Bisse J. et al. HFC 47236* (HAJB); Palenque. Bernardo, Sierra de Frijol, loma Bernardo 21.V.1983, *Arias I. et al. HFC 49946* (HAJB, JE); Sierra de Imías Loma Jubal 19.VII.1975, *Álvarez A. et al. HFC 27534* (HAJB, JE); Sierra de Imías. Camino entre Tres Piedras y La María 8.IV.1984, *Bisse J. et al. HFC 52496* (HAJB, JE); Sierra de Imías: cabezadas del río Jojo 15.IV.1984, *Bisse J. et al. HFC 53005* (HAJB, JE); **Yateras:** Altiplano de Monte Cristi, sobre suelo calizo V.1968, *Bisse J. HFC 9291* (HAJB, JE); Felicidad de Yateras, pinar en la zona de Monte Cristi sobre caliza 23.VIII.1971, *Bisse J. HFC 20215* (HAJB, JE); Felicidad de Yateras: Monte Cristi 12.V.1983, *Arias I. et al. HFC 49827* (HAJB, JE); Sierra de Frijol, en el camino de Riíto a Cayo Fortuna, pluviosilva de montaña destruida, suelo ultrabásico, 700 m 12.V.1983, *Arias I. et al. HFC 49320* (JE). **Holguín: Mayarí:** Cayo Mujeres 11.V.2016, *Palmarola A. et al. HFC 89213* (HAJB); Pinares de Mayarí, río Piloto en su curso medio 1.VI.1983, *Arias I. et al. HFC 50616* (HAJB, JE); Río Piloto, curso medio cerca de la Poza de Rafael 22.III.2018, *Gómez J.L. et al. HFC 89901* (HAJB); Rio Seboruco to Falls of Rio Mayarí, Oriente 23.I.1912, *J.A. Shafer 3661* (NY); Sierra de Nipe, en charrascales 25.VII.1914, *E.L. Ekman 2189* (S); Sierra de Nipe: chemin de la mine Woodfred, terrain sec et ouvert 14-18.III.1944, *Hnos. Marie-Victorin & Clément 22087* (US); **Moa:** Arroyo Bueno, Alto de la Melba 11.VIII.2017, *Palmarola A. et al. HFC 89584* (HAJB); arroyo Bueno, Alto de la Melba 11.VIII.2017, *Palmarola A. et al. HFC 89585* (HAJB); camino desde Moa hacia La Melba I.1969, *Bisse J. HFC 12473* (HAJB); Cayo Guam: entre el campismo y la poza del Ché 1.VII.2016, *Palmarola A. et al. HFC 89243* (HAJB); Cayo Guam: entre el campismo y la poza del Ché 1.VII.2016, *Palmarola A. et al. HFC 89244* (HAJB); km 10 camino a La Melba 30.XII.1968, *Bisse J. HFC 12289-A* (HAJB); km 10 de la carretera de "La Melba" pluviosilva 19.IV.1981, *Bisse J. et al. HFC 44507* (HAJB); Los Farallones, sur de la Sierra de Moa 31.IV.1972, *Bisse J. &*

*Berzaín R. HFC 22238* (HAJB, JE); Río Yamanigüey, cerca de la toma de agua, en la carretera Moa-Baracoa 16.VII.2017, *Bécquer E.R. et al. HFC 89449* (HAJB); Río Yamanigüey, cerca de la toma de agua, en la carretera Moa-Baracoa 16.VII.2017, *Bécquer E.R. et al. HFC 89450* (HAJB); Río Yamanigüey, cerca de la toma de agua, en la carretera Moa-Baracoa 16.VII.2017, *Bécquer E.R. et al. HFC 89452* (HAJB); Yamanigüey 15.V.2016, *Palmarola A. et al. HFC 89230* (HAJB); **Sagua de Tánamo:** Cupeyal del Norte, Camino a Castro 26.VII.2016, *Falcón B. et al. HFC 88870* (HAJB); Cupeyal del Norte, Hoyo de Mola 29.VII.2016, *Falcón B. et al. HFC 88955* (HAJB); Cupeyal del Norte, Hoyo de Mola 29.VII.2016, *Falcón B. et al. HFC 88957* (HAJB); Cupeyal del Norte, Hoyo de Mola 29.VII.2016, *Falcón B. et al. HFC 88958* (HAJB); Cupeyal del Norte. Hoyo de Mola 29.VII.2016, *Falcón B. et al. HAC 88953* (HAJB). **Santiago de Cuba: Segundo Frente:** Sierra Cristal, Cabezadas Río San Miguel, restos de pluviosilva 23.II.1976, *Areces A. et al. HFC 30554* (HAJB, JE); Sierra Cristal, Orillas Río Levisa IV.1968, *Bisse J. HFC 7248* (HAJB).

***Magnolia oblongifolia* (León) Palmarola**

**Cuba: Guantánamo: Baracoa:** A 1 km del margen occidental del Toa, entre Tabajó y Bernardo, bosque pluvial montano, 18.IV.1986, *Arías I. et al. HFC 58971* (HAJB); Camino al sur de las Delicas después de la toma de agua del río del Duaba, 21.VII.2017, *Bécquer E.R et al. HFC 89538* (HAJB); Camino al sur de las Delicas después de la toma de agua del río del Duaba, 21.VII.2017, *Bécquer E.R et al. HFC 89556* (HAJB); Camino entre arroyo Claro y Vega de la Palma, alrededores del río Arroyo Blanco, 19.II.1979, *Bisse J. et al. HFC 39615* (HAJB, JE); Cuchillas del Toa, Sierra del Maguey, pluvisilva y charrascales, 24.III.1972, *Bisse J. et al. HFC 22687* (HAJB, JE); Loma Los Guineos, bosque pluvial montano, 14.IV.1986, *Arías I. et al. HFC 58693* (HAJB); Mirador del Iberia, 5.VII.2016, *Palmarola A. et al. HFC 89261* (HAJB); Mirador del Iberia 5.VII.2016, *Palmarola A. et al. HFC 89262* (HAJB); Mirador del Iberia, 5.VII.2016, *Palmarola A. et al. HFC 89263* (HAJB); Orillas del Río Báez, cerca de los Naranjos, 21.I.1977, *Bisse J. et al. HFC 33839* (HAJB, JE); Palenque: Cuchillas del Toa, Cayo Fortuna pluvisilva cerca del arroyo Manajú, 30.III.1972, *Bisse J. & Berzaín R. HFC 22701* (HAJB, JE); Pluvisilva al sur del Yunque de Baracoa, camino desde el charrascal de la Ermita, 19.VII.2017, *Bécquer E.R et al. HFC 89528* (HAJB); Pluvisilva al sur del Yunque de Baracoa, camino desde el charrascal de la Ermita, 19.VII.2017, *Bécquer E.R et al. HFC 89529* (HAJB); Valle al noreste del Yunque de Baracoa, II.1968, *Bisse J. & Köhler E. HFC 5169* (HAJB); Valle del río Duaba “La Ermita”, 2.III.2012, *Greuter W. et al. WG 27639* (HAJB); Vega de la Palma, alrededores del río Duaba, 27.II.1979, *Bisse J. et al. HFC 40068* (HAJB, JE); **Imías:** Falda oeste de la loma de Majagua Hueca, 16.IV.1984, *Meyer F.K. et al. HFC 53146* (HAJB); Monte Oscuro, 3.V.1998, *Arías I. et al. HFC 76464* (HAJB); Palenque, Cuchillas del Toa, charrascos de la Loma de Yarey, 26.III.1972, *Bisse J. et al. HFC 22169* (HAJB); Sierra de Imías Palmarito de Yamagua, 17.IV.1984, *Bisse J. et al. HFC 53300* (HAJB, JE). **Holguín: Frank País:** Falda norte de la Sierra Cristal subida a

Palenque en la zona de Brazo Grande, 7.IV.1987, *Bassler M. et al. HFC 60533* (HAJB); Falda norte de la Sierra Cristal, al sureste de El Culebro, en la subida al Alto del Cuncuní, 10.IV.1987, *Bassler M. et al. HFC 60851* (HAJB); Falda norte de la Sierra Cristal, al sureste de El Culebro, subida al Pico el Cielo, 13.IV.1987, *Bassler M. et al. HFC 61180* (HAJB); **Mayarí:** Meseta el Toldo, al oeste del primer campamento de los americanos en los alrededores de las cabezadas del río Piloto, 28.VII.2015, *Falcón B. et al. HFC 88424* (HAJB); **Moa:** Alto de La Melba, 10.VIII.2017, *Palmarola A. et al. HFC 89586* (HAJB); Alto de La Melba, 10.VIII.2017, *Palmarola A. et al. HFC 89587* (HAJB); Alto de La Melba, 10.VIII.2017, *Palmarola A. et al. HFC 89588* (HAJB); Alto de La Melba, 10.VIII.2017, *Palmarola A. et al. HFC 89589* (HAJB); Camino de Mina Iberia a La Melba, 30.XII.1968, *Bisse J. & Lippold H. HFC 16527* (HAJB, JE); Camino entre las Comadres y La Melba, 12.VIII.2017, *Palmarola A. et al. HFC 89582* (HAJB); Camino entre las Comadres y La Melba, 12.VIII.2017, *Palmarola A. et al. HFC 89583* (HAJB); Cayo Guam, 15.II.2016, *Palmarola A. et al. HFC 89228* (HAJB); Cayo Guam, margen izquierdo del río a 2 km del campismo en dirección al campamento de pioneros, 16.VII.2017, *Bécquer E.R & Testé E. HFC 89438* (HAJB); Cayo Guam, margen izquierdo del río a 2 km del campismo en dirección al campamento de pioneros, 16.VII.2017, *Bécquer E.R & Testé E. HFC 89439* (HAJB); Cayo Guam, margen izquierdo del río a 2 km del campismo en dirección al campamento de pioneros, 16.VII.2017, *Bécquer E.R & Testé E. HFC 89440* (HAJB); Cayo Guam: detrás del campamento de los pioneros, 27.VI.2016, *Palmarola A. et al. HFC 89239-48*; Cayo Guam: entre el campismo y la poza del Ché, 27.VI.2016, *Palmarola A. et al. HFC 89250* (HAJB); En el sendero de El Alto de La Melba, 19.VI.2017, *Falcón B. et al. HFC 89377* (HAJB); En las orillas del río Jiguaní, cerca del segundo aserrío de la Melba, IV.1968, *Bisse J. & Köhler E. HFC 6715* (JE); En las orillas del río Jiguaní, cerca del segundo aserrío de la Melba, IV.1968, *Bisse J. & Köhler E. HFC 6802* (HAJB, JE); Falda norte del Pico El Toldo, 21.I.1988, *Berazaín R. et al. HFC 63401* (HAJB); La Melba: falda oeste de la Sierra de Moa, 800-1000 m alto, pluviosilva, 23.XII.1968, *Bisse J. & Lippold H. HFC 11612* (JE); Mina Delta, VII.1949, *Hnos. León & Clemente 910* (US); Monte de la Breña, 300-500 m alto, pluviosilva, III.1968, *Bisse J. & Köhler E. HFC 6390* (JE); Monte La Breña, alrededores del campamento "Los Carboneros", camino al nacimiento del río Yagrumaje, 17.IV.1981, *Bisse J. et al. HFC 44378* (HAJB, JE); Orillas del río Yagrumaje, cerca del puente en el camino a Moa, II.1968, *Bisse J. & Köhler E. HFC 6002* (HAJB, JE); Playa Moa, 15.IV.1945, *J.L. Acuña 12417* (US); Pluviosilva 8-10 km camino de la Melba, 1.V.1980, *Álvarez A. et al. HFC 42531* (HAJB); Sierra de Moa, al oeste del Toldo 800-900 m, monte nublado, 14.VIII.1970, *Bisse J. & Lippold H. HFC 17897* (JE); **Sagua de Tánamo:** Cupeyal del Norte, Hoyo de Mola, 29.VII.2016, *Falcón B. et al. HFC 88947* (HAJB); Cupeyal del Norte, Hoyo de Mola, 29.VII.2016, *Falcón B. et al. HFC 88948* (HAJB); Cupeyal del Norte, Hoyo de Mola, 29.VII.2016, *Falcón B. et al. HFC 88950* (HAJB); Cupeyal del Norte, Hoyo de Mola, 29.VII.2016, *Falcón B. et al. HFC 88952* (HAJB); Cupeyal del Norte, Hoyo de Mola, 29.VII.2016, *Falcón B. et al. HFC 88954* (HAJB). **Santiago de Cuba: Segundo Frente:** Sierra Cristal, Cabezadas Río San Miguel, IV.1968, *Bisse J. HFC 8367* (HAJB, JE).



A close-up photograph of a branch of Magnolia minor. The branch is covered with large, glossy, green leaves that have prominent veins. A small, light-colored flower bud is visible in the center of the branch, partially obscured by the leaves. The background is a soft, out-of-focus green, suggesting a dense foliage environment.

**Jardín Botánico Nacional**

Universidad de La Habana, Cuba

**Institut Diversité Écologie et Évolution du Vivant**

Université Paris-Saclay, France

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