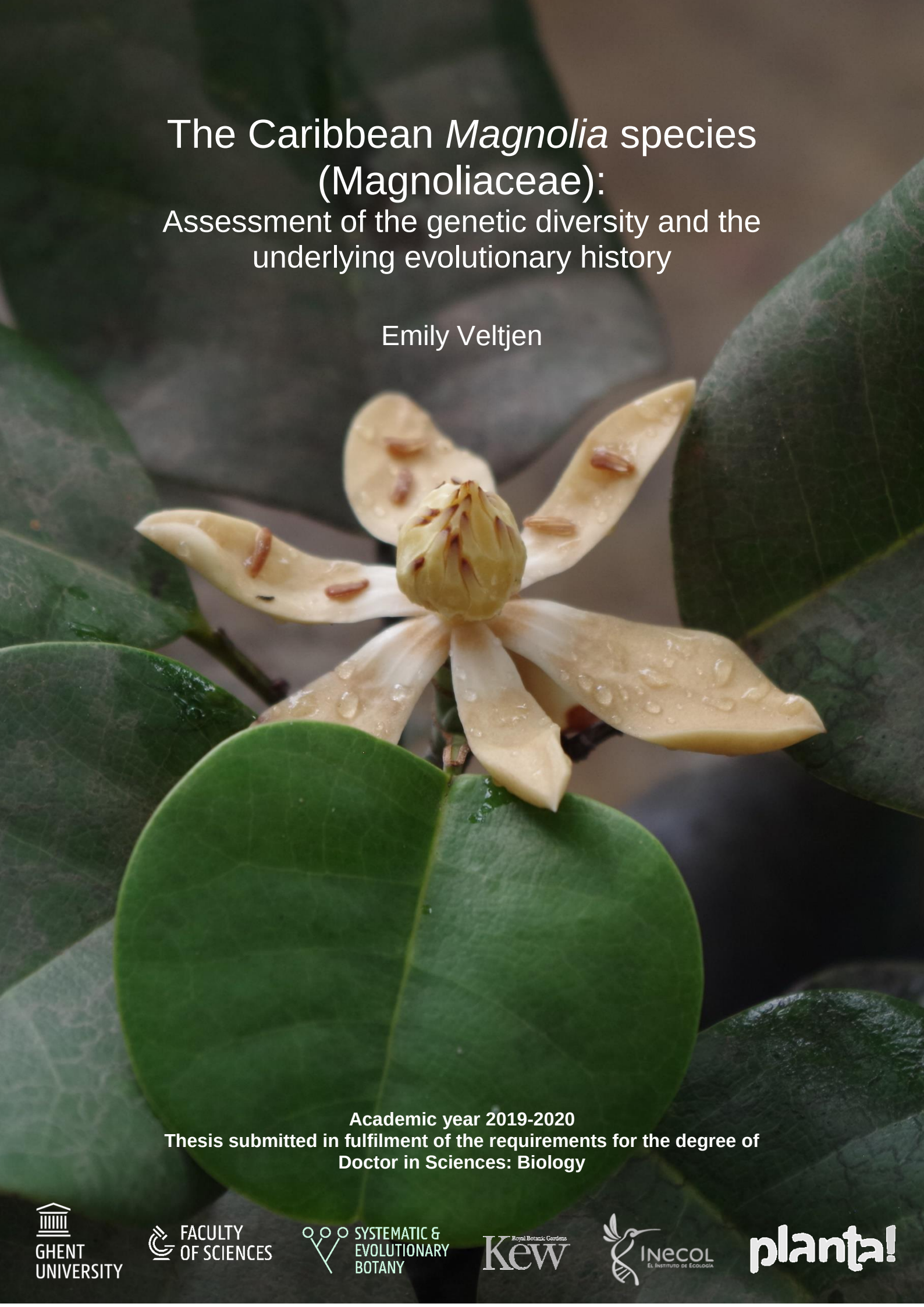
The Caribbean *Magnolia* species (Magnoliaceae):
Assessment of the genetic diversity and the underlying evolutionary history

Emily Veltjen



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Acknowledgements

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Summary

Magnolias are commonly associated with Asia due to the popularity of precocious[1] flowering hardy species found in gardens all over the northern hemisphere. However, a significant proportion of the diversity of the genus *Magnolia*[2] is found in the Americas, of which most are evergreen trees residing in the Neotropics. Conserving this biodiversity is important, not only given the general mission to conserve Earth's biodiversity in its totality, but also because the Magnoliaceae contribute to human welfare by providing a range of services, such as cultural services as garden ornamentals, regulating services by shaping biodiverse and unique habitats like primary forests, and (potential) provisioning services as a source of timber, medicines, and ingredients for fragrances. Even more so, *Magnolia* trees act as a flagship and umbrella species, whereby a focus on and conservation of their biodiversity brings forward awareness and conservation for the ecosystem they co-constitute. Unfortunately, 48% of the 300 currently assessed *Magnolia* species are listed as threatened on the IUCN[3] Red List, and 31% are Data Deficient (DD). An area contributing to this high number of threatened Magnolias is the Caribbean, a secondary biodiversity hotspot for the Magnoliaceae family. The threatened status of all 15 Caribbean *Magnolia* taxa is not surprising, given that each species is endemic to a specific Caribbean island, or island arc in the case of *M. dodecapetala*, resulting in a small area of occupancy and extent of occurrence. Furthermore, the Caribbean islands have a high degree of natural (e.g. hurricanes) and human (e.g. land conversion) disturbance.

We conducted extensive fieldwork during this PhD project and sampled all 15 Caribbean *Magnolia* taxa, except *M. emarginata* endemic to northern Haiti. The 14 located taxa were sampled at population level; however, *M. domingensis* endemic to Hispaniola could only be collected from the Dominican Republic. For these two species, none of the Haitian historical localities could be located and hence the presence of *Magnolia* populations in the north and centre of Haiti remains unverified since 1925.

Using molecular data, we zoomed in on the genetic diversity of the 15 threatened Caribbean Magnolias using two main scientific disciplines: biogeography and conservation genetics. To ensure proper usage and data interpretation we addressed the ploidy of the Caribbean Magnolias based on chromosome counts and flow cytometry. All studied Caribbean taxa were confirmed to be diploid, which puts forward allopatric, rather than sympatric *Magnolia* speciation in this biogeographic region.

[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1.
[2] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.
[3] Abbreviations are explained in Appendix 1.2.

The current Caribbean islands are isolated land masses that have a complex geological and environmental history, while maintaining a historical and current geographical proximity to the American mainland – an interesting setting for a biogeographic study. Sanger sequencing data comprising eleven DNA regions (i.e. five nuclear genes, three chloroplast genes and three chloroplast intergenetic spacers) of 62 Magnoliaceae taxa were used to prepare a calibrated Bayesian phylogenetic species hypothesis and execute ancestral range estimations. The data delivered evidence for four colonisation events of *Magnolia* into the Caribbean, and the Caribbean Magnolias form clades of within-island relatives. The latter with one exception: *M. ekmanii* from Haiti was more related to the Cuban instead of the Dominican taxa of subsection *Cubenses*. The *Cubenses* species in the Greater Antilles followed a south to north colonisation trajectory, while the trajectory of *M. dodecapetala* in the Lesser Antilles followed the concept of the island progression rule. The colonisation of *Magnolia* was estimated to have occurred maximum 16 million years ago which provided support for overwater dispersal as the most plausible dispersal hypothesis for their presence in the Caribbean islands, while excluding the vicariance and the GAARlandia hypotheses. This with the caveat that the low sequence divergence appears to hamper a convincing translation of molecular data to divergence time estimates.

The inclusion of additional nuclear markers and the usage of coalescent theory species tree building methods did not deliver a more conclusive family-level phylogenetic hypothesis, neither more insight into Magnoliaceae classification. Our results contradict the currently accepted Magnoliaceae classification by strongly contrasting placement of the major clades within the family depending on the genetic marker; even for clades previously defined with high support. One important example is section *Talauma*, which includes all Caribbean *Magnolia* species, except *M. virginiana* subsp. *oviedoae*. The section clade is ill-supported, as subsections *Cubenses, Dugandiodendron* and *Talauma* are not retrieved as sister clades in many of the gene trees. The data also put forward that the subsection *Cubenses* is nested in subsection *Dugandiodendron*. Future studies will benefit from using phylogenomic data and a broad taxon sampling to elucidate the continued problem of low support for the relationships between the main clades of the Magnoliaceae.

Caribbean *Magnolia* taxon delimitations were mainly tested by means of phylogenetic hypotheses complemented with a haplotype network analysis. Genetic synapomorphies of the eleven studied markers confirmed the delimitation of 14 out of 15 Caribbean Magnolias with the exception of the *M. minor* / *M. oblongifolia* species complex from Cuba, which urgently needs further investigation given the high genetic variation that does not match the morphological concepts of the species. Genetic distance between the two subspecies of *M. cubensis* were similar to the genetic distance found between pairs of other Caribbean *Magnolia*

species, hence, a taxonomic revision of the *M. cubensis* subspecies is advised. The intraspecific genetic variation of *M. dodecapetala* was notably higher than the other Caribbean species, as well did the fruits show high morphological variation categorizable per island. The different island populations were labelled as worthy candidates for evolutionarily significant units (ESU) and we advised no taxonomic changes as yet.

A first conservation-genetic study comprising eight of the fourteen Caribbean species and one Mexican *Magnolia*, each represented by two predefined populations with the exception of *M. splendens* for which only one predefined population is known, genotyped with SSR (Single Sequence Repeat) markers, retrieved 16 out of the 17 populations as separate entities. This suggests that the *Magnolia* populations do not experience extensive gene flow. Even more so, two out of the 17 predefined populations were genetically subdivided even more than anticipated, showing that dynamics of *Magnolia* species can occur at a fine spatial scale (i.e. 3–5 km). The SSR studies found little evidence of inbreeding (exceptions: *M. dodecapetala* and *M. portoricensis*), indicating ample gene flow within populations and mechanisms favouring cross-pollination. We state that the reproductive biology of the studied Magnolias appears resilient yet limited in their animal-mediated dispersal. Other patterns that were found indicate recent bottlenecks (*M. domingensis*), unexpectedly high genetic diversity (*M. hamorii*) and low genetic diversity (*M. ekmanii* and *M. domingensis*). In general, the genetic diversity showed better patterns than expected for threatened endemic species. This puts forward that maintenance or an increase of forest connectivity would be the most effective conservation management for the studied *Magnolia* populations.

Two detailed population level case studies were conducted, re-addressing their conservation genetics with a more elaborate sampling. The first study executed on two populations of *M. cubensis* subsp. *acunae* delivered evidence of the influence of fragmentation to pollen dispersal and showed unexpectedly little genetic differentiation between populations, which is most likely a result of their shared evolutionary history. The results of the study rendered a new conservation management strategy of reinforcement and reintroductions. The second study executed on five island populations of *M. dodecapetala* delivered overall evidence of inbreeding, which emphasized that even though the species is denoted as VU by the IUCN Red List, the highly structured island system has severe consequences to the species' genetic diversity. The data highlight the species for further investigation and management.

The overall results, discussion and recommendations in this PhD study illustrate how scientific research can contribute to on-the-ground conservation, while also addressing bigger questions concerning evolution and biogeography. A strong attribute of this study is the translation of research into applied conservation via translational science.

Samenvatting

Magnolia's worden geassocieerd met Azië vanwege hun populariteit in het noordelijk halfrond als vroegbloeiende, winterharde tuinplanten. Een aanzienlijk deel van de *Magnolia*-diversiteit bevindt zich echter op het Amerikaanse continent, waarvan het merendeel altijdgroene Neotropische magnolia's zijn. Conservatie van de *Magnolia* soortendiversiteit, samen met alle biodiversiteit op Aarde, wordt nagestreefd, des te meer omdat deze groep van (voornamelijk) bomen bijdraagt aan het welzijn van de mens door een scala aan diensten, zoals culturele diensten als sierplanten, regulerende diensten door het vormen van unieke habitats zoals primaire bossen, en (potentiële) bevoorradingsdiensten als een bron van hout, medicijnen en ingrediënten voor geuren. Daarbovenop treden de magnolia's ook op als "flagship" en "umbrella" soorten, waarbij ze aandacht brengen naar, en eveneens ook zorgen voor, conservatie van het habitat waarvan ze een onderdeel zijn. Helaas zijn 48% van de ca. 300 magnolia's bedreigd in hun voortbestaan volgens de Rode Lijst van IUCN en is 31% Data Deficient (DD). Een gebied dat bijdraagt aan dit hoge aantal bedreigde magnolia's is de Caraïben, een secundaire "hotspot" voor de Magnoliaceae. De bedreigde status van alle 15 Caraïbische *Magnolia* taxa is niet verwonderlijk aangezien elke soort endemisch is voor één eiland, of in het geval van *M. dodecapetala*: één eilandenboog. Dit resulteert in een klein "gebied van bezetting" (area of occupancy) en "mate van voorkomen" (extent of occurrence). Behalve hun klein areaal, ondervinden de Caraïbische magnolia's ook een hoge mate van natuurlijke en menselijke verstoring door orkanen en land conversie in functie van landbouw.

Tijdens dit doctoraatsproject werden er verscheidene expedities ondernomen en alle 15 Caraïbische *Magnolia* taxa werden bemonsterd, met uitzondering van *M. emarginata* uit het noorden van Haïti. De 14 overige taxa werden op populatieniveau bemonsterd, waarbij *M. domingensis* enkel in de Dominicaanse Republiek. Geen van de historische *Magnolia* locaties in het noorden en het centrum van Haïti werden dus teruggevonden en bijgevolg blijft de aanwezigheid van *Magnolia* hier sinds 1925 ongeverifieerd.

Met behulp van moleculaire data bestudeerden we de 15 bedreigde Caraïbische magnolia's in twee wetenschappelijke disciplines: biogeografie en conservatie-genetica. De ploïdie van de Caraïbische magnolia's werd allereerst bepaald op basis van chromosoomtellingen en flowcytometrie met als doel het correct gebruik en interpretatie van deze moleculaire data. Alle bestudeerde Caraïbische taxa blijken diploïd. Dit ondersteunt de hypothese van allopatrische, eerder dan sympatrische, speciatie van de Caraïbische magnolia's.

De huidige Caraïbische eilanden zijn geïsoleerde landmassa's gekenmerkt door een complexe geologische en ecologische geschiedenis, met een historische en huidige geografische nabijheid tot het Amerikaanse vasteland: een interessante context voor een biogeografische

studie. Sanger-sequeneringsdata, bestaande uit elf DNA-regio's (vijf nucleaire genen, drie chloroplast genen en drie chloroplast "intergenetic spacers"), van 62 Magnoliaceae taxa, werden gebruikt om een gekalibreerde Bayesiaanse fylogenie en een hypothese over de historische verspreiding op te stellen. De gegevens leverden bewijs op voor vier kolonisaties van *Magnolia* in het Caraïbisch gebied. Daarbovenop vormen de Caraïbische magnolia's clades van verwante soorten per eiland, met één uitzondering: *M. ekmanii* uit Haïti is meer verwant met de Cubaanse dan met de Dominicaanse taxa van subsectie *Cubenses*. De *Cubenses* soorten in de Grote Antillen volgden een zuid-noord kolonisatie traject, terwijl het traject van *M. dodecapetala* in de Kleine Antillen het concept van eiland-progressie volgt. De kolonisaties vonden volgens de fylogenetische hypothese maximaal 16 miljoen jaar geleden plaats, hetgeen steun biedt voor over-water-verspreiding als de meest plausibele dispersie-hypothese. Dit met de kanttekening dat de datering en daaruit volgende conclusies worden beïnvloed door de lage sequentie-diversiteit in deze plantenfamilie, wat een overtuigende vertaling van moleculaire data naar schattingen van de divergentie-tijd belemmert.

De toevoeging van extra nucleaire merkers en het gebruik van de coalescentie-theorie leverden geen betere of nieuwe inzichten in de Magnoliaceae classificatie: onze resultaten zijn zelfs in tegenspraak met de momenteel geaccepteerde diepere Magnoliaceae classificatie door sterk contrasterende plaatsing van de belangrijkste clades, afhankelijk van de genetische merker. Een belangrijk voorbeeld is sectie *Talauma*, die alle Caraïbische magnolia's behalve *M. virginiana* subsp. *oviedoae* omvat. De sectie verliest zijn ondersteuning, omdat subsecties *Cubenses*, *Dugandiodendron* en *Talauma* niet worden bevestigd als zuster-clades in veel van de aparte fylogenetische bomen per merker. De data stellen ook dat subsectie *Cubenses* genest is in subsectie *Dugandiodendron*. Toekomstige studies zullen baat hebben bij het gebruik van fylogenomische data en een grotere staalname aan soorten, om zo het aanhoudende probleem van lage ondersteuning voor de relaties tussen de voornaamste clades van de Magnoliaceae op te lossen.

Taxonafbakeningen van de Caraïbische magnolia's werden voornamelijk getest door middel van fylogenetische hypothesen, aangevuld met een haplotype netwerkanalyse. Genetische synapomorfieën van de elf bestudeerde merkers bevestigden de afbakening van 14 van de 15 Caraïbische magnolia's met uitzondering van het *M. minor* / *M. oblongifolia*-soortscomplex uit Cuba, dat dringend verder onderzoek nodig heeft, gezien de grote genetische variatie die niet overeenkomt met de morfologische concepten van de soorten. De genetische afstand tussen de twee ondersoorten van *M. cubensis* was vergelijkbaar met de genetische afstand gevonden tussen paren van andere Caraïbische *Magnolia* soorten, daarom wordt een taxonomische revisie van de ondersoorten van *M. cubensis* geadviseerd. De intraspecifieke genetische variatie van *M. dodecapetala* was hoger dan die van de andere Caraïbische soorten, en de

vruchten vertoonden ook een grote morfologische variatie die per eiland kan worden gecategoriseerd. De verschillende eilandpopulaties werden bestempeld als waardige kandidaten voor de status van “evolutionair significante eenheden” en we adviseren (voorlopig) geen taxonomische veranderingen.

In een studie waarbij acht van de 14 Caraïbische *Magnolia* soorten en één Mexicaanse *Magnolia*, elk vertegenwoordigd door twee vooraf gedefinieerde populaties met uitzondering van *M. splendens* waarvoor er maar één vooraf gedefinieerde populatie bestaat, werden gegenotypeerd met SSR-data, werden 16 van de 17 populaties bevestigd als een aparte eenheid, wat suggereert dat de populaties geen verreikende gene flow ervaren. Dit werd zelfs benadrukt doordat twee van de 17 populaties een onverwachte genetisch substructuur vertoonden, wat aangeeft dat de dynamiek van deze soorten op een fijne ruimtelijke schaal kan optreden (3 tot 5 km). De SSR-studies vonden weinig aanwijzingen voor inteelt (met uitzondering van *M. dodecapetala* en *M. portoricensis*), wat wijst op een voldoende gene flow binnen populaties en mechanismen die kruisbestuiving bevorderen. We stellen dat de reproductieve biologie van de Caraïbische magnolia's veerkrachtig maar beperkt lijkt in hun door dieren gemedieerde verbreiding. Andere gevonden patronen duiden op recente bottlenecks (*M. domingensis*), onverwacht hoge genetische diversiteit (*M. hamorii*) en lage genetische diversiteit (*M. ekmanii* en *M. domingensis*). Over het algemeen vertoonde de genetische diversiteit betere patronen dan verwacht voor bedreigde endemische soorten en we concluderen dat een toename van bosconnectiviteit de meest effectieve maatregel zou zijn voor het behoud en beheer van de Caraïbische *Magnolia* populaties.

Verder werden er twee gedetailleerde studies op populatieniveau gerealiseerd. De eerste studie, uitgevoerd op twee populaties van *M. cubensis* subsp. a*cunae*, leverde bewijs van de invloed van fragmentatie op pollenverbreiding en vertoonde weinig genetische differentiatie tussen populaties, hoogstwaarschijnlijk het resultaat van gedeelde evolutionaire geschiedenis. De resultaten van de studie stelden een nieuw beheer voor, bestaande uit zowel versterking als herintroductie. De tweede studie, uitgevoerd op vijf eilandpopulaties van *M. dodecapetala*, leverde aanzienlijk bewijs van inteelt. Er werd benadrukt dat, hoewel de soort door de IUCN Rode Lijst wordt aangeduid als VU, het sterk gestructureerde eilandsysteem ernstige gevolgen heeft voor de genetische diversiteit van de soort. De studie adviseert verder (conservatie-genetisch) onderzoek en de start van actief conservatiebeheer.

De algemene resultaten, discussie en aanbevelingen in dit doctoraatsonderzoek illustreren hoe wetenschappelijk onderzoek kan bijdragen aan biodiversiteitsbehoud en grotere vraagstellingen over evolutie en biogeografie. Een sterk attribuut van deze studie is de vertaling van onderzoek naar toegepast beheer en behoud via translationele wetenschap.

Thesis outline

Project history

The PhD is part of two, subsequently occurring, overarching projects funded by the Fondation Franklinia called: “PLAN(E)T: Plants for the future – A future for plants” (2012–2016) and “Magnolias of the Caribbean and Mesoamerica” (2016–2021). The first project aimed to investigate the genetic diversity in selected taxa of three plant families, i.e. Cactaceae, Hydrangeaceae and Magnoliaceae, using molecular data. The second project aims to trace the evolutionary and biogeographic history of the Caribbean and Mesoamerican *Magnolia* species, and to apply conservation genetic studies on a selection of these species to advise conservation practitioners and undertake specific conservation actions.

The PhD study is linked to an international working group of researchers, botanists, students and conservation practitioners. To guarantee good planning, collaboration and transfer of skills, the core of the working group had yearly meetings: a 2014 meeting in Havana, Cuba; a 2015 meeting in Pátzcuaro, Mexico; a 2016 meeting in Havana, Cuba; a 2017 meeting in Ghent, Belgium; and a 2018 meeting in Quito, Ecuador. The meetings coincided twice with important symposia, relevant to the working group: the third international symposium on the family Magnoliaceae (Cuba, 2016) and the XII Congreso Latinoamericano de Botánica (Ecuador, 2018).

The choice to focus on *Magnolia*[1] was driven by the fact that Magnolias act as umbrella species[2]: their *in situ* conservation safeguards other species in the habitat in which they occur; and as flagship species: their emblematic reputation attracts greater local and international interest. The family and species are also of great importance in horticulture, enhancing potential to establish living *ex situ* collections both in their country of origin and in botanic gardens and arboreta around the world.

The focus on the American continent was due to practical considerations for project execution given a more workable language and expertise of the supervisors (in contrast to Asia), as well as a complete lack of prior conservation genetic or phylogenetic focus in this area. The proposed PhD project originally focused solely on Cuba: “The Cuban *Magnolia* species (Magnoliaceae): assessment of the genetic diversity and the underlying evolutionary history”. The Cuban Magnolias were targeted given the unresolved taxonomic problems and the literature study of Cires et al. (2013) that highlighted this region for conservation genetic study.

[1] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.
[2] A selection of less commonly used words is explained in the glossary: Appendix 1.1

However, once the contact with the Cuban botanists was made during the abovementioned, crucial visit to Cuba in 2014 it became clear to us that the taxonomic work on these species was already underway by a PhD study of Alejandro Palmarola Bejerano. Even more so, due to limitations linked to the political climate of the country, it appeared to be impossible for foreign researchers to conduct fieldwork and research in the country. Hence, we teamed up with the Cuban researchers and expanded the focus of the PhD to incorporate the entire Caribbean region.

Chapter overview & PhD student contributions

Chapter 1: General introduction | This chapter gives an overview of the Magnoliaceae family and in particular the Caribbean Magnolias by means of a profound literature study and fieldwork observations. It discusses aspects such as classification, morphology and reproduction known about the (Caribbean) *Magnolia* species and their populations. In the summary of the different Magnolias per island, pictures, maps and demographic data per species are provided. These data were collected, on the one hand by our Cuban collaborators over many years of intensive fieldwork in Cuba, and on the other hand by our team during three expeditions to Hispaniola, Puerto Rico and the Lesser Antilles executed as part of this PhD and the overarching Franklinia project. At the end of the chapter a number of research hypotheses for this PhD study are put forward. | *This chapter is written by Emily Veltjen, whereby Ernesto Testé Lozano helped with the maps. Photo credits are provided in the figure captions*.

Chapter 2: Ploidy of Caribbean Magnolias | This chapter summarizes results on the ploidy level of the Caribbean Magnolias, by means of a literature study, chromosome counts and flow cytometry. | *This chapter is partly based on the Bachelor thesis of Koen Claeys: "Plantengenomen onder de loep. Het bepalen van de ploïdiegraad met flowcytometrie en chromosoomtellingen", for which Prof. Dr. em. Paul Goetghebeur and Prof. Dr. Tom Beeckman were the supervisors, and Emily Veltjen and Dr. Olivier Leroux were the day-to-day tutors. Emily Veltjen contributed to this chapter in terms of study design and acquisition of plant material (i.e. dried leaves, seeds), aiding Guy Van der Kinderen in planting and maintenance of the seedlings, aiding in testing of the chromosome count protocol as designed by Dr. Leroux, aiding Koen Claeys execution and analyses of the flow cytometry, and supervision of the thesis writing.*

Chapter 3: Biogeography of the Caribbean Magnolias | This chapter uncovers the phylogenetic relationships of the Caribbean Magnolias in a time-calibrated Bayesian framework, using coalescent theory to combine nuclear and chloroplast Sanger sequencing data. | *This chapter is modified version of a manuscript submitted to Molecular Phylogeny and*

Evolution: Veltjen et al. "The evolutionary history of the Caribbean Magnolias (Magnoliaceae): testing species delimitations and biogeographical hypotheses using molecular data". Emily Veltjen collected the samples and field data of Hispaniola, Puerto Rico and the Lesser Antilles, executed most of the lab work under supervision of Pieter Asselman, analysed the data and drafted the manuscript.

Chapter 4: SSR patterns of Neotropical Magnolias | This chapter represents a study that employs 63 *de novo* developed SSR markers in different datasets of nine Neotropical *Magnolia* species to study patterns of genetic structure and inbreeding. | *This chapter is a modified from a paper published in Heredity*: *Veltjen et al. (2019) "Genetic patterns in Neotropical* Magnolia *species using* de novo *developed SSR markers". Emily Veltjen collected the samples for Hispaniola, Puerto Rico and the Lesser Antilles, executed most of the lab work together with, and under supervision of Pieter Asselman, analysed the data and drafted the manuscript.*

Chapter 5: SSR study of *Magnolia cubensis* subsp. *acunae* | This chapter represents a case study which employs 11 SSR markers on a dataset 67 individuals of *M. cubensis* subsp. *acunae*, divided over two populations and two maturity classes. The study correlates the degree of fragmentation of the populations with their genetic diversity and studies the genetic patterns across generations. | *This chapter is modified from a published in Oryx: Hernández et al. (2020) "Population structure and genetic diversity of* M. cubensis *subsp.* acunae *(Magnoliaceae): effects of habitat fragmentation and implications for conservation". Emily Veltjen developed the SSR markers used in this study together with Pieter Asselman, organised and taught "Conservation Genetics" the 2015 workshop in Pátzcuaro, Mexico, where knowledge in the form of theory and practice on how to analyse SSR data was transferred to the Cuban author, and was involved in the writing and preparation of the manuscript.*

Chapter 6: SSR study & biogeography of *M. dodecapetala* | This chapter represents a study employing 19 SSR markers on a dataset of 195 individuals, distributed over five different islands in the Lesser Antilles, together with a calibrated phylogenetic Bayesian hypothesis of 11 Sanger sequencing markers, to elucidate questions on their biogeography, genetic structure and genetic diversity. | *This chapter is a manuscript in preparation: Veltjen et al. 2020 "An integrative approach to understand the diversity of* M. dodecapetala *(Magnoliaceae:* Talauma *subsect.* Talauma*) in the Lesser Antilles".* | *Emily Veltjen collected the data, executed most of the lab work together with and under supervision of Pieter Asselman, analysed the data and drafted the manuscript.*

Chapter 7: General discussion and conclusions | This chapter integrates the executed studies and results in a wider context whereby the contribution of evolutionary studies,

classification, species delimitation and conservation genetics to effective conservation is critically analysed in the context of *Magnolia* research. In the general conclusions the answers to all the research hypotheses from Chapter 1 are recapitulated. The chapter ends with listing the future perspectives, based on the knowledge and data gathered within the framework of this PhD thesis. | *This chapter is written by Emily Veltjen*.

Chapter 8: Lessons learned: conservation genetics in practice | This chapter critically reviews challenges adherent to conservation genetic research; while reflecting on how the challenges were tackled in the PhD study. | *This chapter is written by Emily Veltjen*.

References | References are listed in alphabetical order.

Appendices | Appendices are numbered according to their chapter: e.g. Appendix 1.1 is the first Appendix of Chapter 1, Appendix 3.1 is the first Appendix of Chapter 3. **Appendix 1** contains appendices that belong to Chapter 1: General introduction. Appendix 1.1 gives an alphabetical glossary of terms used throughout the thesis. Appendix 1.2 lists the abbreviations mentioned in the PhD. Appendix 1.3 lists the taxa used throughout the PhD thesis in with their taxonomic authorities, following the classification of Figlar and Nooteboom (2004). Appendix 1.4 encompasses an alphabetical list of all the Magnoliaceae species listed in this PhD. Appendix 1.5 is a Dutch publication for the journal of De Vrienden van de Plantentuin Gent that describes the first field experience to Hispaniola and Puerto Rico in 2015. Appendix 1.6 is a publication for The Journal of Magnolia Society International that describes the fieldwork in Hispaniola. **Appendix 2** contains all the appendices related to Chapter 2: Ploidy of the Caribbean Magnolias. Appendix 2.1 compiles the fluorescence histograms of the flow cytometry measurements. **Appendix 3** contains all the appendices related to Chapter 3: Biogeography of the Caribbean Magnolias. Appendix 3.1 summarizes the sample information of the phylogenetic analyses. Appendix 3.2 is a list of the used primers of the phylogenetic analyses. Appendix 3.3 tabulates the GenBank accession numbers of the sequences used in this study. Appendix 3.4 compiles the different Bayesian phylogenetic hypotheses per alignment. Appendix 3.5 tabulates the pairwise distance matrix for each sequence. Appendix 3.6 lists the partitioning schemes for all six alignments. Appendix 3.7 tabulates the output of the BioGeoBEARS analysis. **Appendix 4** contains all the appendices related to Chapter 4: SSR patterns of Neotropical Magnolias. Appendix 4.1 tabulates the results of the SSR amplification tests. Appendix 4.2 tabulates the results of the SSR polymorphism tests. Appendix 4.3 lists the microsatellite primer information. Appendix 4.4 tabulates the population statistics per (sub)species, marker and location. Appendix 4.5 shows the STRUCTURE ΔK and mean likelihood plots. Appendix 4.6 illustrates and tabulates the confidence intervals of the pairwise F_{ST} and D_{JOST} values from Table 4.4. Appendix 4.7 tabulates the geographic distance and the pairwise F_{ST} values per dataset. **Appendix 5** contains all the appendices

related to Chapter 5: SSR study of *Magnolia cubensis* subsp. *acunae*. Appendix 5.1 tabulates the summary statistics per locus, subdivided per adult and juvenile population. **Appendix 6** contains all the appendices related to Chapter 6: SSR study & biogeography of *M. dodecapetala*. Appendix 6.1 lists the summary statistics per locus, subdivided per island population and found STRUCTURE subpopulations. Appendix 6.2 shows the STRUCTURE ΔK and mean likelihood plots. Appendix 6.3 shows the DAPC results and plots of the D(15) and D(7) datasets. Appendix 6.4 is a compilation of tables with the F_{ST}, G_{ST} and D_{JOST} estimates, their ranges and a correlating graphical representation. Appendix 6.5 visualises the individual variation in fruit morphology. **Appendix X** is the Curriculum vitae of Emily Veltjen.

1. General introduction

1.1 The Magnoliaceae: biodiversity and classification

Magnolias are well-known due to their evolutionary, ecological and economic importance. The evolutionary significance of these plants is based on being well-represented in the fossil record (Azuma et al., 2001 and references herein; Dilcher and Crane, 1984; Kim et al., 2004; Romanov and Dilcher, 2013), their early-diverging position in the angiosperm tree of life (Ruhfel et al., 2014), their intriguing intercontinental disjunct biogeography (Li, 1952; Qiu et al., 1995a; Qiu et al., 1995b), and their flower morphology formerly interpreted as primitive which shaped previous morphology-based reconstructions of angiosperm classification (Cronquist, 1981; Takhtajan, 1969). The ecological value of the Magnolias is derived from the trees constituting an important part of (unique types of) (former) primary forest (e.g. cloud forest; (Dieringer and Espinosa, 1994)) and their association with certain flora (e.g. epiphyte communities; (Morales et al., 2019)) and fauna (e.g. specialist pollinators; (Gottsberger et al., 2012)). Their economic worth lies mainly in the high horticultural value of the large, showy flowers and beautiful tree shape, complemented by derived medicinal, perfume, food or timber products (Sánchez-Velásquez et al., 2016).

In the classification of the plant kingdom, the Magnoliaceae family is found within the flowering plants or angiosperms, where it classified in the Magnoliids (APG IV, 2016). Members of this family are evergreen[1] or deciduous trees and shrubs that can be easily recognised on a macro-morphological basis. The plants have annular scars on the nodes of the branches, left behind by the ephemeral, dehiscent stipules; petiolate, alternate and simple leaves with entire margins; conspicuous, solitary, and (mostly) bisexual flowers with spirally arranged flower parts, i.e. free tepals, stamens and carpels on an elongated receptacle; and conspicuous fruits being an aggregate of follicles or an aggregate of winged samaras – the first bearing seeds with a reddish coloured sarcotesta, labelling the seeds to be arilloid.

According to the most recent Magnoliaceae classification followed in this manuscript (Figlar and Nooteboom, 2004) as applied by Govaerts et al. (2019), the family contains two extant monogeneric subfamilies: subfamily Liriodendroideae[2] with the genus *Liriodendron* and subfamily Magnolioideae with the genus *Magnolia*. The use of the subfamilies was first proposed by Law (1984) and retained ever since. However, some authors argument to recognise the subfamily Liriodendroideae (and hence the genus *Liriodendron*) as a separate family “Liriodendraceae”, closely related to Magnoliaceae s.s. (Barkely, 1975; Romanov and

[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1
[2] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.

Dilcher, 2013). The main morphological differences between the two subfamilies can be found in leaf morphology, anther dehiscence, fruit morphology and seed coat anatomy. Firstly, the subfamilies differ in their leaves being entire in the Magnolioideae and lobed in the Liriodendroideae. Secondly, they can be distinguished by the dehiscence of the anthers: introrse or latrorse in the Magnolioideae and extrorse in Liriodendroideae. Thirdly, the fruits are an aggregate of follicles in the Magnolioideae (also called a multifollicle; (Romanov and Dilcher, 2013), and an aggregate of samaras in the Liriodendroideae (also called a multinutlet; (Romanov and Dilcher, 2013). Each fruit type differs in its carpel dehiscence: longitudinally or circumscissile in Magnolioideae, with at least the base remaining attached to the torus, *versus* indehiscent, caducous and samaroid in Liriodendroideae. Fourthly, the seed coat is thick, fleshy and free from the endocarp in the Magnolioideae and thin, dry and adherent to the endocarp in Liriodendroideae. The genus *Liriodendron* comprises only two species, while *Magnolia* includes more than 300 (Rivers et al., 2016).

Wild, extant populations of species of the two subfamilies are distributed in the Americas (the New World) and Asia (the Old World). About 40–50% of the species diversity is found in the Americas and the rest in Asia (Pérez et al., 2016; Rivers et al., 2016). It might seem surprising that about half of the *Magnolia* diversity is found in the Americas; we usually associate Magnolias with Asia, an image shaped by the Asian hardy, often precocious flowering Magnolias sold in garden centres (e.g. *Magnolia x soulangeana*, *Magnolia stellata*). However, even for a genus mainly composed of trees, there are still new *Magnolia* species discovered every year as more botanical explorations and studies of herbarium specimens reveal undescribed biodiversity. This is especially the case for the Magnolias of the Americas, and more specifically the Neotropics, i.e. 15 new *Magnolia* species have been described the past five years (Aguilar-Cano et al., 2018; Cogollo-Pacheco et al., 2019; de Azevedo et al., 2018; Domínguez-Yescas and Vázquez-García, 2019; García-Morales et al., 2017; Pérez et al., 2016; Vázquez-García et al., 2015a; Vázquez-García et al., 2016a; Vázquez-García et al., 2015b; Vázquez-García et al., 2017a; Vázquez-García et al., 2016b; Vázquez-García et al., 2018; Vázquez-García et al., 2017b). This is in strong contrast with only three new *Magnolia* species that have been described in Asia in the past five years (Hu et al., 2019; Liu and Zhang, 2019; Zhou et al., 2018). Although the current distribution of the family and of both subfamilies is disjunct, fossil records, found in the northern parts of North America, Alaska, Greenland, Spitzbergen, Kazakhstan and Europe dated from the Cretaceous and the Tertiary, prove that the Magnoliaceae were previously distributed across most of the northern hemisphere (Azuma et al., 2001 and references herein; Dilcher and Crane, 1984; Frumin and Friis, 1996; Frumin and Friis, 1999; Manchester, 1994; Tiffney, 1977).

The classification within subfamily Magnolioideae has undergone a fair number of changes (Azuma et al., 2001; Chen and Nooteboom, 1993; Dandy, 1927, 1978; Figlar and Nooteboom, 2004; Keng, 1978; Kim and Suh, 2013; Law, 1984, 1996; Nooteboom, 1985, 1987; Sima and Lu, 2012; Spongberg, 1976; Xia et al., 2008). The topic is controversial up to this date given that taxonomists have a different opinion on the taxonomic ranks that should be given to the clades retrieved in molecular studies. Molecular systematics revealed that many of the characters previously used, are phylogenetically uninformative because they evolved in parallel (Nooteboom, 2000). As modern classifications should attempt to reflect phylogeny rather than superficial resemblance, the taxonomic revision of the family went in two directions: lumping all the genera into one genus *Magnolia* (e.g. Figlar and Nooteboom, 2004) *versus* instating new genera per clade (e.g. Xia et al., 2008). Lumping the segregate genera into one single genus *Magnolia* is taxonomically justified given: a) phylogenetic reconstructions (Azuma et al., 2001; Azuma et al., 1999b; Kim et al., 2001; Kim and Suh, 2013; Nie et al., 2008; Qiu et al., 1995a; Qiu et al., 1995b) show that the main genera formally recognised were merely small clades within much bigger clades; b) macro- and micro-morphological observations of living plants (Baranova, 2000; Figlar, 2002a, b; Figlar and Nooteboom, 2004; Nooteboom, 1985, 1998; Xu, 2003); c) low sequence divergence in comparison with other angiosperm families; d) the relative high frequency of man-made inter-clade hybrids; and e) nomenclatural stability and nomenclatural efficiency. The main arguments against lumping the genera of subfamily Magnolioideae are 1) that the morphological diversity and coherent evolutionary patterns and relationships are not reflected in the species names and 2) that the new combinations are causing nomenclatural instability (Callaway, 1994; Xia, 2009). Given that Figlar and Nooteboom (2004) delivered a convincing set of arguments and a robust solution, most authors, such as Govaerts et al. (2019) and myself, follow the “lumping” classification and recognise one genus: *Magnolia*. Interestingly, Baillon (1866) in his *Mémoire sur la famille des Magnoliacées* already stated that the conservative morphology within the Magnolioideae could be translated in the diversity being classified in one single genus.

Another point of discussion is the evolutionary relationships among the clades in *Magnolia*. Up to this date, the deeper relationships among the different clades remain greatly unresolved, which adds to the instability of the classification of the Magnoliaceae. Phylogenetic hypotheses (Azuma et al., 2001; Azuma et al., 1999b; Kim et al., 2001; Qiu et al., 1995a) published before the classification of Figlar and Nooteboom (2004) inspired the creation of 12 sections and 15 subsections coinciding with the clades. However, the three described subgenera (i.e. subgenus *Magnolia*, subgenus *Yulania* and subgenus *Gynopodium*) were phylogenetically unsupported, and remained so in phylogenetic hypotheses conducted after this classification (Kim and Suh, 2013; Nie et al., 2008). In the latest published phylogenetic study, Kim and Suh

(2013) conclude there are 11 major clades in the subfamily which align with various groups at the rank of section and subsection of Figlar and Nooteboom (2004) given in Table 1.1.

The controversy in classification of the subfamily Magnolioideae was and still is mainly a product of its, relatively to other plant families, conservative morphology. *Magnolia* leaves are always petiolate, simple and alternate and protected by a pair of ephemeral stipules. Variation of vegetative characters and subsequent classification and identification are found in the leaf shape, leaf texture, leaf apex, leaf base, phyllotaxis (spiral or distichous) and stipule adnation (varying from 0% to 100% adnate: see Figure 1.1). Once molecular data were included and new relationships were revealed, new vegetative characteristics such as growth form, i.e. syllepsis vs. prolepsis (Figlar, 2000), and leaf development, i.e. conduplicate or open leaf prefoliation, were put forward as synapomorphies at deeper classification levels (Figlar and Nooteboom, 2004). These two characters remained undetected for long, given the difficulty to register them in herbarium collections. The flower morphology is quite rigid for the family overall (see Figure 1.2): the flowers are solitary, large, showy and trimerous, with basic structures such as the perules, pedicle, tepals and floral axis with its numerous, spirally arranged pistils and stamens. Here, important synapomorphic characters at the lower taxonomic levels are the position of the thecae (i.e. introrse vs. latrorse) and the structure of the floral axis. Other variation in the reproductive structures is found in the colour of the tepals, number of ovules per carpel and the number of carpels, stamens and pistils. Lastly, the fruit, which is a follicetum in *Magnolia*, varies in its texture, shape, and as a consequence of the number of ovules and carpels, in its number of follicles and seeds per follicle.

Table 1.1 Classification within the subfamily Magnolioideae. An overview of the publication recognizing the most genera i.e. 16 (Xia, 2009) *versus* the publication recognizing the least genera i.e. one (Figlar and Nooteboom, 2004). Names with taxonomic authorities can be found in Appendix 1.3 and Appendix 1.4.

Xia (2009)		**Figlar and Nooteboom (2004)**				**Kim and Suh (2013)**
Tribe	**Genus**	**Genus**	**Subgenus**	**Section**	**Subsection**	**Clade**
Magnolieae	*Magnolia*	*Magnolia*	*Magnolia*	*Magnolia*		THEORHODON
				Auriculata		FRASERI
				Macrophylla		MACROPHYLLA
	Lirianthe			*Gwillimia*	*Gwillimia*	GWILLIMIA
					Blumiana	
	Talauma			*Talauma*	*Talauma*	TALAUMA
	Dugandiodendron				*Dugandiodendron*	
					Cubenses	
	Manglietia			*Manglietia*		MANGLIETIA
	Woonyoungia			*Kmeria*		KMERIA
	Kmeria					
	Houpoëa			*Rhytidospermum*	*Rytidospermum*	RYTIDOSPERMUM
	Oyama				*Oyama*	
	Parakmeria		*Gynopodium*	*Gynopodium*		GYNOPODIUM
	Pachylarnax			*Manglietiastrum*		
Michelieae	*Yulania*		*Yulania*	*Yulania*	*Yulania*	YULANIA
					Tulipastrum	
	Michelia			*Michelia*	*Michelia*	MICHELIA
	Elmerillia				*Elmerillia*	
	Alcimandra				*Maingola*	
	Aromadendron				*Aromadendron*	

Note: Figlar and Nooteboom (2004) used the name *Magnolia* section *Talauma* subsection *Splendentes* instead of the nomenclaturally correct name subsection *Cubenses*. Imchanitzkaja (1991) published the name subsection *Cubenses* before Vázquez (1994) published section *Splendentes* and hence according to the International Code of Nomenclature Article 11.3 (Turland et al., 2018) this name is the correct one. This information was unaccounted for in the publication of Figlar and Nooteboom (2004), who changed the rank of section *Splendentes* Dandy ex A. Vazquez to the rank of subsection *Splendentes* (Dandy ex A. Vazquez) Figlar & Noot. The classification of Figlar and Nooteboom (2004) has since been updated on the website of the Magnolia Society International. From here on, the correct name: subsection *Cubenses* Imch. – Type: *Magnolia cubensis*, will be used.

Figure 1.1 A selection of Magnolias illustrating the variation in stipule adnation, detectable by the stipular scar. **A** *M. coronata*: no scar (0%). **B** *M. portoricensis*: no scar (0%). **C** *M. pacifica*: short scar (5%). **D** *M. striatifolia*: short scar (5%). **E** *M. chiguila*: 30% scar. **F** *M. mindoensis*: 40–50% scar. **G** *M. jardinensis*: 75% scar. **H** *M. espinalii*: 100% scar. Photo credits: A, C, D, G, H: Richard Figlar; B, E, F: Emily Veltjen.

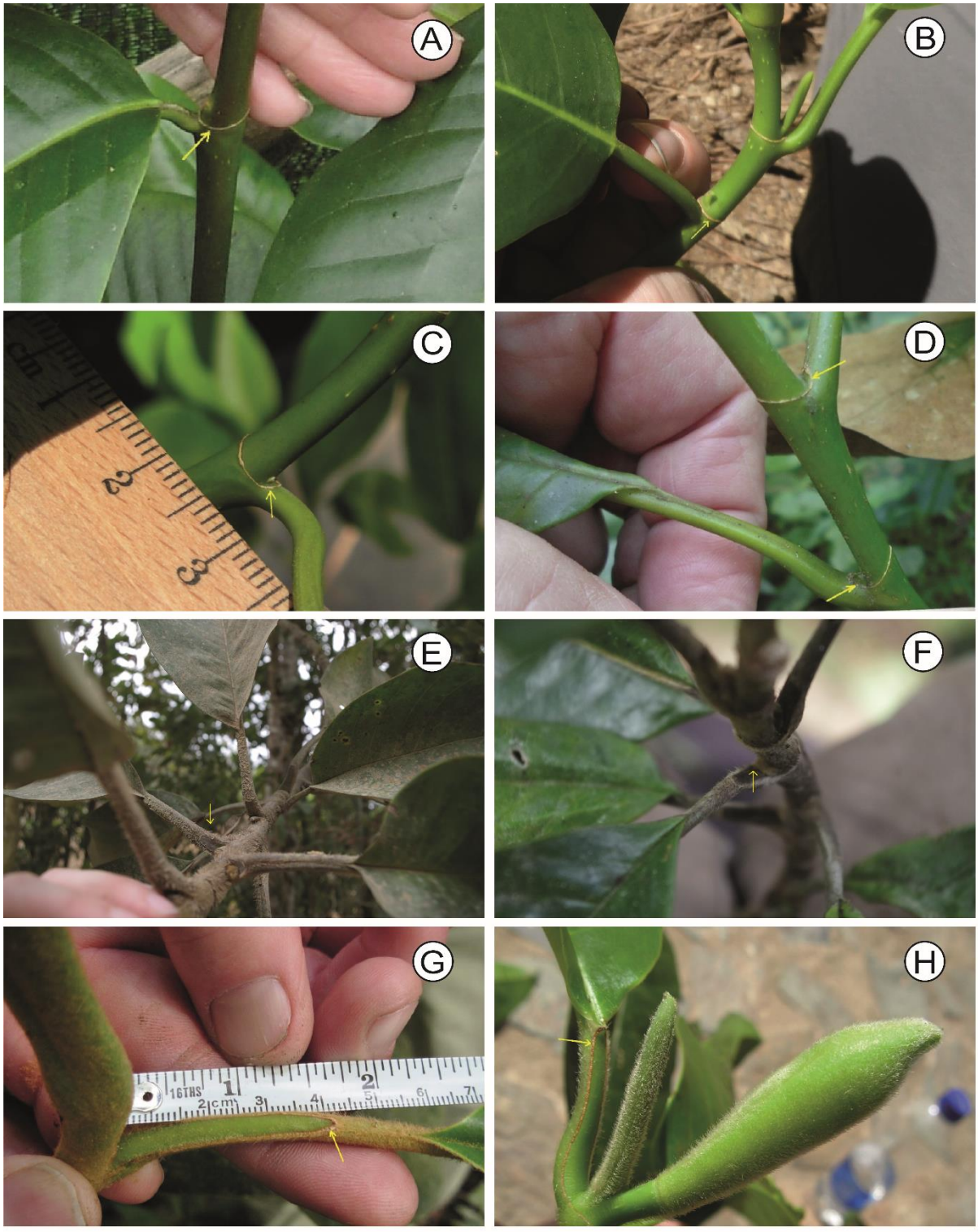


Figure 1.2 A selection of Magnolias illustrating variation in flower morphology. **A** *M. insignis* (sect. *Manglietia*): pink flowers in male phase (left) and female phase (right). **B** *M. cubensis* subsp. *cubensis* (subsect. *Cubenses*): stamen connectives are embedded in the gynoecium, flower in male phase; flower deliberately inverted. **C** *M. chiguila* (subsect. *Chocotalauma*): stamens caducous, detaching at the base and stamen connective is not embedded in the gynoecium. **D** *M. lotungensis* (sect. Gynopodium): cup-shaped male flower (androdioecious trees), stamens with purplish red filaments. **E** *M. fulva* (sect. *Michelia*): flowers initially white but turning pale yellow, stamens yellowish brown, gynoecium stipitate with pubescent carpels. **F** *M. virginiana* subsp. *oviedoae* (sect. *Magnolia*): white flowers. Notice the glaucous abaxial side of the leaves. Photo credits: A, D, E: Richard Figlar; B, F: Mikhail S. Romanov. C: Lou Jost.

1.2 Reproductive biology of Magnolias

In the study of plant biogeography, systematics and conservation genetics, genetic data can provide first insights in the extent of gene flow. However, to comprehend and explain these data and translate them to conservation management, species-specific direct observations of phenology, pollinators and seed dispersers, which collectively can be referred to as the reproductive biology, are valuable information. Needless to say, mainly from a practical point of view (i.e. high trees, remote populations, scattered individuals), such observations are generally missing for most *Magnolia* species.

The few species of which the flowers were studied in greater detail already provided many interesting insights. Studies have demonstrated that the flowers of extant Magnoliaceae are specialised with ultraviolet patterns (Thien et al., 1995; Yasukawa et al., 1992), secretions (Heiser, 1962; Thien, 1974; Yasukawa et al., 1992), fragrances (Azuma et al., 1999a; Azuma et al., 1997; Thien, 1974; Yasukawa et al., 1992) and thermogenesis (Dieringer et al., 1999; Gottsberger et al., 2012; Seymour et al., 2010; Wang et al., 2014). With the exception of the few unisexual flower-bearing *Magnolia* species, the bisexual *Magnolia* flowers are protogynous. The female and male floral reproductive structures are not (distinctly) spatially separated, but the temporal separation makes self-pollination within one flower unlikely, as proven by flower bagging experiments (Chen et al., 2016; Dieringer et al., 1999; Dieringer and Espinosa, 1994; Ishida, 1996).

Pollinator studies in temperate (Delphino, 1875; Heiser, 1962; Ishida, 1996; Kikuzawa and Mizui, 1990; Thien, 1974; Yasukawa et al., 1992; Zhao and Sun, 2009) and (sub)tropical regions (Chen and Nooteboom, 1993; Dieringer et al., 1999; Dieringer and Espinosa, 1994; Gibbs et al., 1977; Gottsberger et al., 2012; Vázquez-García et al., 2015b; Zhao and Sun, 2009) indicated beetles as the pollinators of *Magnolia* flowers. However, the abovementioned studies also list bees, flies, moths, paper wasps and brown lacewings to visit the flowers. Here, the question on the pollinator community remains controversial as a flower visit does not equal effective pollination and most insects other than the beetles “don’t seem to fit the flowers”; they are reported to visit the flowers only in the male phase of the flowering sequence (Chen et al., 2016; Heiser, 1962; Thien, 1974) or are reported as occasional pollinators (Wang et al., 2014).The flower visitors consume the pollen and (species-dependent) also nectar, stigmas, secretions of the tepals, or the (inner) tepals (Chen et al., 2016; Dieringer et al., 1999; Dieringer and Espinosa, 1994; Heiser, 1962; Seymour et al., 2010; Thien, 1974; Yasukawa et al., 1992). In species where no apparent reward exists for visiting the female phase, the concept of automimicry (Bernhardt, 1987) has been proposed to explain effective pollination (Ishida, 1996; Kikuzawa and Mizui, 1990). At least for the beetles, the “mess and spoil” principle,

whereby beetles tramp around in *Magnolia* flowers and accidental pollination occurs, is described (Faegri and Van Der Pijl, 1971). However, given the observed specialised structures and timing sequence, this concept of accidental pollination does not entirely fit for *Magnolia* (Thien, 1974). Most observations of *Magnolia* pollination are made in the male phase of the flowers, as the flowers are then open, often more profoundly and always for a much longer period compared to the female phase: here the detached stamens fall into the concave *Magnolia* tepals, which results in the tepals becoming covered with large quantities of pollen where after the pollinators eat and mate in this setting, while becoming coated with pollen. Here, it must be mentioned that in the Caribbean Magnolias of subsection *Cubenses*, and the species of subsection *Dugandiodendron*, the setaceous stamen tips (also called the connective appendages) are embedded in the gynoecium, which deviates from the litter of stamens and pollen that accumulate in the concave tepals. However, Howard (1948) did mention that the tepal surfaces become covered with pollen, and the general concept of a "feeding frenzy" is not violated. Selection for this characteristic of stamen embedment could be due to the characteristic facilitating pollen transfer, providing a form of protection to the pollinators, or/and providing higher reproductive success as the falling of stamens on the ground is prevented (Figlar, 2015).

Studies on seed dispersers are scarce, executed on the species level and sometimes not published for an international audience (Cazetta et al., 2002; Chen et al., 2016; Gottsberger et al., 2012; Gutiérrez Zúñiga, 2018; Martinez, 1996; Wang et al., 2019). Observations so far report birds to be the main dispersers, but there are also reports of rodents and ants eating the red *Magnolia* seeds (Martinez, 1996). Most often a more generalist bird species is observed, rather than a specialist.

The phenology can be derived from the label data of herbarium specimens or documented field observations. This with the caveat that herbarium records might be misleading as some collections may represent a single (or a few) individuals that represent off-season flowering or fruiting stages, or as the climatic conditions in the year of collection might differ from the present-day conditions. The most accurate and valuable records of flowering and fruiting time are those from students, researchers or NGOs that frequently visit populations or have observed the trees for a longer period of time (e.g. Chen et al., 2016; Dahua Machoa, 2018; Dieringer et al., 1999; Gómez Restrepo, 2011; Gottsberger et al., 2012; Kikuzawa and Mizui, 1990; Martinez, 1996; Setsuko et al., 2008; Vázquez-García et al., 2015b; Wang et al., 2010). Other than the documentation of time of flowering and fruiting in the year, there is often also variation documented in the timing of male and female structures being receptive in combination with the movement of tepals (i.e. characters that can be observed with the naked eye). The nastic-like tepal movement (Figlar, 2019) is documented in detail for ca. 10 *Magnolia*

species (Chen et al., 2016; Dahua Machoa, 2018; Dieringer and Espinosa, 1994; Figlar, 2019; Gottsberger et al., 2012; Heiser, 1962; Ishida, 1996; Losada et al., 2014; Thien, 1974; Wang et al., 2014). In such reports, the number of functional days for an individual flower is reported to be between 24 hours and nine days. The timing of flower opening differs among species: some flowers open in the morning i.e. they are diurnal, others in the evening i.e. they are nocturnal. The timing of tepal movements is suggested to be dependent on climatic conditions (Thien, 1974) and sexual selection of the pollinator community. The evolutionary advantage of the tepal movements is suggested to be that the closure prevents other types of insects from gaining access to the flower until the stigmas and stamens become non-functional (Thien, 1974).

Self-incompatibility is rarer in Magnoliaceae compared to self-compatibility. Due to the temporal separation of the female and male phase, self-pollination occurs (naturally) in the form of geitonogamy (Heiser, 1962), unless the flowers on an individual tree are synchronous (Bernhardt, 1987). Studies suggested that geitonogamy considerably reduces the seed set (Ishida and Ito, 2003; Wang et al., 2010; Zhao and Sun, 2009). Even more so, compatibility of Magnolias does not necessarily end at the species boundary: there are over a hundred *Magnolia* artificial hybrids in cultivation, some even across clades (Callaway, 1994), yet few hybrids have been found in nature (Thien, 1974).

1.3 The Caribbean Magnolias

We zoom in on the Magnolias of a biogeographically interesting and quite extensively explored area: the Caribbean. In this PhD, the Caribbean is synonymized with the Caribbean islands, which, due to their colonial past, are also referred to as the West Indies. The Caribbean islands are found in the Caribbean Sea that lies southeast from the North American mainland, east of Central America and north of South America. The Caribbean is recognised as a priority for conservation due to its high level of endemism (Santiago-Valentín and Olmstead, 2004) in a mosaic of different vegetation types (Areces-Mallea et al., 1999) which is overall vulnerable due to the small areas and a high degree of human induced and natural disturbance (Myers et al., 2000; Olsen and Dinerstein, 1998; Rodrigues et al., 2004; Smith et al., 2004). The difficulty and wonder of conducting research and conservation on these islands is the astonishing cultural diversity of the Caribbean: a vibrant cultural, political and linguistic mosaic of nations and peoples that reflects a turbulent colonial past (Maunder et al., 2011). The colonial past, together with the geographical characteristic of episodic hurricanes in the region contribute to the political vulnerability of the region, which can lead to extreme poverty of which Haiti, the poorest country in the Western hemisphere, is the most notorious example.

Currently, fourteen Caribbean *Magnolia* species, of which one consists of two subspecies, are accepted to occur in this area. The 15 Caribbean *Magnolia* taxa reside in eight different Caribbean islands, i.e. Cuba, Hispaniola, Puerto Rico, Saint Vincent, Saint Lucia, Dominica, Martinique and Guadeloupe. There are no records of *Magnolia* occurring naturally on any of the other Caribbean islands, with the exception of an erroneous report of *M. dodecapetala* on Trinidad and Tobago (See Chapter 1.7 for a more in-depth discussion). The geographical boundary of most of these islands aligns with their political boundary, except for Haiti and the Dominican Republic, two countries located on the island of Hispaniola; and Martinique and Guadeloupe which are two different French overseas departments in the Lesser Antilles. For each taxon, its morphology, distribution and demographic data collected during the field trips, are compiled in the following subchapters.

The taxonomic history of the Caribbean Magnolias starts together with the taxonomic history of the Magnoliaceae family given that *Magnolia dodecapetala* from Martinique was the first species of Magnoliaceae described to science (see Box 1). The Caribbean Magnolias were reviewed in 1948 by Richard A. Howard who published an extensive description on the morphology and systematics of this group. In this work, he summarised the information on a total of eleven native *Magnolia* species for the Caribbean islands. Eight of these eleven species, i.e. the species that occur on Hispaniola, Puerto Rico and the Lesser Antilles, are still delineated as in 1948. In contrast to the straightforward taxonomic history of these eight

species, the taxonomical history of the Cuban Magnolias is more complicated (Bisse, 1988; Imchanitzkaja, 1991; Imchanitzkaja, 1993; Palmarola et al., 2016). The number of Cuban *Magnolia* (sub)species recognised, increased to twelve (Bisse, 1988; Imchanitzkaja, 1991, 1993), including seven species and five heterotypic subspecies. Following the work of Imchanitzkaja different opinions were expressed by other authors concerning the number of Cuban *Magnolia* taxa, and several names have been placed in synonymy (e.g. Acevedo-Rodríguez and Strong, 2019). The most recent revisions (González Torres et al., 2016; Palmarola et al., 2016) recognise six native Cuban *Magnolia* taxa. These six Cuban Magnolias include a recently found population of *M. virginiana* from the Majaguillar Swamp in the north of the Cuban province of Matanzas (Oviedo Prieto et al., 2008) that was described as a subspecies due to its distinctive morphology: *M. virginiana* subsp. *oviedoae* (Palmarola-Bejerano et al., 2008) and one heterotypic subspecies: *Magnolia cubensis* subspecies *acunae*.

BOX 1: The history behind the name *Magnolia* takes us on a voyage to the Caribbean islands![1]

The taxonomic history of the name *Magnolia* brings us to the island of Martinique, an overseas department of France located in the Lesser Antilles. Charles Plumier (1646–1704), a famous French botanist, named a flowering tree on the island Martinique after the French botanist Pierre Magnol (1638–1715) in his botanical work *Nova plantarum americanarum genera* (1703). The tree is nowadays known under the name *Magnolia dodecapetala*. The name "*Magnolia*" was later adopted by William Sherard (1659–1728), who was responsible for the nomenclatural parts of *Hortus Elthamensis* (1732) by Johann Jacob Dillenius and the *Natural History of Carolina, Florida and the Bahama Islands* (1730) by Mark Catesby. Carl Linnaeus (1707–1778) referred to the work of both Plumier and Sherard and adopted the genus name *Magnolia* in the *Systema Naturae* (1735) and later on in the *Species Plantarum* (1753), where he described it as a monotypic genus, of which the type species was *Magnolia glauca*, later known as *Magnolia virginiana*. Hence Linnaeus unintentionally transferred the name *Magnolia* from a tropical genus to a temperate genus.

Until now, the 14 Caribbean *Magnolia* species (i.e. 15 *Magnolia* taxa) have been delineated on morphological grounds, with little (phylo)genetic information available. *Magnolia virginiana* subsp. *oviedoae* belongs to a different section of subgenus *Magnolia* (i.e. section *Magnolia*) and is morphologically easily distinguished from the other Caribbean Magnolias: the shrubs or trees have tender, non-coriaceous, lanceolate leaves, covered with sparse silvery hairs on the abaxial side, resulting in a glaucous colour (see Figure 1.2F); caducous stamens without

[1] Treseder, N.G., 1978. Magnolias. Faber and Faber, London.

gynoecium embedment; and the fruit is an apocarpous aggregate of follicles which open via longitudinal slits. The other 13 species (i.e. 14 taxa) belonging to section *Talauma* can be subdivided according to the morphological characters that define their two subsections: subsection *Cubenses* and subsection *Talauma*. The nine Caribbean Magnolias of subsection *Cubenses* (hereafter shorted as *Cubenses*) are the only members of this subsection. For section *Talauma* subsection *Talauma* (hereafter shortened as *Talauma*), the Caribbean has four representatives out of the ca. 90 (Vázquez-García et al., 2018) currently described *Talauma* species.

Morphologically, *Cubenses* and *Talauma* can be separated from each other by differences in the following characters: adnation of the stipules to the petiole, the shape of the stamen apices, position of the thecae, and the fruit morphology. Firstly, the species classified in *Cubenses* have stipules that are completely free from the petiole while the species classified in *Talauma* have adnate stipules. Secondly, species of *Cubenses* have stamens with an elongated apex which are embedded in the gynoecium and hence remain attached to the flower when their filaments detach from the floral axis, whereas the species of *Talauma* have stamens with a short apex that are completely shed from the floral axis and flower upon dehiscence. Thirdly, the carpels of the apocarpous fruits of *Cubenses* dehisce longitudinally, whereby the carpels of the syncarpous fruits of *Talauma* are characterised by circumscissile dehiscence. Although these characters are very useful to distinguish the main subsections of the Caribbean Magnolias, they do recur in other subsections of Magnoliaceae, and it is mainly the combination of the three characters that make morphology-based identification of the subsections possible. The character of stamen embedment of *Cubenses* is quite unique, as it is only found in one other subsection of the subgenus *Magnolia* section *Talauma* subsection *Dugandiodendron* (hereafter shortened as *Dugandiodendron*), which is composed of species found on the South American mainland i.e. Colombia, Ecuador, Peru and Venezuela. However, the members of *Dugandiodendron* have fruits of which the carpels dehisce circumscissile, in contrast to the longitudinal dehiscence of the carpels of *Cubenses.* The elongated anther apex morphology found in *Cubenses* and *Dugandiodendron* is also found in subgenus *Yulania* section *Michelia* subsection *Aromadendron*. However, there the stamen appendages are not embedded into the gynoecium; as well do the stamens persist during the male phase of the flower in contrast to the caducous stamens of *Cubenses*. The circumscissile fruit dehiscence of *Talauma* is the most distinctive character for identifying the subsection. However, it is also found in *Dugandiodendron* and in two Asian subsections: subgenus *Magnolia* section *Gwillimia* subsection *Blumiana* and subgenus *Yulania* section *Michelia* subsection *Aromadendron*. In the pre-molecular days, all species with circumscissile fruit dehiscence were merged in the segregate genus *Talauma* (Dandy, 1927, 1978), but molecular

data showed that this character evolved multiple times independently (Kim and Suh, 2013), which was already predicted by Nooteboom (1985) when considering more characters altogether. As previously mentioned, subsections *Dugandiodendron* and *Aromadendron* can be easily distinguished from *Talauma* given the anther morphology. Other than using geographical information (Asia vs. America) or micro-morphological characters (different stomata groups, (Baranova, 2000)), subsection *Blumiana* from Asia and *Talauma* from the Americas cannot be easily distinguished morphologically.

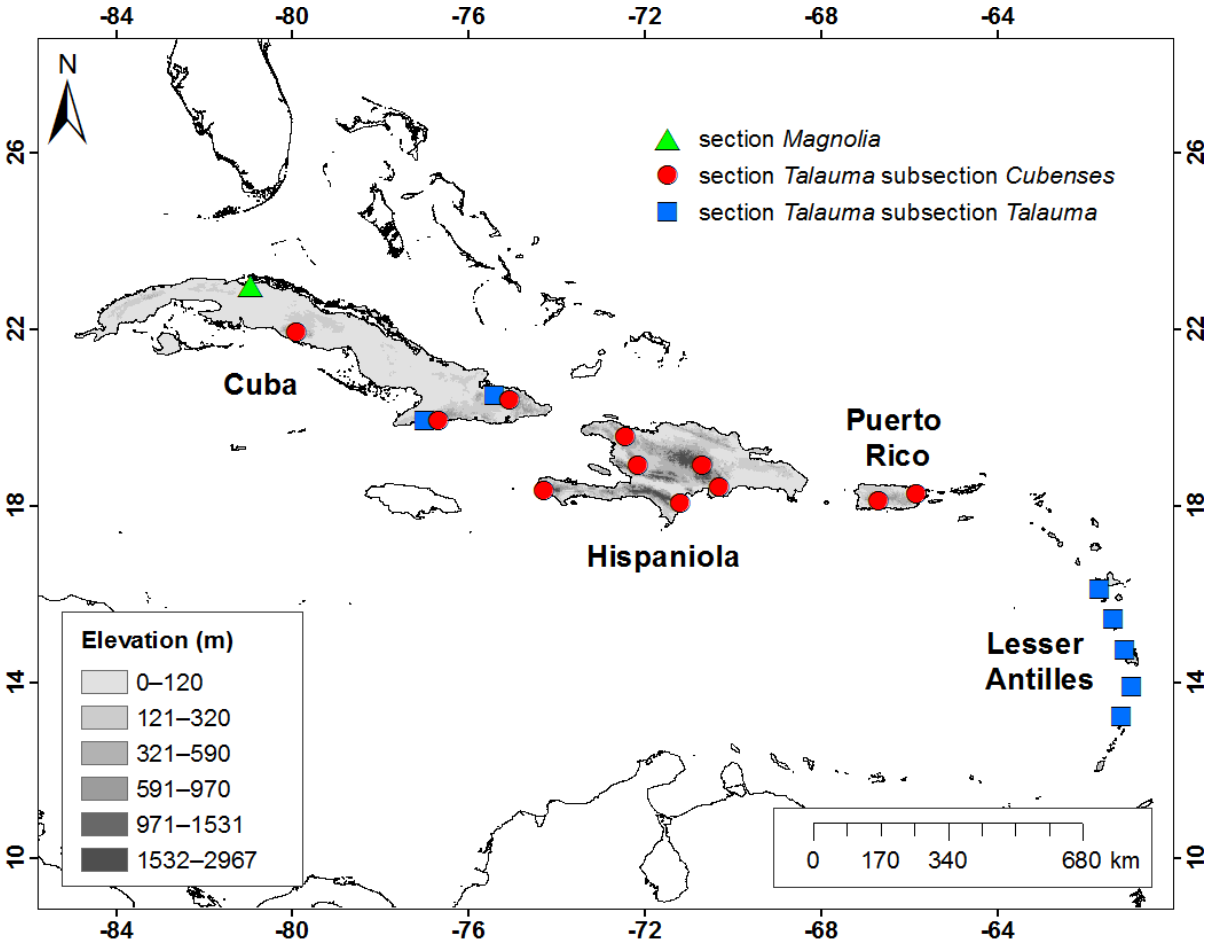
Morphological differentiation among Caribbean *Magnolia* species within the subsections is made by using characters such as leaf size, shape, texture and margin type; absence or presence of pubescence; number of perianth parts; and number of carpels (Howard, 1948; Imchanitzkaja, 1991; Imchanitzkaja, 1993; Palmarola et al., 2016). Box 2 contains an identification key of all 15 Caribbean *Magnolia* taxa. Figures 1.3–1.9; 1.14–1.15 and 1.18 illustrate this morphological variation and illustrate a selection of distinguishing morphological characters. Although the morphological characters are defined as distinct in the species descriptions and identification keys, variation in the distinguishing characters has been reported (Howard, 1948; Palmarola et al., 2016; Stehlé and Marie, 1947), introducing doubt and debate over the species entities. High intraspecific morphological variation is also reported in general for Magnoliaceae (Chen and Nooteboom, 1993) and overall hampers convincing morphological species delimitations.

Given that morphological differences alone can make it hard to identify the Caribbean Magnolias species, i.e. intraspecific morphological variation can cast doubts and there is often a need of flowering or fruiting structures for a more certain identification, the discrete geographical distribution per species makes a quick identification possible: the fourteen species occur either on a different island or a distinct mountain chain (or swamp in the case of *M. virginiana* subsp. *oviedoae*) within an island. Maps 1.1–1.13 illustrate the known distribution of all species. Neighbouring allopatric Caribbean *Magnolia* species are found between approximately 30 and 150 km apart and hence, in theory and not knowing the seed disperser communities of the species, long-distance dispersal of (most likely) seeds (Petit and Hampe, 2006) between the “alleged” species remains optional given that (generalist) seed dispersers could migrate between the species localities taking the seed with them, or natural disasters such as hurricanes could translocate seeds over that distance. Yet, to date no reports of such migrants or subsequently, hybrids are found, and overall the geographic distance between the species appears to prevent exchange of genetic material. There are, however, two exceptions to this rigid geographical division: two sets of Caribbean *Magnolia* species are sympatric, both in Cuba: *M. cubensis* subsp. *cubensis* and *M. orbiculata* in the Sierra Maestra Mountain Range, and *M. cristalensis*, *M. minor* and *M. oblongifolia* in the Nipe-Sagua-Baracoa Massif. The first

set of species i.e. *M. cubensis* subsp. *cubensis* and *M. orbiculata* in the Sierra Maestra Mountain Range, consists of one member of *Cubenses* and one of *Talauma*, making morphological discrimination among the two species easy and the probability of gene flow amongst them very unlikely. Similarly, the second set, i.e. *M. cristalensis*, *M. minor* and *M. oblongifolia*, consists of one representative of *Cubenses* (*M. cristalensis*) and two of *Talauma* (*M. minor* and *M. oblongifolia*). Of the latter, recent intense fieldwork has found intermediate morphologies between the discretely described morphospecies.

Locality data of the 15 Caribbean taxa usually are confined to knowledge gathered via a few herbarium records, and in the case of the Cuban Magnolias, some intensive surveys by local botanists and NGO's to new, remote areas in the mountains. Similarly, demographic data of the 15 Caribbean taxa are usually lacking or consist of estimates based on the forest area around a herbarium record, which is now complemented with more in-depth surveys by the Cuban NGO Planta!, the National Botanical Garden (University of Havana) and the University of Havana, with whom we work closely together; and three expeditions that were executed during the framework of this PhD. Table 2.1 summarizes all the known localities of the Caribbean *Magnolia* taxa (as illustrated in Maps 1.1–1.13) and the demographic information that is known of these localities. From this table it is apparent that little effort is allocated to exploring new areas and actively documenting population demographics; which is understandable given the intensity of the work.

Map 1.1 Distribution of *Magnolia* on the Caribbean islands. The nine *Magnolia* species of *Cubenses* reside on three different islands on the Greater Antilles i.e. Cuba, Hispaniola and Puerto Rico and encompass 10 different mountain ranges i.e. the Guamuhaya mountains in central Cuba, the Sierra Maestra in the south of Cuba, the Nipe-Sagua-Baracoa mountains in the southeast of Cuba, the Massif de la Hotte in the southwest of Haiti, the Montagnes Noires in central Haiti, the Massif du Nord in Haiti, the Cordillera Central in the centre of Hispaniola, the Sierra de Bahoruco in the south of the Dominican Republic, the Cordillera Central in Puerto Rico and El Yunque in Puerto Rico. The four *Magnolia* species of *Talauma* reside on either Cuba of the Greater Antilles, where one species can be found in the Sierra Maestra in the south of Cuba, and two species in the Nipe-Sagua-Baracoa mountains in the southeast of Cuba; or five islands in the Lesser Antilles i.e. Guadeloupe, Dominica, Martinique, Saint Lucia and Saint Vincent. There is one *Magnolia* species from the subgenus *Magnolia* section *Magnolia* that resides in the north of Cuba. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: Fick and Hijmans (2017); Protected Areas: CNAP (2014); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



BOX 2: Identification key to the native *Magnolia* taxa of the Caribbean islands.

This identification key is based on morphological data compiled from Howard (1948), Palmarola et al. (2016) and observations documented during our field expeditions. [1,2,3,4] The geographical origin is each time given next to the species name between brackets: Cuba (C), the Dominican Republic (DR), Haiti (H), the Lesser Antilles (LA) and Puerto Rico (PR).

1a. Stipules clearly adnate to the petiole: conspicuous petiolar scars present 2

1b. Stipules free from the petiole: petiolar scars absent..... 6
(+ Stamen appendages long (female stage of the flowers) and stamens become embedded in the gynoecium with their tips, while the base becomes detached (male stage of the flowers). Fruits apocarpous, non-woody, each carpel dehiscent along a dorsal suture = section *Talauma* subsection *Cubenses*)

2.a Fruits apocarpous, non-woody, each carpel dehiscent along a dorsal suture .....***M. virginiana* subsp. *oviedoae*** (C)
(= section *Magnolia* + Lamina lanceolate or narrowly elliptic, abaxially glaucous due to pubescence. Carpel number 20–40.)

2.b. Fruit syncarpous, woody with dehiscence circumscissile 3
(+ Stamen appendage short (female stage of the flowers) and stamens completely detaching from flower (male stage of the flowers) = section *Talauma* subsection *Talauma*)

3.a. Lamina length 18–20 cm. Carpel number 22–84 ..... ***M. dodecapetala*** (LA)
(+ Gynoecium and fruit glabrous. Lamina elliptic to obovate, never orbicular, oblong or oblong-elliptic.)

3.b. Lamina length 5–18 cm. Carpel number 5–27 4

4.a. Lamina widely obovate to orbicular. Carpel number 15–27..... ***M. orbiculata*** (C)
(+ Gynoecium and fruit pubescent)

4.b. Lamina oblong-elliptic to widely elliptic. Carpel number 5–20 5

5.a. Carpel number 8–20. Lamina oblong, oblong-elliptic (to elliptic), coriaceous. Gynoecium pubescent, not persisting in the fruit. .....***M. oblongifolia*** (C)

5.a. Carpel number 5–8. Lamina elliptic to widely elliptic, coriaceous. Gynoecium and fruit glabrous. ..... ***M. minor*** (C)

6.a. Pubescence on abaxial side of (young) leaves conspicuous, dense..... 7

6.b. Pubescence on abaxial side of (young) leaves absent or inconspicuous 9

[1] Whenever pubescence is used for identification, this is to be studied on young structures. Hence, it is recommended to observe the leaves and stems, or if present: perulae, peduncle and pedicle, at the tip of the branches. When *in situ*: manually open a pair of stipules (preferably near to opening, i.e. the largest pair of stipules within reach) and look for pubescence on the petiole, stem, and abaxial side of the young leaf and the new pairs of stipules within. If possible, look at younger structures that are exposed to sunlight; exceptionally structures have been found to be glabrous, presumed to be correlated to their more shaded location on the tree.
[2] In red: morphological characteristics that need more data given discrepancies between observations *in situ* or on photos/notes acquired via the collaboration with the Cuban botanists, and species descriptions.
[3] Leaf texture: three categories were given here: coriaceous, subcoriaceous and papery; however, in reality this follows a more continuous scale; A. Palmarola distinguishes more categories, R.A. Howard only mentions coriaceous native *Magnolia* species in the Caribbean.
[4] In blue: deeper classification of the species with the listed morphological synapomorphies.

7.a. Pubescence long: villose. ..... ***M. domingensis*** (DR+H?)
(+ Pubescence is found on the full abaxial side of the leaves, the petioles, the stipules, young branches; Leaves are widely elliptic to orbicular, coriaceous, with a rounded to even slight emarginate apex. Carpel number 14.)

7.b. Pubescence short: sericeous or tomentose 8

8.a. Pubescence densely sericeous, resulting in a golden/silvery coloration. Leaf apex acute to acuminate. Lamina ovate, ovate-elliptic, subcoriaceous. Carpels 15–18.
..... ***M. splendens*** (PR)

8.b. Pubescence densely short-tomentose, resulting in a yellow/brown, on abaxial sides of very young leaves sometimes golden, coloration. Leaf apex obtuse, rounded, truncate, shortly cuspidate or occasionally emarginate. Lamina elliptic/obovate to widely elliptic/obovate, coriaceous. Carpel number 10–30..... ***M. pallescens*** (DR)

9.a. Pubescence conspicuously present on the stipules, the petioles, the perules, the peduncle and pedicle 10

9.b. Conspicuous pubescence absent on the abaxial sides of the leaves, the stipules, the petioles, the perules, the peduncle and pedicle 11

10.a. Leaf apex acute to acuminate. Lamina (ovate-)elliptic to widely elliptic, coriaceous. Pubescence short-tomentose, giving a yellow-golden coloration. Carpel number 4–13
..... ***M. cristalensis*** (C)

10.b. Leaf apex emarginate, asymmetrically bilobed, or shortly cuspidate. Lamina elliptic, with two unequal halves, subcoriaceous. Pubescence white/golden-sericeous. Carpel number 18–21. ..... ***M. hamorii*** (DR)

11.a. Carpel number ≥15. ..... ***M. portoricensis*** (PR)
(+ Lamina widely elliptic, to widely obovate, subcoriaceous. Leaf apex acute, rounded or cuspidate.)

11.b. Carpel number <15 12

12.a. Lamina coriaceous which translates in the conduplicate prefoliation still being visible in the mature lamina. ..... ***M. ekmanii*** (H)
(+ Lamina generally elliptic. Leaf apex mostly short cuspidate, sometimes rounded. Carpel number 10–15.)

12.b. Lamina subcoriaceous which translates in the mature lamina being flat. 13

13.a. Areoles diameter ≤1 mm. ..... ***M. cubensis* subsp. *cubensis*** (C)
(+ Leaves generally elliptic. Carpel number 5–8.)

13.b. Areoles diameter 1–2 mm. ..... ***M. cubensis* subsp. *acunae*** (C)
(+ Lamina generally elliptic, yet more widely elliptic compared to *M. cubensis* subsp. *cubensis*. Carpel number 5–13.)

Table 1.2 The recorded locality and demographic information of the 15 Caribbean *Magnolia* taxa. An important yet, consequently unreported valuable number is that of the sampling effort undertaken, which can be expressed as number of days (#days) in the field actively searching for *Magnolia* trees. DR = Dominican Republic. #trees: number of trees explicitly counted.

Caribbean island	Species	Population	#trees	#days	Reference
Cuba	*M. cristalensis*	Cayo Mujeres	2	2	Palmarola et al. expedition 2017
Cuba	*M. cristalensis*	Cayo San José	2	1	Gómez-Hechevaría et al. expedition 2016
Cuba	*M. cristalensis*	Pico Cristal	38	6	Testé et al. expedition. 2018
Cuba	*M. cristalensis*	Cupeyal del Norte	90	15	Falcón et al. expedition 2016 + Becquer et al. expedition 2018 + Testé et al. expedition 2019
Cuba	*M. cristalensis*	El Toldo (= ±Yamanigüey)	48	4	Hernández et al. expedition 2019
Cuba	*M. cristalensis*	Mina Iberia	31	3	Palmarola et al. (2017)
Cuba	*M. cubensis* subsp. *acunae*	Lomas de Banao	70	10	Palmarola et al. (2018)
Cuba	*M. cubensis* subsp. *acunae*	Alturas de Trinidad (Topes de Collantes, Tres Palmas, Hanabanilla, Cumanayagua)	416	30	Palmarola et al. (2018)
Cuba	*M. cubensis* subsp. *cubensis*	Pico Caracas	50	10	Hernández et al. expedition 2016
Cuba	*M. cubensis* subsp. *cubensis*	Turquino	804	15	Palmarola et al. expedition 2014
Cuba	*M. cubensis* subsp. *cubensis*	Pico La Bayamesa	319	15	Palmarola et al. expedition 2014
Cuba	*M. cubensis* subsp. *cubensis*	El Gigante	62	15	Molina-Pelegrín et al. (2014)
Cuba	*M. cubensis* subsp. *cubensis*	Loma del Gato	1	2	Becquer et al. expedition 2018
Cuba	*M. cubensis* subsp. *cubensis*	Gran Piedra	60	20	Testé et al. (2019)
Cuba	*M. minor*	Cayo Mujeres	1	2	Palmarola et al. expedition 2017
Cuba	*M. minor*	Río Piloto (=±Cayo Mujeres)	1	1	Gómez et al. expedition 2018
Cuba	*M. minor* & *M. oblongifolia*	Pico Cristal	33	6	Testé et al. expedition 2018
Cuba	*M. minor* & *M. oblongifolia*	Cupeyal del Norte	210	15	Falcón et al. expedition 2016 + Becquer et al. expedition 2018 + Testé et al. expedition 2019
Cuba	*M. minor* & *M. oblongifolia*	Cayo Guam, Moa	34	5	Palmarola et al. expedition 2016
Cuba	*M. oblongifolia*	Cayo Guam	133	5	Testé et al. expedition 2016
Cuba	*M. minor* & *M. oblongifolia*	La Melba	19	5	Palmarola et al. expedition 2017
Cuba	*M. minor* & *M. oblongifolia*	Yamanigüey	83	3	Testé et al. expedition 2018

Cuba	*M. minor*	Piedra la Vela	20	4	Bécquer et al. expedition 2019
Cuba	*M. minor & M. oblongifolia*	Mina Iberia	83	3	Palmarola et al. (2017) + Testé et al. Expedition 2018
Cuba	*M. minor*	Naranjo del Toa	15	1	Hernández et al. expedition 2018
Cuba	*M. minor*	Guantánamo (Cañón del Yumurí)	9	2	Testé et al. expedition 2017
Cuba	*M. minor*	Guantánamo (Yumurí del Sur)	21	4	Rodríguez-Meno et al. expedition 2018
Cuba	*M. minor*	Guantánamo (Río Báez)	15	1	Testé et al. expedition 2017
Cuba	*M. minor*	Guantánamo (Río Minas)	2	1	Galano et al. expedition 2017
Cuba	*M. minor*	Guantánamo (la Delicias del Duaba)	3	1	Galano et al. expedition 2017
Cuba	*M. minor / M. oblongifolia*	Guantánamo (la Delicias del Duaba)	14	1	Testé et al. expedition 2017
Cuba	*M. oblongifolia*	Guantánamo (la Delicias del Duaba)	33	3	Galano et al. expedition 2017
Cuba	*M. minor*	Guantánamo (Yunque de Baracoa)	3	1	Galano et al. expedition 2019
Cuba	*M. minor / M. oblongifolia*	Guantánamo (Yunque de Baracoa)	4	1	Hernández et al. expedition 2018
Cuba	*M. minor*	Guantánamo (El Recreo)	4	1	Testé et al. expedition 2018
Cuba	*M. minor*	Guantánamo (Arroyo Yarey, Arroyo la Hoya, Mina la Hoya)	39	4	Galano et al. expedition 2018
Cuba	*M. orbiculata*	Pico Caracas	44	4	Molina-Peregrín et al. expedition 2017 and 2018
Cuba	*M. orbiculata*	Turquino	41	6	Molina-Peregrín et al. expedition 2017 and 2018
Cuba	*M. orbiculata*	Pico La Bayamesa	6	6	Molina-Peregrín et al. expedition 2017 and 2018
Cuba	*M. orbiculata*	Reserva Ecológica El Gigante	4	1	Molina-Peregrín et al. expedition 2018
Cuba	*M. virginiana* subsp. *oviedoae*	Majaguillar swamp	1350	30	Testé (2018)
Hispaniola (Haiti)	*M. ekmanii*	Morne Grand Bois	133	3	Veltjen et al. expedition 2015
Hispaniola (Haiti)	*M. ekmanii*	Morne Mansinte	21	2	Veltjen et al. expedition 2015
Hispaniola (Haiti)	*M. ekmanii*	Ti Letan	<10	0	Timyan pers. comm. (2018)
Hispaniola (Haiti)	*M. emarginata*	Type locality of Haiti unknown	0	0	
Hispaniola (Haiti)	*M. emarginata*	Pilate, Port Margot, Morne Maleuvre	0	1	Veltjen et al. expedition 2015
Hispaniola (Haiti)	*M. domingensis*	Petit Rivière de l'Artibonite	0	1	Veltjen et al. expedition 2015
Hispaniola (Haiti)	*M. domingensis*	Morne Colombeau (type locality)	0	0	
Hispaniola (DR)	*M. domingensis*	Loma Barbacoa	24	1	Veltjen et al. expedition 2015

Hispaniola (DR)	*M. domingensis*	Loma Rodríguez	50	1	Veltjen et al. expedition 2015
Hispaniola (DR)	*M. hamorii*	Cortico	51	1	Veltjen et al. expedition 2015
Hispaniola (DR)	*M. hamorii*	Cachote	52	1	Veltjen et al. expedition 2015
Hispaniola (DR)	*M. hamorii*	Loma Pie de Palo	?	?	Castillo et al. (2018)[1]
Hispaniola (DR)	*M. hamorii*	La Trocha de Pei	?	?	Castillo et al. (2018)[1]
Hispaniola (DR)	*M. hamorii*	Monteada Nueva	?	?	Castillo et al. (2018)[1]
Hispaniola (DR)	*M. hamorii*	Provincia Barahona	?	?	Castillo et al. (2018)[1]
Hispaniola (DR)	*M. pallescens*	Ebano Verde: Loma de la Sal	40	1	Veltjen et al. expedition 2015
Hispaniola (DR)	*M. pallescens*	Ebano Verde: Casabito	110	1	Veltjen et al. expedition 2015
Hispaniola (DR)	*M. pallescens*	Valle Nuevo: National Park	60	2	Veltjen et al. expedition 2015
Hispaniola (DR)	*M. pallescens*	Valle Nuevo: La Siberia	41	1	Veltjen et al. expedition 2015
Puerto Rico	*M. portoricensis*	Guilarte State Forest	35	2	Veltjen expedition 2015+2016b
Puerto Rico	*M. portoricensis*	Toro Negro State Forest	33	5	Veltjen expedition 2015+2016b
Puerto Rico	*M. portoricensis*	Maricao State Forest	51	3	Veltjen expedition 2015+2016b
Puerto Rico	*M. portoricensis*	Carite State Forest	10	5	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	Orocovis	24	1	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	Cerro Morales	25	1	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	La Silla de Calderon	20	1	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	Tres Picachos State Forest	25	1	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	Yauco	23	1	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	Cerro Roncador	31	1	Veltjen expedition 2016b
Puerto Rico	*M. portoricensis*	Bosque Del Pueblo	14	1	Veltjen expedition 2016b
Puerto Rico	*M. splendens*	El Yunque	187	13	Veltjen expedition 2015+2016b
Lesser Antilles	*M. dodecapetala*	Saint Vincent	32	4	Veltjen expedition 2016a
Lesser Antilles	*M. dodecapetala*	Saint Lucia	40	3	Veltjen expedition 2016a
Lesser Antilles	*M. dodecapetala*	Martinique	75	5	Veltjen expedition 2016a
Lesser Antilles	*M. dodecapetala*	Dominica	57	4	Veltjen expedition 2016a
Lesser Antilles	*M. dodecapetala*	Guadeloupe	47	5	Veltjen expedition 2016a

[1] In the publication of Castillo et al. 2018 more locality names of *M. hamorii* are mentioned and more datapoints within the known localities of *M. pallescens* in Ebano Verde and Valle Nuevo are visible on the published maps. However, only few locality names are expliclity mentioned, and for all GPS data points or localities number of counted, or estimated, individuals is not published. In 2021 a new expedition is planned to the Dominican Republic (See Chapter 7.4) where new localities shall be visited and/or discussed, as for now, e-mail communication goes slow.

1.4 Magnolias of Cuba

CLASSIFICATION: Cuba, the largest island of the Caribbean islands (109 880 km² (World Bank, 2019)), is home to seven of the fifteen described Caribbean *Magnolia* taxa. Three of them belong to *Cubenses*: *M. cristalensis*, *M. cubensis* subsp. *acunae* and *M. cubensis* subsp. *cubensis*; three of them to *Talauma*: *M. minor*, *M. oblongifolia* and *M. orbiculata*; and one to section *Magnolia*: *M. virginiana* subsp. *oviedoae.*

TAXONOMY: Taxonomically the seven taxa were recently revised in the PhD study of Alejandro Palmarola Bejerano (Palmarola-Bejerano et al., 2008; Palmarola et al., 2016), taking into consideration the past taxonomic history.

MORPHOLOGY: The species are morphologically well described in the PhD thesis of Alejandro Palmarola Bejerano (Palmarola-Bejerano et al., 2008; Palmarola et al., 2016). The three Cuban *Talauma* species can be distinguished from each other morphologically as *M. oblongifolia* has a distinct oblong-elliptic leaf shape, compared to the orbicular leaves from *M. orbiculata* and the widely elliptic leaves of *M. minor*. *Magnolia oblongifolia* has pubescence on its gynoecium that does not persist in the fruit; while *M. orbiculata* is characterised by pubescence on its gynoecia that does persist in the fruits, and *M. minor* shows glabrous gynoecia and fruits. *Magnolia oblongifolia* has a more rhombic fruit shape, compared to the ellipsoid fruits of *M. minor* and *M. orbiculata*. The ellipsoid fruits of *M. minor* and *M. oblongifolia* can be distinguished from each other by number of carpels: *M. orbiculata* has between 20 and 27 carpels and *M. minor* between five and eight. Lastly, the small tree size of *M. oblongifolia* (up to 12 m) contrasts with the larger tree size of *M. minor* and *M. orbiculata* (up to 20–25 m). A selection of morphological variation in the three Cuban *Talauma* species is presented in Figure 1.3. The three Cuban *Cubenses* species are distinguishable from the other *Cubenses* species, given their elliptic leaves with a more often acute leaf apex (in contrast to the obtuse-rounded leaf apex of other *Cubenses* species) in combination with small number of carpels (5–13). *Magnolia cristalensis* has a more coriaceous V-shaped lamina compared to *M. cubensis*: the lamina of *M. cubensis* is flat and less coriaceous. *Magnolia cristalensis* has clear pubescence on newly developed parts, while the two subspecies of *M. cubensis* are (mostly) glabrous. Morphological discrimination among the two subspecies of *M. cubensis* is difficult (Hernández Rodríguez, 2014): *M. cubensis* subsp. *cubensis* and *M. cubensis* subsp. *acunae* can only be clearly distinguished based on their leaf areole size, which is significantly smaller in *M. cubensis* subsp. *cubensis* (<1 mm) than in *M. cubensis* subsp. *acunae* (>1 mm and ≤ 2mm). A selection of morphological variation in the three Cuban *Cubenses* species is presented in Figure 1.4.

DISTRIBUTION: The seven Cuban *Magnolia* taxa occur in four main localities: 1) the Majaguillar swamp in the province of Matanzas (i.e. *M. virginiana* subsp. *oviedoae*); 2) the Guamuhaya mountains in the provinces Cienfuegos, Sanctí Spíritus and Villa Clara (i.e. *M. cubensis* subsp. *acunae*); 3) the Sierra Maestra in the provinces Granma and Santiago de Cuba (i.e. *M. cubensis* subsp. *cubensis* and *M. orbiculata*); 4) Nipe-Sagua-Baracoa mountains in the provinces Holguín, Guantánamo and Santiago de Cuba (i.e. *M. cristalensis*, *M. oblongifoli*a and *M. minor*). Map 1.2 depicts the four main regions where the species can be found. Map 1.3–1.5 show the known populations in greater detail.

DEMOGRAPHICS: All seven taxa are under monitoring of the Cuban NGO Planta!, the National Botanical Garden (University of Havana) and the University of Havana, with whom we work closely together. The group of investigators and students working on the species is large, which also results in recent and persistent data acquisition on the demography of the different populations. Current data acquisition is still ongoing, especially for the populations in the Nipe-Sagua-Baracoa Mountains. See Table 1.2 for number of known localities and the explicit number of *Magnolia* trees recorded at these localities.

CONSERVATION: The IUCN Red List status of all species was recently revised (González Torres et al., 2016). Planta! (website: https://www.planta.ngo/en/) already works intensively with the conservation of *M. cubensis* (Hernández and Palmarola, 2016). In 2018, the red list project also started a collaboration with Planta! (website: https://www.theredlistproject.org). This initiative integrates economic, conservation and community empowerment focused on threatened island plant species.

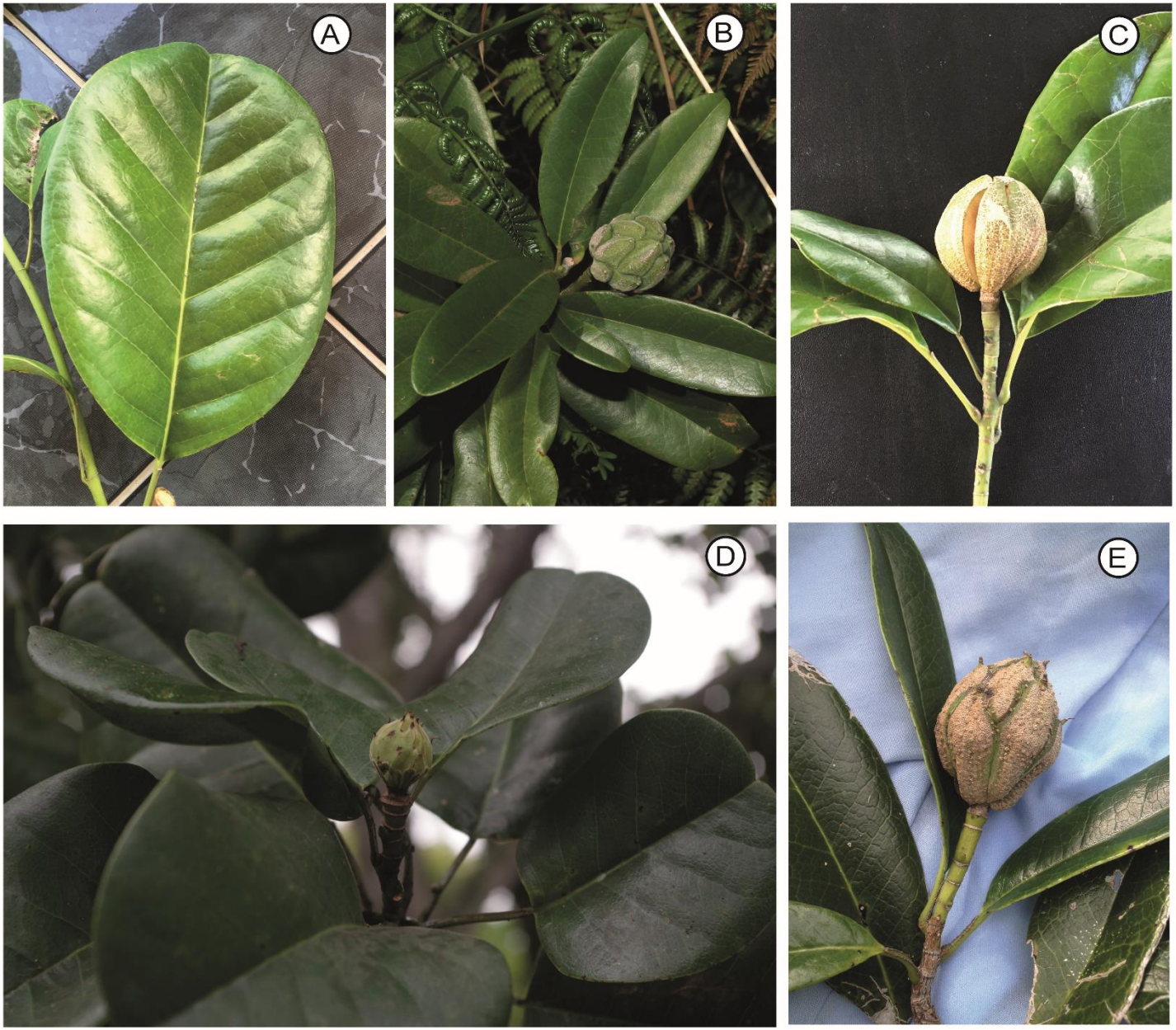


Figure 1.3 Morphology of the three Cuban *Talauma* species. **A**: widely elliptic leaves of *M. minor*. **B**: oblong-elliptic leaves of *M. oblongifolia*. **C**: glabrous, ellipsoid fruit of *M. minor*. **D**: orbicular leaves of *M. orbiculata* and the pubescent gynoecium. **E**: glabrous, rhombic fruit of *M. oblongifolia*. Photo credits: A, C, E: Ernesto Testé Lozano; B: Alejandro Palmarola Bejerano; D: Emily Veltjen.

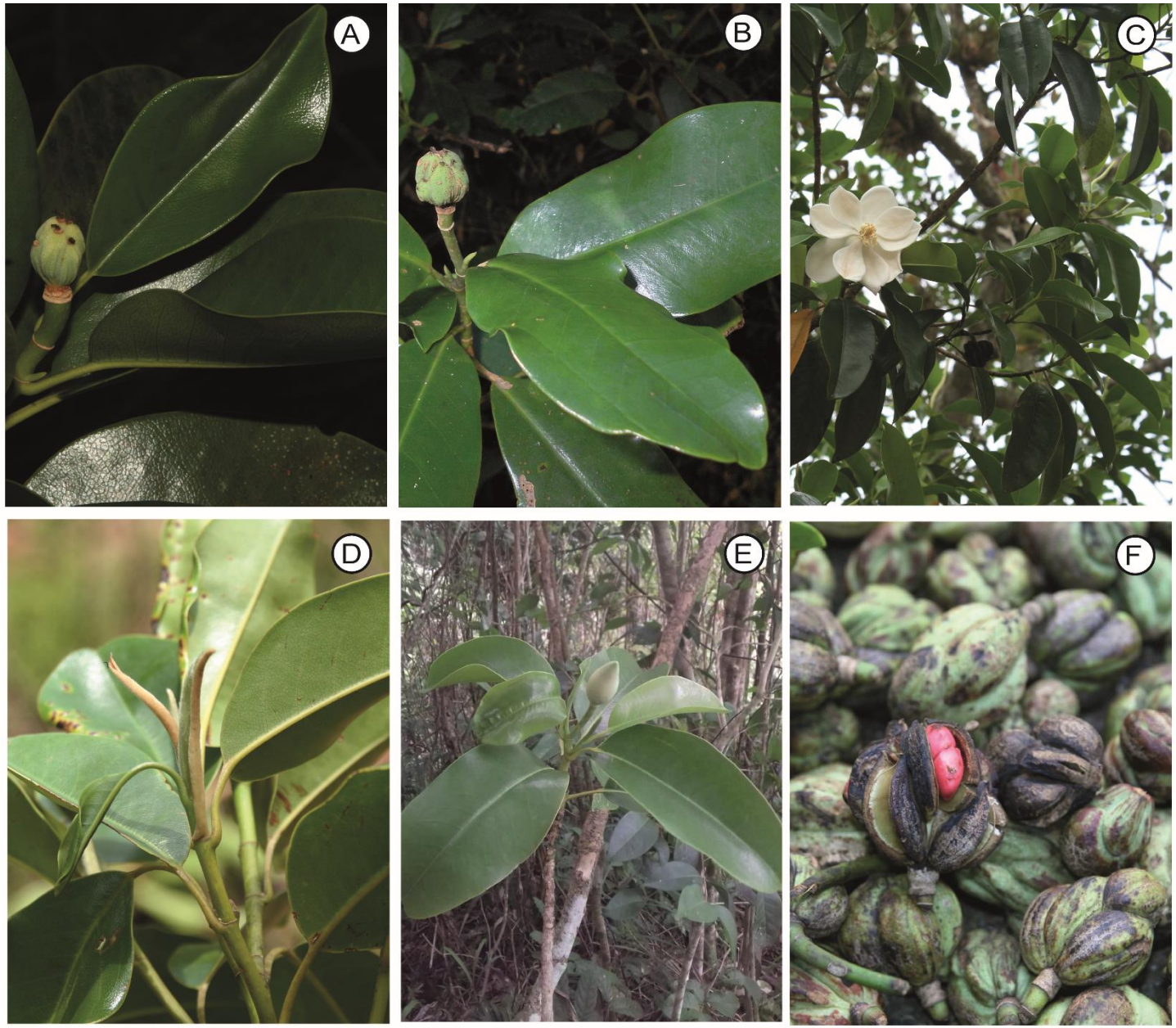
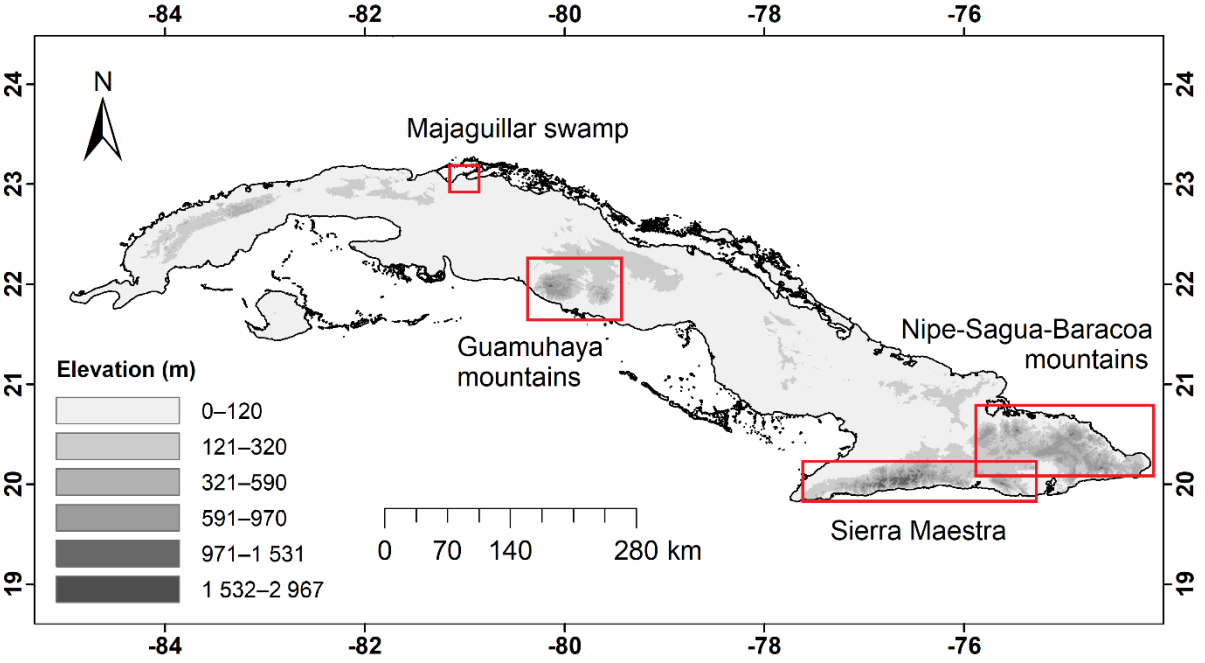


Figure 1.4 Morphology of the three Cuban *Cubenses* taxa. **A**: coriaceous, V-shaped lamina of *M. cristalensis* with acute apex. **B**: less coriaceous and flat lamina of *M. cubensis* subsp. *cubensis*. **C**: less coriaceous leaves and flat lamina of *M. cubensis* subsp. *acunae* and a flower in the male phase. **D**: pubescence on the stipules and newly exposed leaves in *M. cristalensis*. **E**: glabrous newly exposed leaves and flower bud of *M. cubensis* subsp. *acunae*. **F**: fruits of *M. cubensis* subsp. *cubensis* exposing the red seeds. Photo credits: A: Mikhail S. Romanov; B: José Luis Gómez; C, F: Alejandro Palmarola Bejerano; D: Banessa Falcón; E: Majela Hernández.

Map 1.2 Distribution of *Magnolia* in Cuba. The seven Cuban *Magnolia* taxa occur in four main localities: 1) the Majaguillar swamp in the province of Matanzas: *M. virginiana* subsp. *oviedoae*; 2) the Guamuhaya mountains in the provinces Cienfuegos, Sancti Spíritus and Villa Clara: *M. cubensis* subsp. *acunae*; 3) the Sierra Maestra mountains in the provinces Granma and Santiago de Cuba: *M. cubensis* subsp. *cubensis* and *M. orbiculata*; 4) Nipe-Sagua-Baracoa mountains in the provinces Holguín, Guantánamo and Santiago de Cuba: *M. cristalensis*, *M. oblongifolia* and *M. minor*. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: Fick and Hijmans (2017); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



Map 1.3 Distribution of *M. cubensis* subsp. *acunae* in the Guamuhaya Mountains in the provinces Cienfuegos, Sancti Spíritus and Villa Clara of Cuba. Management categories of the Protected Areas (González-Torres et al., 2016) and corresponding IUCN Protected Area Management Category (Dudley, 2008): **ER:** Ecological Reserve = Reserva Ecológica (II); **NPL:** Natural Protected Landscape = Paisaje Natural Protegido (V). Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (CNAP, 2014); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

Note: in this PDF version of the PhD dissertation, the maps of the Cuban Magnolias (Fig. 1.3–1.5) are omitted due to the sensitivity of the locality data.

Map 1.4 Distribution of *M. cubensis* subsp. *cubensis* and *M. orbiculata* in the Sierra Maestra in the provinces Granma and Santiago de Cuba of Cuba. Management categories of the Protected Areas (González-Torres et al., 2016) and corresponding IUCN Protected Area Management Category (Dudley, 2008): **ER**: Ecological Reserve = Reserva Ecológica (II); **NP**: National Park = Parque Nacional (II); **NPL**: Natural Protected Landscape = Paisaje Natural Protegido (V). Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (CNAP, 2014); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

Note: in this PDF version of the PhD dissertation, the maps of the Cuban Magnolias (Fig. 1.3–1.5) are omitted due to the sensitivity of the locality data.

Map 1.5 Distribution of *M. cristalensis*, *M. oblongifolia* and *M. minor* in the Nipe-Sagua-Baracoa Mountains in the provinces Holguín, Guantánamo and Santiago de Cuba, of Cuba. Management categories of the Protected Areas (González-Torres et al., 2016) and corresponding IUCN Protected Area Management Category (Dudley, 2008): **DNE**: Distinct Natural Element = Elemento Natural Destacado (III); **NP**: National Park = Parque Nacional (II); **NPL**: Natural Protected Landscape = Paisaje Natural Protegido (V); **PAMR**: Protected Area of Managed Resources = Área Protegida de Recursos Manejados (VI). Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (CNAP, 2014); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

Note: in this PDF version of the PhD dissertation, the maps of the Cuban Magnolias (Fig. 1.3–1.5) are omitted due to the sensitivity of the locality data.

1.5 Magnolias of Hispaniola

CLASSIFICATION: Hispaniola, the second largest island of the Caribbean islands (76 420 km² (World Bank, 2019)), is home to five different *Magnolia* species, which all belong to *Cubenses*: *M. domingensis*, *M. ekmanii*, *M. emarginata*, *M. hamorii* and *M. pallescens.*

TAXONOMY: Howard (1948) summarizes the taxonomic history of the species, which remains unchanged since then. However, there are some questions raised after a revision of the species distributions and morphology, using herbarium collections, undertaken within the framework of this PhD. Firstly: *Magnolia domingensis* provides an interesting case as the lectotype for this species was assigned by Howard (1948) to *G.V. Nash 1081* (NY), collected in 1905 on "The Road from Camp No.1 to La Barrière Couchant" in Haiti. The exact location of this collection is unknown until this date. One additional Haitian collection of *Magnolia* is currently identified as *M. domingensis*: *E.L. Ekman H2810* (S) collected a *Magnolia* in Port Margot on the slopes of Morne Maleuvre in 1924. The species description of Urban (1914) for *M. domingensis*, mentioning densely villose young branches, was matched with *Magnolia* populations found on Loma Barbacoa and Loma Rodríguez of the Dominican Republic. Hence these collections are all currently labelled *M. domingensis*. On the one hand, the identifiable collection sites for the species are in the same mountain chain, namely the Cordillera Central of the Dominican Republic which is connected to the Massif du Nord in Haiti. At first sight this does not raise any questions, as it appears a case of a more widespread distribution of the species throughout the mountain chain on the same island. On the other hand, *M. pallescens* interrupts this widespread distribution, making the two *M. domingensis* populations disjunct in its distribution. Hence the identification of the Haitian *M. domingensis* and the Dominican *M. domingensis* under the same species name is questionable. Secondly, the taxonomical concept of *M. emarginata* is scarcely documented: the lectotype for this species was assigned by Howard (1948) to the collection *Ekman H4439* (S), collected in 1925 on the "Massif du Nord, Anse-á-Foleur, top of Morne Colombeau" in Haiti. This collection site is geographically close to Morne Maleuvre, labelled as *M. domingensis* (*E.L. Ekman H2810*). There is one additional collection labelled *M. emarginata*: *E.L. Ekman H3442* (S) in the Massif de Cahos, Pétite-Rivière de l'Artibonite, Pérodin, near Ingram. Massif du Cahos is part of what is generally considered as the Montagnes Noires in Central Haiti. Previously, E.L. Ekman named all its collections in Hispaniola *M. domingensis*, which can also be retrieved from the original label of this herbarium specimen. The *E.L. Ekman H3442* specimen lacks the emarginate leaf apices as described from the type specimen of *M. emarginata* and it also lacks the villose hairs as described for *M. domingensis*. Given that in total there are only four collections in the Massif du Nord and Massif du Cahos in Haiti, of which the locality of the type collection of *M. domingensis* is unclear, it is hard to revise and determine the morphological or geographical

species concepts for *Magnolia* in these two Haitian mountain chains. The lack of new herbarium collections or other forms of documentation of the *Magnolia* species of north and central Haiti since 1925 makes it impossible to revise the species taxonomically with the available information. The questions on the Haitian *Magnolia* species from the Massif du Nord and Massif du Cahos are in contrast with the *Magnolia* populations that have a more elaborate herbarium record collected in the Massif de la Hotte of Haiti (i.e. *M. ekmanii*), the Cordillera central of the Dominican Republic (i.e. *M. pallescens* and *M. domingensis*) and the Sierra Bahoruco of the Dominican Republic (i.e. *M. hamorii*).

MORPHOLOGY: The morphology of the five Hispaniolian species is provided in Howard (1948) and during the 2015 expedition undertaken within the framework of this PhD (Appendix 1.5 and 1.6), we were able to document and verify this morphology of four species: (the Dominican populations of) *M. domingensis*, *M. ekmanii*, *M. hamorii* and *M. pallescens*. All the described Hispaniolian Magnolias have rounded, apiculate or emarginate leaf apices, in contrast to Puerto Rican or Cuban *Cubenses* Magnolias that show acute or acuminate leaf apices (Howard, 1948). The Dominican populations of *M. domingensis* can be differentiated morphologically from other *Cubenses* species by its widely elliptic to orbicular leaves with dense, villose pubescence. In the original description of *M. domingensis* the carpels are described as pubescent persisting into the fruiting stage, yet this is not verified for the Dominican population of *M. domingensis* (see Figure 1.5D). Carpels of the other *Cubenses* species are described and verified to be glabrous. *Magnolia pallescens* also has pubescence on its leaves; however, hairs of *M. pallescens* are tomentose compared to the villous hairs of *M. domingensis*. Even more so the leaves of *M. pallescens* are elliptic, while those of *M. domingensis* are widely elliptic to orbicular. *Magnolia hamorii* and *M. ekmanii* also have elliptic leaves, but compared to *M. domingensis* and *M. pallescens,* these two species have glabrous leaves (with the exception of *M. hamorii* that can occasionally still have some pubescence on its petiole and base of the midvein on the abaxial side of the leaf). *Magnolia hamorii* is distinguishable from the other *Cubenses* species, given its emarginate leaf apex and unequal leaf lobes; yet, *in situ* it was also apparent that some leaves did not express this morphology as distinctly. *Magnolia ekmanii* has glabrous, elliptic leaves with a rounded or apiculate leaf apex and fruits with ca. 10 carpels, which is significantly less than the 18–30 carpels per fruit reported for the Dominican *Cubenses* species *M. hamorii* and *M. pallescens* (Castillo et al., 2018; Howard, 1948). The morphology of the four species documented *in situ* is illustrated in Figures 1.5–1.8. Looking at the morphologically distinguishing characters summarised for the four species we were able to study and document *in situ* and from literature, a short overview of the visible characteristics for the four species of the North and Central of Haiti as observed on the herbarium collections and in relevant literature, is provided as well. All four collections

have elliptic leaves; whereby the collection of *E.L. Ekman H4339* (i.e. the type of *M. emarginata* from the Massif du Nord) has glabrous leaves that are more widely elliptic - compared to the three other collections - with emarginate leaf apices and leaves with lobes of an equal size; the collection of *E.L. Ekman H3442* (labelled *M. emarginata* from the Massif du Cahos) has glabrous, elliptic leaves with a rounded leaf apex and glabrous fruits; the collection of *G.V. Nash 1081* (i.e. type of *M. domingensis* ["Massif undetermined"]) has sericeous hairs on its leaves, stems and fruit; and the collection of *E.L. Ekman H2810* (labelled *M. domingensis* from the Massif du Nord) has elliptic, glabrous leaves with apiculate apices. The four Haitian herbarium vouchers of the Massif du Nord and the Massif du Cahos are depicted in Figure 1.9.

DISTRIBUTION: The five Hispaniolian *Magnolia* species occur in five main localities: 1) the Massif de La Hotte in Southern Haiti (i.e. *M. ekmanii*); 2) the Massif du Nord in Northern Haiti (i.e. *M. emarginata* (*E.L. Ekman H4339*) and *M. domingensis* (*E.L. Ekman H2810*)); 3) the Massif de Cahos in Central Haiti, part of the Montaignes Noires in Central Haiti (i.e. *M. emarginata* (*E.L. Ekman H3442*)); 4) the Cordillera Central in Central Dominican Republic (i.e. *M. pallescens* and the Dominican populations of *M. domingensis*); 5) the Sierra Bahoruco (i.e. *M. hamorii*). Map 1.6 depicts all the localities that are currently known for the five Hispaniolian *Magnolia* species. In the Massif de La Hotte there are currently three populations known of *M. ekmanii*: Morne Mansinte, Morne Grand Bois and a more recent expedition in 2018 has also found trees at Ti Letan, which is 5 km from what is presumed to be Morne-Pain-de-Sucre, the type-locality of the species on the herbarium voucher of *E.L. Ekman H10395* (GH, S) (Joel Timyan, Societé Audubon de Haiti, pers. comm.). In the Sierra Bahoruco there are two localities that were visited in the expedition of 2015, close to each other: Cortico and Cachote. There are four more localities reported for this species in Castillo et al. (2018): Loma Pie de Palo, La Trocha de Pei, Monteada Nueva and Provincia Barahona. All six localities occur in the Monumento Natural Padre Miguel Domingo Fuerte. *Magnolia pallescens* in the Cordillera Central occurs in the Scientific Reserve Ebano Verde (La Reserva Científica Ebano Verde) and the National Park Valle Nuevo. The Dominican populations of *M. domingensis* are known from two localities: Loma Barbacoa and Loma Rodríguez, both in the National Park Padre Luis Quinn, within an area that has the status "Área de protección estricto de Loma Barbacoa" (Castillo et al., 2018).

DEMOGRAPHICS: Within the framework of this PhD one expedition to Hispaniola was undertaken in 2015. We documented the number of individuals for populations of *M. ekmanii*, *M. hamorii*, *M. pallescens* and the Dominican populations of *M. domingensis* and were not able to find the historical localities: Morne Maleuvre and Petite Rivière De l'Artibonite. See Table 1.2 for number of known localities and the explicit number of *Magnolia* trees recorded at these localities. See Figures 1.10–1.13 for the number of individuals encountered per DBH class.

CONSERVATION: Haiti National Trust (website: https://www.haititrust.org) identified the Morne Grand Bois as one of the biodiversity hotspots of Haiti and acquired the land for the conservation of its biodiversity, including *M. ekmanii* to establish it as a private nature reserve. They work closely together with the Société Audubon de Haiti (website: http://audubonhaiti.org), which since 2018 is engaged in conservation management of *M. ekmanii* at Grand Bois together with Fundación PROGRESSIO from the Dominican Republic (website: http://www.fundacionprogressio.com/) by means of growing the plants *ex situ* in two nurseries. Next to the execution of conservation work in Haiti, Fundación PROGRESSIO is involved in the management of La Reserva Científica Ebano Verde in the Dominican Republic, where it has focused for many years on the *ex situ* propagation of *M. domingensis*, *M. pallescens* and *M. hamorii*. Ramón Castillo provided an overview of Dominican *Magnolia* germination percentages during the third symposium on the family Magnoliaceae in Cuba (2016) (website presentation: https://www.magnoliasociety.org/page-1813213). In 2018, the Jardín Botánico Nacional, Dr. Rafael M. Moscoso and Fundación PROGRESSIO published a book: "*Plan de acción de conservación integrada de las Magnolias (Magnoliaceae) amenazadas de República Dominicana* – Magnolia domingensis, M. hamorii *y* M. pallescens" (Castillo et al., 2018). It summarizes the morphology of the Dominican *Magnolia* species, their distribution, threats and conservation strategies (website: https://issuu.com/magikpublicidad/docs/muestra_libro_funadacion_progressio). In this book, it is reported that in 2004, 2500 *M. hamorii* trees were planted in Cachote. The planted trees are still being monitored as a survival rate of 70% is reported.

Figure 1.5 Morphology of *M. domingensis* from the Dominican Republic. **A**: leaves with dense villose hairs at the abaxial side. **B**: plump appearance of the thick coriaceous leaves and stipules. Stipules also densely covered with the villose hairs. Gynoecium without any pubescence, although expected for the species. **C**: open flower in the male phase with the tips of the stamens embedded in the gynoecium, characteristic for the *Cubenses*. **D**: fruit without any pubescence, although expected for the species. Photo credits: A: Emily Veltjen; B–D: Ramón Elias Castillo.

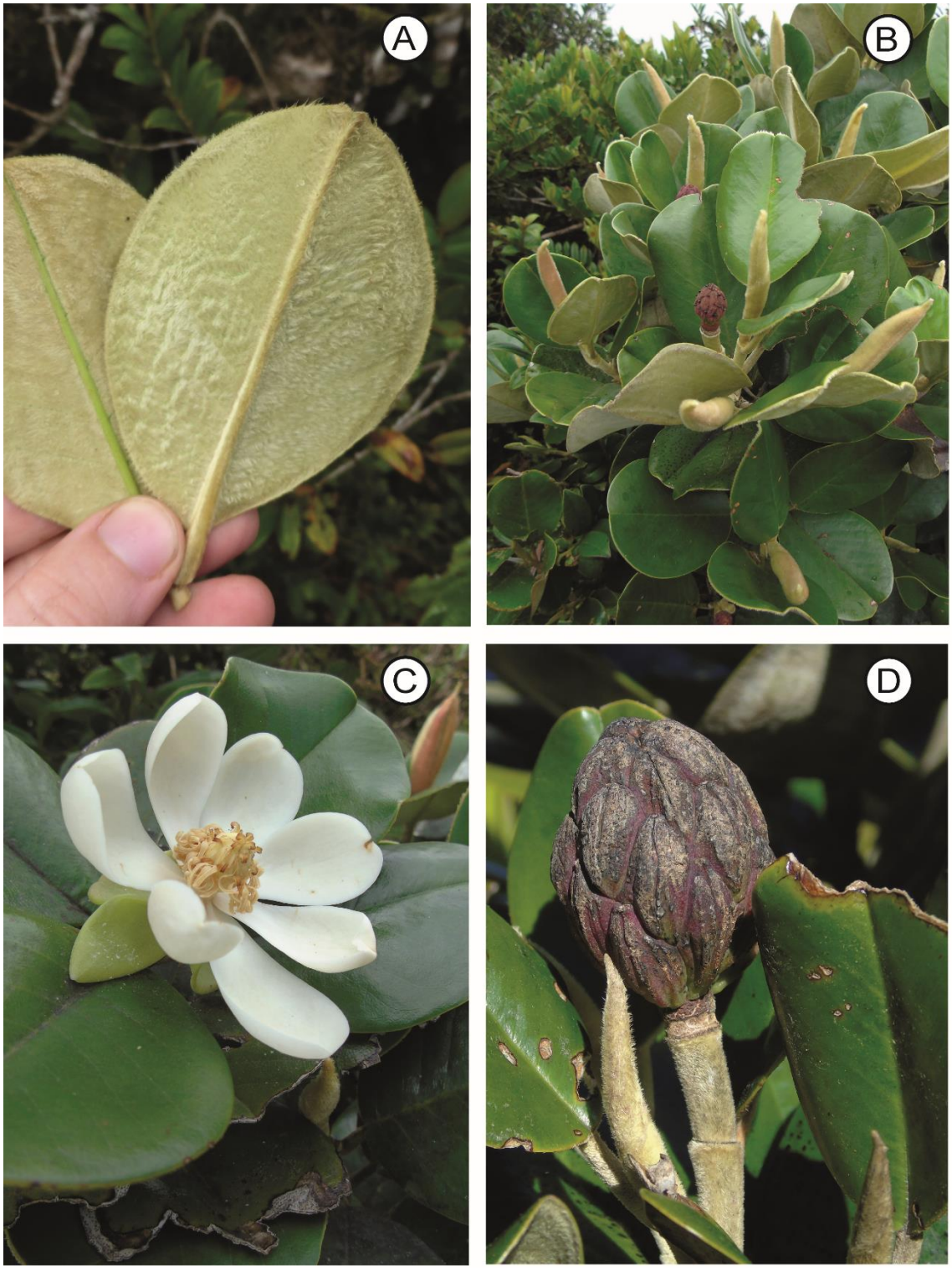


Figure 1.6 Morphology of *M. ekmanii* from Haiti. **A**: glabrous leaves with apiculate apex. **B**: general habit of *M. ekmanii* leaves. **C**: open(ed?) flower in the female phase. **D**: fruit. Photo credits: A, B: Emily Veltjen; C: Eladio Fernández; D: Jean-François Orilién Beauduy.

Figure 1.7 Morphology of *M. hamorii* from the Dominican Republic. **A**: glabrous leaf with the unequal leaf halves and emarginate leaf apex. **B**: general habit of *M. hamorii* leaves, illustrating conduplicate leaf prefoliation. **C**: flower in the male phase. **D**: fruits that were removed and dried, exposing the reddish seeds. Photo credits: A, B: Emily Veltjen; C, D: Ramón Elias Castillo.

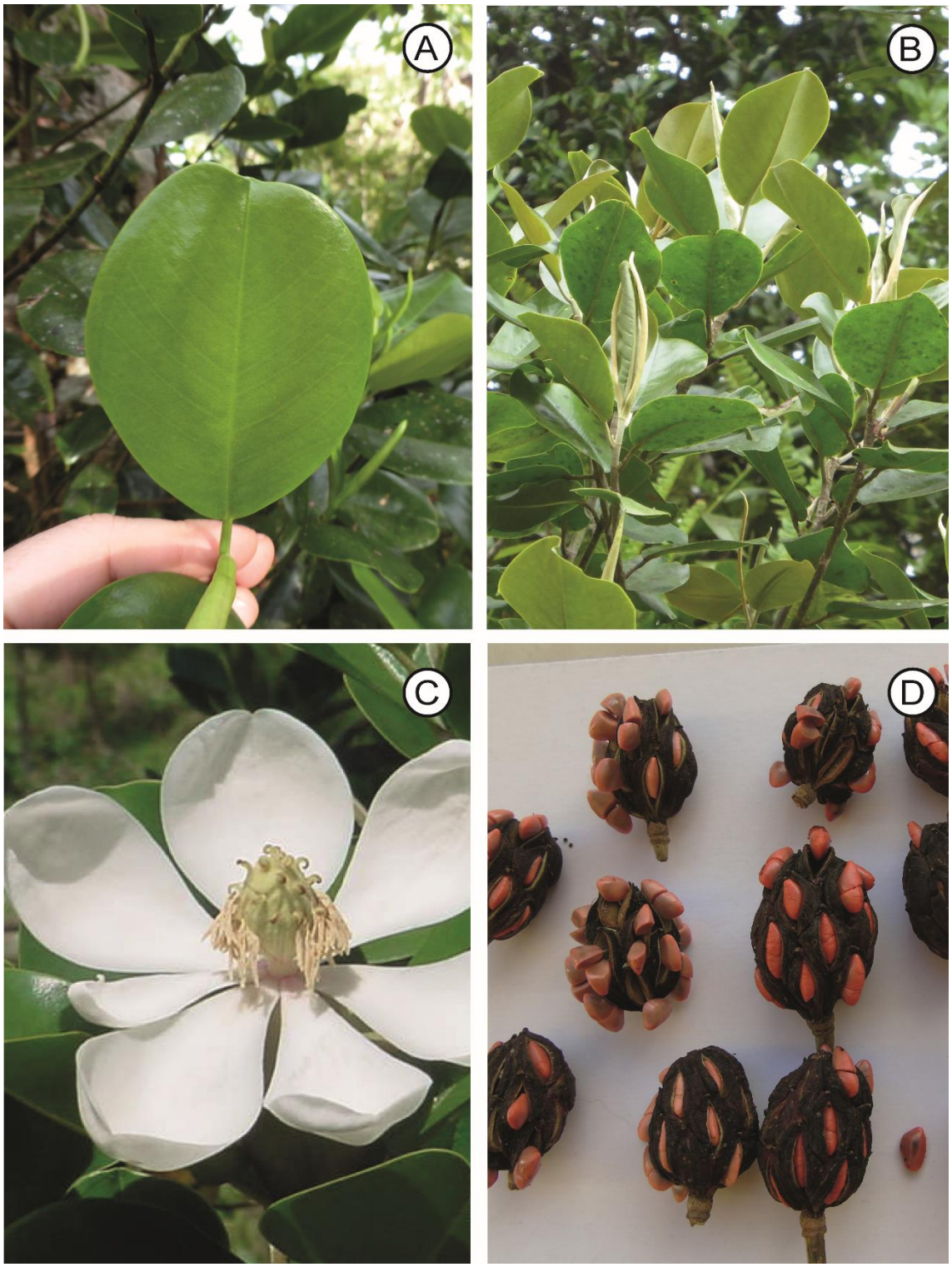


Figure 1.8 Morphology of *M. pallescens* from the Dominican Republic. **A**: elliptic, abaxially sericeous pubescent leaves. **B**: Branches with two dried fruits. **C**: forced-open, immature flower showing immature stamens and pistils. The brownish colouration on the peduncle is due to the dense sericeous pubescence. **D**: glabrous fruit. Photo credits: A–D: Emily Veltjen.

Figure 1.9 Morphology of *M. domingensis* and *M. emarginata* in the Massif de Cahos in Central Haiti, part of the Montaignes Noires and Massif du Nord of Haiti. **A**: Lectotype of *M. domingensis*: G.V. Nash 1081 (NY), Road from Camp No.1 to La Barrière Couchant (1905). **B**: Lectotype of *M. emarginata*: E.L. Ekman H4339 (S), Massif du Nord, Anse-á-Foleur, top of Morne Colombeau (1925). **C**: *M. domingensis*: E.L. Ekman H2810 (S), Départ. Du Nord, Port Margot, Morne Maleuvre (1924). **D**: *M. emarginata*: E.L. Ekman H3442 (S), Massif du Cahos, Pétite-Rivière De l'Artibonite, Pérodin, near Ingram (1925).

Map 1.6 Distribution of *Magnolia* in Hispaniola. The five *Magnolia* species occur in five main localities: 1) the Massif de La Hotte in Southern Haiti (i.e. *M. ekmanii*); 2) the Massif du Nord in Northern Haiti (i.e. *M. emarginata* (E.L. Ekman H4339) and *M. domingensis* (E.L. Ekman H2810)); 3) the Massif de Cahos in Central Haiti, part of the Montaignes Noires (i.e. *M. emarginata* (E.L. Ekman H3442)); 4) the Cordillera Central in Central Dominican Republic (i.e. *M. pallescens* and the Dominican populations of *M. domingensis*); 5) the Sierra Bahoruco (i.e. *M. hamorii*). Management categories of the Protected Areas (Reyna Alcántara and Polonia Martínez, 2012) and corresponding IUCN Protected Area Management Category (Dudley, 2008): **NP**: National Park (II); **NPL**: Natural Protected Landscape (V); **SR**: Scientific Reserve = Reserva Científica (Ia). Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (Caribbean protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

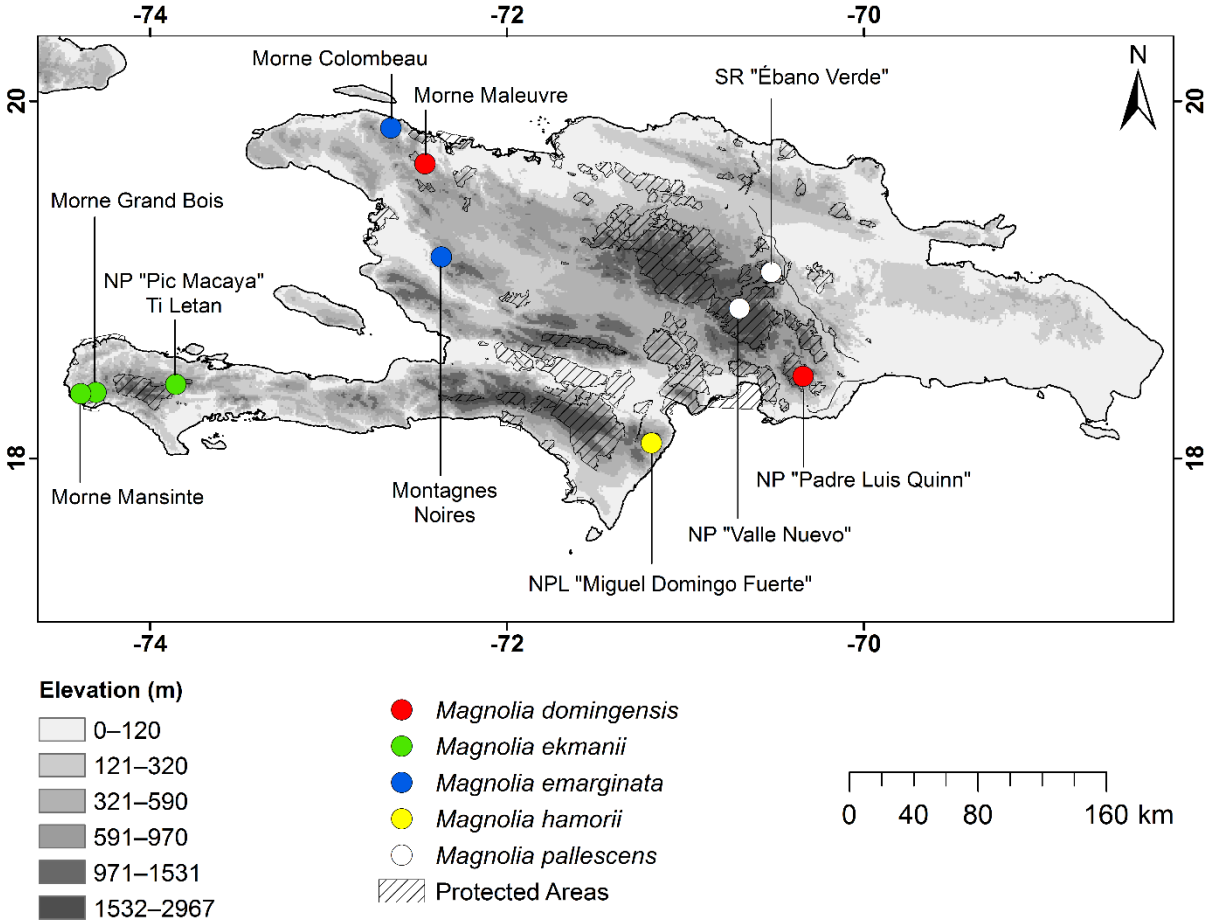


Figure 1.10 DBH (in cm) classes of the two populations of *Magnolia domingensis* measured during the 2015 expedition in the Dominican Republic.

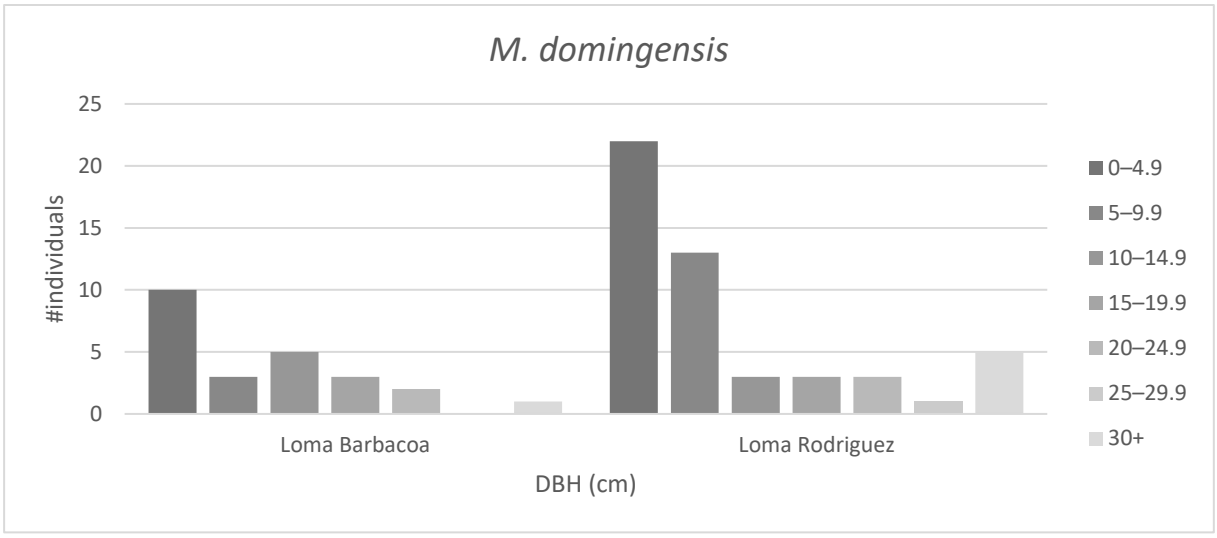


Figure 1.11 DBH (in cm) classes of the two populations of *Magnolia ekmanii* measured during the 2015 expedition in Haiti.

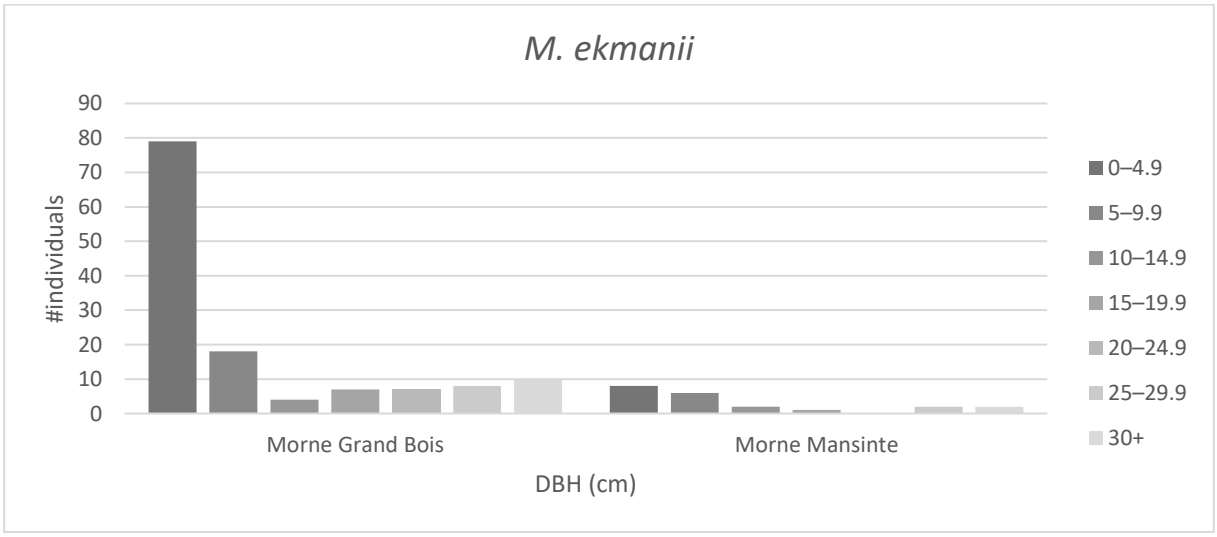


Figure 1.12 DBH (in cm) classes of the two populations of *Magnolia hamorii* measured during the 2015 expedition in the Dominican Republic.

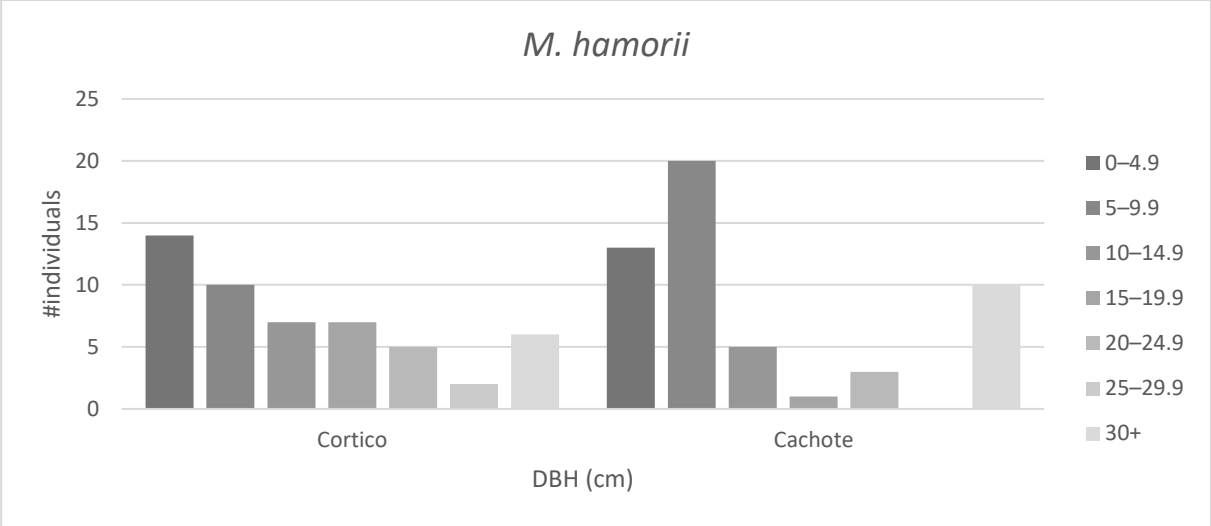
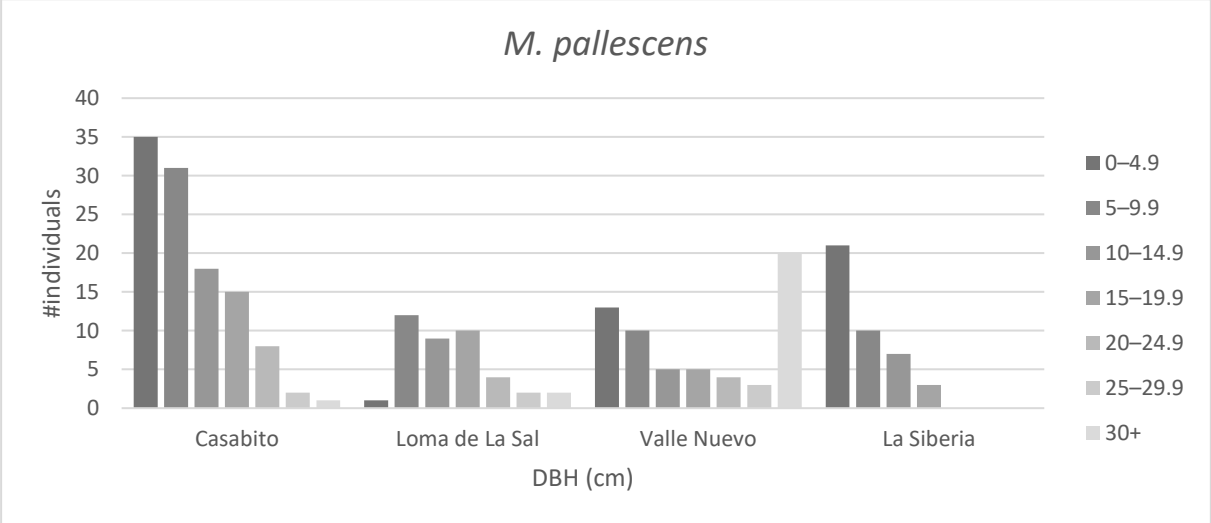


Figure 1.13 DBH (in cm) classes of the four populations of *Magnolia pallescens* measured during the 2015 expedition in the Dominican Republic.



1.6 Magnolias of Puerto Rico

CLASSIFICATION: Puerto Rico, although being a smaller Caribbean island (8870 km² (World Bank, 2019)), is home to two different *Magnolia* species, which both belong to *Cubenses*: *M. portoricensis* and *M. splendens*.

TAXONOMY: Howard (1948) provided the taxonomic history of the species, to which a neotype for *M. portoricensis* was added a recent publication (Santiago-Valentín et al., 2015). During the fieldwork of 2015 and 2016 both common names "jagüilla" (*M. portoricensis*) and "laurel sabino" or "laurel" (*M. splendens*) were still actively being used by locals.

MORPHOLOGY: The two species are distinguished from the other *Cubenses* species by the combination of two characters: acute leaf apices and a higher number of carpels (i.e. 20–25) (Howard, 1948). However, *in situ* observations showed that the leaves of *M. portoricensis* can also have rounded or short cuspidate tips. The leaves of both Puerto Rican species are also significantly less coriaceous than those of Hispaniola. The two Puerto Rican species can be distinguished from each other by the golden sericeous hairs present on the abaxial side of the ovate leaves and generally on newly developed organs (e.g. stipules, young stems) of *M. splendens*. *Magnolia portoricensis* is glabrous overall and its leaves are broadly elliptic. The morphology of *M. portoricensis* is illustrated in Figure 1.14 and the morphology of *M. splendens* is illustrated in Figure 1.15.

DISTRIBUTION: The species each occur on a different mountain on the island. *Magnolia portoricensis* is widespread throughout the Cordillera Central and *M. splendens* grows in El Yunque (see Map 1.7).

DEMOGRAPHICS: Within the framework of this PhD, two expeditions to Puerto Rico were undertaken. One followed immediately after the 2015 expedition to Hispaniola and was for the period of one week. However, the week proved to be inadequate for both the number of trees and number of populations to be sampled. Hence, in 2016 Puerto Rico was visited again for a duration of two months, after which an extensive sampling and monitoring could be executed. See Table 1.2 for number of sampled localities and the explicit number of *Magnolia* trees recorded at these localities. See Figures 1.16–1.17 for the number of individuals encountered per DBH class.

CONSERVATION: Populations of *M. portoricensis* occur in Puerto Rican State Forests, managed by the Puerto Rican government. The population of *M. splendens* grows in El Yunque National forest, which is managed by the U.S. Forest Service of the U.S. government. Seeds of *M. portoricensis* were collected and grown at the Guilarte State Forest under the initiative of Ruben Padrón Vélez, the former forest manager of that state forest (Ruben Padrón Vélez,

pers. comm.) and these were planted near the Forest Manager office and distributed among local *Magnolia* enthusiasts such as Bryan Brunner of Montoso Gardens in Maricao (Bryan Bunner, pers. comm.). It is generally accepted that there was also reinforcement of the *M. splendens* population in El Yunque during the late 1900s. During the 2016 expedition in Puerto Rico, we collected seeds that were brought to Para La Naturaleza (website: http://www.paralanaturaleza.org/en/), the Arboretum Parque Doña Ines of the Fundación Luis Muñoz Marín (website: http://www.flmm.org/) and the Ghent University Botanical Garden. All seedlings that grew in Puerto Rico unfortunately were destroyed by/after the hurricanes Irma (September 2017) and Maria (October 2017) that followed one year after the seed collection (Christian Torres Santana, pers. comm.). Many of the seedlings germinated with success in Ghent University Botanical Garden, which is a similar result as the study of Mejía (1990). Unfortunately, almost all died in the course of the following three years either due to problems with water provision or perhaps even the lack of the adequate (symbiotic) microorganisms such as mycorrhiza (Alemañy-Merly, 1999; Serna-González et al., 2019). We now have five seedlings left in the Ghent University Botanical Garden that remain very small and struggle to survive, which is similar to the reports of Figlar (1982); Figlar (1984).

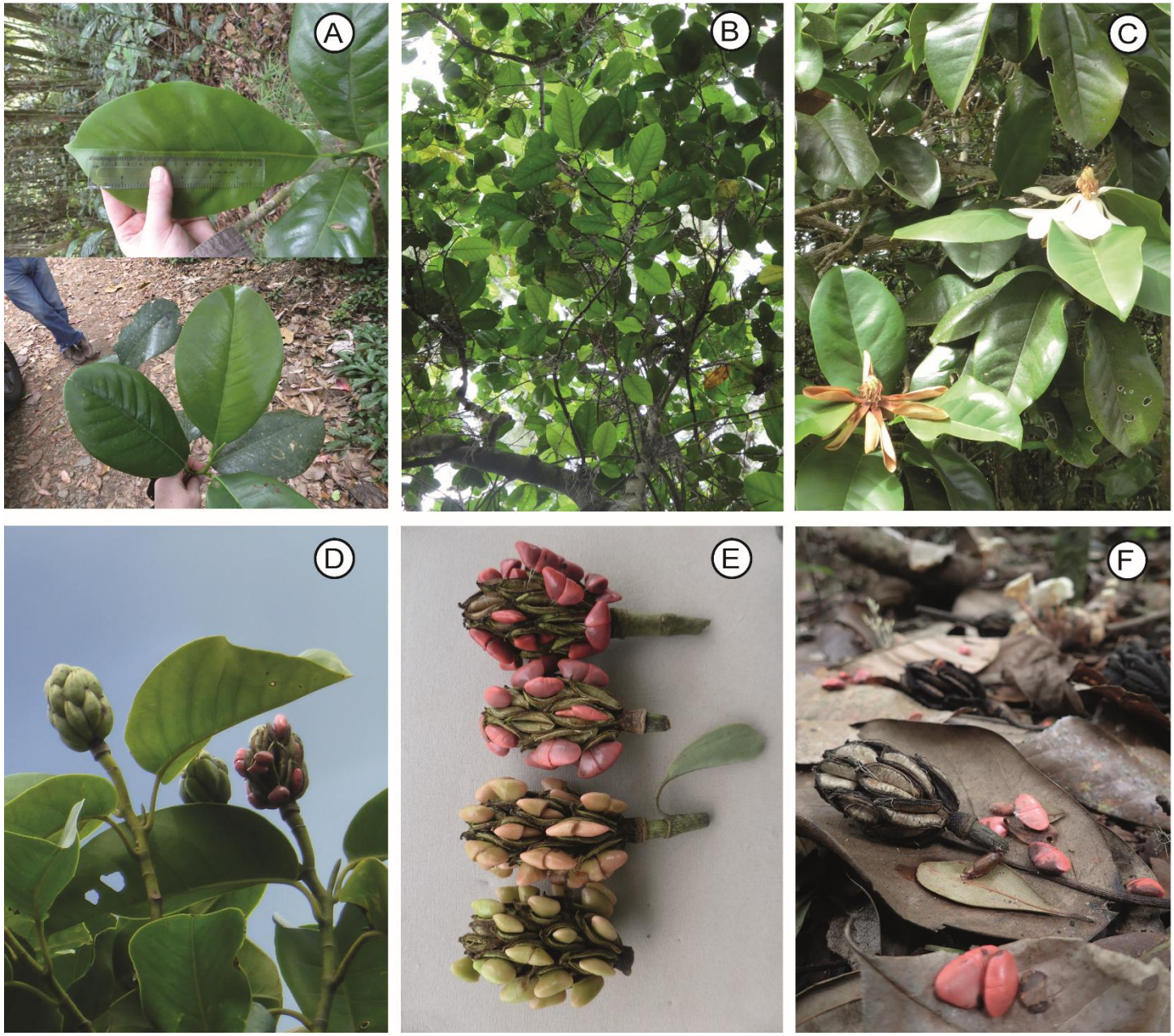


Figure 1.14 Morphology of *M. portoricensis* from the Cordillera Central in Puerto Rico. **A**: Variation in leaf apices: top photo: acuminate/apiculate; bottom photo: rounded. **B**: View from under a *M. portoricensis* tree. **C**: Flowers, one old flower and one flower in the male phase. Both flowers have the setaceous tips of the stamens embedded in the gynoecia. Also visible: acute leaf apices. **D**: Closed, developing fruit and dehisced fruit. **E**: Variation in seed colour depending on maturity; carpels were forced to dehisce with a heating fan. **F**: understory view of a *M. portoricensis* tree: ripe, pink seeds and empty follicles. Photo credits: A–C, E: Emily Veltjen; D, F: Carlos Rodríguez – Arbonautas.

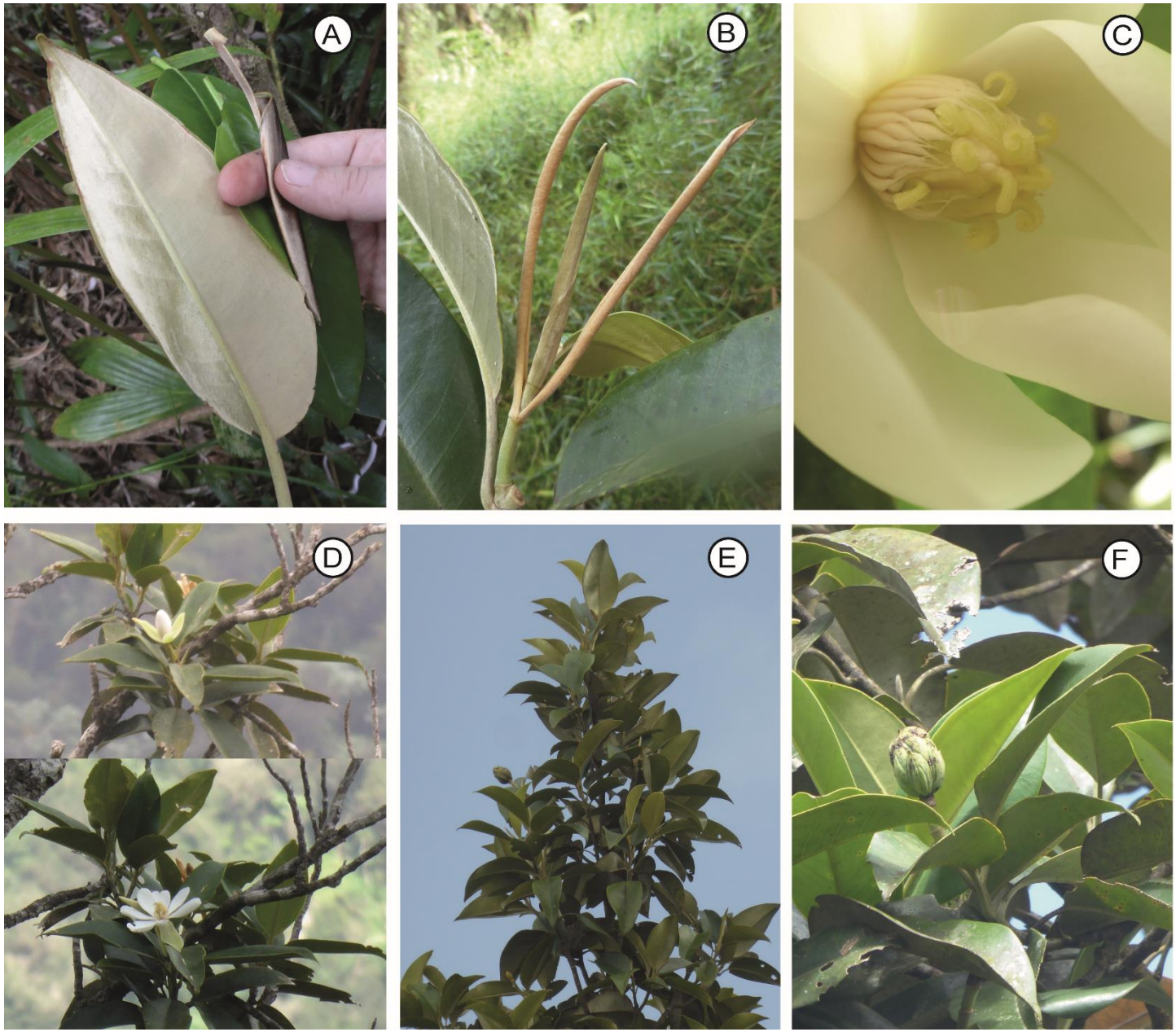


Figure 1.15 Morphology of *M. splendens* from the Luquillo Mountains in El Yunque National Forest, Puerto Rico. **A**: Abaxial side of leaf with the white sericeous hairs that give a silvery to golden appearance. The leaf apex is clearly acute. **B**: opening of stipules, revealing a new pair of stipules and a young leaf. All newly exposed structures clearly covered in sericeous hairs. **C**: close-up of a female phase flower. **D**: the same flower photographed approximately 24 hours apart. Top photo: the first morning the flower opens only its outer tepals: the flower is in its female phase. Bottom photo: the next morning the flower opens completely: the flower is in its male phase. **E**: top of the tree that has a pyramidal shape that typifies Neotropical Magnolias when having an undisturbed growth. Also shown: a developing fruit. **F:** close-up of a developing fruit. Photo credits A–F: Emily Veltjen.

Map 1.7 Distribution of *M. portoricensis* and *M. splendens* in Puerto Rico. *Magnolia portoricensis* occurs throughout the mountains of the Cordillera Central. *Magnolia splendens* occurs in the Sierra de Luquillo mountains. Management categories of the Protected Areas (Caribbean Landscape Conservation Cooperative, 2015). **SF**: State Forest; **NF**: National Forest. Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (Caribbean protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

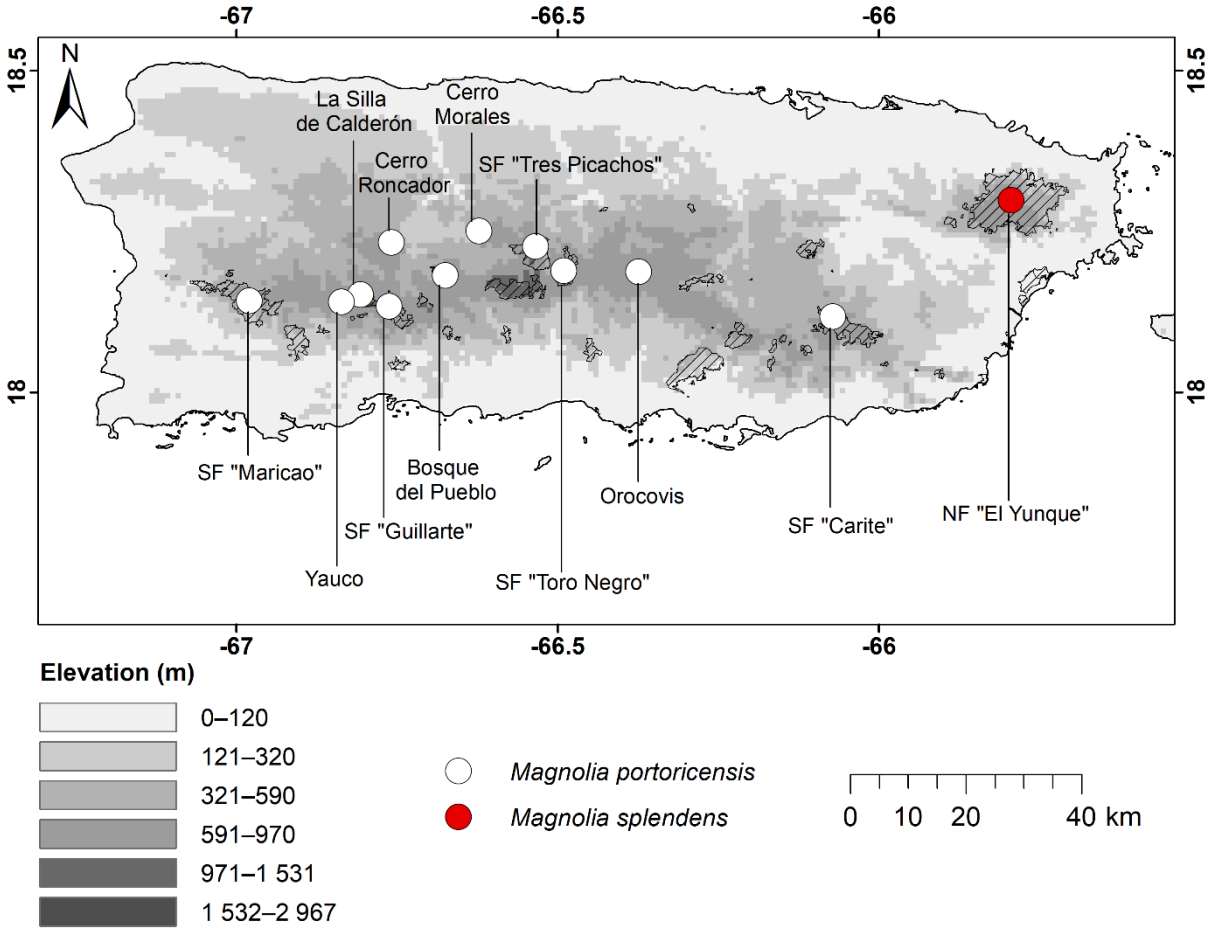


Figure 1.16 DBH (in cm) classes of the eleven populations of *Magnolia portoricensis* measured during the 2015 and 2016 expeditions to Puerto Rico.

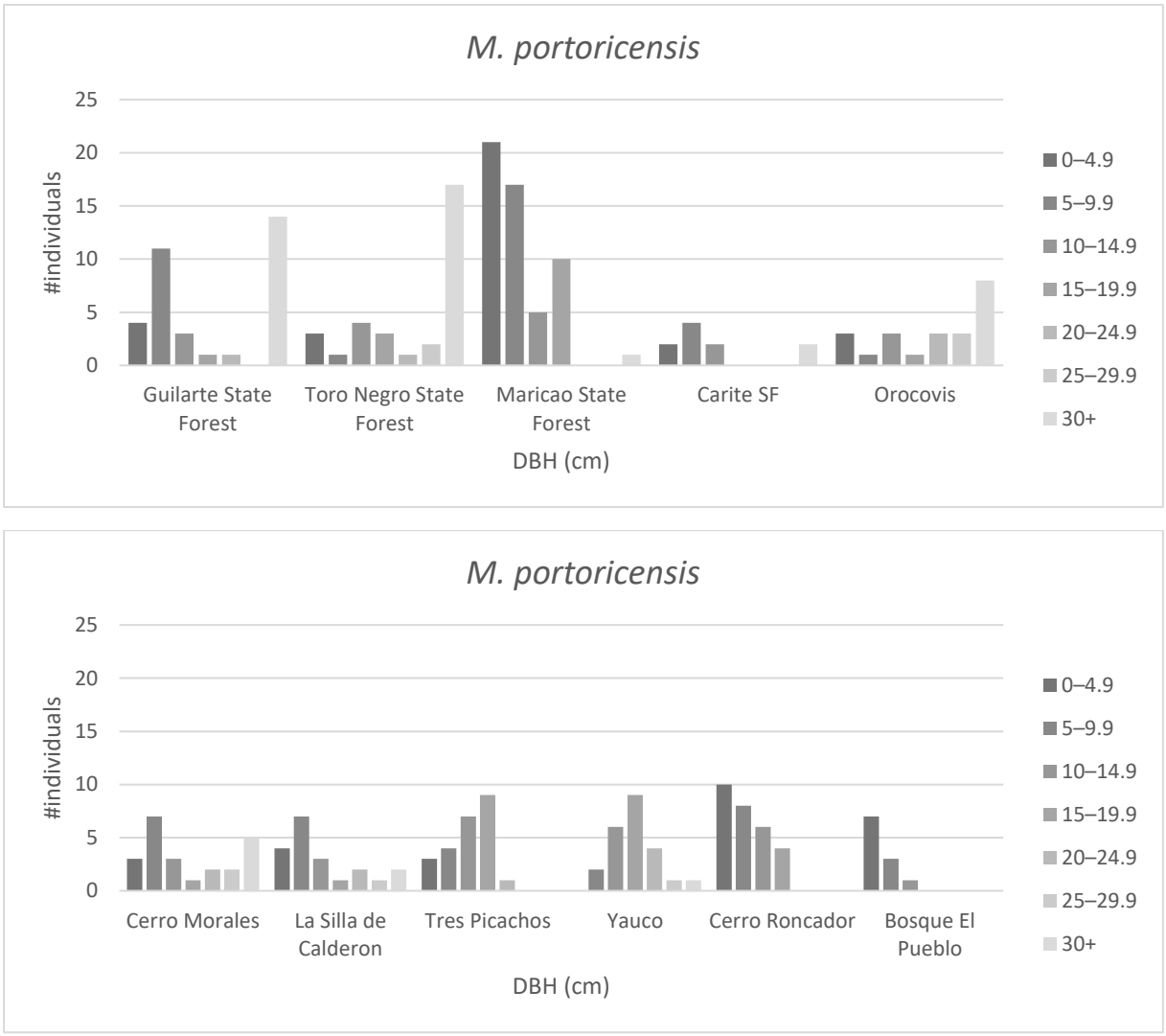
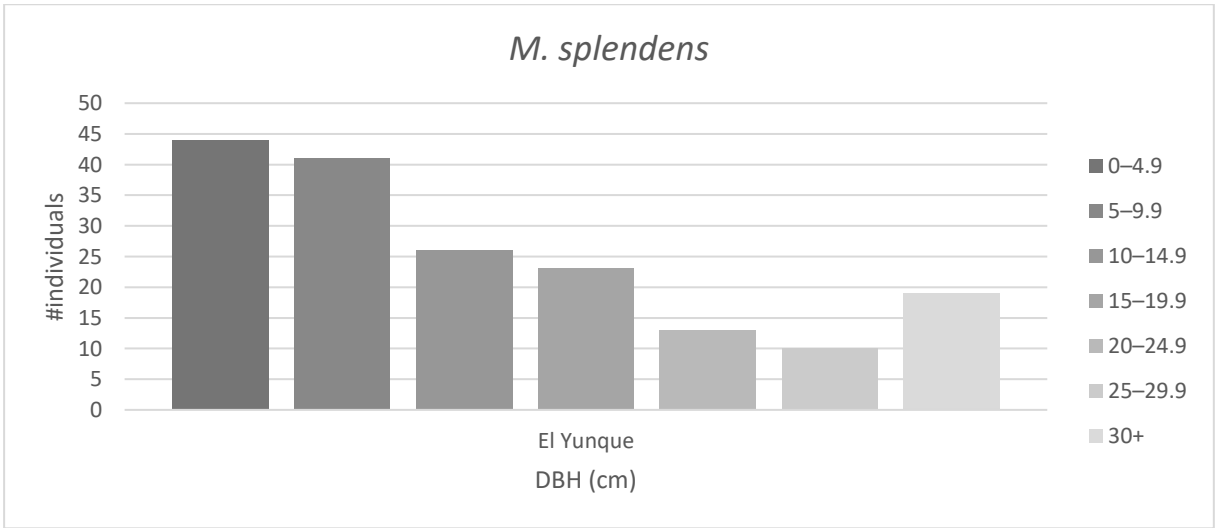


Figure 1.17 DBH (in cm) classes of the population of *Magnolia splendens* measured during the 2015 and 2016 expeditions to Puerto Rico.



1.7 *Magnolia dodecapetala* of the Lesser Antilles

CLASSIFICATION: One *Magnolia* species occurs in the Lesser Antilles: *M. dodecapetala*, which belongs to *Talauma*.

TAXONOMY: The taxonomic history of *M. dodecapetala* was provided by Howard (1948). The species is famous as it is the first *Magnolia* described by Plumier in 1703, establishing the genus and giving its name to the family (See Box 1). In literature, there are many common names listed for this species, however, during the expedition in 2016 the only common names (besides *Magnolia* or *Talauma*) that were in use were the following: Wild Almond (Saint Vincent) and Bwapen mawon (Saint Lucia).

MORPHOLOGY: The morphology of *M. dodecapetala* is summarised in more detail in Stehlé and Marie (1947), Howard (1948) and Lozano Contreras (1994). Howard compares *M. dodecapetala* morphologically with the *Talauma* species from Cuba, which is easily done given the distinctly higher number of carpels in *M. dodecapetala* and coinciding significantly bigger fruit and flower size. Leaves of *M. dodecapetala* are reported to be variable in size in Stehlé and Marie (1947) and this was verified during the 2016 expedition to the Lesser Antilles. The morphology of *M. dodecapetala* is illustrated in Figure 1.18. *Magnolia dodecapetala* can be distinguished from other Neotropical *Talauma* species given the combination of the following characteristics: globose fruits with more than 22–84 carpels, a 100% petiole scar, a glabrous habit, one perule and six to nine inner tepals. In the key of Lozano Contreras (1994), the petiole scar is noted to be partial (9/10 of its length); which was not verified in field observations and the extent of the scar (i.e. partial, full) is lacking in descriptions of the species prior to that of Lozano (i.e. Howard (1948); Stehlé and Marie (1947)). Even more so, in the morphological study conducted in Chapter 6 of this PhD we found that the number of carpels varied between 22–84, while in the key of Lozano, a minimum of 35 carpels is denoted. If the key of Lozano is followed with the *M. dodecapetala* morphology observed *in situ*: *M. dodecapetala* would either be identified it as *M. hernandezii* from Colombia when holding a fruit with more than 35 carpels; however, once the species descriptions are read, it is clear that they are different species as *M. dodecapetala* has a distinctly lower number of carpels (i.e. 22–84) than *M. hernandezii* (i.e. 176–222). Alternatively, when holding a *M. dodecapetala* fruit of less than 35 carpels, de species would be identified as *M. gloriensis* from Nicaragua, Costa Rica and Panama, following the key, yet again the two perules of this species and short pubescence do not align with the one perule and glabrous habit of *M. dodecapetala*. *Magnolia venezuelensis* is the present-day closest *Talauma* to the Lesser Antilles, which according to the descriptions compiled in Lozano Contreras (1994) differs most significantly in the number of perules: *M. dodecapetala* only has one pair while *M. venezuelensis* has three; and *M. dodecapetala* has

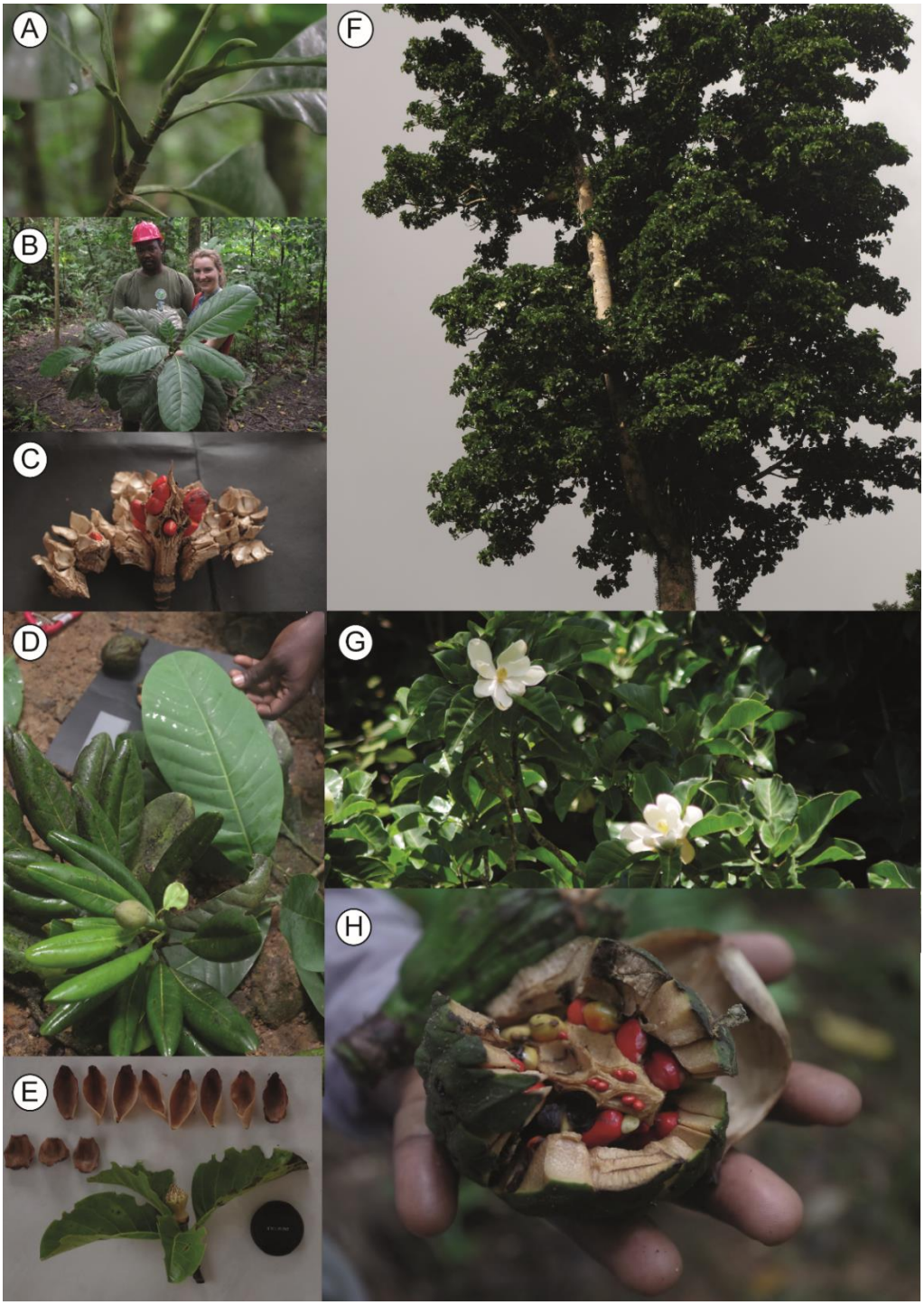
a distinctly higher number of carpels (i.e. 22–84) compared to *M. venezuelensis* (i.e. 11). Since Lozano´s work, no other taxonomist working on the Neotropical Magnolias has provided a new, complete key to identify the *Talauma* species, nor a complete critical review of all the *Talauma* species, while there have been approximately 59 new species (Vázquez-García et al., 2018 report 90 *Talauma* species) added to this subsection since that publication. It becomes more complicated as most new species that are described are often only compared to a few morphologically similar species of the same country.

DISTRIBUTION: There are *Magnolia* herbarium collections from the islands Saint Vincent, Saint Lucia, Martinique, Dominica and Guadeloupe. These five locations were all verified in the expedition of 2016 (see Map 1.8–1.13). There is a herbarium collection *F.W. Sieber 293* (MO) that states “Trinidad” on its label, as well as a herbarium collection of *Parmentier s.n.* (P), with then again, a recurring label (not the original label) mentioning Fl. Trinidatis No. 293; hence, Trinidad was/is often also mentioned as a locality for the species. F.W. Sieber did not visit Trinidad himself: on JSTOR it is reported that his herbarium collections of Trinidad (*Flora Trinidatis*) were collected by F. Wrbna (1822). Communication with Yasmin Baksh-Comeau from the National Herbarium of Trinidad and Tobago claims this to be an erroneous report and stated that most of his collections according to the literature came from French Guiana. Alternatively, it is also possible that a mistake in labelling happened, given that F.W. Sieber also has herbarium collections from Martinique (*Flora Martinicensis*), that were collected by F. Kohaut (1819-1821). Hence the presence of *Magnolia* on Trinidad could not be verified by consulting local floras, botanists, or during the 2016 expedition. There are three possibilities that explain this: 1) the species is still present on Trinidad, yet there are no (reliable) extant records; 2) the species was present on Trinidad but is extinct by now; 3) the collection is actually from Martinique or French Guiana and was labelled incorrectly. Option 1 and 2 are deemed more unlikely given that Trinidad and Tobago have a long history of botanical exploration and no other records of *Magnolia* are present.

DEMOGRAPHICS: Within the framework of this PhD, one expedition to Trinidad, Saint Vincent, Saint Lucia, Martinique, Dominica and Guadeloupe was undertaken in June-July 2016. See Table 1.2 for number of known localities and the explicit number of *Magnolia* trees recorded at these localities. See Figure 1.19 for the number of individuals encountered per DBH class.

CONSERVATION: The five different islands did not have any active conservation programs running with a focus on Magnolia. In one locality: Hermitage of Saint-Vincent the former forest manager did actively plant some of the trees present (Amos Glasgow, pers. comm.).

Figure 1.18 Overview of the morphology of *M. dodecapetala* from the Lesser Antilles. **A**: Adnate stipule, adnation over the full length of the petioles leaving a clear petiole scar (St. Vincent); **B**: Large leaf size (St. Lucia); **C**: Fruit exposing red seeds, dried *ex situ* (Guadeloupe); **D**: Branch with a flower bud, note the difference in leaf size within the same population (St. Lucia); **E**: Old flower with all the 8 inner tepals and 3 outer tepals removed (Guadeloupe); **F**: Habit of a flowering *M. dodecapetala* tree in an open, logged area (Dominica); **G**: Two post-male stage flowers (Guadeloupe); **H**: Fruit exposing red seeds and "ripened" ovules. Fruit opened *in situ* (St. Lucia). Photo credits: Emily Veltjen.



Map 1.8 Distribution of *M. dodecapetala* on five different islands in the Lesser Antilles in the Caribbean. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

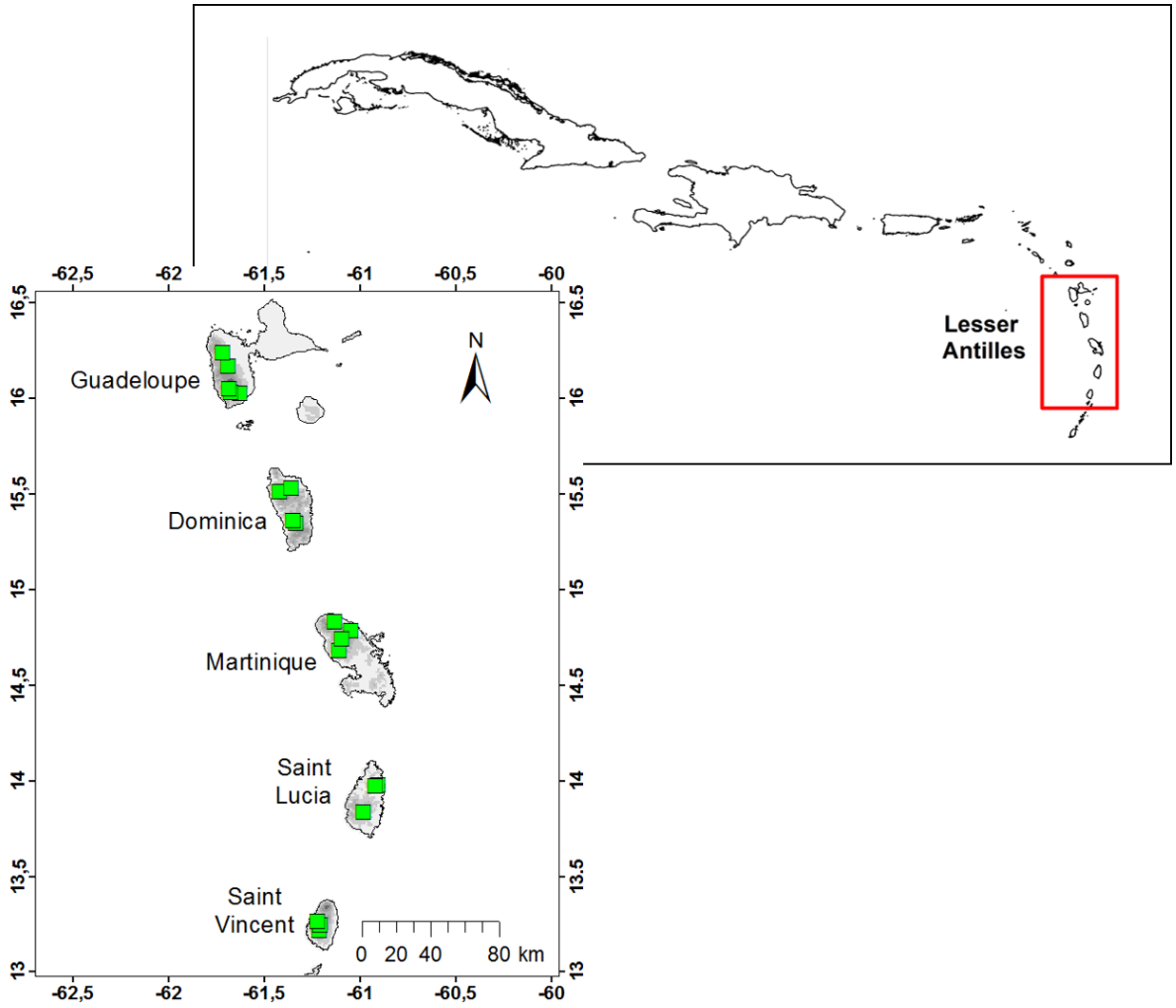
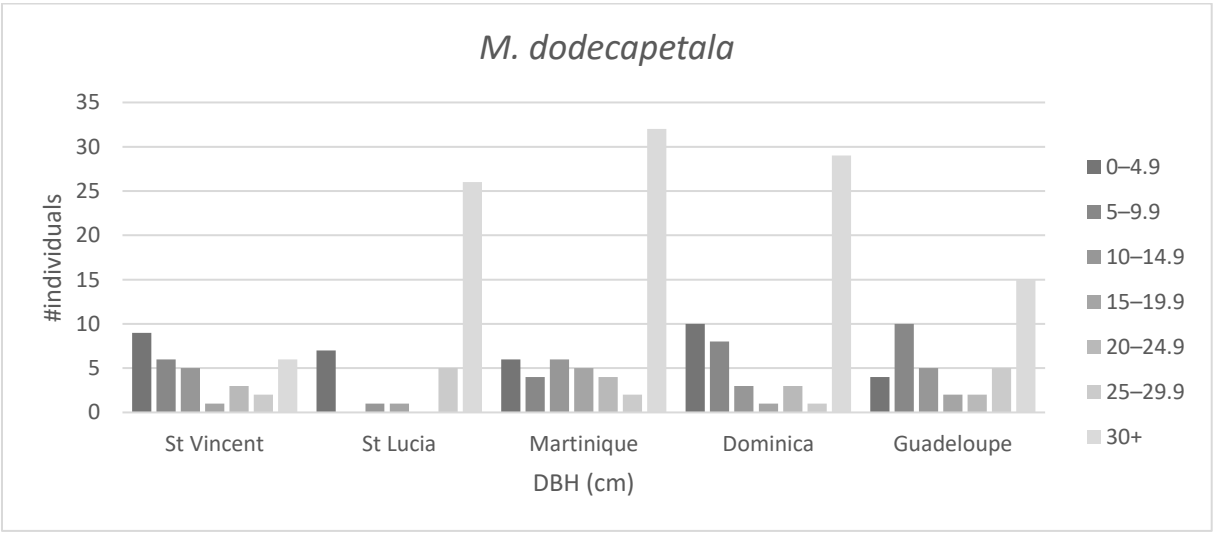
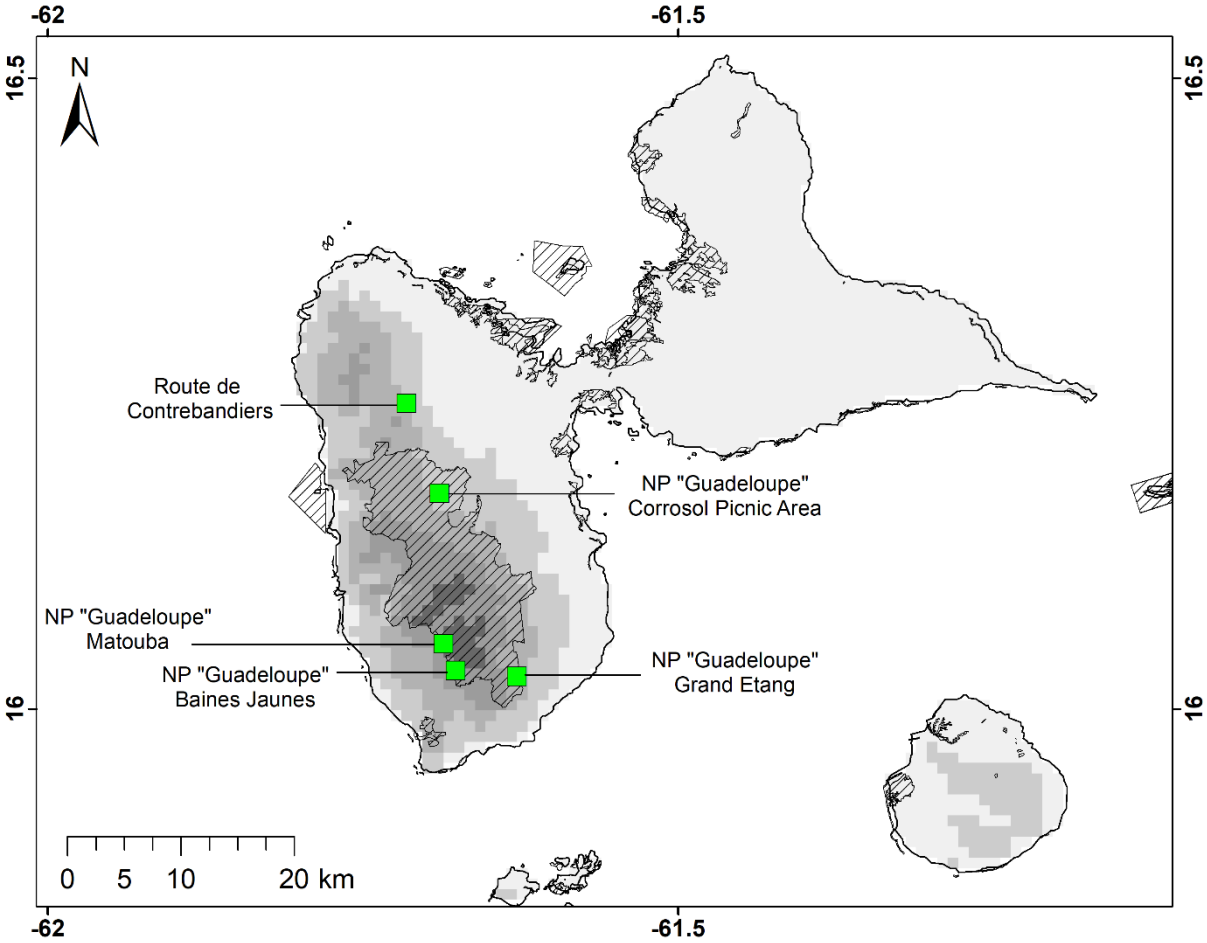


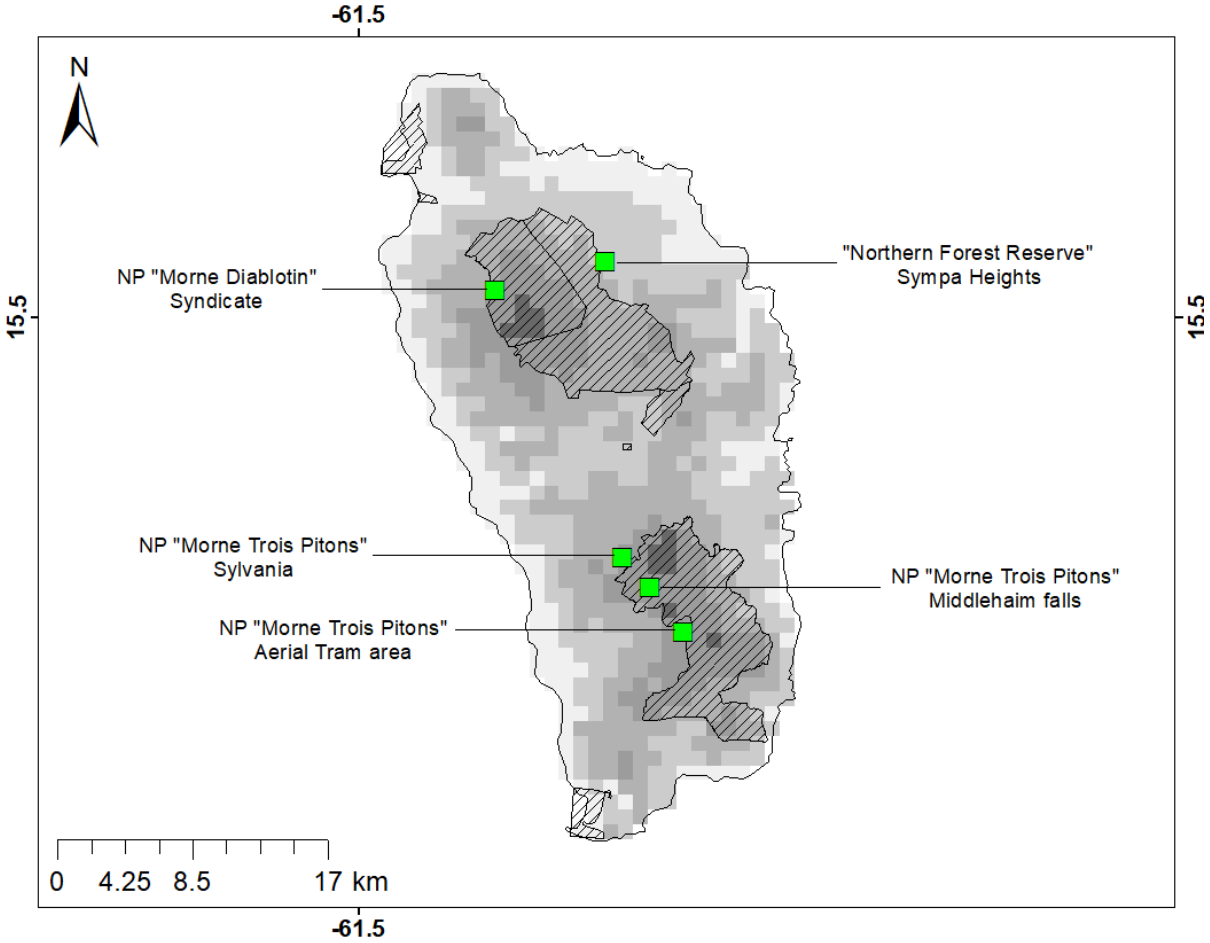
Figure 1.19 DBH (in cm) classes of the five island populations of *Magnolia dodecapetala* measured during the 2016 expedition to the Lesser Antilles.



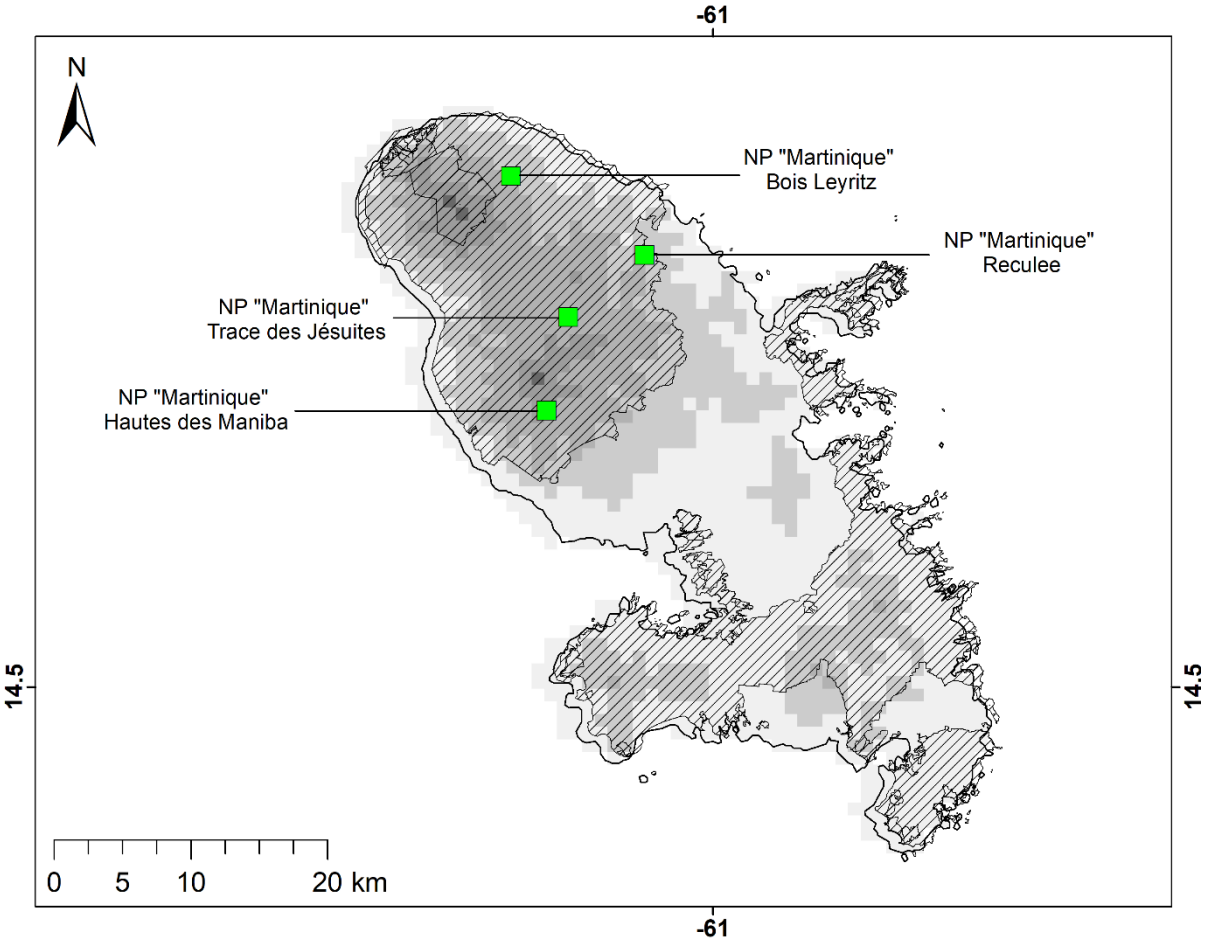
Map 1.9 Distribution of *M. dodecapetala* in Guadeloupe, the northernmost of the five islands where the species occurs in the Lesser Antilles in the Caribbean. **NP:** National Park. Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (French Antilles protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



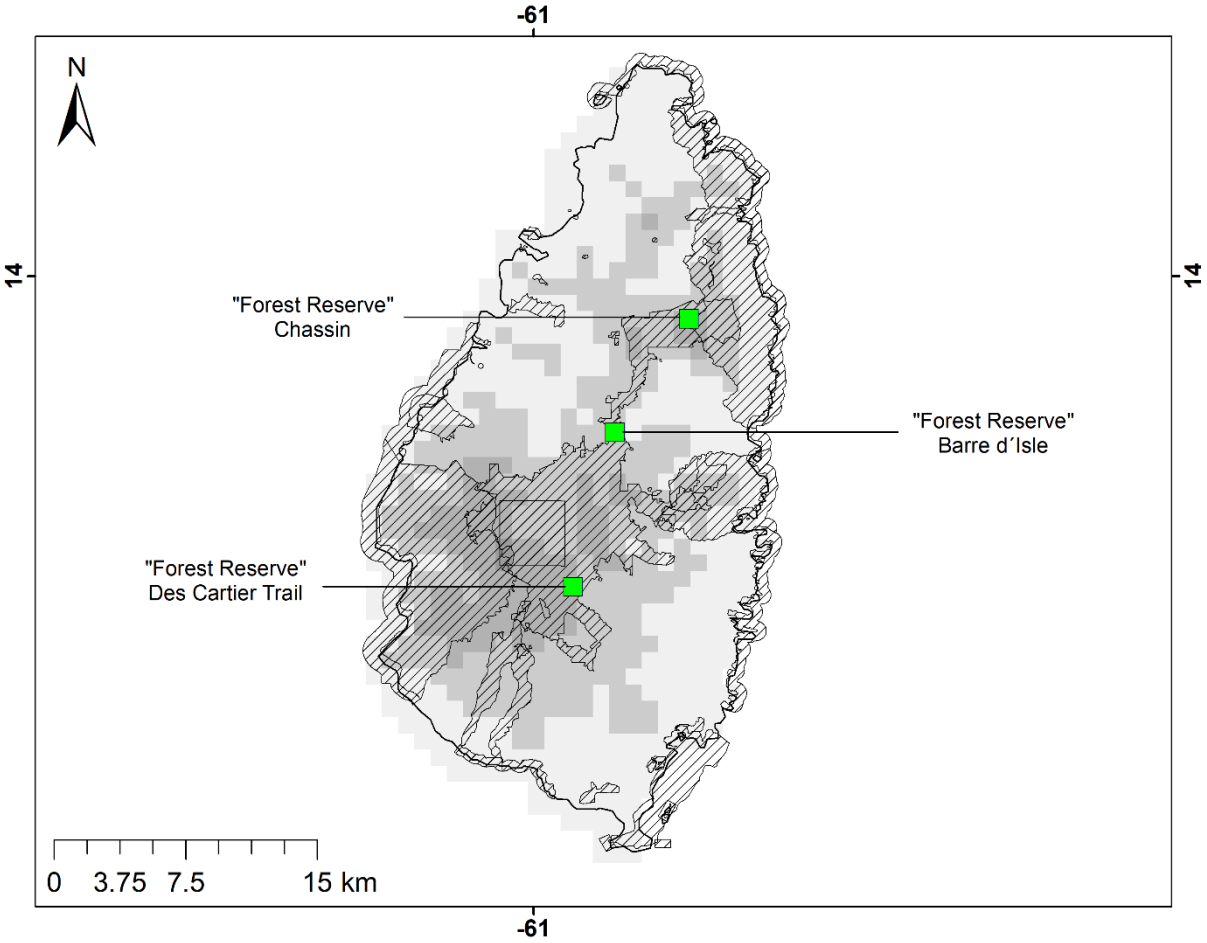
Map 1.10 Distribution of *M. dodecapetala* in Dominica, the second of the five islands where the species occurs in the Lesser Antilles in the Caribbean, when following the islands from north to south. **NP:** National Park. Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (Caribbean protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



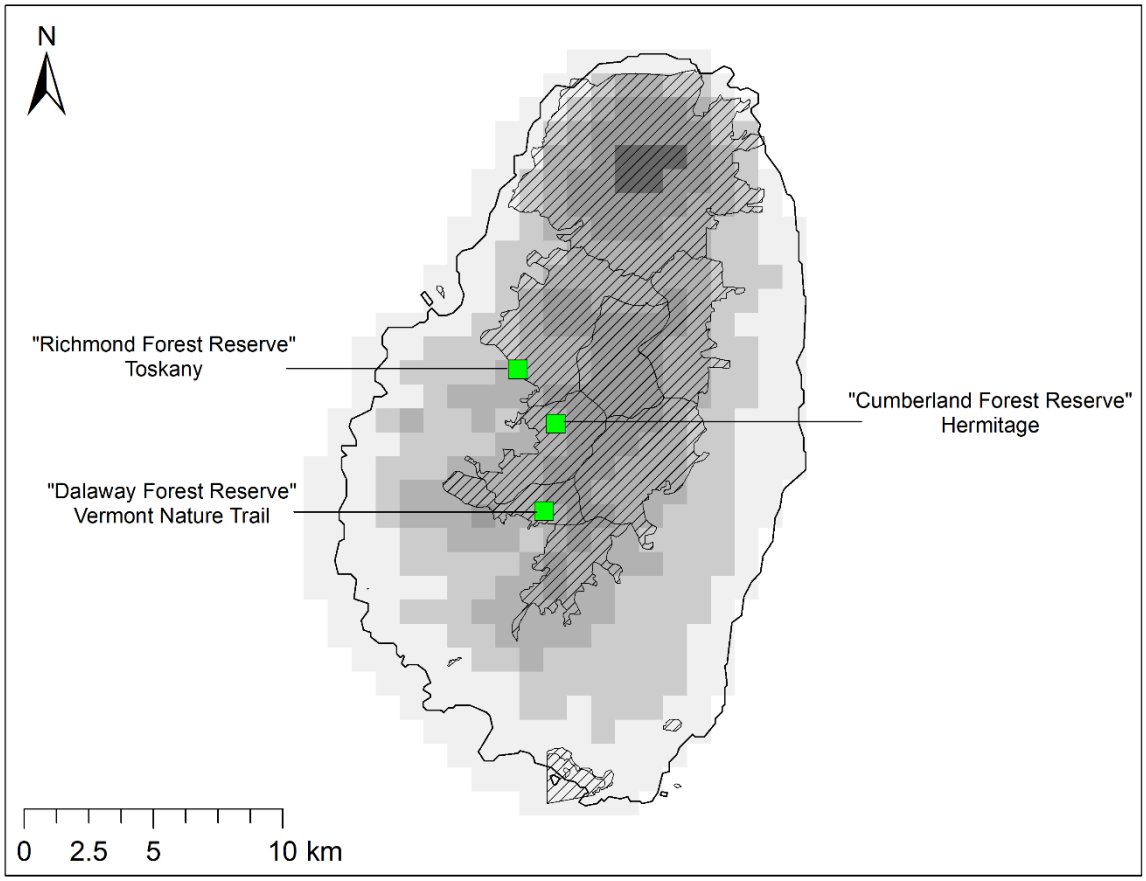
Map 1.11 Distribution of *M. dodecapetala* in Martinique, the third of the five islands where the species occurs in the Lesser Antilles in the Caribbean, when following the islands from north to south. **NP:** National Park. Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (French Antilles protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



Map 1.12 Distribution of *M. dodecapetala* in Saint Lucia, the fourth of the five islands where the species occurs in the Lesser Antilles in the Caribbean, when following the islands from north to south. Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (Caribbean protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



Map 1.13 Distribution of *M. dodecapetala* in Saint Vincent, the fifth of the five islands where the species occurs in the Lesser Antilles in the Caribbean, when following the islands from north to south. Saint Vincent is the main island of Saint Vincent and the Grenadines. Locality names that refer to a Protected Area are annotated with quotation marks. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (Caribbean protected areas: UNEP-WCMC, 2019); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



1.8 Conservation of (Caribbean) Magnoliaceae

Conservation of biodiversity can be defined as an attempt to protect the variability among living organisms on our planet, produced over the years of evolution (Eisner et al., 1995). Ideally, it is aimed to manage at least entire ecosystems, if not whole landscapes, by unified methods designed to save all their inhabitants at one time (Simberloff, 1998). Because it is so difficult to monitor and manage every aspect of biodiversity, several shortcuts have been proposed, whereby on the one hand we monitor and/or protect single species (Simberloff, 1998), i.e. single-species conservation, or on the other hand, we protect a selection of areas and hope this safeguards the biodiversity within. Although resources are limited, research and conservation focussed on both the ecosystem level and the species level should be regarded as complementary, rather than the two being in competition with one another (Lindenmayer et al., 2007). As briefly mentioned in the thesis outline of this PhD (see: Thesis outline), the choice to focus research on *Magnolia* does not provide valuable knowledge for *Magnolia* conservation only. Magnolias act as umbrella species (Roberge and Angelstam, 2004): their *in situ* conservation safeguards other species in the habitat in which they occur; and as flagship species (Caro, 2010): their emblematic reputation attracts greater local and international interest. This makes conservation of *Magnolia* a case of single-species conservation with a higher potential of also conserving its habitat and its associated fauna and flora. While the maintenance of (*Magnolia*) biodiversity primarily depends upon the protection of the environment and maintenance of habitat (Allendorf et al., 2013), conserving, monitoring and understanding the underlying genetic diversity play an important role, as in essence, the genetic diversity of populations is the source of their evolutionary potential: their resilience, their adaptability (McNeely et al., 1990).

Mainly due to degradation of natural habitats, but also because of overharvesting for timber and medicinal uses, as well as a consequence of low natural regeneration, the survival of many Magnoliaceae, including the Caribbean Magnolias, is considered under threat (Rivers et al., 2016). BGCI and partners have recently executed conservation assessments of the members of the Magnoliaceae following the IUCN Red List of threatened Species Categories and Criteria version 3.1 (IUCN, 2012) to accentuate priorities and inspire researchers and NGOs in their conservation efforts (Rivers et al., 2016). The three IUCN Red List categories of threatened species are Critically Endangered (CR), Endangered (EN) and Vulnerable (VU). The Magnoliaceae have a fair number of taxa labelled as Data Deficient (DD) as well. This status is assigned to poorly known taxa either due to poorly formulated species descriptions or a lack of (recent) explorations, which are desperately in need of more research or more distribution data. Of the 304 species assessed, 48% was considered threatened, 20% Data Deficient and 32% not threatened. All Caribbean *Magnolia* species were evaluated, and an even more recent

publication of the Red List of the Cuban Flora (González Torres et al., 2016) re-evaluated the Cuban Magnolias in greater detail, with information that was not included in the BGCI Red Listing. The IUCN Red List status of each species is given in Table 1.3.

Table 1.3 IUCN Red List status (RL) of the Caribbean *Magnolia* species and subspecies summarised from González Torres et al. (2016) and Rivers et al. (2016). See Appendices 1.2 and 1.3 for the taxonomic authorities linked to each species. RL categories: Critically Endangered (CR), Endangered (EN) and Vulnerable (VU) and criteria are assigned following the IUCN Red List of Threatened Species Categories and Criteria version 3.1 (IUCN, 2012).

Taxon	**RL**	**Criteria**
M. cristalensis	CR	B2ab(i,ii,iii,iv,v);C1+2a(i)
M. cubensis subsp. *cubensis*	VU	B2ab(i,ii,iii,iv,v);C2a(i)
M. cubensis subsp. *acunae*	CR	B2ab(ii,iii,v)
M. dodecapetala	VU	B1ab(iii)
M. domingensis	CR	A2ac
M. ekmanii	CR	A2ac
M. emarginata	CR	A2ac
M. hamorii	EN	B1ab(i,iii)
M. minor	EN	B1ab(ii,iii,v)+2ab(ii,iii,v)
M. oblongifolia	CR	B2ab(ii,iii,v);C2a(i)
M. orbiculata	VU	B2ab(I,ii,iii,v);C2a(i)
M. pallescens	EN	B1ab(i,iii)+2ab(i,iii)
M. portoricensis	EN	B1ab(iii,v)
M. splendens	EN	B1ab(iii,v)+2ab(iii,v)
M. virginiana subsp. *oviedoae*	CR	B1ab(iii)

The main threats at the family level (Rivers et al., 2016) have been reported for the Caribbean Magnolias, i.e. degradation of natural habitats (Hedges et al., 2018), overharvesting for timber (Alemañy-Merly, 1999) and low natural regeneration (Castillo et al., 2018). Even more so, the natural setting of the Caribbean itself adds to the species' vulnerability, given the small areas and natural disturbance in the form of seasonal hurricanes (Myers et al., 2000; Olsen and Dinerstein, 1998; Rodrigues et al., 2004; Smith et al., 2004); to which in extension climate change can be added, given that tropical cyclones are expected to intensify under a warming climate, with uncertain effects on tropical forests (Uriarte et al., 2019). It is not yet known whether the Caribbean Magnolias will be able to cope, or even whether they are currently coping well, with the climatic changes, and how severe the impact will be on their survival.

1.9 Research hypotheses

The general aim of this PhD research was to unravel the genetic diversity and the underlying evolutionary history of the Caribbean *Magnolia* species (Magnoliaceae), to support their conservation. Using molecular data, we zoom in on the genetic diversity of the 15 threatened Caribbean Magnolias using two main scientific disciplines: biogeography and conservation genetics. We thoroughly introduced the study group and area in the previous subchapters of Chapter 1 and an introduction to Caribbean biogeographic hypotheses, conservation genetics of trees, and genetic patterns of island populations can be found in the introductions of the Chapters 3, 4 and 6, respectively. Throughout the chapters of this PhD study, twelve hypotheses centred around the Caribbean Magnolias were tested. They are formulated as null hypotheses to illustrate the expectance based on prior knowledge of literature, presented in the introductions of different chapters in which these hypotheses are addressed.

H01: The Caribbean *Magnolia* species are all diploid. | *Chapter 2: Ploidy of the Caribbean Magnolias.*

H02: The delimitation of Caribbean *Magnolia* species as described by Howard (1948) and Palmarola (González Torres et al., 2016; Palmarola-Bejerano et al., 2008; Palmarola et al., 2016) based on the Morphological Species Concept (Cronquist, 1978) with their underlying, (mostly) discrete geographic separation is confirmed by the Phylogenetic Species Concept (Cracraft, 1989). | *Chapter 3: Biogeography of Caribbean Magnolias.*

H03: Section *Talauma* (Figlar and Nooteboom, 2004) is monophyletic. | *Chapter 3: Biogeography of Caribbean Magnolias.*

H04: Subsections *Talauma* and *Cubenses* (Figlar and Nooteboom, 2004) are monophyletic. | *Chapter 3: Biogeography of Caribbean Magnolias.*

H05: Magnolias colonised the Caribbean islands in three independent events: one of subsection *Talauma*, one of subsection *Cubenses* and one of section *Magnolia*. | *Chapter 3: Biogeography of Caribbean Magnolias.*

H06: The Caribbean Magnolias are an example of dispersal via a temporary land bridge as proposed by the GAARlandia biogeographic hypothesis (Iturralde-Vinent and MacPhee, 1999), not vicariance (Rosen, 1975, 1985) or overwater dispersal (Hedges, 1996). | *Chapter 3: Biogeography of Caribbean Magnolias.*

H07: The *Magnolia* species from each Caribbean island form a clade as proposed by the stepping-stone dispersal biogeography hypothesis (MacArthur and Wilson, 1967). | *Chapter 3:*

Biogeography of Caribbean Magnolias; Chapter 6: SSR study & biogeography of M. dodecapetala*.*

H08: Caribbean *Magnolia* populations show patterns of extensive gene flow (i.e. within a species there is no population structuring) as expected for trees (Petit and Hampe, 2006). | *Chapter 4: SSR patterns of Caribbean Magnolias; Chapter 5: SSR study of* M. cubensis *subsp.* acunae; *Chapter 6: SSR study & biogeography of* M. dodecapetala*.*

H09: Caribbean *Magnolia* populations have an inbreeding coefficient (F_{IS}) that significantly differs from zero, given their small population sizes and the endemic and threatened status of the Caribbean *Magnolia* taxa. | *Chapter 4: SSR patterns of Caribbean Magnolias; Chapter 5: SSR study of* M. cubensis *subsp.* acunae; *Chapter 6: SSR study & biogeography of* M. dodecapetala*.*

H10: Caribbean *Magnolia* populations show a correlation between degree of habitat fragmentation and genetic diversity. | *Chapter 5: SSR study of* M. cubensis *subsp.* acunae*.*

H11: Genetic differentiation of Caribbean *Magnolia* populations correlates with morphological differentiation. | *Chapter 6: SSR study & biogeography of* M. dodecapetala*.*

H12: The IUCN Red List Status of the Caribbean *Magnolia* taxa correlates with their genetic diversity. | *Chapter 7: General discussion and conclusions.*

2. Ploidy of the Caribbean Magnolias

MODIFIED FROM: Claeys K. 2016 “Plantengenomen onder de loep. Het bepalen van de ploïdiegraad met flowcytometrie en chromosoomtellingen” (“Plant genomes examined; in search of ploidy using flow cytometry and chromosome counts”). Bachelor thesis, Ghent University.

2.1 INTRODUCTION

Questions on the evolutionary history of plants cannot be discussed without the concept of speciation by polyploidization (Alix et al., 2017; Rieseberg and Willis, 2007). A successful increase in ploidy level results in a reproductive barrier between the seedlings and its parental population(s) after which the progeny can follow its own evolutionary path (Rieseberg and Willis, 2007). There are two general types of polyploids: those involving the multiplication of one chromosome set (autopolyploidy[1]) and those resulting from the merger of structurally different chromosome sets and subsequent genome duplication (allopolyploidy). The doubling of genetic material, or whole-genome-duplication (WGD), often brings in harmful effects on fertility and fitness owing to genomic instability, mitotic and meiotic abnormalities, changes in gene expression and epigenetic changes (Comai, 2005). Hence, it is claimed that most neopolyploids are evolutionary ‘dead ends’ (Arrigo and Barker, 2012; Mayrose et al., 2011). However, as proven by the plethora of polyploid plants thriving, as well as the growing evidence of ancient WGD events (e.g. the *Amborella* Genome Project (2013)): polyploids can also have adaptive potential both on the short and long term, ensuring their survival (Van de Peer et al., 2017). Recent estimates suggest that up to 25–30% of extant flowering plant species are neopolyploids (Barker et al., 2016; Mayrose et al., 2011; Scarpino et al., 2014; Wood et al., 2009) and recent evidence indicates there to be signals of the occurrence of tens, or even hundreds, of WGD events during the past 500 million years of evolution (Van de Peer et al., 2017).

The plant family of interest in this PhD project: the Magnoliaceae[2], contributes to this high reported percentage of neopolyploids in flowering plants (Wood et al., 2009). Even more so their high chromosome number, together with the high chromosome number of other members of the basal angiosperms, even inspired first concepts of ancient WGD events (Soltis and Soltis, 2000). Whittaker (1933) carried out the first cytological study of the Magnoliaceae and reported that the basic chromosome number is x = n = 19. This basic chromosome number has been confirmed in all Magnoliaceae ploidy studies (Biswas and Sharma, 1984; Chen et

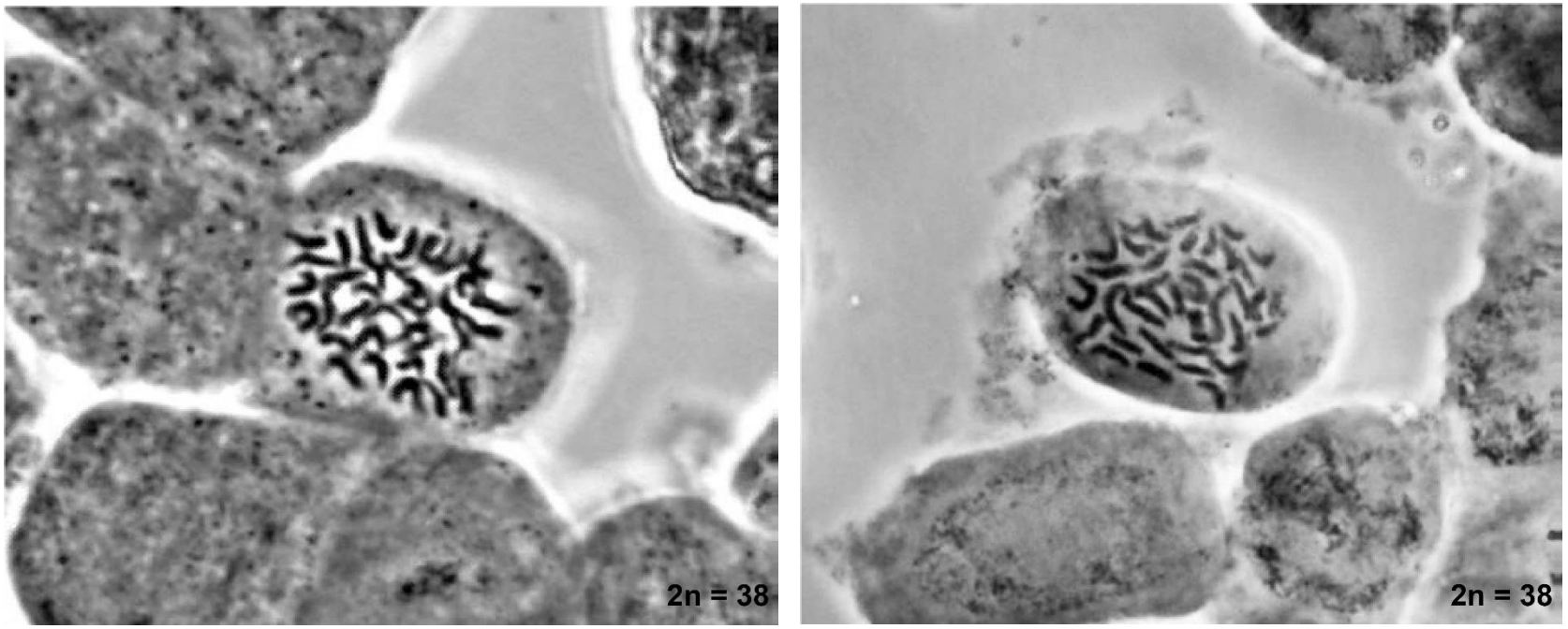
[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1
[2] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.

al., 2000; Howard, 1948; Lepper, 1979, 1982; Parris et al., 2010). Although first studies claimed that ploidy has the potential to contribute substantially to the evolution in this family (Whittaker, 1933), more recent studies found that neopolyploid (wild) Magnolias are rather rare when compared to the diversity of the Magnoliaceae family and restricted to certain clades only (Parris et al., 2010). Tetraploids (n = 76) and hexaploids (n = 116) have been found in the sections *Gynopodium*, *Macrophylla*, *Magnolia* and *Yulania* (Figlar and Nooteboom, 2004; Parris et al., 2010). In all other sections, the species studied were found to be diploid (Parris et al., 2010). The ploidy of the species of section *Talauma* (Figlar and Nooteboom, 2004), to which all but one (i.e. *M. virginiana* subsp. *oviedoae*) of the Caribbean Magnolias belong, has been scarcely documented: chromosome counts of three out of the roughly 150 *Talauma* species have been found in (internationally accessible) literature. All three reported species studied are Caribbean Magnolias: *M. cubensis* (Lepper, 1979), *M. oblongifolia* (Lepper, 1982) and *M. hamorii* (Howard, 1948), all three are diploid. For the other eleven Caribbean *Magnolia* species of section *Talauma* this data are still missing. Hence, the research executed in this chapter was aimed at providing this missing ploidy data by methods of direct chromosome counts and flow cytometry. On the one hand, these data are important for executing genetic research on, and interpreting genetic data of the studied species (Meirmans et al., 2018; Rothfels et al., 2017). On the other hand, if one of the Caribbean Magnolias is a neopolyploid this would indicate potential ongoing or past sympatric speciation and hence provide more insight on the evolutionary history of the species (Van de Peer et al., 2017).

2.2 MATERIAL AND METHODS

Root tips from *Magnolia dodecapetala* and *Magnolia portoricensis* seedlings grown in the Ghent University Botanical Garden were used to execute chromosome counts. The *M. dodecapetala* seedlings were from seeds of mother tree number 28.52.; which belongs to the Dominica island population at the Sylvania sampling location (See Chapter 6; Table 6.1) and the *M. portoricensis* seedlings were from seeds collected in the Cordillera Central; however, the exact mother tree was not specified. Root tips were collected before noon and stored in darkness at 4°C for 2h in a microcentrifuge tube containing a mitotic inhibitor, consisting of 45 ml demineralised water, 50 mg colchicine, 25 mg 8-hydroxyquinoline dissolved in 5 ml 95% ethanol, and 1 ml DMSO (dimethylsulfoxide). After the mitotic inhibition, root tips were placed in Carnoy-fixative (3:1 (v/v) 99.8% ethanol:45% acidic acid) for 15 minutes. Subsequently, they were first soaked in 2:1 (v/v) 99.8% ethanol:37% HCl solution for ten minutes, which was then rinsed by a 10-minute soak in demineralised water. The root tips were stained with aceto-carmine at 60°C for one hour. The latter staining solution was composed of 0.5% w/v carmine, dissolved in 45% v/v acidic acid. After staining, root tips were rinsed with 45% v/v acidic acid, after which the calyptra was removed and the apical meristem

Figure 2.1 Cytological preparations of root tips of *Magnolia dodecapetala* (left) and *Magnolia portoricensis* (right).



excised from the rest of the root body. Root tip meristem was placed on glass slides in a drop of 45% v/v acidic acid, on which cover slips were mounted and the preparations were squashed. The squashed cells were studied using a light microscope equipped with phase contrast optics (Zeiss Axioplan 2) and a Plan-neofluar 100×/1.3 oil objective lens for phase-contrast observations. Images were obtained using a Nikon DXM1200 camera.

Flow cytometry was executed on dried leaf material of 13 *Magnolia* taxa, collected on silica-gel during the various expeditions executed prior 2017 (see Chapter 1), and fresh leaf material of two *Magnolia* species that served as internal standards. Internal standard 1 comprised of fresh leaf tissue of the hexaploid *Magnolia grandiflora* (IPEN number: GENT-1900-2395). Internal standard 2 comprised of fresh leaf tissue of the diploid *Magnolia virginiana* subsp. *australis* (IPEN number: GENT-2008-0619). A small amount of dried and fresh leaf material for each Caribbean *Magnolia* species and the internal standard 1, respectively, were chopped in a petri dish with 200 µl PARTEC Nuclei Extraction Buffer (NEB). Using 800 µl of PARTEC DAPI Staining Solution, the chopped leaves and the NEB were poured over a clean PARTEC Celltrics™ filter with pore size of 50 µm. The filtered solution was analysed by the Cyflow MB flow cytometer (PARTEC, Germany). The data were analysed using the software Cyflogic v.1.2.1 (CyFlo Ltd, http://www.cyflogic.com) where 50 n/s was acquired and the gain was set using a measurement of the two internal standards together (diploid/hexaploid mixture of *M. grandiflora* and *M. virginiana*). The axes were set to the logarithmic scale: the y-axis showing the side scatter (SSC), and the x-axis portraying the relative fluorescence (FL1). Measurements were stopped after a minimum of 7500 registered particles. Peaks were gated manually and the average fluorescence (Xmean of FL1) was recorded. If only one peak was recorded, the process was repeated, using the internal standard 2.

2.3 RESULTS

Results of the chromosome counts of *Magnolia dodecapetala* and *Magnolia portoricensis* are presented in Figure 2.1. For both species we counted 38 chromosomes in the diploid cells. Results of the flow cytometry measurements are summarized in Table 2.1 and gated fluorescence histograms are compiled in Appendix 2.1.

2.4 DISCUSSION

The overall data of the Magnoliaceae, now complemented with the ploidy data of 11 Caribbean Magnolias, confirm the statement that there is a minor role of neopolyploidy in the evolution of Magnoliaceae (Biswas and Sharma, 1984). For the Caribbean, the overall diploid status puts forward the hypothesis that the species arose due to allopatric speciation after successful colonization of emerged land blocks, from one or multiple diploid ancestors. Given that the

Table 2.1 Ploidy derived from flow cytometry measurements on leaf material of 12 *Magnolia* samples and fresh leaf material of two *Magnolia* samples that served as internal standards.

Tested Sample (TS)	Subsection	Ratio (TS/IS)	Ratio/CS	Ploidy
M. cristalensis (dried)	*Cubenses*	0.72	1.01	2
M. cubensis subsp. *acunae* (dried)	*Cubenses*	0.72	1	2*
M. cubensis subsp. *cubensis* (dried)	*Cubenses*	0.73	1.03	2*
M. dodecapetala (fresh)	*Talauma*	0.77	1 (=$CS_{Talauma}$)	2
M. domingensis (dried)	*Cubenses*	0.72	1.02	2
M. ekmanii (dried)	*Cubenses*	NA	NA	NA
M. hamorii (dried)	*Cubenses*	0.71	0.99	2*
M. minor (dried)	*Talauma*	0.71	0.92	2
M. oblongifolia (dried)	*Talauma*	0.74	0.95	2*
M. orbiculata (dried)	*Talauma*	0.78	1.01	2
M. pallescens (dried)	*Cubenses*	0.66	0.93	2
M. portoricensis (dried)	*Cubenses*	0.68	0.96	2*
M. portoricensis (fresh)	*Cubenses*	0.71	1 (=$CS_{Cubenses}$)	2*
M. splendens (dried)	*Cubenses*	NA	NA	NA

The internal standard (IS) for all measurements was the internal standard 1: the hexaploid *Magnolia grandiflora* (IPEN number: GENT-1900-2395). The counted standard (CS) was *Magnolia dodecapetala* for the species that belong to subsection *Talauma* and *Magnolia portoricensis* for the species that belong to subsection *Cubenses*. NA = not available. The Ratio (TS/IS) is that of the mean X-value as depicted per gated peak in Appendix 2.1. An asterisk (*) indicates all species for which ploidy was also derived via direct cell counts.

Caribbean is not associated with cold or a history of glaciation (i.e. cold environments are associated with the production of more unreduced gametes and hence a higher frequency of polyploidy (Bretagnolle and Thompson, 1995)), the frequency of polyploids is overall reported to be low for the Caribbean, and that the Caribbean Magnolias are woody perennials (i.e. being components of climax stages of plant succession, which advance into new regions only when both climatic and soil conditions have become similar to those in their previous homes, not coping with drastic environmental differences, polyploid individuals do not have an adaptive superiority over their diploid progenitors (Stebbins, 1971)); the result of diploidy for the Caribbean Magnolias is not surprising (Rice et al., 2019).

The flow cytometry measurements were executed on dried leaf samples, not fresh leaf samples, which made flow cytometry data interpretation more difficult given the damaged nuclei (Dolezel and Bartos, 2005 but see also; Suda and Trávníček, 2006), yet not impossible.

2.5 CONCLUSION

For *Magnolia cubensis*, *M. dodecapetala*, *M. hamorii*, *M. oblongifolia* and *M. portoricensis*, the five Caribbean Magnolias of which direct chromosome counts have been undertaken, we can state with high certainty that they are diploid. For *M. cristalensis*, *M. domingensis*, *M. minor*, *M. orbiculata* and *M. pallescens* we have a strong indication for their diploidy by the indirect method of flow cytometry performed on dried leaves. For *M. ekmanii* and *M. splendens* the dried leaf data did not render a clear peak in the flow cytometry measurements.

3. Biogeography of the Caribbean Magnolias

MODIFIED FROM: Veltjen E., Testé E., Palmarola Bejerano A., Asselman P., Hernández Rodríguez M., González Torres L. R., Chatrou L.W., Goetghebeur P., Larridon I., Samain M.-S. (submitted 9 December 2019) The evolutionary history of the Caribbean Magnolias (Magnoliaceae): testing species delimitations and biogeographical hypotheses using molecular data. Molecular Phylogenetics and Evolution. Impact factor 2018: 3.992.

ABSTRACT

The Caribbean islands provide a hard-to-beat setting for studying (historical) biodiversity, given its complex geological and environmental history and its historical and current geographical proximity to the American mainland. We reconstruct phylogenetic relationships of the Caribbean Magnolias to: (1) reveal their evolutionary history, (2) test the current largely morphology-based classification and assess species limits, and (3) investigate major biogeographic hypotheses proposed for the region. Nuclear and chloroplast DNA sequence data of all 15 Caribbean *Magnolia*[1] taxa are included, supplemented by a selection of American mainland species, and species representing all major clades[2] of the Magnoliaceae family. We constructed phylogenetic hypotheses in a time-calibrated Bayesian framework, supplemented with haplotype network analyses and ancestral range estimations. Genetic synapomorphies found in the studied markers confirm the species limits of 14 out of 15 morphologically recognizable Caribbean *Magnolia* taxa. However, our results challenge the currently accepted Magnoliaceae classification by strongly contrasting placement of the well-supported clades within the family depending on the genetic marker, resulting in low support values for the deeper classification in the species tree. This study delivers evidence for four colonization events of *Magnolia* into the Caribbean from the American mainland and puts forward overwater dispersal as the most plausible dispersal hypothesis, given age estimates of maximum 16 mya for their presence on the Caribbean islands.

3.1. INTRODUCTION

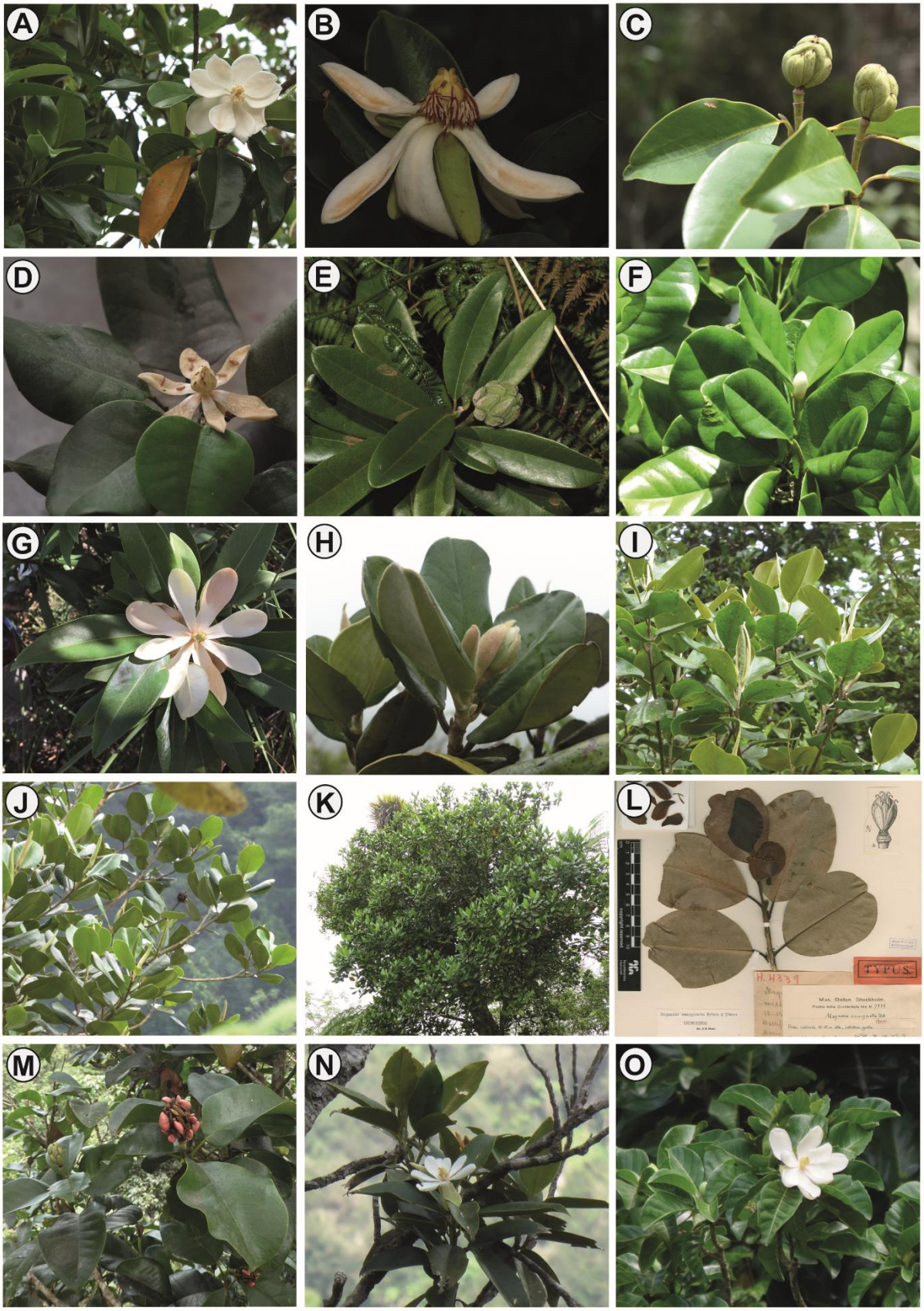
The Caribbean islands, also known as the West Indies, have a rich endemic biodiversity (Mittermeier et al., 2011; Myers et al., 2000; Smith et al., 2004) and complex geological and environmental history (Draper, 2008; Pindell et al., 2011), inspiring different biogeographic hypotheses on the evolutionary history of their present and past biodiversity (e.g. Graham, 2003b; Hedges, 2006; Iturralde-Vinent, 2006; Maunder et al., 2011; Ricklefs and Bermingham, 2008). There are three main hypotheses, or models, explaining the distribution of the

[1] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.
[2] A selection of less commonly used words is explained in the glossary: Appendix 1.1

Caribbean biodiversity in relation to related species on the American mainland: (1) vicariance (Rosen, 1975, 1985), (2) land bridges (Iturralde-Vinent and MacPhee, 1999), or (3) overwater dispersal (Hedges, 1996). The vicariance model proposes that the "Proto-Antilles" volcanic archipelago that existed between North and South America in the Mesozoic moved by plate tectonics in the Late Cretaceous (ca. 100–66 mya), resulting in the separation of biota. The land bridges model proposes a "landspan" between South America and the Greater Antilles for a short time during the Late Eocene–Early Oligocene (35–33 mya); this model is also called the GAARlandia hypothesis: Greater Antilles + Aves Ridge (Iturralde-Vinent, 2006; Iturralde-Vinent and MacPhee, 1999). The overwater dispersal model suggests that organisms dispersed by flying or flotsams from the mainland to the Caribbean islands during the Cenozoic (65.5 mya–present day). Most of the framework and testing of these biogeographic hypotheses were inspired by, and executed on, empirical data of vertebrates, which have distinct limitations in their dispersal (e.g. Alonso et al., 2012; Hedges, 2006). Data on the biogeography of the Caribbean flora, however, support the notion that all three hypotheses are valid (Francisco-Ortega et al., 2007; Nieto-Blazquez et al., 2017; Santiago-Valentín and Olmstead, 2004). This is not surprising given that seed dispersal greatly enhances the probability and geographical extent of plant colonization (Cano et al., 2018; Gugger and Cavender-Bares, 2013).

Biogeographic research in the region does not only cover questions on the relationship of the plant diversity in the Caribbean islands compared to the diversity on the continent, but also queries on the interplay between the different island masses, such as emergence versus submergence, land bridges and land block composition and movement, delivering the present day distribution of the Caribbean biodiversity (Oleas et al., 2013; Santiago-Valentín and Olmstead, 2004).

A flagship tree genus that offers an excellent case study to empirically test the array of Caribbean biogeographical hypotheses is *Magnolia* (Magnoliaceae). The tree genus is present on many of the different Caribbean islands and its reproductive biology is animal-mediated, i.e. seed dispersal by birds (Testé, 2018) and pollen dispersal by (large) beetles (Thien, 1974). A total of 15 accepted *Magnolia* taxa (i.e. species and subspecies) occur in the Caribbean (Figure 3.1, Table 3.1). Of these 15 taxa, 10 taxa make up the complete subsection *Cubenses*, delimited in previous family-wide phylogenetic studies by inclusion of 1–2 representatives (Azuma et al., 2001; Kim and Suh, 2013). Four out of these 15 Caribbean *Magnolia* taxa, belong to subsection *Talauma*, which in previous family-wide phylogenetic studies showed to be a well-supported sister clade to subsection *Cubenses* (Kim and Suh, 2013). The abovementioned subsections, together with subsections *Dugandiodendron* and *Chocotalauma* (Pérez et al., 2016), make up section *Talauma* (Figlar and Nooteboom, 2004). The last,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◄ **Figure 3.1** *Magnolia* diversity of the Caribbean. (A) *M. cubensis* subsp. *acunae* (Cuba). Photo: Alejandro Palmarola Bejerano; (B) *M. cubensis* subsp. *cubensis* (Cuba). Photo: Mikhail S. Romanov; (C) *M. cristalensis* (Cuba). Photo: Banessa Falcón; (D) *M. orbiculata* (Cuba). Photo: Emily Veltjen; (E) *M. oblongifolia* (Cuba). Photo: Alejandro Palmarola Bejerano; (F) *M. minor* (Cuba). Photo: Ernesto Testé Lozano; (G) *M. virginiana* subsp. *oviedoae* (Cuba). Photo: Ernesto Testé Lozano; (H) *M. domingensis* (Dominican Republic). Photo: Emily Veltjen; (I) *M. hamorii* (Dominican Republic). Photo: Emily Veltjen; (J) *M. pallescens* (Dominican Republic). Photo: Emily Veltjen; (K) *M. ekmanii* (Haiti). Photo: Emily Veltjen; (L) *M. emarginata* (Haiti). Scan: S herbarium; (M) *M. portoricensis* (Puerto Rico). Photo: Carlos Rodríguez, Arbonautas; (N) *M. splendens* (Puerto Rico). Photo: Emily Veltjen; (O) *M. dodecapetala* (Lesser Antilles: Saint-Vincent, Saint-Lucia, Martinique, Dominica and Guadeloupe). Photo: Emily Veltjen.

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fifteenth taxon, namely *M. virginiana* subsp. *oviedoae*, is placed in section *Magnolia*, unrelated to the other 14 Caribbean Magnolias. Two “calibrated” phylogenetic hypotheses on the Magnoliaceae generated in previous studies provide insufficient data to answer questions on Caribbean biogeography: one study included very few Caribbean taxa and potential mainland relatives (Nie et al., 2008) and a second one was based on limited chloroplast data (Azuma et al., 2001). Nie et al. (2008) included only the Caribbean *M. dodecapetala* and three Mexican *Talauma* species and no members of any of the other *Talauma* subsections (e.g. the Caribbean subsection *Cubenses*) in their phylogenetic hypothesis based on three nuclear genes using a Maximum Likelihood (ML) retrieved topology and a Penalized likelihood (PL) and Bayesian dating based on a relaxed-clock model. In their results exact dates for all the non-disjunct nodes are not tabulated nor given in the supplementary data; yet with the scale bar the MRCA of *M. dodecapetala* and the Mexican *Talauma* species is deducted to be about 31.6 mya. Azuma et al. (2001) included *M. portoricensis* and *M. splendens* in their ML phylogenetic hypothesis of the single chloroplast gene *matK*, whereby nodes were calibrated using the substitution rate of *matK* and the calibrated node of what according to the classification of Figlar and Nooteboom (2004) is considered section *Yulania*, set at 25 mya. In their phylogenetic hypothesis the crown node of the *Cubenses* clade is (deducted from the figure) dated around 30 mya and the stem group around 36 mya. For the members of subsection *Talauma* again *M. dodecapetala* was included, which forms a clade together with *M. ovata* from Brazil and has their crown node dated around 24.5 mya and their stem node at 35.6 mya. They also have one accession of *M. minor* included, which forms a clade with *M. mexicana* from Mexico, with their crown node dated around 37.5 mya.

Table 3.1 List of the 15 Caribbean *Magnolia* taxa (i.e. species and subspecies) and the currently accepted classification (at sectional and subsectional level).

Accepted taxa	**Synonyms**
Section *Talauma*	
Section *Talauma* subsection *Cubenses*	Subsection *Splendentes*
M. cristalensis	*M. cacuminicola*
	M. cacuminicola subsp. *cacuminicola*
	M. cacuminicola subsp. *bissei*
	M. cristalensis subsp. *cristalensis*
	M. cristalensis subsp. *baracoana*
	M. cristalensis subsp. *moana*
	M. cubensis subsp. *cacuminicola*
	M. cubensis var. *baracoensis*
M. cubensis subsp. *cubensis*	*M. cubensis* subsp. *turquinensis*
M. cubensis subsp. *acunae*	
M. domingensis	
M. ekmanii	
M. emarginata	
M. hamorii	
M. pallescens	
M. portoricensis	
M. splendens	
Section *Talauma* subsection *Talauma*	
M. dodecapetala	
M. minor	*Talauma minor*
	Talauma truncata
	Svenhedinia minor
	Svenhedinia truncata
M. oblongifolia	*Talauma minor* subsp. *oblongifolia*
	Talauma minor var. *oblongifolia*
	Talauma oblongifolia
	Talauma opithicola
M. orbiculata	*Talauma minor* subsp. *orbiculata*
	Talauma orbiculata
Section *Magnolia*	
M. virginiana subsp. *oviedoae*	

Howard (1948) revised the Caribbean *Magnolia* diversity and provided detailed information on 11 native species. The eight *Magnolia* species of Hispaniola, Puerto Rico and the Lesser Antilles are still delineated as such. In contrast to the straightforward taxonomic history of these eight species, the taxonomical history of the Cuban Magnolias is more complicated (Bisse, 1988; Imchanitzkaja, 1991; Imchanitzkaja, 1993). The number of Cuban taxa recognised raised to eleven in the studies of Imchanitzkaja (1991, 1993), i.e. five species and six heterotypic subspecies. Following the work of Imchanitzkaja, other authors have expressed a different opinion about the number of Cuban *Magnolia* taxa, and several names have been placed in synonymy (e.g. Acevedo-Rodríguez and Strong, 2019). The most recent revisions (González Torres et al., 2016; Palmarola et al., 2016) recognize six native Cuban *Magnolia* species, comprising seven native Cuban *Magnolia* taxa. These include a recently found population of *M. virginiana* from the Majaguillar Swamp in the north of the Cuban province of Matanzas (Oviedo Prieto et al., 2008), that was described as a subspecies due to its distinctive morphology: *M. virginiana* subsp. *oviedoae* (Palmarola-Bejerano et al., 2008). One of the species, i.e. *M. cubensis*, contains two subspecies: *Magnolia cubensis* subsp. *cubensis* and *M. cubensis* subsp. *acunae.*

Caribbean Magnolias have been distinguished based on morphological characters such as leaf size, shape and texture; leaf margin type; absence or presence of pubescence; stipules deciduous or not; number of perianth parts; and number of carpels (Howard, 1948; Imchanitzkaja, 1991; Imchanitzkaja, 1993). Although the morphological characters are defined as distinct in the species descriptions and identification keys, variation in many of the distinguishing characters has been reported (Howard, 1948; Palmarola et al., 2016; Stehlé and Marie, 1947). Coinciding with the morphological delimitations made, most populations occur as discrete geographical entities, either on a different island or a distinct, separate mountain chain within an island, whereby populations of adjacent species (within one island) are roughly between 30 km and 400 km apart. However, there are two sets of Caribbean *Magnolia* species that occur in sympatry, both of them in Cuba: *Magnolia cubensis* subsp. *cubensis* and *Magnolia orbiculata* in the Sierra Maestra Mountain Range, and *Magnolia cristalensis*, *Magnolia minor* and *Magnolia oblongifolia* in the Nipe-Sagua-Baracoa Massif.

By generating phylogenetic hypotheses including all the Caribbean Magnolias and a selection of mainland American Magnolias, based on both nuclear and chloroplast data, this study aims to test (1) **species delimitations**: Do chloroplast and nuclear DNA regions support the 15 Caribbean *Magnolia* taxa? (2) **classification**: Does the classification in which 14 Caribbean Magnolias are placed in section *Talauma* hold? Are there two (sister) clades, following subsections *Talauma* and *Cubenses*? (3) **phytogeography of the mainland versus the Caribbean**: Which of the three biogeographical hypotheses is most likely for the Caribbean

Magnolias: vicariance, land bridges or overwater dispersal? Which are the most likely source areas for the Caribbean *Magnolia* species? and (4) **phytogeography within the Caribbean islands**: Did the historic dispersal of *Magnolia* species follow any of the known Caribbean phytogeographic patterns?

3.2. MATERIALS AND METHODS

3.2.1 Taxon and data sampling

DNA sequence data were obtained from leaf samples collected from wild populations and *ex situ* collections dried in silica gel, supplemented by GenBank accessions (www.ncbi.nlm.nih.gov/genbank/, Clark et al., 2016). The molecular phylogenetic analyses comprise 62 Magnoliaceae taxa represented by 100 accessions. An overview of the (sub)species, populations, herbarium vouchers and reference publications is summarised in Appendix 3.1. To provide a good outgroup sampling, we aimed to represent all the different sections of *Magnolia* by at least two representatives where possible, given that there is no consensus on relationships between *Magnolia* sections as yet. However, herbarium vouchers and *ex situ* collections of section *Talauma*, usable for Sanger sequencing, proved to be scarce. Hence in the final sampling, all species of subsection *Cubenses* were represented at the population level, subsection *Dugandiodendron* was represented by three accessions, subsection *Talauma* by 13 accessions (of which four are Caribbean species) and subsection *Chocotalauma* has no representative in this study. DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle, 1987) with MagAttract Suspension G solution (Qiagen, Germantown, USA) (Xin and Chen, 2012) mediated cleaning (Larridon et al., 2015). Sequences were obtained via Sanger sequencing using forward and reverse primers summarised in Appendix 3.2. New primers were developed using the 1KP transcriptome data from *Magnolia maudiae*: XQWC and *Magnolia grandiflora*: WBOD (Matasci et al., 2014); and the transcriptome data of *Liriodendron tulipifera* from the Floral Genome Project (Liang et al., 2006). Eleven DNA regions were targeted and amplified: five (partial) nuclear genes: AGT1, GAI1, LEAFY, PHYA, SQD1; three (partial) chloroplast genes: *ndhF*, *rbcL* and *trnK*; and three chloroplast intergenic spacers: *atpB-rbcL*, *ndhF-rpl32* and *psbA-trnH*. GenBank accession numbers per DNA region can be found in Appendix 3.3. The nuclear regions were reconfirmed to be single copy in the *Magnolia* genome by BLAST searches against the *Magnolia kobus* genome (Park et al., 2017). PCRs were performed on a total volume of 25 µl under the following conditions: 2 min at 95°C; 35 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 90 s; 72°C for 6 min. The PCRs contained 0.2 µM forward primer, 0.2 µM reverse primer, approximately 5 ng/ml of DNA (suspended in 1× TE buffer) and 2× DreamTaq MasterMix (Thermo Fisher Scientific, Waltham, MA, USA) per reaction. PCR

products were run on a 1% agarose gel, stained with ethidium bromide and visualised under UV-light. Sanger sequencing was executed by Macrogen Europe (Amsterdam, the Netherlands).

3.2.2 Phylogenetic analyses and divergence time estimation

The raw abi-files obtained from Macrogen were assembled in Geneious v. 8.1.9 (https://www.geneious.com, Kearse et al., 2012). The forward and reverse reads were trimmed with an error probability limit of 0.01, sequence read direction was set, and the reads were assembled *de novo*. Ambiguous regions were annotated using the "Find Heterozygotes" plugin set to a peak similarity of 50%. Visual inspection of the assembly was executed while checking the disagreements between forward and reverse strand, as well as annotated heterozygous sites. The sequences were aligned using the MUSCLE plugin in Geneious. The previously marked inversions in *trnK* and *psbA-trnH* (Azuma et al., 1999b; Kim and Suh, 2013) were replaced by their reverse complement and coded as binary characters to acknowledge their presence while not biasing the result given the low sequence divergence of the family overall (Kim and Suh, 2013). The chloroplast genes were concatenated in Geneious as these fragments are linked on the circular chloroplast genome. The distance matrix of each multiple sequence alignment was extracted using Geneious, meaning that ambiguous sites and gaps (in their full length) were taken into account. PartitionFinder v.2.1.1 (Lanfear et al., 2017) was used to partition data. Candidate data blocks in the partitioning analyses respected coding and non-coding regions, including the three codon positions within the coding regions. Branch lengths were set to linked and the comparison of partitioning schemes used the greedy algorithm (Lanfear et al., 2012). Gaps were coded using Seqstate v.1.4.1 (Müller, 2005), whereby IndelCoder was set to Modified Complex Indel Coding (MCIC). Summary statistics for each of the eleven separate fragments were obtained using PAUP v.4.0a164 (Swofford, 2002). To acquire the separate gene trees, phylogenetic analyses for each of the six alignments (i.e. a single concatenated chloroplast and five nuclear alignments) were run with MrBayes v.3.2.6 (Ronquist et al., 2012) through the CIPRES web portal (Miller et al., 2010) and visualised using TreeGraph2 v.2.15 (Stöver and Müller, 2010). In the analyses of the gene trees, *Liriodendron tulipifera* was used as outgroup and partitions followed those found using PartitionFinder. Substitution models for each data partition were estimated during the MCMC runs (so-called "model jumping") by sampling across model space and integrating over all possible models. Two independent runs were performed, each with four MCMC chains of 10 000 000 generations, of which every 5000th generation was sampled. MCMC diagnostics of the gene trees were run using the package RWTY v.1.01 (Warren et al., 2017) in R v.3.6.1. (R Core Team, 2019). The first 25% of the sampled trees were discarded as burn-in (i.e. 500 out of 2001 trees).

The six alignments (i.e. a single concatenated chloroplast and five nuclear alignments) were also used to infer calibrated phylogenetic hypotheses using BEAST v.2.5.2. (Bouckaert et al., 2019) for all 100 accessions. Given the incongruences found among the six alignments that generated the six gene trees, the package *BEAST2 (Ogilvie et al., 2017) was used to estimate the underlying species tree. The total of 24 partitions (AGT1: 3 partitions; chloroplast: 11 partitions; GAI1: 3 partitions; LFYB: 2 partitions; PHYA: 3 partitions; SQD1: 2 partitions) were unlinked for substitution parameters and linked per alignment for clock and tree parameters, estimating six clocks and six gene trees that underlie the species tree. Substitution models for each data partition were estimated by model jumping using bModelTest in *BEAST2 (Bouckaert and Drummond, 2017): all the site model parameters were allowed to vary. All six clock models were estimated using random local clocks (Drummond and Suchard, 2010). Both the *Magnolia* stem node and crown node were calibrated. We used fossils of seeds and fructifications because they are the most diagnostic and reliably identified (Azuma et al., 2001; Hebda and Irving, 2004). Firstly, a uniform prior was put on the *Magnolia* crown node using *Magnolia tiffneyi*, described from fossilised seeds of the Oligocene Clarno Formation of Oregon (Manchester, 1994). This fossil taxon has seed morphology synapomorphies with the extant *Magnolia grandiflora* and extinct *Magnolia septentrionalis* (Manchester, 1994; Tiffney, 1977). However, because the sister clade of section *Magnolia* remains unresolved in the family-wide phylogeny (Azuma et al., 2011; Kim and Suh, 2013; Nie et al., 2008), the stem node of section *Magnolia* coincides with the crown node of the genus *Magnolia*; hence, for the crown node of the genus *Magnolia* we used a uniform prior with the minimum set to 44 mya. The maximum bound of this uniform prior for the *Magnolia* genus was set to be 70 mya as this is the estimated age for the Magnoliaceae family by Wikström et al. (2001). Secondly, a prior was set on the stem node of *Magnolia* using the *Archaeanthus* fossil (Dilcher and Crane, 1984), which is (one of) the oldest, well-documented and studied fossil collections assigned to Magnoliaceae (Doyle and Endress, 2010; Romanov and Dilcher, 2013), placed in the uppermost Albanian-mid-Cenomanian of the Cretaceous (ca. 98 mya). Because the fossil is most convincingly placed as a sister lineage to the Magnoliaceae (Doyle and Endress, 2010; Massoni et al., 2015b), its age was set as the maximum age for the crown node of the family Magnoliaceae. To allow for younger ages, the minimum bound for this split was set to 44 mya, again conforming to the oldest, morphologically well-studied fossil linked to the extant members of the *Magnolia* genus. To determine if any of the set priors interact significantly, the analysis was run by sampling from the prior for 700 00 000 MCMC generations.

*BEAST runs were set to continue indeterminately, and the resulting parameter values were tested periodically for convergence as indicated by the effective sample sizes (ESS) using Tracer v.1.7.1 (Rambaut et al., 2018). A final number of 2 000 000 000 generations was

needed to reach ESS values >100 and a burn-in of 10% was shown to be necessary. To study the topology, the species trees were visualised using DensiTree v2.5.2 (Bouckaert and Heled, 2014) for which a resampling of 20 000 was allowed using LogCombiner v.2.5.2 (Rambaut and Drummond, 2019), due to memory constrains of the DensiTree software. To visualize the estimated age of each node, the 2 000 000 000 species trees were summarised using TreeAnnotator v1.8.2 (Rambaut and Drummond, 2015) with a burn-in of 10% as found by Tracer, into a maximum-clade-credibility summary tree whereby the node heights represent the mean heights. The summarised tree was visualised using Figtree v. 1.4.2 (Rambaut, 2014).

3.2.3 Testing of biogeographical hypotheses

Ancestral range estimation was conducted using the R package 'BioGeoBEARS' (Matzke, 2013; Matzke, 2014). Because the focus of the biogeographical hypotheses on the Caribbean islands, we used the calibrated subtree only with the members of subsections *Cubenses* and *Talauma*, excluding the *M. virginiana* subsp. *oviedoae* accession of Cuba. We defined six geographic areas: from North to South: Mesoamerica (M), Cuba (C), Hispaniola (H), Puerto Rico (P), the Lesser Antilles (L) and South America (S). We analysed our dataset under three models: the DEC model (Dispersal-Extinction-Cladogenesis; Ree et al., 2005; Ree and Smith, 2008), the "DIVALIKE" model and the "BAYAREALIKE" model. For our data the models with the "jump dispersal" or founder (j) parameter were not taken into account, as the dispersal events under the J parameter occur in the nodes and were not penalised in the AICc scores (Ree and Sanmartín, 2018). The fit of the three models to the dataset was compared using the AICc criterion (Burnham and Anderson, 2002).

3.2.4 Haplotype network analyses

We conducted a network analysis of the (derived) haplotypes using the R package pegas (Paradis, 2010) focused on *M. minor* and *M. oblongifolia* accessions to contest their species delimitation. This alternative method was executed, as heterozygous sites represented by ambiguous IUPAC characters were unaccounted for in the Bayesian phylogenetic analyses, yet clearly present in a set of the sequenced DNA regions when looking at the raw alignments. For the nuclear single copy genes, the genotypes were phased to their haplotypes using DnaSP v.6.12.03 (Rozas et al., 2017) using the PHASE algorithm (Stephens and Donnelly, 2003; Stephens et al., 2001) run per gene with the default MCMC settings and the assumption of no recombination within one gene. Each of the included samples was coded by its species identification and population. The first consisted of the options *M. minor*, *M. oblongifolia* or mixed morphology, whereas the latter was composed of a three-letter abbreviation representing its collection site: CGU, CMU, CUP, LME, MIB, NDT, PCR, YAM and YUM (Appendix 3.1).

3.3. RESULTS

3.3.1 Phylogenetic analyses and divergence time estimation

The six alignments comprised 12257 base pairs in total. The concatenated chloroplast sequence comprised 8351 base pairs, which corresponds to about 5.21–5.28% of the full *Magnolia* chloroplast genome (Shen et al., 2018). The percentage of parsimony informative characters (PIC) of the different amplified regions are depicted in Figure 3.2.

Gene trees for each of the amplified regions and for the concatenated chloroplast alignment are compiled in Appendix 3.4, whereby the pairwise distance matrix of the Caribbean Magnolias is tabulated in Appendix 3.5. Partitioning schemes for all analyses are summarised in Appendix 3.6. The DensiTree species tree and the summarised calibrated multi-species coalescent tree are depicted in Figure 3.3 and Figure 3.4, respectively. The time calibrations of significant clades: all the main nodes of containing Caribbean taxa as well as the supported nodes of the non-Caribbean taxa (i.e. with a posterior probability higher than 0.95) from Figure 3.4 are depicted in Table 3.2.

3.3.2 Testing of biogeographical hypotheses

The raw output of the BioGeoBEARS analysis is tabulated in Appendix 3.7. According to the AICc criterion, the “DIVALIKE” model best fits the data on the Caribbean Magnolias out of the three tested models. Ancestral range estimation results constructed using the “DIVALIKE” model and a schematic overview of de six defined geographic regions are visualised in Figure 3.5.

3.3.3 Haplotype network analyses

Figure 3.6 illustrates the relationships among the sequenced chloroplast and simulated nuclear haplotypes present in the data for the *M. minor* and *M. oblongifolia* species complex. In the chloroplast haplotypes we can allocate the H_IV haplotype to *M. oblongifolia* given the pure population of MIB and the H_II to *M. minor* given the pure population of YUM (Figure 3.6A). CGU, YAM, LME, all surrounding populations of MIB with individuals morphologically identified as *M. minor*, have the same haplotype as defined for *M. oblongifolia*. In AGT1 (Figure 3.6B), two more derived haplotypes are found in the MIB population (i.e. H_VIII and H_VII). For GAI1 (Figure 3.6C) the haplotype of the MIB population is found in all populations around (CGY, YAM, CUP. LME, NDT). For PHYA (Figure 3.6D) there is one haplotype only found in MIB (i.e. H_III).

Figure 3.2 Parsimony informative characters (PIC) of the Magnoliaceae Sanger sequencing alignments used in this study. This count includes both parsimony informative substitutions, gaps and inversions.

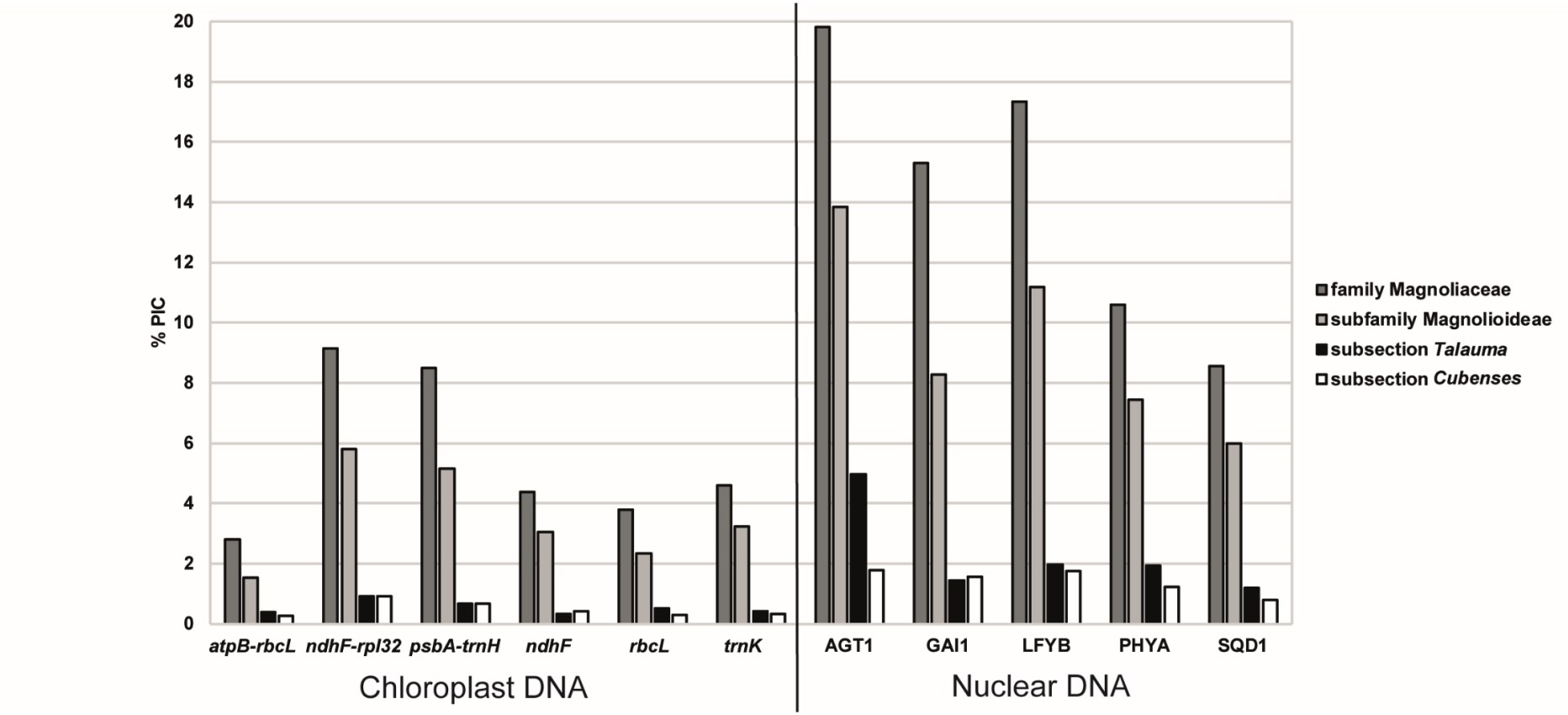


Figure 3.3 Species trees of the Magnoliaceae species comprising both the nuclear (i.e. AGT1, GAI1, LFYB, PHYA, SQD1) and the chloroplast (i.e. *atpB-rbcL, ndhF*, *ndhF-rpl32*, *psbA-trnH*, *rbcL*, *trnK*) sequences visualised using DensiTree (900001 species trees after resampling). *Liriodendron* and *Magnolia* species are classified according to their lowest possible rank published in Figlar and Nooteboom (2004), represented by a three-letter abbreviation.

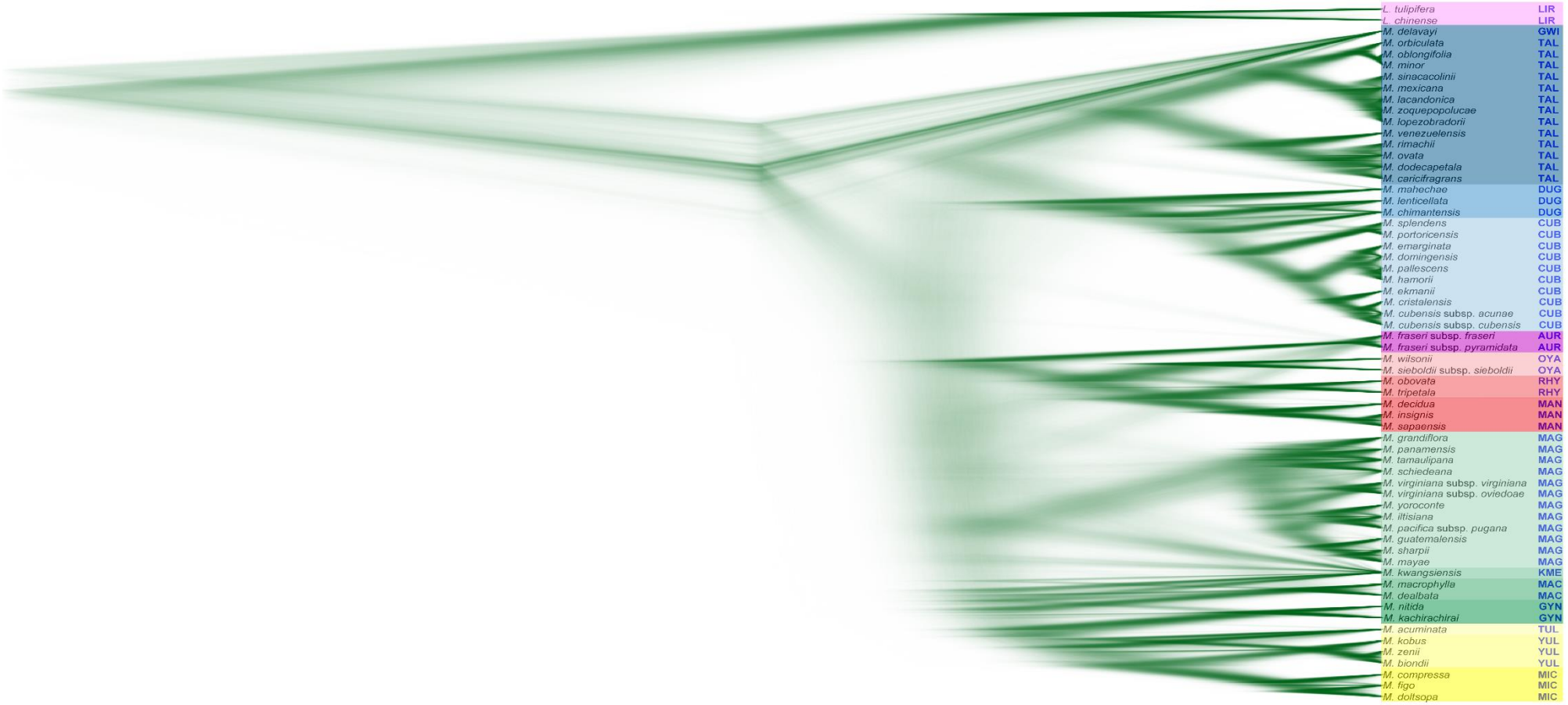


Figure 3.4 Calibrated phylogenetic hypothesis of the Magnoliaceae species comprising both the nuclear (i.e. AGT1, GAI1, LFYB, PHYA, SQD1) and the concatenated chloroplast (i.e. *atpB-rbcL, ndhF*, *ndhF-rpl32*, *psbA-trnH*, *rbcL*, *trnK*) sequences. Node labels represent the posterior probabilities. Supported nodes are highlighted by a green circle. The tree was calibrated with the *Archaeanthus* fossil, placed as a maximum age of the Magnoliaceae crown node and the fossil *Magnolia tiffneyi* placed as a minimum age on the crown node of *Magnolia* (= Magnolioideae subfamily). The x-axis represents time (mya). Node bars represent the 95% interval of the age estimates. *Liriodendron* and *Magnolia* species are classified according to their lowest possible rank published in Figlar and Nooteboom (2004), represented by a three-letter abbreviation.

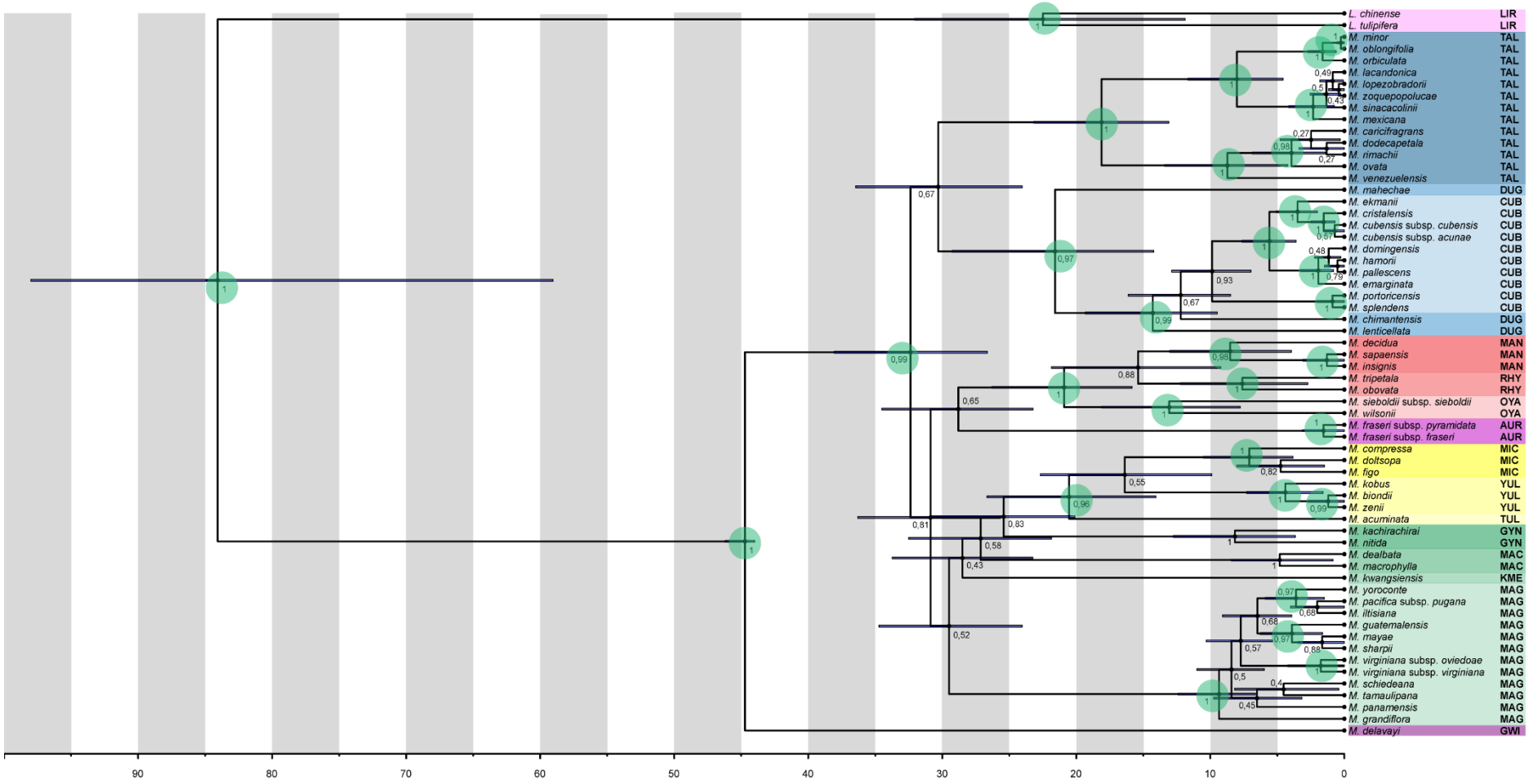


Figure 3.5 Subtree of the ancestral range estimation results of *Magnolia* taxa from subsection *Cubenses* and subsection *Talauma*, constructed in BioGeoBEARS using the "DIVALIKE" model. Each colour represents one of the six defined geographic regions, illustrated by the map in the top of the figure.

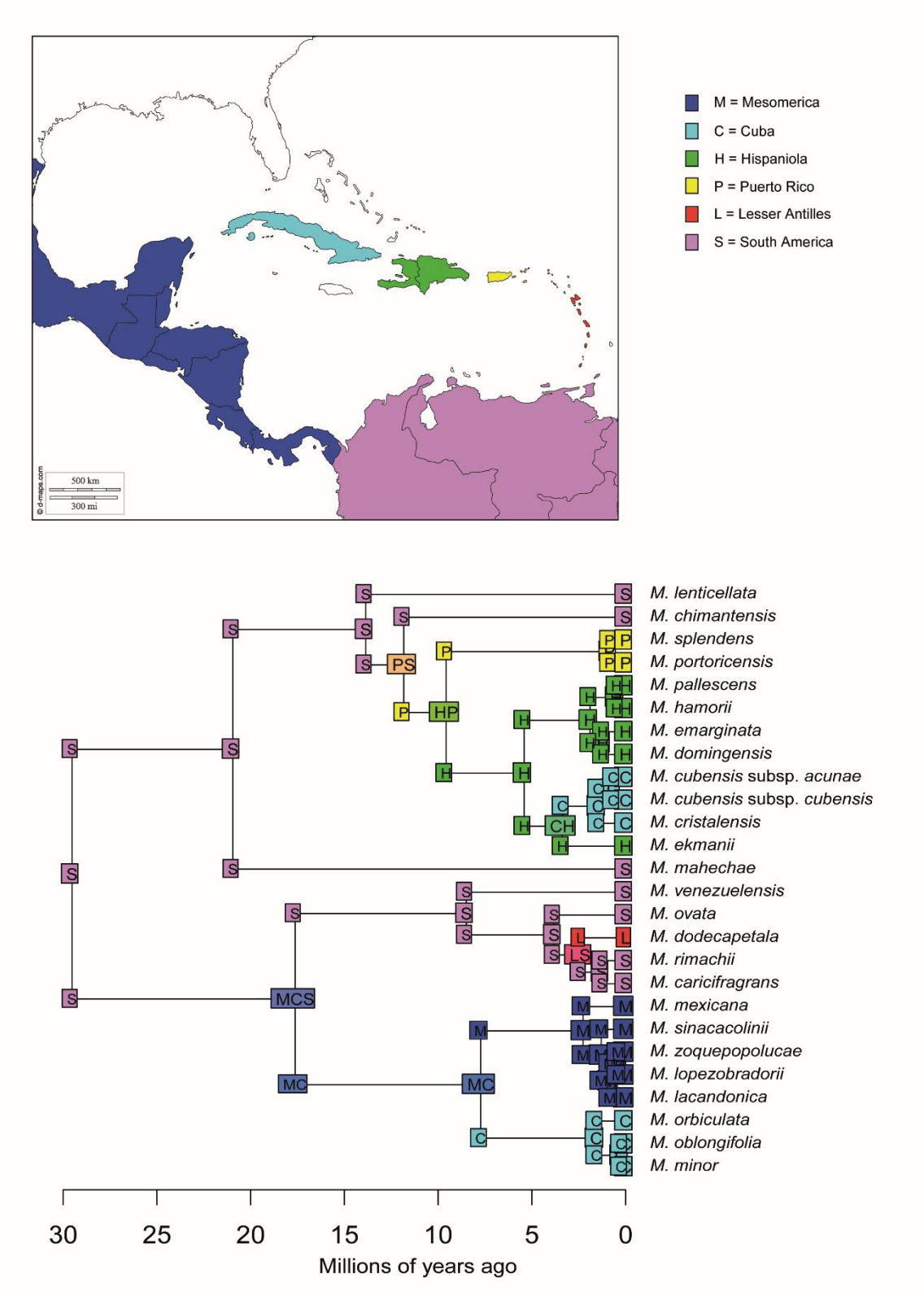


Table 3.2 Important clades of the generated calibrated Bayesian framework phylogenetic hypothesis of 100 Magnoliaceae accessions representing 62 species, with their estimated age and posterior probabilities (pp). Ages are expressed as mya (million years ago). All the clades retrieved in the phylogeny for the Caribbean Magnolias are discussed. At family level, only the clades supported by pp higher than 0.95 are tabulated. For each tabulated clade, we sum up the underlining gene trees with pp higher than 0.95 for that node from Appendix 3.4. An asterisk * indicates which node is calibrated.

Clade	**pp**	**Age: mean**	**Age: range**	**Gene trees**
Family Magnoliaceae	1	84.06*	98*–59	1–6
Genus *Liriodendron*	1	22.49	32–12	1–6
Genus *Magnolia*: *M. delavayi* most basal	1	44.73*	46–44*	2, 3
Genus *Magnolia sine M. delavayi*	0.99	32.37	38–27	2, 3
Section *Talauma*	0.67	30.37	36–24	1
Subsection *Talauma*	1	18.05	23–13	1, 2–6
Subsection *Talauma*: split Cuba & Mexico	1	7.09	12–5	1–2, 4–5
Subsection *Talauma*: split Lesser Antilles & South-Am.	0.99	3.76	7–1	1
Subsection *Cubenses* + *Dugandiodendron*	0.97	21.46	29–14	1 (2, 4)
Subsection *Cubenses*: split from *M. chimantensis*	0.68	12.11	16–8	1–2, 4
Subsection *Cubenses*	0.93	9.79	13–7	1–3, 5
Subsection *Cubenses*: MRCA of Cuba and Hispaniola	1	5.53	8–4	1
Subsection *Cubenses*: MRCA of *M. ekmanii* and Cuba	1	3.44	5–2	1, 5–6
Section *Auriculata*	1	1.46	3–2	1–6
Section *Rhytidospermum* + section *Manglietia*	1	20.81	26–16	3, 5
Subsection *Oyama*	1	13.21	18–8	1–5
Subsection *Rhytidospermum*	1	7.72	12–3	1, 3–5
Section *Manglietia*	0.99	8.53	13–4	1, 3
Section *Macrophylla*	1	4.85	8–1	1–2, 5
Section *Tulipastrum* + *Yulania* + *Michelia*	0.96	20.68	27–14	2
Section *Gynopodium*	1	8.19	13–4	1–3, 5–6
Section *Yulania*	1	4.34	7–2	1–5
Section *Michelia*	1	7.02	11–4	1, 3–6
Section *Magnolia*	1	9.23	12–6	1–2, 5

3.4. DISCUSSION

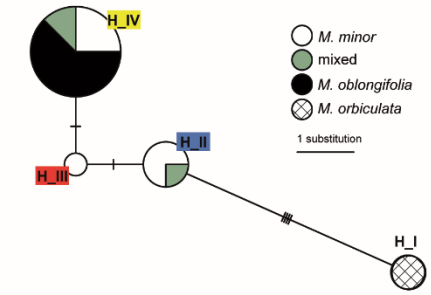
3.4.1 Species delimitations

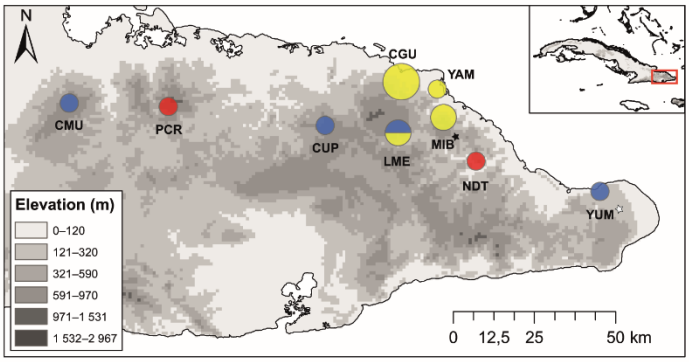
We find genetic synapomorphies delimitating 14 out of 15 Caribbean taxa across the different gene trees (Appendix 3.4 and 3.5), the exception being the *M. minor* and *M. oblongifolia* species complex. As there is no scientific consensus on how many genetic synapomorphies define a clear species, and as speciation is a continuous process (de Queiroz, 2007), their distinctive morphology, geography and (limited) genetic synapomorphies support their delineation as separately evolving metapopulation lineages.

For all the Caribbean taxa we included (minimally) one accession per population in our sampling, which in most cases rendered no to very little intraspecific variation (Appendix 3.5). Most of the chloroplast intraspecific variation that shows high pairwise distance numbers denoted in Appendix 3.5 represents gaps, especially in the non-coding DNA such as the poly-A sequence in *psbA-trnH*. Irrespective of the gaps, the intraspecific variation in substitutions between the populations of *M. dodecapetala* raise attention. *Magnolia dodecapetala* is noteworthy given that the genetic differences between the population of Martinique and Guadeloupe in all six alignments, even for the conserved chloroplast sequences, is in a similar extent as between within-island sister species pairs (Appendix 3.5).

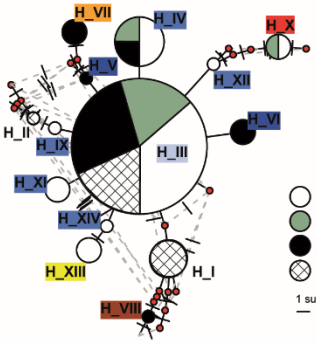
► **Figure 3.6** Haplotype networks and haplotype pie charts per population based on the parsimony informative characters (PIC) found in four alignments of the Cuban sympatric species *Magnolia minor* and *Magnolia oblongifolia*. The size of the pie charts corresponds to the sample size of that haplotype. *Magnolia orbiculata* was included as an outgroup in the haplotype analyses, as its species delimitation is not questioned due to its geographical, phylogenetic and morphological distinctness. The MIB population (black star) is labelled to have individuals only with a *M. oblongifolia* morphology and the CMU, YAM and YUM populations were labelled to have only individuals with the *M. minor* morphology, of which YUM is the most isolated (white star). For the chloroplast (A), GAI1 (C) and PHYA (D) haplotypes, each haplotype is assigned a different colour. For the AGT1 haplotypes (B) H_III was the most frequent found haplotype (light blue). The AGT1 haplotypes that differ with only one substitution from H_III are given shades of blue (medium blue for those with the *M. minor* morphology and dark blue for those with the *M. oblongifolia* morphology). The AGT1 haplotypes that differ with more than one base pair (i.e. H_XIII, H_VII, H_VIII and H_X) are coloured from yellow to dark red with increasing redness according to increased number of substitutions compared to H_III. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017); Protected Areas: (CNAP, 2014); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).

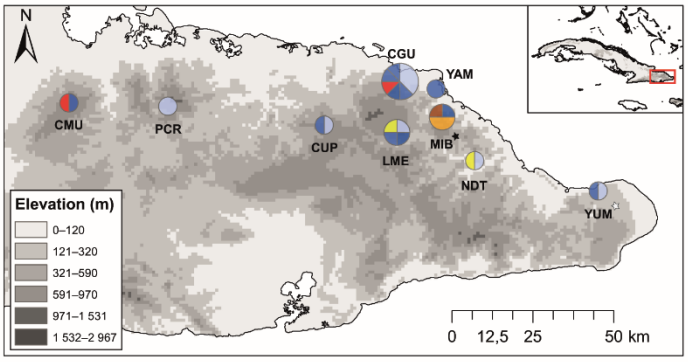
A) Chloroplast haplotypes



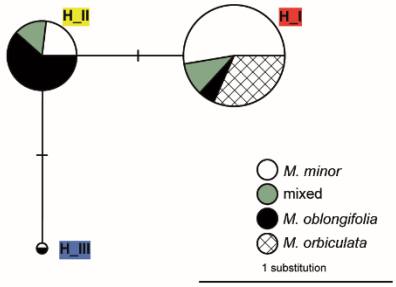


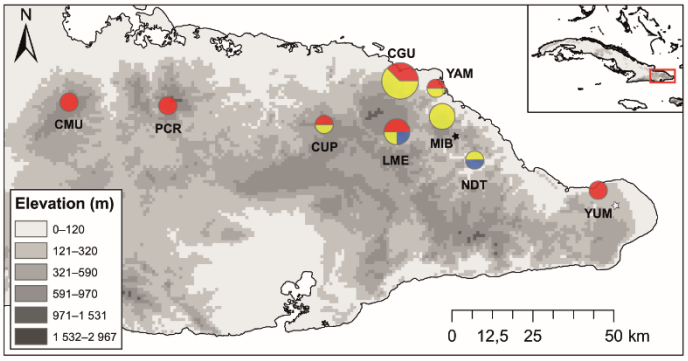
B) AGT1 haplotypes



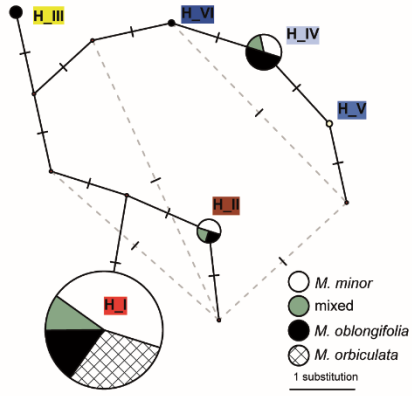


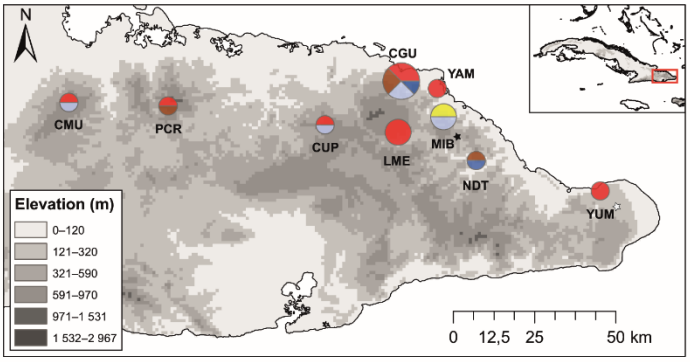
C) GAI1 haplotypes





D) PHYA haplotypes





It is not surprising that this intraspecific genetic variation is greater given the distinct oceanic boundaries between the islands of the Lesser Antilles. The relationships between *M. domingensis*, *M. emarginata, M. hamorii* and *M. pallescens*, all four occurring in the Cordillera Central Mountains of Hispaniola, show a very clouded network (Figure 3.3) and low posterior probabilities in the species tree (Figure 3.4). This could be partly due to the species circumscription of *M. domingensis* which needs a revision: we see in the gene trees that the Haitian accession identified as *M. domingensis* MA2167 (*Ekman 2180*, B) is not inferred as sister to the *M. domingensis* accessions from the south of the Cordillera Central in the Dominican Republic (Appendix 3.4.1, 3.4.3, 3.4.4). Unfortunately, the DNA extracted from the Haitian type specimen (*Nash 1081*, BM) was fragmented and could not be used for this study. Since the collections of Ekman in 1925, no new collections of *Magnolia* have been made in the Cordillera Central of Haiti. It is clear from both this study and a previous one using microsatellite markers (Veltjen et al., 2019) that, although the species are geographically, morphologically and (partly) genetically discrete, they represent recent speciation events. Similarly, the relationships among the three Cuban taxa of subsection *Cubenses* show a blurred relationship in the DensiTree figure (Figure 3.3), and hence, their relationship is unresolved, visible by the low posterior probability in the summary tree (Figure 3.4). Interestingly, based on chloroplast data (Appendix 3.4.1), *M. cubensis* subsp. *acunae* and *M. cristalensis* appear to be sister taxa – which comes down to a signal of six synapomorphies shared between *M. cubensis* subsp. *acunae* and *M. cristalensis*, of which four are found in the *ndhF* gene, that outweigh the single one found between the two *M. cubensis* subspecies and the single synapomorphy that brings together *M. cubensis* and *M. cristalensis* (Appendix 3.5). One synapomorphy in favour of the two *M. cubensis* subspecies being sister taxa in the GAI1 gene (Appendix 3.4.3), without any contrasting signals, outweighs the chloroplast data in the summary tree, albeit with low support (Figure 3.4). It was expected that there would be a great genetic similarity between the two subspecies based on morphology (Hernández Rodríguez, 2014; Imchanitzkaja, 1991; Imchanitzkaja, 1993) and other phytogeographical studies (Borhidi, 1996). As the current data cannot give us a straightforward answer on their sister relationship, they do show that the genetic discrimination among the two subspecies is similar to that between other recognised sister species pairs (Appendix 3.5). This information, together with the large geographical distance between them (ca. 400 km), does call for a revision of their subspecies status to be lifted to the species level.

Magnolia minor and *M. oblongifolia* show a very recent yet supported sister species relationship in the summary tree (Figure 3.4), but this is not expressed as a clear alignment of genetic synapomorphies and morphology in any of the gene trees (Appendix 3.4, 3.5). This contrasts with the morphological differentiation between the two, which is at least equally

distinct as between most of the other sister-species pairs of Caribbean Magnolias (see Figure 1.3): *M. oblongifolia* has distinct oblong-elliptic leaf shape, compared to the orbicular or obovate leaves from the other Cuban Magnolias; a rhombic fruit shape, compared to the ellipsoid fruit of *M. minor* and the small tree size to the large tree size of *M. minor* (Palmarola et al., 2016). The high presence of ambiguous characters in the sequences of the species' representatives also did not translate into a clear haplotype pattern that aligns with their morphological identifications (Figure 3.6). The occurrence of a range of different haplotypes (Figure 3.6) and the long branches of the accessions of these species in many of the gene trees (Appendix 3.4.3–3.4.6) do show that this species complex has a high amount of genetic variation compared to the other Caribbean accessions; yet most of this genetic variation seems to be mixed rather than align with the currently defined morphological differences. Two possible explanations for this genetically variable species complex are that we either have two former species that are now hybridizing successfully for already more than one generation (Schley et al., 2019), or that we are looking at sympatric speciation in process, whereby only the genes under selection will give a clear differentiation for the two morphological entities we observe (Smadja and Butlin, 2011).

An intraspecific *M. virginiana* study of Azuma et al. (2011) proved that for the chloroplast data *M. virginiana* subsp. *oviedoae* did not have a specific haplotype linked to its population, in contrast to other haplotypes found in the wider distribution of the species. Given the study of Azuma et al. (2011), we are aware that using a combination of GenBank sequences and the new sequences from Conrad s.n. (Appendix 3.1) make the discussion of the taxon delimitation difficult. However, for the fragment of LFYB and *ndhF-rpl32* from *M. virginiana* of Florida (Appendix 3.1) we do find one and two substitutions, respectively, that allow discrimination between the two sequences. A more profound sampling of the species and, preferably, the usage comparative genomics data would be necessary for a more conclusive answer on its species delimitation. At this point we cannot exclude the possibility that the difference in morphology, and thus the status of a subspecies, is merely phenotypic plasticity given the colonised marsh habitat (Oviedo Prieto et al., 2008; Palmarola-Bejerano et al., 2008; Testé, 2018).

3.4.2 Classification

Talauma is the section of interest when discussing Magnolias in the Caribbean. Surprisingly, in all the nuclear alignments (i.e. Appendix 3.4.2–3.4.6) this section does not hold as a supported clade, which results in a low posterior probability for the clade in the summarised tree (Figure 3.4, Table 3.2), which questions the current classification (Figlar and Nooteboom, 2004) and is not in line with the previous study executed with chloroplast DNA only (Kim and

Suh, 2013). The results demonstrate that subsection *Cubenses* is nested within a paraphyletic subsection *Dugandiodendron*. It is expected that the support of the clade comprising subsection *Dugandiodendron* and *Cubenses* would be even higher, should *M. mahechae* be resequenced, given that we observe a conflicting position of this herbarium type specimen between the gene tree of *ndhF* (Kim et al., 2001), where it is in the clade comprising of members of subsection *Talauma* and the gene tree of *matK* (Azuma et al., 2001), where it is in the clade comprising of members of subsection *Dugandiodendron*. However, the evidence for this paraphyletic relationship is rather scarce due to few accessions of subsection *Dugandiodendron* sequenced so far (Rivers et al., 2016) and the limited number of genetic regions sequenced for the included taxa of subsection *Dugandiodendron* (Appendix 3.2). It would not be surprising that subsection *Cubenses* is merely the crown group of subsection *Dugandiodendron* given their synapomorphy of anther tip embedment (Figlar and Nooteboom, 2004). Yet, given their difference in woodiness of the fruit pericarp, there was a synapomorphic signal expected for the members of subsection *Dugandiodendron* as well. Within subsection *Talauma*, the sampling across the family is far from complete (Rivers et al., 2016), yet this dataset so far separates the Mesoamerican taxa from the South American ones in two well-supported clades.

Zooming out on the classification of the Magnoliaceae family as depicted by the phylogenetic hypotheses, the sister-relationships among well-supported and recognised clades (Table 3.2) other than section *Talauma* are quite inconsistently placed when comparing the different gene trees (Appendix 3.4), hence, their relationships remain unresolved in the species tree (Figure 3.4), apparent from low support values as previous studies had concluded as well (Azuma et al., 2011 and precursors; Kim and Suh, 2013 and precursor; Nie et al., 2008). The classification of Figlar and Nooteboom (2004) overall follows the robust and (then) supported clades found in chloroplast phylogenetic studies executed prior to their taxonomical revision (Azuma et al., 1999a; Azuma et al., 1999b; Kim et al., 2001). The phylogenetic hypothesis published here (Figure 3.4) shows that the lowest possible ranks of Figlar and Nooteboom (2004) remain supported when adding nuclear and more chloroplast data. The results of this phylogenetic hypothesis challenge the limited number of higher classifications (e.g. subgenera within the *Magnolia* genus and subsections within sections) that imply there to be relationships between the lowest possible ranks that group the *Magnolia* species (Figlar and Nooteboom, 2004). Namely, the subsections of the section *Yulania*, i.e. subsection *Yulania* and subsection *Tulipastrum*, are not found in a supported sister relationship; the subgenus *Magnolia* is unsupported: the sections that are classified within this subgenus show no genetic synapomorphies; section *Rhytidospermum* is unsupported, i.e. subsection *Rhytidospermum* and subsection *Oyama* are not found as supported sister clades. Interestingly, the data puts

forward a supported relationship between the members of subsection *Rhytidospermum*, subsection *Oyama* and section *Manglietia*; a relationship that previously was not found (Kim and Suh, 2013). Lastly, the only deeper node that remains supported is that of subgenus *Yulania*, here represented by section *Tulipastrum*, section *Yulania* and section *Michelia*. Although all the included lowest possible taxonomic ranks are retrieved in the species tree, we did notice that even some of these robust groups (i.e. *Magnolia*, *Manglietia* and *Rhytidospermum*) do not withhold in each gene tree (See Appendix 3.4), which could be attributed to more complex evolutionary trajectories for that species×gene combination, or homoplasies. Surprisingly, *M. delavayi* has a supported basal position in the genus *Magnolia* in the summary tree (Figure 3.4). When aligning this result with the gene trees, this position results from a very strong signal from the AGT1 and GAI1 nuclear genes. The inconsistent topology of the clades in the Magnoliaceae family depending on the genetic region studied, in contrast to the robustness of the separate clades, puts forward the hypothesis that either we are dealing with such a low sequence divergence that genetic homoplasies combined with few gene fragments quickly disturb the analyses to recover the true relationships between species, or that evolutionary novelties defining the clades as we see today, evolved in a rapid evolutionary timespan, giving an extreme case of incomplete lineage sorting to this day forward. Either way, with the era of phylogenomics (McKain et al., 2018; Young and Gillung, 2019) knocking on the door of the Magnoliaceae phylogeny research (Park et al., 2017; Veltjen et al., 2018), solving the overall relationships among the clades is within reach.

3.4.3 Robustness of the fossil calibrations

When calibrating a phylogenetic hypothesis, the priors can greatly influence the results, as they, together with the effective sequence data, determine the range in which the posterior values can be found. In the calibrated phylogenetic analysis (Figure 3.4) we observe that for both calibrated nodes the set maximum for the crown node of the Magnoliaceae, and set minimum for the crown node of *Magnolia*, of their uniform prior distributions are within the 95% HPD (Table 3.2). The Magnoliaceae node, calibrated by the prior setting *Archaeanthus*, has a large 95% HPD, while the *Magnolia* node, calibrated by the prior setting of *M. tiffneyi*, shows a very short 95% HPD: the data push the posterior towards younger ages, towards the minimum bound of the prior distribution.

We placed *Archaeanthus* on the crown node of the Magnoliaceae, representing the maximum age of the node, following the placement of the fossil being sister to the MRCA of the extant Magnoliaceae (Doyle and Endress, 2010). This reasoning is followed by other authors, however, translated differently given different research questions and subsequently sample design: other studies often position *Archaeanthus* on the stem node of the Magnoliaceae,

representing the minimum age of the node (e.g. Massoni et al., 2015b; Pirie and Doyle, 2012). In contrast, a detailed morphological study of the fruit pericarp, executed by Romanov and Dilcher (2013), consider *Archaeanthus* to be a stem relative of *Liriodendron*, rather than of Magnoliaceae, which implies the fossil could be placed on the crown node of the Magnoliaceae family as a minimum age, not a maximum age. According to Massoni et al. (2015b), this conclusion was the result of redundant characters usage and inappropriate outgroups, and hence invalid. A last argument in the debate on the fossil calibration of the Magnoliaceae node is a less commonly used Cretaceous fossil: *Liriodendroidea*, which is reported to be reliably linked to the family (Nie et al., 2008) and estimated to be 93.5 mya (Frumin and Friis, 1996; Frumin and Friis, 1999). The fossil is associated with *Liriodendron* which implies it could be used as a minimum age for the Magnoliaceae crown node as well. However, given the more in-depth study of the morphology of *Archaeanthus*, the higher amount of fossil structures available and the more frequent usage by a range of researchers who each assessed its reliability for fossil calibration linked to the Magnoliaceae or its related plant-families, we decided to use *Archaeanthus* instead of *Liriodendroidea*.

The crown node age of *Magnolia*, calibrated with the minimum age of 44 mya, represented by the fossil *M. tiffneyi*, is being pushed towards the minimum bound of the prior distribution, apparent from its narrow confidence interval (Table 3.2). This could either be due to a discrepancy between the placement and/or age of the fossil and the sequence data, or an unforeseen interaction with the first calibrated node which forces a younger age downstream in the tree, given that 98 mya was set as a maximum bound, rather than a minimum bound for the node calibration as discussed in the previous paragraph. Considering the potential discrepancy in sequence diversity and the fossil placement/date: although morphologically resembling the extant *M. grandiflora*, it is possible that the fossil belongs to an extinct *Magnolia* stem lineage with homoplasious seed morphology characters. Considering the potential influence of the two calibration points: it is possible that the two calibrations interact, yet when the analysis was run sampled from the prior only (hence the sequence data are empty alignments) the ages of the nodes have 95% HPD ranges that encompass their full prior range. It is also possible that the fossil placement and dating is correct, and that there is no interaction between the set priors, whereby the low sequence evolution is in such extent that we obtain underestimations of the clade ages based on the sequence data alone (Barba-Montoya et al., 2018).

Lastly, although Nie et al. (2008) also used the *Archaeanthus* fossil in their calibration, we decided not to work with the Miocene *M. latahensis* fossil in our analysis, given the conflicting results retrieved from two amplified regions of this fossil, casting doubt on its placement (Golenberg et al., 1990; Kim et al., 2004).

3.4.4 Biogeographical history of the Magnoliaceae revised

Although not the aim of the current biogeographical study, the wider inclusion of taxa over the Magnoliaceae family enables to revise some biogeographical patterns with the calibration and topology of Figure 3.4. Overall, the assumption is made that the current affinity of most Magnolias with humid, warm temperate habitats, which occur at low altitudes in low to mid-altitudes and at higher altitudes in low latitudes, is also their past climatic "preference" (Hebda and Irving, 2004). The Cretaceous fossil record and Eocene *Magnolia* fossils found in Europe, together with the long branch leading up to the MRCA of the extant Magnolias, is associated with the concept of the "Boreotropical Flora" (Tiffney, 1985), or in this case "Boreotemperate" flora (Hebda and Irving, 2004). In this concept North Atlantic intercontinental connections allowed migration, most likely via the Thulean isthmus, rather than the Beringian and De Geer land-bridges, as fossil records are not found at such high latitudes (Hebda and Irving, 2004) and a mass-extinction of this flora occurred around the Oligocene cooling, forcing a southward migration. Despite the long history of Magnoliaceae, it is generally accepted that the diversification of extant taxa within *Magnolia* occurred recently. The MRCA of the Magnolioideae is dated to the Eocene coinciding with the fossil record and previous molecular analyses (Azuma et al., 2001; Nie et al., 2008). Although this was verified in this analysis, it was markedly determined by the set prior for that node, and after the bifurcation of *M. delavayi*, the sole representative of the Asian tropical section *Gwillimia*, the data jump towards the younger age of 32 mya for the MRCA of the rest of subfamily. The age of 32 mya emphasizes the Terminal Eocene Event at the Eocene-Oligocene boundary (Wolfe, 1978) and the subsequently southward migration as major drivers in the formation of the current main clades, rather than the preceding Early Eocene Climate Optimum as suggested by the calibration of Nie et al. (2008). The unsupported deeper nodes of the current phylogenetic hypothesis do not allow us to revise the concept of tropical intercontinental disjunctions within the family, for which it would be expected that their MRCA is dated before the Terminal Eocene Event. However, we do see that the tropical clades (in this study: section *Gwillimia* and *Talauma*) branch of first and are roughly dated before 30 mya.

When examining the concept of the temperate intercontinental disjunctions in the family, we can revise data of three supported clades that have extant Magnoliaceae of both the American and Asian continent (Table 3.2 and Figure 3.4). The first is the *Rhytidospermum* clade, containing *M. obovata* (Asian) and *M. tripetala* (American), that have their MRCA dated around 7.6 mya (12–3 mya) in this calibration. The second is the clade containing section *Michelia*, section *Yulania* and section *Tulipastrum*, where the MRCA of *M. acuminata* (American) with the rest of the clade (Asian) is dated at 20.54 mya (27–14 mya). The third is the clade containing the two *Liriodendron* species: *L. tulipifera* (American) and *L. chinense* (Asian),

which have their MRCA dated at 22.49 mya (32–12 mya). Tiffney (1985a) suggested five major periods of migration between eastern Asia and eastern north America based on the fossil record: pre-Tertiary, Early Eocene, Late Eocene-Oligocene, Miocene, and Late Tertiary-Quaternary, whereby the evergreen taxa were proposed to have migrated during the Early Eocene through both the Bering and North Atlantic routes, and the deciduous lineages during the Miocene. The disjunct *Yulania* and *Liriodendron* clades, calibrated at around 20 mya, challenge the assumption that the Bering land bridge at the latitude of 70° did not contribute to *Magnolia* migration, as suggested by Hebda and Irving (2004). In previous discussions on the biogeography of the family, the involvement of the Bering land bridge was already suggested, as it was the most elegant hypothesis to explain the disjunct *Rhytidospermum* species pair, which previously was calibrated around 20 mya (Hebda and Irving, 2004). Considering this *Rhytidospermum* clade, the very young age found in this calibration is dated around 7.6 mya (12–3 mya). This age challenges previous migration concepts of the family even more.

Overall, the combined nuclear and chloroplast data, in combination with the setting of the local random clocks, pushes to younger ages than previously discussed for the family, still respecting the general patterns of sequentially represented by the sequence divergence (e.g. older tropical sections *Talauma* and *Gwillimia*, youngest disjunction remains that of subsection *Rhytidospermum*, …). As the discrepancy between the fossil record and the molecular data is substantial and the younger dates challenge many of the former biogeographical concepts previously described for the family, the further data interpretation of the calibration is treated with caution. We will work with the full range of the 95% confidence interval to discuss the age of the nodes, to make conclusions about the questioned colonization hypothesis of the Caribbean Magnolias. Future biogeographical studies on the family need resolved deeper nodes by acquisition of more genetic data (i.e. phylogenomic data), whereby we would suggest a wider variation in fossil calibration schemes to address their influence as a prior on the calibration, as this study already indicates that the addition of more sequence data together with quite broad prior settings on the fossil calibrations, invokes surprisingly younger ages for the nodes than previously assumed.

3.4.5 Biogeographical history of the Caribbean *Magnolia*: mainland vs. islands

The obtained molecular phylogenetic hypotheses, visualised by either the bulk of species trees in DensiTree (Figure 3.3) or the calibrated summary tree (Figure 3.4), illustrate four different colonization events of *Magnolia* from the mainland to the Caribbean islands that occurred since 16 mya (Table 3.2) i.e. 12.11 (16–8) mya for subsection *Cubenses*, 7.09 (12–5) mya for the Cuban *Talauma* species, 3.75 (7–1) mya for *M. dodecapetala* and 1.76 (4–1) mya for *M. virginiana* subsp. *oviedoae*. In this time frame simulations of the position and state of

submergence of the Caribbean islands resemble that of the current geography (Iturralde-Vinent, 2006). The found young ages coincide with the view of Gentry (1982) which lists the Magnoliaceae as Laurasian-derived taxa, which are primarily montane, higher altitude plant groups that are not very species-rich in the Caribbean, a fact interpreted by Gentry as evidence of their recent (Late Tertiary-Quaternary) arrival.

Members of section *Talauma* subsection *Talauma* in the Caribbean have colonised the islands twice, most likely from two different source areas (Figure 3.5). On the one hand, the Cuban Magnolias from subsection *Talauma*, i.e. *M. orbiculata*, *M. oblongifolia* and *M. minor*, form a well-supported clade together with the Magnolias currently distributed in Mexico. On the other hand, *M. dodecapetala* from the Lesser Antilles shows a well-supported clade with the species currently residing in South America: *M. venezuelensis*, *M. ovata*, *M. caricifragrans* and *M. rimachii*. For both cases, the exact sister relationship cannot be deducted from this dataset due to an incomplete sampling of the mainland taxa both in taxon sampling (Rivers et al., 2016) and in data sampling (Appendix 3.3). The young age of the dispersal of *M. dodecapetala* from the South American mainland seems plausible, given a) that the Panama isthmus was already formed (either being Middle Miocene ca. 20–15 mya (Bacon et al., 2015; Montes et al., 2015) or ca. 3.5 mya (Graham, 2003a; Iturralde-Vinent, 2006)allowing the ancestral lineage of *Talauma* to cross from Mesoamerica to the South American mainland – after which it colonised the Lesser Antilles, and b) age estimates of the formation of the Lesser Antilles range from the Middle Miocene: ca. 15 mya until present day (Draper et al., 1994). With this reasoning the estimates of Azuma et al. (2001) that date the MRCA of *M. ovata* and *M. dodecapetala* to be round 24.5 mya would imply the presence of *Talauma* in South America at that time, which is an older age than the oldest ages associated with the Panama isthmus formation (Bacon et al., 2015).

Similarly, the members of subsection *Dugandiodendron* included in this analysis, now all residing in South-America, are found to be closely related to subsection *Cubenses* and show estimated ages (Figure 3.4) between 22 and 8 mya, or when excluding the doubtful *M. mahechae:* between 14–8 mya suggesting *Magnolia* to be present in South America at that time (Figure 3.5). Assuming that the formation of the Panama isthmus is a prerequisite for *Magnolia* to disperse to the South American mainland, this phylogenetic hypothesis coincides the more ancient timing (i.e. Middle Miocene ca. 15 mya) of the formation of the Panama isthmus (Bacon et al., 2015; Montes et al., 2015), rather than the relatively younger estimations (i.e. 3 mya) of, for example Graham (2003a) and Iturralde-Vinent (2006). Alternatively, long-distance overwater dispersal between Mesoamerica and South American land masses remains optional – which for the calibrated study of Azuma et al. (2001) would be a necessary option to explain the older ages together with an affiliation with extant South-American taxa. In

the latter study the crown node of the *Cubenses* clade is estimated at ca. 30 mya and its stem node at ca. 35 mya, which does coincide with the proposed land bridge of the GAARlandia hypothesis (Iturralde-Vinent, 2006).

The fourth colonization of *Magnolia* into the Caribbean islands is from *M. virginiana* subsp. *oviedoae*, which shows a very recent colonization event, estimated around 4 mya to the present time. This is not surprising, given the well-documented botanical records of Cuba that did not record the species prior to 2006 (Oviedo Prieto et al., 2008) and overall it is not estimated to have been in Cuba prior 1950 (pers. comm. Ernesto Testé Lozano).

For the four colonization events the produced data (Figure 3.4, Table 3.2, Figure 3.5) support significantly younger timing than proposed by the vicariance theory or the GAARlandia hypothesis. By exclusion, overwater dispersal remains the most likely candidate for all four colonization events of *Magnolia* on the Caribbean islands. Similar as for the dates found on the family-level, these suggested dispersal dates are also young compared to former biogeographic analysis of the family (Azuma et al., 2001; Nie et al., 2008), yet with the assumption that the Panama isthmus is a hard limit to *Magnolia* dispersal, the younger ages found in this phylogenetic hypothesis are plausible.

The validity of the younger ages found for *Magnolia* dispersal into the Caribbean is hard to contest when comparing with dispersal dates of other plant lineages, and little effort has been undertaken to compile different biogeographical studies and look for general patterns related to Caribbean biogeography. One study, however, executed by Nieto-Blazquez et al. (2017) focuses on patterns within endemic Caribbean seed plant genera. In their results the range of ages and source areas were found to be diverse, and general patterns of dispersal waves were not deducted. The age estimates of *Magnolia* dispersal into the Caribbean found (Figure 3.4, Table 3.2) are in the younger ranges of dispersal dates in the review of Nieto-Blazquez et al. (2017) such as the stem node age of 11.12 mya of the endemic cactus genus *Leptocereus* sister to lineages from South America, or the stem node age of 8.64 or the endemic legume genus *Stahlia* with sister lineages from Central America. Similarly, the arrival of the genus *Buxus* to the Caribbean from Central America (Mexico) is also situated to be younger of age: around the Middle to Late Miocene (ca. 12.3 mya) (González Gutiérrez, 2014), as well as the arrival of the genus *Amphilophium* to the Caribbean (ca. 10 mya) from Central America (Thode et al., 2019) or the arrival from different Euphorbiaceae genera from either Central or South America also situated around the Miocene (Cervantes et al., 2016).

3.4.6 Biogeography within the Caribbean islands

Within the Caribbean islands, the phytogeographic relationships between the *Magnolia* species of subsection *Cubenses* illustrate a stepping-stone dispersal (MacArthur and Wilson,

1967) colonization trajectory from South America to Puerto Rico (between 16–8 mya), the southernmost island of the Greater Antilles, to Cuba (between 5–2 mya), the northernmost of the Greater Antilles. The Caribbean Magnolias from subsection *Cubenses* within each island are more closely related to each other, than to Magnolias from the other islands, with the exception of *Magnolia ekmanii*, residing in the Massif de La Hotte of Haiti, that has a well-supported sister relationship with the Cuban Magnolias of subsection *Cubenses*, rather than with the other *Magnolia* species belonging to that section, occurring in Hispaniola. To date, we are not aware of literature discussing the intra-Caribbean relationships by means of calibrated phylogenetic hypotheses, yet compared to the compilation of historical biogeography studies in Santiago-Valentín and Olmstead (2004), *Magnolia* colonization appears to be simple, with no recolonizations of continental land masses or Caribbean islands. The genetic affinity between the most western tip of Hispaniola and eastern Cuba was not found elsewhere in literature.

3.5. CONCLUSIONS

In conclusion, the generated phylogenetic hypotheses, provide genetic synapomorphies supporting the taxon limits of 14 of the 15 Caribbean Magnolias; the exception being *M. minor* and *M. oblongifolia* species complex, which occur sympatrically in Cuba. Furthermore, genetic differences were found between the two subspecies of *M. cubensis* and between the two included populations of *M. dodecapetala* to the same extent as between other Caribbean *Magnolia* species. Therefore, we advise for the taxonomy of the two *M. cubensis* subspecies to be revised and *Magnolia dodecapetala* requires further investigation over its full geographic extent to re-evaluate its diversity and taxonomy. The classification within section *Talauma* is unsupported due to the discrepancies between gene trees, yet subsection *Talauma* and subsection *Cubenses* + *Dugandiodendron* each have high support on their own. The data support four colonization events of *Magnolia* from the mainland to the Caribbean islands since 16 mya, which indicate overwater dispersal to be the most likely explanation for the presence of *Magnolia* on the Caribbean islands. Within subsection *Cubenses*, we see an upward migration pattern and within island diversification. The exception to this pattern is *Magnolia ekmanii* that has a sister relationship with the Cuban Magnolias of subsection *Cubenses*. Future studies will benefit from using phylogenomic data to elucidate the continued problem of low support between the well-supported clades within the Magnoliaceae family, and need to incorporate a broader mainland taxon sampling. Similarly, genomic-level data may help elucidate whether or not we have sympatric speciation or hybridization in the, for now, genetically undistinguishable species complex of *M. minor* and *M. oblongifolia*.

4. SSR patterns of Neotropical Magnolias

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ABSTRACT

Conserving tree populations safeguards forests since they represent key elements of the ecosystem. The genetic characteristics underlying the evolutionary success of the tree growth form: high genetic diversity, extensive gene flow[1] and strong species integrity, contribute to their survival in terms of adaptability. However, different biological and landscape contexts challenge these characteristics. This study employs 63 *de novo* developed microsatellite or SSR (Single Sequence Repeat) markers in different datasets of nine Neotropical *Magnolia*[2] species. The genetic patterns of these protogynous, insect-pollinated tree species occurring in fragmented, highly-disturbed landscapes were investigated. Datasets containing a total of 340 individuals were tested for their genetic structure and degree of inbreeding. Analyses for genetic structure depicted structuring between species, i.e. strong species integrity. Within the species, all but one population pair were considered moderate to highly differentiated, i.e. no indication of extensive gene flow between populations. No overall correlation was observed between genetic and geographic distance of the pairwise species' populations. In contrast to the pronounced genetic structure, there was no evidence of inbreeding within the populations, suggesting mechanisms favouring cross pollination and/or selection for more genetically diverse, heterozygous offspring. In conclusion, the data illustrate that the Neotropical Magnolias in the context of a fragmented landscape still have ample gene flow within populations, yet little gene flow between populations.

4.1 INTRODUCTION

Conservation genetics utilises a representative sample of DNA and organisms to quantify and study genetic diversity to preserve species as dynamic entities capable of coping with environmental change (Frankham et al., 2010). A collection of DNA fragments representing the genome is realised by employing molecular markers: fragments of DNA associated with a certain location within the genome, providing information about the allelic variation at the given

[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1
[2] Taxonomic authorities: Appendix 1.3 and Appendix 4.

locus (Schlötterer, 2004). Microsatellite or SSR (Simple Sequence Repeat) markers are often the preferred type of molecular marker in conservation genetics because they are codominant, highly polymorphic, ubiquitous, reproducible and neutral; and they have a high mutation rate, as well as an easy sample preparation (Selkoe and Toonen, 2006). Although it is labour and cost intensive to develop and test SSR primer pairs, these can often be employed across species, with success decreasing proportionally to relatedness (Kalia et al., 2010). A representative sampling of organisms can be interpreted at different levels: e.g. individuals for populations, populations for species, and species for ecosystems. The latter strategy makes use of the umbrella species concept (Roberge and Angelstam, 2004).

An exemplar group of umbrella species are trees: they maintain the structure and function of forest ecosystems, and create resource niches and patches for other organisms (Pautasso, 2009). Trees also provide various ecosystem services and resources for human use (Neale and Kremer, 2011) and their genetics and evolution have paradoxical features (Petit and Hampe, 2006). Trees were found to maintain high levels of genetic diversity (Hamrick et al., 1992), but experience low nucleotide substitution rates and low speciation rates when compared to annual plant lineages (e.g. Bousquet et al., 1992; Petit and Hampe, 2006; Whittle and Johnston, 2003). They combine high local differentiation for adaptive traits (Aitken et al., 2008) with extensive gene flow (Austerlitz et al., 2000; Kremer and Le Corre, 2012). Furthermore, they maintain species integrity, while expressing abundant interspecific gene flow (Ellstrand et al., 1996). The abovementioned features provide an expected capacity for tree survival, as they create resilience against threats such as climate change or habitat fragmentation (Aitken et al., 2008; Hamrick, 2004). However, the interplay of the biological and landscape context challenges these generalised characteristics and creates the need for context-oriented tree conservation genetic studies and subsequent management guidelines (Aparicio et al., 2012; Dick et al., 2008).

To investigate the general patterns of tree genetics in an empirical setting, and to contribute to the conservation of the species and forests under study, we focus on New World representatives of the tree genus *Magnolia* (Magnoliaceae) occurring at tropical latitudes, hereafter named Neotropical Magnolias. *Magnolia* trees provide an interesting case-study with bisexual, protogynous flowers, specialised beetle pollination with tepal movement, variable flowering phenology and seed dispersal by animals (Thien, 1974). The Red List of Magnoliaceae (Rivers et al., 2016) states that 76% of the Neotropical Magnolias are threatened, with an additional 16% listed as Data Deficient. Neotropical *Magnolia* populations have not been studied from a molecular point of view (Cires et al., 2013) and their species are delineated based on morphological and distributional argumentation (e.g. Howard, 1948; Palmarola et al., 2016; Vázquez-García et al., 2013c). Many of the *Magnolia* species and

populations occur in fragmented, highly-disturbed, relict primary forest landscapes, such as the cloud forests of the Caribbean islands and the cloud and rain forests of Mexico (Rivers et al., 2016).

This study aims to (1) provide *de novo* developed SSR markers for Neotropical *Magnolia* species; (2) employ the SSR markers for genetic species delimitation between Caribbean *Magnolia* species; (3) search for patterns of extensive gene flow between Caribbean *Magnolia* (sub)species and populations; and (4) test for signs of inbreeding within the Neotropical *Magnolia* populations.

4.2 MATERIAL AND METHODS

4.2.1 Sampling and DNA extraction

Sample information of the 17 different taxa (i.e. 16 species, of which one species consists of two subspecies) and 17 populations included in this study are given in Table 4.1. A map, showing the location information of the wild collected accessions of Neotropical *Magnolia* from the Caribbean and Mexico, is given in Map 4.1. The wild collected samples comprise 346 samples, of which 340 represent the 17 populations. The additional six wild collected samples represent single collections of different species. One further sample is from an *ex situ* collection of *M. dealbata*.

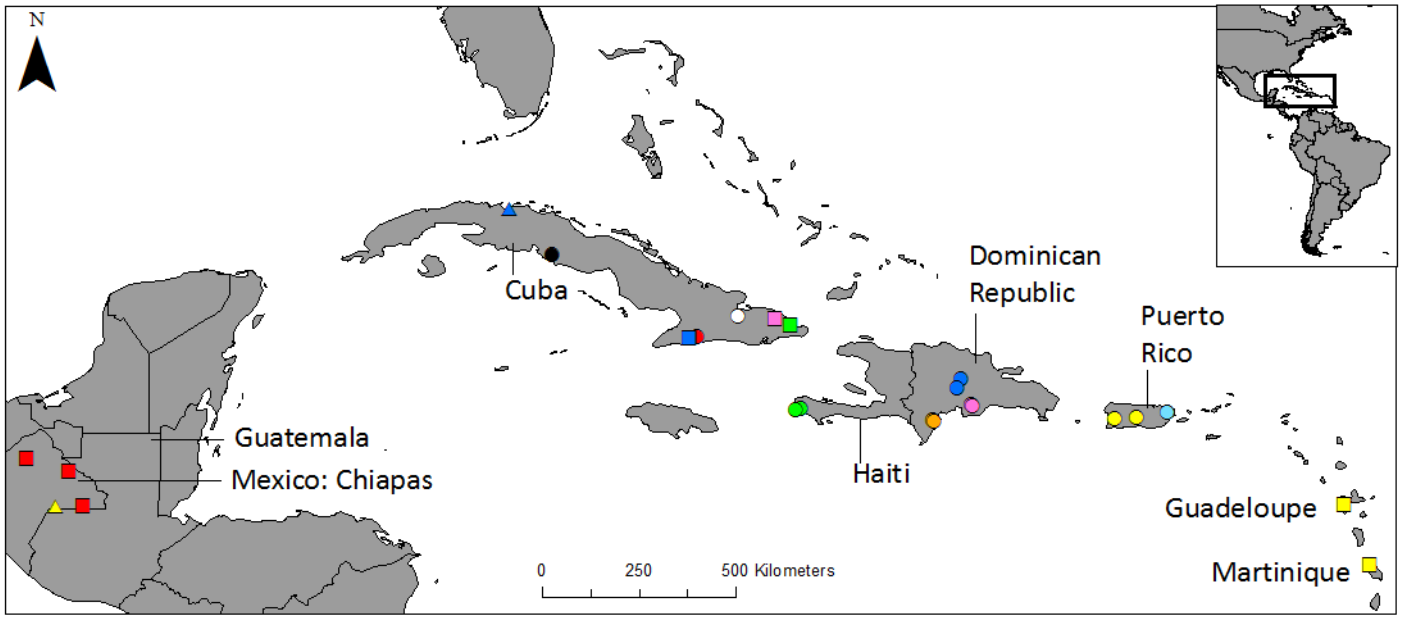
For the 17 populations included in the full genetic analyses, Average Pairwise Distance between individuals (APD), Maximum distance between consecutive individuals (Max), Spatial extent of the populations (SpE) and number of sampled individuals per populations (N_S) are given in Table 4.2. Pairwise distances were calculated using the fossil package (Vavrek, 2011) in R v.3.4.3 (R Core Team, 2016), taking into account all known georeferenced individuals.

All 347 leaf samples were dried in silica gel and their DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) (Doyle and Doyle, 1987) extraction protocol, with MagAttract Suspension G solution (Qiagen, Germantown, USA) (Xin and Chen, 2012) mediated cleaning (Larridon et al., 2015). DNA quantity and quality control was executed using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, USA) and Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific), respectively.

4.2.2 SSR markers: development and testing

Primer pairs were developed to amplify sequences containing SSR repeats based on four Neotropical *Magnolia* species: *Magnolia lacandonica* (MA39), *M. mayae* (MA40), *M. dealbata* (MA41), and *M. cubensis* subsp. *acunae* (MA42). The development of the enriched

Map 4.1 Location map of 16 *Magnolia* taxa (i.e. 15 *Magnolia* species, of which one species consists of two subspecies) from the Caribbean and Mexico, collected in the wild. Circles represent the species of section *Talauma* subsection *Cubenses*. Squares represent species of the *Talauma* subsection *Talauma*. Triangles represent species of section *Magnolia*. Classification is according to Figlar and Nooteboom (2004); Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



Taxa	Tax.	Population	Pop.	Class.	Country	RL	Herbarium reference
M. cristalensis	CRI	-	-	TAS	Cuba	EN	Falcón et al. HFC-88423 (HAJB)
M. cubensis subsp. *acunae**	ACU	Topes de Collantes	TOP	TAS	Cuba	CR	Palmarola & González-Torres HFC-89432 (HAJB)
M. cubensis subsp. *cubensis*	CUB	Pico Turquino	PIC	TAS	Cuba	VU	Palmarola & González-Torres HFC-89418 (HAJB)
*M. dealbata**	DEA	-	-	MAC	Mexico	NT	Veltjen 2018-001 (Wespelaar)
M. dodecapetala	DOD	Martinique	MART	TAT	Lesser Antilles	VU	Veltjen et al. 2016-010 (GENT, K, MTK)
		Guadeloupe	GUA				Veltjen et al. 2016-015 (GENT, GUAD)
M. domingensis	DOM	Loma Barbacoa	BAR	TAS	Hispaniola	CR	Veltjen et al. 2015-011 (GENT, JBSD)
		Loma Rodríguez	ROD				Veltjen et al. 2015-012 (GENT, HAJB, JBSD)
M. ekmanii	EKM	Morne Grand Bois	GRA	TAS	Haiti	CR	Veltjen et al. 2015-001 (EHH, IEB, GENT)
		Morne Mansinte	MAN				Veltjen et al. 2015-003 (EHH, IEB, GENT, JBSD, K)
M. hamorii	HAM	Cortico	COR	TAS	Dominican Republic	EN	Veltjen et al. 2015-009 (GENT, HAJB, JBSD, K)
		Cachote	CAC				Veltjen et al. 2015-010 (GENT, JBSD)
*M. lacandonica**	LAC	Lacanjá	LAC	TAT	Mexico	CR	Samain et al. 2013-039 (IEB, MEXU)
		Yajalón	YAJ				Samain & Martínez 2017-016 (IEB, MEXU)
*M. mayae**	MAY	-	-	MAG	Mexico	CR	Samain 2013-048 (IEB, MEXU)
M. minor	MIN	-	-	TAT	Cuba	EN	Palmarola et al. HFC-84609 (HAJB)
M. oblongifolia	OBL	-	-	TAT	Cuba	CR	Falcón et al. HFC-89377 (HAJB)
M. orbiculata	ORB	-	-	TAT	Cuba	VU	Palmarola & González-Torres HFC-89393 (HAJB)
M. pallescens	PAL	Loma de la Sal	SAL	TAS	Dominican Republic	EN	Veltjen et al. 2015-004 (GENT, JBSD)
		Montellano	MON				Veltjen et al. 2015-007 (GENT, JBSD)
M. portoricensis	POR	Toro Negro	TOR	TAS	Puerto Rico	EN	Veltjen & Rodríguez-Guzmán 2015-015 (GENT, K, UPRRP)
		Maricao	MARI				Veltjen 2015-016 (GENT, UPRRP)
M. splendens	SPL	El Yunque	YUN	TAS	Puerto Rico	EN	Veltjen et al. 2015-013 (GENT, UPRRP)
M. virginiana	VIR	-	-	MAG	US	LC	Conrad s.n. (GENT)

◄ **Table 4.1** Sample information of 17 *Magnolia* taxa (i.e. 16 species, of which one species consists of two subspecies) and 17 populations included in the SSR testing and/or genotyping. The four taxa used for microsatellite marker development are denoted with an asterisk. Taxa according to García-Morales et al. (2017); González Torres et al. (2016); Howard (1948); Vázquez-García et al. (2013c); Vázquez-García et al. (2013d). **Tax.**: three letter code to represent the (sub)species. **Pop.**: three- or four-letter code to represent the population. When there is no population code this means that only one DNA sample was present, used for amplification testing only. **Class.**: classification according to Figlar and Nooteboom (2004); **MAC**: section *Macrophylla*; **MAG**: section *Magnolia*; **TAS**: section *Talauma* subsection *Cubenses*; **TAT**: section *Talauma* subsection *Talauma*. **RL**: IUCN Red List status according to González Torres et al. (2016) and Rivers et al. (2016); **CR**: Critically Endangered; **EN**: Endangered; **VU**: Vulnerable. All three (i.e. E, CR and VU) are considered to be threatened. Herbarium acronyms are according to the Index Herbariorum (Thiers, (continuously updated)). Samples were collected in 2013 (Mexico, Cuba), 2014 (Cuba), April-May 2015 (Hispaniola, Puerto Rico), June 2016 (Lesser Antilles), August-October 2016 (Puerto Rico) and February 2017 (Mexico).

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microsatellite library was outsourced to Allgenetics® (A Coruña, Spain) where enrichment was performed using the Nextera XT DNA kit probes (Illumina, California, USA) with the following motifs: AGG, ACG, AAG, AAC, ACAC and ATCT. The library was sequenced on an Illumina MiSeq® platform.

From the 4 × 500 predetermined SSR primer pairs provided by Allgenetics®, 176 were selected for further testing: 49 developed from MA39-reads, 20 developed from MA40-reads, 20 developed from MA41-reads and 87 developed from MA42-reads. Selection of the 176 SSR markers was carried out randomly, respecting the characteristics specified in Guichoux et al. (2011). The forward primers were linked with a universal tail to accomplish multiplex pooling in a three-primer PCR (Vartia et al., 2014). The following universal tags were used: T3: 5′ AATTAACCCTCACTAAAGGG 3′, M13(-20): 5′ GTAAAACGACGGCCAGT 3’, Hill: 5’ TGACCGGCAGCAAAATTG 3′ (Tozaki et al., 2001) and Neomycin reverse: 5′ AGGTGAGATGACAGGAGATC 3’. The reverse primers had a PIG-tail (Brownstein, 1996).

All 176 markers were screened for amplification success on the 17 taxa, each represented by one randomly selected sample. PCRs were performed on a total volume of 13µL under the following conditions: 2 min at 95°C; 35 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 90 s; 72°C for 6 min. The Master Mix contained 0.2µM forward primer, 0.2 µM reverse primer, 5ng/ml DNA (suspended in 1× TE buffer), 1× TrueStart Taq Buffer (Thermo Fisher Scientific), 1.5 µM $MgCl_2$ (Thermo Fisher Scientific), 0.125 µM dNTP, 5U of TrueStart Hot Start DNA polymerase (Thermo Fisher Scientific), and 0.4 mg/ml BSA (bovine serum albumin) per reaction. PCR products were run on a 1% agarose gel, stained with ethidium bromide and visualised under UV-light. Every (sub)species × primer combination was scored. Amplification scores of the 63 published SSR markers are given in the Appendix 4.1. The (sub)species × primer combinations which were scored to have a single band were submitted to polymorphism testing.

Table 4.2 Population statistics of Caribbean and Mexican Magnolias.

Tax.	Pop.	N_S	SpE	Max	APD	M		P		N_G		A		H_O		H_E		F_{IS}	
						T	S	T	S	T	S	T	S	T	S	T	S	T	S
ACU	TOP	20	3.78	1.8	1.44	31	10	69.565	90	19.871	20	5.452	5.9	0.594	0.610	0.591	0.647	0.021	0.083
CUB	PIC	20	5.32	3.9	1.85	30	10	70.455	100	19.967	20	5.833	6.6	0.597	0.625	0.613	0.674	0.052	0.098
DOD	MART	20	17.92	10.2	8.62	21	-	65.517	-	19.857	-	6.714	-	0.451	-	0.528	-	0.170*	-
DOD	GUA	20	26.08	10.4	12.39	21	-	68.966	-	19.905	-	7.238	-	0.515	-	0.573	-	0.127*	-
DOM	BAR	20	0.16	0.05	0.06	19	10	62.500	100	19.947	20	4.263	5.4	0.625	0.750	0.573	0.673	-0.065	-0.089
DOM	ROD	20	0.28	0.09	0.10	19	10	62.500	100	20.000	20	3.368	3.8	0.503	0.600	0.482	0.577	-0.018	-0.014
EKM	GRA	20	1.02	0.28	0.47	28	10	57.447	100	20.000	20	4.536	4.3	0.482	0.520	0.464	0.496	-0.013	-0.024
EKM	MAN	20	1.52	0.88	0.40	28	10	59.574	80	19.929	19.9	3.786	3.4	0.475	0.465	0.458	0.449	-0.012	-0.01
HAM	COR	20	0.98	0.79	0.15	22	10	60.000	90	20.000	20	6.682	6.2	0.723	0.650	0.712	0.668	0.011	0.053
HAM	CAC	20	1.70	0.60	0.71	22	10	60.000	90	20.000	20	6.591	6.5	0.707	0.635	0.704	0.661	0.021	0.064
LAC	LAC	20	-	-	-	20	-	64.706	-	20.000	-	4.500	-	0.638	-	0.603	-	-0.032	-
LAC	YAJ	20	0,23	0.81	0.10	20	-	67.647	-	20.000	-	4.750	-	0.688	-	0.592	-	-0.135	-
PAL	SAL	20	0.62	0.19	0.20	18	10	59.375	100	20.000	20	4.611	5.5	0.514	0.625	0.511	0.638	0.021	0.046
PAL	MON	20	0.16	0.05	0.05	18	10	59.375	100	20.000	20	4.278	5.2	0.464	0.580	0.483	0.594	0.066	0.049
POR	TOR	20	10.45	6.1	3.43	28	10	70.000	100	20.000	20	6.286	6.4	0.525	0.510	0.607	0.625	0.160*	0.209*
POR	MARI	20	1.95	1.4	0.90	28	10	67.500	90	19.964	20	5.357	6.0	0.566	0.645	0.564	0.622	0.022	-0.011
SPL	YUN	20	8.08	3.7	3.31	23	10	69.444	100	19.957	20	5.391	6.2	0.580	0.630	0.602	0.662	0.063	0.073

Tax.: abbreviations of (sub)species according to Table 4.1. **Pop.**: population abbreviations according to Table 4.1. **N_S**: number of sampled individuals. **SpE**: Spatial Extent (in km): the greatest pairwise distance in the population. **Max**: Maximum distance (in km) between two consecutive individuals of a population (i.e. with no other (recorded) individual(s) in between). **APD**: Average Pairwise Distance between individuals (in km). **M**: number of microsatellite markers employed. **T**: taxon-datasets, which include all the markers out of the 63 published microsatellite markers that were polymorphic and unambiguous to score for the species at hand (Appendix 4.2: A), omitting the markers with high probability of containing null alleles (Appendix 4.4). **S**: The *Cubenses*-normalised dataset (dataset 3) which contains ten microsatellite markers that could be genotyped for all the 8 taxa of section *Talauma* subsection *Cubenses* present in this study (See Appendix 4.2: all the microsatellite markers indicated with an asterisk). **P**: percentage of polymorphic loci (%). **N_G**: average number of genotyped individuals. **A**: average number of alleles. **H_O**: average observed heterozygosity. **H_E**: average expected heterozygosity. **F_{IS}**: population inbreeding coefficient, significant deviations from Hardy-Weinberg proportions are indicated with * (p = 0.05).

Polymorphism tests were executed on eight individuals per *Magnolia* species, comprising four individuals per predefined population. The individuals for the test-multiplexes were selected to be spatially spread throughout the populations and have 260/230 and 260/280 OD (Optical Density) ratios approximating 2. The (sub)species × primer combinations were scored: 63 were considered polymorphic and unambiguous SSR markers in at least one of the ten tested taxa (Appendix 4.2). These 63 SSR markers were used for species-specific multiplex design and final genotyping. Their primer information can be found in Appendix 4.3.

Genotyping of individuals was executed by a multiplex pooling with a three-primer PCR (Vartia et al., 2014). The fluorescent labels FAM, NED, PET and VIC were linked to the tails T3, Hill, Neo and M13, respectively. The multiplex pools were designed using Multiplex Manager (Holleley and Geerts, 2009). Multiplex PCRs were performed on a total volume of 5 µL, under the following conditions: 15 min at 95°C; 35 cycles of 94°C for 30 s, 57°C for 90 s, 72°C for 90 s; 72°C for 10 min. Each multiplex reaction contained 2× QIA Multiplex PCR Master Mix (Qiagen), 5 ng/µL DNA, 0.025 µM for each forward primer, 0.1 µM for each reverse primer and 0.1 µM for each specified dye, carrying the same universal tail as the selected forward primer of the chosen primer pairs. Fragment analyses were executed by Macrogen Inc. (Seoul, South Korea) on an ABI 3730XL fragment analyser (Thermo Fisher Scientific) with a GeneScan™ 500 LIZ™ ladder (Thermo Fisher Scientific). The results were analysed in Geneious v.8.1.9 (Kearse et al., 2012) using the microsatellite plugin. When the test on the subset of individuals appeared promising (i.e. one set of clear peaks, good amplification and more than one allele), 20 individuals per population were genotyped for that marker. The ten taxa were genotyped for 21–36 polymorphic markers, delivering ten separate taxon-datasets (Appendix 4.2: one taxon-dataset = one column with the markers coded “A”).

Error rates (Selkoe and Toonen, 2006) for the markers (Appendix 4.3) across all ten taxon-datasets were calculated, but were not actively and consistently tested for: duplicate genotyping was produced as a side-product during testing for polymorphism, optimizing multiplexes, re-genotyping a complete multiplex for (a) low/unclear peak(s), or as positive control between PCR batches.

The ten taxon-datasets were submitted to MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004) and ML-NullFreq (Kalinowski and Taper, 2006) to test for null alleles. MICRO-CHECKER was run with 1000, and ML-NullFreq was run with 100 000 repetitions. Based on the results, markers with a high probability of representing null alleles were discarded from all downstream analyses.

To ensure that all amplified genetic regions were independent samples of the genome, linkage disequilibrium (Lewontin and Kojima, 1960) per population was analysed in each of the ten

taxon-datasets using the software program GENEPOP v.4.3 (Rousset, 2008) with the dememorization number set to 10 000, batches set to 1000 and 50 000 iterations per batch. Evaluation of linkage disequilibrium was executed by examining both the uncorrected (Waples, 2015) and (sequential Bonferroni) corrected p-values (Holm, 1979) with nominal p-values of 0.05 per species and per population.

4.2.3 Genetic structure

To assess the utility of the SSR markers for genetic species delimitation between closely located Caribbean *Magnolia* species and to search for patterns of extensive gene flow between Caribbean *Magnolia* (sub)species, five different supraspecific (i.e. above species level) datasets were instated. Dataset 1 comprises 340 individuals representing 17 populations all the polymorphic genotyped for all their polymorphic, and the genotyped and assumed monomorphic loci (see Appendix 4.2: all marker × taxon combinations coded A, B and C). Hence, for this dataset it was assumed that the loci that tested to be monomorphic for four or eight individuals were monomorphic for all 20 individuals. Dataset 2 comprises 340 individuals representing 17 populations genotyped for all the polymorphic loci, the genotyped monomorphic loci, but not the assumed monomorphic loci (See Appendix 4.2: all marker × taxon combinations coded A and B). Dataset 3, or the *Cubenses*-normalised-dataset, comprises ten loci (see Appendix 4.2: SSR markers labelled with an asterisk) that were genotyped for 260 individuals representing 13 populations and eight taxa of section *Talauma* subsection *Cubenses* (Table 4.1: Class. = TAS). Added to datasets 1, 2 and 3, two smaller supraspecific datasets were instated, representing the apparently closely related species i.e. the two species of Puerto Rico: the PR-dataset; and the three species of the Dominican Republic: the DR-dataset. To search for patterns of extensive gene flow between Caribbean *Magnolia* population pairs within the defined species, the 17 populations were studied on the infraspecific (i.e. below species) level using nine species-datasets (i.e. the taxon-datasets for the two *M. cubensis* subspecies were joined) and 17 population-datasets.

A first batch of analyses was conducted in STRUCTURE v.2.3.4 (Pritchard et al., 2000) on datasets 1, 2 and 3, the PR- and DR-datasets, the nine species-datasets and the 17 population-datasets. STRUCTURE analyses were run with a burn-in of 100 000, 100 000 MCMC steps after the burn-in and the admixture model as ancestry model. Datasets 1, 2 and 3 were run with the allele frequency model set to independent allele frequencies. They were expected to consist of 13 or 17 populations and were run with K set from 1 to 25. The PR- and DR-datasets were run both with the independent allele frequency model and the correlated allele frequency model and their results were compared. They were expected to have between 2 and 6 populations and K set from 1 to 15. The nine species-datasets and 17 population-

datasets were run with the allele frequency model set to correlated allele frequencies. They were run with K set from 1 to 10. For all datasets, each value of K was run 10 times. The results were visualised with Structure Harvester Web v.0.6.94 (Earl and vonHoldt, 2012). The best K-value was selected using the ΔK statistic (Evanno et al., 2005) and the results for mean likelihood (mean L(K)). The latter was taken into consideration because the DK statistic appointed K-values with unstable replicate results for datasets 1, 2 and 3 and because the ΔK statistic cannot detect single clusters: an outcome expected at the infraspecific level (i.e. population-datasets and possibly the species-datasets). Barplots were visualised using DISTRUCT v.1.1 (Rosenberg, 2003).

DAPC analyses (Discriminant Analysis of Principal Components) on datasets 1, 2 and 3 were executed in R using the package adegenet (Jombart, 2008). In the find.clusters function we retained 300 PCs for dataset 1 and 2, and 140 PCs for dataset 3. The number of PCs to retain for the PCA eigenvalues was determined using cross-validation. All discriminant functions (DA eigenvalues) were kept.

Pairwise F_{ST} (Weir and Cockerham, 1984), G_{ST} (Nei, 1973; Nei and Chesser, 1983) and D_{JOST} (Jost, 2008) values and their confidence intervals were calculated in R using the package diveRsity (Keenan et al., 2013). To visualize the genetic distances for dataset 1, 2 and 3, an unrooted network applying the Neighbour-joining (NJ) method based on Nei's genetic distance: D_A (Nei et al., 1983), was constructed using Populations v.1.2.32 (http://bioinformatics.org/populations/) using 1000 bootstrap replicates as a confidence measure.

Mantel tests on the supraspecific level were performed in GenAlEx v.6.5 (Peakall and Smouse, 2012; Peakall and Smouse, 2006) on the pairwise log-transformed geographic distance and pairwise F_{ST} values using 9999 permutations. Coordinates of one individual were taken as a representative of its population. Species geographic distance was averaged over the populations of the species.

4.2.4 Inbreeding and population statistics

To test for inbreeding within the Caribbean *Magnolia* populations, the inbreeding coefficient (F_{IS}) for each locus and population was calculated in FSTAT. Tests to detect significant deviations from Hardy-Weinberg proportions (HWP) were calculated in GENEPOP, performing 2-tailed exact tests for each locus in each population. Complete enumeration was performed whenever possible (Louis and Dempster, 1987), otherwise MCMC chains were run with 200 batches and 50 000 iterations (Guo and Thompson, 1992). Deviations of both the uncorrected and sequential Bonferroni corrected p-values were used to evaluate if populations were truly deviating from HWP (Waples, 2015). To frame and discuss the results, different statistical

parameters were calculated for each locus and population within the ten taxon-datasets using GenAlEx, i.e. the percentage of polymorphic loci (P), the number of genotyped individuals (N), mean number of alleles (A), expected heterozygosity (H_E), and observed heterozygosity (H_O).

4.3 RESULTS

4.3.1 SSR markers

Overall, 82–92% of the primer pairs amplified, of which 53–67% were scored to be a single amplification product (Appendix 4.1). The polymorphism tests of the markers giving a single amplification product classified 16–37% of the primer pairs unambiguous and polymorphic (Appendix 4.2). The reported SSR primers all have heterozygote states in at least one individual and a perfect motif (Weber, 1990). For 56 SSR markers, the duplicate runs rendered the same genotypes (Appendix 4.3: error rate: 0%). For one SSR marker no genotypes were duplicated. The error rates of the other six SSR markers ranged from 1–3.85%.

Results of detection and frequency of null alleles per marker × population combination are given in Appendix 4.4. Twelve marker × species combinations were considered to have a high probability of showing null alleles: *M. cubensis* (MA42_028), *M. domingensis* (MA39_199), *M. ekmanii* (MA39_023, MA42_087), *M. hamorii* (MA40_223, MA42_413), *M. lacandonica* (MA39_182), *M. pallescens* (MA39_023, MA42_472), *M. portoricensis* (MA42_481) and *M. splendens* (MA39_023, MA42_481).

Associated alleles per marker × species combination are given in Appendix 4.4. *Magnolia domingensis* and *M. lacandonica* showed a number of SSR markers with associated alleles that were higher than expected for the number of pairwise tests executed. The other eight taxa fell within their confidence intervals of false positives, whereby one significantly associated pair of SSR markers was detected in *M. pallescens* (MA40_045 × MA42_472).

4.3.2 Genetic structure: supraspecific level

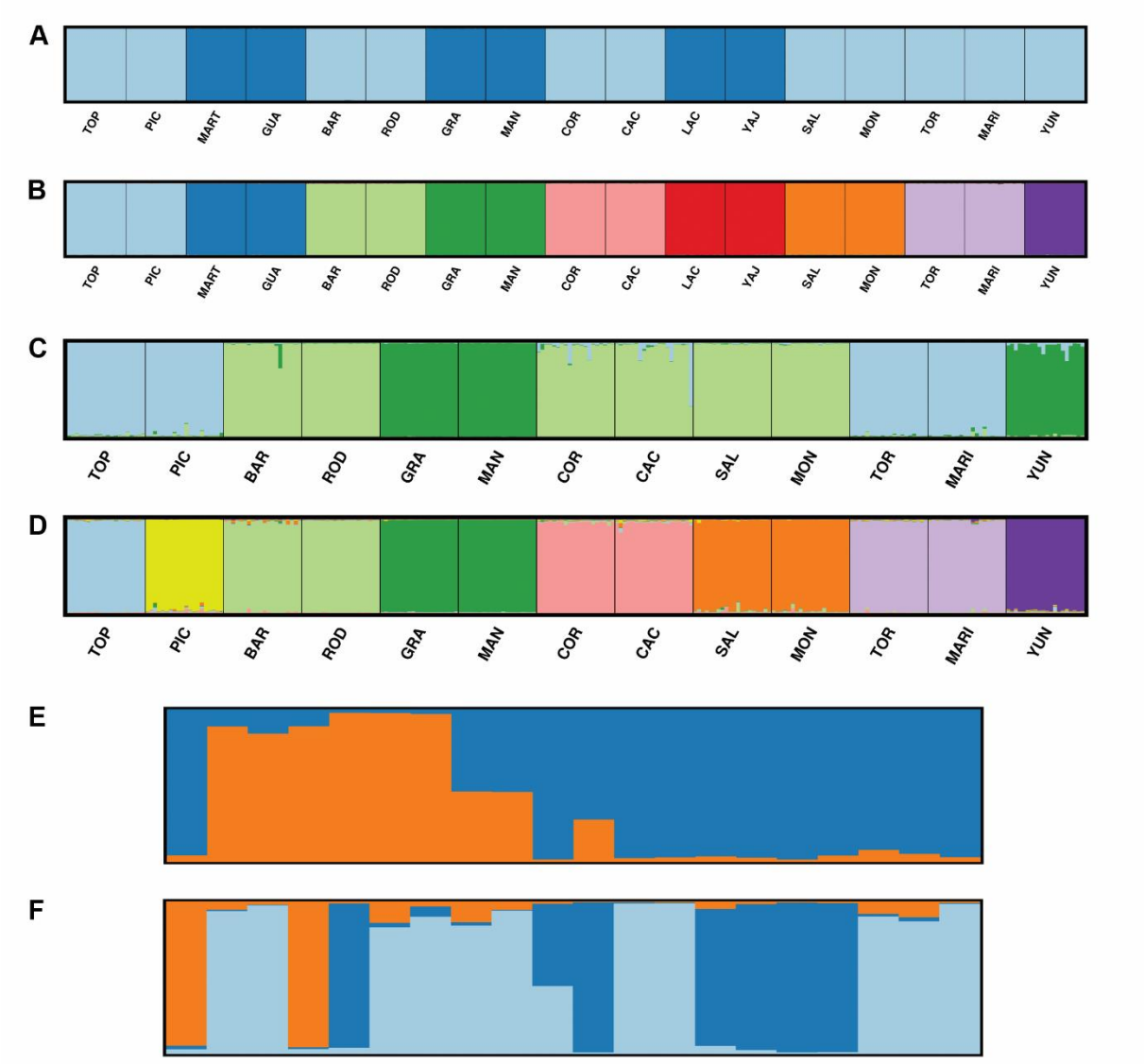
Supraspecific ΔK and mean L(K) plots are depicted in Appendix 4.5 and their interpretation is summarised in Table 4.3. Barplots of the STRUCTURE analyses on the three full supraspecific datasets are depicted in Figure 4.1A–D. The DR-dataset and PR-dataset structured according to the species given both criteria and correlation frequency models. In the DAPC analysis, the "true" K in the replicate runs of the find.clusters algorithm was not univocal, and ranged between 9–13 for dataset 1, 9–15 for dataset 2 and 8–11 for dataset 3. For each dataset, a representative DAPC analysis is visualised in Figure 4.2. Supraspecific pairwise F_{ST} values range from 0.216 to 0.618 for dataset 1, 0.166 to 0.472 for dataset 2 and 0.130 to 0.308 for dataset 3 (See Table 4.4).

Table 4.3 Number of STRUCTURE clusters of Magnolias from the Caribbean and Mexico.

	D1	D2	D3	DR(i)	DR(c)	PR(i)	PR(c)			
ΔK	2	2	3	3	3	2	2			
mean L(K)	9	10	8	7	4	3	3			
S5	A	B	C	D1	D2	E1	E2			
	CU	DOD	DOM	EKM	HAM	LAC	PAL	POR	SPL	
ΔK	2	2	2	2	2	2	2	2	7	
mean L(K)	2	2	3	2	1	2	2	5	1	
S5	F	G	H	I	J	K	L	M	N	
	TOP	PIC	GUA	MART	BAR	ROD	GRA	MAN	CAC	COR
ΔK	2	2	2	2	3	5	2	6	5	2
mean L(K)	1	1	2	1	1	1	1	1	1	1
S5	O1	O2	P1	P2	Q1	Q2	R1	R2	S1	S2
	LAC	YAJ	SAL	MON	MARI	TOR	YUN			
ΔK	7	7	5	8	3	3	7			
mean L(K)	1	1	1	1	1	3	1			
S5	T1	T2	U1	U2	V1	V2	N			

D1 = dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **D2** = dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **D3** = dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk). **DR:** DR-dataset comprising the 120 individuals comprising 6 populations and 3 species of the Dominican Republic for all the markers of which data was generated (See Appendix 4.2: categories A, B and C in the columns DOM, HAM and PAL). **PR**: PR-dataset comprising 60 individuals representing three populations and two species of Puerto Rico for all the markers of which data was generated (See Appendix 4.2: categories A, B and C in the columns POR and SPL). The DR- and PR-dataset were run with the independent allele model **(i)** and the correlated allele model **(c)**. Abbreviations of species and populations are according to Table 4.1; **CU**: *Magnolia cubensis*. **ΔK** according to Evanno et al. (2005). **mean L(K)** = mean likelihood. **S5**: the corresponding plots in Appendix 4.5.

Figure 4.1 STRUCTURE barplots of Magnolias from the Caribbean and Mexico. The replicate with the highest likelihood score is given. **A** STRUCTURE barplot of dataset 1 and dataset 2, K = 2. **B** STRUCTURE barplot of dataset 1: K = 9. **C** STRUCTURE barplot of dataset 3, K = 3. **D** STRUCTURE barplot of dataset 3, K = 8. **E** STRUCTURE barplot of the Guadeloupe population of *Magnolia dodecapetala*. **F** STRUCTURE barplot of the Toro Negro population of *Magnolia portoricensis*.



Dataset 1 comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **Dataset 2** comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **Dataset 3** comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk).

Table 4.4 Pairwise F_{ST} values (Weir and Cockerham, 1984), pairwise D_{JOST} values (Jost, 2008) and Pairwise Geographic Distance of Magnolias from the Caribbean and Mexico.

Sp.		CU	DOD	DOM	EKM	HAM	LAC	PAL	POR	SPL
CU	D1	**0.154 / 0.046**	0.093	0.069	0.127	0.100	0.115	0.062	0.088	0.092
	D2	**0.154 / 0.046**	0.038	0.034	0.089	0.045	0.027	0.028	0.048	0.044
	D3	**0.160 / 0.339**	**-**	0.513	0.721	0.320	**-**	0.365	0.447	0.607
	PGD	**408.404**	1897.652	890.127	513.501	817.711	1481.214	843.4194	1259.906	1353.569
DOD	D1	0.513	**0.181 / 0.028**	0.046	0.086	0.070	0.058	0.064	0.074	0.075
	D2	0.360	**0.181 / 0.028**	0.015	0.033	0.024	0.037	0.021	0.032	0.022
	D3	**-**	-	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	PGD	1897.652	**168.881**	1009.428	1418.235	1088.315	3245.707	1057.382	647.440	567.164
DOM	D1	0.428	0.499	**0.138 / 0.012**	0.053	0.020	0.049	0.012	0.054	0.053
	D2	0.262	0.264	**0.138 / 0.012**	0.032	0.016	0.013	0.014	0.024	0.014
	D3	0.196	**-**	**0.093 / 0.130**	0.511	0.305	**-**	0.353	0.525	0.456
	PGD	890.127	1009.428	**4.540**	424.854	100.864	2274.335	66.576	379.509	479.761
EKM	D1	0.455	0.618	0.486	**0.223 / 0.040**	0.118	0.108	0.080	0.145	0.126
	D2	0.387	0.472	0.380	**0.223 / 0.040**	0.055	0.035	0.036	0.072	0.045
	D3	0.272	**-**	0.296	**0.226 / 0.198**	0.512	**-**	0.601	0.599	0.467
	PGD	513.501	1418.235	424.854	**10.079**	333.286	1849.511	399.205	803.612	904.498
HAM	D1	0.389	0.520	0.216	0.497	**0.044 / 0.009**	0.078	0.023	0.099	0.083
	D2	0.187	0.339	0.166	0.325	**0.044 / 0.009**	0.019	0.019	0.050	0.024
	D3	0.130	**-**	0.132	0.275	**0.035 / 0.037**	**-**	0.363	0.494	0.376
	PGD	817.711	1088.315	100.864	333.286	**3.785**	2181.049	114.939	471.798	573.613
LAC	D1	0.539	0.471	0.573	0.611	0.570	**0.185 / 0.029**	0.074	0.112	0.095
	D2	0.316	0.373	0.318	0.423	0.307	**0.185 / 0.029**	0.017	0.037	0.018
	D3	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	PGD	1481.214	3245.707	2274.335	1849.511	2181.049	**109.658**	2244.901	2652.663	2753.896
PAL	D1	0.466	0.557	0.318	0.574	0.279	0.607	**0.163 / 0.009**	0.060	0.085
	D2	0.300	0.346	0.230	0.416	0.216	0.283	**0.163 / 0.009**	0.021	0.023
	D3	0.152	**-**	0.164	0.301	0.150	**-**	**0.115 / 0.124**	0.395	0.631
	PGD	843.4194	1057.382	66.576	399.205	114.939	2244.901	**27.064**	418.427	515.043
POR	D1	0.409	0.489	0.422	0.535	0.404	0.541	0.534	**0.101 / 0.031**	0.077
	D2	0.246	0.352	0.236	0.396	0.240	0.316	0.314	**0.101 / 0.031**	0.047
	D3	0.152	**-**	0.226	0.308	0.218	**-**	0.210	**0.105 / 0.211**	0.481
	PGD	1259.906	647.440	379.509	803.612	471.798	2652.663	418.427	**52.916**	102.892
SPL	D1	0.437	0.559	0.487	0.564	0.461	0.580	0.549	0.338	**-**
	D2	0.264	0.373	0.237	0.402	0.208	0.266	0.282	0.233	**-**
	D3	0.227	**-**	0.226	0.290	0.223	**-**	0.257	0.239	**-**
	PGD	1353.569	567.164	479.761	904.498	573.613	2753.896	515.043	102.892	**-**

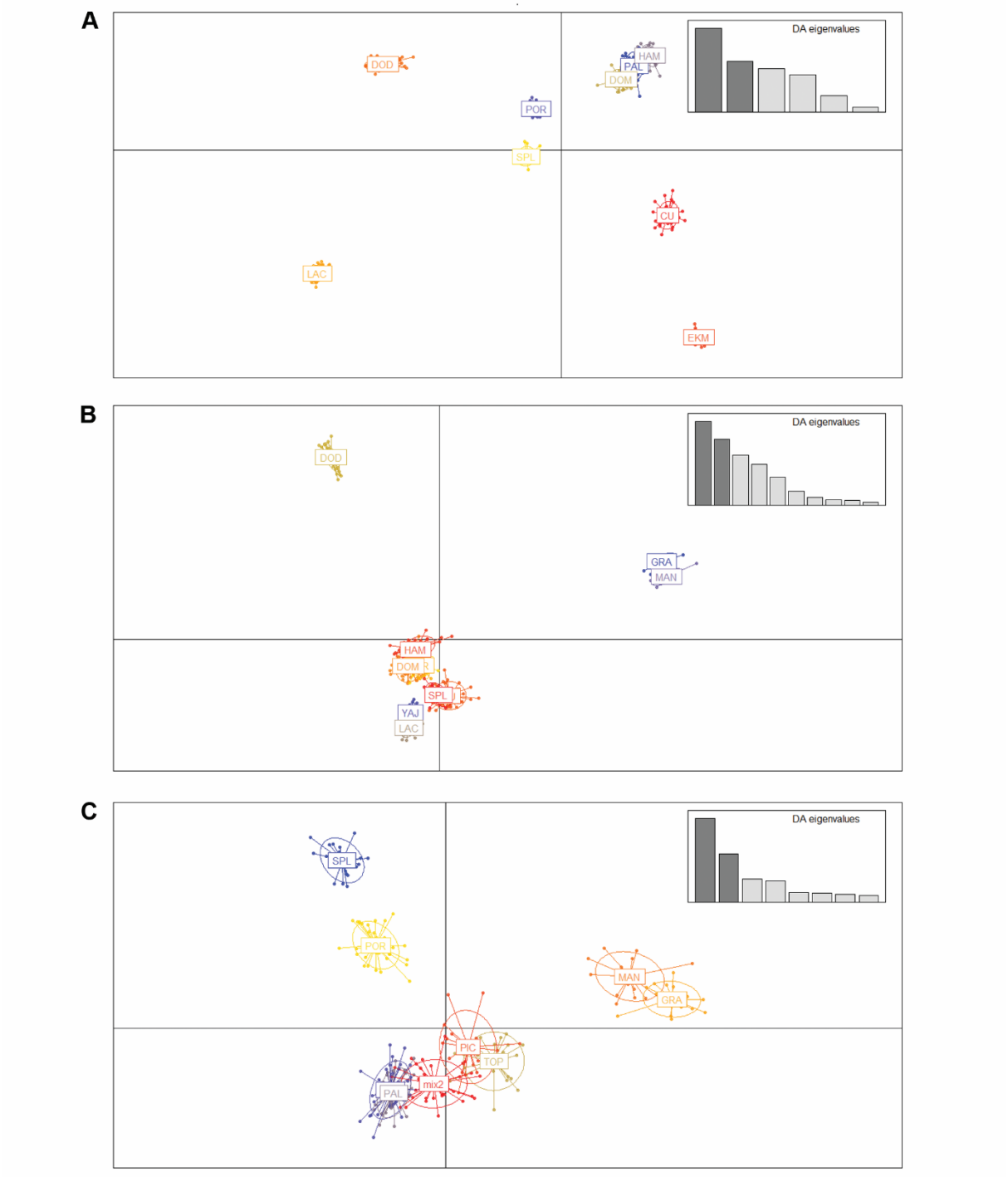
PGD = Pairwise Geographic Distance (in km). Species (**Sp.**) are abbreviated according to Table 4.1 and **CU** = *Magnolia cubensis*. **D1** = dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **D2** = dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **D3** = dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk). On the diagonal (in **bold**): the pairwise infraspecific F_{ST} / D_{JOST} values and the pairwise distances between the pairs of populations per species. Below the diagonal supraspecific pairwise F_{ST} values. Above the diagonal supraspecific pairwise D_{JOST} values.

Supraspecific pairwise D_{JOST} values range from 0.012 to 0.145 for dataset 1, 0.013 to 0.089 for dataset 2 and 0.305 to 0.721 for dataset 3 (See Table 4.4). The F_{ST} confidence intervals are visualised in Appendix 4.6. The unrooted NJ trees based on D_A are depicted in Figure 4.3. The Mantel tests for all three datasets including all population-pairs were significant (p = 0.000–0.003). Mantel tests on the supraspecific pairwise distances were significant for dataset 1 (p = 0.000), but not for dataset 2 (p = 0.080) and dataset 3 (p = 0.256). See Appendix 4.7 for visualisation of the relationship between geographic and genetic distance and Table 4.4 for the Pairwise Geographic Distance (PGD) between the population pairs.

4.3.3 Genetic structure: infraspecific level

Infraspecific ΔK and mean L(K) plots are depicted in Appendix 4.5 and their interpretation is summarised in Table 4.3. Barplots of the two infraspecific STRUCTURE analyses exceeding the predefined clusters: GUA & TOR are given in Figure 4.1E & 4.1F, respectively. Infraspecific pairwise F_{ST} values can be found in Table 4.4 and range from 0.044 to 0.222 for the species-datasets and 0.035 to 0.226 when standardised according to dataset 3. Confidence intervals of the infraspecific pairwise F_{ST} values are depicted in Appendix 4.6. Infraspecific pairwise D_{JOST} values can be found in Table 4.4 and range from 0.009 to 0.046 for the species-datasets and 0.037 to 0.339 when standardised according to dataset 3. Mantel tests at the infraspecific level were not significant (dataset 1 and dataset 2: p = 0.084, dataset 3: p = 0.080): see Appendix 4.7.

► **Figure 4.2** DAPC plots of Magnolias from the Caribbean and Mexico. DAPC: Discriminant Analysis of Principal Components. The x-axis represents the first linear discriminant (LD) and the y-axis the second linear discriminant. Populations and (sub)species are abbreviated according to Table 4.1 and **CU:** *Magnolia cubensis*. **A** DAPC plot of dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). Nine clusters are visualised following the nine species: CU, DOD, DOM, EKM, HAM, LAC, PAL, POR, SPL. **B** DAPC plot of dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). Eleven clusters are visualised: CU (behind SPL), DOD, DOM, HAM, GRA, LAC (population), MAN, PAL (behind POR), POR (behind DOM), SPL, YAJ. **C** DAPC plot of dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk). **mix1**: all 40 individuals of DOM and 3 individuals of SAL. **mix2**: all 40 individuals of PAL and 1 individual of PIC. Nine clusters are visualised: GRA, MAN, mix1 (behind PAL), mix2, PAL, PIC, POR, SPL, TOP.


A
DA eigenvalues
DOD
HAM
PAL
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POR
SPL
CU
LAC
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B
DA eigenvalues
DOD
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HAM
DOM
SPL
YAJ
LAC
C
DA eigenvalues
SPL
POR
MAN
GRA
PIC
TOP
PAL
mix2

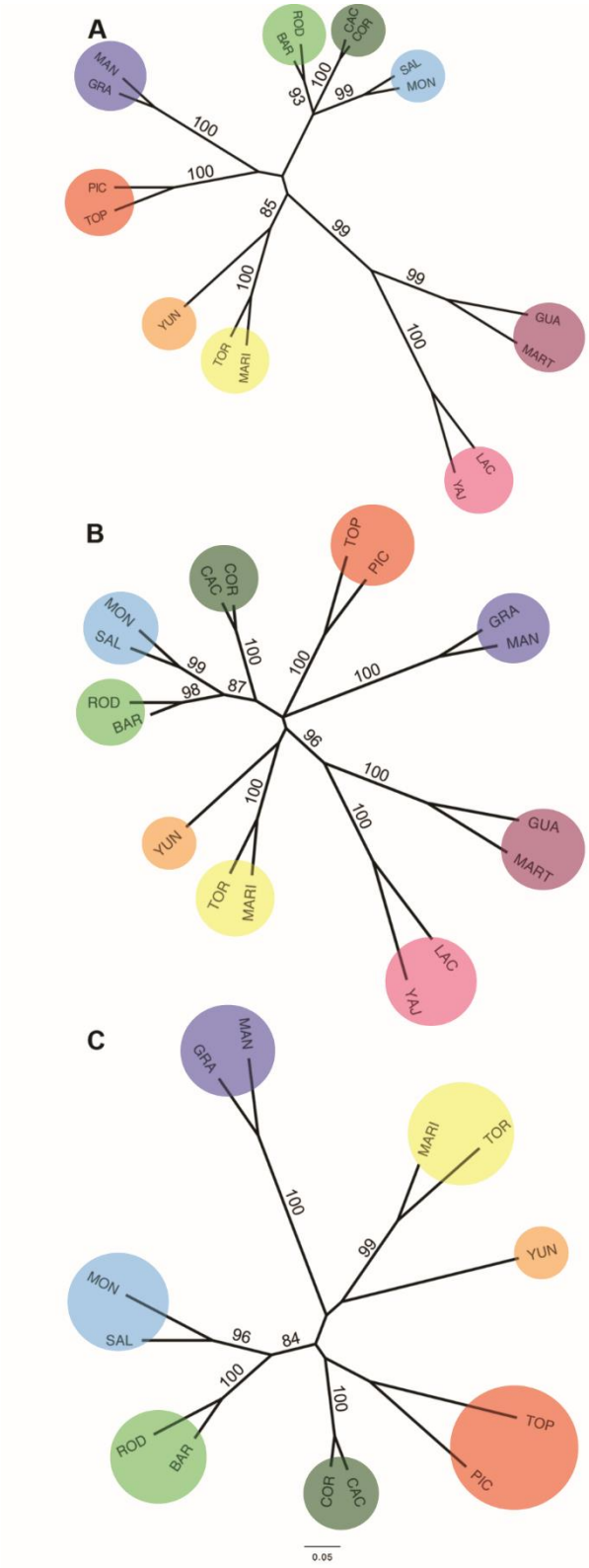

Figure 4.3 NJ trees of the Magnolias from the Caribbean and Mexico. Unrooted networks are constructed by the Neighbour-joining (NJ) method based on Nei's genetic distance: D_A (Nei et al., 1983). Bootstrap values above 70 are depicted. **A** NJ-tree of dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **B** NJ-tree of dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **C** NJ-tree of dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk).

4.3.4 Inbreeding: infraspecific level

Detailed results on the population statistics calculated on the ten taxon-datasets are listed per marker, population and subset in Appendix 4.4. Population statistics of the most representative subset are listed in Table 4.2. Three populations: GUA, MART and TOR showed significant departure from HWP. GUA and MART presented significant deviation from HWP for 5/21 and 4/21 loci (1.45 [0, 3] expected to test false positive when p = 0.05). TOR showed significant deviation from HWP for 7/29 loci (1.45 [0, 3] expected to test false positive when p = 0.05).

4.4 DISCUSSION

4.4.1 SSR markers

The data on marker development show an attrition of usable SSR primer pairs during post-sequencing marker development, which is a general issue in SSR development (Hodel et al., 2016). Patterns in success of the polymorphism tests should be treated with caution because (1) multiplexing SSR markers can lead to marker interaction; (2) testing with more individuals or populations can show more markers to be polymorphic; (3) massive parallel testing was executed, for which some SSR marker-species combinations were not replicated; (4) random isolated PCR artefacts have been observed. Because all 63 reported SSR markers had a heterozygous state in at least one individual and contain di- or tri-repeats, they are labelled nuclear SSR loci (Wheeler et al., 2014).

4.4.2 Sampling design

The sampling design renders a dataset which is standardised yet limited in the number of individuals per population and populations per species (Hoban et al., 2013; Ward and Jasieniuk, 2009). It is possible that the limited number of samples invoked false positives or false negatives due to random sampling error (Waples, 2015), hence, we recommend including SSR markers that reported to have null alleles when genotyping more individuals and populations in further analyses, except for the markers that have very strong evidence i.e. MA42_028 for *M. cubensis*, MA39_182 for *M. lacandonica* and MA42_481 for *M. portoricensis*.

4.4.3 Genetic structure: supraspecific level

In general, results of all supraspecific analyses (Table 4.3–4.4, Figure 4.1–4.3, Appendix 4.5A–4.5E2, 4.6, 4.7) are influenced by the datasets used. Firstly, due to the resolution: inclusion of more differentiated species/populations conceals the signal of the lower genetic structural levels (e.g. Figure 4.1A vs. Figure 4.1B). Secondly, due to inclusion or exclusion of the assumed monomorphic SSR loci or fixed alleles (e.g. Figure 4.2A vs. Figure 4.2B). On the one hand, fixed alleles determined a higher genetic differentiation among species. This is apparent

in the NJ-tree when comparing branch lengths and bootstrap values in Figure 4.3A and 4.3B and in the DAPC plots when comparing Figure 4.2A with Figure 4.2B. On the other hand, the monomorphic loci strengthen genetically similar species groups, illustrated by the three species of the Dominican Republic to be clustered together in Figure 4.3A, while when omitting the assumed monomorphic data (Figure 4.3B), *M. hamorii* is differentiated from the other two Dominican Magnolias.

Currently, a molecular phylogenetic analysis including a representative sampling of section *Talauma* and its four subsections (Figlar and Nooteboom, 2004; Pérez et al., 2016) is not available. On the basis of the SSR results, it can be stated that the species delineations of the studied seven species of subsection *Cubenses* are genetically confirmed. Clustering methods placed individuals and populations in their respective species genetic cluster (Figure 4.1B, 4.1D, 4.2A, 4.2B and 4.3). However, the likelihood of clustering according to the species was not significant enough for the ΔK method to recognize the K corresponding to the number of species (Figure 4.1A, 4.1C) and species-clusters often overlap in the two-dimensional visualization of the DAPC analysis (Figure 4.2) or even consistently cluster with another species (Figure 4.2C: mix1, mix2).

Although the SSR data is able to deliver evidence for species boundaries, there can be little conclusions drawn on their evolutionary relationships (Figure 4.2, Table 4.4, Figure 4.3). The data illustrates that the set of three Dominican Magnolias and the set of two Puerto Rican Magnolias are the least genetically differentiated (Table 4.4, Figure 4.2A, 4.3A), which is visible as a gap in pairwise F_{ST} values (Appendix 4.6) as well as the main driver for the significant results of the Mantel tests (Appendix 4.7). The pairwise F_{ST} values (Table 4.4, Appendix 4.6A, 4.6B) suggest (*M. domingensis* + *M. hamorii*) + *M. pallescens*; however, D_{JOST} and Figures 4.2, 4.3B and 4.3C put forward (*M. domingensis* + *M. pallescens*) + *M. hamorii*. Although native to the same island as the three Dominican Magnolias, *M. ekmanii* is conspicuously differentiated from them, as well as from all other species. There is a hint that *M. ekmanii* is most closely related to the Cuban Magnolias: their pairwise F_{ST} calculated on dataset 1 is significantly lower compared to the other *M. ekmanii* pairwise comparisons (Table 4.4, Appendix 4.6A), the DAPC analyses (Figure 4.2) place them more closely together according to the two most explanatory in the ordination space, and the NJ-tree of dataset 1 and 2 display shared ancestry, albeit unsupported (Figure 4.3A, 4.3B). For *M. ekmanii* and species relationships across the different Caribbean islands, the SSR loci have accumulated too many (homologous) mutations for supported relationships to be deducted (Calonje et al., 2008). Therefore, studying more conservative DNA fragments by phylogenetic studies (e.g. on chloroplast DNA or single copy nuclear genes) would be valuable.

4.4.4 Genetic structure: infraspecific level

GUA, MART and TOR are suspected to suffer from the Wahlund effect given the larger spatial distances (Table 4.2: SpE, Max, APD), significantly high number of null alleles (Appendix 4.4), significant F_{IS} values (Table 4.2), high number of alleles (Table 4.2: A) and their population STRUCTURE (Figure 4.1E, 4.1F). The absence of genetic HWP-based structure in the MART population could be due to unequal mixture fractions (Waples, 2015) combined with a small sample size. For more in-depth study of these populations, it is recommended to invoke more substructure in future sampling design and analyses.

The range of pairwise infraspecific F_{ST} values (Table 4.4) is large and the genetic fixation can be labelled: little (HAM), moderate (DOM, PAL: dataset 3, POR), great (CUB, DOD, EKM, LAC, PAL: dataset 1&2*)* (Hartl and Clark, 1997) or significant (CUB, DOD, EKM, LAC, PAL: dataset 1&2) (Frankham et al., 2010). Similarly, the range of intraspecific pairwise D_{JOST} values (Table 4.4) can be labelled little for all the comparisons under dataset 1&2; and little (HAM), moderate (DOM, PAL) or great (CU, EKM, POR). The large range of pairwise, infraspecific F_{ST} and D_{JOST} values reminds us of the conflict between the continuity of lineage separation and the discrete entity of a species (de Queiroz, 1998). Theoretically, infraspecific genetic differentiation was expected to be counteracted by extensive gene flow between populations: either by long-distance pollen dispersal (Petit and Hampe, 2006) or seed dispersal by natural disturbances (Lugo et al., 1981).

The Wahlund effect and moderate to great genetic differentiation indicate that the population dynamics of the studied Neotropical Magnolias occur at a fine spatial scale; in this sampling design suggested to be limited in the spatial extent of 4 km (Table 4.4: PGD of HAM) to 6 km (Table 4.2: SpE of TOR). The Mantel tests on the infraspecific level (Appendix 4.7) and comparisons with *Magnolia* SSR literature (Kikuchi and Isagi, 2002; Setsuko et al., 2007; Zhao et al., 2012) show no correlations or trends between pairwise geographic and infraspecific genetic distance. For this result, the biological context (i.e. different animal vectors), different evolutionary histories (i.e. recent long-distance dispersal), and different landscape context (i.e. less fragmented landscapes vs. highly disturbed landscapes) cannot be decoupled from one another. However, given the conservative flower and fruit morphology within the Magnoliaceae family and the extensive deforestation history of the studied populations, the landscape context is expected to be the main driver.

Unexpectedly, the two subspecies of *M. cubensis* express low genetic differentiation combined with a high geographic distance, while we find high structuring overall for the other Magnolias. Here, the hypothesis of relatively recent long-distance dispersal is put forward as the most likely explanation to be tested in further research. Similarly, MAR and GUA, the “populations”

of *M. dodecapetala*, were expected to have a higher degree of genetic differentiation compared to the other infraspecific genetic differentiation regardless of the Wahlund effect, given that the populations are separated by ocean and that a “population” on Dominica lies in between that of Guadeloupe and Martinique.

4.4.5 Population statistics: infraspecific level

The high amount of linkage disequilibrium found in three populations (ROD, LAC, YAJ) is most likely due to a major reduction in population size: a recent bottleneck. This is concluded given that (a) there is genome-wide linkage disequilibrium for all three populations, in contradicting strengths when compared across populations pairs per species; and (b) the visited locations had a high degree of disturbance. The samples studied of the ROD and LAC populations indicate that they have not been able to recombine their genetic material since the bottleneck. For the YAJ population it cannot be excluded that a high degree of kinship between the samples produced the results. The 20 samples of this population could only be collected at the border of, what is expected to be, a much larger population and include two adults and 18 juveniles. It is recommended to either exclude the population from species-focused analyses, or to recollect a better representation of the population.

We cannot easily label the observed genetic diversity (Table 4.2) to be healthy, high or low, as there is no related, non-threatened *Magnolia* species studied for comparison (Spielman et al., 2004). However, comparisons of the population statistics between the studied threatened species can be made. Firstly, when comparing the statistics of the taxon-datasets, the two populations of *M. hamorii* from the Dominican Republic show a high mean number of alleles (A), in the same extent as the three populations suspected to experience the Wahlund effect. They also have the highest reported values of H_o and H_e compared with the other Magnolias of this dataset. In the *Cubenses*-normalised-dataset (dataset 3), the statistics of *M. hamorii* do not stand out anymore. However, they remain in the higher range of values, now similar to the statistics found for *M. cubensis*, *M. portoricensis* and *M. splendens*. The latter three species also show A- and H-values in the higher range of values in the calculations of their full taxon-datasets.

Secondly, GRA, MAN and ROD report the three lowest A values in their taxon-datasets, and MAN and ROD show lower A and H values than the GRA and BAR populations, respectively. The lower statistics of the GRA and MAN populations confirm that conservation management of Magnolias in the last remaining forests of Haiti is urgent. Interestingly, even though MAN appeared deforested in an equal, or even higher extent than the ROD population, its alleles tested to be independently associated. LD decreases after recombination events at a rate that depends on the recombination frequency and generally takes more than one generation of

random mating to restore, even for (physically) unlinked loci (Slatkin, 2008). Hence, the combination of highly disturbed forest and independently associated alleles indicates successful pollination events and surviving new recruits for the MAN population.

Thirdly, the population inbreeding coefficients (F_{IS}) of the 14 populations not suspected to be under the Wahlund effect, do not significantly differ from zero. Taking the reproduction biology of Magnolias into consideration, both arguments in favour and against this result can be listed. No (apparent) inbreeding seems likely given that (1) *Magnolia* flowers are reported to be protogynous (Gibbs et al., 1977; Gottsberger, 1977; Thien, 1974); (2) trees have characteristics that promote cross-pollination (Petit and Hampe, 2006); and (3) high cross-pollination rates have been found in other *Magnolia* species (Tamaki et al., 2009). However, (some degree of) inbreeding was expected given that (1) geitonogamy is theoretically possible (Gibbs et al., 1977; Ishida and Ito, 2003) provided that they express asynchronous flowering and no self-incompatibility mechanisms; (2) the species are classified as threatened due to small population sizes, high disturbance, and small estimations of extent of occurrence (Rivers et al., 2016); and (3) significant inbreeding has been reported for other Magnolias (Kikuchi and Isagi, 2002; Sun et al., 2011). It is possible that recent inbreeding remains undetected due to a time-lag (Kramer et al., 2008).

4.5 CONCLUSIONS

In conclusion, the data showed structuring on three different levels. Firstly, the supraspecific structuring confirms high species integrity with no extensive gene flow between species. Secondly, species sets within islands express lower genetic structuring but no signs of current gene flow, which is interpreted as a more recent shared ancestry. Thirdly, the populations within species also show moderate to strong differentiation, uncorrelated with the distance between the population pairs. The generalisation of extensive gene flow in trees does not withhold in the studied species. Our data support the hypothesis that the generalised concept of extensive gene flow in trees mainly applies to wind pollinated trees or trees that have larger animal vectors such as mammals (Dick et al., 2008). In contrast to the strong structuring, there is no sign of inbreeding, indicating ample gene flow within populations and mechanisms favouring cross-pollination. Hence, the reproductive biology of the Neotropical Magnolias appears resilient yet limited in their animal-mediated dispersal. A fragmented landscape is expected to strengthen this limitation. Hence, in terms of forest conservation, maintenance of – or preferably: an increase of – connectivity between forest patches would be the most effective strategy to ensure the survival of the species. To practically outline and further investigate the forest connectivity for Magnoliaceae, *Magnolia* SSR research would benefit from studying (1) the reproductive biology of the *Magnolia* trees (pollinators, seed dispersers

and phenology) and its limits, shaping the high genetic differentiation between, and high gene flow within populations; (2) the genetic diversity of closely related non-threatened *Magnolia* species, either in fragmented or continuous landscapes, placing past and future SSR *Magnolia* studies on threatened populations in perspective; and (3) splitting *Magnolia* conservation genetic studies according to age, to exclude this potential time-lag and detect whether or not the younger generation of *Magnolia* trees are genetically depauperate (e.g. Graignic et al., 2016; Watanabe et al., 2017).

4.6 DATA ARCHIVING

Data available from Dryad: https://doi.org/10.5061/dryad.0m625h4.

GenBank accession numbers for the 63 original sequences on which the primers were developed range from MH923371 to MH923433.

5. SSR study of *Magnolia cubensis* subsp. *acunae*

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ABSTRACT

Genetic data on threatened[1] plant populations can facilitate the development of adequate conservation strategies to reduce extinction risk. Such data are particularly important for species affected by habitat fragmentation such as *Magnolia cubensis* subsp. *acunae*[2], a Critically Endangered *Magnolia* subspecies endemic to Cuba. Using genetic data from 67 individuals, we aimed to evaluate the effect of habitat fragmentation on two populations in the Guamuhaya mountain range, in Topes de Collantes Protected Natural Landscape and Lomas de Banao Ecological Reserve. We characterised the structure and genetic diversity of these populations, with the objective of managing their conservation more effectively. We used Landsat satellite images to determine land-cover types at the two locations and calculated indices of habitat fragmentation. For genetic analyses, we extracted DNA from the leaf tissue of individuals from the two populations and used 11 microsatellite markers to genotype them. We calculated heterozygosity, allelic richness and genetic differentiation measures to evaluate genetic variability. The montane rainforest in Topes de Collantes was most affected by habitat fragmentation, with smaller patches of more irregular shapes, compared to submontane forest at this location and both montane and submontane forests in Lomas de Banao. Genetic diversity was higher in Topes de Collantes and we found little genetic differentiation between the populations. Our findings suggest considering the two populations as a single conservation unit. We propose to use individuals from both populations for reinforcement and translocations to increase the overall genetic diversity of the subspecies.

5.1 INTRODUCTION

Genetic data on threatened plant populations can facilitate the development of adequate conservation strategies to reduce extinction risk (Hedrick, 2001). This is particularly important for species affected by habitat fragmentation, which reduces the number of individuals per population and leads to isolation between populations, raising the probability of local extinction (Heinken and Weber, 2013). The genus *Magnolia* is represented in Cuba by seven endemic

[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1
[2] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.

taxa (Palmarola et al., 2016). One of them being the Critically Endangered subspecies *Magnolia cubensis* subsp. *acunae*, which is endemic to the Guamuhaya mountain range (González-Torres et al., 2013; González Torres et al., 2016) and threatened by deforestation and land conversion for cattle farming and coffee production. Recent studies on the distribution and conservation status of the subspecies (Granado, 2015; Palmarola et al., 2012) reported that the two main populations Topes de Collantes and Lomas de Banao have 416 and 70 individuals, respectively. However, there is no information on genetic diversity, the degree of habitat fragmentation or the interaction between these factors. Our study aimed to evaluate the effect of habitat fragmentation on the structure and genetic diversity of the *M. cubensis* subsp. *acunae* populations in the Guamuhaya mountain range, to support effective conservation management.

5.2 STUDY AREA

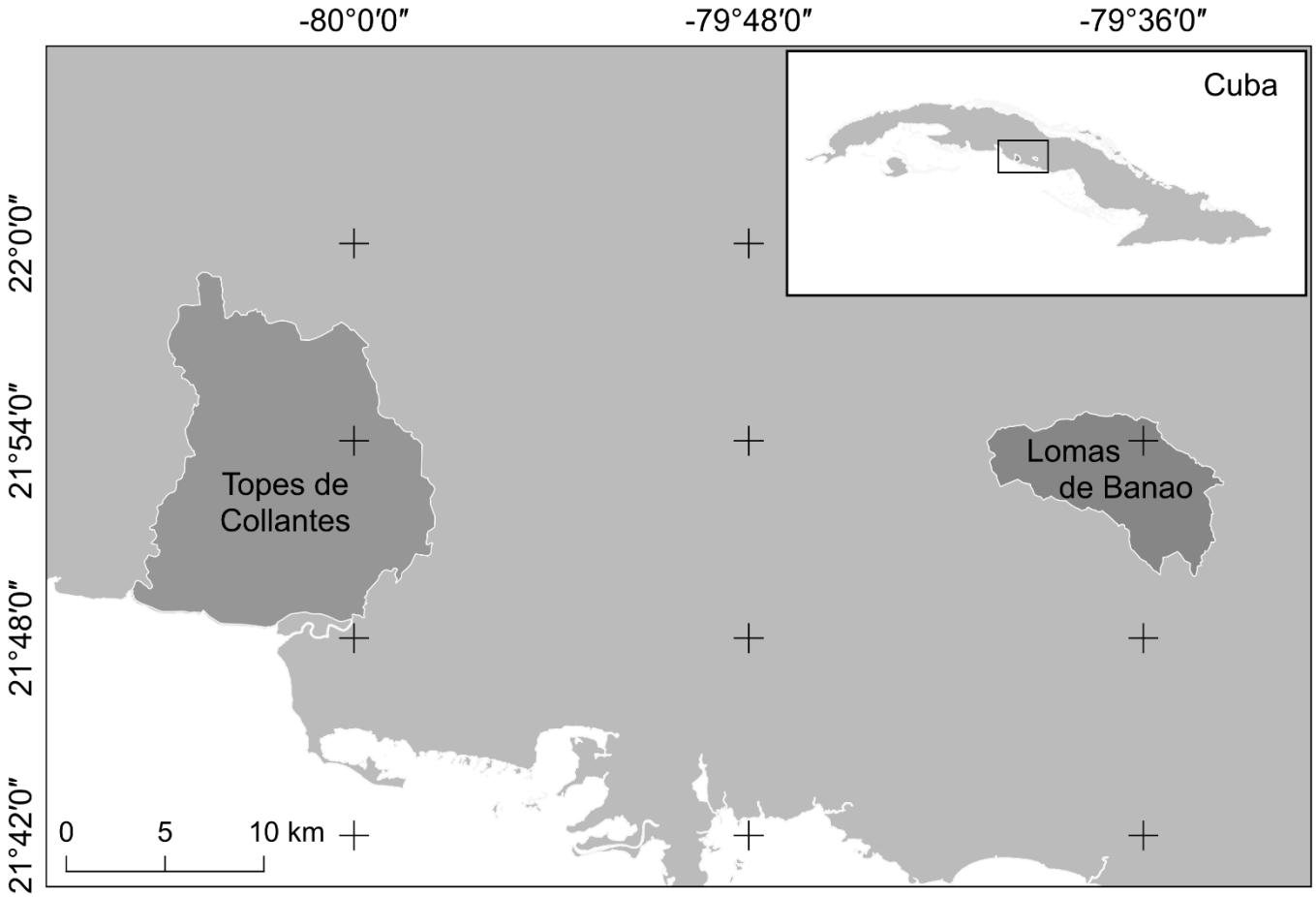
The study was carried out in the 201.35 km^2 Protected Natural Landscape Topes de Collantes and the 60.91 km^2 Ecological Reserve Lomas de Banao, in the Guamuhaya mountain range in central Cuba (Map 5.1 (CNAP, 2014)). Each of these protected areas contains one of the two main populations of *M. cubensis* subsp. *acunae* (Granado, 2015). The Topes de Collantes population has the highest number of individuals, representing 75% of known individuals of the species whereas Lomas de Banao represent 15% of the known individuals of the species (Granado, 2015). The climate of Guamuhaya corresponds to the Western Caribbean subregion and is classified as 'humid tropical' although at high elevations it could be considered 'mild warm' (Domínguez and Acosta, 2012). The variety of ecosystems that make up the region contain high levels of biodiversity which, especially considering the flora, makes the Guamuhaya one of the most biodiverse and endemics-rich localities in Cuba (Ruiz et al., 2011).

5.3 METHODS

5.3.1 Fragmentation analyses

We used a Landsat 8 satellite image of Guamuhaya mountain range, taken in February 2014, with 30 m spatial resolution and 11 spectral bands (Roy et al., 2014) and cropped the areas of interest using a map of protected areas (CNAP, 2014). We extracted georeferenced points of the habitat types in which *M. cubensis* subsp. *acunae* occurs from the vegetation map of Estrada et al. (2012). The points were used for supervised categorization of satellite images using the maximum likelihood method. In this process, pixels with a known land-cover type that are located within the training areas are used to categorize pixels of unknown land-cover type. We used four land-cover categories: submontane rainforest, montane rainforest, water

Map 5.1 The two study areas in Guamuhaya, Cuba. Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



and matrix, the latter defined as non-forested areas, including other vegetation, and agricultural and urban areas.

We converted the raster land-cover map to vector format and calculated seven fragmentation indices (Table 5.1). The variety of habitat patch shapes was classified according to Henao (1988) for the mean shape index and Hargis et al. (1998) for the mean patch fractal dimension index. We used IDRISI Selva v.12.1 (Eastman, 2012) for the supervised categorization and the Patch Analyst extension of ArcGis v.9.3 (Esri, Redlands, USA) for the fragmentation analyses.

Table 5.1 Description of the fragmentation indices.

Index	Description
Number of patches	Number of patches of a particular land-cover type
Mean patch size (km^2)	Average size of patches of a particular land-cover type
Patch size standard deviation (km^2)	Variability in relation to average size of patches in a particular class
Mean shape index	Average shape of patches of a particular land-cover type. The index varies from 1 to infinity, whereby 1 represents a compact patch and infinity an irregular patch
Mean perimeter-area ratio	Ratio of patch perimeter length to patch area
Mean patch fractal dimension	Mean shape of the patch, considering how much the perimeter of the patch represents in relation to the area
Edge density	Ratio of actual patch edge in relation to total length of edge possible

5.3.2 Population genetic analyses

5.3.2.1 Sampling

We collected leaf samples from 67 *M. cubensis* subsp. *acunae* individuals and stored them in self-sealed bags with silica gel. Of these, 58 were from Topes de Collantes (39 leaf samples from mature plants, 19 leaf samples from juveniles obtained from seeds) and 9 from Lomas de Banao (all from mature plants), representing 10% and 13% of all individuals in these populations, respectively. We collected seeds from fruits randomly selected from the Topes de Collantes population, using only one fruit per tree and one seed per fruit. The seeds were planted in nurseries in December 2014 and after two months, when the seedlings had six leaves, one leaf was collected for genetic analysis.

5.3.2.2 DNA extraction and genotypification

We extracted DNA from dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle, 1987), with MagAttract Suspension G solution mediated cleaning (Xin and Chen, 2012). We genotyped individuals with 11 microsatellite or Simple Sequence Repeats (SSR) markers developed on four Neotropical *Magnolia* species (Veltjen et al., 2019). PCR conditions and primer labelling followed Veltjen et al. (2019). Fragment analyses were executed by Macrogen Inc. (Seoul, South Korea) and we analysed the results in Geneious v.8.0.5 (https://www.geneious.com, Kearse et al., 2012), using the microsatellite plugin.

5.3.2.3 Simple sequence repeats marker testing

We calculated the deviation from Hardy–Weinberg proportions, linkage disequilibrium and the inbreeding coefficient (F_{IS}) for each locus using 10 000 dememorization steps, 100 batches and 5000 iterations per batch in Genepop v.4.3 (Rousset, 2008). We calculated deviations of both the uncorrected (Waples, 2015) and (sequential Bonferroni) corrected p-values to the nominal level of $\alpha = 0.05$ for both analyses.

5.3.3.4 Population structure and genetic diversity

We classified individuals into genetic populations using STRUCTURE v.2.3 (Pritchard et al., 2000) under the following conditions: 10 000 Markov chain Monte Carlo replicates after an initial burn-in of 10 000, using correlated allelic frequencies and assuming the admixture model. To obtain probability values of allocation of individuals to each genetic group K, we used five repetitions for each evaluated value of K, set to run from 1 to 8. We determined the most probable number of groups from the value of ΔK obtained according to the method of Evanno et al. (2005). The results were analysed in Structure Harvester Web v.0.6.94 (Earl and vonHoldt, 2012). An individual was considered to be a member of a genetic group when its probability of belonging to that group was > 0.5.

We quantified genetic diversity of each population and maturity class by the following parameters: the number of alleles per locus (A), number of private alleles (A_P), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), percentage of polymorphic loci (P) and inbreeding coefficient (F_{IS}). We estimated genetic differentiation between populations and across generations through pairwise comparisons of F_{ST} (Weir and Cockerham, 1984), G_{ST} (Nei, 1973; Nei and Chesser, 1983) and D_{JOST} (Jost, 2008) values calculated in R using the fastDivPart function of the package diveRsity (Keenan et al., 2013). Interpretation of genetic differentiation followed the criteria of Hartl and Clark (1997). Measures

of genetic diversity were calculated with GenAlEx v.6.1 (Peakall and Smouse, 2012). We calculated allelic richness and significance of F_{IS} with FSTAT v.2.9.3.2 (Goudet, 1995).

We estimated genetic distances between individuals and populations and carried out a Principal Coordinates Analysis (PCoA) from the matrix obtained. To identify possible patterns of isolation by distance in the genetic differentiation of the studied populations, we performed Mantel correlation tests with 10 000 permutations between genetic distances and geographical distances for all pairs of mature individuals of both populations. We used GenAlEx for both analyses.

To investigate the occurrence of any bottlenecks in the sampled populations, we characterised allele frequency distribution by locus, and evaluated deficit or excess of heterozygotes for the Infinite Alleles Model (IAM), the Two Phase Model (TPM) and the Stepwise Mutation Model (SMM) using the Wilcoxon test in Bottleneck v.1.2.02 (Cornuet and Luikart, 1996).

5.4 RESULTS

5.4.1 Fragmentation analyses

The supervised categorization projected on the distribution map of the habitat of *M. cubensis* subsp. *acunae* in Topes de Collantes and Lomas de Banao showed that submontane rainforest covered a larger area than montane rainforest (Figure 5.1). Lomas de Banao had greater landscape homogeneity and a smaller area categorised as matrix (i.e. other vegetation, and agricultural and urban areas). In Topes de Collantes the matrix land-cover type was primarily in the south and to a lesser extent at higher altitudes in the central part of the protected area. In Lomas de Banao, matrix areas were mainly in the submontane rainforest regions and the montane rainforest was less fragmented.

Table 5.2 depicts the results of the fragmentation indices of submontane and montane rainforest in the two populations. The number of patches in the four land-cover categories ranged between 107 and 169. In Topes de Collantes, patches of both forest types were smaller compared to Lomas de Banao. The mean shape index indicated that for the submontane rainforest, the most common patch form was rectangular-oblong, whereas for the montane rainforest, most patches could be considered amorphous. The mean path fractal dimension for both forest types showed complex forms analogous to fractal objects.

Figure 5.1 Land-cover types in (**a**) Protected Natural Landscape Topes de Collantes, (**b**) Ecological Reserve Lomas de Banao, Cuba, resulting from satellite Landsat image classification. Matrix refers to other vegetation, agricultural and urban areas.

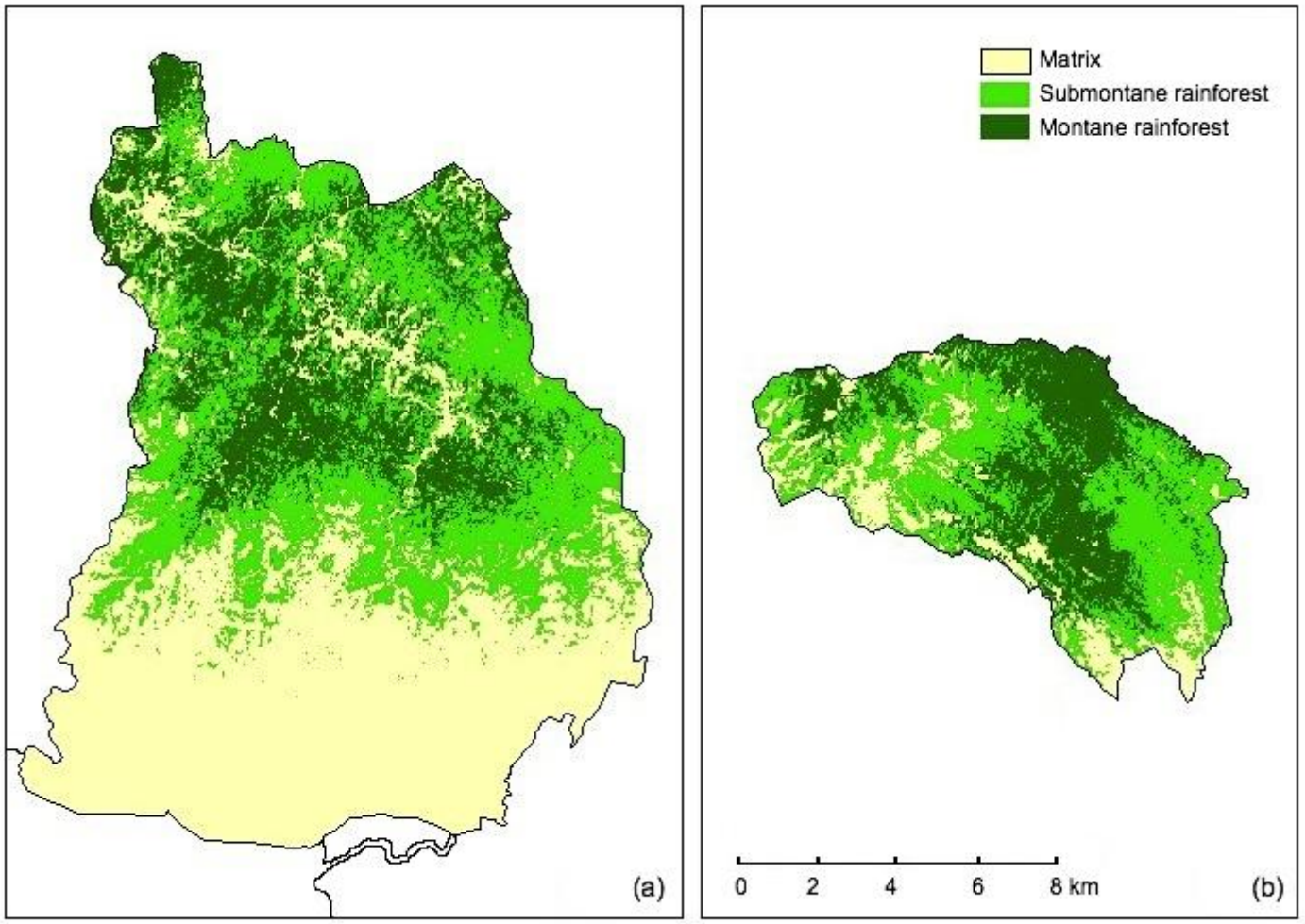


Table 5.2 Calculated fragmentation indices of submontane and montane rainforest in the Protected Natural Landscape Topes de Collantes and the Ecological Reserve Lomas de Banao in the Guamuhaya mountain range, Cuba.

Location and land-cover categories	No. of patches	Mean±SD patch size (km^2)	Mean shape index	Mean perimeter–area ratio	Mean path fractal dimension	Edge density	Total area (km^2) of all patches
Topes de Collantes							
Submontane rainforest	114	0.0224±0.15	1.87	2405.17	1.52	94.29	64.59
Montane rainforest	115	0.0029±0.01	2.36	2289.99	1.54	53.93	39.20
Lomas de Banao							
Submontane rainforest	169	0.1671±1.12	1.98	1003.87	1.40	78.88	28.24
Montane rainforest	107	0.2054±1.89	2.06	1775.94	1.40	52.39	21.91

5.4.2 Population genetic analyses

5.4.2.1 Simple sequence repeats marker testing

We found no significant deviations from Hardy–Weinberg proportions for 10 out of the 11 tested loci. Only MA42_279 in Topes de Collantes significantly deviated from Hardy–Weinberg proportions. The loci were not in linkage disequilibrium, with the exception of MA40_045 and MA42_279. Consequently, MA42_279 was discarded from all subsequent analyses.

All the 11 microsatellite loci were polymorphic at least in one of the two populations. MA41_076 was monomorphic in Lomas de Banao. We found 68 alleles among all loci (1–11 per locus), with a mean of 6.18 alleles per locus. Of the 24 rare alleles found, 22 (91.67%) were private alleles of Topes de Collantes. Summary statistics on the genetic diversity estimates per locus, analysed per population are given in Table 5.3 and summary statistics on the genetic diversity estimates per locus, analysed per population and maturity class are given in Appendix 5.1.

Table 5.3 Summary statistics of the 11 SSR markers in the Topes de Collantes and Lomas de Banao populations of *Magnolia cubensis* subsp. *acunae*.

	Topes de Collantes						Lomas de Banao					
SSR locus	A	A_R	H_O	H_E	P	F_{IS}	A	A_R	H_O	H_E	P	F_{IS}
MA39_333	3	2.358	0.351	0.333	1.000	−0.042	2	2.000	0.222	0.346	0.341	0.407
MA41_264	9	7.020	0.718	0.858	0.032	0.156	3	2.993	0.333	0.438	0.106	0.294
MA41_076	2	1.205	0.026	0.025	NI		1	1.000	NI			
MA42_255	6	4.228	0.513	0.695	0.012	0.174	3	3.000	0.625	0.617	0.229	0.054
MA42_274	6	3.783	0.564	0.512	0.029	−0.088	2	2.000	0.000	0.219	0.067	1.000
MA42_083	9	4.807	0.711	0.618	0.507	−0.137	6	5.765	0.778	0.722	0.414	−0.018
MA40_045	9	5.985	0.684	0.758	0.240	0.111	4	3.882	0.444	0.599	0.109	0.312
MA42_166	4	3.048	0.541	0.449	0.806	−0.191	2	1.993	0.222	0.198	1.000	−0.067
MA42_063	11	7.710	0.895	0.853	0.567	−0.035	5	4.765	0.667	0.525	1.000	−0.215
MA42_279	5	3.104	0.385	0.575	0.002*	0.342*	3	2.889	0.444	0.512	0.638	0.189
MA42_265	2	1.889	0.179	0.204	0.405	0.134	2	2.000	0.222	0.346	0.341	0.407

A: number of alleles per locus; A_R: allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; P: exact probability of the Hardy–Weinberg proportions test; F_{IS}: inbreeding coefficient calculated according to Weir and Cockerham (1984); when $p < 0.005$ this is highlighted with an asterisk *; NI, non-informative comparison because it is a monomorphic locus or presents low values of H_O and H_E.

5.4.2.2 Population structure and genetic diversity

The optimal ΔK was 2 (Figure 5.2). Of the mature trees, 75% were assigned to a genetic group that aligned with their sampling location. All individuals of Lomas de Banao clustered in genetic group 2, together with 30.77% of the mature individuals and 100% of the juveniles of Topes de Collantes. This means that 12 of 39 adult individuals from Topes de Collantes had a probability of > 50% to belong to genetic group 2, which aligns with the individuals of Lomas de Banao (Figure 5.3).

Figure 5.2 ΔK values obtained with K (number of groups) from K=2 to K=8, and five simulations per analysis, analysed in Structure Harvester Web v.0.6.94 (Earl and vonHoldt, 2012), to determine the most probable number of groups of *Magnolia cubensis* subsp. *acunae* individuals from the Topes de Collantes and Lomas de Banao populations, according to the method of Evanno et al. (2005).

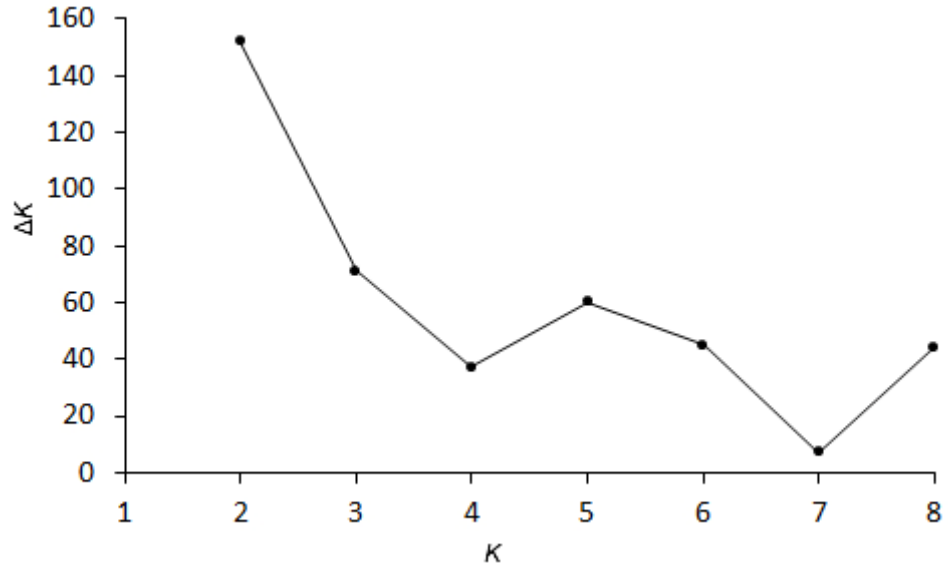
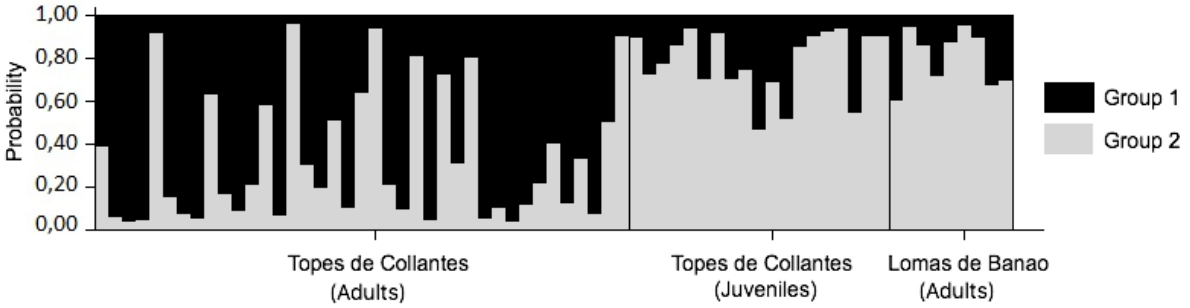


Figure 5.3 Probability of genetic group allocation of *M. cubensis* subsp. *acunae* individuals of Topes de Collantes and Lomas de Banao populations, inferred at K=2 based on allelic frequencies of SSR data.



Values for all genetic diversity measures were lower in Lomas de Banao than Topes de Collantes. Similarly, juveniles of Topes de Collantes were less genetically diverse than the mature population of this locality. The inbreeding coefficient (F_{IS}) was higher, and statistically significant, in Lomas de Banao compared to Topes de Collantes (Table 5.4).

Table 5.4 Measures of genetic diversity of *M. cubensis* subsp. *acunae* in the Topes de Collantes and Lomas de Banao populations, calculated per maturity class.

		Genetic diversity measures						
Population	N	A (range)	A_R	H_O	H_E	HW	F_{IS}	P
Topes de Collantes (adults)	39	6.000 (2–11)	4.103	0.506	0.535	2	0.037	100
Topes de Collantes (juveniles)	19	3.545 (2–8)	3.100	0.435	0.461	2	0.067	100
Lomas de Banao (adults)	9	3.000 (1–6)	2.935	0.360	0.411	0	0.183*	90.91

N: number of sampled individuals; A: mean number of alleles; A_R: allelic richness; H_O: mean observed heterozygosity; H_E: average expected heterozygosity; HW: number of loci that deviated from Hardy–Weinberg proportions; F_{IS}: population inbreeding coefficient, significant deviations from zero are indicated with * (p = 0.05); P: percentage of polymorphic loci.

There is little genetic differentiation between the two populations according to the F_{ST} and G_{ST} fixation indices and little allelic differentiation according to the D_{JOST} statistic (Table 5.5). The PCoA showed no differential clustering for individuals from different populations or maturity class (Figure 5.4). The Mantel correlation test had a low correlation coefficient (r = 0.034), considered statistically non-significant (p = 0.32), indicating no correlation between distance and genetic differentiation.

Table 5.5 Genetic differentiation between populations of *M. cubensis* subsp. *acunae*, respecting the subdivision of maturity class. Pairwise fixation indices: F_{ST} (Weir and Cockerham, 1984) and G_{ST} (Nei, 1973; Nei and Chesser, 1983), are place above the diagonal; and allelic differentiation: D_{JOST} (Jost, 2008) is placed below the diagonal.

Population (generation)	Topes de Collantes (adults)	Topes de Collantes (juveniles)	Lomas de Banao (adults)
Topes de Collantes (adults)		0.0373 (F_{ST}) 0.0205 (G_{ST})	0.0435 (F_{ST}) 0.0262 (G_{ST})
Topes de Collantes (juveniles)	0.0151		0.0245 (F_{ST}) 0.0155 (G_{ST})
Lomas de Banao (adults)	0.0148	0.0066	

Figure 5.4 Principal coordinates analysis of genetic distances matrix with 10 microsatellite markers in *M. cubensis* subsp. *acunae* in Guamuhaya, Cuba.

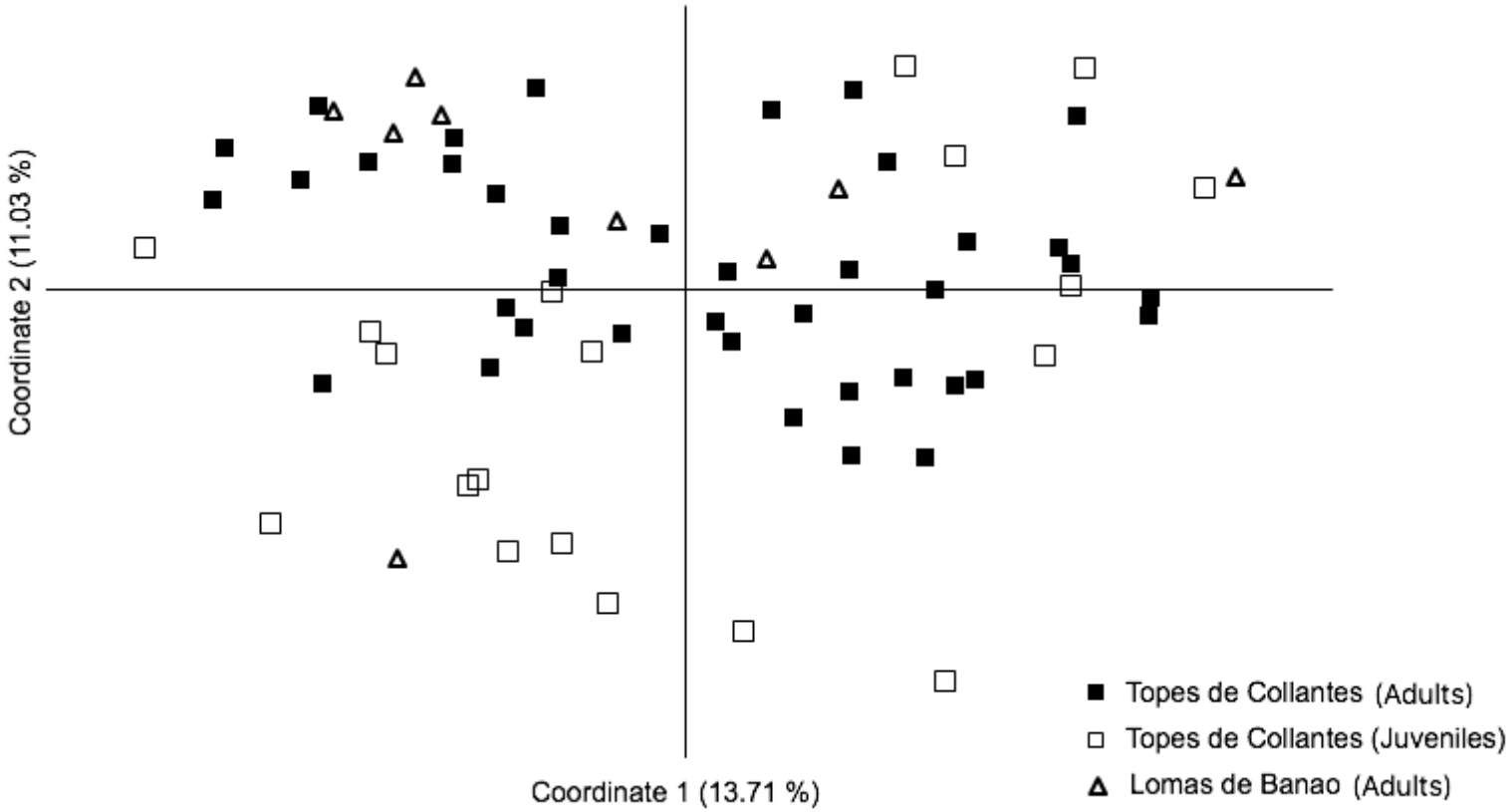
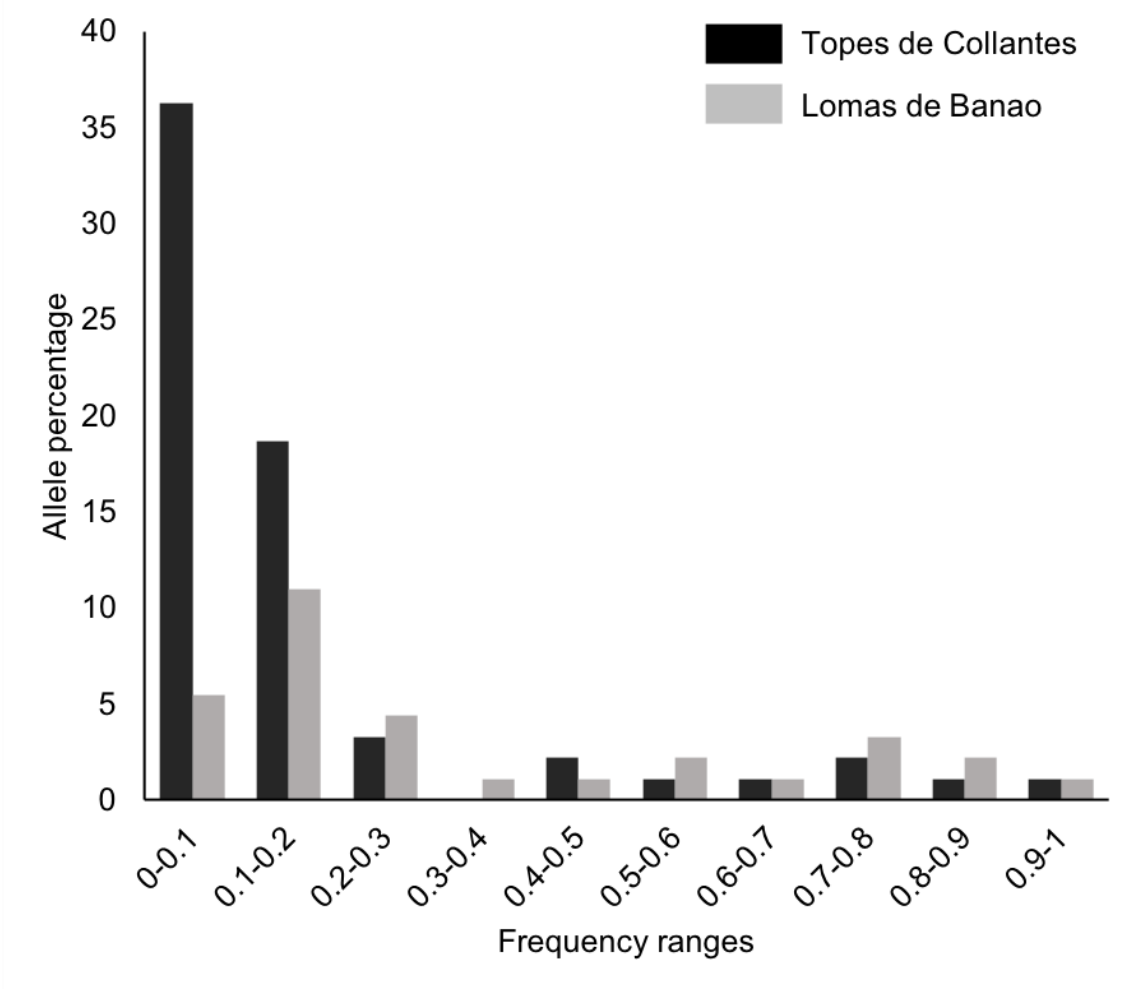


Figure 5.5 Allele percentage distribution by allele frequency ranges for 48 adult individuals of populations of *M. cubensis* subsp. *acunae* in Guamuhaya, Cuba.



The Wilcoxon test for Topes de Collantes showed differences between the estimated heterozygosity value at equilibrium and that obtained by simulating diversity under the IAM. For Lomas de Banao, the analysis also revealed differences simulated under the TPM and SMM (Table 5.6). The distribution of allelic frequencies displays an L-shaped distribution for Topes de Collantes, which is not the case for Lomas de Banao (Figure 5.5).

Table 5.6 Probability of deficiency and excess heterozygosity (H_E) observed at equilibrium, compared to that estimated under the Infinite Alleles Model (IAM), Two Phase Model (TPM) and Stepwise Mutation Model (SMM) for the populations of *M. cubensis* subsp. *acunae.*
*$p < 0.01$ was considered statistically significant.

Model	Topes de Collantes Deficiency H_E	Excess H_E	Lomas de Banao Deficiency H_E	Excess H_E
IAM	0.995	0.007*	1.000	0.001*
TPM	0.984	0.042	1.000	0.001*
SMM	0.903	0.116	1.000	0.001*

5.5 DISCUSSION

5.5.1 Fragmentation analyses

Habitat fragmentation was most severe in the montane rainforest of Topes de Collantes, with smaller, irregularly shaped patches and greater patch perimeter to area ratio. Edge effects and the quality of the surrounding area influence the characteristics of remnant habitat patches (Heinken and Weber, 2013). In the montane rainforest, where many fragments are surrounded by submontane rainforest, the matrix effect is smaller, whereas the submontane rainforest is mainly surrounded by matrix areas (Fischer and Lindenmayer, 2007).

The high degree of forest fragmentation was expected, given the history of land-use changes in the area. Coffee has been produced in Topes de Collantes since the 19th century, resulting in selective logging in large spaces and the replacement of natural vegetation by a monoculture. Furthermore, the wood of native tree species such as *M. cubensis* subsp. *acunae* is also used to build coffee plantation infrastructure (Domínguez et al., 2012).

5.5.2 Population genetic analyses

5.5.2.1 Population structure and diversity

The STRUCTURE analysis (Figure 5.3), the PCoA (Figure 5.4) and F_{ST}, G_{ST} and D_{JOST} values (Table 5.5) indicated low genetic differentiation between the two adult populations. There are three potential explanations for this:

(1) Recent/current gene flow between the populations. Geographical proximity of populations increases the chance of gene flow. The studied populations are 33 km apart and such distance would be hypothesised to be overcome by seed dispersal rather than pollen dispersal, because there are only few reports of pollen travelling distances > 10 km for insect-pollinated species (Petit and Hampe, 2006), such as *Magnolia* (Thien et al., 1996). The potential seed dispersers of this subspecies are the Cuban trogon *Priotelus temnurus*, fieldfare *Turdus* and western spindalis *Spindalis zena*, which are permanent or seasonal residents in Cuba (Garrido and Kirkconnell, 2011). However, detailed studies of the ecology, behaviour and foraging strategies of potential seed dispersers in this mountainous region are lacking to verify this possibility further.

(2) Evolutionary history of between the populations, reflected by past gene flow. The simplest interpretation of small genetic distances between populations is that they share a recent common ancestor. The low genetic diversity and inbreeding found in Lomas de Banao (Table 5.4) could either be an indication of the founder effect (Slatkin, 2004), or deleterious effects of genetic drift after isolation due to fragmentation of the once single, larger population that enclosed both forest patches.

The adult population in Topes de Collantes was more genetically diverse compared to the adult population in Banao (Table 5.4). Although both sample sizes are considered comprehensive given their equal proportion to the known individuals per population (Granado, 2015), the result could be due to the low sample size of the Lomas the Banao population. Allelic richness should overcome this problem using rarefaction, yet the A_R of Topes de Collantes remained substantially higher than that of Lomas de Banao.

The diminution of the diversity across generations in Topes de Collantes (Table 5.4) is indicative of a decrease in pollen dispersal. As pollen dispersal distances for trees visited by small insects such as beetles in closed-canopy forests often do not exceed 300 m (Dick et al., 2008), this mediator of gene flow is most sensitive to the fragmented habitat. Although the data deliver evidence of a direct consequence of the fragmented habitat in gene flow, it must be kept in mind that this is one sample in time; hence, one reproduction event in a species that has a with multiple chances of reproducing throughout its long lifespan (Petit and Hampe, 2006). Even more so, a few migrants shared across the populations by seed dispersers can reset the deleterious effects of low pollen mixture by habitat fragmentation (Holsinger and Weir, 2009). When the two genetic assignments of the maturity classes of the Topes de Collantes population are compared, the result of the STRUCTURE analysis is puzzling (Figure 5.3). Given the mix of ca. two-thirds genetic group 1 and one-third genetic group 2 in the mature generation of Topes de Collantes, a similar representation was expected in the juveniles. Potential explanations are cross-fertilization with father trees that make the juvenile population genetically more similar to the adult population of Lomas the Banao or positive selection (in the nursery) for juveniles of genetic group 2.

Topes de Collantes tested positive for a past bottleneck, only when assuming the IAM, while the population of Lomas de Banao tested positive for a past bottleneck under all three models (Table 5.6). Because on the one hand, for microsatellite markers, the SMM is considered more appropriate (Putman and Carbone, 2014), and the distribution of allelic frequencies in Topes de Collantes, as shown in Figure 5.5, does not show a high percentage of alleles with high frequencies (Allendorf et al., 2013), we conclude that this population did not suffer from a recent bottleneck. Together with the result of inbreeding and low genetic diversity (Table 5.4), and the known past forest extent of the area - it seems likely that the population in Lomas de Banao did suffer from a recent bottleneck. However, the results in Table 5.6 should be interpreted with caution because the Wilcoxon test requires at least 10 individuals (Cornuet and Luikart, 1996) and hence could be compromised by the small sample size from Lomas de Banao.

5.5.3 Implications for conservation

The principal consequences of ongoing habitat fragmentation are progressive reduction of population sizes and increased distance between habitat fragments, which can affect genetic variation, heterozygosity, inbreeding, gene flow and genetic divergence between populations (Heinken and Weber, 2013). The listed genetic consequences of fragmented habitats are reflected in our results, with high levels of inbreeding and low genetic diversity in Lomas de Banao, reduction of the genetic diversity across generations in Topes de Collantes and low genetic differentiation between populations. The studied populations are currently losing genetic resilience and hence, conservation actions are appropriate.

Our findings merit a new approach to the conservation management of *M. cubensis* subsp. *acunae*. The results of the structure analyses, PCoA plots and genetic differentiation parameters predict that there is no marked genetic structure in the populations. Hence, the two populations can be considered a single Evolutionarily Significant Unit (ESU) and conservation entity (Moritz, 1994). We suggest using individuals from both populations for on the one hand reinforcement actions, whereby the Lomas de Banao population should be considered a priority over the Topes de Collantes population, and on the other hand reintroductions in Guamuhaya as proposed by Granado (2015), considering the modelled (potential) distribution of the species, as well as protection status and landscape use of the area.

Despite past habitat fragmentation and loss of natural vegetation cover in these protected areas, the improvement of the legal framework on biological diversity in Cuba of the recent years prohibit agricultural, forestry and fruit production in protected areas and set goals to reduce the impact of agroforestry. This will support the recovery of the studied populations and the ecosystems they are part of. Even more so, awareness has increased after 12 years of conservation projects focussing on this subspecies. People are now interested in protecting this subspecies and its habitat because it represents a symbol for the community and its cultural identity. Many farmers cultivate coffee in the shade of *M. cubensis* subsp. *acunae* and claim that coffee quality is superior when shade is provided by this native species. Restoration of the montane and submontane rainforest, reinforcement and reintroductions, and support of the local communities are essential for the long-term improvement of the conservation status of this endemic Cuban subspecies.

6. SSR study & biogeography of *M. dodecapetala*

MODIFIED FROM: Veltjen E., Asselman P., Goetghebeur P., Samain M.-S., Larridon I. (in preparation) An integrative approach to understand the diversity of *Magnolia dodecapetala* (Magnoliaceae: *Talauma* subsect. *Talauma*) in the Lesser Antilles. Frontiers in Plant Science. Impact factor 2018: 4.106.

ABSTRACT

Five of the volcanic islands of the Lesser Antilles are home to the first described Magnoliaceae species, now known as *Magnolia dodecapetala*[1], and provide a distinct study system for investigating biodiversity in the context of biogeographic and conservation genetic patterns. We have characterised the genetic diversity of *M. dodecapetala* in the Lesser Antilles using Sanger sequencing of 21 individuals amplified[2] for 11 DNA markers, plus Single Sequence Repeat (SSR) data of 195 individuals genotyped with 19 SSR markers, and we have aligned this genetic diversity with the variation present in its fruit morphology. The results provide different lines of evidence for the underlying biogeography: a first colonization of Saint Lucia or Martinique is suggested by the calibrated Bayesian phylogenetic hypothesis based on the Sanger sequencing data, and alternatively, a north to south colonization is indicated by the DIYABC analysis based on the SSR data. Both biogeographic scenarios hint towards more complicated patterns than a recent stepwise colonization from the South American mainland to the Lesser Antilles. Both data types provide support for treating at least the different island populations as distinct Management Units (MU) with and suggest defining them as Evolutionarily Significant Units (ESU). Furthermore, the SSR data showed significant inbreeding in all populations except the population from Saint Lucia. Also, conservation management and co-aligning continued conservation genetic studies of the population from Saint Vincent and the southern subpopulation from Dominica are suggested given the low genetic diversity, and an unexpectedly high amount of genetic linkage, respectively. Lastly, no correlation between pairwise morphological and genetic distance was found, yet we report great variation in fruit morphology that can be grouped into discrete clusters per island population, whereby the Saint Vincent (southernmost) and Guadeloupe (northernmost) populations have the smallest fruits.

[1] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.
[2] A selection of less commonly used words is explained in the glossary: Appendix 1.1

6.1 INTRODUCTION

Islands are often dubbed “nature’s laboratories of evolution” (Ricklefs and Bermingham, 2008; Whittaker et al., 2017) given that they frequently are relatively young, replicated, discrete “experiments” varying in size and distance from the mainland or neighbouring islands, often with simplified biota and a wide diversity of habitats that promote diversification and speciation (Emerson, 2002; Losos and Ricklefs, 2009; Ricklefs and Bermingham, 2008; Whittaker et al., 2017). Two emblematic works inspired by patterns derived from insular systems are *On the Origin of Species* by Darwin (1859) focussed on speciation, and *Core Island Biogeography Theory* by MacArthur and Wilson (1967) focussed on community ecology. These works provided inspiration and foundation for different lines of research, studied on island systems and beyond.

A textbook island study system is the Lesser Antilles: a classic island arc of volcanic origin within the Caribbean, known as a biogeographical sweet spot in terms of colonization dynamics due to its “close enough, yet isolated enough” location to the mainland (Ricklefs and Bermingham, 2008). Starting north of Martinique, the Lesser Antilles comprise two distinct island arcs. The older, outer or eastern arc is called the “Limestone Caribbees” estimated to be of Eocene to Miocene origin (Draper et al., 1994). The younger, inner or western arc, known as the “Volcanic Caribbees”, comprises the present-day volcanic front and the origin of its islands is dated from the Miocene to the present (Draper et al., 1994). Both arcs are a result of subduction of the Atlantic oceanic crust under the Eastern Caribbean Plate and in the southern half of the chain, the two arcs are superimposed on one another to form the islands of Grenada, the Grenadines, St. Vincent, St. Lucia and Martinique (Draper et al., 1994). The Lesser Antilles never have had continental connections (with the exception of a few shallow banks), nor have the different islands ever been connected to each other, hence it is accepted that the Lesser Antilles have been colonized by flora and fauna entirely by over-water dispersal (Hedges, 1996; Ricklefs and Bermingham, 2008), making the Lesser Antilles islands a perfect model system for testing the concept of stepping-stone dispersal (MacArthur and Wilson, 1967): islands are most likely to be colonised first from their neighbouring island, most likely in a sequence starting from the mainland.

Although the islands of the Lesser Antilles are geologically relatively young, they are home to the first described Magnoliaceae species, now known as *Magnolia dodecapetala* (Figure 1.18). Magnoliaceae, part of the Magnoliids (APG IV, 2016), counts over 300 species worldwide (Rivers et al., 2016), and provide a range of services such as cultural services as popular garden ornamentals; regulating services by shaping diverse and unique habitats like primary forests; and provisioning services as a (potential) source of timber, medicines or ingredients

for fragrances (Sánchez-Velásquez et al., 2016). Within the family, *Magnolia dodecapetala* is historically emblematic, yet other than the 'silvicultural and botanic monograph' of Stehlé and Marie (1947) and the work of Howard (1948), the species has not received any attention from researchers. *Magnolia dodecapetala* is classified in subgenus *Magnolia* section *Talauma* subsection *Talauma* (Figlar and Nooteboom, 2004) and it occurs on St. Vincent, St. Lucia, Martinique, Dominica and Guadeloupe; five islands of the Lesser Antilles (Map 6.1). Two herbarium records are known from Trinidad: *F.W. Sieber 293* (MO) and *Parmentier s.n.* (P), yet this locality has not been verified by any other sources (e.g. herbarium records, local floras, local botanists and a visit to the island in 2016 by the first author) and it is most likely an erroneous report (see Chapter 1.7 for a more elaborate discussion).

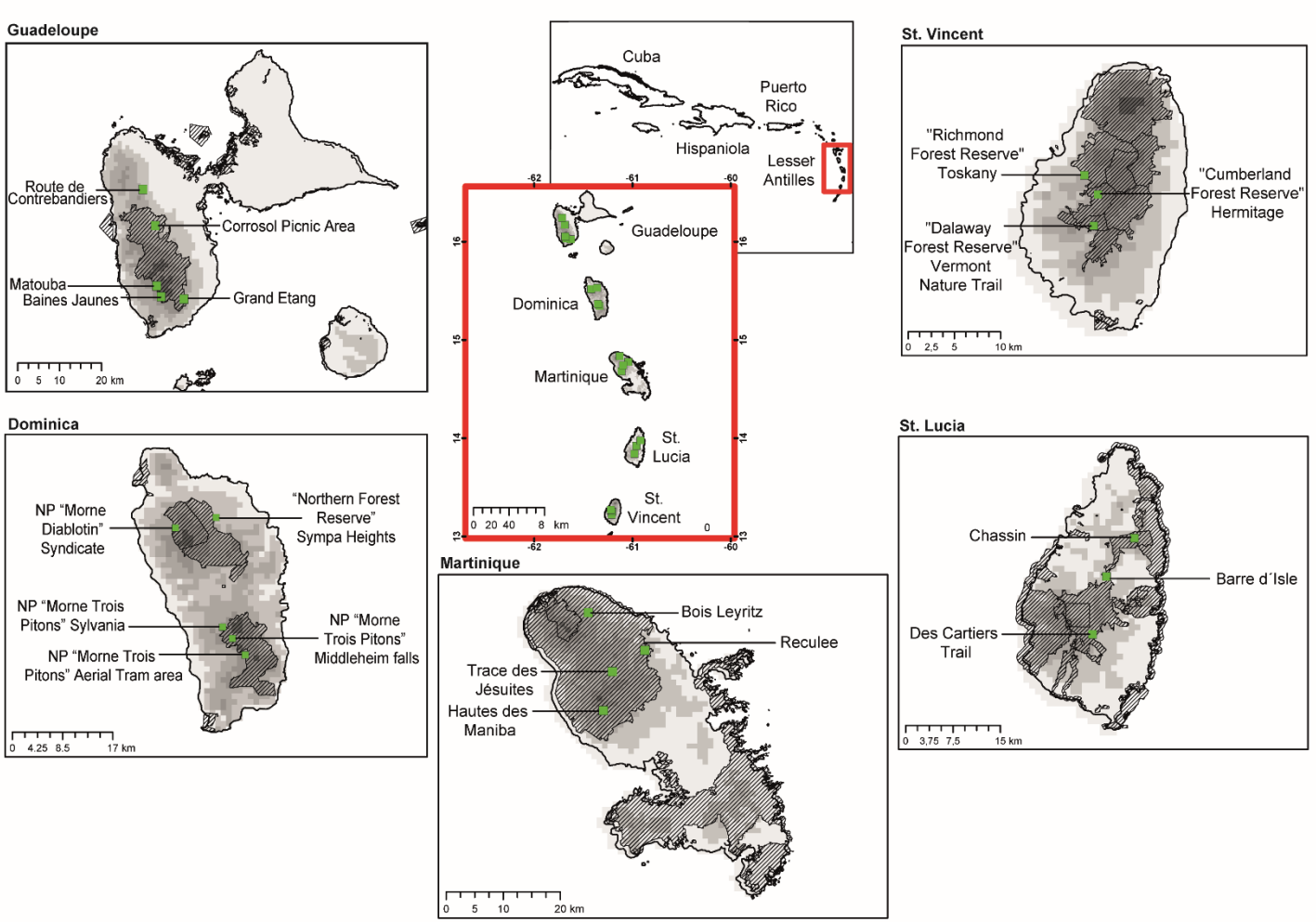
In a previous biogeographical study (see Chapter 3), the species was found to be a sister lineage to South American mainland Magnolias and the age of its dispersal from the South American mainland to the islands of the Lesser Antilles was estimated at around 3.75 (7–1) mya. As the full distribution range of *M. dodecapetala* was not yet included in the sampling design of that study, it did not allow conclusions on the sequence and timing of island colonisation within the Lesser Antilles, let alone discuss possibilities of 'reverse colonisation' (Bellemain and Ricklefs, 2008) and 'repeated colonisations' (Silvertown, 2004). Interestingly, the detailed documentation of the geology of the Lesser Antilles allows a study of the colonisation sequence and dates of *M. dodecapetala* in its full range. Known dates of the five islands on which *M. dodecapetala* occurs, are the following: The oldest rocks of Saint Vincent (SV) are estimated to be younger than 5 mya (Wadge, 1994); Saint Lucia (SL) contains exposures of Miocene rocks dated to be from 18–5 mya (Wadge, 1994); Martinique (M) partly consists of Oligocene age rocks, dated to be maximally 38 mya old (Birden et al., 1979), however, the bulk of rocks building up this island are dated to be 16 mya in the east to 6 mya in the west; a result from the two arcs that together form the island (Wadge, 1994); The rocks of Dominica (D) are estimated to be not older than 7 mya (Bellon, 1988; Monjaret, 1985); Basse-Terre, the part of Guadeloupe (G) that belongs to the inner arc, is estimated to have a similar age as the rocks of St. Vincent: younger than 5 mya (Wadge, 1994). Grande Terre, the part of Guadeloupe that belongs to the outer arc, has one isolated known date of 11 mya, however older, Miocene ages were expected (Nagle et al., 1976). Currently, Grande Terre does not appear to be home to any *M. dodecapetala* populations.

Beyond presenting an ideal case study to investigate the biogeographical and evolutionary history underlying the current distribution of *M. dodecapetala*, knowledge of the genetic diversity of this species is also of interest for conservation. The species is currently assessed as Vulnerable on the IUCN Red List (Rivers et al., 2016), and adds to the high percentage of threatened species of the family. A previous preliminary SSR study (Veltjen et al., 2019)

included two populations with each 20 individuals and found genetic signatures of inbreeding, substructure and relatively compared to the other studied populations - higher levels of genetic diversity, which altogether highlighted the species for further conservation genetic investigation (see Chapter 4). Given that it is factual that the species reached the islands via overwater dispersal, and that the research in Chapter 3 already indicated that this happened in a geologically recent time frame, it is very likely that the stochastic genetic processes such as inbreeding, low genetic diversity and founder effects had to be overcome by this species at the start of each colonization event, and even more so, that they are still actual. The founder effect is a special case of genetic drift describing successful immigrants arriving by chance dispersal, bringing with them only a small portion of the genetic variation that existed in the parental population (Allendorf et al., 2013). Founder effects are generally associated with limitations restraining the new settled population to genotypic and phenotypic "load" from the past (Kolbe et al., 2012), or, if genetic drift and inbreeding interplay in such a way in subsequent generations after the successful colonization, the low genetic diversity of the founder population can lead to extinction (Matute, 2013). Quite controversially, in the right set of conditions involving adaptation and major genetic shifts, founder effects are hypothesized to be a main driver of speciation after colonization, which is called founder speciation (Templeton, 2008). Regardless of the influence of the founder effect in the evolutionary history of the *Magnolia* populations of the Lesser Antilles, the finite island populations are bound by the small island size, which sets the stage for concepts such as inbreeding and genetic differentiation by isolation, adding to the species' vulnerability to extinction (Frankham, 1998).

In this chapter, we characterise the genetic diversity of *M. dodecapetala* in the Lesser Antilles using Sanger sequencing and Single Sequence Repeat (SSR) data, and align the genetic diversity with the variation present in its fruit morphology, to (1) reveal the biogeographical and evolutionary history of the species; (2) establish the conservation units for this species and by proxy the connectivity of the hygrophytic forests; (3) assess the level of inbreeding and genetic diversity *M. dodecapetala* and by proxy its evolutionary resilience; and (4) test whether the genetic diversity measured with SSR markers can be correlated to morphological variation in the fruits.

Map 6.1 Distribution of *Magnolia dodecapetala* in the Lesser Antilles in the Caribbean. Map data: Caribbean outline: (National Imagery and Mapping Agency, 2011); Elevation data: (Fick and Hijmans, 2017). Protected areas are shaded (French Antilles protected areas: UNEP-WCMC, 2019). Software: ArcGIS v10.6 (ESRI, Redlands, CA, USA).



6.2 MATERIAL AND METHODS

6.2.1 Sampling, genetic and morphological characterization

Leaf samples corresponding to 195 *Magnolia dodecapetala* individuals with their respective geographical coordinates, 31 herbarium vouchers and 170 fruits were collected in June–July 2016 on the islands St. Vincent, St. Lucia, Martinique, Dominica and Guadeloupe. The distribution of *M. dodecapetala* and the sampling localities are visualised in Map 6.1. Number of sampled individuals per population and herbarium vouchers are depicted in Table 6.1. Leaves were dried in silica gel and their DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle, 1987) with MagAttract Suspension G solution (Qiagen, Germantown, USA) (Xin and Chen, 2012) mediated cleaning (Larridon et al., 2015).

In total, 21 individuals were sequenced for phylogenetic hypothesis construction (Table 6.1). For each island three to five individuals were sequenced, representing the main sampling sites within each island. A sample from *M. lacandonica* was used as outgroup, as this was the geographically closest species of which sequences of all markers were available through the study of Chapter 3. Sequencing occurred via Sanger sequencing using forward and reverse primers, PCR protocols and assembly methods from Chapter 3, resulting in a total of 11 sequence alignments. The chloroplast data were concatenated and together with the five nuclear genes, a total of six final alignments were assembled.

For each island, the individuals of *M. dodecapetala* were genotyped using 19 SSR primer pairs and PCR protocols from Veltjen et al. (2019); to which a new extra SSR primer MA39_191 was added[24]. Peak calling was executed in Geneious v.8.1.9 (https://www.geneious.com, Kearse et al., 2012) and transferred to the necessary data formats using CONVERT (Glaubitz, 2004) and PGDSpider v.2.0.8.2 (Lischer and Excoffier, 2012). We used MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004) with the default settings, and ML-NullFreq (Kalinowski and Taper, 2006) with 100 000 randomizations, 1000 batches and 50 000 iterations per batch to test for null alleles, assuming each island was one population. After null allele detection two extra datasets were instated besides the **D(19)**: dataset of all the 19 SSR markers, namely **D(15)**: dataset of 15 SSR markers excluding all the markers with a potential null allele in two or more out of five populations; and **D(7)**: dataset of 7 SSR markers excluding all the markers with a potential null allele in one or more out of five populations. Calculations of the null frequencies (A_0) were repeated with the D(15) and D(7) datasets.

[24] F: TCCAACGAGTACTTGGGCAG; R: GATGCGTCCTTGAGTCCCAA; repeat motif: AG(22); size range: 164–204.

Table 6.1 The 19 localities of *Magnolia dodecapetala* sampled in the Lesser Antilles and one outgroup sample of *Magnolia lacandonica* from Mexico.

Species	Location	Locality	Loc	Herbarium voucher	Lab	N
M. dodecapetala	St. Vincent	Vermont Nature trail	VER	Veltjen 2016-002 (GENT)	MA1100	5
M. dodecapetala	St. Vincent	Hermitage	HER	Veltjen & Glasgow 2016-003 (GENT)	MA1104	16
M. dodecapetala	St. Vincent	Toskany	TOS	Veltjen et al. 2016-004 (GENT)	MA1128	8
M. dodecapetala	St. Lucia	Chassin	CHA	Veltjen et al. 2016-005 (GENT, SLUC)	MA1318+ MA1334	5+7
M. dodecapetala	St. Lucia	Barre d'Isle	BAR	Veltjen & Sealys 2016-006 (GENT)	MA1316	10
M. dodecapetala	St. Lucia	Des Cartier trail	CAR	Veltjen et al. 2016-007 (GENT, SLUC)	MA1322	6
M. dodecapetala	Martinique	Reculee	REC	Veltjen & Pitoula 2016-010 (GENT)	MA1139	12
M. dodecapetala	Martinique	Bois Leyritz	LEY	Veltjen & Pitoula 2016-072 (GENT)	MA1144	10
M. dodecapetala	Martinique	Hautes des Maniba	HAU	Veltjen & Giraud 2016-008 (GENT, MTK, K, IEB)	MA1168	11
M. dodecapetala	Martinique	Trace des Jésuites	JES	Veltjen & DelBlond 2016-009 (GENT, MTK, K, IEB)	MA1182	16
M. dodecapetala	Dominica	Syndicate	SYN	Veltjen & Stedman 2016-011 (GENT)	MA1195	11
M. dodecapetala	Dominica	Trois Pitons: Aerial tram	TPA	Veltjen & Stedman 2016-082 (GENT)	MA1219	11
M. dodecapetala	Dominica	Trois Pitons: Middleheim	TPM	Veltjen & Stedman 2016-086 (GENT)	-	11
M. dodecapetala	Dominica	Sympa Heights	SYM	Veltjen & Stedman 2016-016 (GENT, ATREC)	MA1230	4
M. dodecapetala	Dominica	Sylvania	SYL	Veltjen & Stedman 2016-017 (GENT)	MA1235	11
M. dodecapetala	Guadeloupe	Corrosol + Mammeles	COR	Veltjen et al. 2016-015 (GENT, GUAD)	MA1245	1+2
M. dodecapetala	Guadeloupe	Grand Etang	GRA	Veltjen et al. 2016-018 (GENT)	MA1248	5
M. dodecapetala	Guadeloupe	Baines Jaunes	BAI	Veltjen & Van-Laere 2016-020 (GENT)	MA1262	15
M. dodecapetala	Guadeloupe	Route de Contrebandiers	CON	Veltjen & Rousteau 2016-025 (GENT)	MA1272	4
M. dodecapetala	Guadeloupe	Matouba	MAT	Veltjen 2016-030 (GENT)	MA1286	14
M. lacandonica	Mexico	Yajalón	YAJ	Samain & Martínez 2017-016 (IEB, MEXU)	MA1831	1

The samples of *M. dodecapetala* are sorted per island from south to north, which was the trajectory during the sampling of June-July 2016. For each locality its abbreviation ("**Loc**" column), corresponding herbarium voucher, lab code of the sample used ("**Lab**" column) for Sanger sequencing and number of sampled trees ("**N**" column) are given. Herbarium abbreviations follow Index Herbariorum (Thiers, continuously updated), except **SLUC**, which stands for the herbarium of the Department of Forestry (Ministry of Agriculture) of Saint Lucia and **ATREC**, which stands for the herbarium of the Archbold Tropical Research & Education Center in the Commonwealth of Dominica.

We used GENEPOP v.4.3 (Rousset, 2008) with the dememorization number set to 10 000, batches set to 1000 and 50 000 iterations per batch, to test for linkage disequilibrium (LD) (Lewontin and Kojima, 1960). Analyses were repeated with the unaccounted substructure found in STRUCTURE analyses (see further).

For the 170 fruits, we tabulated the number of carpels, fruit length (in cm), individual number and island population from which they were collected. Fruit length was measured from the inner fruit cores, starting from the lowest point of the tepal scar on this woody structure, to the tip. The choice using fruits for morphological characterization is twofold; firstly, it represents data that could be collected without tree climbing, and secondly, it represents an important structure that has been used in alpha-taxonomy for discriminating species within section *Talauma*, throughout its distribution area (Figlar and Nooteboom, 2004; Lozano Contreras, 1994; Pérez et al., 2016; Vázquez-García et al., 2016b; Vázquez-García et al., 2013c). We chose not to work with morphological variation of leaves, because leaf morphology has been reported by Stehlé and Marie (1947) to be very variable, which indeed was observed and confirmed during the 2016 expedition (see Figure 1.18B & 1.18D). The 170 fruits were collected over a period of one month and the sampling was highly dependent of the number of fruits found on the forest floor for that time. In total, the 170 fruits represented 48 individuals: 4 from Saint Vincent, 7 from Saint Lucia, 12 from Martinique, 14 from Dominica and 11 from Guadeloupe.

6.2.2 Evolutionary history and biogeography: phylogenetic hypotheses and DIYABC

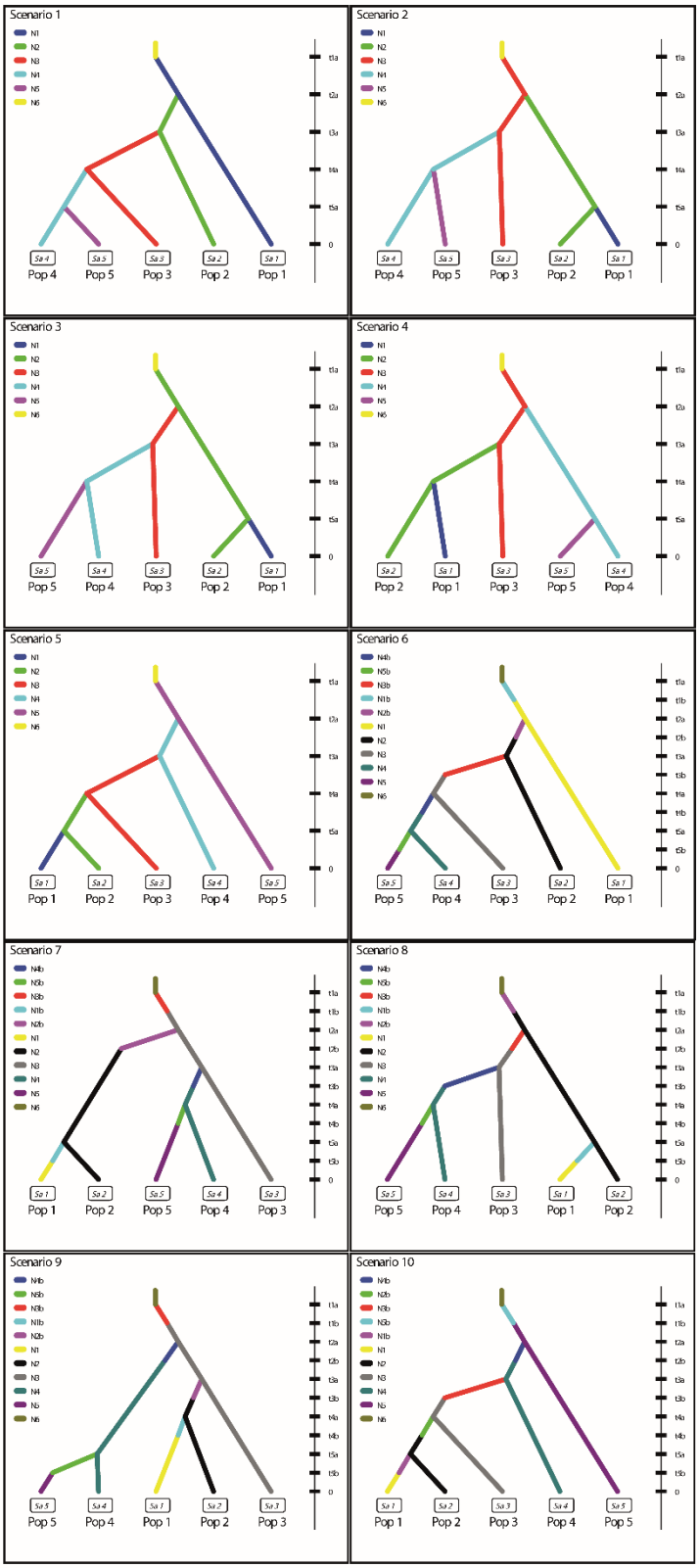
To test whether the island populations of *M. dodecapetala* most likely fit the model of stepping-stone dispersal based on Sanger sequence data, the six alignments were used to construct calibrated phylogenetic hypotheses using BEAST v.2.5.2. (Bouckaert et al., 2019). Partitioning of alignments occurred via the AICc selection criteria in PartitionFinder v.2.1.1 (Lanfear et al., 2017), whereby the branch lengths were set to linked and the comparison of partitioning schemes occurred via the greedy algorithm (Lanfear et al., 2012). Partitioning was executed for each of the six alignments separately. Within each alignment, potential partitions were allowed by marker; within a marker by coding and non-coding segments; and within coding sequences according to codon position. In the concatenated chloroplast alignment (i.e. *atpB-rbcL* and *ndhF*) and in LFYB, phylogenetically informative gaps were present. These gaps were coded using Seqstate v.1.4.1 (Müller, 2005), whereby IndelCoder was set to Modified Complex Indel Coding (MCIC); however, Simple Coding (Simmons and Ochoterena, 2000) would render the same result given the simple absent-present status of the gaps in these alignments. In BEAUTI v.2.5.2. (Bouckaert et al., 2019) we used the Standard template. The total of 19 significant sequence partitions and two morphological partitions were unlinked for

substitution parameters, linked for clock parameters per sequence alignment (hence six clocks were allowed), and linked over all 21 partitions for the tree parameters, rendering one final tree. Substitution models for each data partition were averaged by model jumping using bModelTest (Bouckaert and Drummond, 2017), whereby all the site model parameters were allowed to vary. The six clock models were run with a strict clock and the tree prior was set to the Birth Death Model. We first calibrated the root of the tree which, given that we included *M. lacandonica* as well as *M. dodecapetala*, represents *Magnolia* section *Talauma* subsection *Talauma* (Figlar and Nooteboom, 2004). The root, and hence the stem node of *M. dodecapetala*, was given a uniform prior with the minimum set to 13 mya, following the result from Chapter 3, and the maximum set to 38 mya as this is the age of the Oligocene rocks of Martinique (Birden et al., 1979; Wadge, 1994). This calibration is referred to as **calibration 1**. Once the topology was known, two alternative calibrations were made. **Calibration 2**: a uniform prior on the stem node of *M. dodecapetala* with minimum of 38 mya (according to the age of the oldest dated Martinique rocks) and the maximum of 70 mya (the age of the Magnoliaceae family, estimated by Wikström et al. (2001)). **Calibration 3**: a uniform prior set on the stem node of the sequences from Saint Vincent with a minimum of 5 mya (maximum age of Saint Vincent) and a maximum of 38 mya (maximum age of Martinique) and, similarly, a uniform prior set on the stem node of the sequences from Guadeloupe, with a minimum of 5 mya and a maximum of 38 mya. Calibration 3 assumes that the sequences from Saint Vincent and Saint Lucia, and sequences from Dominica and Guadeloupe each form a clade. The analyses were evaluated for burn-in and convergence (as indicated by the effective sample sizes, ESS) using Tracer v.1.7.1 (Rambaut et al., 2018): the analyses were allowed to run for 300 000 000 MCMC which rendered ESS > 200, and the appropriate burn-in was 10%. To visualize the estimated time of each node and study the biogeography, the trees were summarised using TreeAnnotator v1.8.2 (Rambaut and Drummond, 2015) with the found burn-in of 10% into a maximum-clade-credibility summary tree, whereby the node heights represent the mean heights. The summarised, calibrated tree was visualised using Figtree v. 1.4.2 (Rambaut, 2014).

To test whether the populations of *M. dodecapetala* most likely fit the model of a south to north stepping-stone dispersal using the generated SSR data, the likelihood of this model (Figure 6.1: **hypothesis 1**) was calculated using an approximate Bayesian computation (ABC) statistical approach using the software DIYABC v.2.1 (Cornuet et al., 2014) against four out of many alternative hypotheses. The first two alternative hypotheses followed the sequence of colonization according to the age of each of the five islands: we pose the alternative dispersal hypotheses where *Magnolia* colonised the Lesser Antilles in the time frame where either Saint Lucia and/or Martinique were already formed and the other islands were formed later (Figure

6.1: hypothesis 2, 3 and 4). **Hypothesis 2**: a first arrival to Martinique, with the first bifurcation between Saint Lucia and Martinique, followed by further stepwise colonization north- and southwards. **Hypothesis 3**: a first arrival to Saint Lucia, with the first bifurcation between Saint Lucia and Martinique; followed by further stepwise colonization north- and southwards. **Hypothesis 4**: a first arrival to Martinique, with the first bifurcation between Martinique and Dominica; followed by further stepwise colonization north- and southwards. The last and fifth hypothesis assumes that *M. dodecapetala* had a north to south stepwise migration, starting from Guadeloupe (Figure 6.1: **hypothesis 5**). Because colonization of the islands most likely invoked bottlenecks due to founder populations, each of the five hypotheses was also evaluated with a bottleneck event with a population size (N) following the prior of a log uniform distribution set between 1 and 500 for the first 5–25 generations of its presence on the new island: **hypothesis 6–10**. Other values of the historical model (N, t) parameters and mutation model parameters were left at their defaults under the Generalised Stepwise Mutation Model (GSM) (Cornuet et al., 2014), allowing a broad sampling from the prior. In total, this rendered 21 historical parameters for 10 scenarios. One Sample summary statistics under evaluation were: mean number of alleles, mean genic diversity, mean size variance and mean Garza-Williamson's M. Two Sample summary statistics under evaluation were: F_{ST}, classification index, shared allele distance, and $(d\mu)^2$ distance. In total, 70 summary statistics were considered for the 19 microsatellite loci. Under the ten different models, 10 000 000 datasets and their summary statistics were simulated as a reference. The direct estimate of the posterior probabilities was calculated based on 500 datasets and local linear regression on the closest 1% of simulated data (i.e. 100 000) sets to the observed data with ten intermediate values.

Figure 6.1 Ten potential (DIYABC) scenarios for the dispersal of *Magnolia dodecapetala* between the islands of the Lesser Antilles. **Pop 1**: Saint Vincent (SV) with a population size of **N1**. **Pop 2**: Saint Lucia (SL) with a population size of **N2**. **Pop 3**: Martinique (M) with a population size of **N3**. **Pop 4**: Dominica (D) with a population size of **N4**. **Pop 5**: Guadeloupe (G) with a population size of **N5**. **N6** represents an ancestral source population. **t**: time.



6.2.3 Testing for genetic conservation units: genetic structure

To address the assumption that each island represents one population, and hence one conservation unit, rather than a collection of subpopulations (or sample localities) within an island, we ran a STRUCTURE analysis (Pritchard et al., 2000) on the D(19) dataset comprising all the 195 individuals, with a burn-in of 100 000, 100 000 MCMC steps after the burn-in, the admixture model as ancestry model, and the correlated allele frequency model. K was expected to be between 5 (i.e. the number of islands) and 20 (i.e. the number of sample localities), hence we set K from 1 to 25. Each value of K was run 10 times. The results were visualised with Structure Harvester Web v.0.6.94 (Earl and vonHoldt, 2012). The best K-value was selected using the ΔK statistic (Evanno et al., 2005) and the mean likelihood (mean L(K)). Barplots were visualised using DISTRUCT v.1.1 (Rosenberg, 2003). To further exclude any undetected sub-structure due to "grain", we repeated the analyses for each island separately, with the same MCMC setting; and K run for the number of sample localities within an island + 2. All analyses were also repeated for the D(15) and D(7) datasets, which rendered a total of 18 STRUCTURE analyses.

DAPC analyses (Discriminant Analysis of Principal Components) were executed in R v.3.6.1 (R Core Team, 2019) using the package adegenet (Jombart, 2008). For the D(19) dataset we retained 150 PCs in the find.clusters function, where after we selected the number of groups to be five according to the BIC; placing the individuals in each island. For the DAPC analysis we determined the number of PCA (Principal Components Analysis) eigenvalues using cross-validation with 1000 replicates. The PCA of the DAPC analysis was set to 40 given this was the number of PC's associated with the lowest MSE (Mean Squared Error). All four discriminant functions (DA eigenvalues) were kept. Analyses were also repeated for the D(15) and D(7) datasets, which rendered a total of 3 DAPC analyses.

To test for genetic differentiation between the five islands, pairwise F_{ST} (Weir and Cockerham, 1984), G_{ST} (Nei, 1973; Nei and Chesser, 1983) and D_{JOST} (Jost, 2008) values; as well as their confidence intervals using 1000 bootstraps were calculated in R using the fastDivPart function of the package diveRsity (Keenan et al., 2013). Calculations were made for the D(19), D(15) and D(7) datasets which rendered a total of nine clusters of pairwise genetic differentiation measures. Pairwise genetic differentiation measures were also calculated for the two times two subpopulations found via the STRUCTURE analyses.

6.2.4 Genetic resilience of *M. dodecapetala*: inbreeding and population statistics

To assess the level of inbreeding of *M. dodecapetala* we calculated the inbreeding coefficient (F_{IS}) in FSTAT v. 2.9.3.2 (Goudet, 1995). Tests to detect significant deviations from Hardy-Weinberg proportions (HWP) were calculated in GENEPOP, performing 2-tailed exact tests for

each locus in each population. Complete enumeration was performed whenever possible (Louis and Dempster, 1987), otherwise MCMC chains were run with 200 batches and 50 000 iterations (Guo and Thompson, 1992).

To assess the genetic diversity of *M. dodecapetala*, different statistical parameters were calculated for each locus and population using GenAlEx v.6.5 (Peakall and Smouse, 2012; Peakall and Smouse, 2006), i.e. the percentage of polymorphic loci (P), the number of genotyped individuals (N_G), (mean) number of alleles (A), (mean) number of private alleles (A_P), (mean) expected heterozygosity (H_E), and (mean) observed heterozygosity (H_O). FSTAT was used to calculate allelic richness (A_R).

6.2.5 Correlation between morphology and genetic diversity

The individual variation in number of carpels and fruit length was visualised with boxplots using the function boxplot2 of the gplots package in R. The mean number of carpels and mean fruit length per individual were tabulated, which rendered a matrix of 48 values for each. The mean and the 95% confidence interval of both morphological characteristics grouped per island were calculated and visualised using the package gplots in R. The correlation chart was calculated in R using the chart.Correlation function of the package PerformanceAnalytics. The pairwise morphological distance between the islands was represented by the Euclidean distance for both the number of carpels (MDC) and the length of the fruits (MDL). On the one hand, the pairwise genetic distance between the islands was represented by the distance matrix of the Sanger sequencing concatenated alignment (GD1SS) of five representative island sequences (i.e. MA1104 for Saint Vincent, MA1316 for Saint Lucia, MA1144 for Martinique, MA1195 for Dominica and MA1245 for Guadeloupe – See Table 6.1 for metadata) extracted from Geneious. These five sequences were selected so that they had maximum amount of sequence data available for distance calculation, i.e. as little as possible (partly) missing sequence data. On the other hand, the pairwise genetic distance between the islands was represented by the pairwise F_{ST} values (GD2FST) calculated from the D(19) dataset of SSR markers. Lastly, we calculated the pairwise geographic distance (in km) between the two closest known *M. dodecapetala* individuals of each island (GEO).

6.3 RESULTS

6.3.1 SSR characterization

Different SSR marker × population combinations for all five islands gave hits in the MICRO-CHECKER and ML-NullFreq analyses, summarised in the column A_0 of Appendix 6.1. MA39_023, MA39_182, MA39_259, MA39_442, MA40_136, MA42_274, MA42_421 did not have an excess of homozygotes, and hence do not potentially contain null alleles. MA39_159,

MA39_185, MA39_191, MA39_199, MA42_072, MA42_255, MA42_333 and MA42_495 had an excess of homozygotes in one of the five island populations. MA39_287, MA42_231 and MA42_471 had an excess of homozygotes in two of the five island populations. MA40_282 had an excess of homozygotes in three out of five island populations. None of the 19 markers had an excess of homozygotes for all five island populations.

In the tests for LD, respecting the island populations, 69 of the 855 tested SSR marker pairwise comparisons had a p-value lower than 0.05 of which 7, 12, 12, 29 and 9 out of the 171 pairwise tests per island were from the Saint Vincent, Saint Lucia, Martinique, Dominica and Guadeloupe population, respectively. For 855 and 171 pairwise tests it would be expected that 42.75 [33, 53] and 8.55 [4, 13], respectively, of the tests are positive due to Type I errors, given a p-value of 0.05. After Bonferroni correction, four sets of alleles remain in LD, all in the population of Saint Vincent: MA42_231 × MA42_471, MA39_191 × MA42_471; MA39_185 × MA42_231 and MA39_185 × MA42_471.

6.3.2 Evolutionary history and biogeography: phylogenetic hypotheses and DIYABC

The Bayesian maximum-clade-credibility summary tree for calibration 3 is depicted in Figure 6.2. The summary trees for calibration 1 and calibration 2 had the same topology, yet different estimates of node ages depending on the set priors. Posterior probabilities, mean age and 95% confidence interval of each node age for all three calibrations are summarised in Table 6.2.

► **Figure 6.2** The Bayesian phylogenetic hypothesis of the *M. dodecapetala* populations in the Lesser Antilles, based on Sanger sequencing data of 21 individuals and 11 markers, calibrated according to calibration 3. **SV**: Saint Vincent; **SL**: Saint Lucia; **M**: Martinique; **D**: Dominica; **G**: Guadeloupe. Codes in the tip labels and their metadata can be found in Table 6.1. Values on the x-axis are expressed in mya.

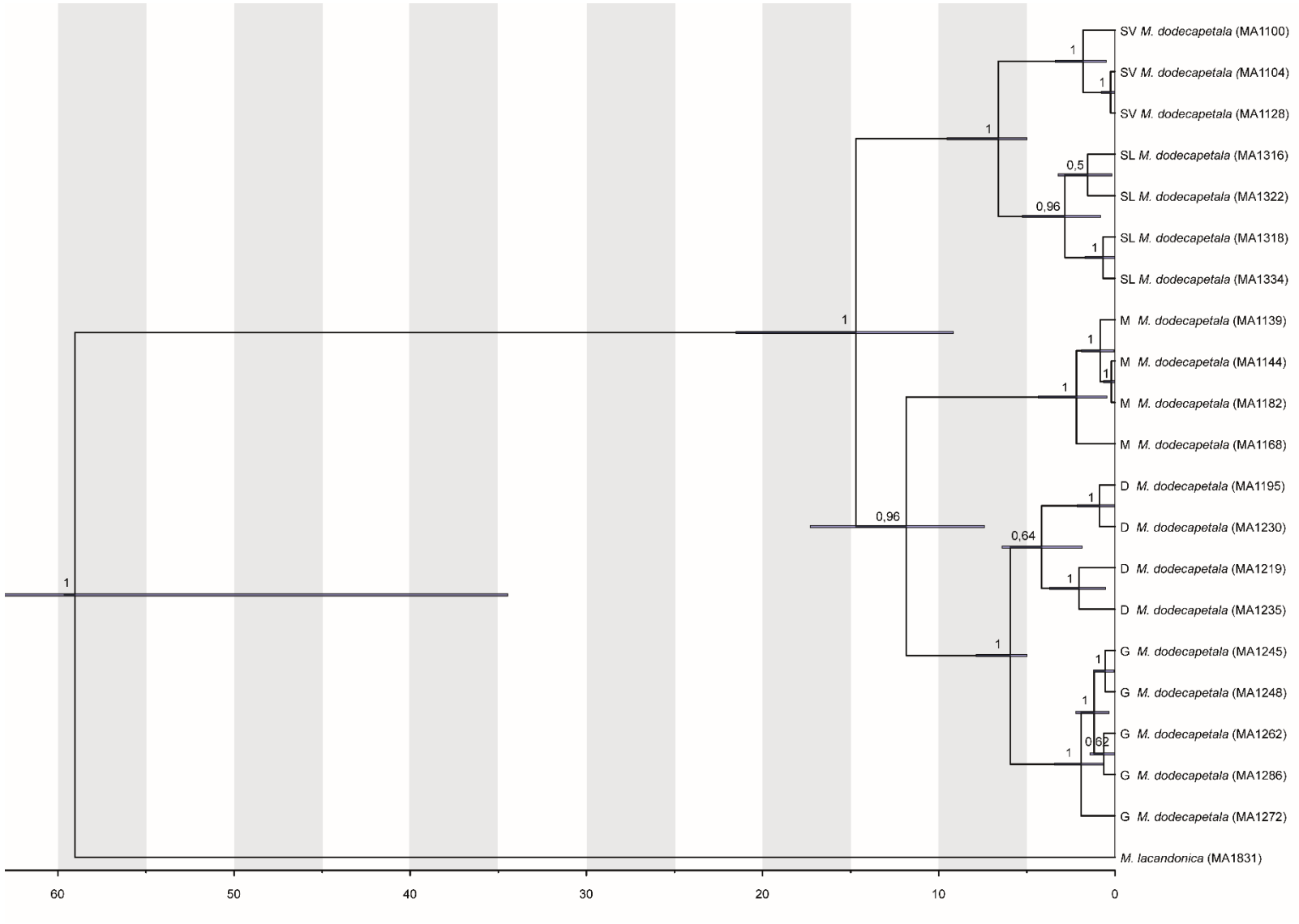

SV M. dodecapetala (MA1100)
SV M. dodecapetala (MA1104)
SV M. dodecapetala (MA1128)
SL M. dodecapetala (MA1316)
SL M. dodecapetala (MA1322)
SL M. dodecapetala (MA1318)
SL M. dodecapetala (MA1334)
M M. dodecapetala (MA1139)
M M. dodecapetala (MA1144)
M M. dodecapetala (MA1182)
M M. dodecapetala (MA1168)
D M. dodecapetala (MA1195)
D M. dodecapetala (MA1230)
D M. dodecapetala (MA1219)
D M. dodecapetala (MA1235)
G M. dodecapetala (MA1245)
G M. dodecapetala (MA1248)
G M. dodecapetala (MA1262)
G M. dodecapetala (MA1286)
G M. dodecapetala (MA1272)
M. lacandonica (MA1831)
1
0,5
0,96
0,64
0,62
60
50
40
30
20
10
0

Table 6.2 Clades of the generated calibrated Bayesian phylogenetic hypothesis of 21 Magnoliaceae accessions representing two *Magnolia* species: *Magnolia dodecapetala* in the Lesser Antilles as the ingroup and *M. lacandonica* from Mexico as outgroup.

Calibration		**1**	**2**	**3**	**1**	**2**	**3**
Clade	**Pp**	**Age: mean**			**Age: range**		
Subsection *Talauma*	1	14.85	43.15	59.03	13–18.90*	38–54.37*	34.47–88.44
M. dodecapetala	1	3.11	9.02	14.69	1.89–4.50	5.52–12.98	9.19–21.51
M + D + G	0.96	2.46	7.14	11.84	1.39–3.63	4.10–10.53	7.40–17.29
SV + SL	1	1.29	3.76	6.61	0.60–2.09	1.73–6.06	5–9.52*
D + G	1	1.04	2.98	5.92	0.51–1.64	1.46–4.73	5–7.87*
D	0.64	0.77	2.19	4.16	0.32–1.28	0.87–3.64	1.85–6.41
SL	0.96	0.58	1.65	2.84	0.17–1.08	0.47–3.12	0.81–5.25
M	1	0.44	1.26	2.18	0.10–0.88	0.26–2.55	0.46–4.35
G	1	0.36	1.02	1.93	0.11–0.66	0.31–1.88	0.64–3.44
SV	1	0.36	1.03	1.82	0.09–0.69	0.24–1.99	0.49–3.37

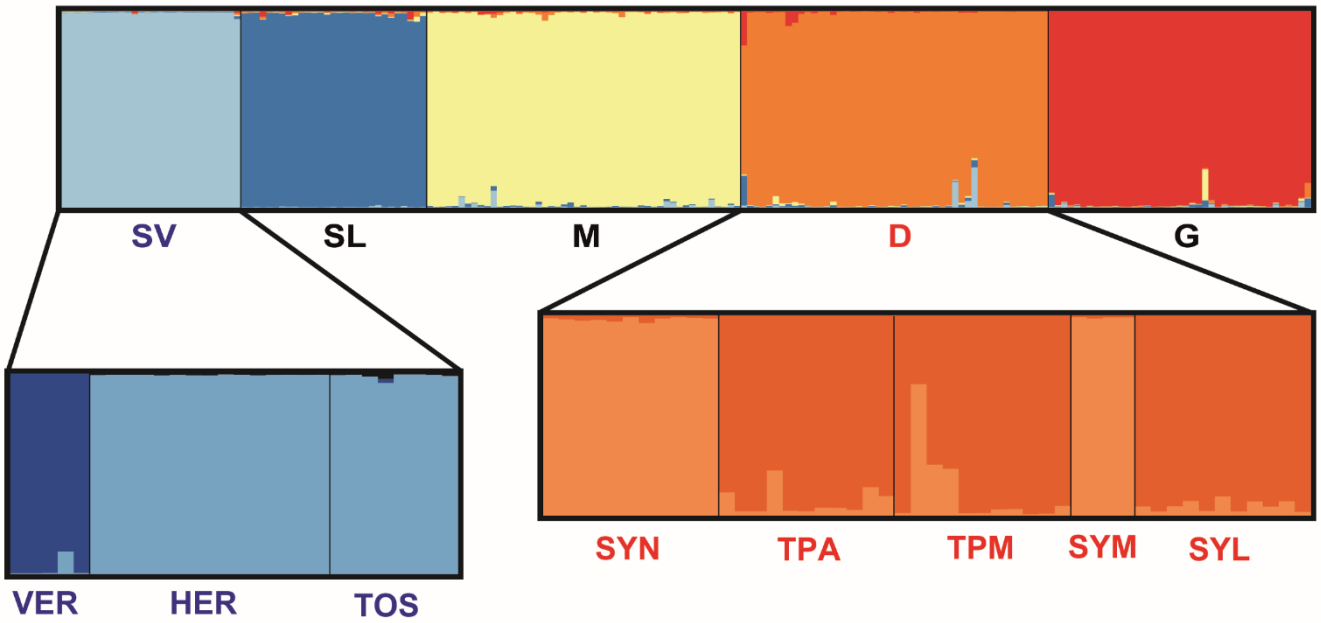
Magnolia dodecapetala sequences were collected within its full known range in the Lesser Antilles of the Caribbean: **SV**: Saint Vincent; **SL**: Saint Lucia; **M**: Martinique; **D**: Dominica; **G**: Guadeloupe. **Pp**: posterior probability. Age is expressed in million years ago (mya). **Age: range** depicts the 95% confidence interval of the **Age: mean**. **Calibration 1:** a uniform prior on the stem node of *M. dodecapetala* with the minimum set to 13 mya and the maximum set to 38 mya. **Calibration 2**: a uniform prior on the stem node of *M. dodecapetala* with minimum of 38 mya and the maximum of 70 mya. **Calibration 3**: a uniform prior set on the stem node of the sequences from Saint Vincent with a minimum of 5 mya and a maximum age of 38 mya and a uniform prior set on the stem node of the sequences from Guadeloupe, with a minimum of 5 mya and a maximum of 38 mya. This calibration assumed a monophyletic relationship between SV and SL sequences and a monophyletic relationship between D and G sequences. An **asterisk *** indicates which nodes are calibrated.

The DIYABC model had the highest posterior probability for scenario 5 (Figure 6.1), both in the direct and the logistic approach.

6.3.3 Testing for genetic conservation units: genetic structure

For the D(19) dataset the ΔK values were 5, 2, 3, 2, 2 and 2 for all the 195 individuals, the Saint Vincent population, the Saint Lucia population, the Martinique population, the Dominica population and the Guadeloupe population, respectively. For the Saint Lucia, Martinique and Guadeloupe populations the mean L(K) showed no clear asymptotic curve, nor a barplot that split according to individual rather than within an individual. Hence, for their optimal ΔK and thus the result of K = 1 is placed forward as their optimal K-value. Barplots, aligning with the K-value that was interpreted to be best suited for the D(19) datasets, are given in Figure 6.3. For the D(15) dataset and D(7) dataset no new sub-structuring was revealed. ΔK and mean L(K) plots per run STRUCTURE analysis are compiled in Appendix 6.2. When allelic

Figure 6.3 STRUCTURE barplots of *Magnolia dodecapetala* in the Lesser Antilles ran on dataset D(19). The analyses comprising all 195 individuals delivered a barplot where the populations followed the five island populations; **SV**: Saint Vincent; **SL**: Saint Lucia; **M**: Martinique; **D**: Dominica; **G**: Guadeloupe. Separate per island STRUCTURE analyses for SL, M and G did not render any further substructure; SV and D had an optimal K of 2. Abbreviations of the subpopulations and metadata: see Table 6.1 and Map 6.1.



association tests were re-run, respecting the newly detected subpopulations of the Saint Lucia and Dominica populations found by STRUCTURE analyses, none of the pairs of alleles in LD remained significant after Bonferroni correction, and 67 of the 1197 tested SSR marker pairwise comparisons had a p-value lower than 0.05 of which 0, 2, 12, 4, 28 and 9 out of the 171 pairwise tests per (sub)population were from the VER subpopulation, the HER+TOS subpopulation, the Saint Lucia population, the Martinique population, the SYN+SYM subpopulation, the TPA+TPM+SYL subpopulation, and the Guadeloupe population, respectively. For 1197 and 171 pairwise tests it would be expected that 59.85 [43, 72] and 8.55 [4, 13], respectively, of the tests are positive due to Type I errors, given a p-value of 0.05.

The D(19) DAPC analysis on all 195 individuals recognised the islands as the best number of clusters in the find.clusters function (Figure 6.4). Results of the DAPC analyses on the more conservative datasets are given in Appendix 6.3.

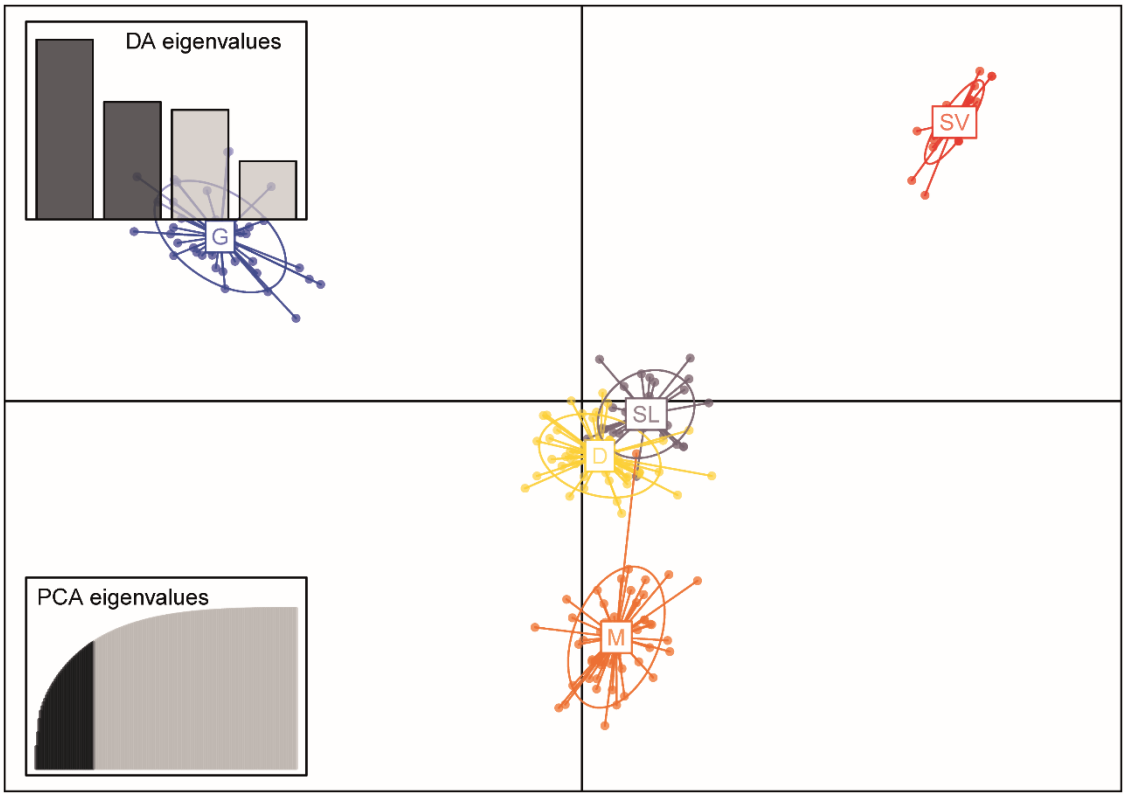
Genetic differentiation measures of the D(19) dataset are summarised in Table 6.3. Tables and graphs depicting the pairwise, bootstrapped, bias corrected genetic differentiation measures and their 95% confidence intervals of all three datasets are compiled in Appendix 6.4. D(19) pairwise genetic differentiation measures on the two subpopulations of Saint Vincent are 0.595 (F_{ST}), 0.5138 (G_{ST}), and 0.0493 (D_{JOST}). D(19) pairwise genetic differentiation measures on the two subpopulations of Dominica are 0.11 (F_{ST}), 0.0603 (G_{ST}), and 0.0945 (D_{JOST}).

Table 6.3 D(19) pairwise genetic differentiation measures: fixation indices (F_{ST}, G_{ST}) and allelic differentiation index (D_{JOST}) calculated for the *Magnolia dodecapetala* island populations in the Lesser Antilles.

G_{ST} / F_{ST}	**SV**	**SL**	**M**	**D**	**G**
SV		0.2061	0.2359	0.2535	0.2623
SL	0.3381		0.0884	0.1004	0.1059
M	0.3492	0.1635		0.1062	0.1157
D	0.3702	0.1810	0.1910		0.0993
G	0.3895	0.1903	0.2077	0.1794	
D_{JOST}	**SV**	**SL**	**M**	**D**	**G**
SV					
SL	0.1893				
M	0.2210	0.2051			
D	0.2683	0.2282	0.2232		
G	0.3048	0.2207	0.2297	0.2534	

D(19): Dataset of 19 SSR loci. **SV**: Saint Vincent; **SL**: Saint Lucia; **M**: Martinique; **D**: Dominica; **G**: Guadeloupe. **F_{ST}** values (Weir and Cockerham, 1984). **G_{ST}** (Nei, 1973; Nei and Chesser, 1983). **D_{JOST}** (Jost, 2008).

Figure 6.4 D(19) DAPC of *Magnolia dodecapetala* populations in the Lesser Antilles of the Caribbean. **SV**: Saint Vincent; **SL**: Saint Lucia; **M**: Martinique; **D**: Dominica; **G**: Guadeloupe. DA eigenvalues: 4. PCA eigenvalues: 40.



6.3.4 Genetic resilience of *M. dodecapetala*: inbreeding and population statistics

Detailed results on the locus, population and subpopulation statistics per dataset are listed in Appendix 6.1. Population statistics of the D(19) dataset are listed in Table 6.4. Significant deviations from HWP are reported for all populations but the one from Saint Lucia, and all subpopulations but VER.

Table 6.4 D(19) Summary statistics of the five island populations (SV, SL, M, D, G) and four subpopulations (VER, HER+TOS, SYN+SYM, TPA+TPM+SYL) found with STRUCTURE for *Magnolia dodecapetala* in the Lesser Antilles.

D(19)	N_G	P	A	A_R	A_P	H_O	H_E	F_{IS}	A_0
SV	28	36.84	1.895	1.895	0.105	0.389	0.440	0.551**	6
SL	28.947	89.47	5.789	5.760	0.421	0.516	0.515	0.015	(1)
M	49	89.47	7.579	6.676	1.316	0.449	0.476	0.067*	2(1)
D	47.684	84.21	7.526	6.631	1.474	0.418	0.518	0.204**	6(2)
G	40.947	94.74	7.579	6.964	1.632	0.494	0.538	0.093*	3(2)
VER	5	15.79	1.158	NA	NA	0.021	0.044	0.600	(1)
HER+TOS	23	31.58	1.684	NA	NA	0.080	0.114	0.319*	2(2)
SYM+SYN	14.789	68.42	5.158	NA	NA	0.399	0.472	0.203*	3(5)
TPA+TPM+SYL	32.895	84.21	6.158	NA	NA	0.426	0.510	0.143**	4(4)

D(19): Dataset with 19 SSR markers i.e. the full dataset. **SV**: Saint Vincent. **SL**: Saint Lucia. **M**: Martinique. **D**: Dominica. **G**: Guadeloupe. For the abbreviations of the subpopulations. and the metadata: see Table 6.1. N_G: mean number of genotyped individuals. **P**: percentage of polymorphic loci (%). **A**: mean number of alleles. A_R: allelic richness (rarefaction to 28 individuals) – not given, nor calculated with inclusion of, the subpopulations. A_P: mean number of private alleles – not given, nor calculated with inclusion of, the subpopulations. H_O: mean observed heterozygosity. H_E: mean expected heterozygosity. F_{IS}: inbreeding coefficient, significant deviations from Hardy-Weinberg proportions (HWP): ***** ($p = 0.05$) and ****** ($p = 0.05$ Bonferroni corrected). A_0: number of loci with null alleles estimated by ML-NullFreq ($p > 0.05$), of which the number of recognised by MICRO-CHECKER is given between brackets. **NA**: Not Available.

6.3.5 Correlation between morphology and genetic diversity

For *Magnolia dodecapetala*, the number of carpels ranged from 22 to 84 and the fruit length from 4 to 13 cm. Individual morphological variation for the number of carpels and the fruit length is visualised using barplots in Appendix 6.5. Multiple fruits were collected for 34 out of 48 individuals. The difference between maximum and minimum recorded number of carpels of one individual tree ranged between 0 and 15. The difference between maximum and minimum fruit length of one individual tree ranged between 0.7 and 4.5 cm. Mean number of carpels per island, mean fruit length grouped per island, and the correlation chart can be found in Figure 6.5. The sampling recorded lower mean fruit sizes for the fruits from Saint Vincent and Guadeloupe, intermediate fruit sizes for the fruits of Saint Lucia and Martinique, and a high mean fruit size for the fruits from Dominica, with 95% confidence intervals in the three categories not overlapping. No significant correlation between fruit morphology and genetic distance was recorded.

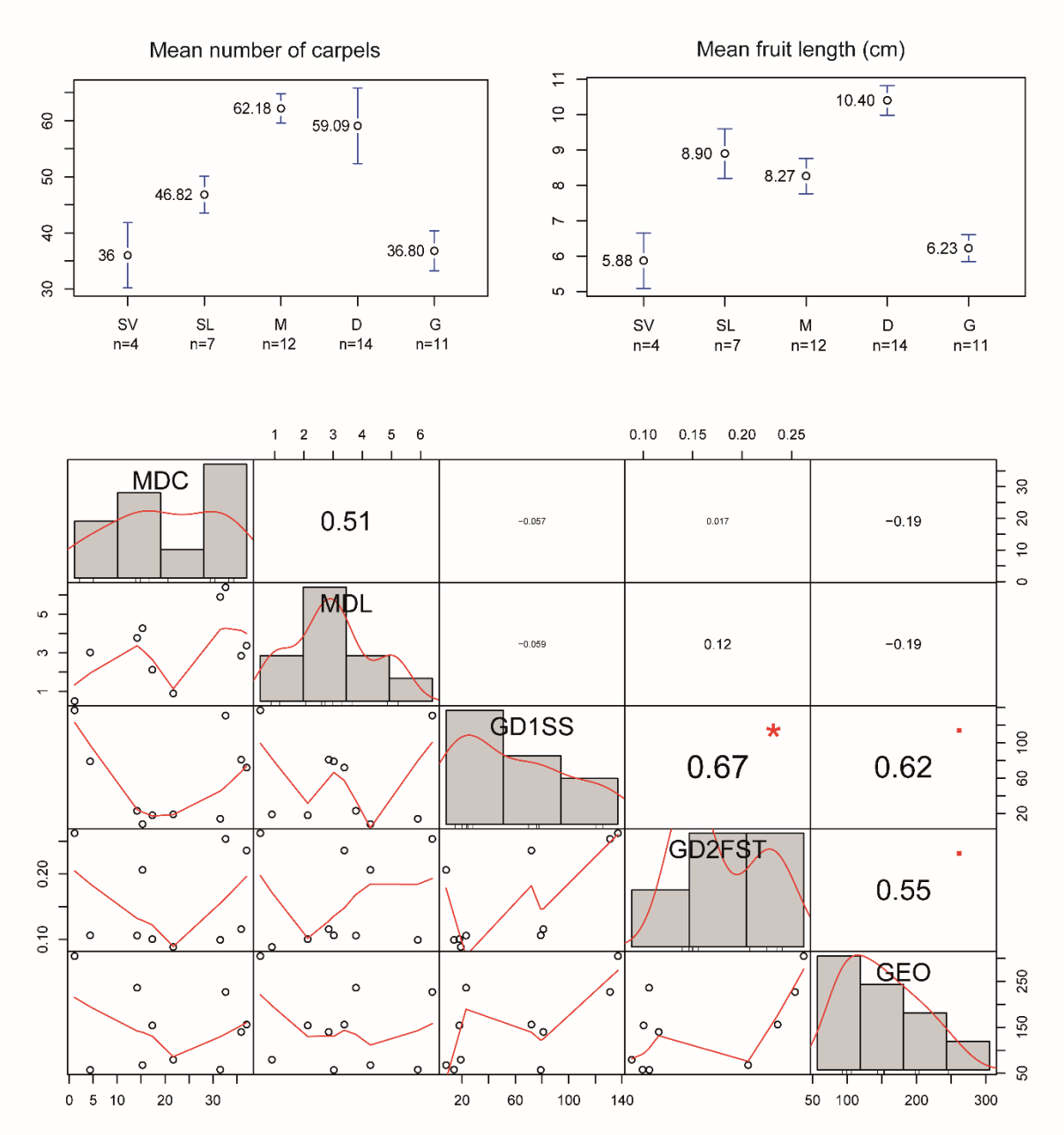
6.4 DISCUSSION

6.4.1 SSR characterization

Calculations for null alleles and linkage disequilibrium are routinely executed to exclude markers that interfere with the statistics of the populations, however, they can also reveal biologically significant information (Waples, 2015). For this dataset, we interpret the deviation from HWP as biologically significant given that there is no marker that shows an excess of homozygotes over the five populations, and even for the markers deviating from HWP the pattern of significant hits is random. Unfortunately, we can never completely exclude the possibility of null alleles, especially given the fact that mutations in the primer sites can be allele × population specific. The execution of analyses with a full evidence dataset vs. a conservative dataset partly overcomes this insecurity.

The recorded LD is interpreted as being a signal from the population biology of the species, rather than actual physical linkage of, or simultaneous selection on, the associated loci (Slatkin, 2008). The overall number of alleles in LD is higher than what would be expected given the pairwise testing that respected the island populations, yet after respecting the subpopulations of Saint Vincent and Dominica (Figure 6.3), they fall within the 95% confidence interval and no significance remains after Bonferroni correction. The significant signal of linkage from the Dominica population in the initial analysis, however, remains present in the TPA+TPM+SYL subpopulations. This flags this subpopulation for potential ill-recovery of a recent bottleneck or even more undiscovered/undetectable sub-structure (Slatkin, 2008).

Figure 6.5 Morphological variation of fruits from *Magnolia dodecapetala* of the Lesser Antilles. **Top:** Mean number of carpels and mean fruit length grouped per island population. Error bars represent 95% confidence intervals. **n** = number of individuals sampled per population. **Bottom**: Correlation charts, correlation coefficients and significance between the pairwise Morphological Distance of the mean number of Carpels (**MDC**), pairwise Morphological Distance of the mean fruit Length (**MDL**), pairwise Genetic Distance 1 based on Sanger Sequencing data (**GD1SS**), pairwise Genetic Distance 2 based on FST (**GD2FST**) and pairwise GEOgraphical distance (**GEO**).



6.4.2 Evolutionary history and biogeography: phylogenetic hypotheses and DIYABC

Similar to Chapter 3, the posterior of the time estimates in the phylogenetic hypotheses (Table 6.2) go to their minima set by the priors, for each calibrated node in each of the three calibration schemes, due to the limited sequence divergence reported overall in *Magnolia* (Kim and Suh, 2013; Nie et al., 2008). On the one hand, the recent colonization ages found in calibration 1 and 2 (Table 6.2) could reflect the true young age of the colonization of the Lesser Antilles. On the other hand, the low sequence information can provide the bias towards younger ages (Brown and Yang, 2010; Marin and Hedges, 2018; van Tuinen and Torres, 2015).

The topology of the phylogenetic hypothesis (Figure 6.2) does not support the expected topology under a unidirectional stepping-stone hypothesis (Figure 6.1: hypotheses 1 and 5). The phylogenetic hypothesis shows a topology as expected under hypotheses 2 and 3 (Figure 6.1), which assume a first arrival of the Most Recent Common Ancestor (MRCA) of *M. dodecapetala* on Saint Lucia or Martinique. Two independent colonization events from the same ancestral population/species would also deliver the retrieved topology. The node bars (Figure 6.2) and co-aligning 95% confidence intervals (Table 6.2) for the two clades are overlapping, hence we cannot state which of the two is estimated to be older and was therefore colonised first. This topology puts forward either the time scheme of calibration 2 or 3, compared to the hypothesis of very recent colonization (according to calibration 1) given the assumption made that colonization of Martinique and/or Saint Lucia occurred first because there was no suitable habitat present (yet) on the other three, younger islands.

The DIYABC hypothesis, based on SSR data, does find the highest posterior probability for a stepping-stone hypothesis. However, the highest posterior distribution was found for the north to south hypothesis (Figure 6.1: hypothesis 5). Notwithstanding the main objection to ABC: inference is limited to a finite set of phylogeographical models (Csillery et al., 2010) – which is also applicable to this study, i.e. we only tested ten possible hypotheses; the result was unexpected. We expected either hypothesis 1 to be most likely given the overall affiliation with South America and young ages for the *M. dodecapetala* clade found in Chapter 3; or we expected hypothesis 2 or 3 to be retrieved by the analysis, given the found topology of the phylogenetic analysis in this study based on Sanger sequencing. Here, we suspect the higher allelic diversity of the Guadeloupe population (Table 6.4, Appendix 6.1) to be one of the factors contributing to this result. The north to south trajectory and affinity to the South American mainland could be plausible in evolutionary scenarios that invoke a source population of *Magnolia* section *Talauma* subsection *Talauma* in the Greater Antilles (e.g. Puerto Rico) or on any of the other, older islands of the Lesser Antilles (i.e. the limestone Caribbees) that are now submerged or unsuitable for the species; or that there was overwater dispersal due to birds

starting in Guadeloupe. The GAARlandia hypothesis (Iturralde-Vinent, 2006; Iturralde-Vinent and MacPhee, 1999) could be an elegant explanation for this pattern: the estimated age of the Aves Ridge is 35–33 mya (Eocene-Oligocene transition). This scenario implies that the current populations of *M. dodecapetala* are not older than 5 mya, which is the maximum estimated age of Guadeloupe and hence, the transition and extinction of the unknown northern source population cannot be less than 5 mya. Overall, this discrepancy in results between the DIYABC and the phylogenetic analyses raises more questions than answers, with both outcomes on the biogeography of *M. dodecapetala* in the Lesser Antilles hinting towards more complicated patterns than a recent stepwise colonization from the South American mainland to the Lesser Antilles.

As each island population does each form a clade per island, there is no evidence of (successful and/or detectable) 'reverse colonisation' (Bellemain and Ricklefs, 2008) or (successful and/or detectable) 'repeated colonisations' (Silvertown, 2004); dispersal patterns of *Magnolia* appear to be simple.

6.4.3 Testing for genetic conservation units: genetic structure

When the five islands are analysed together in STRUCTURE, the analyses result in an optimal K=5 (Appendix 6.2). However, given the high amount of linkage and deviations of HWP found in the Saint Vincent and Dominica populations, we expected there to be within-island clustering. Only when running the per island analyses this suspicion was confirmed: both the Saint Vincent and Dominica populations tested to be consisting of two subpopulations. For the individuals from Dominica, this result is not surprising: the STRUCTURE analysis separates the two northern subpopulations from the three southern ones that are ca. 18 km apart (Map 6.1). For the Saint Vincent individuals, the found subpopulations were surprising as STRUCTURE separates the more southern subpopulation VER from the HER + TOS subpopulation, which are only separated by a distance of 3.7 km (Map 6.1). On the one hand, the VER subpopulation is very small, and it is possible that the five sampled individuals do not adequately represent this subpopulation and have a higher degree of relatedness. On the other hand, the five individuals are the only currently known trees in the area and hence the pattern has an equal probability of representing low gene flow between the forest patches.

In the D(19) DAPC analysis (Figure 6.4), it is observed that along the primary, most explanatory axis the Guadeloupe and Saint Vincent populations are genetically differentiated from the Dominica, Saint Lucia and Martinique populations. Along the secondary axis the Martinique population is genetically differentiated from the Saint Lucia and Dominica populations, with one individual that is not placed together with the other Martinique individuals. This analysis indicates a main differentiation from the Saint Vincent and Guadeloupe population, which is

consistent with the pattern of the phylogenetic hypothesis (Figure 6.2) that places both this southern and northern, respectively, population as the most recent colonised islands.

Overall, the different D(19) pairwise comparisons, for all different measures of genetic differentiation (Table 6.3) have at least great fixation or allelic differentiation (> 0.15), with exception of most values found for G_{ST} that would be classified as moderate fixation (0.05–0.15) (Hartl and Clark, 1997). The D(19) measures of genetic differentiation showed the highest fixation and genetic differentiation in pairwise comparisons for the Saint Vincent population. This difference is visible as a distinct gap in the 95% confidence intervals in the D(19) and D(15), F_{ST} and G_{ST} pairwise comparison graphs (e.g. Appendix 6.4A, 6.4B, 6.4D, 6.4E). However, in the D_{JOST} graphs (e.g. Appendix 6.4C, 6.4F) this distinction disappears, yet the pairwise comparisons between the Saint Vincent and Dominica population and the Saint Vincent and Guadeloupe population remain values of very great allelic differentiation. For D(7), overall values of genetic differentiation (Appendix 6.4G–I) are lower and less outspoken, compared to the D(15) and D(19) values, because of the loss in statistical power with only seven SSR loci (Appendix 6.1). The usage of genetic differentiation measures is controversial and, situation and application dependent (Jost et al., 2018; Whitlock, 2011). In general, their information should be regarded as complementary rather than interchangeable: F_{ST} and G_{ST} are measures of fixation and D_{JOST} measures allelic differentiation (Jost et al., 2018). The fixation indices (F_{ST}, G_{ST}) show that the *M. dodecapetala* populations have moderate to very great fixation; and the differentiation index (D_{JOST}) shows that each population is doing so with great to very great allelic differentiation, hence different sets of alleles. For the subpopulations of Saint Vincent and Dominica detected in STRUCTURE (Figure 6.3), the difference among the two families of genetic differentiation measures is nicely illustrated: the Saint Vincent subpopulations show great fixation, yet little allelic differentiation – meaning that many alleles became fixed, yet the two subpopulations do not differ that profoundly in allelic diversity. In contrast, the Dominica subpopulations show little fixation, yet moderate allelic differentiation – meaning that not so many alleles got fixed, yet the allelic composition does vary.

Compared to the F_{ST} values on *Magnolia* SSR datasets throughout the Caribbean (Veltjen et al., 2019), the trend of overall high fixation for *Magnolia* populations – and in this case island populations, remains. The F_{ST} values between the islands of the Lesser Antilles are comparable to those found for sister species pairs (not population pairs!) residing on the Dominican Republic, i.e. *M. domingensis*, *M. hamorii* and *M. pallescens* and on Puerto Rico i.e. *M. splendens* and *M. portoricensis*, yet lower than or similar to those found for sister species pairs residing on different islands in the Greater Antilles and comparable to some intraspecific populations, e.g. the populations of *M. ekmanii* (0.223) and the populations of *M. pallescens* (0.163). This comparison provides both arguments for recognizing the island

populations at a higher taxonomic rank, as well as to maintain them at their current taxonomic rank. This given that the reference set is not straightforward in its correlation between delimited unit (species/population) and genetic differentiation, and due to the values of genetic differentiation of this dataset which are not convincingly grouped with either the clear quantified cases of between-island species genetic differentiation (e.g. *M. cubensis* subsp. *acunae* versus *M. ekmanii*: F_{ST} = 0.455), nor of population differentiation (e.g. the populations of *M. portoricensis* F_{ST} = 0.101).

At least the combined information of the STRUCTURE (Figure 6.3) and genetic differentiation analyses (Table 6.3, Appendix 6.4) calls for each *M. dodecapetala* island population being treated as a separate MU (Moritz, 1994).

6.4.4 Genetic resilience of *M. dodecapetala*: inbreeding and population statistics

All the island populations except the one from Saint Lucia showed significant inbreeding (Table 6.4, Appendix 6.1), with the Saint Vincent and Dominica populations being the most significant and high, and the Martinique and Guadeloupe populations having lower F_{IS} values, yet still significantly different from zero. Although the possibilities of higher kinship and unaccounted sub-structure (Waples, 2015) cannot be excluded given the more clustered sampling at sampling localities within each island, the inbreeding coefficient in the Saint Lucia population and 12 other Caribbean *Magnolia* populations (Veltjen et al., 2019), which were sampled in the same manner, do not significantly differ from zero. Hence, overall there is significantly more homozygosity found in the populations of Saint Vincent, Martinique, Dominica and Guadeloupe. The population summary statistics (Table 6.4, Appendix 6.1) highlight the lower genetic diversity of the Saint Vincent population as the P, A, A_R and A_P statistics are low compared to the other Lesser island populations, and other Caribbean Magnolias.

6.4.5 Correlation between morphology and genetic diversity

The amount of variation in carpel number and fruit length within *M. dodecapetala* is very large when taking into account the variation over all five island populations, and even some of the recorded individual variation is very high. Although the two characteristics are used often in alpha-taxonomy, they are never applied on their own for species discrimination (e.g. Pérez et al., 2016; Vázquez-García et al., 2013c). Other characteristics are number of tepals, leaf shape, number of lateral veins of the leaves, pubescence, and number of perules. However, the results do emphasize the merit of studying more (*in situ*) individuals for alpha-taxonomy: Lozano Contreras (1994) distinguished *M. dodecapetala* from other members of subsection *Talauma* given its number of carpels higher than 35, while this study found many individuals of which the fruits do not fit this description. The variation found within one individual is also found to be larger than expected, especially when compared to reports of the studied

characteristics in other species descriptions. The Saint Vincent and Guadeloupe population have smaller fruits with less carpels compared to the other three populations, yet genetically they are the most differentiated from each other (Table 6.3), resulting in no correlation between the fruit morphology and genetic diversity (Figure 6.5). The Saint Lucia population has an intermediate number of carpels with an intermediate fruit length, the Martinique population has a large number of carpels with an intermediate fruit length and the Dominica population has a large number of carpels and a large fruit length. The correlation coefficient of number of carpels with fruit length is high (0.51), yet not significant; most likely due to low sample size and not taking into account the percentage of matured seeds in the measurements, which unmistakably is expected to be a confounding variable in this relationship. If the phylogenetic hypotheses delivered the true sequence of colonization (Figure 6.2 and Figure 6.1: hypotheses 2 and 3), this result would imply that the species has a reduction in fruit size and number of carpels in its most "derived" island populations. If the DIYABC hypothesis delivered the true sequence of colonization (Figure 6.1: hypothesis 5), this would imply an increase of fruit size and subsequent decrease in fruit size, in the course of 5 mya of evolution.

6.4.6 Replicates of *Magnolia* colonisation in the Lesser Antilles

Our chosen study system of *Magnolia* in the Lesser Antilles most likely provides a case study for the "island progression rule" (Whittaker et al., 2017): the older islands of Martinique and Saint Lucia were colonised first, and the younger islands of Saint Vincent and Guadeloupe were colonised last (Figure 6.2), whereby we see that the populations of the younger islands have both smaller fruits (Figure 6.5) and stronger pairwise genetic differentiation (Table 6.3); yet present at both ends of the second most explanatory linear discriminant (Figure 6.4). The clustering per island is interpreted as each island being colonised once, following concepts of niche pre-emption (Silvertown, 2004). Although assumed that founder effects were in place at the colonisation of each island, the species did become and remained established in the five successive islands, without any of the separate replicates going extinct, i.e. we see no "island gap". Of course, we cannot exclude that more northward or more southward colonisations of the species did fail, or occurred yet led to extinction. Even more so, it is interesting that the species exhibited up to three successive colonization events over, for the within-Lesser-Antillean colonisations time intervals of 0.79–1.82 mya (calibration scheme 1); 1.98–5.26 mya (calibration scheme 2) and 3.77–8.08 mya (calibration scheme 3) (Table 6.2), whereby SSR marker loci tested only on the population of Martinique and Guadeloupe (Veltjen et al., 2019) were applicable over all five replicates. In the five replicates of colonisation and settlement of the species, we do see varying fruit morphology, moderate to high genetic differentiation, inbreeding and higher genetic diversity in the sequence data, which are genetic signatures indicative of founder effects, or at the least: a high influence of genetic drift. We are only tuning

in an unknown number of generations after the colonisation of the islands and, although likely, it is also possible that the founder populations were not genetically depauperate or small in number and that the current genetic patterns are simply signatures of random genetic drift due to isolation and small population sizes restricted to the smaller island sizes. Most interesting is even that Guadeloupe, the island that is assumed to have to most successive colonisations when following Figure 6.2, shows genetic diversity equal to the formerly colonised islands Martinique and Dominica (Table 6.4); which was also noted by the run DIYABC analysis. This could be indicative of the founder effect already being counteracted in the surpassed timeframe, since its colonisation by for example local adaptation. This would indicate the inbreeding being mostly due to small population sizes still present, given the island setting and that for the replicate of Saint Vincent either local adaptation is absent, or human disturbance is of such a great extent that genetic diversity is being lost. The possibility of local adaptation overcoming deleterious stochastic genetic effects after colonisation invoke a more careful study of the island populations biology as for now the forest habitats of the species are perceived similar between the islands, the island populations' ecological interactions are still unknown (e.g. seed disperser community, pollinator community) and between-island morphological variation is poorly documented. As the data support no (recent) exchange of genetic material between the islands, the discussion of revising their delimitation and status commences. On the one hand, the gathered data in this study i.e. monophyly per island (Figure 6.2), higher genetic differentiation between islands (Figure 6.3 and Table 6.3) and significant differences in fruit morphology, make each island population a worthy candidate of the label of "evolutionarily significant unit (ESU)": they are products of long-term reproductive isolation (Waples, 1991) with reciprocal monophyly (Moritz, 1994). On the other hand, there is no proof (yet) for their "evolutionary legacy" (Waples, 1991) or non-exchangeability (Crandall et al., 2000), we provided little proof for phenotypic divergence (Robertson et al., 2014) by looking at one morphological character with little sampling (Figure 6.5) for which some pairwise island comparisons the ranges overlap, and the genetic differentiation found is intermediate between genetic differentiation of Caribbean *Magnolia* within-island sister species and *Magnolia* between-island sister species (Veltjen et al., 2019). These counterarguments are indicative that the five island replicates are still in the beginning of their lineage divergence (speciation) and that we are currently in the grey, or disagreement zone, in the discussion of what is a species. Both arguments and counterarguments aside in the discussion of the label of an ESU, in theory the five island populations would be considered a separate species under the unified species concept (de Queiroz, 1998, 2007) given the evidence of monophyly. In practice, we decide not (yet) to formalize this result into a taxonomical change. To formally split the species in five different species, a more profound study of the *Magnolia* biodiversity on the Lesser Antilles and its delimitation is desired. Until more evidence is gathered we recommend

adopting a more conservative approach when addressing conservation unit status (and by proxy: the taxonomic status) of the *M. dodecapetala* island populations: we advise to delimit the species as one, with each *M. dodecapetala* island population definitely being treated as a separate MU.

6.5 CONCLUSIONS

(1) The biogeographic history of *M. dodecapetala* was explored using two different lines of evidence: a calibrated Sanger sequencing phylogeny and a DIYABC analysis based on SSR data. They provide very different potential biogeographic scenarios that hint towards more complicated patterns than a recent, stepwise or stepping-stone colonization from the South American mainland to the Lesser Antilles. Given the more plausible fit in timing, the information on genetic differentiation by SSR data, the data on the fruit morphology, and the insecurity of oversimplification of the true evolutionary trajectory of the SSR markers in the DIYABC scenarios, we believe that the highest probability of representing the true biogeographic history is that of the phylogenetic hypothesis – whereby either Saint Lucia or Martinique is put forward as the first colonised island of the Lesser Antilles. (2) We advise to continue to delimit the species as one, with each *M. dodecapetala* island population being treated as a separate MU, yet there are first lines of evidence to recognize the five different island populations as different species. (3) The results show inbreeding on all the islands except Saint Lucia; and substructuring in Saint Vincent and Dominica. We recommend further investigation and translational science (Enquist et al., 2017) to be prioritised for the populations on these two islands, where based on our results it would be advised to enhance gene flow among the Saint Vincent populations, and search for the cause of the high amount of linkage and inbreeding found for the southern subpopulation of Dominica (e.g. more substructure or a recent bottleneck?). Overall more surveys of the species on all five islands are recommended to add unaccounted populations and/or satellite individuals to subsequent analyses and have a better insight in the population dynamics and their evolutionary resilience. Conservation genetic research is not finite, and the results of this study can serve as a baseline to which more data can be added. (4) The great variation measured in fruit morphology of this one species questions its usage for species delimitation in *Magnolia* section *Talauma* subsection *Talauma*. The Saint Vincent and Guadeloupe populations have smaller fruits with fewer carpels compared to the other three island populations, yet genetically they are the most differentiated from each other. These data suggest a more profound re-evaluation is needed of the morphological variation in *M. dodecapetala* and by proxy a re-evaluation of diagnostic characteristics and intraspecific morphological variability in subsection *Talauma.*

7. General discussion and conclusions

7.1 Caribbean Magnolias: integrating results

Considering the evolutionary history of the Caribbean Magnolias, results of the PhD point towards the main patterns of diploid[1] (Chapter 2), allopatric speciation events per island, whereby islands were colonized by stepwise colonization via over-water dispersal from its closed land mass. For the Greater Antilles data point towards stepping-stone dispersal from the American mainland (Chapter 3), while for the Lesser Antilles colonisation followed the island progression rule (Chapter 6). Overall young ages correlating with little phylogenetically informative characters (Chapter 3 and 6) were found, indicating recent speciation and/or slow divergence and evolution, which together with sometimes variable morphological intraspecific (Chapter 1 & 6) variation and intermediate genetic diversification (Chapter 3, 4 and 6), invoke debate on Caribbean *Magnolia* species concepts.

Considering the genetic diversity of the populations, results of the PhD point towards overall strong population structure between and within islands (Chapter 4 and 6), with the exception of the two studied *M. cubensis* subsp. *acunae* populations (Chapter 5) and the two populations of *M. hamorii* (Chapter 4). Overall the high population structure (Chapter 4, 5, 6) and also the found genetic erosion between generations (Chapter 5) are most likely results of the fragmented landscape and highlight landscape connectivity as a main driver in the population's resilience and survival. Surprisingly for endemic, threatened taxa, there was little inbreeding detected for most populations (Chapter 4), which led to the conclusion that the Caribbean *Magnolia* reproductive biology seems resilient yet limited in its dispersal. Yet, it appeared that for the small island populations (or species?) of *Magnolia dodecapetala* in the Lesser Antilles, the resilience does not withhold as inbreeding was ubiquitous (Chapter 6). Here the importance of stochasticity (i.e. genetic drift) on finite populations is highlighted, while going deeper into the potential influence of the founder effect, given that the colonisations of this species occurred most recently of all Caribbean Magnolias.

7.1.1 Supraspecific data: ploidy

In Chapter 2, we confirmed null hypothesis **H01** that the Caribbean *Magnolia*[2] species are all diploid with direct chromosome counts for *M. cubensis*, *M. dodecapetala*, *M. hamorii*, *M. oblongifolia* and *M. portoricensis* and indirect flow cytometry measurements for *M. cristalensis*, *M. domingensis*, *M. minor*, *M. orbiculata* and *M. pallescens*. For *M. ekmanii* and *M. splendens*

[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1
[2] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.

the dried leaf data did not render a clear peak in the flow cytometry measurement, however, in later genetic studies did not find any evidence of polyploidy in neither the Sanger sequencing (Chapter 3) nor the SSR genotyping (Chapter 4). Hence, we preliminary conclude that also these two species are most likely diploids. Similarly, the Sanger sequencing on herbarium samples of *Magnolia emarginata* (Chapter 3) did not indicate there to be multiple copies of nuclear and/or chloroplast amplified DNA. Lastly, *M. virginiana* subsp. *oviedoae* has not been studied cytologically, yet the species *M. virginiana* is known to be diploid and previous work of Azuma et al. (2011) did not report any suspicion of polyploidy.

Studies on (Neotropical) *Magnolia* species are advised to verify the assumption of diploidy (wherever possible) as more polyploid species may be present, yet undetected, in the family (Parris et al., 2010). Polyploidy has severe consequences on the interpretation of reproductive isolation, adaptation and speciation based on retrieved evolutionary trajectories (Baduel et al., 2018). If detected, a different approach for studying conservation genetics (Meirmans et al., 2018) and biogeography by means of phylogenetic hypothesis (Rothfels et al., 2017) is recommended.

7.1.2 Supraspecific data: classification

In Chapter 3, a phylogenetic hypothesis that refutes the posed null hypothesis **H03**: "section *Talauma* (Figlar and Nooteboom, 2004) is monophyletic" was produced, while confirming the posed null hypothesis **H04**: "subsections *Talauma* and *Cubenses* (Figlar and Nooteboom, 2004) are monophyletic". The latter with the caveat that the data so far show that subsection *Cubenses* is monophyletic, but nested in the paraphyletic subsection *Dugandiodendron*. In addition to providing answers about the sections and subsections including the Caribbean *Magnolia* species, the newly generated phylogenetic hypothesis based on more DNA data, and hence having more statistical power, followed the pattern retrieved in earlier Magnoliaceae phylogenies (Azuma et al., 2011 and precursors; Kim and Suh, 2013 and precursor; Nie et al., 2008), considering the relationships between the deeper nodes (i.e. sections and subsections): the clades are well-supported, yet inconsistently placed between the gene trees, resulting in unresolved relationships in the species trees. Hence, since adding more markers to the Sanger-sequencing data analyses does not resolve the deeper nodes, the results show that phylogenomic data are necessary to answer further questions concerning the family-level classification as it does not render to continue adding more markers to the analysis. It is also recommended to use a profound sampling of section *Talauma* including species from across the Neotropics to reveal more insights into the evolutionary history of the family. Our data already provided additional insights: 1) two clades are found within subsection *Talauma*, separating the Mexican and Cuban species from the South American ones and those from the

Lesser Antilles; and 2) subsection *Cubenses* evolved from an ancestor of subsection *Dugandiodendron*. An important question awaiting an answer is whether this pattern holds when more species and genetic data are added.

In light of conservation management, the classification above the species level does not directly inform the conservation status or management of the taxa. However, conservation of certain species can be prioritised based on their evolutionary importance represented by the topology of the phylogenetic tree and derived measures with more evolutionary distinct species awarded a higher degree of importance (Mishler et al., 2014).

7.1.3 Supraspecific data: historical biogeography

Caribbean island colonization was studied at two temporal and geographical scales: the larger geographic scale and expected older colonization times of the Greater Antilles (Chapter 3) and the smaller geographic scale and expected younger colonization times of the Lesser Antilles (Chapter 6).

In Chapter 3, the topology of the phylogenetic hypotheses illustrates that Magnolias colonised the Caribbean islands in four independent events since 16 mya, which disproved the expectation of null hypothesis **H05**. Most species form a clade of within-island relatives, following the stepping-stone dispersal biogeography hypothesis (MacArthur and Wilson, 1967), confirming null-hypothesis **H07**. There was one exception: *M. ekmanii* from Haiti forms a clade with the Cuban species of subsection *Cubenses*. While the taxa of the *Cubenses* clade followed a south to north trajectory of bifurcations, the island populations of *M. dodecapetala* did not (Chapter 6). The trajectory of *M. dodecapetala* follows the concept of the "island progression rule" in which older land masses donate colonists to younger islands (Whittaker et al., 2017).

All *Magnolia* colonisation events to the Caribbean are most likely examples of overwater dispersal (Hedges, 1996), given the recent timing of the bifurcations in the phylogenetic hypotheses, which refutes the posed null hypothesis **H06**. In all phylogenetic analyses, the data push towards the set minima of the youngest calibrated node. Given the result of the *M. dodecapetala* topology (Chapter 6), which sets the first bifurcation on the two oldest islands (Martinique and Saint Lucia) of the Lesser Antilles, it is expected that at least for this species, and by proxy for the (Caribbean) Magnoliaceae in general, true ages could be older than the proposed estimates: the molecular clock with the current settings and datasets does not adequately translate the low sequence variation to correct divergence times (Marin and Hedges, 2018).

Dispersal patterns of *Magnolia* appear to be very simple. Based on the retrieved interspecies relationships, it appears that every island was colonised once, most likely from the closest neighbouring island in unidirectional trajectories from the first colonised island, where after no 'reverse colonisation' (Bellemain and Ricklefs, 2008) or (detectable) 'repeated colonisation' occurred (Silvertown, 2004). The most obvious propagule for overwater dispersal is the seed and the most obvious candidate for mediating this dispersal are birds. A more complicated pattern was expected given that bird-mediated dispersal imposes a softer barrier to overwater colonisation events (Ricklefs and Bermingham, 2008). As geographic distances between the mainland are not as large as for example to the Galapagos or the Hawaiian Islands, the geographic distances and found monophyletic within-island radiation patterns seem in line with theories on niche pre-emption (Silvertown, 2004).

In general, the occurrence of species on islands depends on five basic factors: island geography, island heterogeneity, regional conditions, species biology and the source pool of species (Whittaker, 2007). Further lines of study that may help elucidating the historical biogeographic patterns of this group more in depth would be: a) more knowledge on current (Caribbean) *Magnolia* seed dispersers and pollinators (Chapter 1), their dispersal abilities and historical biogeographic patterns; and b) inclusion of more outgroup taxa in phylogenetic hypotheses, to determine the source pool of species with more precision; together with a more certain and accurate estimate of the node ages.

Overall, the study on the biogeography of *Magnolia* in the Caribbean provides very specific knowledge on the studied group, while also presenting empirical data that can be added to studies of bigger evolutionary and ecological processes, given the "laboratory" setting of the islands (Maunder et al., 2011; Ricklefs and Bermingham, 2008), and hence refute or confirm important theories (Losos et al., 2009; MacArthur and Wilson, 1967).

7.1.4 Supraspecific data: taxon delimitations and the species concept debate

In Chapter 3, we confirmed null hypothesis **H02** by the evidence of genetic synapomorphies that support delimitation of 14 out of 15 Caribbean *Magnolia* taxa. Similarly, the taxa studied in Chapter 4 were also clearly structured according to previous morphological taxon delimitations. In Chapter 3, four red flags are raised considering taxon delimitation: a first for the *M. minor* and *M. oblongifolia* species complex, which urgently needs further investigation; a second for the taxonomic delineation of *M. domingensis* and *M. emarginata* in Haiti, which urgently need more field explorations (see also: Chapter 1), a third for the subspecies of *M. cubensis*, that were genetically equally variable as the other Caribbean *Magnolia* sister species, and a fourth: the higher intraspecific variation of *M. dodecapetala* compared to the almost absence of intraspecific variation for the other Caribbean Magnolias. The latter was

addressed in Chapter 6 where the five island populations were labelled as different CU and even more so as worthy candidates for ESU (Crandall et al., 2000; Moritz, 1994), given their current “intermediate” genetic differentiation status and variable fruit morphology.

Overall the PhD thesis follows the “unified species concept” as discussed in de Queiroz (1998, 2007) whereby species are regarded as separately evolving metapopulation lineages, that acquire properties (e.g. reproductive isolation, monophyly, morphological discrimination) during the course of divergence. Both the morphological (Chapter 1) and genetic (Chapter 3, 4 and 6) data illustrate that all the Caribbean *Magnolia* have somewhat weaker lines of evidence supporting their existence as separately evolving metapopulation lineages. However, they are present and hence it is justified to (continue to) acknowledge them as species, following the unified species concept. For three out of four of the “red flag cases” a more conservative approach was adopted in the decision of formalizing the found patterns into a taxonomic change, the exception being the request to lift the two subspecies of *M. cubensis* to the species level (Chapter 3). For the “red flag case” of *M. minor* and *M. oblongifolia*, the current taxonomic delimitation of them being two species appears justified as there is morphological discrimination possible. However, the mismatch between the morphological discrimination and monophyly advocates for further studies focussed on identifying ongoing hybridization or ongoing sympatric speciation. If the future data illustrate there to be hybridization and the discrete morphospecies have no “pure” genetic populations/individuals left for which monophyly can be found, the proposed taxonomic change would be to lump the species again after the re-instatement by Palmarola et al. (2016). If there would be signatures of ongoing sympatric speciation in future research, evolution would be caught “red handed” in its early divergence and the recognition of the two species remains valid under the unified species concept, with the assumption that the sympatric speciation will render to be “successful” after an undefined amount of evolutionary time. For the “red flag case” of *M. emarginata* and *M. domingensis* from Haiti, changing the taxonomic status of the species at the present time was decided to be futile, given three reasons: 1) the Haitian populations of the species have not been confirmed to be extant, 2) the data so far result in monophyly for *M. emarginata* making this species valid under the unified species concept, and for the Haitian *M. domingensis*, only one out of the two Haitian herbarium collections could be included, with contrasting placements (e.g. compare Appendices 3.4.1 3.4.3 and 3.4.5), however, so far not placing the (non-type!) herbarium collection as sister to the Dominican *M. domingensis*; 3) both species already have the highest possible IUCN Red Status: CR, and this seems more than justified for the Dominican population of *M. domingensis* as well, hence a taxonomic change will not highlight them even more for conservation efforts. Lastly, the “red flag case” of the island populations of *M. dodecapetala* in theory would be considered a separate species under

the unified species concept given the found monophyly. As there are also some counterarguments casting doubt on other lines of evidence, we decided to be more conservative and in practice not (yet) formalize this result into a taxonomical change. To formally split the species in five different species, a more profound study of the biodiversity of *Magnolia* in the Lesser Antilles and its delimitation is desired. The study of Chapter 6 did recognize that as a minimum, the five different islands have to be considered five different MU, given the found genetic and morphological patterns so far.

Resolving the taxonomic uncertainties and defining management units is the crucial first step in biodiversity conservation (Frankham et al., 2010), as species are the most important taxonomic rank used for conservation legislation and management (Convention on Biological Diversity, 2007; IUCN, 2012; Mace, 2004). The low Sanger-sequence divergence (Chapter 3) between *Magnolia* species is correlated with a higher possibility of low reproductive isolation and hence makes the species sensitive to naturally or unnaturally induced crossing, which can reset the ongoing genetic drift or selection of the separately evolving metapopulation lineages (de Queiroz, 1998, 2007).

7.1.5 Supraspecific data: the PIC in the Magnoliaceae

The phylogenetically informative characters (PIC) studied in this PhD were found to be insufficient to resolve the deeper nodes of the Magnoliaceae phylogenetic hypothesis on the one hand and, on the other hand, many of the pairwise species comparisons within the defined clades of the lowest possible ranks were formed on a little amount of PIC. In Chapter 3 we hence advised to switch to NGS based techniques which will provide more data to address both issues. Chloroplast plastome data are becoming available over the recent years and have already indicated that the plastid genome evolves very slowly in Magnoliaceae species (Li et al., 2019).

To frame the pattern of unresolved deeper nodes (i.e. polytomies, “phylogenetic pitchforks” or “phylogenetic bushes”) in the Magnoliaceae family phylogeny, we compare this pattern with general explanations of unresolved deeper nodes (Whitfield and Lockhart, 2007). Given the slow evolution of the Magnoliaceae in general, this could simply be a numbers game, whereby adding more data (and taxa) will resolve the nodes (e.g. Larridon et al., 2020). Even more so, it is likely that the family experienced bursts of accelerated evolution (i.e. rapid speciation) at the formation of each of the robust clades whereby the number of PIC for a stem is proportional to its short time span (Rokas and Carroll, 2006) and incomplete lineage sorting can be at hand (Whitfield and Lockhart, 2007). As at this stage we cannot exclude the possibility of a soft polytomy (Maddison, 1989), hence, a further and substantial increase in data is the proposed first remedy.

To frame the little amount of PIC and hence, slow molecular evolution within the clades, we look at the biology of the studied group. The variation in rates of molecular evolution among plants remains both unexplored an unexplained, yet there are some trends and co-aligning hypotheses at hand which we can evaluate for *Magnolia*. The most general and widely accepted trend is the one discussed by Lanfear et al. (2013) as a correlation with "body size": taller plants have lower rates of evolution. For this the two strongest explanations are the "generation-time effect hypothesis" and the "rate of mitosis hypothesis" (Lanfear et al., 2013). The first states that species with shorter generation times copy their genomes more often, and consequently accrue more replication errors per unit time, resulting in higher mutation rates (Li et al., 1996). Hence, heritable mutations due to meiosis are correlated with generation time, which determines the absolute timescale of genetic drift (i.e. the fixation of the substitutions), whereby it is probable that fixation of alleles is slowed down even more due to overlapping generations (Balloux and Lehmann, 2012; Ellner and Hairston, 1994). Although this might explain the slow evolutionary rates partly, it must also be kept in mind that for plants meiosis might be less relevant given that they grow from apical meristems, which undergo continuous mitosis and from which reproductive tissues are derived late in the development (Petit and Hampe, 2006). This brings us to the second explanation: the long-term rate of mitosis in the apical meristem is likely to be lower in taller plants because growth slows as plants increase in size, and because there are physical limits to the delivery of water and nutrients to apical meristems as they increase in distance from the root system (Lanfear et al., 2013). Stating that all tropical trees have a similar standard slow rate of evolution given their taller habit, the question remains why *Magnolia* evolves more slowly than related tree lineages. A potential explanation could be that of a different strength of selection and adaption: within the *Magnolia* clades that are well-supported, the floral and fruit morphology seems quite rigid (e.g. within *Talauma* reproductive structures we observe mainly differences in numbers, yet little variation in shape/colours), indicating perhaps a less significant role of selection and co-evolution with pollinators (but: what about the "invisible" phenotypic variation such as floral scent?) and seed dispersers to influence diversification in the family, and a larger role of allopatric speciation and genetic drift, compared to for example the Annonaceae, which also have a tree habit, but a more diverse flower and fruit morphology, more intraspecific diversity and faster evolutionary rates (Massoni et al., 2015a).

7.1.6 Population data: species biology (demographics, field data, conservation)

The field data compiled in Chapter 1 show characteristics adherent to the Caribbean Magnolias which provide hope for their long-term survival: we observed vegetative regeneration (synonym: resprouting); we counted a fair numbers of individuals for the time spent in the field; we found juvenile trees for all species (Figures 1.10–13, 1.16–1.17 and 1.19) we observed a

good seed set and germination for *M. portoricensis ex situ*, and received reports of good *ex situ* seed set of *M. cubensis* subsp. *acunae* and *M. ekmanii*. Resprouting has been reported for other *Magnolia* species, such as *M. dealbata* (Sánchez-Velásquez and Pineda-López, 2006). The reported number of Caribbean *Magnolia* individuals per population was mostly higher compared to explicitly reported demographic data for other Neotropical species that reported only a few up to about 25 individuals per species observed *in situ* (Aguilar-Cano et al., 2018; Dahua Machoa, 2018; Serna González and Urrego Giraldo, 2016; Vásquez-Morales et al., 2017; Vázquez-García et al., 2013a; Vázquez-García et al., 2013b; Vázquez-García et al., 2015b; Vázquez-García et al., 2018; Vázquez-García et al., 2013c). A comparable or a higher explicitly counted number of individuals per species has also been reported e.g. *M. equatorialis* (Vázquez-García et al., 2013b), *M. dealbata* (Gutierrez and Vovides, 1997; Sánchez-Velásquez and Pineda-López, 2006), *M. perezfarrerae* and *M. sharpii* (Vásquez-Morales and Ramírez-Marcial, 2019) and *M. pedrazae* and *M. schiedeana* (Rico and Gutierrez Becerril, 2019*)*. Many of these numbers are strongly impacted by the amount of sampling effort by the researchers and the population density of the species. The reported numbers of mature fruits and germination rates of the Caribbean Magnolias are also promising compared to reports of other endemic, threatened Magnolias (Chen et al., 2016). Germination success was not actively recorded during this PhD study, yet preliminary observations are not worrisome. A potential further line of investigation is to document these data more precisely. Based on current observations and reports, it is expected that results will be in line with other Neotropical Magnolias, i.e. medium to high germination percentages (Corral-Aguirre and Sánchez-Velásquez, 2006; Saldaña-Acosta et al., 2001; Toledo-Aceves, 2017; Vásquez-Morales and Ramírez-Marcial, 2019; Vásquez-Morales and Sánchez-Velásquez, 2011).

7.1.7 Population data: structure

Rejection or confirmation of null hypothesis **H08: "**Caribbean *Magnolia* populations show patterns of extensive gene flow (i.e. within a species there is no population structuring) as expected for trees (Petit and Hampe, 2006)" was proven to be depending on the population of study as indicated by the various STRUCTURE analyses and genetic differentiation coefficients calculated (Chapter 4, 5, 6). For most populations, we found no evidence of extensive gene flow. This conclusion is mostly based on the values for genetic fixation (F_{ST}): we found evidence of little (*M. hamorii* populations, *M. cubensis* subsp. *acunae* populations), moderate (*M. domingensis* populations, *M. portoricensis* populations, *M. dodecapetala*: Dominica subpopulations), great (*M. pallescens* populations) and very great (*M. ekmanii* populations, *M. dodecapetala*: Saint Vincent subpopulations) genetic fixation. The genetic differentiation measured through D_{JOST}, i.e. allelic differentiation, however, shows that the allelic compositions of the pairwise population comparisons do not (yet) differ that substantially:

we found evidence of little (populations of *M. domingensis*, *M. ekmanii*, *M. hamorii*, *M. pallescens* and *M. portoricensis* as well as the Saint Vincent subpopulations of *M. dodecapetala*) and moderate allelic differentiation (Dominica subpopulations of *M. dodecapetala*). Substructure was detected by STRUCTURE analyses in *M. dodecapetala* from Dominica, *M. dodecapetala* from Saint Vincent and *M. portoricensis*. Hereby it must be mentioned that the Saint Vincent subpopulations of *M. dodecapetala* had the highest F_{ST} value of those categorised into the D_{JOST} little genetic differentiation category, close to the cut-off of 0.05 to be placed into the D_{JOST} moderate genetic differentiation category. Interestingly, although experiencing little genetic differentiation expressed by a low fixation index (F_{ST}) and little allelic differentiation index (D_{JOST}), the K = 2 STRUCTURE analyses of *M. cubensis* subsp. *acunae* (Chapter 5) had two genetic clusters that did not follow the two sampled populations. On the one hand, this could indicate extensive gene flow over the 33 km distance between the two studied patches; however, with this data the gene flow would seem to be unidirectional: no individuals of the Banao population were clustered in the genetic cluster 1. On the other hand, this is likely a result of the evolutionary history of the populations, where the Banao population is represented by one of the two genetic clusters of the Topes population due to founder effects or by genetic drift and inbreeding after the two populations, in the past being part of one former, larger population, became isolated from one another. The latter option is considered more likely, especially given the inbreeding detected for the Banao population, as well as the knowledge on the former forest cover of the region.

When the observed genetic patterns are roughly translated to the landscape context, they suggest that moderate genetic fixation and moderate allelic differentiation of *Magnolia* can already occur within the geographic extent of 3.7 km (*M. dodecapetala*: Saint Vincent subpopulations) to 6 km (*M. portoricensis*: Toro Negro subpopulation); while, although hypothesised to be unlikely, the data in Chapter 5 indicated that gene flow could be occurring within the extent of 33 km (*M. cubensis* subsp. *acunae*). Hence, overall the data point towards the importance of landscape connectivity for Magnolias, confirmed by other conservation genetic studies on members of the family (Rico and Gutierrez Becerril, 2019). In further studies on the Caribbean *Magnolia* species we recommended including more data wherever possible, such as the species biology (pollinators, seed dispersers), the patchiness of the forests, more individuals or populations that can act as stepping stones for gene flow between known populations, and population demographics (juveniles, adults). The results presented here, together with future conservation genetic studies of other (threatened) *Magnolia* species, can elucidate which specific set of conditions limit gene flow, with the eventual goal of establishing general conservation management guidelines for Magnoliaceae.

Interestingly, the pairwise F_{ST} value between the Martinique and Guadeloupe island populations increases from 0.118 (Chapter 4) to 0.2077 (Chapter 6); as well as do the D_{JOST} values increase from 0.028 (Chapter 4) to 0.2297 (Chapter 6). Although the two SSR datasets are not completely comparable because of the inclusion of one extra marker (i.e. MA39_191) and the exclusion of three other markers (i.e. MA42_077, MA42_372 and MA42_397) that did not render unambiguous interpretation when the sampling was expanded in the dataset of Chapter 6, all other 18 (out of the 21 in Chapter 4 and 19 out of Chapter 6) SSR markers were the same. Hence, the main difference for the different value for F_{ST} can be (mainly) appointed to the addition of more individuals in both populations – from 20 to 49 for the Martinique island population and from 20 to 41 for the Guadeloupe island population. This stresses the importance of the influence of sampling design (Hoban et al., 2013; Ward and Jasieniuk, 2009), while also illustrating the importance of re-assessments and continuous data acquisition beyond one conservation genetic study.

7.1.8 Population data: inbreeding and genetic diversity

Chapters 4 and 5 showed that most of the Caribbean *Magnolia* populations do not demonstrate significant signs of inbreeding, which was in contrast to the expectation posed in null hypothesis **H10** and data of other endemic and threatened Magnolias (Kikuchi and Isagi, 2002; Sun et al., 2011). However, Rico and Gutierrez Becerril (2019) and Budd et al. (2015) also did not report significant inbreeding in *Magnolia*.

In Chapter 3, populations of two species, *M. dodecapetala* and *M. portoricensis*, did show signs of inbreeding in the first conservation genetic study. However, their detected inbreeding was interpreted first as a consequence from the Wahlund effect (Waples, 2015), given the relatively small, but normally adequate, sample sizes of that study; haphazard sampling at discrete localities (Ward and Jasieniuk, 2009); and higher allelic diversity. Nevertheless, the populations of *M. dodecapetala* continue to show signs of inbreeding in the more elaborate study of the genetic diversity of the species (Chapter 6), where increased sample sizes, equal locality fractions, and tests for population structure did deliver the conclusion that this species is currently experiencing inbreeding depression. When expanding the sampling of *M. cubensis* subsp. *acunae*, inbreeding was also detected for the Banao population (Chapter 5).

In Table 7.1, the overall interpretation of the genetic health of the Caribbean *Magnolia* populations is compiled. Quite ironically and unexpectedly, the results of Chapter 6 indicate that of the nine Caribbean *Magnolia* species which were studied genetically, one of the two species with the lowest IUCN Red List Status (IUCN, 2012; Rivers et al., 2016) shows inbreeding in four out of five of its known island populations, which already violates the proposed null-hypothesis **H12: "**The IUCN Red List Status of the Caribbean *Magnolia* species

correlates with their genetic diversity". The four species listed as Endangered range from the "most genetically healthy" Caribbean Magnolia species to one of the "most genetically unhealthy" Caribbean *Magnolia* species. The three species categorised as Critically Endangered, however, do show lower SC_F scores either due to detected inbreeding (*M. cubensis* subsp. *acunae*) or low relative allelic diversity (*M. domingensis* and *M. ekmanii*), which is in line with the expectations.

Table 7.1 Scoring scheme to evaluate the overall genetic health of the Caribbean *Magnolia* populations (SC_F), expressed by inbreeding (IN_P) and allelic diversity (SC_A), compared to their Red List Status (RL). The scoring was applied to the nine Caribbean *Magnolia* taxa for which conservation genetic data were produced during the framework of this PhD.

Taxa	IN_P	SC_A	SC_F	RL	Criteria
M. cubensis subsp. *cubensis*	0/1	12	+1	VU	B2ab(i,ii,iii,iv,v);C2a(i)
M. dodecapetala	4/5	11	-1	VU	B1ab(iii)
M. hamorii	0/2	17.5	+2	EN	B1ab(i,iii)
M. pallescens	0/2	6	0	EN	B1ab(i,iii)+2ab(i,iii)
M. portoricensis	1/2?	11.5	-1	EN	B1ab(iii,v)
M. splendens	0/2	9	+1	EN	B1ab(iii,v)+2ab(iii,v)
M. cubensis subsp. *acunae*	1/2	10	-1	CR	B2ab(ii,iii,v)
M. domingensis	0/2	3	0	CR	A2ac
M. ekmanii	0/2	4.5	0	CR	A2ac

Species are sorted from the least severe threatened IUCN Red List category, to the most severe threatened IUCN Red List category. Within one IUCN Red List category they are listed alphabetically. **IN_P** = number of populations of which significant inbreeding was reported. **SC_A** = Allelic diversity score. The latter was calculated as follows: the dataset of the five island populations of *M. dodecapetala* was re-examined, whereby the A_R of a sample size of 20 individuals was calculated. Then all 18 populations for which an A statistic with a sample size of 20 individuals was available, were ranked from lowest to highest values. The ranks were averaged over the populations and tabulated. **SC_F** = Final score (IN_P + SC_A whereby yellow = 0; red = -1; green = +1). **RL**= IUCN Red List status (González Torres et al., 2016; IUCN, 2012; Rivers et al., 2016).

The IUCN Red List criteria capture information on population decline (A-category) and range loss (B-category) (IUCN, 2012), which implicitly assumes loss of genetic variation; however, the lack of an explicit genetic dimension means that IUCN assessments may not adequately reflect a species' potential for adapting to future environmental change (Rivers et al., 2014). The mismatch between IUCN Red List category (RL) and final score (SC_F) displayed in Table 7.1 can be explained partly by a) past conservation management: *M. hamorii*, *M. cubensis* subsp. *acunae* and *M. splendens* are scored to have better allelic diversity than expected for their estimated ranges because here reinforcement and/or relocations were executed in the past (Chapter 1.4 and 1.5); b) the preliminary status of the Chapter 3 results: *M. portoricensis* has a low score because we found evidence of inbreeding, and *M. pallescens* is scored quite

low because of its low allelic diversity; however, there are more populations known of these species (Chapter 1.5 and 1.6) and it is advised to see whether this pattern withholds in more elaborate studies – nonetheless; this result flags the species for prioritization. The mismatch found for *M. dodecapetala* implies that for a highly structured system such as small islands within the Lesser Antilles, the combined area of the islands as the total range should need a form of "penalization" given that genetic exchange between islands is most unlikely with consequences for the species' genetic diversity. It is advisable to review this suggestion on a larger scale (i.e. across different studied animal and plant species; and different discrete geographic systems) to see if this is practically feasible. We also suggest further *Magnolia* conservation research to include related, non-threatened Magnolias as a null-hypothesis (Spielman et al., 2004), wherever possible, as this makes rankings and comparisons more valuable and outspoken.

7.1.9 Population data: correlations

In the test-case of *M. cubensis* subsp. *acunae* (Chapter 5), we could not confirm that Caribbean *Magnolia* populations show a direct correlation between degree of habitat fragmentation and genetic diversity (null hypothesis **H10**). This because the more fragmented population of Topes had higher values for genetic diversity compared to the less fragmented Banao population – even for the standardised measure of allelic richness (A_R). However, the Banao population had a significantly smaller area and consequently is a smaller population; which most likely are confounding variables. On the one hand, these results could indicate that the overall patch size of the population, and subsequently, the number of individuals in a population, has a more significant influence on the genetic diversity compared to the degree of patch fragmentation. On the other hand, it is also possible that regardless of the patch and sampling size, a time-lag between the degree of fragmentation and genetic diversity measures obscures the pattern (Kramer et al., 2008). The latter possibility was confirmed by the lower genetic diversity of the Topes juveniles compared to the Topes adults; however, the genetic diversity of the Topes juveniles was not lower than that of the Banao adults. Hence, we conclude that both small population size and fragmentation are clearly negatively influencing the genetic diversity of the studied species, which is in line with intuitive expectations and other studies (Schlaepfer et al., 2018). Agriculture, livestock farming and urbanization are listed as the second, third and fourth most reported threats for the family after logging (Rivers et al., 2016) and all three are contributors to habitat fragmentation. Further conservation genetic studies are encouraged to integrate the landscape context in their analyses as the compilation of more data can elucidate general patterns for the sensitivity of Magnolias, or by proxy: insect-pollinated and seed-dispersed tropical trees, to habitat fragmentation – or their landscape in general. Here the most desired information would be to extract habitat thresholds (van der Hoek et al., 2015) that can

be translated into general landscape management policy, allowing a balance between conservation of biodiversity and landscape development.

Although the morphology of the species was not actively revised in this PhD work, the collection of population data also creates the opportunity to collect morphological data *in situ* over a very broad population sampling. In Chapter 6 fruits were sampled over the five island populations of *M. dodecapetala* and we did not find direct correlation between the morphology and genetic diversity, disproving null-hypothesis **H011**. However, great variation was recorded in the fruit morphology both within one individual as within the species; and the data showed that almost each island population was characterised by its own fruit morphology. These insights suggest a more profound re-evaluation is needed of the morphological variation in *M. dodecapetala* and by proxy a re-evaluation of diagnostic characteristics and intraspecific morphological variability in subsection *Talauma.*

7.1.10 The importance of integrative science

Overall the PhD thesis illustrates the strength and the importance of integrating different disciplines to grasp the concepts of biodiversity and evolution, here executed on the study system of Caribbean *Magnolia* species, populations and individuals. This especially in the light of species conservation: effective conservation comes forth not only of species-specific knowledge, but also from deducting more general patterns from species-specific case-studies to anticipate proper management of species and ecosystems not (yet) under investigation. The work here stresses the importance of 1) defining what to conserve wherein the role of (integrative!) taxonomy is unmistakable (Mace, 2004); 2) the knowledge of evolutionary history shaping the existing diversity, and more specifically its changes in pace and past success in coping with a changing climate and/or empty niches (Forest et al., 2015); and 3) the contribution of conservation genetic research ranging from “basic” data such as the documentation of populations, individuals and demographics (Chapter 1) to effective changes in conservation management based on genetic structure and diversity (e.g. Chapter 5).

7.2 Next steps for the conservation of the Caribbean Magnolias

Once the severity of the species' threats is identified, the next task at hand is to instate actions that ensure that at least the species do not continue to decline (Rivers et al., 2016), with extinction as the most final and irreversible result. Conserving species is often categorised in two ways; *in situ* or "on-site" conservation, where the species is protected in its natural habitat, and *ex situ* or "off-site" conservation, where individuals of the species are brought outside their natural habitats to ensure their survival. *In situ* plant conservation can be achieved by upgrading the status of the habitat to a protected status, such as a reserve and a national park. Other *in situ* conservation management options are reinforcing plant populations, translocating individuals between populations, actively aiding in cross-pollination, managing natural threats (herbivores), reinstating connections between population patches, reintroductions... *Ex situ* conservation management often resides in botanical gardens, but can also be in the form of seedbanks, DNA banks, cryopreservation or field gene banks. The choice of which conservation management to invest in greatly depends on the national and international political climate and legislation, the manpower that can enforce the management, and the current knowledge of the species. In some cases, conservation action has to be undertaken at any provided opportunity, while in other cases, the "political climate" is stable enough to make more informed decisions on what conservation management to invest in. In the Caribbean for example, the rate of forest degradation in Haiti provides such an unstable and imminent threat to the survival of the *Magnolia* species that any form of action should be undertaken wherever possible – not only to ensure the survival of *Magnolia* but also as much of the Haitian flora as possible. In other Caribbean countries, such as the countries of the Lesser Antilles, Cuba or Puerto Rico, which have Magnolias under threat in a more "politically stable" environment, scientific research can aid policy makers by providing knowledge on detailed distribution data, delimitation of conservation units, amount of genetic differentiation found, knowledge on the reproductive biology (phenology, pollinators, seed dispersers), estimates on the actual population sizes, estimates on the connectivity between populations, identification of appropriate material for *ex situ* conservation collections, monitoring of the impact of conservation management, identification of management priorities, ...

From observations and the results of the conservation genetic studies conducted so far, we formulate the following 7 advices:

Advice 1	**Do NOT maintain adult *ex situ* collections of Caribbean Magnolias of species X in close proximity to natural populations of species Y.**
Clarification	This is most applicable to Caribbean *Magnolia* within-island relatives (i.e. the three Cuban *Cubenses* Magnolias, the two-three Cuban *Talauma* Magnolias, the three Dominican *Cubenses* Magnolias or the two Puerto Rican *Cubenses* Magnolias). Should this already be the case, then it is advised to remove the planted, adult trees as soon as possible, prohibiting and further genetic down-break of the naturally occurring species in the area by recurrent crossing. A temporary *ex situ* collection of species X close to natural populations of species Y (e.g. a nursery) does not invoke any problems, as long as the seedlings are brought to their *in situ* or *ex situ* locality before they reach maturity and no seeds "escape" the nursery (e.g. due to rodents that move the planted seeds from the nursery out to unsupervised suitable habitat that, if the plant remains undetected, can reach maturity and cross with the natural populations). As the island populations of *M. dodecapetala* were found to be worthy of the label of evolutionarily significant units, we advise to respect the island boundaries of the Lesser Antilles and hence not translocate *M. dodecapetala* individuals between different islands.
Data	Although species boundaries are confirmed genetically, the genetic differentiation between the Caribbean *Magnolia* species is not outspoken. We currently have no evidence for boundaries to hybridization between the Caribbean Magnolias, other than geographic distance and subsequently isolation. Similarly, the genetic differentiation between island populations of *M. dodecapetala* are not that outspoken, yet in the same range of other within-island sister species-pairs. We currently have no evidence for actual ongoing adaptation of the different island populations, but it is probable and hence outbreeding depression after translocation of individuals between islands is possible.

Advice 2	**To further investigate the *M. minor* and *M. oblongifolia* species complex, and already safeguard genetic diversity of some of the most isolated, "pure" populations of each species *ex situ*.**
Clarification	One of the two possible explanations for the found data is that we have two former species that are now hybridizing successfully for more than one generation. If it is desired to maintain the two species, a temporary rescue can be executed, while more data is gathered e.g. whether or not it is hybridization (and not ongoing speciation), what is causing the hybridization/speciation, whether this pattern is also happening in other species in the region, etc. Then once the situation is clearer, the intensity of the intervention can be decided; however, already with an *ex situ* stock of the two potential former species present.
Data	We found high genetic variation within this species complex and a high amount of ambiguous characters within the sequences, compared to other Caribbean Magnolias. The genetic patterns found do not align with that of morphologically delimited entities; and little with the population distributions.

Advice 3	**To maintain at least, or preferably increase, connectivity between forest patches that hold *Magnolia* population(s) of one *Magnolia* species – wherever possible.**
Clarification	The most practically feasible approach would be to work up from the smallest geographic scale to the largest natural geographic scale possible. For example: start with efforts to increase the connectivity between patches of forest on one private property or protected forest (geographic scale of max 5 km for example) and follow with an increase in landscape connectivity between different larger patches within one naturally occurring entity e.g. one mountain. With the gathered data so far, we think that one mountain (e.g. “Loma”, “Morne”) would be the most practical MU for most Caribbean *Cubenses* species; and for *M. dodecapetala* one island would be the most practical MU – for now, with the exception of the populations on Dominica (see Advice 6). This advice does not apply to the *M. minor* and *M. oblongifolia* species complex.
Data	Given the small sizes of the Caribbean islands, the human-induced fragmented landscape, the slow rates of evolution, little gene flow among populations, i.e. high amount of fixation, however, with lower allelic differentiation, and little amount of detected inbreeding, we believe it to be practically most feasible to manage the populations in their largest possible natural units. The reproductive biology of the Caribbean Magnolias appears resilient, yet limited in their animal-mediated dispersal, hence once seed dispersers and/or pollinators can find their way between individuals and/or populations, the species have a high probability to maintain their own genetic diversity in healthy proportions and no other management (other than monitoring – see advice 3) would be necessary.

Advice 4	**To continue to monitor and report the demographics of the Caribbean Magnolia populations.**
Clarification	This work can be executed in the form of general floristic surveys of Caribbean forest (patches) with as a minimal, yet extremely valuable effort: the collection of herbarium vouchers. It is expected that there will be many unaccounted Caribbean *Magnolia* populations and/or scattered, isolated individuals. Actively documenting and reporting locality and/or demographic information is key information for further effective conservation genetic research and conservation management.
Data	The difference in number of recorded individuals per population, and recorded number of populations depended greatly on sampling effort. Almost no information on localities and number of individuals was published prior this PhD, with the exception of the intensive field exploration executed by the Cuban botanists.

Advice 5	**To prioritize the following species for conservation genetic research: *M. domingensis*, *M. pallescens* and *M. portoricensis* (given in order of urgency).**
Clarification	Although conservation management/action is advised for all Caribbean *Magnolia* species and their forests wherever there is capacity to do so, and if prioritization would be necessary: following the prioritization of the Red List given that these categories are based on trends such as population decline and habitat loss; we advise **conservation genetic research** to focus on the species highlighted in red in Table 7.1. With the preliminary data based on 20 individuals and two populations per species, populations of *M. domingensis*, *M. pallescens* and *M. portoricensis* raised red flags and their genetics have not been explored further (yet – but see Chapter 7.4). Given the two species that were already analysed in more detail (i.e. *M. dodecapetala* and *M. cubensis* subsp. *acunae*), we see that including more individuals and populations can greatly nuance or further stress the red flags; or even raise new red flags. Hence, next conservation genetic research on these three species, while already reaching out to on-the-ground conservation initiatives, is currently being conducted.
Data	*Magnolia domingensis* showed a low genetic diversity, relatively high genetic fixation between the populations that are only 4.5 km apart, signs of a recent bottleneck of which it has not recovered, and a highly disturbed habitat during the visit of 2015 – making this species the most urgent target for conservation management, even more so than the Haitian *M. ekmanii* Grand Bois population. *Magnolia pallescens* showed a low amount of genetic diversity for its IUCN Red List Status and the geographic extent of its two populations which are 27 km apart. Unexpectedly, *Magnolia portoricensis* showed inbreeding and substructure in one of its studied populations.

Advice 6	**To conserve the Haitian Magnolias, focusing on exploration and counter-acting further loss of habitat in the known populations. During explorations a minimum of seeds can be collected for *ex situ* conservation.**
Clarification	Given that (what is left of) the forest of Haiti is ill-explored in past and especially recent times, we believe that one of the most valuable contributions is exploring the biodiversity of found forest patches, trying to obtain a protected status for the most biodiverse patches and investing in direct communication to the local people. The political climate is very unstable, which limits the effectiveness of most *in situ* conservation management actions – yet it does not make them ineffective, wherever undertaken! During such botanical explorations, we advise whenever seeds of local flora are presented, these are collected for germination trails in (far enough: see Advice 1!) *ex situ* collections.
Data	The discovery of *M. ekmanii* in Morne Grand Bois was due to a helicopter mission in 2011, mainly focused on reptile biodiversity exploration; similarly, the recently discovered population, close to the type locality (Ti Letan) was also found due to exploration efforts. These results are hopeful, and it is very likely that there are more populations of *M. ekmanii* in Massif de La Hotte – and by proxy in remnants of Haitian primary forest. For *M. emarginata* and *M. domingensis* in the north and center of Haiti it is now often suggested that the species are extinct. However, there are still remnant forest patches present in the northern and central mountains and the possibility remains that there is biodiversity, including Magnolias, left to safeguard. During our visit in 2015, and during following visits by Societé Audubon d'Haiti and Fundación PROGRESSIO, it is reported that the local people show a keen interest in the conservation and participation.

Advice 7	**To prioritize for *Magnolia dodecapetala* in the Lesser Antilles explorations and conservation genetic studies on Dominica; and explorations and conservation actions on Saint Vincent.**
Clarification	Considering the size of the Saint Vincent island, we believe that it does not render to respect the found structure of the Saint Vincent island population in subpopulations: we set the MU to the island. Within the island, it would render to promote connectivity between the forests overall, and for the species in particular it would render to start reinforcement actions. However, reinforcement actions would be most valuable if the known stock population is first expanded by executing explorations for more seed donators. Considering the island population of Dominica, explorations and further conservation genetic research are advised, prior undertaking effective conservation management actions. This is the only island of the Lesser Antilles were the combination of the distance and genetic substructure with the current data does suggest respecting the two subpopulations.
Data	The population and subpopulations of *M. dodecapetala* on Dominica shows a high degree of inbreeding, yet non-alarming amount of genetic diversity. The subpopulations of *M. dodecapetala* in Saint Vincent show genetic fixation and inbreeding and the island population shows low genetic diversity compared to the other island populations.

Executing conservation management on other species and/or populations than those proposed, are, of course, not discouraged, especially not, given that “increasing connectivity among forest patches” is not always practically feasible and all species and population remain to be of a threatened status. By a rule of thumb: the one-migrant-per-generation rule (Wang, 2004), or by extension, a-few-migrants-per-generation-rule could be applied by means of human intervention – however, with the trade-off or necessary continued effort to unnaturally maintain connectivity between patches of forest which does not solve the problem of fragmented habitats, it only “buys more time”. Generally, it is advised to consider the effort versus the merit of the chosen actions, as well as to take in account the potential counter-effective results that could happen in the “worst-case” scenario examples i.e. learning from mistakes made by other conservation practitioners (Catalano et al., 2019). As an example, we share some considerations to be taking into account when executing reinforcements and/or translations i.e. collecting seeds, growing them in nurseries and planting the seedlings *in situ*, which is the commonly most (practically) appealing form of conducting (*Magnolia*) tree conservation management.

1) A conservative approach is the safest one: restrain to reinforcements and only execute translocations between “populations” once a form of natural connectivity is confirmed, e.g. conservation genetic research that demonstrates recent evolutionary history or gene flow (e.g. the *M. cubensis* subsp. *acunae* example – Chapter 5) or intensive explorations that indicate that what was once believed to be two populations, can actually be considered/managed as one big population. Similarly, relocations are the “safest” when there are no natural populations of the same species occurring in close proximity (e.g. 20 km – however: more research is

needed to know what a “safe” distance would be!) to avoid outbreeding depression in the natural population close by.

2) The parent stock of the reinforcement should be as genetically diverse as possible – hence it is advisory to include as many parent trees as possible, over the full scope of the population aimed to be reinforced. Here it is important that there is adequate knowledge of the true population boundaries.

3) We believe that a proper way of conduct is the “minimal intervention” approach (Götmark, 2013). Preferably, the result of the intervention is monitored to evaluate its success and extrapolate this result for decision making of comparable case-studies (Ferraro and Pattanayak, 2006).

4) It is riskier, yet not ineffective, to reinforce populations with seedlings in more unstable *in situ* localities, such as areas where the forest is still actively and/or severely being logged. Effort should be prioritised to reach a more stable *in situ* locality, or relocate/reintroduce the species to (effective) protected habitat.

7.3 General conclusions

All posed research hypotheses (Chapter 1.9) were solved:

- **H01**: All Caribbean *Magnolia* species are diploid.
- **H02**: Genetic data confirm the current taxa delimitations, with the exception of the *M. minor* and *M. oblongifolia* species complex.
- **H03**: Section *Talauma* is not monophyletic due to gene tree incongruences.
- **H04**: Subsection *Talauma* is monophyletic, yet with apparent substructure; and subsection *Cubenses* is monophyletic, yet clustered within the paraphyletic subsection *Dugandiodendron*.
- **H05**: *Magnolia* colonised the Caribbean at least four times from the mainland.
- **H06**: All *Magnolia* colonisation events to the Caribbean are most likely examples of overwater dispersal.
- **H07**: Most Caribbean *Magnolia* species form a clade of within-island relatives with one exception: *M. ekmanii*, in the overall pattern of unidirectional, stepwise island colonization.
- **H08**: We found little evidence for extensive gene flow among the Caribbean *Magnolia* populations.
- **H09**: Six out of twenty studied Caribbean *Magnolia* populations demonstrate significant signs of inbreeding.
- **H10**: The genetic diversity of *M. cubensis* subsp. *acunae* was not directly correlated to the degree of habitat fragmentation of its populations, yet the genetic diversity did decline in subsequent studied generations of the fragmented population.
- **H11**: The pairwise genetic distance between island populations of *M. dodecapetala* was not directly correlated to the pairwise morphological distance between island populations, yet there was a clear pattern of island genetic and morphological diversity.
- **H12**: The relatively scored genetic diversity of the nine Caribbean *Magnolia* species does not directly correlate to their IUCN Red List status.

The produced data are translated into conservation guidelines which express species and population priorities for conservation genetic studies, recommendations in types of conservation management and species and population priorities for conservation management actions to be instated.

7.4 Suggested and planned future research

During the execution of this PhD work it became clear that many data are still missing for the family, section and species. We suggest the following lines of future research:

For the Magnoliaceae, there is need for a **phylogenomic hypothesis** to elucidate relationships among major clades to understand the evolutionary history of the family; and to provide a robust calibration of the major nodes with more accuracy for understanding the biogeography of the family (and by proxy the biogeography of the Caribbean Magnolias) with more precision.

1) For section *Talauma*, there is need for a more **comprehensive framework on species delimitation**, whereby on the one hand morphological characteristics are critically reviewed in relation to their evolutionary significance and a key to the species is provided; and whereby on the other hand, the genetic differentiation among different sister species pairs is evaluated in its strength to provide guidelines for future genetic species delimitations.

2) Studies on other (Neotropical) *Magnolia* species are advised to **verify the assumption of diploidy** (wherever possible).

3) At all times important "basic" information on the **species' biology or demographics** of Caribbean Magnolias is valuable and encouraged to be gathered. For questions on the species' biology, we would prioritize studies on the ecological interactions i.e. seed dispersers, pollinators, mycorrhizae, etc.

4) It is encouraged to utilize the published and unpublished SSR markers on **a wider scope of conservation studies in the family**, so that general patterns can be compiled and predictions of the underlying genetic diversity can be made with higher accuracy starting from more quickly assessed data such as pairwise population distances, the degree of habitat fragmentation, (estimated) population size, etc.

5) A crucial, yet hardly practiced line of further research, is the evaluation of conservation management by a pre- and post- conservation genetic study. We hence encourage to include conservation genetic studies in **monitoring of the effectiveness of an intervention**.

6) In the light of **climate change**, a further line of study is to look at the impact of stronger and more frequent hurricanes, and changing temperature and moist regimes on the survival of the Caribbean *Magnolia* species by means of modelling or even, in the case of the hurricanes, before- and after- population (genetic) surveys.

There are many field data on Caribbean Magnolias collected in the framework of this PhD and by the Cuban team of researchers that still await further conservation genetic analysis and publication. The following projects and collaborations are currently still ongoing in the framework of the overarching project (See Thesis outline: Project history):

1) The three Caribbean *Cubenses* species have been intensively monitored and sampled and their genetic diversity will be further investigated in the PhD of one of our collaborators: Majela Hernández Rodríguez.

2) The three Caribbean *Talauma* species have been intensively monitored and sampled and their genetic diversity will be further investigated in the PhD of one of our collaborators: Ernesto Testé Lozano.

3) The data of the 2015 expedition of the Dominican Magnolias will be analysed in 2020 in the framework of a master thesis of Tim Claerhout. In 2021 more population surveys in collaboration with Fundación PROGRESSIO are planned.

4) The data of the 2015 and 2016 expeditions on the Puerto Rican Magnolias will be analysed in 2020 and in collaboration with Para La Naturaleza, a second attempt at successful seed germination and seedling establishment is foreseen, with help of professor Eugenio Santiago Valentin of the University of Puerto Rico Rio Piedras.

5) The results of the *M. dodecapetala* study (Chapter 6) are currently being communicated to the collaborators on the five islands of the Lesser Antilles, with the aim to onset conservation efforts and find on-site conservation practitioners that want to collaborate on further genetic characterization and monitoring of the populations.

8. Lessons learned: conservation genetics in practice

Although the aims of conservation genetics are noble, and the science has much potential for applicability and impact, there are pitfalls and few overall guidelines for proper conduct of conservation genetic research, which can cause difficulties and doubts along the way as scientists try to contribute to conservation. We discuss the different challenges by providing relevant literature, while also reflecting on how we tackled the challenges during the studies executed in the framework of this PhD project and how to address these better in future studies of the ongoing overarching project (See Thesis outline: Project history).

8.1 The sampling strategy challenge

In conservation genetics, the sampling of individuals and populations needs to be representative of the populations and the target species, respectively. The sampling should be random within the pool of possible samples and in substantial number, to deliver a correct estimation of the true genetic diversity of the studied species. A sampling strategy is designed to approach this criterion. Firstly, the sampling strategy considers which samples to include on a spatial and temporal scale; secondly, it contemplates how many samples to include; and thirdly, it examines which type of and how many molecular markers to include. Overall, it is acknowledged that the effect of sampling strategy on conservation genetic analyses is especially strong for sessile organisms such as plants (Suzuki et al., 2005), which is most applicable to the component of which samples to include on a spatial scale. At all times, it is important to acknowledge the influence of the sampling strategy on the outcome and interpret these results accordingly. Even more so, the concept of the sampling strategy can be expanded to include not only genetic sampling of the species, populations and individuals, yet also a more profound sampling of, and correlation with, data on landscape features at finer temporal and spatial scales i.e. the young scientific discipline of Landscape genetics (Richardson et al., 2016; Segelbacher et al., 2010; Storfer et al., 2010).

8.1.1 How to define a population?

For most threatened species the distributional information is scattered at best, or practically absent at worst. For plants the definition of a locality and hence, pre-defined or presumed population is often guided by herbarium records, sometimes complemented by knowledge of local contacts or in the lucky few cases there is time in the study design and expedition to execute random walks and surveys in suitable habitat for the species of interest. In the end, researchers have visited a number of (potential) localities in which they sampled a representative number of individuals of their species to study its genetics: within the full

landscape of the species clustered sampling of pre-defined populations is often the default sampling design. More trees and populations are possible to exist, yet this information is unknown, given that it is most often impossible to survey the full potential habitat of a species.

BOX 3: Populations in the PhD project study design.

In the conservation genetic studies executed in the framework of this PhD project, the sampling design led us to visit pre-defined populations, assumed not to be connected. Yet the possibility of the different pre-defined populations being clustered sampling events within a larger population cannot be ruled out. One should consider that the null-hypothesis in this case is that, with the information at hand, we expect the sampled "populations" to be genetically differentiated, given that they have a distance between them which makes genetic exchange less likely. The STRUCTURE analysis that is then run either confirms or refutes this null-hypothesis. As our analyses was run on *Magnolia*: a tree species, we expected there to be extensive gene flow that would overcome even the potential influence of the clustered sampling and hence we expected that for most cases the STRUCTURE analyses would refute the null-hypothesis (Chapter 4). For *Magnolia*, we did refute the pre-defined populations of *M. hamorii* (Chapter 4) and *M. cubensis* subsp. *acunae* (Chapter 5) being approximately 4 km and 30 km apart, respectively, regardless the sample strategy being more prone to over-split the populations due to expected neighbour mating. However, for most Caribbean *Magnolia* species the structure we found followed that of the sampling design. For these species, we cannot exclude the possibility that this is an artefact due to neighbour mating, but with the currently available information the populations are categorised as distinct and decisions on their management should be made with this assumption, until this structure is disproven by new data. For management the safest default course of action is also to reinforce at the population scale first, and only once the populations are labelled with certainty as to be considerable as one (i.e. there is no local adaptation going on), it is justifiable to translocate individuals between patches, should this be a desired conservation management strategy for the threatened species; this all in avoidance of introducing outbreeding depression. To exclude that the found high structure is an artefact of the sampling design, one could survey suitable habitat patches between the presumed, and for now confirmed, populations for more *Magnolia* trees and adding them to the dataset. Lastly, the data show even higher structuring for some presumed populations than anticipated for in two species, i.e. *Magnolia dodecapetala* (Chapter 4 and 6) and *M. portoricensis* (Chapter 4). In this case we are certain that there is influence of the landscape on the structuring of the visited localities, presumed to be one random mating population

Neighbour mating (mating within proximal individuals) will lead to patterns of close relatedness at fine scales and, conversely, larger gradients of change in gene frequencies at larger scales (Schwartz and McKelvey, 2008). Additionally, the landscape, defined by isolating barriers separates the individuals of a species into populations, on which the evolutionary forces of genetic drift, counteracted by (occasional) (past) migration are acting. Neighbour mating and isolation by barriers occur simultaneously within a species. This can hamper interpretation of genetic structure of a sampling design of grouped samples within a landscape: do we see genetic structure because of isolating barriers in the landscape, or is it purely an artefact of the grouped sampling design of the pre-defined populations that are genetically more similar due to neighbour mating? As for most species, it is not known with certainty that the sample localities (aka the predefined populations) are truly separated by a gap in distribution: the possibility remains that the pre-defined populations are merely two clustered sampling events within a larger population. Simulations studies, explicitly focussed on landscape genetics have tried to quantify the extent of the influence of sampling design within a landscape and found influence of clustered sampling on the data interpretation (Oyler-McCance et al., 2012; Schwartz and McKelvey, 2008).

8.1.2 Which samples of a "population" should be included?

On a **spatial scale**, recommended practical sampling strategies on how to decide which - of all possible individuals of the population - to include, are: simple random sampling, stratified and hierarchical sampling and systematic sampling (Ward and Jasieniuk, 2009). An optimal sampling strategy is based on prior knowledge of the distribution of the individuals (Ward and Jasieniuk, 2009). However, for most tree conservation geneticists the number and location of trees within a population, the boundaries of the populations, and even the number of populations for the species are unknown prior to the start of a research project. Hence, for species that are not yet surveyed (thoroughly) at the population level prior to the conservation genetic study, initial analyses should be considered preliminary and depending on the results obtained, an additional round of sampling may be recommended (Lowe et al., 2004). With this general lack of comprehension of the species´ distribution, most conservation genetic research executes haphazard sampling: **"**sampling by an investigator collecting plants at will while wandering within a study area" (Ward and Jasieniuk, 2009). In most cases haphazard sampling occurs along a transect i.e. a path in the forest. Although this sampling strategy is non-random and hence not recommended, from a practical point of view it is most often selected. There are three arguments of why it is acceptable conducting this "malpractice". Firstly, although the data might be incomplete, it is an informative first analysis of the genetic health of the species, and hence, it provides valuable new information. Secondly, one conservation genetic study of a species should not be regarded as a finite event; there is always the option to expand and

re-evaluate the study on the species of interest. Data addition and re-evaluation make the subsequent studies even more valuable in terms of reporting the found results of the re-evaluation to the community of conservation geneticists. Thirdly, most of the time the haphazard sampling is the only demographic data collected so far on the species of interest and that contribution alone is already a great step forward in the insight of the health of the populations.

BOX 4: Spatial scale of the PhD project study design

In the conservation genetic studies executed in the framework of this PhD project, we mainly executed haphazard (transect) sampling and the limitations of the sampling strategy were taken into account for data interpretation. In the case of haphazard (transect) sampling, it is expected that there is a higher chance that the sampled individuals from the population are a non-random representation (e.g. more related) which leads to an overestimation of structure and allelic differentiation between the sampled populations and an overestimation of inbreeding[1] within a population. Hence, where little allelic differentiation and/or fixation was/were recorded between populations, we are very certain that there is no substructure (e.g. *M. hamorii* in Chapter 4; *M. cubensis* subsp. *acunae*[2] in Chapter 5; the *M. dodecapetala* population from Saint Lucia in Chapter 6). Wherever inbreeding is not detected, the sampling strategy also invokes more certainty to conclude that for these species there are mechanisms in place that promote cross-fertilization (see Chapter 4: 14/17 populations did not have an F_{IS} significantly higher than zero). Wherever we obtained evidence for inbreeding in a population, we were more cautious with the results, given that we knew this could be an artefact from the sampling strategy, and flagged the species for further research (e.g. populations of *M. dodecapetala*, *M. lacandonica* and *M. portoricensis* in Chapter 4). However, with the argument that for the other 14/17 equally, non-randomly sampled populations of Magnolias this result was not found, we believe that this red flag of inbreeding is most likely a true effect of the species' biology.

Although it is in a way "forgivable" to work with haphazard sampling, given the practical considerations and limitations, we should not neglect its impact and try to mitigate this problem as much as possible in future studies. It is hence worthwhile to invest in ways to execute a sampling design that enables a more random sampling and more certainty that the population was sampled to its full extent. A first possible execution mitigation is to create more opportunities for intensive monitoring of species of interest. This requires strong on-the-ground capacity and a structured approach of survey strategy. For the latter, "Brief 1: How to survey

[1] A selection of less commonly used words is explained in the glossary: Appendix 1.1
[2] Taxonomic authorities: Appendix 1.3 and Appendix 1.4.

an area for threatened tree species" on the website of the Global Trees Campaign: https://globaltrees.org/resources/resource-type/practical-guidance is a recommended guideline. Herein, it is acknowledged that we are limited in what we can survey, where after the concepts of intuitive controlled surveys and minimum convex polygon are proposed. A second approach to mitigate this problem is to integrate modern technology, e.g. the usage of drones (Käslin et al., 2018), and complementary types of analyses such as species distribution modelling (Sofaer et al., 2019).

On a **temporal scale** the sampling can vary – it can be a sampling of a population at a single point in time, or can be a comparison of a population throughout different time intervals (Aravanopoulos, 2016; Fussi et al., 2016).

BOX 5: Temporal scale of the PhD project study design

In the conservation genetic studies executed in the framework of this PhD project, we only worked with one sampling of each population at one point in time. Given the recent catastrophic events in the Caribbean during the course of this PhD, i.e. hurricane Matthew in 2016 went straight through the Massif de La Hotte in Haiti (Lai and Peçanha, 2016), where the *M. ekmanii* populations are located; the trajectory of hurricane Maria in 2017 in Puerto Rico hit populations of both *M. splendens* and *M. portoricensis* (Uriarte et al., 2019): all the population data collected are now pre-hurricane impact situations. A post-hurricane study of the same populations would enable to quantify the impact of the recent hurricanes on the demographics and genetics of these *Magnolia* species.

8.1.3 How many samples to include?

In most cases, it is impossible to study the genetics of all individuals from a population because costs constrain the number of samples that can be analysed (Suzuki et al., 2005). Population genetic studies are generally based on statistical rules of thumb that guide sample size selection (Nazareno et al., 2017). For microsatellite markers it is recommended to include 25–30 individuals per population (Hale et al., 2012). Kalinowski (2005) suggest that for cases where F_{ST} is larger than 0.05, sampling fewer than 20 individuals per population should be sufficient and the simulation studies of Cavers et al. (2005) on tree population structure suggest that 100 individuals is the lower limit for a 0.9 correlation. Hoban et al. (2013) created a tool (SPOTG) for sample strategy planning, defining the number of individual samples in a study to be very low, low, medium and high for 10, 20, 50 and 100 individual samples.

BOX 6: Sample quantity of the PhD project study design

In the conservation genetic studies realised in the framework of this PhD project, we executed a more standardised study on 20 individuals per population (Chapter 3), although for many populations we had collected more individuals. This number is limited but expected to be adequate for a first overall study. The sample size was chosen because there were two populations sampled, i.e. Morne Mansinte and Loma Barbacoa that only had 21 and 24 individuals, respectively (See Chapter 1, Table 1.2). For two out of the 15 Caribbean Magnolia taxa, we executed more elaborate studies (i.e. Chapter 5 and Chapter 6). For the *M. cubensis* subsp. *acunae* study (Chapter 5), for many of the interpretations of the results we had to admit that sampling size might be inadequate for realizing correct interpretations, because the Banao population had a sample size of only nine individuals. For the *M. dodecapetala* study (Chapter 6), we found an adequate number of individuals to represent the island populations, with the minimum at 28 individuals.

8.1.4 Which type of, and how many markers to include?

The type of molecular marker to include partly depends on the questions asked, the expertise of the researchers and the resources available. Using genetics for conservation management is upcoming and still experiences difficulties adherent to the translation of science to practice (Holderegger et al., 2019). Here, there is also the appealing possibility to work with NGS data instead of SSR data given that big data provides higher accuracy. In NGS analyses, Single Nucleotide Polymorphisms (SNPs) are used to quantify genetic diversity to address questions linked to conservation management of species of interest (McMahon et al., 2014). However, it is claimed that although appealing in the academic context, working with SNPs and conservation genomics is not yet of interest for conservation practitioners (Shafer et al., 2015) and that SNP data need researchers to make a higher financial investment in a species-by-species case, while SSR data need a one-time-only higher financial investment (Puckett, 2016). Even more so, cross-species amplification tests ensure usage of the generated tools (i.e. the SSR loci) over a broader taxonomic scope and subsequent analyses such as expanding the sampling in number of individuals, loci or populations is possible without making a significant much higher financial investment (Puckett, 2016). Consequently, for this PhD study we decided to work with SSR markers (Chapter 4, 5, 6), given that the aim was to execute conservation genetic research on different Caribbean *Magnolia* species and to provide data that could facilitate research on other Neotropical *Magnolia* species outside the scope of this PhD. Although working with conservation genomics would have provided a more profound understanding of the sampled populations, microsatellite data are an elegant tool which is easily reproducible (Kalia et al., 2010) and provide adequate answers that deliver key insights

of the genetic health of the Caribbean Magnolias. We recommend the continued usage of SSR markers to study general patterns of genetic diversity in conservation priority species. However, once questions such as adaptive potential or ongoing sympatric speciation need to be addressed, it is recommended to switch from conservation genetics to conservation genomics. In all cases, it is preferable to work in close collaboration with conservation practitioners who require such information (Enquist et al., 2017).

Furthermore, we recommend the article of Allendorf (2017) for more insights in the history of molecular marker usage and recommend the articles of Agarwal et al. (2008); Puckett (2016); Shafer et al. (2015) for arguments why SSR markers are valuable, even in the era of genomics.

Few studies have searched for the minimal number of SSR loci to respect for proper conduct of conservation genetic research. According to the simulation study of Cavers et al. (2005), the lower limit for a 0.9 correlation was 10 SSR loci, while empirical examples have shown that even six SSR markers can deliver the desired answers (Koskinen et al., 2004). Hoban et al. (2013) define the number of SSR markers in a study to be very low, low, medium and high for 3, 8, 15 and 30 genetic markers, respectively.

BOX 7: SSR quantity of the PhD project study design

In the conservation genetic studies executed in the framework of this PhD project, the number of SSR markers used to genotype a population ranged from 10 (Chapter 5) to 31 (Chapter 3, Table 3.2) – ironically both for *M. cubensis* subsp. *acunae,* yet in different stages of the development and testing processes. Namely, the *M. cubensis* subsp. *acunae* study of Chapter 5 was part of a Master thesis that was carried out in 2015-2016, at the beginning of the project, while Chapter 3 aimed to perform a more intensive testing over all the Caribbean taxa and hence was carried out over more time, i.e. 2015-2018. In the planned PhD thesis of Majela Hernández (See Chapter 7.4), the opportunity will hence present itself to not only expand the conservation genetic analyses in terms of the number of species, but also in terms of number of SSR markers, and to report the influence on the results and subsequent decision making. The standardised dataset also contained 10 SSR markers (Chapter 3), which can be considered as (too) few.

8.2 The comparability challenge

The genetic diversity measures: A, A_R, A_P, H_O, H_E, only make sense when they are interpreted in comparison to other populations or species, that preferably were quantified with the same molecular markers and the same number of individuals (Kalinowski, 2004; Ward and Jasieniuk, 2009). Hence, the interpretation of these measurements is relative: there is need of "a baseline", "a reference", "a null-hypothesis" to compare parameter values to.

On the one hand, conservation genetic studies can actively and explicitly set a baseline in the sampling design. Some options are the comparison of: a) the genetics of a threatened to a related non-threatened species (e.g. Spielman et al., 2004); b) comparisons over different time scales; and c) comparisons between different maturity classes or, in tree species: DBH classes (e.g. Graignic et al., 2016; Watanabe et al., 2017). On the other hand, conservation studies without an explicit comparison in the research question of the study can compare the parameters of different studied populations to each other in function, or even to the parameter values of other conservation genetic studies on (related) populations of related taxa. This last practice must be executed with great caution, given the dependency of the parameters on the number of individuals and specific set of markers (Kalinowski, 2004; Ward and Jasieniuk, 2009). However, theoretically all conservation genetic studies should have a set of genetic markers and an adequate number of samples to effectively represent the genetic diversity of the populations they study and hence parameter values should be comparable across different studies – which assumes proper conduct of research and complete confidence in the methodology.

BOX 8: Comparisons in the PhD project study design

In the conservation genetic studies executed in the framework of this PhD project, the statistics of the different threatened populations were compared to one another in Chapter 3 and 6. There is no Caribbean Magnolia that is not threatened, yet we evaluated the variation of the different IUCN Red List statuses (often a proxy for their range size as most plant species are assessed using criteria B or D) with the observed genetic variation (See Chapter 7.1.8). The study in Chapter 3 was designed as a preliminary assessment to assign priorities for more in depth conservation genetics studies. The results of the study can serve as a baseline for comparing the effect of increase in individuals and/or markers over the conclusions made of subsequent studies. The study in Chapter 6 was mainly aimed to assess the genetic differentiation of *M. dodecapetala* between the islands. In the study of *M. cubensis* subsp. *acunae* (Chapter 5), we compared a less fragmented habitat (= baseline) with a more fragmented one and we contrasted adults with juveniles of the Topes population.

8.3 The conservation unit challenge

This challenge revolves around the turbulent field of species concepts (Hey et al., 2003), diagnostic methods in systematic biology and the concept of what is a population (Waples and Gaggiotti, 2006), subpopulation, Conservation Unit (CU), Evolutionarily Significant Unit (ESU) (de Guia and Saitoh, 2006) or Management Unit (MU) (Taylor and Dizon, 1999). The detection of both species and populations is one of the main goals in conservation genetics; yet drawing the line for effective conservation is often a judgement call after data interpretation. This call needs to take in account practical limitations that come with the species of interest, country, area and (number of) conservation practitioners. The two main consequences of taxonomy or conservation genetics making wrong delimitations, are “over-splitting” or “over-lumping”. Over-splitting of conservation units can potentially restrict management flexibility and consign small genetically divergent populations to inbreeding and eventual extinction (Frankham et al., 2012). Over-lumping can result in outbreeding depression and loss of species entities. For the delimitation of species, the scientific community applies the consensus of the General Lineage Species Concept (de Queiroz, 1998, 2007), where multiple lines of evidence are compiled to decide what is a species. However, the most conclusive evidence is direct evidence of a form of reproductive isolation allowing limited to no gene exchange. This evidence is often missing and replaced by a proxy such as the Phylogenetic Species Concept (Campillo et al., In press.) and/or the Morphological Species Concept. For the delimitation of populations there are no general guidelines or concepts on how to make a formal decision on what is a population or conservation unit, given the flexible nature of the population concept and the limited number of guidelines available (Waples and Gaggiotti, 2006). The concept of a population in the IUCN guidelines even more complicates usage of the term: a population is defined as the total number of individuals of the taxon and a subpopulation is the geographically or otherwise distinct groups in that population between which there is little demographic or genetic exchange (IUCN, 2012).

BOX 9: Species delimitations of the Caribbean Magnolias

The phylogenetic genetic studies executed in the framework of this PhD project were confronted with the challenge of species delimitation and tackled it as a hypothesis to test (Chapter 1.9). The geographical separation of the species in different mountain chains was the strongest line of evidence, and the morphological and phylogenetic concepts were confirmed, albeit with little power given a more conservative morphology (i.e. low supraspecific morphological variability, see Chapter 1) and low sequence divergence, while maintaining a fair amount of morphological variability within an individual, population and/or species (i.e. high infraspecific morphological variability e.g. Chapter 6). The incongruence between the genetic and morphological data for the *M. minor* / *M. oblongifolia* species complex greatly influences current advised conservation management and prioritization of research (Chapter 7.2). The usage of microsatellites for species delimitation is generally not recommended; however, quite interestingly some patterns put forward in the first executed study of Chapter 4 were confirmed in the later study of Chapter 3. Data on the direct evidence of reproductive isolation between Caribbean Magnolias are still lacking.

BOX 10: Species delimitations of the Neotropical Magnolias

In this study the boundaries between islands and between mountain chains were distinct, which provided more certainty in the species delimitations. For conservation of the mainland Neotropical Magnoliaceae, however, we have reservations on whether or not current species delimitations will hold once studied more profoundly. This because taxonomists working on the Magnoliaceae family often rely solely on the Morphological Species Concept by using only a few *in situ* found individuals or herbarium vouchers (Arroyo et al., 2013; Pérez et al., 2016; Vázquez-García et al., 2015a; Vázquez-García et al., 2016a; Vázquez-García et al., 2013a; Vázquez-García et al., 2012; Vázquez-García et al., 2015b; Vázquez-García et al., 2017a; Vázquez-García et al., 2016b; Vázquez-García et al., 2013c; Vázquez-García et al., 2013d). Hence, morphological variation within a species is hardly considered when describing new *Magnolia* species. Even more so, many of the former widespread *Magnolia* species are being split in a number of new species with an unknown to small distribution, which coincides with them being given a higher or DD IUCN Red List category. Over-splitting leads to effort and resources being used on a perhaps unnecessary target, neglecting other species or populations; and conservation management being executed on a too small scale with all potential disastrous genetic consequences for the species at hand. This is exemplified by the study of Rico and Gutierrez Becerril (2019), who conclude that the genetic differentiation between *M. pedrazae* and *M. schiedeana* localities did not correspond with two species.

BOX 11: Conservation units in the PhD project study design

Considering the delimitations of populations: we did not invoke MU in Chapter 4 given that the study was preliminary in number of individuals and populations. In Chapter 5, a more profound conservation genetic study on *M. cubensis* subsp. *acunae*, we suggested to lump the former separately managed populations of the two nature reserves. The data in Chapter 6, a more profound conservation genetic study on *M. dodecapetala*, suggest a strong structuring according to the islands of the Lesser Antilles; however, we remained conservative in proposing taxonomic changes or ESUs, while making a clear statement on the recognition of each island as a MU.

Although international conservation policy recognizes biodiversity at three levels: ecosystem, species and genetic (Convention on Biological Diversity, 2007) – from a practical point of view, conservation management involves mainly the species level, whereby the changes in number of individuals and geographic ranges are considered a proxy for the genetic health of the species (Rivers et al., 2014). An effective study of the conservation genetics is only for the lucky few. The general advice to taxonomists and conservation geneticists is to be conservative with interpretations of morphological variation and genetic data (Coates et al., 2018). Additionally, a more regulated approach to taxonomy is desirable that mitigates malpractice (e.g. the unnecessary splitting or lumping of species) (Garnett and Christidis (2017); but see also Thomson et al. (2018)). Although this might be impossible in practice for at the levels of biodiversity, we do believe that a more regulated approach on a lower taxonomical level e.g. the family Magnoliaceae, would already avoid malpractice and confusion. Examples of a more regulated approach to *Magnolia* taxonomy are: the publication of new species whereby a minimal number of individuals is studied *in situ* to describe the morphology, publishing the amount of survey effort that was undertaken to find more individuals/populations of the species *in situ*, the co-publication of a variable DNA marker that shows the species to be genetically differentiated from other sequenced Magnolias, a consensus on the most diagnostic morphological characters and how to report them adequately in a publication of a new species, etc. The importance and influence of taxonomy on conservation, or actually any field of the biological sciences, is of such great extent (Mace (2004); but see also Morrison et al. (2009)), yet the science is currently placed in a predicament (Ebach et al., 2011; Wheeler, 2014).

8.4 The challenge of limited resources

In the general debate on what to conserve, there is interesting literature available on the "ecological triage" approach, which advocates directing the limited resources (funding, time, expertise) at problems where management success, being either single-species or ecosystem conservation, is most likely (Bottrill et al., 2008; Wilson and Law, 2016). Not everyone agrees: this approach makes species extinction and landscape degradation acceptable and allows decision-makers to get away with allocating insufficient resources to address environmental problems (Parr et al., 2009).

BOX 12: Conservation priorities in this PhD

The research in this PhD thesis provides data for single-species conservation, yet with the underlying assumption that conservation of Magnoliaceae safeguards the conservation of the primary forest (remnants) they are part of (See Thesis outline: Project history and Chapter 1.8). In our efforts to guide conservation with the produced genetic data, we put emphasis on *in situ* conservation by increasing connectivity between forest patches and the increase of more (general!) surveys; options such as reinforcement, translocations and collection of seeds for *ex situ* conservation (Chapter 7.2), in an attempt to make conservation efforts beneficial for as many other species as possible and hence extrapolate the results to a larger scale. Nonetheless, focussing conservation management on the establishment of the *Magnolia* trees themselves is already beneficial due to the concept of the trees being important constituents of the ecosystem (e.g. they are the habitat for animals and epiphytes). Although the problematic situation in Haiti should not be ignored, we did (unknowingly) follow the ecological triage approach, whereby we argued to use future expertise resources, i.e. conservation genetic studies, on for example *M. domingensis* rather than *M. ekmanii*, which had comparably "bad" genetic diversity, because of the more politically stable climate of the Dominican Republic over that of Haiti and hence the higher chance of management success (see Chapter 7.2).

8.5 The science vs. practice challenge

Conservation practitioners and conservation genetic scientists are bound by the common goal of conserving biodiversity, yet communication among the two appears to be hampered (Fabian et al., 2019; Holderegger et al., 2019) and even more so there is a general mismatch between many aspects of science vs. practice (Arlettaz et al., 2010; Cook et al., 2013). One way to overcome this challenge, is by executing translational science (Enquist et al., 2017), where the conservation practitioner and conservation genetic scientist closely work together from the very start of the conservation project. Another proposed solution to close the science-practice gap is to work with centralised platforms and initiatives such as the Conservation Genetics Specialist Group (CGSG) of the IUCN Species Survival Commission (SSC: https://www.iucn.org/ssc-groups/disciplinary-groups), the Genetic Composition Working Group: GEO BON (https://geobon.org/ebvs/working-groups/genetic-composition/), ConGRESS: Conservation Genetic Resources for Effective Species Survival (www.congressgenetics.eu/Default.aspx), and the website "Conservation Evidence" (www.conservationevidence.com).

From the viewpoint of a scientist it will always be difficult to ensure continuity and involvement, given that the academic scene does not provide long-term job security, research projects are of short timeframes and the impact of a scientist is commonly measured by number of publications and citations (Arlettaz et al., 2010). Although challenging, both the conservation practitioners and the scientists should acknowledge the problems adherent to their occupation, remain realistic and most of all keep communicating directly. Both parties have great merit of symbiosis and hence at all times it is advised to collaborate wherever possible.

BOX 13: Science vs. practice for the Caribbean Magnolias

The studies executed in the framework of this PhD thesis are examples of either translational science (Chapter 3 and Chapter 5) or science still in search of on-the-ground practitioners (Chapter 6). Unaware at first, we performed translational science together with the Cuban NGO Planta!, whereby we worked closely together to achieve more knowledge on the Cuban Magnolias in the form of a phylogenetic and conservation genetic study and by providing training for the Cuban students (see PhD outline: project history). Even more so, two future PhD studies are planned to continue the research on the Cuban Magnolias in this translational science framework (see Chapter 7.4). From our hands-on experience of the successful collaboration with Planta! in the first years of this PhD project, we aimed to search for more on-the-ground practitioners for further in-depth studies of the Caribbean Magnolia species and are currently collaborating with Fundación PROGRESSIO from the Dominican Republic and Para La Naturaleza from Puerto Rico (see Chapter 1 and Chapter 7.4). Although the data prioritised a further study on *M. dodecapetala* (Chapter 3 and Chapter 6) and we have potential collaborators in the five islands of the Lesser Antilles, we currently lack a demand from local conservation practitioners and are experiencing the difficulties in finding conservation practitioners to focus on the studied species post-hoc.

In all fairness, during our five years of running a conservation genetic themed project while communicating with already more established initiatives such as BGCI, people from IUCN and the GTSG, the initiatives from conservation geneticists listed above remained undiscovered up until scientific literature addressing the science-practice gap was actively searched for. Although this could be due to our research group being new to the topic of conservation genetics, the outreach of such initiatives appears not to be effective.

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Appendices

Appendix 1: General Introduction

Appendix 1.1 Glossary. This glossary is a selection of terms used in this PhD thesis. The explanations for the botanical terms mainly follow Beentje (2016), however sometimes the definitions are slightly adapted. Where other publications were used to explain terms, a reference is provided.

1KP transcriptome project	The 1000 plants (oneKP of 1KP) initiative is an international multi-disciplinary consortium that has generated large-scale gene sequencing data for over 1000 species of plants. Website: https://sites.google.com/a/ualberta.ca/onekp/
Abaxial	The side of an organ that faces away from the axis that bears it; for example, the lower surface of a leaf. OPPOSITE: adaxial.
Acuminate	Tapering to a long tip (usually of leaf tips).
Acute	Sharp, sharply pointed, the margins near the tip being almost straight and forming an angle of <90°. OPPOSITE: obtuse.
Adaxial	The side of an organ towards the axis on which it is inserted, (e.g. the upper surface of a leaf). OPPOSITE: abaxial.
Adherent	(of different organs) Sticking to, attached to, but not fused with.
Adnate	Attached to, surface to surface; usually said of different organs or structures (e.g. stamen adnate to a petal).
Akaike Information Criterion (AIC)	An estimate of the amount of information lost when we use a model to represent a stochastic process. The AIC for a model is –2*ln *likelihood* + 2*k, where k is the number of estimated parameters. Models with smaller AIC provide a better fit to the data. (Lemey et al., 2009)
Allelic diversity (A)	Average number of alleles per locus, a measure of genetic diversity within a population. (Frankham et al., 2010)
Allelic richness (A_R)	Allelic diversity standardised to a particular sample size. (Frankham et al., 2010)
Allopatric	Of related taxa that do not overlap in geographical range. OPPOSITE: sympatric.
Allopolyploid	A polyploid formed from a combination of two genetically different genomes (usually considered to originate from two different species): AABB as opposed to autotetraploid, AAAA.
Alpha-taxonomy	The most fundamental taxonomy: finding, describing and grouping organisms.
Amplify	To use PCR to make many copies of a segment of DNA. (Allendorf et al., 2013)
Androdioecious	With male flowers growing on some plants, and bisexual flowers.
Androecium	A collective term for the male sexual organs, the stamens.
Annular	In the shape of a ring; used of any organs arranged in a circle.
Anther	The part of the stamen containing the pollen.
Anthesis	Time of fertilization of the flower; time of receptivity of stigma or distribution of pollen; used more loosely for the time when the flower opens.
Apex, Apices	Distal end, tip. OPPOSITE: base.
Apiculate	Ending in an abrupt, short point.

Apocarpous	A multiple fruit with free carpels, or a simple fruit consisting of a single carpel. OPPOSITE: syncarpous.
Area of occupancy	The area within its extent of occurrence, which is occupied by a taxon, excluding cases of vagrancy. (IUCN, 2012)
Areole	± Circular areas on a surface that are separated from similar areas by a division line such as a vein.
Arilloid	(Of seed appendages) false aril, a structure that, like the aril (wholly or partly) envelops the seed, but unlike the aril does not derive from the placenta or funicle.
Automimicry	(in *Magnolia*) Non-rewarding female-stage flowers mimic the rewarding male-stage flowers (Hirayama et al., 2005). More generally, the term can be applied to any form of intraspecific mimicry; however, the original usage, which is still applied most in literature, is being that related to infraspecific mimicry to avoid predation (e.g. Svennungsen and Holen, 2007).
Autopolyploid	A polyploid with three or more sets of chromosomes, all from the same taxon.
Axis	Main line of development of a plant or organ.
Base	Usually the point of attachment of any organ.
Biodiversity hotspot	Exceptional concentrations of endemic species that are undergoing exceptional loss of habitat (Myers et al., 2000). For *Magnolia* the primary hotspots are China in Asia and Colombia in America, and the secondary hotspots are Vietnam in Asia and Cuba, Mexico and Panama in America (Cires et al., 2013).
Bottleneck	A sudden restriction in population size. (Frankham et al., 2010)
Bract	A modified and specialised leaf in the inflorescence, standing below partial peduncles, pedicels or flowers.
Burn-in	Term that describes the initial phase of a MCMC run, when the sampled values are still influenced by the starting point. The samples collected during this phase are typically discarded. (Lemey et al., 2009)
Caducous	Falling off soon after formation, not persistent. The use of 'early' caducous or 'quickly' caducous' is incorrect, 'falling early' would be better. See also deciduous (falling seasonally).
Carpel	1. The basic unit of the female sexual organ; 2. One of the cells or locules of the syncarpous ovary.
Circumscissile	Opening by a slit running around the circumference or equator, and with the upper part coming off like a lid.
Clade	A sub-group of organisms from among a larger group sharing common ancestry, not shared by the other organisms in the larger group. (Frankham et al., 2010)
Coalescent theory	A mathematical framework describing the times at which extant lineages had their most recent common ancestors as a function of population size. First developed by J.E.C. Kingman and later expanded by other researchers to include migration, recombination, growth, selection, population divergence, and other forces. (Lemey et al., 2009)
Conduplicate	Folded together lengthwise with the upper surfaces closely parallel and facing each other (e.g. unfolding leaves). OPPOSITE: reduplicate.
Connective	The part of a stamen between and connecting the anther cells, distinct from the filament; sometimes called the filament extension between the thecae.
Conservation Unit (CU)	A population of organisms that is considered distinct for purposes of conservation, such as a management unit (MU), distinct population segment (DPS), or evolutionarily significant unit (ESU). (Allendorf et al., 2013)

Coriaceous	Leathery, tough.
Critically Endangered (CR)	A species with a very high probability of extinction within a short time, e.g. 50% probability of extinction within 10 years, or three generations, whichever is longer. (Frankham et al., 2010)
Crown	(In a phylogenetic tree) When used with “group”, i.e. crown group: the crown group of a phylum consists of the last common ancestor of all living forms in the phylum an all of its descendants (Budd and Jensen, 2000). When used with “node”, i.e. crown node: the node at the base of a crown group.
Data Deficient (DD)	A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or publication status. A taxon in this category may be well studied, and its biology well known, but appropriate data on abundance and/or distribution are lacking. Data Deficient is therefore not a category of threat. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that future research will show that threatened classification is appropriate. It is important to make positive use of whatever data are available. In many cases great care should be exercised in choosing between DD and a threatened status. If the range of a taxon is suspected to be relatively circumscribed, and a considerable period of time has elapsed since the last record of the taxon, threatened status may well be justified. (IUCN, 2012)
Deciduous	Falling seasonally, losing all its leaves for part of the year, not evergreen.
Dehisce	To open when ripe.
Dehiscence	Mode of opening (of a fruit or anther).
Dehiscent	Splitting; opening spontaneously when ripe, as of fruits and anthers.
Diploid	(2n) With twice the haploid (n) (somatic) number of chromosomes.
Distichous	In two opposite rows, one on each side of the stem.
Dorsal	1. Literally ‘regarding the back’; 2. The surface facing away from the axis, = abaxial, which is preferred, so for any lateral organ the upper or inner face; OPPOSITE: ventral, adaxial.
Elliptic	Broadest at the middle with two equal rounded ends.
Ellipsoid	A 3-dimensional shape that is elliptic in the vertical plane.
Emarginate	(of apices) With a distinct sharp notch.
Endangered (EN)	A species or population with a high probability of extinction within a short time, e.g. a 20% probability of extinction within 20 years, or 10 generations, whichever is longer. (Frankham et al., 2010)
Endemic	1. Native to; 2. (when used with ‘to’) Restricted to, unique to, not naturally found elsewhere (e.g. “endemic to Mt Hanang” means occurring only on Mt Hanang and nowhere else). The term is meaningless unless a native area or habitat is specified.
Endemism	Restriction of distribution to one particular area or habitat.
Endocarp	The innermost layer of a multi-layered fruit wall (e.g. the stone or putamen in a drupe).
Entire	1. Not divided; 2. (of margins) Smooth, unbroken by serrations, teeth or other irregularities.
Ephemeral	1. Short-lived annual plant; 2. Soon disappearing or remaining for a very short time.
Evergreen	Retaining its leaves throughout the year. OPPOSITE: deciduous.
Evolutionarily Significant Unit (ESU)	A classification of populations that have substantial reproductive isolation which has led to adaptive differences so that the population represents a significant evolutionary component of the species. The

	original term was “evolutionarily” (Ryder, 1986). However, both evolutionarily and evolutionary are currently used in the literature. (Allendorf et al., 2013)
Ex situ	Off site, away from the natural location.
Ex situ conservation	“Off-site conservation”; the conservation of species outside the species natural habitat. (Allendorf et al., 2013)
Expected heterozygosity (H_E)	The heterozygosity expected for a random mating population with the given allele frequencies according to the Hardy-Weinberg principle. (Frankham et al., 2010)
Extent of occurrence	The area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon, excluding cases of vagrancy. (IUCN, 2012)
Extrorse anther	Opening outwards, away from the centre of the flower; OPPOSITE: introrse.
Filament	A stalk that bears an anther, usually distinct from the connective.
Fixation index (F_{ST})	The proportional increase of homozygosity through population subdivision. (Allendorf et al., 2013)
Flagship species	Species chosen for their charisma, to increase public awareness of conservation issues and rally support for the protection of that species’ habitat. Protection of other species is accomplished through the umbrella effect of the flagship species. (Caro, 2010)
Flotsam	Also called ocean debris; resources that can be found floating in the ocean. Term used by Hedges (1996) in the context of Caribbean biogeography.
Flow cytometry	A technology to study cellular populations with high precision (Picot et al., 2012) that uses a flow cytometer, an instrument that illuminates cells (or other particles) as they flow individually in front of a light source and then detects and correlates the signals from those cells that result from the illumination (Givan, 2011).
Follicetum	An aggregate of follicles, representing the outcome of an apocarpous multi-pistillate gynoecium.
Follicle	A fruit arising from a single carpel, opening along the inner (adaxial) suture to which the seeds are attached. NOTE: In *Magnolia* this term is actually incorrectly applied: the “follicles” either split longitudinally, or they have a circumscissile dehiscence. Even more so, when the “follicles” split longitudinally this is usually along the dorsal (= abaxial) suture, but in subgenus *Magnolia* section *Kmeria* and in subgenus *Gynopodium* section *Manglietiastrum* the “follicles” split along the ventral (= abaxial) suture. As the term follicle has been used for a long period of time for Magnolioideae, its use is generally accepted.
Founder effect	A loss of genetic variation in a population that was established by a small number of individuals that carry only a fraction of the original genetic diversity from a larger population. A special case of genetic drift. (Allendorf et al., 2013)
GAARlandia	A “landspan” (i.e. a subaerial connection between a continent and one or more off shelf islands) comprising the Greater Antilles + Aves Ridge. (Iturralde-Vinent and MacPhee, 1999)
Geitonogamy	Where the flowers of a plant are fertilised by pollen from another flower on the same plant.
Gene flow	Movement of alleles between populations via migrants. (Frankham et al., 2010)

General Lineage Species Concept	Species are separately evolving metapopulation lineages and multiple criteria (e.g. reproductive isolation, diagnostic characters) may be used to identify them. The order in which properties of lineages appear during cladogenesis, or whether they appear, cannot always be predicted, thus the application of several different criteria may be necessary. (de Queiroz, 1998, 2007)
Genetic drift	Random changes in allele frequencies in a population between generations due to sampling individuals that become parents and binomial sampling of alleles during meiosis. Genetic drift is more pronounced in small populations. (Allendorf et al., 2013)
Glaucous	Covered with a waxy bluish grey or seagreen bloom (as on plum or cabbage), which rubs off easily.
Globose	Round, spherical.
Gynoecium, gynoecia	The female element of a flower, the pistil(s).
Haploid	With one set of chromosomes.
Haplotype	Allelic composition for several different loci in a chromosomal region, e.g. $A_1B_3C_2$. (Frankham et al., 2010)
Hardy-Weinberg proportions (HWP)	The equilibrium genotype frequencies achieved in a random mating population with no perturbing forces from mutation migration, selection or chance. If two alleles A_1 and A_2 have frequencies of *p* and *q*, the Hardy-Weinberg proportions for the A_1A_1, A_1A_2 and A_2A_2 are p^2, $2pq$ and q^2, respectively (Frankham et al., 2010).
Heterotypic subspecies	A subspecies based on a different type. If a new subspecies is described, the former type for the species remains with subspecies which repeats the species name (autonym), e.g.; *Magnolia cubensis* subspecies *cubensis*. The new subspecies, e.g. *Magnolia cubensis* subspecies *acunae* is based on a different type specimen and is regarded as heterotypic.
Heterozygosity	A measure of genetic variation that estimates either the observed, or expected proportion of individuals in a population that are heterozygotes. (Allendorf et al., 2013)
Heterozygote	An organism that has different alleles at a locus (e.g., *Aa*). (Allendorf et al., 2013)
Homologous	In biology, homology refers to similarity due to shared ancestry. (Lemey et al., 2009)
Homoplasy, homoplasies	Sharing of identical states that cannot be explained by inheritance from the common ancestor of a group of taxa. (Lemey et al., 2009)
Homozygosity	A measure of the proportion of individuals in a population that are homozygous, and is the reciprocal of heterozygosity. (Allendorf et al., 2013)
Homozygote	An organism that has two or more copies of the same allele at a locus (e.g. AA). (Allendorf et al., 2013)
Hybrid	A cross between two species.
Hybridization	The process of producing a hybrid.
Hybridize (to)	To cross-breed (individuals of two different species).
In situ	In the original place.
In situ conservation	The conservation of a population or species in its natural habitat. (Allendorf et al., 2013)
Inbreeding	The production of offspring from mating of individuals related by descent, e.g. self-fertilization, brother × sister, or cousin matings**.** (Frankham et al., 2010)
Inbreeding coefficient (F_{IS})	A measure of the level of inbreeding in a population, developed by Sewall Wright, that determines the probability that an individual

	possesses two alleles at a locus that are identical by descent. It can also be used to describe the proportion of loci in an individual that are homozygous. (Allendorf et al., 2013)
Inbreeding depression	The relative reduction in fitness of progeny from matings between related individuals compared with progeny from unrelated individuals. (Allendorf et al., 2013)
Incomplete lineage sorting	Failures of lineages in a population to coalesce, leading to the possibility that at least one of the lineages first coalesces with a lineage from a less closely relation population; in genealogical studies expressed as a gene tree – species tree discordance (Degnan and Rosenberg, 2009).
Indehiscent	(of fruits) Not splitting open.
International Plan Exchange Network (IPEN)	This network establishes a system of facilitated exchange for a network of gardens that have signed up to a common Code of Conduct on ABS (Access and Benefit Sharing) (Davis, 2008)
International Union of Pure and Applied Chemistry (IUPAC)	An organisation that formalized a nomenclature for incompletely specified nucleic acids. (Johnson, 2010)
Internode	The part of the stem between two nodes.
Introrse anther	Opening inwards, towards the centre of the flower; OPPOSITE: extrorse.
Lanceolate	Narrowly ovate and tapering to a point at the apex.
Latrorse anther	Opening sideways or laterally, not inwards.
Lectotype	One specimen or illustration designated from the original material (Art. 9.4) as the nomenclatural type, in conformity with Art. 9.11 and 9.12, if the name was published without a holotype, or if the holotype is lost or destroyed, or if a type if found to belong to more than one taxon (see also Art. 9.14). (Turland et al., 2018)
Linkage	The non-random segregation of two loci. (Allendorf et al., 2013)
Linkage disequilibrium (LD)	Non-random association of alleles at different loci within a population. Also known as gametic disequilibrium. (Allendorf et al., 2013)
Local clock	A local clock model allows different rates in different parts of the phylogenetic tree. In this model, different substitution rate parameters are assigned to different branches or collections of branches. (Lemey et al., 2009)
Lobed	1. Divided into lobes; 2. A rounded margin split in two or more sub-divisions.
Management Unit (MU)	Populations of conspecific individuals among which the degree of connectivity is sufficiently low so that each population should be monitored and managed separately. (Taylor and Dizon, 1999)
Markov chain Monte Carlo (MCMC)	A statistical technique for integrating a function by drawing samples at random (“Monte Carlo”) from the function, basing each sample on the previous one (“Markov chain”). This stochastic technique is useful when the function cannot be integrated directly, but can fail if the sample drawn is not big enough or does not explore all important regions of the function. (Lemey et al., 2009)
Molecular clock	Constancy of evolutionary rate among lineages in a genealogy or phylogeny. In a genealogy with a molecular clock, any two lineages samples at the same time should show about the same amount of genetic divergence from their common ancestor. (Lemey et al., 2009)
Monomorphic	A locus at which only one allele is present, generally taken to mean the most common allele is at a frequency of greater than 99%, or 95%. Contrast with polymorphic. (Frankham et al., 2010)

Monophyletic / Monophyly	In phylogenetics, a group of taxa is monophyletic or represents a monophyly if the group includes all descendants from its inferred common ancestor. (Lemey et al., 2009)
Morphological Species Concept	A species is a community, or a number of related communities, whose distinctive morphological characters are, in the opinion of a competent systematist, sufficiently definite to entitle it, or them, to a specific name. (Regan, 1925)
Nastic movements	Movements of plant organs in response to external stimuli that are (largely) independent of the direction of the stimuli. (Braam, 2005)
Neopolyploids	Newly formed polyploids. (Ramsey and Schemske, 2002)
Neotype	A specimen or illustration selected to serve as nomenclatural type if no original material exists, or as long as it is missing. (Turland et al., 2018)
Next-generation sequencing (NGS)	Highly parallel or high-output sequencing methods that produce data at or beyond the genome scale. (Levy and Myers, 2016)
Node	1. (morphological definition) The area of a stem where a leaf is attached or used to be attached; see also internode. 2. (phylogenetic definition) A point on a phylogeny where a single ancestral lineage breaks into two or more descendent lineages.
Null allele	Allele that does not produce a functional product, or a mutation in a primer site that precludes PCR amplification. (Frankham et al., 2010)
Oblong	(of a plane shape) Longer than broad, with the margins parallel for most of their length.
Obovate	Egg-shaped (2-dimensional) with the broadest part near the apex.
Obtuse	(of an apex or base) Not pointed, blunt, ending in an angle of between 90–180°.
Orbicular	(2-dimensional) Flat with a circular outline.
Outbreeding depression	The reduction in fitness of hybrids compared with parental types. (Allendorf et al., 2013)
Outgroup	A taxon that is used to root a phylogenetic tree and thus providing directionality to the evolutionary history. An outgroup taxon is not considered to be part of the group in question (the ingroup), but preferably, it is closely related to that group. In cladistics analysis, an outgroup is used to help resolve the polarity of characters, which refers to their state being original or derived. (Lemey et al., 2009)
Ovate	Egg-shaped (2-dimensional), about 1.5 × as long as broad, with the wider part below the middle.
Paraphyletic	In phylogenetics, a group of taxa is paraphyletic or represent a paraphyly if the group does not include all descendants from its inferred common ancestor. (Lemey et al., 2009)
Pedicle	(*Magnolia* specific term) Small stalk-like structure – used here for the internodes at the apex of the peduncle immediately below the flower. This is the internode between the tepal and the uppermost bract**.** The uppermost internode, between the perianth and the bract (or uppermost bract), varies in length. When G.H. Johnstone was writing his book: *Asiatic Magnolias in Cultivation* (1955), one of the first monographs published on Magnolias, he wanted to call this internode the pedicel, but J.E. Dandy, a British botanist that was the acknowledged world authority on the Magnoliaceae in the 1900s, pointed out to him that that this would be incorrect, as this term is generally applied to the ultimate stalk of a single flower within an inflorescence, a collective arrangement which does not occur in Magnoliaceae. Consequently, Johnstone compromised by adopting the term '*pedicle*'. (Treseder, 1978)

Peduncle	Stalk of a single flower or of an inflorescence. In *Magnolia* the internodes between the upper-most foliage leaf and the perianth of the flower, with the node which carries the spathaceous bract, or the annular scar where it grew. (Treseder, 1978)
Perianth	Collective term for the calyx and corolla.
Pericarp	The wall of the ripened ovary.
Perule	In *Magnolia*, modified leaf (formed by stipules) enclosing and protecting the flower bud (Treseder, 1978). Howard (1948) uses the term spathaceous bract and Lozano Contreras (1994) uses the term hypsophylls – we discourage usage of both terms.
Petiolate	With a leaf stalk, not sessile.
Petiole	Leaf stalk, the basal and usually narrowly cylindrical part of the leaf which carries the vascular bundles and is intermediate in position between stem and blade.
Phenology	(abbreviated from phenomenology) Study of the timing of recurring natural phenomena, e.g. flowering times, fruiting times.
Phyllotaxis	In Beentje (2016): Phyllotaxy. Arrangement of the leaves along a stem.
Phylogenetic Species Concept	A species is a group of organisms that share at least one uniquely derived character, perhaps with a shared pattern of ancestry and descent or monophyly. (Agapow et al., 2004 and references herein)
Pistil	1. (in apocarpous flowers) the unit of separate ovary, style and stigma (Bell, 2008; Hickey and King, 2000); 2. (in syncarpous flowers) the whole gynoecium (Bell, 2008; Hickey and King, 2000); 3. The female organ of a flower, consisting when complete of ovary, style and stigma (Jackson, 1928).
Ploidy level	Relating to the number of chromosome sets.
Polymerase Chain Reaction (PCR)	Method used to make replicate copies (amplify) of a specific segment of DNA. The DNA is heated, primers (short segments of DNA flanking the segment of interest) added and the intervening DNA copied over 30–40 cycles using thermostable *Taq* polymerase enzyme. (Frankham et al., 2010)
Polymorphic	A locus at which more than one allele is present, generally taken to mean the most common allele is at a frequency of less than 99%, or 95%. Compare with monomorphic. (Frankham et al., 2010)
Polymorphism	The presence of more than one allele at a locus. Polymorphism is also used as a measure of the proportion of loci in a population that are genetically variable or polymorphic (P). (Allendorf et al., 2013)
Polyploid	With more than twice the normal haploid set of chromosomes.
Polyploidization	The process of producing a polyploid.
Precocious	Appearing or developing early, often used of flowers which appear before the leaves.
Prefoliation	The folding or packing of leaves in bud.
Primer	A short nucleotide sequence that pairs with one strand of DNA and provides a free end at which DNA polymerase enzyme begins synthesis of a complementary segment of DNA. (Frankham et al., 2010)
Private allele(s) (A_P)	An allele present in only one of many populations sampled. (Allendorf et al., 2013)
Prolepsis	Growth of a bud from a dormant stage into a lateral shoot [unusual term].
Protogynous	(of a flower) With the stigma receptive before the anthers open, i.e. first functionally female and afterwards functionally male.
Pubescence	Hairiness, indumentum [not recommended].

Pubescent	With dense fine, short, soft hairs; downy. (This term has been used in various ways, sometimes meaning any kind of hair covering).
Receptacle	1. The expanded part at the end of the flower stalk on which the organs of a flower (i.e. sepals, petals, stamens and carpels) are inserted.
Rhombic	(of plane shapes) In the shape of an equilateral parallelogram (generally excluding the square), lozenge-shaped.
Reinforcement	The intentional movement and release of an organism into an existing population of conspecifics. (IUCN SSC, 2013)
Reintroduction	The intentional movement and release of an organism inside its indigenous range from which it has disappeared. (IUCN SSC, 2013)
Samara	A dry indehiscent fruit with a wing (longer than the seed-bearing part) developed to one side (as in *Acer pseudoplatanus*, the sycamore).
Samaroid	Resembling a samara, although the wing may surround the seed chamber.
Sarcotesta	Fleshy layer developed from the outer seed coat.
Self-incompatibility	The inability of an individual (usually plant) to produce offspring following attempted self-fertilisation. Many plant species have loci that control self-incompatibility. (Frankham et al., 2010)
Sericeous	Silky, with closely appressed soft straight hairs and with a shiny silky sheen.
Setaceous	Bristle-like, narrow and stiff.
Single Nucleotide Polymorphism (SNP)	A nucleotide site (base pair) in a DNA sequence that is polymorphic in a population and can be used as a marker to assess genetic variation within an among populations. Usually only two alleles exist for a SNP in a population. (Allendorf et al., 2013)
Single Sequence Repeat (SSR)	Tandem repeated motifs of 1–6 base pairs which have a frequent occurrence in all prokaryotic and eukaryotic genomes analysed to date (Kalia et al., 2010). SYNONYMS: microsatellite, STR (Short Tandem Repeat).
Spathaceous	Resembling, or with the function of, a spathe (e.g. large bract(s) enclosing the flower(s)).
Stamen	The male organ of a flower, the male sporophyll, consisting of a stalk (filament) bearing the connective and container(s) (anthers) that bear the pollen.
Stem	1. (in plant morphology) The main axis of a plant, bearing roots, leaves and/or flowers. 2. (in a phylogenetic tree) When used with “group”, i.e. stem group: the stem group consists of a series of entirely extinct organisms leading up to the crown group away from the last common ancestor of this phylum and the most closely related phylum (Budd and Jensen, 2000). When used with “node”, i.e. stem node: the node at the base of a stem group.
Stigma	The pollen receptor of the gynoecium, which may be either sessile on the ovary or on top of the style or style arms.
Stipitate	Supported on a special stalk, i.e. not on a petiole, peduncle or pedicel.
Stipule	1. Leaf-like, spine-like or scale-like appendages of the leaf, usually in pairs at the base of the petiole.
Style	The part of the gynoecium between the ovary and the stigma, often slender and sometimes lacking when the stigma is positioned on top of the ovary.
Suture	The line of a junction or seam of union, commonly used of the line of opening of a carpel; dorsal suture (outer or anterior) thought to represent the midrib of the carpellary leaf; ventral suture (inner) thought to represent the united margins on which the ovules and placentas are borne.

Syllepsis	Growth of a bud into lateral shoot without a resting period.
Sympatric	(of two or more taxa) Living in the same area. OPPOSITE: allopatric. SYNONYM: to be in sympatry; sympatrically occurring.
Synapomorphy	(in cladistics) With one or more shared derived character states that identify and define a monophyletic taxon.
Syncarpous	(of a flower) With united carpels. OPPOSITE: apocarpous.
Tepal	A division of the perianth, i.e. a sepal of petal, used especially when it is unclear which is which.
Theca, thecae	The locule(s), usually, two, of an anther.
Threatened	Threatened species are any of those classified as Critically Endangered (CR), Endangered (EN) or Vulnerable (VU). (IUCN, 2012). A population or species that has a finite risk of extinction within a relatively short time frame, say a greater than 10% risk of extinction within 100 years. (Frankham et al., 2010)
Translocation	The movement of an individual from one wild location to another as result of human actions. (Frankham et al., 2010)
Trimerous	In threes (e.g. describing a flower with three sepals and three petals etc.).
Torus	1. Ring-shaped cylinder; 2. The receptacle of a flower, usually used when part of the receptacle is swollen into a distinct cushion (as in many Ochnaceae).
Umbrella species	A single-species shortcut, of which hopefully the location, size and shape of the area covered by a viable population of that one umbrella species will cover sufficient home ranges of individuals or other species so that these too will have viable populations. (Caro, 2010)
Vicariance	Vicariant event: a mode of speciation in which a barrier, such as water or mountains, divides members of a species, the vicariants then evolve separately.
Villose	With long soft weak hairs.
Vulnerable (VU)	A species or population with a tangible risk of extinction within a moderate time, e.g. a 10% probability within 100 years. (Frankham et al., 2010)
Wahlund effect	Reduction in heterozygosity, compared to Hardy-Weinberg expectations, in a population split into partially isolated sub-populations. Named after its discoverer. (Frankham et al., 2010)
Whole Genome Duplication (WGD)	Whole Genome Duplication; a phenomenon by which a whole genome of a cell of an organism is doubled, which results in the acquisition of an additional set of chromosomes (Moriyama and Koshiba-Takeuchi, 2018).

Appendix 1.2 Abbreviations mentioned in the PhD thesis. More in depth explanations and references, are to be found in the glossary (Appendix 1.1).

A	Allelic diversity
A_P	Private alleles
A_R	Allelic richness
AIC	Akaike Information Criterion
BGCI	Botanic Gardens Conservation International (https://www.bgci.org/)
CR	Critically Endangered (IUCN Red List Category)
CU	Conservation Unit
D_{JOST}	Allelic differentiation, a statistic (Jost, 2008).
D_A	Genetic distance, a statistic (Nei et al., 1983).
DA	Discriminant Analysis
DAPC	Discriminant Analysis of Principal Components
DBH	Diameter at breast height
DD	Data Deficient
EN	Endangered (IUCN Red List Category)
ESS	Effective Sample Size
ESU	Evolutionarily Significant Unit
F_{IS}	Inbreeding coefficient
F_{ST}	Fixation index
GTSG	Global Trees Specialist Group (https://globaltrees.org/iucn-ssc-global-tree-specialist-group/).
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HPD	Highest Posterior Density
HWP	Hardy-Weinberg Proportions
IPEN	International Plan Exchange Network
IUCN	International Union for Conservation of Nature and Natural Resources
IUPAC	International Union of Pure and Applied Chemistry
ΔK	Difference in likelihood of K, a statistic (Evanno et al., 2005).
LD	Linkage disequilibrium
L(K)	Mean likelihood of K (Evanno et al., 2005).
MCMC	Markov chain Monte Carlo
ML	Maximum Likelihood
MRCA	Most Recent Common Ancestor
MU	Management Unit
NGS	Next-generation sequencing
PCA	Principal Component Analysis
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PGD	Pairwise Geographic Distance
PIC	Phylogenetically Informative Character(s)
pp	posterior probability
SNP	Single nucleotide polymorphism
SSR	Single Sequence Repeat
VU	Vulnerable (IUCN Red List Category)
WGD	Whole Genome Duplication

Appendix 1.3 Names of the Magnoliaceae taxa mentioned in the PhD thesis whereby the species are ordered alphabetically within their lowest rank in the classification of Figlar and Nooteboom (2004). Taxonomic authorities are given after each taxon name. Species in **bold** are Caribbean Magnolias. Species in grey are synonyms or taxa not accepted in use when following Figlar and Nooteboom (2004).

Family	Subfamily	Tribe	Genus	Subgenus	Section	Subsection	Species, Subspecies, Variety or Hybrid
Magnoliaceae Juss. Liriodendraceae F.A. Barkley	Liriodendroideae Y.W.Law	Liriodendreae Dandy	*Liriodendron* L.				*L. chinense* (Hemsl.) Sarg.
							L. tulipifera L.
Magnoliaceae Juss.	Magnolioideae	Magnolieae	*Magnolia* L.	*Magnolia*	*Magnolia*		*M. grandiflora* L.
							M. guatemalensis Donn.Sm.
							M. iltisiana Vázquez
							M. mayae Vázquez & Pérez-Farrera
							M. pacifica subsp. *pugana* Iltis & Vazquez
							M. panamensis Vazquez & Iltis
							M. pedrazae A.Vázquez
							M. schiedeana Schltdl.
							M. sharpi Meranda
							M. tamaulipana Vazquez
							M. virginiana L.
							***M. virginiana* subsp. *oviedoae* Palmarola, M.S. Romanov & A.V. Bobrov**
							M. yoroconte Dandy

			Magnolia L.	*Magnolia*	*Auriculata* Figlar & Noot.		*M. fraseri* Walt. subsp. *fraseri*
							M. fraseri subsp. *pyramidata* (Bartram) Pampanini
			Magnolia L.	*Magnolia*	*Macrophylla* Figlar & Noot.		*M. dealbata* (Zucc.) D.L. Johnson
							M. macrophylla Michx.
			Magnolia L. = *Lirianthe* Spach	*Magnolia*	*Gwillima* DC.		*M. delavayi* Franchet
			Magnolia L. = *Lirianthe* Spach	*Magnolia*	*Blumiana* (Blume) Figlar & Noot.		
			Magnolia L. = *Talauma* Juss.	*Magnolia*	***Talauma* Baill.**	***Talauma***	*M. caricifragrans* (Lozano) Govaerts
							***M. dodecapetala* (Lam.) Govaerts**
							M. espinalii (Lozano) Govaerts
							M. hernandezii (Lozano) Govaerts
							M. jardinensis M.Serna, C.Velásquez & Cogollo
							M. lacandonica A.Vázquez, Pérez-Farr. & Mart.-Camilo
							M. lopezobradorii A.Vázquez
							M. mexicana DC.
							M. mindoensis A.Vázquez, D.A.Neill & A.Dahua
							***M. minor* (Urb.) Govaerts** = *Svenhedinia minor* (Urb.) Urb. = *Svenhedinia truncata* Moldenke = *Talauma minor* Urb. = *Talauma truncata* (Moldenke) R.A. Howard
							***M. oblongifolia* (León) Palmarola** = *Talauma oblongifolia* (León) Bisse

							= *Talauma minor* subsp. *oblongifolia* (León) Borhidi = *Talauma minor* var. *oblongifolia* León
							***M. orbiculata* (Britton & P. Wilson) Palmarola** = *Talauma orbiculata* Britton & P. Wilson = *Talauma opithicola* Bisse = *Talauma minor* subsp. *orbiculata* (Britton & P. Wilson) Borhidi
							M. ovata (A. St. Hil.) Spreng.
							M. perezfarrerae A.Vázquez & Gómez-Domínguez
							M. rimachii (Lozano) Govaerts
							M. sinacacolinii A.Vázquez
							M. venezuelensis (Lozano) Govaerts
							M. zoquepopolucae A.Vázquez
			Magnolia L. = *Talauma* Juss.	*Magnolia*	*Talauma* Baill.	*Chocotalauma* A.Vázquez, Á.J.Pérez & F.Arroyo	*M. chiguila* F.Arroyo, Á.J.Pérez & A.Vázquez
			Magnolia L. = *Dugandiodendron* Lozano	*Magnolia*	*Talauma* Baill.	*Dugandiodendron* (Lozano) Figlar & Noot.	*M. chimantensis* Steyermark & Maguire
							M. coronata M.Serna, C.Velásquez & Cogollo
							M. lenticellata (Lozano) Govaerts
							M. mahechae (Lozano) Govaerts
			Magnolia L. = *Dugandiodendron* Lozano	*Magnolia*	***Talauma* Baill.**	***Cubenses* Imkhan**. = *Splendentes* (Dandy ex A. Vázquez) Figlar & Noot.	***M. cristalensis* Bisse** = *M. cacuminicola* Bisse = *M. cacuminicola* Bisse subsp. *cacuminicola* = *M. cacuminicola* subsp. *bissei* Imkhan. = *M. cristalensis* subsp. *baracoana* Imkhan. = *M. cristalensis* subsp. *moana* Imkhan. = *M. cubensis* subsp. *cacuminicola* (Bisse) G. Klotz = *M. cubensis* var. *baracoënsis* Imkhan.
							***M. cubensis* subsp. *acunae* Imkhan**.
							M. cubensis* Urb. subsp. *cubensis = *M. cubensis* subsp. *turquinensis* Imkhan.
							***M. domingensis* Urb.**

							***M. ekmanii* Urb.**
							***M. emarginata* Urb. & Ekman**
							***M. hamorii* Howard**
							***M. pallescens* Urb. & Ekman**
							***M. portoricensis* Bello**
							***M. splendens* Urb.**
			Magnolia L. = *Manglietia* Blume	*Magnolia*	*Manglietia* (Blume) Baill.		*M. decidua* (Q.Y. Zheng) V.S. Kumar
							M. insignis Wall.
							M. sapaensis (N.H.Xia & Q.N.Vu) Grimshaw & Macer
			Magnolia L. = *Kmeria* (Pierre) Dandy	*Magnolia*	*Kmeria* (Dandy) Figlar & Noot.		*M. kwangsiensis* Figlar & Noot.
			Magnolia L. = *Woonyoungia* Y.W. Law				
			Magnolia L. = *Houpoëa* N.H. Xia & C.Y. Wu	*Magnolia*	*Rhytidospermum* Spach	*Rhytidospermum*	*M. obovata* Thunb.
							M. tripetala L.
			Magnolia L. = *Oyama* N.H. Xia & C.Y. Wu	*Magnolia*	*Rhytidospermum* Spach	*Oyama* (Nakai) Figlar & Noot.	*M. sieboldii* subsp. *sieboldii* K. Koch
							M. wilsonii (Finet. & Gagnep.) Rehder
			Magnolia L. = *Parakmeria* Hu & W.Y. Cheng	*Gynopodium* Figlar & Noot.	*Gynopodium* Dandy		*M. kachirachirai* (Kanehira & Yamamoto) Dandy
							M. nitida W. W. Smith
			Magnolia L. = *Pachylarnax* Dandy	*Manglietiastrum* (Y.W. Law) Noot.			
		Michelieae Law	*Magnolia* L. = *Yulania* Spach	*Yulania* Spach (Rchb.)	*Yulania*	*Yulania*	*M. biondii* Pampan
							M. kobus DC.
							M. stellata (Siebold & Zucc.) Maxim.
							Magnolia x soulangeana Soul.-Bod.
							M. zenii Cheng
			Magnolia L. = *Yulania* Spach	*Yulania* Spach (Rchb.)	*Yulania*	*Tulipastrum* (Spach.) Figlar & Noot.	*M. acuminata* L.
			Magnolia L.		*Michelia* (L.) Baill.		*M. compressa* Maxim.

			= *Michelia* L.	*Yulania* Spach (Rchb.)		*Michelia* (L.) Figlar & Noot.	*M. doltsopa* (Buch.-Ham. Ex DC.) Figlar
							M. figo (Lour.) DC.
							M. fulva (H.T. Chang & B.L. Chen) Figlar
			Magnolia L. = *Elmerilia* Dandy	*Yulania* Spach (Rchb.)	*Michelia* (L.) Baill.	*Elmerillia* (Dandy) Figlar & Noot.	
			Magnolia L. = *Alcimandra* Dandy	*Yulania* Spach (Rchb.)	*Michelia* (L.) Baill.	*Maingola* Figlar & Noot.	
			Magnolia L. = *Aromdadendron* Blume	*Yulania* Spach (Rchb.)	*Michelia* (L.) Baill.	*Aromdadendron* Figlar & Noot.	

Appendix 1.4 Alphabetical list of all the Magnoliaceae species listed in this PhD. The list includes all synonyms of the Caribbean Magnolias, and commonly used synonyms for the other used species with reference to the currently accepted name. Also given is the lowest assignable taxonomic rank according to the classification of Figlar and Nooteboom (2004).

Taxon	**Synonym of**	**Classification**
Annona dodecapetala Lam.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Dugandiodendron chimantense (Steyerm. & Maguire) Lozano	*Magnolia chimantensis* Steyerm. & Maguire	Subsection *Dugandiodendron*
Dugandiodendron lenticellata Lozano	*Magnolia lenticellata* (Lozano) Govaerts	Subsection *Dugandiodendron*
Dugandiodendron mahechae Lozano	*Magnolia mahechae* (Lozano) Govaerts	Subsection *Dugandiodendron*
Houpoea obovata (Thunb.) N.H.Xia & C.Y.Wu	*Magnolia obovata* Thunb.	Subsection *Rhytidospermum*
Kmeria septentrionalis Dandy	*Magnolia kwangsiensis* Figlar & Noot.	Section *Kmeria*
Lirianthe delavayi (Franch.) N.H.Xia & C.Y.Wu	*Magnolia delavayi* Franch.	Section *Gwillimia*
Liriodendron chinense (Hemsl.) Sarg.		Genus *Liriodendron*
Liriodendron tulipifera L.		Genus *Liriodendron*
Magnolia acuminata (L.) L.		Subsection *Tulipastrum*
Magnolia biondii Pamp.		Subsection *Yulania*
Magnolia cacuminicola Bisse	*Magnolia cristalensis* Bisse	Subsection *Cubenses*
Magnolia cacuminicola Bisse subsp. *cacuminicola*	*Magnolia cristalensis* Bisse	Subsection *Cubenses*
Magnolia cacuminicola subsp. *bissei* Imkhan.	*Magnolia cristalensis* Bisse	Subsection *Cubenses*
Magnolia caricifragrans (Lozano) Govaerts		Subsection *Talauma*
Magnolia chiguila F. Arroyo, Á.J. Pérez & A.Vázquez		Subsection *Chocotalauma*
Magnolia chimantensis Steyerm. & Maguire		Subsection *Dugandiodendron*
Magnolia compressa Maxim.		Subsection *Michelia*
Magnolia coronata M. Serna, C. Velásquez & Cogollo		Subsection *Dugandiodendron*
Magnolia cristalensis Bisse		Subsection *Cubenses*
Magnolia cristalensis subsp. *baracoana* Imkhan.	*Magnolia cristalensis* Bisse	Subsection *Cubenses*
Magnolia cristalensis subsp. *moana* Imkhan.	*Magnolia cristalensis* Bisse	Subsection *Cubenses*
Magnolia cubensis subsp. *acunae* Imkhan.		Subsection *Cubenses*
Magnolia cubensis subsp. Urb. *cubensis*		Subsection *Cubenses*
Magnolia cubensis subsp. *cacuminicola* (Bisse) G. Klotz	*Magnolia cristalensis* Bisse	Subsection *Cubenses*

Magnolia cubensis subsp. *turquinensis* Imkhan.	*Magnolia cubensis* subsp. *cubensis*	Subsection *Cubenses*
Magnolia cubensis var. *baracoënsis* Imkhan.	*Magnolia cristalensis* Bisse	Subsection *Cubenses*
Magnolia dealbata Zucc.		Section *Macrophylla*
Magnolia decidua (Q.Y.Zheng) V.S.Kumar		Section *Manglietia*
Magnolia delavayi Franch.		Section *Gwillimia*
Magnolia dodecapetala (Lam.) Govaerts		Subsection *Talauma*
Magnolia doltsopa (Buch.-Ham. Ex DC.) Figlar		Subsection *Michelia*
Magnolia domingensis Urb.		Subsection *Cubenses*
Magnolia ekmanii Urb.		Subsection *Cubenses*
Magnolia emarginata Urb. & Ekman		Subsection *Cubenses*
Magnolia espinalii (Lozano) Govaerts		Subsection *Talauma*
Magnolia fatiscens Rich. Ex DC.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Magnolia figo (Lour.) DC.		Subsection *Michelia*
Magnolia fraseri Walter subsp. *fraseri*		Section *Auriculata*
Magnolia fraseri subsp. *pyramidata* (Bartram) Pamp.		Section *Auriculata*
Magnolia fulva (Hung T.Chang & B.L.Chen) Figlar		Subsection *Michelia*
Magnolia glauca (L.) L.	*Magnolia virginiana* L.	Section *Magnolia*
Magnolia grandiflora L.		Section *Magnolia*
Magnolia guatemalensis Donn.Sm.		Section *Magnolia*
Magnolia hamorii Howard		Subsection *Cubenses*
Magnolia hernandezii (Lozano) Govaerts		Subsection *Talauma*
Magnolia hypoleuca Siebold & Zucc.	*Magnolia obovata* Thunb.	Subsection *Rhytidospermum*
Magnolia iltisiana Vazquez		Section *Magnolia*
Magnolia insignis Wall.		Section *Manglietia*
Magnolia jardinensis M.Serna, C. Velásquez & Cogollo		Subsection *Talauma*
Magnolia kachirachirai (Kaneh. & Yamam.) Dandy		Section *Gynopodium*
Magnolia kobus DC.		Subsection *Yulania*
Magnolia kwangsiensis Figlar & Noot.		Section *Kmeria*
Magnolia lacandonica A.Vázquez, Pérez-Farr. & Mart.-Camilo		Subsection *Talauma*

Magnolia lenticellata (Lozano) Govaerts		Subsection *Dugandiodendron*
Magnolia linguifolia L. ex Descourt.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Magnolia lopezobradorii A.Vázquez		Subsection *Talauma*
Magnolia macrophylla Minchx.		Section *Macrophylla*
Magnolia macrophylla var. *dealbata* (Zucc.) D.K.Johnson	*Magnolia dealbata* Zucc.	Section *Macrophylla*
Magnolia macrophylla var. *macrophylla*	*Magnolia macrophylla* Minchx.	Section *Macrophylla*
Magnolia mahechae (Lozano) Govaerts		Subsection *Dugandiodendron*
Magnolia mayae Vázquez & Pérez-Farrera		Section *Magnolia*
Magnolia mexicana DC.		Subsection *Talauma*
Magnolia michauxiana DC.	*Magnolia macrophylla* Minchx.	Section *Macrophylla*
Magnolia mindoensis A.Vázquez, D.A.Neill & A.Dahua		Subsection *Talauma*
Magnolia minor (Urb.) Govaerts		Subsection *Talauma*
Magnolia nitida W.W.Sm.		Section *Gynopodium*
Magnolia oblongifolia (León) Palmarola		Subsection *Talauma*
Magnolia obovata Thunb.		Subsection *Rhytidospermum*
Magnolia orbiculata (Britton & P.Wilson)		Subsection *Talauma*
Magnolia ovata (A.St.-Hil.) Spreng.		Subsection *Talauma*
Magnolia pacifica Vazquez subsp. *pacifica*		Section *Magnolia*
Magnolia pallescens Urb. & Ekman		Subsection *Cubenses*
Magnolia panamensis H.H.Iltis & Vazquez		Section *Magnolia*
Magnolia pedrazae A.Vázquez		Section *Magnolia*
Magnolia perezfarrerae A.Vázquez & Gómez-Domínguez		Subsection *Talauma*
Magnolia pilosissimia P.Parm.	*Magnolia macrophylla* Minchx.	Section *Macrophylla*
Magnolia plumieri Sw.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Magnolia portoricensis Bello		Subsection *Cubenses*
Magnolia pyramidata Bartram	*Magnolia fraseri* subsp. *pyramidata* (Bartram) Pamp.	Section Auriculata
Magnolia rimachii (Lozano) Govaerts		Subsection *Talauma*
Magnolia sapaensis (N.H.Xia & Q.N.Vu) Grimshaw & Macer		Section *Manglietia*
Magnolia schiedeana schltl.		Section *Magnolia*

Magnolia sharpii V.V.Miranda		Section *Magnolia*
Magnolia sieboldii K.Koch		Subsection *Oyama*
Magnolia sinacacolinii A.Vázquez		Subsection *Talauma*
Magnolia splendens Urb.		Subsection *Cubenses*
Magnolia stellata (Siebold & Zucc.) Maxim.		Subsection *Yulania*
Magnolia tamaulipana Vazquez		Section *Magnolia*
Magnolia tripetala (L.) L.		Subsection *Rhytidospermum*
Magnolia venezuelensis (Lozano) Govaerts		Subsection *Talauma*
Magnolia virginiana L.		Section *Magnolia*
Magnolia virginiana subsp. *oviedoae* Palmarola, M.S.Romanov & A.V.Bobrov		Section *Magnolia*
Magnolia wilsonii (Finet & Gagnep.) Rehder		Subsection *Oyama*
Magnolia x soulangeana Soul.-Bod.		Subsection *Yulania*
Magnolia yoroconte Dandy		Section *Magnolia*
Magnolia zenii W.C.Cheng		Subsection *Yulania*
Magnolia zoquepopolucae A. Vázquez		Subsection *Talauma*
Manglietia decidua Q.Y.Zheng	*Magnolia decidua* (Q.Y.Zheng) V.S.Kumar	Section *Manglietia*
Manglietia insignis (Wall.) Blume	*Magnolia insignis* Wall.	Section *Manglietia*
Manglietia sapaensis N.H.Xia & Q.N.Vu	*Magnolia sapaensis* (N.H.Xia & Q.N.Vu) Grimshaw & Macer	Section *Manglietia*
Michelia compressa (Maxim.) Sarg.	*Magnolia compressa* Maxim.	Subsection *Michelia*
Michelia doltsopa Buch.-Ham. Ex DC.	*Magnolia doltsopa* (Buch.-Ham. Ex DC.) Figlar	Subsection *Michelia*
Michelia figo (Lour.) Spreng.	*Magnolia figo* (Lour.) DC.	Subsection *Michelia*
Michelia fulva Hung T.Chang & B.L.Chen	*Magnolia fulva* (Hung T.Chang & B.L.Chen) Figlar	Subsection *Michelia*
Michelia kachirachirai Kaneh. & Yamam	*Magnolia kachirachirai* (Kaneh. & Yamam.) Dandy	Section *Gynopodium*
Oyama sieboldii (K.Koch) N.H.Xia & C.Y.Wu	*Magnolia sieboldii* K.Koch	Subsection *Oyama*
Oyama wilsonii (Finet & Gagnep.) N.H.Xi & C.Y.Wu	*Magnolia wilsonii* (Finet & Gagnep.) Rehder	Subsection *Oyama*
Parakmeria kachirachirai (Kaneh & Yamam.) Y.W.Law	*Magnolia kachirachirai* (Kaneh. & Yamam.) Dandy	Section *Gynopodium*
Parakmeria nitida (W.W.Sm.) Y.W.Law	*Magnolia nitida* W.W.Sm.	Section *Gynopodium*
Svenhedinia minor (Urb.) Urb.	*Magnolia minor* (Urb.) Govaerts	Subsection *Talauma*
Svenhedinia truncata Moldenke	*Magnolia minor* (Urb.) Govaerts	Subsection *Talauma*

Talauma caerulea J.St.-Hil.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Talauma caricifragrans Lozano	*Magnolia caricifragrans* (Lozano) Govaerts	Subsection *Talauma*
Talauma dodecapetala (Lam.) Urb.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Talauma espinalii Lozano	*Magnolia espinalii* (Lozano) Govaerts	Subsection *Talauma*
Talauma hernandezii Lozano	*Magnolia hernandezii* (Lozano) Govaerts	Subsection *Talauma*
Talauma mexicana (DC.) G.Don	*Magnolia mexicana* DC.	Subsection *Talauma*
Talauma minor Urb.	*Magnolia minor* (Urb.) Govaerts	Subsection *Talauma*
Talauma minor subsp. *oblongifolia* (León) Borhidi	*Magnolia oblongifolia* (León) Palmarola	Subsection *Talauma*
Talauma minor subsp. *orbiculata* (Britton & P.Wilson) Borhidi	*Magnolia orbiculata* (Britton & P.Wilson)	Subsection *Talauma*
Talauma minor var. *oblongifolia* León	*Magnolia oblongifolia* (León) Palmarola	Subsection *Talauma*
Talauma oblongifolia (León) Bisse	*Magnolia oblongifolia* (León) Palmarola	Subsection *Talauma*
Talauma opithicola Bisse	*Magnolia orbiculata* (Britton & P.Wilson)	Subsection *Talauma*
Talauma orbiculata Britton & P.Wilson	*Magnolia orbiculata* (Britton & P.Wilson)	Subsection *Talauma*
Talauma ovata A.St.-Hil.	*Magnolia ovata* (A.St.-Hil.) Spreng.	Subsection *Talauma*
Talauma plumieri (Sw.) DC.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Talauma plumieri var. *longifolia* DC.	*Magnolia dodecapetala* (Lam.) Govaerts	Subsection *Talauma*
Talauma rimachii Lozano	*Magnolia rimachii* (Lozano) Govaerts	Subsection *Talauma*
Talauma truncata (Moldenke) R.A.Howard	*Magnolia minor* (Urb.) Govaerts	Subsection *Talauma*
Talauma venezuelensis Lozano	*Magnolia venezuelensis* (Lozano) Govaerts	Subsection *Talauma*
Woonyoungia septentrionalis (Dandy) Y.W.Law	*Magnolia kwangsiensis* Figlar & Noot.	Section *Kmeria*
Yulania biondii (Pamp.) D.L.Fu	*Magnolia biondii*	Subsection *Yulania*
Yulania kobus (DC.) Spach.	*Magnolia kobus* DC.	Subsection *Yulania*
Yulania sinostellata (P.L.Chiu Z.H.Chen) D.L.Fu	*Magnolia stellata* (Siebold & Zucc.) Maxim.	Subsection *Yulania*
Yulania stellata (Maxim.) N.H.Xia	*Magnolia stellata* (Siebold & Zucc.) Maxim.	Subsection *Yulania*
Yulania zenii	*Magnolia zenii* W.C.Cheng	Subsection *Yulania*

Appendix 1.5 Magnolia's van de Caraïben. **MODIFIED FROM:** Veltjen E. (2015) Magnolia's van de Caraïben. De Vrienden van de Plantentuin Gent 34(4): 195–211.

Samenvatting: 5 weken, 2 eilanden, 3 landen en 7 soorten – mijn eerste veldreis. Doel: populatiestalen van de Caraïbische Magnolia's inzamelen en inzicht krijgen in de densiteit, het voorkomen en conservatiemogelijkheden in de bezochte landen.

Haïti: 15 dagen: *Magnolia ekmanii*, *Magnolia domingensis (?), Magnolia emarginata (?)*

Marie-Stéphanie neemt haar taak als supervisor niet licht op: ze voegt zich bij me voor het eerste, en ook meeste problematische land: Haïti. Als je denkt aan Haïti, dan denk je aan de aardbeving van 2010 en extreme armoede: Haïti is het armste Westerse land. In lijn met deze kennis is de website van buitenlandse zaken ook niet zo positief over reizen naar dit land. We treffen dus voldoende voorzorgen: met twee sta je sterker dan alleen, we regelen een mannelijke tolk: Roland Trézil die Engels, Frans en Creools spreekt, we nemen de nodige vaccinaties en pillen (malaria!), we vullen onze valies met muggenwerende kledij en contacteren de Haïtiaanse minister van Landbouw.

Ik vlieg uit België naar de Dominicaanse Republiek, Marie komt aan uit Mexico. Samen steken we de grens over met de bus: Caribe Tours. Aan de grens worden we tweemaal gecontroleerd, er zijn "grenshoppers" volop in verkoop, gewapende mannen en veel chaos. Eenmaal de grens over zien we wederopbouw van huizen, veel vuilnis overal, veel straatverkoop en veel drukte. Zeker in Port-au-Prince, de hoofdstad van Haïti. Hier halen we onze jeep van dienst op en vertrokken we naar Les Cayes, een stad in het Zuidwesten.

In Les Cayes ontmoeten we William Cinea, de beheerder van de enige plantentuin in Haïti en een grote hulp in de volledige organisatie. We bezoeken de plantentuin en bespreken onze samenwerking. Later ontmoeten we ook Jean-François Orilién Beauduy, lid van de Société Audubon d´Haiti. Hij zal samen met ons de bergen in het Massif de La Hotte trotseren en de logistiek van deze onderneming regelen. Bij het tonen van de Magnolia-foto´s knikt hij instemmend: deze planten zullen we vinden. Dit is een grote geruststelling, als je weet dat van de vegetatie in heel Haïti er nog maar 1-2 % overblijft wegens ontbossing: houtskool is hier het voornaamste brandstof om te koken.

We slaan eten in voor 4 dagen en rijden naar Tiburon met Roland en Beauduy. Tiburon is een klein dorpje nog meer westelijk gelegen dan Les Cayes. Aangezien dit geen toeristisch dorpje is, wordt er ons een slaapplaats en avondmaaltijd aangeboden bij de schoonmoeder van Beauduy. Geen elektriciteit, een basis maaltijd, véél kakkerlakken en één bed delen. De volgende dag brengt Cétout ons met onze jeep naar Sèvre, een dorpje dat op één uur van Tiburon ligt. Vanuit Sevre start onze klim van de Morne Grand-Bois. In Sevre aangekomen, kwam het hele dorp zich aanbieden om ons water en bagage de berg op te dragen. Er wordt

heel wat geschreeuwd, maar uiteindelijk hebben we een equipe bij elkaar. De beklimming van de Morne Grand-Bois start. De lokale dragers rennen haast de berg op, op half kapotte slippers, terwijl ze amper water drinken. In contrast met onze equipe, doen Marie en ik het trager, met liters water en een regelmatige pauze. Dat vinden de Haïtianen best wel amusant. Na meer dan vijf uur stijgen zijn we dicht bij de top (900m hoogte) en spot ik de eerste *Magnolia ekmanii*. Met veel vreugde verzamelen we de eerste herbariumstaal. Hierna spotten we er nog talrijke meer. Het inzamelen zal voor wat later zijn, we moeten eerst het kamp opzetten en ons inzamelmateriaal klaarmaken. Een dik half uur later hadden we een open plek bereikt waar we de tenten opzetten en de silica-gel in ziplock zakjes gieten. Het was net na de middag, dus we hadden nog tijd om in te zamelen tot aan het tentenkamp. We keren terug naar onze eerste *Magnolia* en beginnen de populatiestalen in te zamelen. Naast het wandelpad vinden we vele jongere exemplaren, die profiteren van het zonlicht. Opvallend is ook, dat er regeneratie gaande is! Individuen die duidelijk omgekapt zijn, hebben vele nieuwe scheuten aangemaakt uit de overgebleven basis van de tronk. Na het inzamelen, genieten we van een lekkere gekookte maaltijd door de kokkin, een vrouw die wat verder op de berg woont.

De volgende dag op Morne Grand-Bois komt Beauduy in de ochtend trots aan met een *Magnolia* bloem, nog-net-niet-open. Tijdens het ontbijt open ik de bloem en bestudeer ik haar morfologie. Vervolgens zamelen we verder in. De locals vinden ons werk erg interessant en helpen naarstig mee: de ene brengt bladeren van bij mij aan de boom tot Marie op het pad en telt luidop mee, de andere is al op zoek naar de volgende boom en nog anderen observeren de show. We maten de diameter op borsthoogte van elke boom, schatten de hoogte en noteren opmerkingen (beschadigd, oude vruchten onder de boom, regeneratie). We vinden in totaal meer dan 100 Magnolia's en moeten goochelen met de beperkte, onderschatte hoeveelheid silica-gel: ´s avonds na het inzamelen stalen versteken, zorgen dat de zakjes luchtdicht zijn en dat de silica-gel goed verspreid rond de staal zit.

De laatste dag van Morne Grand-Bois: de afdaling. Marie te muilezel, Emily te voet. We kunnen het niet laten en zamelen nog een tiental individuen in op de terugweg. Het water was praktisch op en beneden aangekomen in het dorp drinken we menige kokosnoot leeg om onze dorst te lessen. We keren terug naar Tiburon, waar we uitrusten, ons wassen, eten en Marie aan de kindjes stylo`s en schriftjes uitdeelt.

Na de batterijen te hebben opgeladen, beginnen we aan onze tweede, makkelijkere klim: Morne Mansinte, een dichter bij de kust gelegen bergtop. Cétout bracht Marie en mij zover mogelijk met de moto (inderdaad, met 3 op 1 motorfiets) op de berg, de Haitianen komen te voet achter. Eenmaal Marie en ik te voet begonnen waren aan de klim, haalden ze ons al snel in. Opnieuw vinden we Magnolia's, maar niet zo abundant: we overbruggen nogal wat afstand

voor 20 individuen. Het klimaat was ook veel vochtiger dan Morne Grand-Bois en de menselijke verstoring is hier niet enkel houtkap, er is ook landbouw gaande op deze bergtop.

Eenmaal terug, reden we naar Les Cayes, we nemen afscheid van William en we bereiden ons voor om naar het Noorden te reizen. Beauduy gaat mee tot in Port-au-Prince. Daarna rijden Roland, Marie en ik door tot Pilate, een dorpje in Massif du Nord. Onderweg zien we vele kale bergtoppen, de 1% vegetatie zit blijkbaar voornamelijk in het Zuiden. In Pilate zorgt Lucson Ilfrene voor ons: deze agronomie student brengt ons naar een lokaal restaurant en regelt een slaapplaats voor ons bij erg vriendelijke oude Haïtiaanse vrouwtjes. De volgende ochtend maken we ons klaar om Magnolia's te vinden in het Noordelijk gebergte. Het is een frustrerende dag; we kunnen niemand vinden die ons kon helpen, de berg Morne Maleuvre (een herbariumlocatie) is praktisch kaal en de berg die er groen uitzag met een 'cloud forest' (ideale habitat voor de Magnolia's hier) die we wilden exploreren, is niemand bekend noch bij naam, noch van ervaring. Roland, onze tolk en bodyguard voelt zich niet veilig en Lucson houdt zich afzijdig gedurende de hele dag. Op het einde van deze dag besluiten we dat hier in het Noorden niet veel meer succes kunnen boeken. We plannen om de volgende dag terug te keren naar Port-au-Prince, waarbij we Pétite-Rivière de L'Artibonite zullen passeren, een andere herbariumlocatie in het Centraal Massief.

De volgende dag zijn we klaar voor vertrek, maar de oude vrouwtjes van Pilate stonden erop dat we eerst de lokale kerk en school bezochten. Hier hebben Waalse nonnetjes de Haïtianen destijds geholpen met onderwijs en religieuze ondersteuning, wat hun liefde voor ons Belgen verklaart. De oude vrouwtjes vertelden ons dat er in Marmelade, een dorpje in het Massif du Nord, koffieplantages zijn, waardoor we op onze terugweg besloten hier een kijkje te gaan nemen. Jammer genoeg is het landschap volledig kaal en de grond zodanig arm dat er nu ook geen koffie meer verbouwd wordt in deze streek. We reden verder naar Pétite-Rivière de L'Artibonite en hier doet zich hetzelfde probleem zich voor als in Pilate: we geraken niet georganiseerd, de lokale bevolking kan ons niet helpen. Tijd om de staalname in Haïti af te ronden: we rijden in het donker naar Port-au-Prince, een ware hel gezien het verkeer (zéker in de hoofdstad) een complete chaos is. Als afsluiter, rustten we de zondag uit en regelen onze export permit op maandag.

Wat algemene indrukken betreft in Haïti, is er 1 woord dat deze ervaring kan beschrijven: **controverse.** Ondanks dat de mensen zo arm zijn, zijn de prijzen voor bezoekers hoog (hotels, auto, taxi´s, lonen van de lokale helpende handen). Ondanks dat bijna alle vegetatie is kaalgekapt, vonden we toch meer dan we ooit hadden durven dromen (althans in het Zuiden). Ondanks dat we ons zo welkom voelden in het Zuiden door de vriendelijke mentaliteit en gastvrijheid, voelden we ons hierna onveilig en hulpeloos in het Noorden. Blanke bezoekers

zijn in het Noorden een anomalie, wat leidde tot heel wat gestaar, gewijs, gespot en discriminatie. Daarnaast moet ik ook nog vermelden, dat het verkeer in Haïti niet te onderschatten valt! Assertiviteit is troef en hoe groter je auto, hoe veiliger je bent.

Wat de Magnolia's in Haïti betreft: hopelijk kan de *Magnolia ekmanii* in Massif de La Hotte veilig gesteld worden met behulp van dit doctoraat, en hopelijk kunnen we een tweede bezoek organiseren, waarin we de zoektocht door naar populaties van *Magnolia emarginata* of *Magnolia domingensis* in de gebergten van Noord- en Centraal-Haïti kunnen voortzetten. Voorlopig zijn deze twee soorten genoteerd als uitgestorven, voor *Magnolia domingensis* althans in Haïti.

De Dominicaanse Republiek: 11 dagen: *Magnolia pallescens, Magnolia hamorii, Magnolia domingensis*

Na Haïti keren we half voldaan terug naar de Dominicaanse Republiek met de bus. Aan de grenscontrole zien ze onze feloranje gekleurde silica-gel. De controlediensten denken dat deze dienen voor recyclage en maken er verder geen probleem van.

Eenmaal in de DR terug, bezoeken we de Jardín Botánico van Santo Domingo, de hoofdstad. Hier ontmoeten we Rosa, een jonge onderzoekster die aan het hoofd staat van de conservatie-initiatieven in de plantentuin. We maken praktische afspraken met haar en we regelen de autohuur voor dit land. Ik neem afscheid van Marie en wissel haar in voor Majela, mijn Cubaanse collega. Zij komt het team versterken en brengt ook enkele belangrijke stalen mee uit Cuba: win-win! Het team voor de komende dagen is bijna compleet: naast Majela, Rosa en mijzelf voegt ook Victor, een Spaanse student die zijn stage in de Dominicaanse Republiek doet, zich bij ons. Vier jonge onderzoekers beginnen aan het tiendaagse avontuur. Rosa is een vrouw van haar woord en volgt mijn uitgestippelde schema vlekkeloos, ze heeft alle permits geregeld en regelt ook de lokale gidsen. Het is een verademing, en zo kan ik mij volledig op het inzamelen focussen.

We bezoeken Ebano Verde, een beschermd gebied genoemd naar de lokale naam van *Magnolia pallescens*. We installeren ons in het biologisch veldstation, Rosa trommelt een lokale gids op en we bezoeken de eerste plaats: Casabito. We laten ons (iets teveel) gaan: we verzamelen meer dan 100 Magnolia's in op deze dag. De volgende dag bezoeken we Loma de la Sal, ook binnen Ebano Verde. We ontmoeten onze gemotoriseerde gids, die ons leidt tot aan een rivier, die redelijk hoog staat, maar nog over te steken valt volgens zijn inschatting. Mijn eerste echte rivieroversteek met de 4x4 maak ik onvergetelijk: ik rijd onze 4x4 vast in de zachte rivierbodem. Heb ik toch wel de 4x4 stand niet vergeten opzetten zeker? Het water stroomt binnen, er heerst terechte paniek op de achterbak – maar vooraan blijven Rosa en ik kalm: allemaal de auto uit door de ramen en duwen maar! Majela slaat in paniek de deur open

– “No, close the door! Go through the window, Majela!”. Onvergetelijk! Door deze stunt is de auto ook eens goed gewassen aan de binnenkant. De gids snelt ons ook te hulp en met vier personen die duwen, krijgen we de auto uit de rivier. Nadat de auto wat gedroogd is (water in de uitlaat, ai ai!), rijden we voorzichtig verder tot wanneer het pad te smal wordt voor de auto. Vanaf hier gaan we verder te voet. We zamelen een 40-tal Magnolia’s in tijdens deze dag. Opmerkelijk is de extreme groei van varens in dit gebied, wat waarschijnlijk de Magnolia’s en andere planten een gebrek aan juvenielen in hun populaties kan verklaren. Op de terugweg moeten we weer de rivier oversteken, er wordt een man te paard meegestuurd om ons bij te staan, in het geval ik ons weer klemrijd. Gelukkig is dit niet nodig, deze keer geraken we vlot de rivier door – iedereen hield wel zijn adem in natuurlijk. Terug aangekomen in het veldstation maken we ons in de avond klaar om de dag erna naar het volgende gebied te gaan: een lekkere ijskoude douche, spaghetti en zoete dromen onder het muskietennet.

De volgende stop is Valle Nuevo waar we ook *Magnolia pallescens* verwachten. Victor neemt het stuur over en eenmaal we boven aankomen, blijkt dat we de sleutel van het veldstation beneden aan de berg moesten hebben opgehaald. Kleine logistiek foutje. Victor en Rosa keren terug, Majela en ik verzamelen ondertussen al een 30-tal populatiestalen. Onze gids is deze keer aan de passieve kant, waardoor Majela en ik ons ontpoppen tot boomklimmers van dienst. Enkele exemplaren zijn jammer genoeg te hoog om bladeren van te verzamelen. Bij het breken van de avond rijden we verder naar het veldstation, een prachtig gebouw met comfortabele bedden, keuken en gezellig kampvuur. Er is toevallig een natuurfotografencursus aan de gang en we worden uitgenodigd om mee te genieten van een soort kikkererwtenstoofpot aan het kampvuur.

Valle Nuevo krijgt nog een tweede dag: in de voormiddag verzamelen we nog waar we de dag ervoor gestopt waren. In de namiddag rijden we naar de andere kant van de vallei. Hier verzamelen opnieuw een 40-tal stalen in en hebben we een ‘naar adem snakkend’ moment. Onze behulpzame lokale gids valt uit een boom waarbij een scherpe houtspies net tussen zijn benen terecht kwam. Gelukkig zijn we hier er met de schrik vanaf gekomen en we besluiten na dit incident dat het genoeg is geweest voor Valle Nuevo.

Na de staalname van vier populaties *Magnolia pallescens* in het centrum van de Dominicaanse Republiek, trekken we naar het Zuidwesten, naar een gebergte dicht bij de Haïtiaanse grens, met veel armoede: Sierra de Bahoruco. Hier komt *Magnolia hamorii* voor. We rijden naar Polo, zetten onze spullen af in het lokale gemeentegebouw en rijden door tot de Magnolia-populatie van Cortico. We zamelen 50 individuen in, waarbij we spontaan geholpen worden door een vrouw, haar zoontje en puppy, samen met onze twee gidsen. Ik leer hier dat er vuurmieren zijn die uit de boom kunnen vallen en houd aan deze les vervelende brandwonden in mijn nek

over. De namiddag is snel om en we keren terug. Bij het organiseren van de stalen, stuiten we op een vervelend probleem: de silica-gel is allemaal verzadigd of in gebruik. Rosa en Majela verzekeren mij, dat je deze in de microgolf of de kookpan kan “resetten”. Dat staat dus als eerste item op het programma voor de volgende dag.

De volgende dag stoten we op een volgende hindernis: de elektriciteit is uitgevallen in Polo. We rijden alvast door naar een stadje dicht bij de volgende locatie waar we *Magnolia hamorii* wensen in te zamelen. Hier is er jammer genoeg ook geen elektriciteit. Uiteindelijk gaan we aan de slag met de pan bij een vriendelijke vrouw die ons haar keuken liet gebruiken. Het “koken” duurt lang en we spenderen een hele dag aan het opnieuw gebruiksklaar maken van de silica-gel. In de avond rijden we door naar het veldstation van Cachote. Opnieuw een prachtig veldstation, deze keer een rustieke vestiging met stapelbedden van 3 verdiepen hoog, regenwater om je mee te wassen en buitenkeuken. Rosa regelt een kokkin, terwijl we de stalen herorganiseren met de nieuwe, geresette silica-gel. De volgende dag kunnen we eindelijk stalen beginnen verzamelen. Het pad is berijdbaar en we zamelen in van uit de laadbak van onze truck. Opnieuw een unieke ervaring. Na 50-stalen zijn we voldaan en keren we terug naar Santo Domingo. Hier besluit Victor dat het Magnolia-avontuur voor hem stopt, zijn ouders komen namelijk op bezoek uit Spanje. Majela en ik overnachten bij Rosa thuis, waar we opnieuw silica-gel drogen tot in de vroege uurtjes. We starten in de microgolfoven, die het na een zekere tijd begeeft, waarna we weer overschakelen op het kookvuur.

De laatste nog te vinden soort *Magnolia* van de Dominicaanse Republiek is *Magnolia domingensis.* Deze soort komt voor dichtbij Santo Domingo. De rit naar Loma Barbacoa is niet lang, maar we raken ’s ochtends moeilijk georganiseerd, waardoor we een halve dag verliezen. Eenmaal we in het dorpje van dienst aankomen, zo rond 16u, krijgen we een onverwacht tafereel te zien: heel het dorp is zwaar beschonken! We zoeken naar de contactpersoon van Rosa, in de hoop dat deze nuchter zou zijn. De auto wordt van binnenuit gesloten, drie jonge vrouwen in een dorp vol zatte mannen – de eerste keer dat we ons toch wel onveilig voelden hier in de Dominicaanse Republiek. Onze contactpersoon is helaas ook in de wind, maar toch maken we enkele afspraken voor morgen, om de berg op te gaan met muilezels. Hopelijk zal hij het zich de volgende dag nog herinneren. We besluiten enkele dorpen terug te rijden en daar een slaapplaats en mannelijke chaperon te zoeken. Victor heeft duidelijk te vroeg afscheid genomen! Een vriendelijke jongen: Samuel, helpt ons met de een slaapplaats te vinden en we vragen hem met ons mee voor de klim.

De volgende dag staan we zoals afgesproken terug in het ‘zattemansdorp’ dat intussen nuchter is geworden. Onze angsten ebben weg bij het zien van onze nuchtere gidsen. Blijkt dat het tafereel van gisteren te wijten is aan het feit dat het die vrijdag net de dag was dat de

lonen waren toegekomen, wat uitbundig moest worden gevierd. Rosa, Majela, Samuel en ik krijgen elk een muilezel en klimmen de Loma Barbacoa op. Jammer genoeg hebben de gidsen de tocht qua tijd onderschat: de berg opgaan, blijkt moeilijker dan verwacht. Er moet veel begroeiing worden weggekapt. Dit is langs een kant wel positief: de berg was al een jaar onbetreden! We doen er niet 3 uur over, maar 5 uur. Om 13 u komen we bij de eerste *Magnolia*. Een erg mooie soort met wollige beharing onderaan de grote, ronde bladeren. We verzamelen in totaal maar 24 individuen, waarna ik tegen mijn zin moet stoppen, zodat we voor het donker terug beneden zouden zijn. We komen maar net voor het donker terug beneden toe.

Op de laatste locatie die we bezoeken, huist ook *Magnolia domingensis*: Loma Rodriguez. We rijden met de auto zo ver mogelijk de berg op en klimmen erna voor anderhalf uur. De vegetatie van de berg is algemeen erg verstoord, er lopen ezels rond en er is veel weggekapt. We kunnen niet spreken van een bos, we hebben hier en daar een hoopje struiken. Ik heb het gevoel dat we hier geen Magnolia's zullen vinden, maar dit is onterecht! Tussen de struiken vinden we jonge bomen en omgevallen regenererende bomen. We verzamelen 50 individuen op deze sterk verstoorde berg. We bevinden ons nog steeds in cloud "forest" en na een droge 10 dagen, krijgen we als afsluiter een goede regenbui. Al bij al, verloopt de sampling vlot en de gidsen helpen ons goed verder. We keren zoals het hoort "moe maar voldaan" terug naar Rosa's appartement in Santo Domingo.

De laatste dag in de Dominicaanse Republiek staat opnieuw in het teken van organisatie. In de plantentuin hielpen Victor, Majela en Rosa mij met alle stalen om alle stalen te ordenen en klaar te maken voor de export permit, Rosa zal deze regelen terwijl ik in Puerto Rico ga inzamelen. Daarnaast is er weer een sessie 'silica-gel te drogen' – zodat ik in Puerto Rico aan de slag kan. Ik neem (voorlopig) afscheid van Rosa en Victor en rijd met Majela tot aan de luchthaven. We brengen de auto terug en nemen afscheid van elkaar: Majela vliegt terug naar Cuba en ik vlieg verder naar Puerto Rico.

Puerto Rico: 7 dagen: *Magnolia splendens, Magnolia portoricensis*

Ik kwam laat aan in Puerto Rico en moet nog wat wennen aan het alleen te zijn. Na eerst Marie-Stéphanie en Roland als gezelschap 15 dagen, en daarna tien dagen ondersteuning door een geweldig internationaal team van 4 enthousiaste biologen, is dit zwaarder dan verwacht. Ik haal mijn auto af aan de lokale AVIS en word verplicht een nieuwe vaardigheid aan te leren: rijden met een automatiek. Ik vertrek naar mijn hotel en bereid mij mentaal voor op een solo staalname.

De eerste dag bezoek ik de verschillende herbaria en hun curators. Een groot probleem dat mij hier parten speelt, is dat mijn telefoon niet werkte. Het praktisch regelen verloopt hierdoor gedurende de hele week stroef. Gelukkig zijn mijn contacten hier mijn redding: Eugenio, de

curator van het UPR herbarium komt mij halen wanneer ik de weg naar herbarium niet vind, José Sustache van het SJ herbarium en de DRNA forest service heeft de inzamel permit last-minute in orde gebracht en zelfs de kosten ervan voorgeschoten zodat de administratie op tijd klaar is, Christian Torres van het arboretum Parque Doña Ines toont mij het arboretum, regelt een permit voor mij om zaden in te zamelen en leent mij zijn lange knipschaar uit, Jim Ackerman, Franklin Axelrod en Fabiola Areces van het UPRRP herbarium regelen mijn permit voor het inzamelen in El Yunque en bieden hun herbariumfaciliteiten aan om mijn stalen te drogen. Waar zou ik toch gestaan hebben zonder al deze behulpzame botanici?

Zoals vermeld: de rode draad tijdens deze staalname: de organisatie verliep stroef en de tijd was beperkt. Fabiola Areces, een vrouw van Cubaanse afkomst die haar doctoraat doet in Puerto Rico, en haar man: Victor gaan met mij mee naar El Yunque National Forest op de eerste inzameldag. We wandelen El Toro trail af en vinden hier een 30-tal *Magnolia splendens* individuen. Tijdens het inzamelen ontmoet ik enkele hoge Scleria's die mooie snijwonden op mijn handen en zelfs neus achterlaten – mijn respect voor Kenneth (collega Zaadplanten UGent die op deze planten werkt) neemt toe! Het is in het algemeen een dag met meerdere verwondingen: ik verlies mijn evenwicht ergens tijdens het inzamelen, waarna ik mijn hand pal op enkele bromelia's duwde. De stekels van dit exemplaar moet ik de komende dagen hier en daar uit mijn hand prutsen, het geheel is ook gaan ontsteken: niet erg fraai – maar uiteindelijk geraakt alles wel mooi genezen. De avond valt en ik kan Luis Rivera niet bereiken, de man waarmee ik morgen naar een andere plaats in El Yunque wil gaan. Ik moet mijn plannen compleet omgooien, maar het is al te laat om te beginnen rondbellen. First thing in the morning, dus. Het reorganiseren van de silica-stalen alleen uitvoeren, neemt veel tijd in beslag en berooft mij van 2 uren slaap.

De volgende ochtend, vanaf een 'belproof' uur, bel ik eerst naar Gerardo, de forest manager van Toro Negro, het dichtstbijzijnde volgende bos. Hij kan niet deze dag – jammer. Volgende op de lijst: Rubén, de ex-forest manager van Guilarte. Ik heb prijs: Rubén heeft tijd vandaag! Opnieuw wat geklungel: ik vind Rubén niet op de plaats van afspraak, dit in combinatie met een niet werkende telefoon en beperkte Spaanse communicatievaardigheden blijkt echt zeer onpraktisch. Ik beland uiteindelijk in een tegelwinkel, waar de tweetalige kassier zo vriendelijk is om met enkele telefoontjes Rubén tot bij mij te krijgen. Rubén is een zeer vriendelijke, behulpzame, oude man, die weinig Engels kent. Ik haal mijn tien-lessen Spaans boven en Rubén zijn van-het-middelbaar geleden Engels en onze communicatie blijkt toch, op een of andere manier, te lukken. Hij brengt mij naar een plaats waar we een vijftiental Magnolia's vinden, vertelt me over de lokale flora, bezorgt mij spontaan zijn artikel (dat ik nooit op internet zou hebben gevonden, zeer handig!) met observaties over *Magnolia portoricensis* en helpt me

– ondanks zijn leeftijd - met het hanteren van de lange knipschaar. Hierna bezoeken we het kantoor van de huidige forest manager: Amarilys Soto en het begint het te gieten.

Ter informatie: de gehele reis valt in april-mei, de start van het regenseizoen in deze streken. Ik heb tot nu toe geluk gehad: het regenseizoen blijft uit. Voor de lokale bevolking en flora is dit natuurlijk minder: in Puerto Rico is er zelfs waterschaarste op het moment van mijn bezoek en krijgt de helft van San Juan (de hoofdstad) de ene dag water, de andere helft de andere dag. Waar we nu zitten in het verhaal, is het toch al goed begin mei en zoals u las: het regenseizoen is eindelijk begonnen. We schuilen in het kantoor van Amarylis, proberen wat te communiceren en ik probeer al enkele mensen op te bellen om de volgende dagen te regelen. Rubén tekent mij ook een kaartje uit voor een ander domein: Maricao State Forest, waar hij de Magnolia's ook weet staan, maar zich niet bij mij zal kunnen voegen.

Eenmaal de regen mindert, verzamelen we nog 4 Magnolia's maar hierna moeten we stoppen. Een set met 18 Magnolia's is wel aan de karige kant voor een populatie-staalname: ik ben niet tevreden met het aantal. Maar goed, ik neem afscheid van Rubén en rijd verder naar een haciënda in Toro Negro, waar ik de volgende dag een populatie van *Magnolia portoricensis* zal verzamelen, samen met Gerardo. Wanneer ik deze haciënda vind, blijkt deze verlaten te zijn en ik vind ook geen eigenaar. Ik heb mij al neergelegd bij het idee in de auto te slapen, maar bij het verkennen van de mogelijke "entradas", blijkt er toch wel een raampje open te staan. Ik kan de deur van buiten uit openen en zo heb ik toch een lekkere warme douche kunnen nemen en illegale maar comfortabele nacht doorgebracht.

De volgende dag zoek ik het kantoor van Gerardo op. Deze forest manager is bijzonder enthousiast en rijdt mij rond in zijn truck naar verschillende Magnolia's. Hijzelf beschikt over een knipschaar van wel tien meter, die zijn nut goed bewijst tijdens deze staalname. Het regenseizoen is jammer genoeg in volle glorie ondertussen en in de namiddag nemen we stalen in de gietende regen, waarna we ze drogen met de autoverwarming. De regen vertraagt het werk aanzienlijk en we halen ook maar 18 stalen voor deze dag. Ik contacteer Omar Monsegur voor de volgende dag met mij mee te gaan naar Maricao, en daarna zoek ik naar mijn hotel. Een hotel regelen ging behoorlijk moeilijk, en na de gratis nacht betaal ik mij nu blauw aan een kamer in de Holiday Inn, naast het lokale Casino. Ik heb nu wel een zeer comfortabel bed en microgolf: ik kan weer wat silica-gel drogen!

De volgende dag haast ik mij naar de afspraakplaats met Omar in Maricao. Hij is niet te bespeuren. Een bewakingsagent van een naburige camping laat mij zijn telefoon gebruiken, Omar neemt niet op en ik voel me nogal verloren. Ik vraag hulp aan enkele mannen van de camping met mijn Magnolia-foto's en gebrekkig Spaans, maar tevergeefs: de man die mij probeert helpen brengt mij naar een boom die absoluut niet op een *Magnolia* lijkt. Na een

tweede mislukte poging om Omar te bereiken, dringt het tot mij door: ik ga écht alleen op pad moeten gaan. Ik rijd de weg af en probeerde na te denken hoe dit aan te pakken. Plots, naast de autoweg, zie ik een *Magnolia portoricensis,* en niet zomaar een individu, een individu in volle bloei! Ik stopt de auto, neem stalen en metingen van de boom en bestudeer de bloem. Het is dan toch geen verloren dag: ik heb een bloeiende *Magnolia* gevonden. Door deze toevallige vondst begin ik wat te kalmeren en kan ik mijzelf weer bijeenrapen. Naast dit exemplaar vind ik er nog 3 individuen gewoon naast de weg. Ik begin al te hopen: zal ik er op mijn eentje 18 kunnen vinden? Het schiet mij te binnen: Rubén! Ik begin te zoeken tussen mijn papieren en vind de schets terug. Ai, had ik maar beter geluisterd naar zijn uitleg: de schets was nogal abstract. Ik rijd een beetje verder en vind een kantoor. Ik weet nog steeds niet of de aanwezige persoon de huidige forest manager is van deze State Forest, maar hij beweert bij hoog en laag dat hij zijn post niet kan verlaten om mij te helpen. Ik overtuig hem toch om voor mij nog eens te bellen met Rubén, om zo meer Magnolia's te vinden. Zo kom ik te weten dat er nog drie individuen dicht bij het kantoor moeten zijn. Ik vind er uiteindelijk twee terug, en tegelijk krijg ik bezoek van een oude vriend: de stortregen.

Intussen heb ik mijzelf er wel al van overtuigd om alles te geven vandaag. In de auto droog ik mijn stalen met de autoverwarming en bestudeer ik Rubén zijn kaart. De observatietoren vind ik gemakkelijk terug en ik ga het bos achter de toren in: hier zouden er moeten zijn, volgens Rubén. Ik vind vier individuen, maar ik durf niet te ver afdalen: stel dat ik mijn voet verzwik, dan kan ik en de hulpdiensten niet bereiken, en was ik helemaal alleen, ver van de weg. Zeker wanneer het weer begint te gieten, besluit ik toch terug naar de veilige auto te gaan. Ik heb al 10 stalen, ik ben trots op mijzelf.

Op het kaartje van Rubén staan er ook Magnolia's aangeduid tussen de camping en de toren. Terug in de camping waar ik tevergeefs Omar had proberen bellen, ruil ik mijn paspoort in voor de sleutel van dit kampeerterrein en ik begin te zoeken. Op het pad dat leidt naar een prachtig uitkijkpunt vind ik enkele Magnolia's. Jammer genoeg staat hier wel een puntige omheining van anderhalve meter hoog naast het pad. Ik klim hierover en verzamel zo nog drie individuen in. Opnieuw begint het te gieten; wel vervelend zo dat regenseizoen. Ik vind nog een individu in het midden van dit campingterrein, tussen de tenten. Ik zit nu aan 14 individuen, en het is al laat in de namiddag. Ik heb mij er bijna bij neergelegd dat het er 14 gingen blijven, maar bij het verlaten van deze camping, vind ik er nog vier grote individuen langs het grindpad. Ik kan het niet geloven: ik heb 18 Magnolia's gevonden, op mijn eentje!

Volledig uitgeput van deze dag, besluit ik mij toch nog te wagen aan de rit van drie uur, om terug veilig in mijn hotel in San Juan te slapen en niet in de peperdure Holiday Inn. Nadat ik al mijn spullen heb afgezet in het hotel, ga ik nog even langs bij Fabiola om de sleutel van het

UPRRP-herbarium. Daar plaats ik mijn herbariumstalen in de droogoven. Bij het terugbrengen van de sleutel spreken we af voor de volgende dag: Fabiola heeft Luis voor mij kunnen bereiken en enkele locaties gekregen. Luis zelf is niet van plan mij verder te helpen, maar Fabiola bewijst maar nog eens wat voor een behulpzame vrouw ze is: ze gaat de volgende dag nog eens met mij naar El Yunque. Blij dat ik de volgende dag niet alleen moet ronddwalen, ga ik terug naar mijn hotel. Natuurlijk kan ik mijn bed nog niet in: stalen versteken en silica-gel drogen zijn nog steeds deel van de avondlijke routine.

De laatste inzameldag in Puerto Rico eindigen we weer met het zoeken naar *Magnolia splendens.* Deze keer bezoeken we een ander deel van het El Yunque National Forest: het toeristische gedeelte. Het gebied en de wandelingen zijn werkelijk mooi, maar de Magnolia's zijn helaas schaars. We wandelen grote afstanden om uiteindelijk maar 9 individuen te vinden! De dag gaat zo snel voorbij en zo eindigt mijn staalname in Puerto Rico. Ik trakteer Fabiola en Victor op pizza's als bedanking en ga slapen.

De laatste dag in Puerto Rico maak ik mijn valies, ik breng de auto terug naar AVIS en ik onderwerp mezelf weer aan de luchthavenchecks. Vreemd genoeg is er in dit land, deel van de USA, geen equivalent aan een export permit en met veel schrik laat ik mijn bagage inchecken. Terwijl ik zit te wachten aan mijn terminal, krijg ik dan ook bezoek van een Air Marshall. Hij vraagt mijn naam en toont een foto van mijn silica-gel op zijn IPhone: "Can you explain me what this is ma'am?". Na mijn uitleg blijkt alles dan toch in orde en mocht ik het vliegtuig op met mijn dierbare stalen.

Op de luchthaven van Santo Domingo zou Rosa mij opwachten, zoals afgesproken. Over het algemeen zijn ze nogal laat in de Caraïben, dus wanneer Rosa er nog niet is, maak ik mij nog geen zorgen. Na meer dan een half uur wachten, word ik benaderd door een agent van de luchthaven die zich zorgen maakte om deze blanke, jonge, vrouwelijke en vooral eenzame toerist. Hij slaat een babbeltje met mij en belde Rosa voor mij op. Blijkt dat ze dacht dat mijn vlucht vertraging had en ze dus nog niet eens vertrokken is. Na een maand verblijf in de Caraïben neem je de mentaliteit wel wat over en maak je je over zo'n dingen noch amper zorgen: ze komt, dat is het belangrijkste. Ik mag weer bij Rosa thuis slapen op de luchtmatras.

De laatste dag breekt aan, en ook deze dag is niet zonder de nodige spanningen. Ik moet alle stalen met hun permits ordenen in de zéér grote rugzak en daarnaast nog de permit voor de stalen van de Dominicaanse Republiek ophalen. Victor gaat mee, waarmee hopelijke de communicatie soepel zal verlopen. Aangekomen in het ministerie blijkt deze permit helemaal niet in orde te zijn, en wordt er gepreciseerd dat dit proces normaal gezien zeker nog drie dagen nodig had. Jammer genoeg vertrekt mijn vlucht diezelfde avond en heb ik de juiste papieren nodig. Victor en ikzelf weten de ambtenaren te overtuigen om ons papiertje toch snel

te manoeuvreren tussen de departementen door, en zo komen we na twee uur buiten, met papier en door wat smalltalk tijdens het wachten, zelfs met een leuke kalender. Ten zeerste opgelucht ga ik naar de luchthaven en deze keer geraak ik zonder enige problemen het vliegtuig in: terug naar huis toe!

Deze 5 weken zal ik nooit vergeten…

Appendix 1.6 The Magnolias of the Caribbean: adventures in Hispaniola. **MODIFIED FROM: Veltjen E.** (2018) The Magnolias of the Caribbean: adventures in Hispaniola. Magnolia: The Journal of Magnolia Society International 53(103): 8–12.

In April 2015, I set off on my first botanical expedition in the context of my newly started PhD research titled “The Caribbean *Magnolia* species (Magnoliaceae): assessment of the genetic diversity and the underlying evolutionary history”. The aim was to scout for *Magnolia* trees in the Caribbean and collecting leaf samples to study their genetic diversity. My co-supervisor Marie-Stéphanie Samain and I decided to start with what we assumed to be the most challenging destination: Haiti. This country shares the island of Hispaniola with the Dominican Republic, and has rough past and present living conditions. The unstable political climate, reinforcing poverty, has led to natural resources being depleted at a fast rate. Including in these natural resources there are three endemic, native *Magnolia* species described from Haiti: *Magnolia domingensis, Magnolia ekmanii* and *Magnolia emarginata*. All are listed as Critically Endangered on the IUCN Red List (Rivers et al., 2016).

For the sampling in the Massif de la Hotte, in the South-West of Haiti, we were able to organize a lot in advance: our team was strengthened with help from the Société Audubon d’Haiti and Les Cayes Botanical Garden, and advised by botanists who had previously visited this region. For our first collecting location, we drove through rivers with our old rental truck, to get as close as possible to the location of which we knew housed a *Magnolia* population: Morne Grand Bois. We left the truck in a small village, and ascended the 2000 m high mountain ridge on foot starting from sea level, together with a whole team of local helping hands carrying our water, food and tents. It was amazing to see the local people carry all that weight up at such great speed on their worn-down flip-flops. They had some good laughs watching us go up the mountain for six hours at a, for them, slow pace. Slow and steady wins the race: Marie-Stéphanie and I had our first encounter with a *Magnolia ekmanii* before we set up camp that day. It was the first wild *Magnolia* that I ever saw and I could not have been more ecstatic. We spend two nights camping on the mountain and we were spoiled with two delicious dinners, prepared by our personal Haitian cook. During the day, we filled our time with collecting leaf samples, taking GPS coordinates, pressing herbarium voucher specimens and writing down our impressions on the health of this *Magnolia* population. We were pleased to find out that the *Magnolia* population on this mountain seemed to have a good number of individuals, and many young trees and saplings. Perhaps more importantly, the trees proved to have great regenerative strength: stumps of trees which had been completely cut down were shooting up vigorously. A few of the people that helped carrying up the camping gear and our cook were intrigued by our visit and helped us spontaneously with the collecting of leaf samples. On their way from the sampled tree to the road where Marie was labelling and packing the samples,

they loudly proclaimed the number of the sample they brought in: ...numéro trente-huit,…numéro trente-neuf, …quarante!! Locals referred to the *Magnolia ekmanii* trees as “abricôt marron” (brown apricot). We visited Hispaniola in April, which is too early in the season to see flowers or fruits. However, one morning, an enthusiastic team member found a young, closed flower, climbed up the tree to retrieve it and presented it with great pride. We opened and dissected the flower piece by piece, counting and documenting its flower parts. Later on, we found one more flower bud and a young fruit as well. This population in Morne Grand Bois was a beautiful first discovery, and in hindsight, we are very happy that we chose to start here.

PICTURE 1: A young flower of *Magnolia ekmanii*. The glabrous, leathery leaves have the typical twisted shape from being folded conduplicately in the stipules. Photo credit: Marie-Stéphanie Samain.

The unexpected, relatively healthy population of *Magnolia ekmanii* on the first location visited contrasted with the population of *Magnolia ekmanii* on the second location: Morne Mansinte. Here, we were brought up the mountain on the back of a motorcycle as far as possible, followed by ascending it further on foot. The location was retrieved from a herbarium record of T.A. Zanoni collected in 1985, and turned out to be a heavily logged and farmed peak, leaving some *Magnolia ekmanii* trees here and there between farmed areas. We were able to locate about twenty trees, and headed back down the mountain the next day.

Only one other locality record of *Magnolia ekmanii* is currently known, which is the type locality from the collection made by E.L. Ekman and was described as: “Jérémie, ridge between Lapineau and Morne Pain-de-Sucre”. Up to this date, no collections other than E.L. Ekman´s have been made at this type locality. We didn’t allocate time to search for the type location, as we had no certainty on the location of, or expertise to guide us to the Morne Pain de Sucre in the Jérémie department.

We left southern Haiti and continued our journey northward as there were two more species in Central and North Haiti on our list. Other than Marie perfecting her driving skills in a country where bold steering is the only way you can get somewhere, our search did not render any new Magnolias. The deforestation in these areas is even worse than in the South and we could not manage to find the two other recorded species, neither get organised in the same manner that it would be safe to explore mountain peaks that still held some remnants of (primary?) forest. The possibility thus exists that *Magnolia emarginata* and *Magnolia domingensis* are extinct in Haiti.

Although only one out of three endemic *Magnolia* species were found during our ten-day *Magnolia* hunt in Haiti, we should remain hopeful that there are still relict forest patches left that hold more populations of all three species. We severely encourage (well-organised!) botanical expeditions to assess the areas still holding forest in the country and all initiatives trying to protect them. In 2015, Haiti National Trust identified the Morne Grand Bois site as a National Park and raised awareness by announcing it as a biodiversity hotspot in Haiti. Hence, it is fair to say that the conservation of *Magnolia ekmanii* has taken its first baby-steps, although there is still a lot of work to be done as this site is not protected on the ground and no action has been undertaken (yet?!). If you would like to read more information on this specific area or other biodiversity hotspots in Haiti, or if you would like to contribute to the conservation initiative Haiti National Trust, please visit: https://www.haititrust.org/grand-bois. Currently, the trust is focusing its funds to help the families living in Morne Grand Bois to recover from the destruction left by category 5 hurricane Matthew in October 2016.

Following the ten days in Haiti, it was time to visit the Dominican Republic. This country also houses three native, endemic and threatened *Magnolia* species: *Magnolia domingensis*, *Magnolia hamorii* and *Magnolia pallescens*. Marie-Stéphanie had to head back to Mexico, and her place on the expedition was interchanged with Majela Hernández Rodríguez, a colleague from the National Botanical Garden in Havana, Cuba. Majela and I were treated with a well-organised sampling experience in the Dominican Republic. We were hosted by Rosa Rodriguez, who was at that time working as the conservation biologist at the Jardín Botánico Nacional Dr. Rafael M. Moscoso in Santo Domingo. Rosa had arranged everything perfectly

and I was in “expedition-heaven”. Victor, a Spanish student studying the diet of Dominican lizards, was tagging along as he needed to get acquainted with the Dominican flora. We evolved into a dynamic quarto that got well attuned to each other: each having his or her on task during sampling and cheering each other up whenever a hurdle came our way. Speaking of hurdles: no long tree pruner? We’ll hire local people that can freestyle climb trees! Stuck in the river with the 4x4 (oops, I forgot to put on the 4-wheel drive …)? Let’s all jump out of the car through the windows and push the car onto dry land! No dry silica gel left? We will ask a local if we can cook the silica-gel at her home! Tired of ascending all the mountains? Hire mules to get us up there! As I am writing down these adventures and the memories come back, I cannot help but smile from ear to ear.

Now let’s talk about *Magnolia*! The first species we encountered in the Dominican Republic was *Magnolia pallescens*, also called “Ebano Verde”. This species has short, golden hairs on the younger plant parts and on the lower surface of the leathery leaves. We visited four different locations in two protected areas: Ebano Verde Scientific Reserve (yes, named after ...) and Valle Nuevo. Little is known about the distribution of the species other than the four locations mentioned. In three out of four locations recruitment of the species appeared to be low, and forest is still being lost even though the areas are protected. Hence, this species is still assessed as Endangered on the IUCN Red List. Given the timing of this trip, flowers or fruits were not spotted for *Magnolia pallescens*. However, I did observe the interesting feature of the staminal appendages being stuck to the gynoecium of an old flower of a planted *Magnolia pallescens*, making that day extra special.

PICTURE 2: **A**: An old flower (or should I say young fruit?) of *Magnolia pallescens* of which the setaceous tips of the numerous stamens are embedded in the gynoecium, while their bases are already detached. **B**: The stipules that have recently opened showing the young curled up leaf, covered with golden hairs on the lower surface. **C**: A flower bud together with the contrast of the lower and upper leaf surface colours. Photo credit: Emily Veltjen.

Second up: the, also Endangered, *Magnolia hamorii*, a species described in detail by R.A. Howard in his 1948 publication, having glabrous leaves with an emarginate apex and unequal sized leaf lobes. The species occurs in the Sierra de Bahoruco National park and due to the proximity to Haiti and the poverty of the region, the forest in this region, is under a lot of pressure. We visited two different locations and found a good number of trees for the genetic sampling.

PICTURE 3: *Magnolia hamorii* with its glabrous, leathery leaves. Again, look at the twist in the leaves and the long stipules. Photo credit: Emily Veltjen.

Last, but not least is *Magnolia domingensis*, my personal favourite of all the Caribbean *Magnolia* species I have seen. The species is assessed as Critically Endangered, partly due to the fact that the type location of *Magnolia domingensis* is in Haiti, and no collections have been made since E.L. Ekman in 1925 in Haiti, which makes it a possibility that the Haitian populations of *M. domingensis* are now extinct. I have my doubts whether or not the *Magnolia domingensis* described from Haiti is the same as the specimens recorded with this name in the Dominican Republic. Nonetheless, there is a beautiful *Magnolia* in two mountains close to Santo Domingo, covered in fuzzy, golden hairs with a plump appearance and round leaves that needs some urgent conservation management. The *Magnolia* species in the Dominican

Republic, which is currently labelled as *Magnolia domingensis*, has been recorded in Loma Barbacoa and Loma Rodríguez. Both areas are not under any protection or conservation management. Loma Barbacoa is relatively remote and due to an underestimation of the time to go up the mountain, we were able to collect and document 24 trees only. We could not allocate an extra day to stay in this location, hence, all other information on this population remains a mystery until someone manages to spend more time up the mountain. Loma Rodríguez, on the other hand, was easy to access, but heavily logged and grazed. This made it possible to sample about 50 trees on this mountain.

PICTURE 4: *Magnolia domingensis* with long golden hairs covering the lower surface of the leaves, the stipules, the flower bracts and even the tepals. Photo credit: Emily Veltjen.

So, concluding, although the characteristically big white flowers or fruits with the bright red seeds were missing throughout the expedition, identification of *Magnolia* in Hispaniola was fairly easy: the large terminal stipules and typical twist of the leathery leaves made them easy to spot. Once a location was found that harbours one tree, other trees could be spotted easily in the vicinity. We always had to go up the mountains into the cloud forest (or its remnants): a wet environment which is somewhat protected from deforestation due to its more inaccessible nature. Our time in Hispaniola might have been short, but there is hope for all four species found. The known populations should be safeguarded as much as possible and we should continue to scout for more populations of all species known to occur on this island.

In the light of the current events, it must be stressed that the written impressions and findings are from 2015 and that I have not visited Hispaniola since. In the past two years, the Caribbean islands have had a hard time facing the hurricanes Matthew, Irma and Maria. It was heart-breaking watching the hurricanes pass by areas that I visited, which is not only Hispaniola, but also Cuba, Puerto Rico, Guadeloupe, Dominica, Martinique, Saint Lucia and Saint Vincent. My thoughts go out the families that have to recover from their losses. For the *Magnolia* trees, I am positive that they are still there and recovering slowly, as the Magnolias in the Caribbean have faced many storms over the centuries the species has sustained. For seeds and young trees that survived the winds, the years to come will be the time to establish their new territory. It does cross my mind often that, if the frequency and intensity of the hurricanes increase due to climate change, recovery in those islands will become more and more difficult and the Caribbean people and forests (including our Magnolias), have a big challenge ahead.

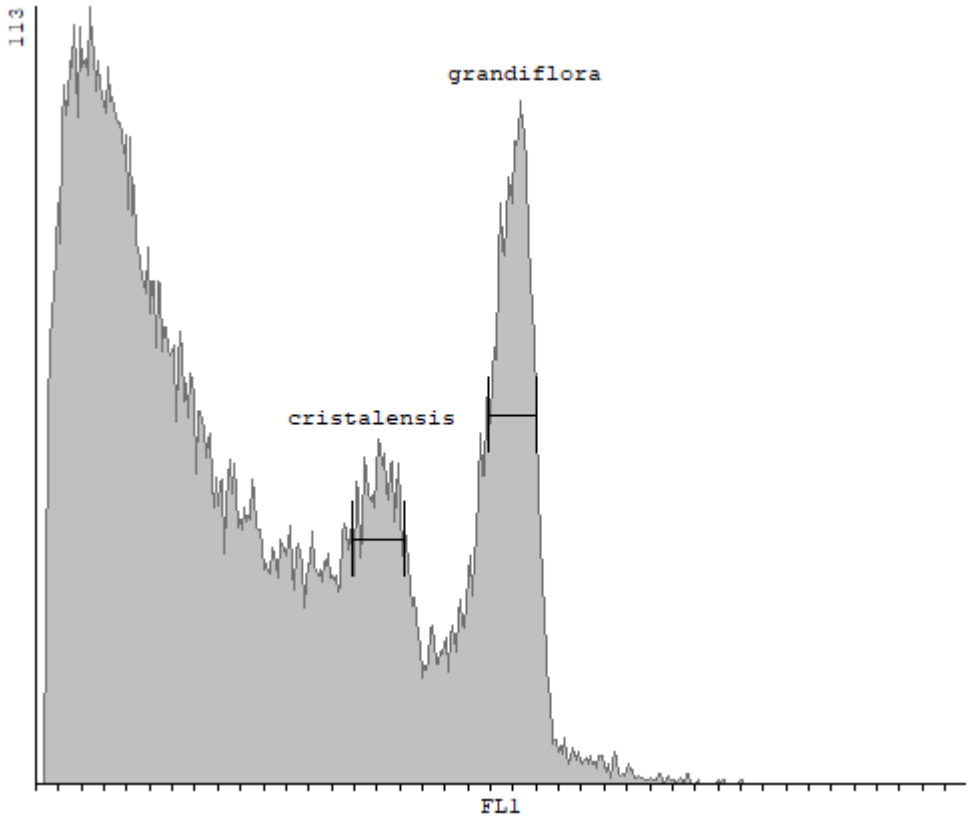
Acknowledgements

All the people and organizations mentioned in the text have been of such great help in the field, so it cannot be stressed enough that I am extremely grateful for all the support they provided. I would like to specifically mention three persons, not mentioned in the storyline; namely Joel Timyan, Roland Trézil and Jean-François Orilién Beauduy – they were indispensable. This fieldwork would not have been possible without the support of the Special Research Fund of the Ghent University, the Research Foundation Flanders and Fondation Franklinia. Also thank you Marie-Stéphanie Samain, Isabel Larridon, Martin Veltjen and Paul Goetghebeur for proof-reading this manuscript.

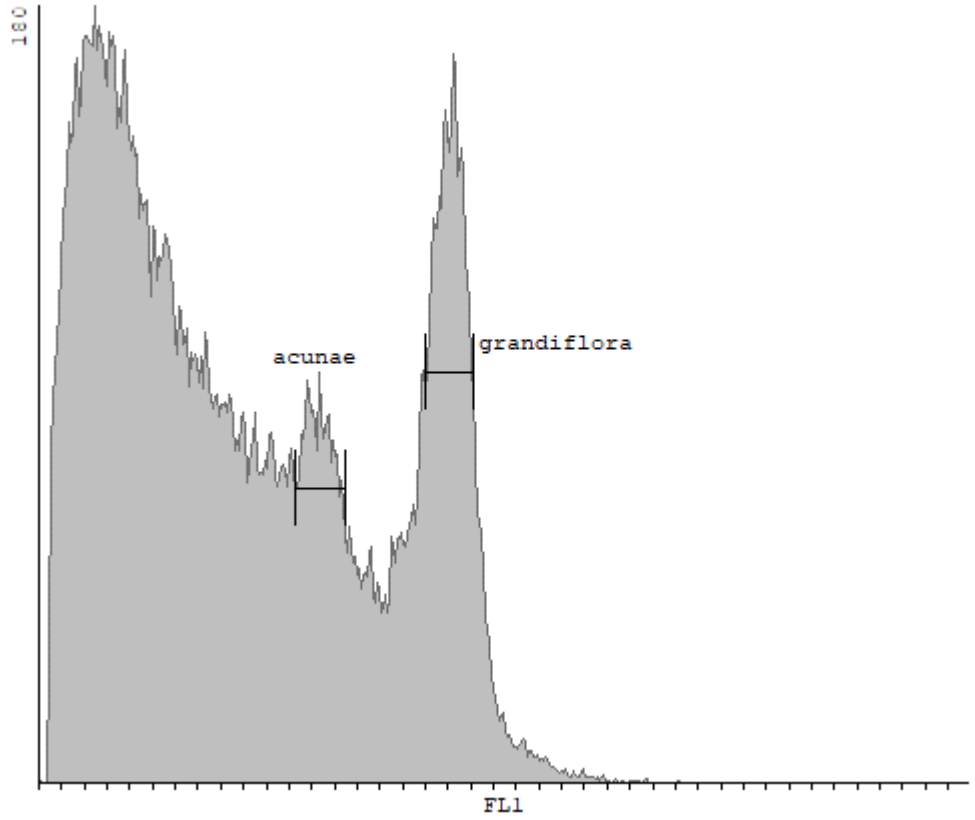
Appendix 2: Ploidy of the Caribbean Magnolias

Appendix 2.1 Raw data of the flow cytometry measurements. A fluorescence histogram is depicted per measurement, where the horizontal axis represents the parameter's signal value in channel numbers (FL1) and the vertical axis represents the number of events (nuclei) per channel number. The x-axis is scaled to be logarithmic. Internal standard: *Magnolia grandiflora* (hexaploid) IPEN-number: GENT-1900-2395 from the Ghent University Botanical Garden.

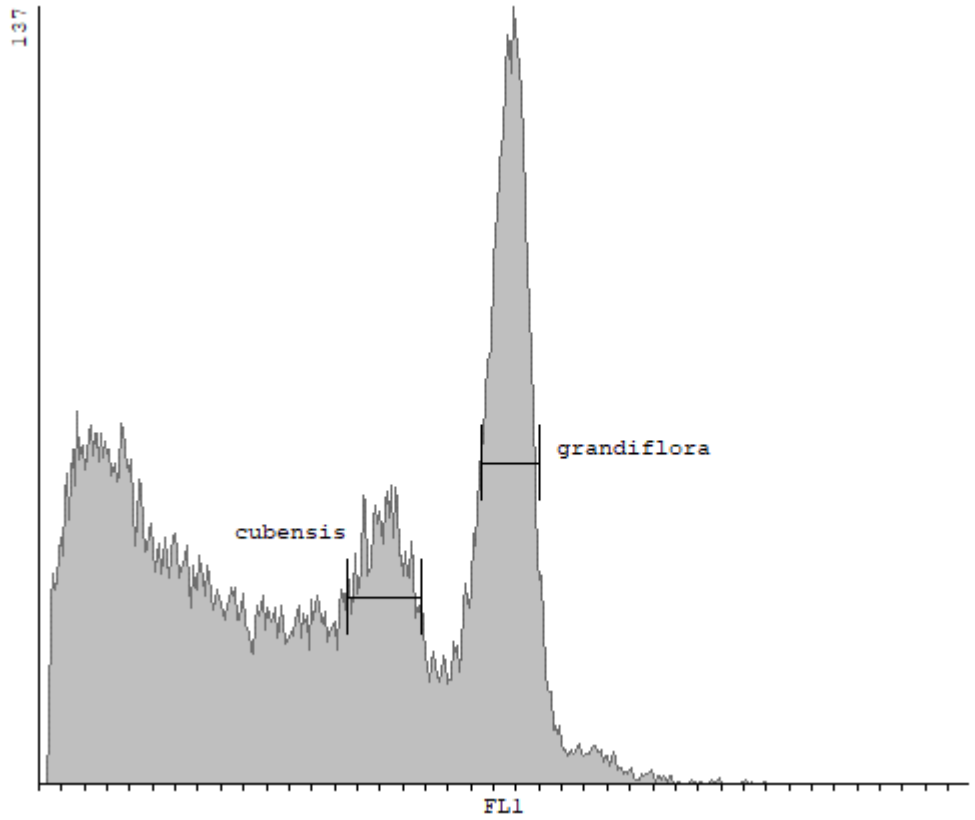
Appendix 2.1A *Magnolia cristalensis*, sample: MA608 (silica-gel dried leaf).



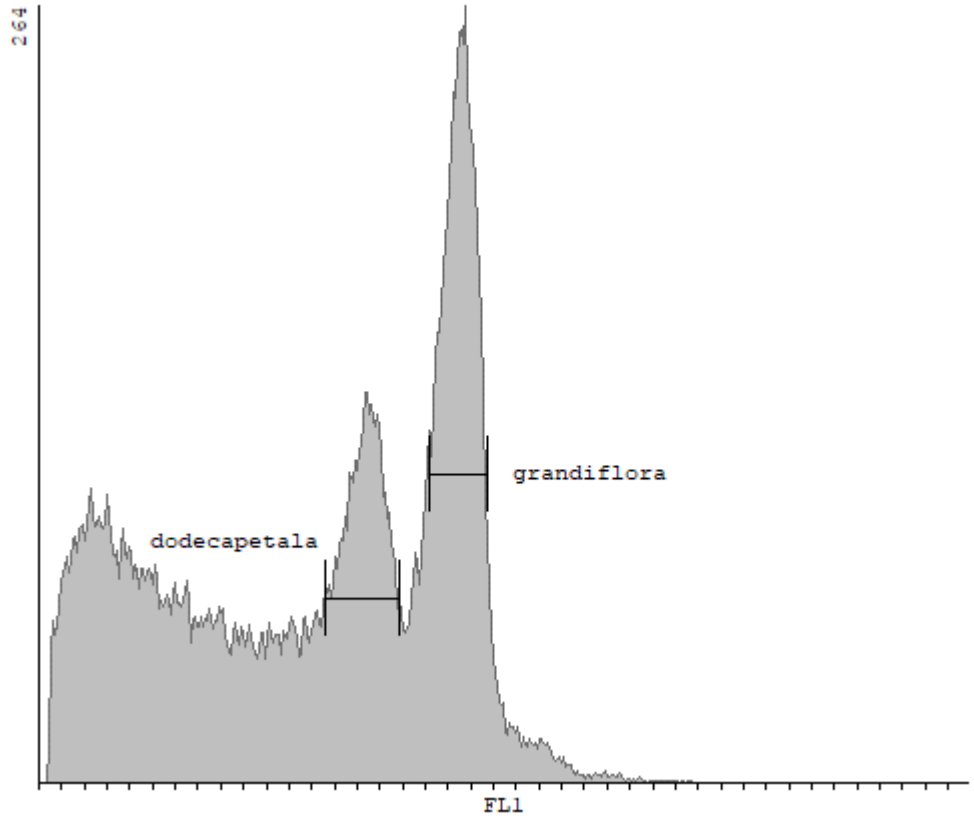
Appendix 2.1B *Magnolia cubensis* subsp. *acunae*, sample: MA184 (silica-gel dried leaf).



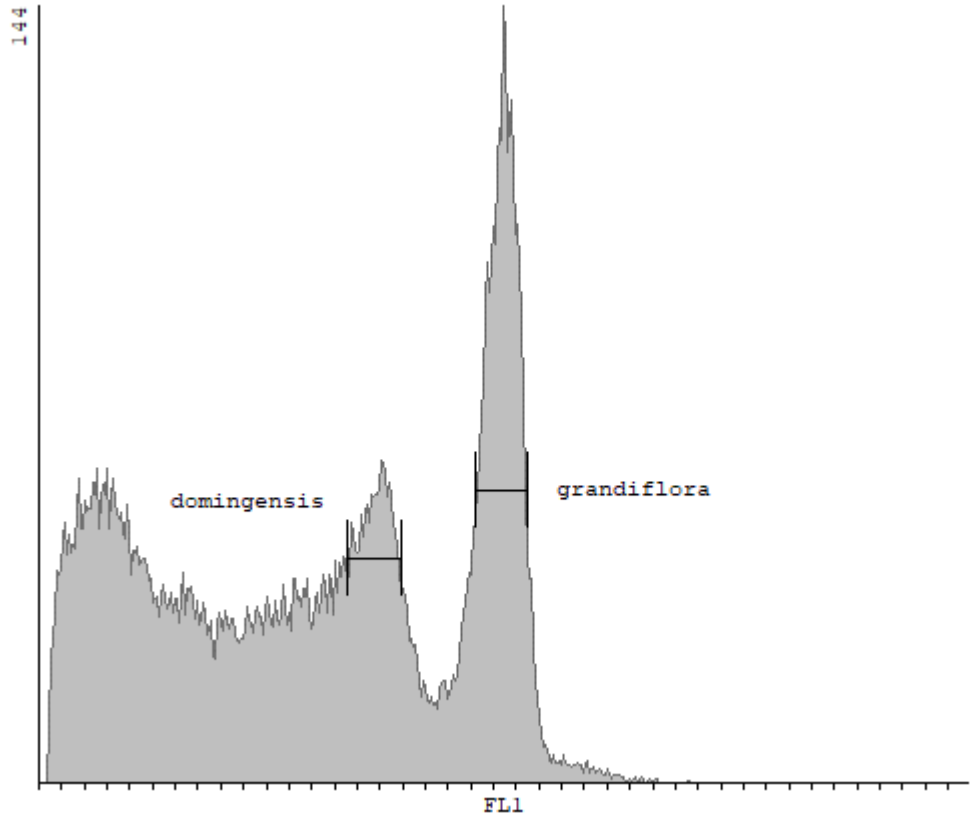
Appendix 2.1C *Magnolia cubensis* subsp. *cubensis*, sample: MA667 (silica-gel dried leaf).



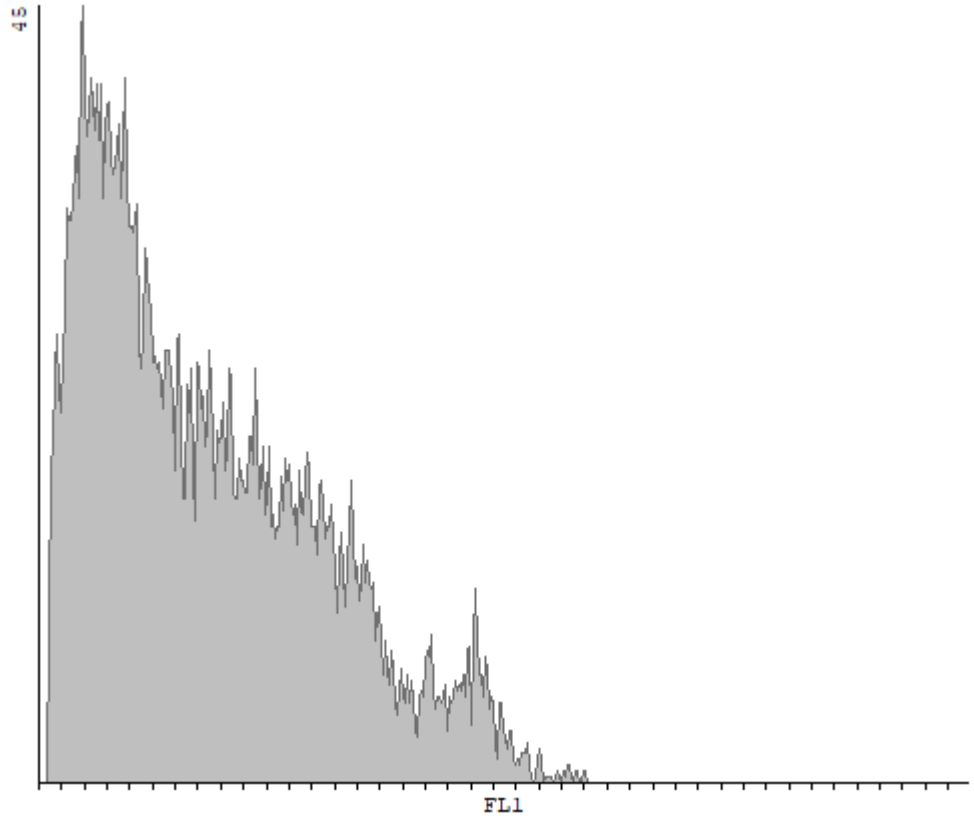
Appendix 2.1D *Magnolia dodecapetala*, sample: fresh leaf sample from seedling.



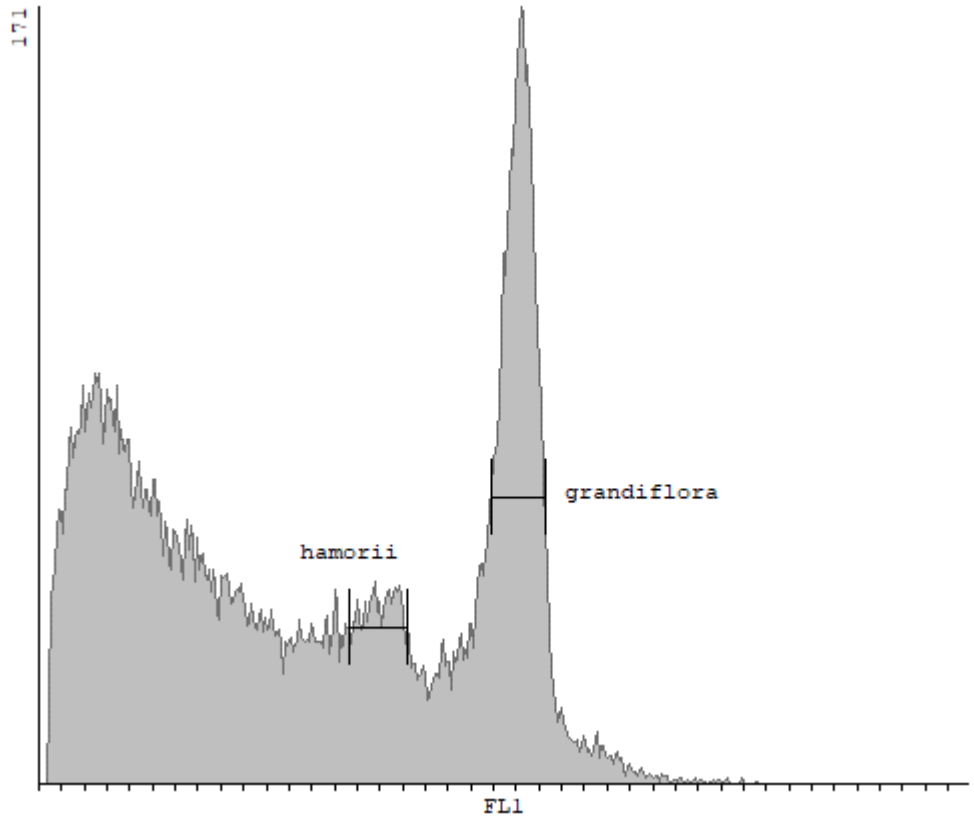
Appendix 2.1E *Magnolia domingensis*, sample: MA533 (silica-gel dried leaf).



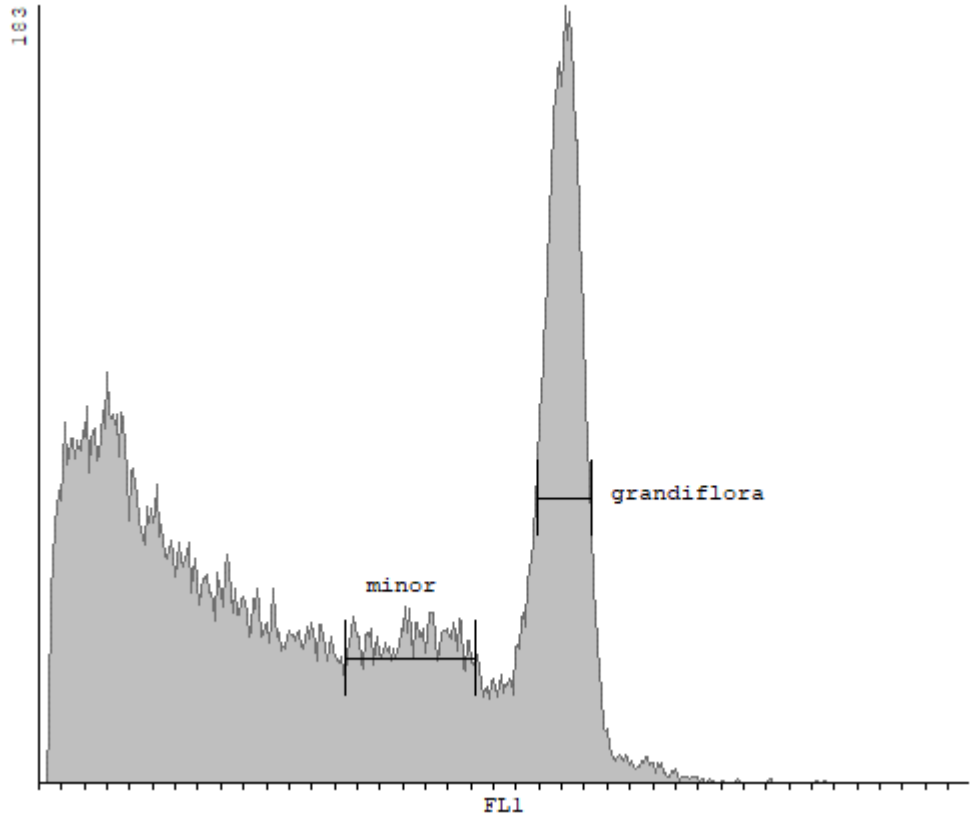
Appendix 2.1F *Magnolia ekmanii*, sample: MA316 (silica-gel dried leaf).



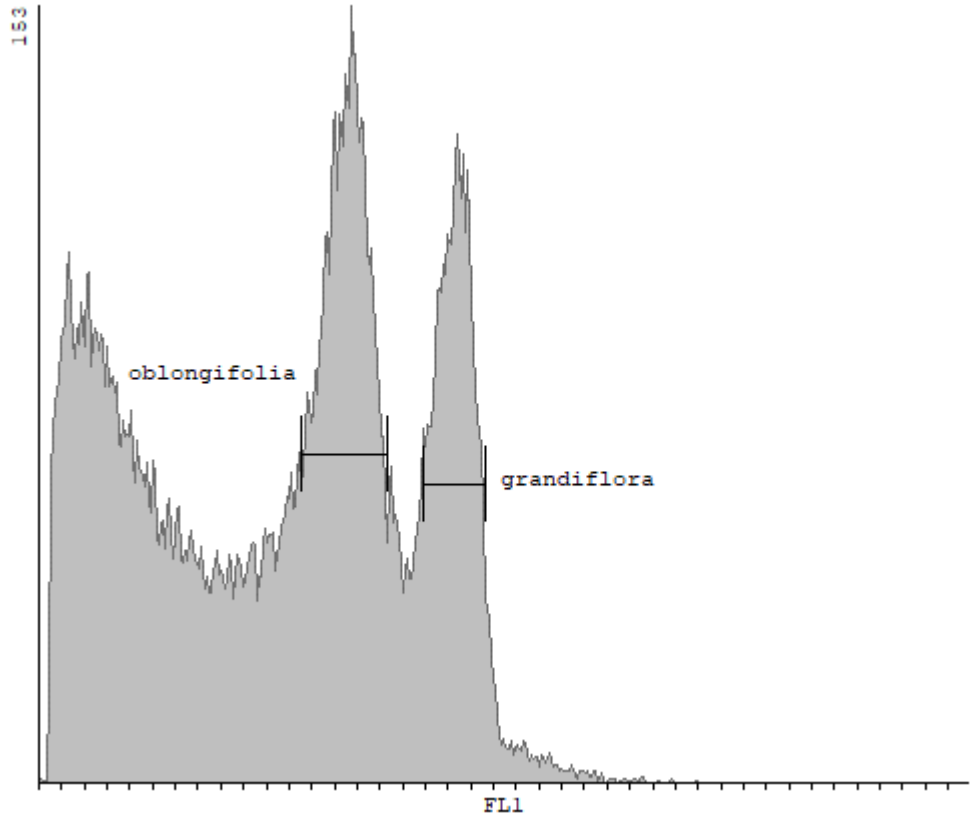
Appendix 2.1G *Magnolia hamorii*, sample: MA881 (silica-gel dried leaf).



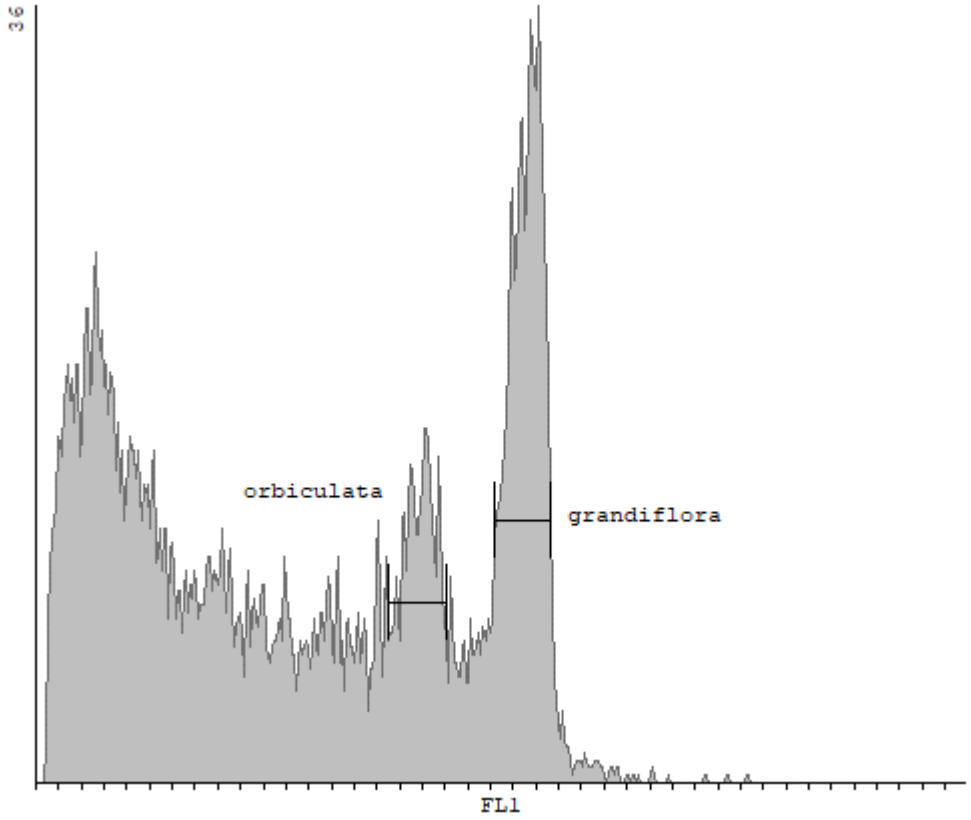
Appendix 2.1H *Magnolia minor*, sample: MA1094 (silica-gel dried leaf).



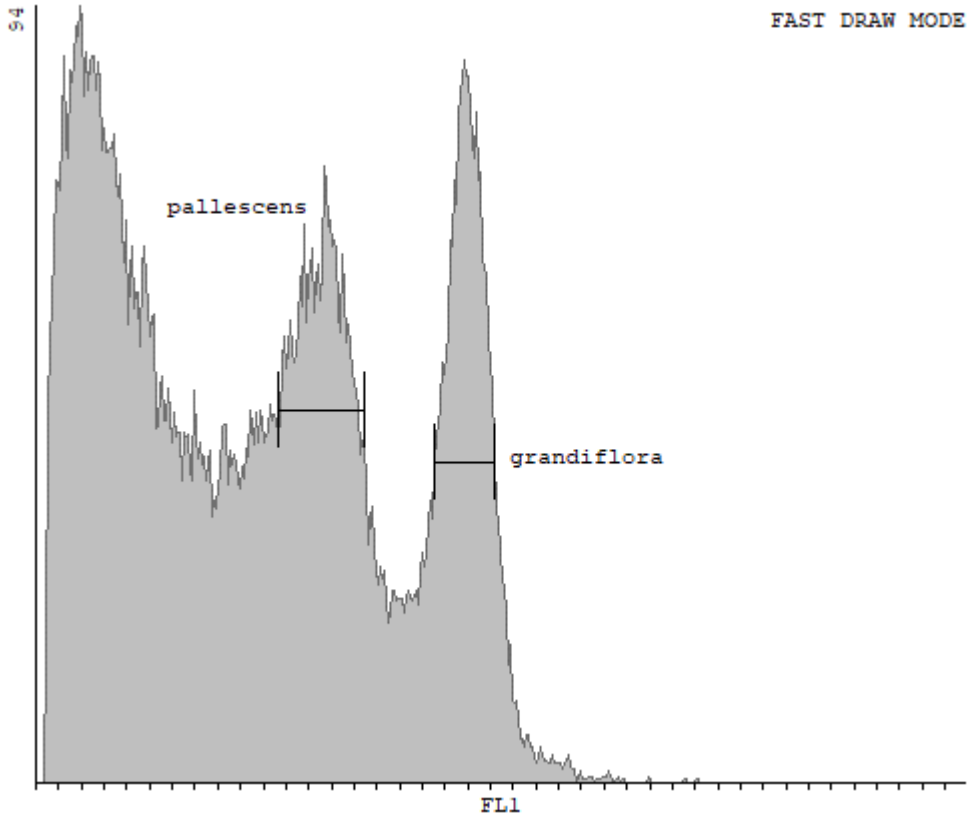
Appendix 2.1I *Magnolia oblongifolia*, sample: MA1090 (silica-gel dried leaf).



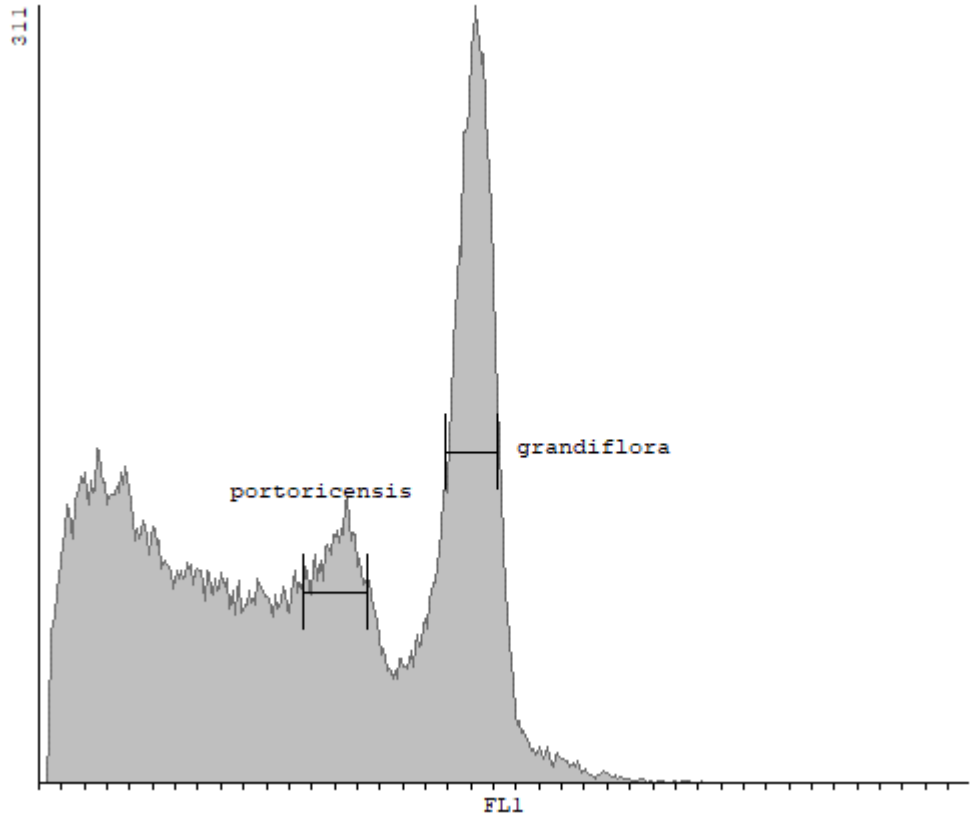
Appendix 2.1J *Magnolia orbiculata*, sample: MA615 (silica-gel dried leaf).



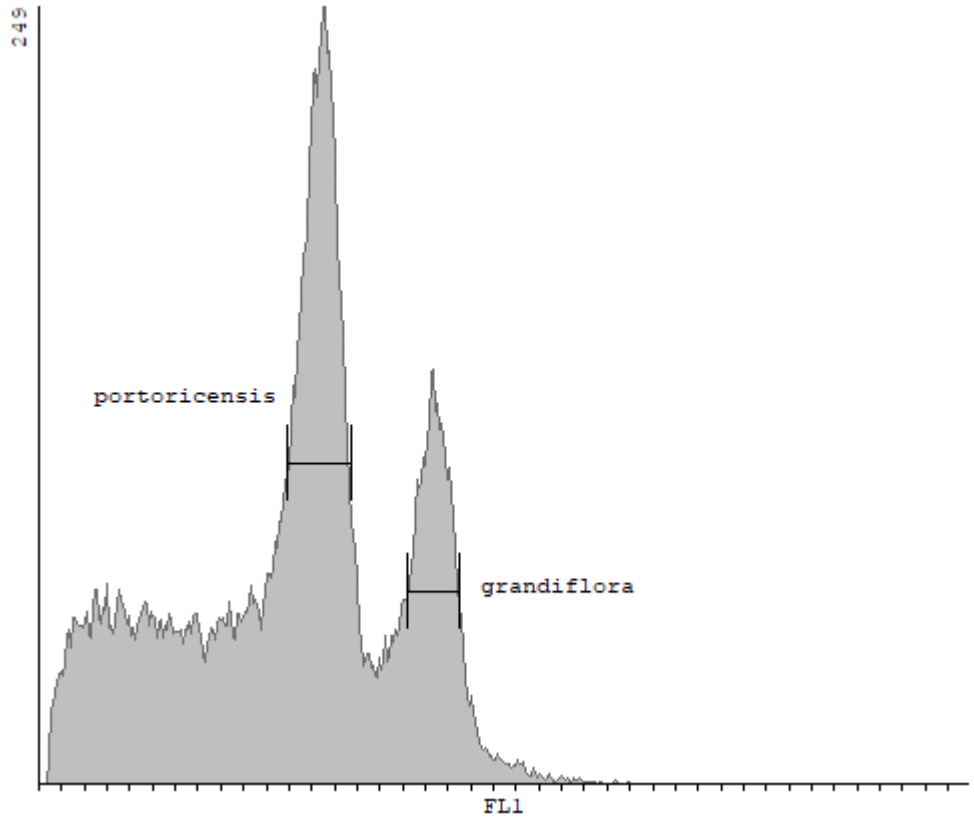
Appendix 2.1K *Magnolia pallescens*, sample: MA480 (silica-gel dried leaf).



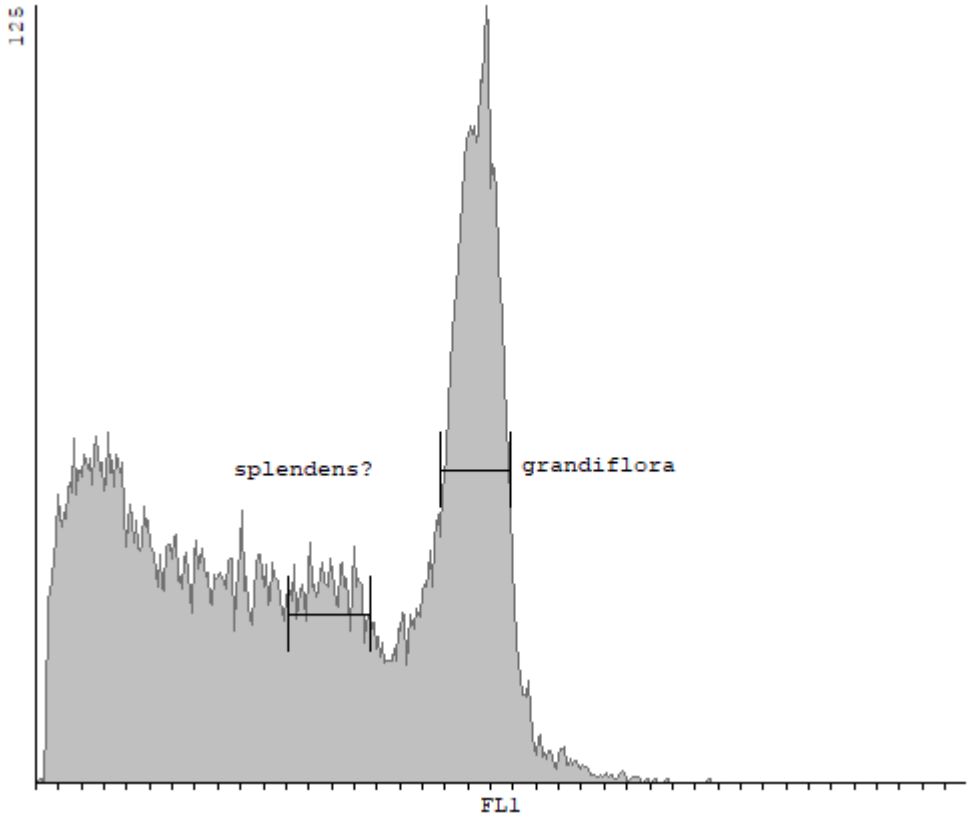
Appendix 2.1L *Magnolia portoricensis*, sample: MA1397 (silica-gel dried leaf).



Appendix 2.1M *Magnolia portoricensis*, sample: fresh leaf sample from seedling.



Appendix 2.1N *Magnolia splendens*; sample: MA1577 (silica-gel dried leaf).



Appendix 3: Biogeography of the Caribbean Magnolias

Appendix 3.1 Sample information of 62 *Magnolia* and *Liriodendron* taxa and their sampled populations. GenBank references (**GB**) from: 1) Azuma et al. (2001); 2) Kim et al. (2001); 3) Nie et al. (2008); and 4) Kim et al. (2013); complemented with newly sequenced data based on silica gel samples collected from *ex situ* collections and herbarium specimens. Herbarium abbreviations follow Index Herbariorum (Thiers, continuously updated).

	Taxa	**Country**	**Population**	**Herbarium reference**	**GB**
Genus *Liriodendron*					
1	*L. chinense* (Hemsl.) Sarg.	China	-	Veltjen & Ossaer 2018-002 (ARWESP)	1,2,3,4
2	*L. tulipifera* L.	USA	-	Veltjen & Ossaer 2018-003 (ARWESP)	1,2,3,4
Genus *Magnolia*					
Subgenus *Gynopodium*					
Section *Gynopodium*					
3	*M. kachirachirai* (Kanehira & Yamamoto) Dandy	Taiwan	-	Hung Kun-Yun s.n. (TAIF)	-
4	*M. nitida* W.W. Smith	China	-	Veltjen & Stappaerts 2018-025 (GENT)	1,2,4
Subgenus *Magnolia*					
Section *Auriculata*					
5	*M. fraseri* subsp. *fraseri* Walt.	USA	-	Wester 1888 (ARWESP)	1,2,3,4
6	*M. fraseri* subsp. *pyramidata* (Bartram) Pampanini	USA	-	Wester 1892 (ARWESP)	1,2,3,4
Section *Gwillima*					
7	*M. delavayi* Franchet	China	-	Veltjen & Ossaer 2018-006 (ARWESP)	1,3
Section *Kmeria*					
8	*M. kwangsiensis* Figlar & Noot.	China	-	-	3,4
Section *Macrophylla*					
9	*M. dealbata* (Zucc.) D.L. Johnson	Mexico	-	Veltjen & Ossaer 2018-001 (ARWESP)	2,3,4
10	*M. macrophylla* Michx.	USA	-	Veltjen & Ossaer 2018-008 (ARWESP)	1,2,3,4
Section *Magnolia*					
11	*M. grandiflora* L.	USA	-	Conrad, Miller & Lewandowski s.n. (GENT)	1,2,3,4
12	*M. guatemalensis* Donn.Sm.	Guatemala	-	-	1,2,3,4
13	*M. iltisiana* Vázquez	Mexico	-	-	1,3
14	*M. mayae* Vázquez & Pérez-Farrera	Mexico	-	Samain 2013-048 (IEB, MEXU)	-
15	*M. pacifica* subsp. *pugana* Iltis & Vazquez	Mexico	-	-	1,3
16	*M. panamensis* Vazquez & Iltis	Panama	-	-	2,4
17	*M. schiedeana* Schltdl.	Mexico	-	-	1,3
18	*M. sharpi* Meranda	Mexico	-	Samain & Martínez 2017-002	1,3
19	*M. tamaulipana* Vazquez	Mexico	-	-	1,2,3,4
20	*M. virginiana* L.	USA	-	Conrad, Miller, Lewandowski s.n. (GENT)	1,2,3,4
21	*M. virginiana* subsp. *oviedoae* Palmarola, M.S. Romanov & A.V. Bobrov	Cuba	-	Oviedo, Palmarola, González HFC84055 (HAJB)	-
22	*M. yoroconte* Dandy	Honduras	-	-	1, 3
Section *Manglietia*					

23	*M. decidua* (Q.Y. Zheng) V.S. Kumar	China	-	Wester 1901 (ARWESP)	1,3
24	*M. insignis* Wall.	China	-	Veltjen & Ossaer 2018-007 (ARWESP)	1,3
25	*M. sapaensis* (N.H.Xia & Q.N.Vu) Grimshaw & Macer	Vietnam	-	Veltjen s.n. (GENT)	-
Section *Rhytidospermum*					
Subsection *Rhytidospermum*					
26	*M. obovata* Thunb.	Japan		Wester 1900 (ARWESP)	1,3
27	*M. tripetala* L.	USA	-	Veltjen & Ossaer 2018-010 (ARWESP)	1,2,3,4
Subsection *Oyama*					
28	*M. sieboldii* subsp. *sieboldii* K. Koch	Korea, China	-	Veltjen & Ossaer 2018-009 (ARWESP)	1,2,3,4
29	*M. wilsonii* (Finet. & Gagnep.) Rehder	China	-	Veltjen & Ossaer 2018-011 (ARWESP)	2,3,4
Section *Talauma*					
Subsection *Cubenses*					
30	*M. cristalensis* Bisse	Cuba	Cayo Mujeres	Palmarola *et al*. HFC-89214 (HAJB)	-
			Cupeyal	Falcón *et al*. HFC-88860 (HAJB)	-
			Mina iberia	Palmarola *et al*. HFC-89255 (HAJB)	-
			Pico Cristal	Bécquer & Testé HFC-89807 (HAJB)	-
31	*M. cubensis* subsp. *acunae* Imkhan.	Cuba	Banao	Arias *et al*. HFC-59766 (HAJB)	-
			Topes	Palmarola & González Torres HFC-89432 (HAJB)	-
32	*M. cubensis* Urb. subsp. *cubensis*	Cuba	Bayamesa	Molina HFC-89593 (HAJB)	-
			El Gigante	Palmarola & González Torres HFC-89429 (HAJB)	-
			Gran Piedra	Palmarola & González Torres HFC-89422 (HAJB)	-
			Loma del Gato	Bécquer *et al*. HFC-89336 (HAJB)	-
			Pico Caracas	Palmarola *et al*. HFC-89195 (HAJB)	-
			Pico Turquino	Palmarola & González Torres HFC-89418 (HAJB)	-
33	*M. domingensis* Urb.	Haiti	Morne Maleuvre	Ekman 2810 (B)	-
		Dominican Republic	Loma Barbacoa	Veltjen *et al.* 2015-011 (GENT, JBSD)	-
			Loma Rodríguez	Veltjen *et al.* 2015-012 (GENT, HAJB, JBSD)	-
34	*M. ekmanii* Urb.	Haiti	Morne Grand Bois	Veltjen *et al.* 2015-001 (EHH, IEB, GENT)	-
			Morne Mansinte	Veltjen *et al.* 2015-003 (EHH, IEB, GENT, JBSD, K)	-
			Morne Pain de Sucre	Ekman 10395 (S)	-
35	*M. emarginata* Urb. & Ekman	Haiti	Massif de Cahos	Ekman 3442 (S)	-
			Massif du Nord	Ekman 4339 (S)	-
36	*M. hamorii* Howard	Dominican Republic	Cortico	Veltjen *et al.* 2015-009 (GENT, HAJB, JBSD, K)	-
			Cachote	Veltjen *et al.* 2015-010 (GENT, JBSD)	-
37	*M. pallescens* Urb. & Ekman	Dominican Republic	Ebano Verde	Veltjen *et al.* 2015-004 (GENT, JBSD)	-
			Valle Nuevo	Veltjen *et al.* 2015-007 (GENT, JBSD)	-
38	*M. portoricensis* Bello	Puerto Rico	Carite	Veltjen *et al.* 2016-033 (GENT)	-
			Guilarte	Veltjen & Padrón Vélez 2015-014 (GENT, UPRRP)	-
			Maricao	Veltjen 2015-016 (GENT, UPRRP)	-
			Toro Negro	Veltjen & Rodríguez Guzmán 2015-015 (GENT, K, UPRRP)	-
39	*M. splendens* Urb.	Puerto Rico	El Toro	Veltjen, Areces & Vega 2015-013 (GENT, UPRRP)	-
			El Yunque	Veltjen & Areces 2015-017 (GENT, UPRRP)	-
Subsection *Dugandiodendron*					
40	*M. chimantensis* Steyermark & Maguire	Venezuela	Chimantá Massif	Steyermark 1191 (K)	-
41	*M. lenticellata* (Lozano) Govaerts	Colombia	-	-	1,2

42	*M. mahechae* (Lozano) Govaerts	Colombia	-	-	1,2
Subsection *Talauma*					
43	*M. caricifragrans* (Lozano) Govaerts	Colombia	-	-	1
44	*M. dodecapetala* (Lam.) Govaerts	Lesser Antilles	Martinique	Veltjen et al. 2016-010 (GENT, K, MTK)	-
			Guadeloupe	Veltjen et al. 2016-015 (GENT, GUAD)	-
45	*M. lacandonica* A.Vázquez, Pérez-Farr. & Mart.-Camilo	Mexico	Lacanjá	Samain et al. 2013-039 (IEB, MEXU)	-
			Yajalón	Samain & Martínez 2017-016 (IEB, MEXU)	-
46	*M. lopezobradorii* A.Vázquez	Mexico	Catemaco	Samain & Martínez 2016-004 (IEB, MEXU)	-
47	*M. mexicana* DC.	Mexico	-	-	2,3,4
48	*M. minor* (Urb.) Govaerts	Cuba	CGU: Cayo Guam	Palmarola *et al.* HFC-89243 (HAJB)	-
			CGU: Cayo Guam	Palmarola *et al.* HFC-89249 (HAJB)	-
			CMU: Cayo Mujeres	Palmarola *et al.* HFC-89213 (HAJB)	-
			CUP: Cupeyal	Falcón HFC-88959 (HAJB)	-
			LME: Arroyo Bueno	Palmarola et al. HFC- 89584 (HAJB)	-
			NDT: Naranjo del Toa	Palmarola et al. HFC- 84609 (HAJB)	-
			PCR: Pico Cristal	Bécquer et al. HFC 89804 (HAJB)	-
			YAM: Yaminigüey	Bécquer et al. HFC 89450 (HAJB)	-
			YUM: Yumurí	Bécquer *et al.* HFC-89829 (HAJB)	-
49	*M. oblongifolia* (León) Palmarola	Cuba	CGU: Cayo Guam	Palmarola *et al.* HFC-89240 (HAJB)	-
			CGU: Cayo Guam	Becquer & Testé HFC-89438 (HAJB)	-
			LME: La Melba	Palmarola *et al.* HFC-89587 (HAJB)	-
			MIB: Mina Iberia	Palmarola *et al.* HFC-89261A (HAJB)	-
			MIB: Mina Iberia	Palmarola *et al.* HFC-89261B (HAJB)	
50	*M. orbiculata* (Britton & P. Wilson) Palmarola	Cuba	Bayamesa	Molina HFC-89590 (HAJB)	-
			Pico Caracas	Palmarola *et al.* HFC-89194 (HAJB)	-
			Pico Turquino	Palmarola & González Torres HFC-89394 (HAJB)	-
51	*M. ovata* (A. St. Hil.) Spreng.	Brazil	-	-	1
52	*M. rimachii* (Lozano) Govaerts	Bolivia	Valle de Sacta	Killeen & Siegle 3579 (K)	-
53	*M. sinacacolinii* A.Vázquez	Mexico	San Andrés Tuxtla	Samain & Martínez 2016-08 (IEB, MEXU)	-
54	*M. venezuelensis* (Lozano) Govaerts	Venezuela	-	Steyermark 97586 (K)	-
55	*M. zoquepopolucae* A.Vázquez	Mexico	San Pedro Soteapan	Samain & Martínez 2016-012 (IEB, MEXU)	-
Subgenus *Yulania*					
Section *Michelia*					
Subsection *Michelia*					
56	*M. compressa* Maxim.	Japan, China	-	Goetghebeur 13206 (GENT)	1
57	*M. doltsopa* (Buch.-Ham. Ex DC.) Figlar	China	-	Veltjen & Stappaerts 2018-022 (GENT)	2,3
58	*M. figo* (Lour.) DC.	China	-	Veltjen & Stappaerts 2018-023 (GENT)	1,2,3,4
Section *Yulania*					
Subsection *Tulipastrum*					
59	*M. acuminata* L.	USA	-	Veltjen & Ossaer 2018-004 (ARWESP)	1,3,4
Subsection *Yulania*					
60	*M. biondii* Pampan	China	-	Veltjen & Ossaer 2018-005 (ARWESP)	1,4
61	*M. kobus* DC.	Japan, Korea	-	Veltjen & Stappaerts 2018-023 (GENT)	1,2,3,4
62	*M. zenii* Cheng	China	-	Veltjen & Ossaer 2018-012 (ARWESP)	3

Appendix 3.2 Primers used to amplify and sequence the 11 markers applied in this study. Names in *italics* are chloroplast markers. Names in CAPITALS are single copy nuclear markers. The approximate length expressed in base pairs (bp) is that of the full fragment, including the forward and the reverse primer. The *Arabidopsis* homolog was acquired through a megablast search (chloroplast DNA), a discontiguous megablast search (nuclear DNA) or a blastn search (*ndhF-rpl32*) of the highest quality *Magnolia* sequence for that fragment against the full *Arabidopsis thaliana* genome on the NCBI GenBank database (GCF_000001735.4). If there is more than one hit for the search, the hit with the highest E-value is underlined.

Name	Primer sequence (5' – 3')	Reference	bp	Arabidopsis gene homologs
atpB-rbcL: intergenetic spacer between ATP synthase CF1 beta-subunit gene and ribulose-bisphosphate carboxylase gene				
AT1	F: AGAACCAGAAGTAGTAGGAT	Azuma *et al.* 1999	838	*atpB* (ArthCp029), *rbcL* (ArthCp030)
RB	R: ACACCAGCTTTGAATCCAAC			
AGT1: alanine:glyoxylate aminotransferase gene				
Agt1_1286F	F: GGAATGGGAATTGTGTGTGC	this publication (& Li *et al.* 2008)	1213	AGT (AT2G13360)
Agt1_1671R	R: CCATTCCTCCTTTTGTGTGCAGTT			
GAI1: gibberellic-acid insensitive gene (GRAS gene family transcription factors)				
GAI1_0710F	F: AGATGGTACTCTGCAACGCG	this publication (& Nie *et al.* 2008)	1150	GAI (AT1G14920), RGA1 (AT2G01570), RGL2 (AT3G3450), RGL3 (AT5G17490)
GAI1_1904R	R: GAGTAGTAGTGCAGTGCTTCG			
LEAFY: floral meristem identity control protein gene				
LFY_F	F: AGGTGACTAACCAGGTGTTC	Nie *et al.* 2008	499	LEAFY (AT5G61850)
LFY_bR	R: CAACCTRGTCTCTATGCACAA			
ndhF: NADH dehydrogenase subunit 5 gene				
1	F: ATGGAACAKACATATSAATATGCGTGG	Kim *et al.* 2001	1179	*ndhF* (ArthCp071)
MF1165R	R: AATTGGCACATATTTGSTTA			
972	F: GTCTCAATTGGGTTATATGATG		1167	
2110R	R: CCCCCTAYATATTTGATACCTTCTCC			
MF1795	F: GTGACAAATGCAATTTATTCA		1237	
P14	R: ACCAAGTTCAATGTTAGCGAGATTACTC			
ndhF-rpl32: intergenetic spacer between NADH dehydrogenase subunit 5 gene & ribosomal protein L32 gene				
ndhF	F: GAAAGGTATKATCCAYGMATATT	Shaw *et al*. 2007	1233	*ndhF* (ArthCp071), *rpl32* (ArthCp072)
rpL32-R	R: CCAATATCCCTTYYTTTTCCAA			
PHYA: phytochrome A gene				
PHYA-F	F: CCTTACGAAGTACCCATGACTG			

PHYA-R	R: TRGCRTCCATYTCATAATCCTT	Nie *et al.* 2008	1177	PHYA (AT1G09570), PHYB (AT2G18790), PHYC (AT5G35840), PHYD (AT4G16250), PHYE (AT4G18130)
psbA-trnH: intergenetic spacer between photosystem II protein D1 gene & $tRNA^{His}$ (GUG) gene				
TRNF	F: CGCATGGTGGATTCACAATC	Azuma *et al.* 1999	470	*psbA* (ArthCp002), *trnH* (ArthCt088)
PSAR	R: AGACCTAGCTGCTATCGAAG			
rbcL: ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit				
Z1	F: ATGTCACCACAAACAGAAACTAAAGCAAGT	Morgan and Soltis 1993	1026	*rbcL* (ArthCp030)
ML6R	R: AGTGATGTCCCGTTCCCC	Kim & Suh 2013		
ML3	F: CCAGGGAATTGGGAGTTC		731	
3`	R: CGGCTCAATCCTTTTAGTAAAAGATTGGGCCGAG	Morgan and Soltis 1993		
trnK: $tRNA^{Lys}$(UUU) gene which contains the *matK* (maturase K gene) ORF (open reading frame)				
trnK-3914F	F: GGGGTTGCTAACTCAACGG	Plunkett *et al.* 1996	1645	*trnK* (ArthCt089)
MR2	R: CAAGGTGAGATTTCCATTTC	Azuma *et al.* 1999		
MF1	F: CTGCTGGATACAAGATBCCC		1303	
trnK-2R	R: AACTAGTCGGATGGAGTAG	Plunkett *et al.* 1996		
SQD1: sulfoquinovosyldiaculglucerol 1 / UDP-sulfoquinovose synthase				
Sqd1_1074F	F: CCCTCACACCCATCTCTTCC	this publication (& Li *et al.* 2008)	580	SQD1 (AT4G33030)
Sqd1_1653R	R: ATGCTGTTCCAAAGACCCCG			

Appendix 3.3 GenBank accession numbers of the sequences used in this study. Sequences from GenBank can be traced back to 1) Azuma et al. (2001); 2) Kim et al. (2001); 3) Nie et al. (2008); and 4) Kim et al. (2013). Herbarium abbreviations follow Index Herbariorum (Thiers, continuously updated). Author names of *Magnolia* and *Liriodendron* species can be found in Appendix 3.1. Submission in progress: for final GenBank numbers, please refer to the Appendices of the manuscript once published.

TAXON	LAB ID	HERBARIUM VOUCHER	CHLOROPLAST DNA						NUCLEAR DNA				
			atpb-rbcL	***ndhF***	***ndhF-rpl32***	***psbA-trnH***	***rbcL***	***trnK***	**AGT1**	**GAI1**	**LEAFY**	**PHYA**	**SQD1**
L. chinense	-	S. Kim 1044 (NPRI)	AB021076	AF107996	-	AB021046	AY008946	AB021016	-	-	-	-	-
	-	Nie & Meng 380 (KUN)	-	-	-	-	-	-	-	EU849705	EU849807	EU849905	-
	MA2151	Veltjen & Ossaer 2018-002 (ARWESP)	-	-	seq147	-	-	-	seq001	-	-	-	seq719
L. tulipifera	-	S. Kim 1045 (NPRI)	AB021077	AF107997	-	AB021047	AY008947	AB021017	-	-	-	-	-
	-	Qiu 52 (NCU)	-	-	-	-	-	-	-	EU849712	EU849812	EU849912	-
	MA2143	Veltjen & Ossaer 2018-003 (ARWESP)	-	-	seq148	-	-	-	seq002	-	-	-	seq720
M. acuminata	-	H. Azuma & S. Kim 95051708 (KYO)	AB021071	AB623370	-	AB021041	-	AB021011	-	-	-	-	-
	-	S. Kim 1001 (NPRI)	-	-	-	-	AY008915	-	-	-	-	-	-
	-	Qiu 4 (NCU)	-	-	-	-	-	-	-	EU849757	EU849856	EU849957	-
	MA2134	Veltjen & Ossaer 2018-004 (ARWESP)	-	-	seq149	-	-	-	seq003	-	-	-	seq721
M. biondii	-	S. Kim 1003 (NPRI)	AY008956	AF107953	-	AY009017	AY008909	AY008986	-	-	-	-	-
	MA2152	Veltjen & Ossaer 2018-005 (ARWESP)	-	-	seq150	-	-	-	seq004	seq498	seq570	seq647	seq722
M. caricifragrans	-	Lozano-C. 2350 (COL)	-	-	-	-	-	AB055533	-	-	-	-	-
M. chimantensis	MA2174	Steyermark 1191 (K)	seq081	seq295	seq151	seq231	seq362	seq434	seq005	-	seq571	-	seq723
M. compressa	MA2129	Goetghebeur 13206 (GENT)	seq082	seq296	seq152	seq363	seq364	seq435	seq006	seq499	seq572	seq648	seq724
M. cristalensis	MA608	Palmarola *et al.* HFC-89214A (HAJB)	seq083	seq297	seq153	seq232	seq365	seq436	seq007	seq500	seq573	seq649	seq725
	MA1093	Palmarola *et al.* HFC-89214B (HAJB)	seq084	seq298	seq154	seq233	seq366	seq437	seq008	seq501	seq574	seq650	seq726
	MA2175	Falcón *et al.* HFC-88860 (HAJB)	seq085	seq299	seq155	seq234	seq367	seq438	seq009	seq502	seq575	seq651	seq727
	MA2180	Palmarola *et al.* HFC-89255 (HAJB)	seq086	seq300	seq156	seq235	seq368	seq439	seq010	seq503	seq576	seq652	seq728
	MA2522	Bécquer & Testé HFC-89807 (HAJB)	seq087	seq301	seq157	seq236	seq369	seq440	seq011	seq504	seq577	seq653	seq729
M. cubensis subsp. *acunae*	MA587	Palmarola & González Torres HFC-89432 (HAJB)	seq088	seq302	seq158	seq237	seq370	seq441	seq012	seq505	seq578	seq654	seq730
	MA596	Arias *et al.* HFC-59766 (HAJB)	seq089	seq303	seq159	seq238	seq371	seq442	seq013	seq506	seq579	seq655	seq731
M. cubensis subsp. *cubensis*	MA189	Molina HFC-89593 (HAJB)	seq090	seq304	seq160	seq239	seq372	seq443	seq014	seq507	seq580	seq656	seq732
	MA190	Palmarola & González Torres HFC-89422 (HAJB)	seq091	seq305	seq161	seq240	seq373	seq444	seq015	seq508	seq581	seq657	seq733
	MA192	Palmarola & González Torres HFC-89429 (HAJB)	seq092	seq306	seq162	seq241	seq374	seq445	seq016	seq509	seq582	seq658	seq734

	MA564	Palmarola & González Torres HFC-89418 (HAJB)	seq093	seq307	seq163	seq242	seq375	seq446	seq017	seq510	seq583	seq659	seq735
	MA2189	Bécquer *et al.* HFC-89336 (HAJB)	seq094	seq308	seq164	seq243	seq376	seq447	seq018	seq511	seq584	seq660	seq736
	MA2190	Palmarola *et al.* HFC-89195 (HAJB)	seq095	seq309	seq165	seq244	seq377	seq448	seq019	seq512	seq585	seq661	seq737
M. dealbata	MA41	Veltjen & Ossaer 2018-001 (ARWESP)	seq096	seq310	seq166	seq245	seq378	seq449	seq020	seq513	seq586	seq662	seq738
M. decidua	-	H. Azuma 386 (KYO)	AB055583	AB623393	-	AB055565	-	AB055542	-	-	-	-	-
	MA1063	Wester 1901 (ARWESP)	-	-	seq167	-	seq379	-	seq021	seq514	seq587	seq663	seq739
M. delavayi	-	Thien 590645 (NO)	AB021065	AB623402	-	AB021035	-	AB021005	-	-	-	-	-
	MA2130	Veltjen & Ossaer 2018-006 (ARWESP)	-	-	seq168	-	seq380	-	seq022	seq515	seq588	seq664	seq740
M. dodecapetala	MA1139	Veltjen et al. 2016-010 (GENT, K, MTK)	seq097	seq311	seq169	seq246	seq381	seq450	seq023	seq516	seq589	seq665	seq741
	MA1245	Veltjen et al. 2016-015 (GENT, GUAD)	seq098	seq312	seq170	seq247	seq382	seq451	seq024	seq517	seq590	seq666	seq742
M. doltsopa	-	S. Kim 1037 (NPRI)	-	AF107963	-	-	-	-	-	-	-	-	-
	MA1065	Veltjen & Stappaerts 2018-022 (GENT)	seq099	-	seq171	seq248	seq383	seq452	seq025	seq518	seq591	seq667	seq743
*M. domingensis**	MA900	Veltjen *et al.* 2015-011 (GENT, JBSD)	seq100	seq313	seq172	seq249	seq384	seq453	seq026	seq519	seq592	seq668	seq744
	MA918	Veltjen *et al.* 2015-012 (GENT, HAJB, JBSD)	seq101	seq314	seq173	seq250	seq385	seq454	seq027	seq520	seq593	seq669	seq745
	MA2167	Ekman 2810 (B)	seq102	seq315	seq174	seq251	seq386	seq455	-	seq521	seq594	seq670	seq746
M. ekmanii	MA204	Veltjen *et al.* 2015-001 (EHH, IEB, GENT)	seq103	seq316	seq175	seq252	seq387	seq456	seq028	seq522	seq595	seq671	seq747
	MA337	Veltjen *et al.* 2015-003 (EHH, IEB, GENT, JBSD, K)	seq104	seq317	seq176	seq253	seq388	seq457	seq029	seq523	seq596	seq672	seq748
	MA2160	Ekman 10395 (S)	seq105	seq318	seq177	seq254	seq389	-	-	-	seq597	-	seq749
M. emarginata	MA1054	Ekman 3442 (S)	seq106	seq319	seq178	seq255	seq390	seq458	seq030	seq524	seq598	seq673	seq750
	MA2164	Ekman 4339 (S)	seq107	seq320	seq179	seq256	seq391	seq459	seq031	seq525	seq599	seq674	seq751
M. figo	-	S. Kim 1039 (NPRI)	AB021075	AF107977	-	AB021045	AY008905	AB021015	-	-	-	-	-
	MA2133	Veltjen & Stappaerts 2018-023 (GENT)	-	-	seq180	-	-	-	seq032	seq526	seq600	seq675	seq752
M.fraseri subsp. *fraseri*	-	S. Kim 1111 (NPRI)	AB021055	AF216256	-	AB021025	AY008940	AB020995	-	-	-	-	-
	-	Qiu 91010 (NCU)	-	-	-	-	-	-	-	EU849761	EU849860	EU849961	-
	MA2126	Wester 1888 (ARWESP)	-	-	seq181	-	-	-	seq033	-	-	-	seq753
M. fraseri subsp *pyramidata*	-	S. Kim 1011 (NPRI)	AB021056	AF107922	-	AB021026	AY008941	AB020996	-	-	-	-	-
	MA2150	Wester 1892 (ARWESP)	-	-	seq182	-	-	-	seq034	seq562	seq601	seq676	seq754
M. grandiflora	-	S. Kim 1012 (NPRI)	AB021050	AF107940	-	AB021020	AY008925	AB020990	-	-	-	-	-
	-	Qiu 6 (NCU)	-	-	-	-	-	-	-	EU849723	EU849823	EU849923	-
	MA2161	Conrad, Miller & Lewandowski s.n. (GENT)	-	-	seq183	-	-	-	seq035	-	-	-	seq755
M. guatemalensis	-	Thien 20007 (TU)	AB021051	AF107941	-	AB021021	AY008926	AB020991	-	EU849793	EU849891	EU849992	-
M. hamorii	MA842	Veltjen *et al.* 2015-009 (GENT, HAJB, JBSD, K)	seq108	seq321	seq184	seq257	seq392	seq460	seq036	seq527	seq602	seq677	seq756
	MA849	Veltjen *et al.* 2015-010 (GENT, JBSD)	seq109	seq322	seq185	seq258	seq393	seq461	seq037	seq528	seq603	seq678	seq757

M. iltisiana	-	Thien 12002 (TU)	AB055569	AB623375	-	AB055551	-	AB055520	-	-	EU849895	EU849996	-
M. insignis	-	Nooteboom 6005 (L)	AB623343	AB623400	-	AB623317	-	AB623291	-	-	-	-	-
	MA2141	Veltjen & Ossaer 2018-007 (ARWESP)	-	-	seq186	-	seq394	-	seq038	seq529	seq604	seq679	seq758
M. kachirachirai	MA13	Hung Kun-Yun s.n. (TAIF)	seq110	seq323	seq187	seq259	seq395	seq462	seq039	seq530	seq605	seq680	seq759
M. kobus	-	S. Kim 1013 (NPRI)	AB021068	AF107954	-	AB021038	AY008910	AB021008	-	-	-	-	-
	MA2153	Veltjen & Stappaerts 2018-023 (GENT)	-	-	seq188	-	-	-	seq040	seq531	seq606	seq681	seq760
M. kwangsiensis	-	S. Kim 1053 (NPRI)	AY008976	AF107930	-	AY009037	AY008943	AY009007	-	-	-	-	-
	-	W.B. Sun 99077 (KUN)	-	-	-	-	-	-	-	EU849802	EU849902	EU849999	-
M. lacandonica	MA49	Samain *et al.* 2013-039 (IEB, MEXU)	seq111	seq324	seq189	seq260	seq396	seq463	seq041	seq532	seq607	seq682	seq761
	MA1831	Samain & Martínez 2017-016 (IEB, MEXU)	seq112	seq325	seq190	seq261	seq397	seq464	seq042	seq533	seq608	seq683	seq762
M. lenticellata	-	G. Lozano Contreras 2272 (COL)	-	AF216261	-	-	-	AB055538	-	-	-	-	-
M. lopezobradorii	MA1302	Samain & Martínez 2016-004 (IEB, MEXU)	seq113	seq326	seq191	seq262	seq398	seq465	seq043	seq534	seq609	seq684	seq763
M. macrophylla	-	S. Kim 1015 (NPRI)	AB021057	AF107923	-	AB021027	AY008944	AB020997	-	-	-	-	-
	-	Qiu 1 (NCU)	-	-	-	-	-	-	-	EU849767	EU849866	EU849967	-
	MA2138	Veltjen & Ossaer 2018-008 (ARWESP)	-	-	seq192	-	-	-	seq044	-	-	-	seq764
M. mahechae	-	G. Lozano Contreras 2161 (COL)	-	AF216262	-	-	-	AB055539	-	-	-	-	-
M. mayae	MA87	Samain 2013-048 (IEB, MEXU)	seq114	seq327	seq193	seq263	seq399	seq466	seq045	seq535	seq610	seq685	seq765
M. mexicana	-	Rico-Gray & Thien 12001 (TU)	AB055580	AB623412	-	AB055562	-	AB055536	-	EU849797	EU849896	-	-
	-	Thien & Azuma s.n. (TI)	AY008973	AF216263	-	AY009034	AY008938	AY009004	-	-	-	-	-
	-	Wen 8726 (US)	-	-	-	-	-	-	-	EU849790	EU849888	EU849989	-
M. minor	MA1088	Bécquer et al. HFC 89450 (HAJB)	seq115	seq328	seq194	seq264	seq400	seq467	seq046	seq536	seq611	seq686	seq766
	MA1092	Palmarola et al. HFC-89213 (HAJB)	seq116	seq329	seq195	seq265	seq401	seq468	seq047	seq537	seq612	seq687	seq767
	MA2192	Palmarola et al. HFC- 89584 (HAJB)	seq117	seq330	seq196	seq266	seq402	seq469	seq048	seq538	seq613	seq688	seq768
	MA2201	Palmarola et al. HFC-89243 (HAJB)	seq118	seq331	seq197	seq267	seq403	seq470	seq049	seq539	seq614	seq689	seq769
	MA2203	Palmarola et al. HFC-89249 (HAJB)	seq119	seq332	seq198	seq268	seq404	seq471	seq050	seq540	seq615	seq690	seq770
	MA2204	Falcón HFC-88959 (HAJB)	seq120	seq333	seq199	seq269	seq405	seq472	seq051	seq541	seq616	seq691	seq771
	MA2631	Palmarola et al. HFC- 84609 (HAJB)	seq121	seq334	seq200	seq270	seq406	seq473	seq052	seq542	seq617	seq692	seq772
	MA2651	Bécquer et al. HFC-89829 (HAJB)	seq122	seq335	seq201	seq271	seq407	seq474	seq053	seq543	seq618	seq693	seq773
	MA2656	Bécquer et al. HFC 89804 (HAJB)	seq123	seq336	seq202	seq272	seq408	seq475	seq054	seq544	seq619	seq694	seq774
M. nitida		L.B. Thien 20005 (TU)	AB021066	-	-	AB021036	-	AB021006	-	-	-	-	-
	-	S. Kim 1017 (NPRI)	-	AF107935	-	-	AY008918	-	-	-	-	-	-
	MA2127	Veltjen & Stappaerts 2018-025 (GENT)	-	-	seq203	-	-	-	seq055	seq545	seq620	seq695	seq775

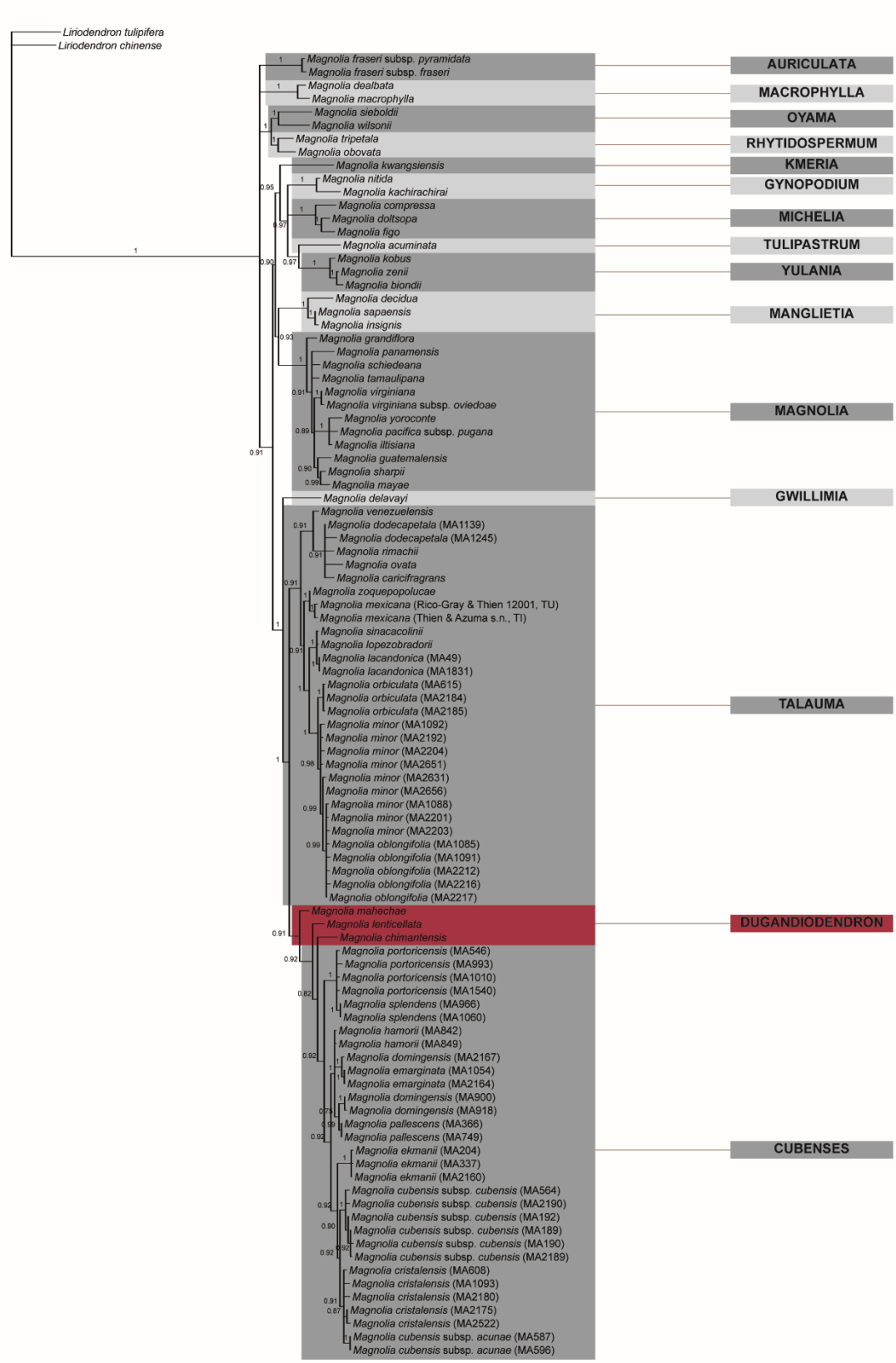
M. oblongifolia	MA1085	Palmarola *et al.* HFC-89587 (HAJB)	seq124	seq337	seq204	seq273	seq409	seq476	seq056	seq546	seq621	seq696	seq776
	MA1091	Palmarola *et al.* HFC-89240 (HAJB)	seq125	seq338	seq205	seq274	seq410	seq477	seq057	seq547	seq622	seq697	seq777
	MA2212	Bécquer & Testé HFC-89438 (HAJB)	seq126	seq339	seq206	seq275	seq411	seq478	seq058	seq548	seq623	seq698	seq778
	MA2216	Palmarola *et al.* HFC-89261A (HAJB)	seq127	seq340	seq207	seq276	seq412	seq479	seq059	seq549	seq624	seq699	seq779
	MA2217	Palmarola *et al.* HFC-89261B (HAJB)	seq128	seq341	seq208	seq277	seq413	seq480	seq060	seq550	seq625	seq700	seq780
M. obovata	-	H. Azuma 96070301 (KYO)	AB021059	AB623384	-	AB021029	-	AB020999	-	-	-	-	-
	MA2124	Wester 1900 (ARWESP)	-	-	seq209	-	seq414	-	seq061	seq551	seq626	seq701	seq781
M. orbiculata	MA615	Palmarola & González Torres HFC-89394 (HAJB)	seq129	seq342	seq210	seq278	seq415	seq481	seq062	seq552	seq627	seq702	seq782
	MA2184	Molina HFC-89590 (HAJB)	seq130	seq343	seq211	seq279	seq416	seq482	seq063	seq553	seq628	seq703	seq783
	MA2185	Palmarola *et al.* HFC-89194 (HAJB)	seq131	seq344	seq212	seq280	seq417	seq483	seq064	seq554	seq629	seq704	seq784
M. ovata	-	Thien 12000 (NO)	AB055581	AB623413	-	AB055563	-	AB055537	-	-	-	-	-
M. pacifica subsp. *pugana*	-	Rico-Gray & Thien 12003 (TU)	AB055570	AB623376	-	AB055552	-	AB055521	-	EU849796	EU849894	EU849995	-
M. pallescens	MA366	Veltjen *et al.* 2015-004 (GENT, JBSD)	seq132	seq345	seq213	seq281	seq418	seq484	seq065	seq555	seq630	seq705	seq785
	MA749	Veltjen *et al.* 2015-007 (GENT, JBSD)	seq133	seq346	seq214	seq282	seq419	seq485	seq066	seq556	seq631	seq706	seq786
M. panamensis	-	G. McPherson 15882 (MO)	AY008965	AF216255	-	AY009026	AY008923	AY008996	-	-	-	-	-
M. portoricensis	MA546	Veltjen & Padrón Vélez 2015-014 (GENT, UPRRP)	seq134	seq347	seq215	seq283	seq420	seq486	seq067	seq557	seq632	seq707	seq787
	MA993	Veltjen & Rodríguez Guzmán 2015-015 (GENT, K, UPRRP)	seq135	seq348	seq216	seq284	seq421	seq487	seq068	seq558	seq633	seq708	seq788
	MA1010	Veltjen 2015-016 (GENT, UPRRP)	seq136	seq349	seq217	seq285	seq422	seq488	seq069	seq559	seq634	seq709	seq789
	MA1540	Veltjen et al. 2016-033 (GENT)	seq137	seq350	seq218	seq286	seq423	seq489	seq070	seq560	seq635	seq710	seq790
M. rimachi	MA2172	Killeen & Siegle 3579 (K)	seq138	-	-	-	-	-	-	-	-	-	-
M. sapaensis	MA2131	Veltjen s.n. (GENT)	seq139	seq351	seq219	seq287	seq424	seq490	seq071	seq561	seq636	seq711	seq791
M. schiedeana	-	Thien & Azuma 12004 (NO)	AB055586	AB623377	-	AB055568	-	AB055550	-	EU849799	EU849898	-	-
M. sharpii	-	Thien 20009 (NO)	AB021053	AB623378	-	AB021023	-	AB020993	-	EU849792	-	EU849991	-
	MA1869	Samain & Martínez 2017-002 (IEB, MEXU)	-	-	seq220	-	seq425	-	seq072	-	seq637	-	seq792
M. sieboldii subsp. *sieboldii*	-	S. Kim 1047 (NPRI)	AB021062	AF107933	-	AB021032	AY008935	AB021002	-	-	-	-	-
	MA2132	Veltjen & Ossaer 2018-009 (ARWESP)	-	-	seq221	-	-	-	seq073	seq564	seq638	seq712	seq793
M. sinacacolinii	MA1296	Samain & Martínez 2016-08 (IEB, MEXU)	seq140	seq352	seq222	seq288	seq426	seq491	seq074	seq565	seq639	seq713	seq794
M. splendens	MA966	Veltjen, Areces & Vega 2015-013 (GENT, UPRRP)	seq141	seq353	seq223	seq289	seq427	seq492	seq075	seq566	seq640	seq714	seq795
	MA1060	Veltjen & Areces 2015-017 (GENT, UPRRP)	seq142	seq354	seq224	seq290	seq428	seq493	seq076	seq567	seq641	seq715	seq796
M. tamaulipana	-	S. Kim 1026 (NPRI)	AB021054	AF107943	-	AB021024	AY008927	AB020994	-	-	-	-	-
	-	Qiu 91021 (NCU)	-	-	-	-	-	-	-	EU849772	EU849871	EU849972	-

M. tripetala	-	S. Kim 1025 (NPRI)	AB021061	AF107928	-	AB021031	AY008934	AB021001	-	-	-	-	-
	-	Qiu 3 (NCU)	-	-	-	-	-	-	-	EU849785	EU849884	EU849985	-
	MA2140	Veltjen & Ossaer 2018-010 (ARWESP)	-	-	seq225	-	-	-	seq077	-	-	-	seq797
M. venezuelensis	MA2170	Steyermark 97586 (K)	seq143	seq355	-	seq291	seq429	seq494	-	-	seq642	-	-
M. virginiana subsp. *virginiana*	-	S. Kim 1027 (NPRI)	AB021048	AF107939	-	AB021018	AY008924	AB020988	-	-	-	-	-
	-	Qiu 7 (NCU)	-	-	-	-	-	-	-	EU849780	EU849879	EU849980	-
	MA1066	Conrad, Miller, Lewandowski s.n. (GENT)	-	-	seq226	-	-	-	-	-	-	-	seq798
M. virginiana subsp. *oviedoae*	MA1016	Oviedo, Palmarola, González-Torres HFC-84055 (HAJB)	seq144	seq356	seq227	seq292	seq430	-	-	-	seq643	-	-
M. wilsonii	MA2142	Veltjen & Ossaer 2018-011 (ARWESP)	seq358	seq357	seq228	seq359	seq431	seq495	seq078	seq563	seq644	seq716	seq799
M. yoroconte	-	Thien 12006 (TU)	AB055571	AB623380	-	AB055553	-	AB055522	-	EU849798	EU849897	EU849997	-
M. zenii	MA1064	Veltjen & Ossaer 2018-012 (ARWESP)	seq145	seq358	seq229	seq293	seq432	seq496	seq079	seq568	seq645	seq717	seq800
M. zoquepopolucae	MA1300	Samain & Martínez 2016-012 (IEB, MEXU)	seq146	seq359	seq230	seq294	seq433	seq497	seq080	seq569	seq646	seq718	seq801

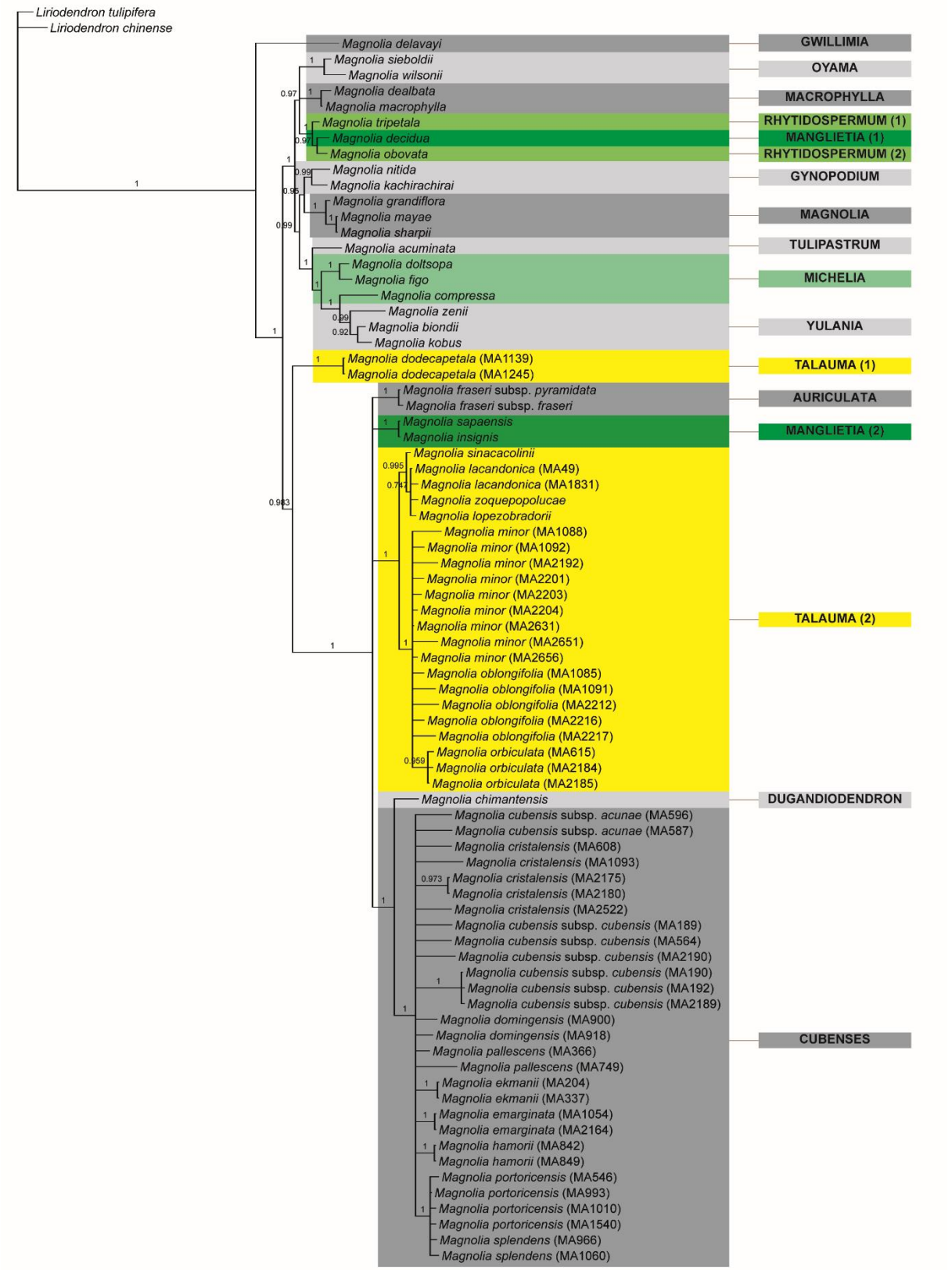
*Note: DNA from the type specimen of *M. domingensis* (Nash 1081, BM) was also extracted to complete the sampling of all collections of *Magnolia* in Haiti. Unfortunately, the little DNA present in this specimen did not have fragments long enough to execute successful Sanger sequencing.

Appendix 3.4 Bayesian phylogenetic hypotheses per sequenced nuclear genes and the concatenated chloroplast. All branches with a posterior probability (pp) lower than 0.70 were collapsed; only pp higher than 0.70 are depicted above the nodes. The branch lengths represent the expected number of substitutions per site. Supported clades matching classification according to Figlar and Nooteboom (2004) are coloured in alternating shades of grey, unsupported clades are highlighted by a specific colour matched to that specific clade over the different gene trees. Naming of the clades follows that of the lowest possible rank in the classification of Figlar and Nooteboom (2004), with an orthographical correction. **3.4.1** Phylogenetic hypothesis of the concatenated chloroplast sequences i.e. *atpB-rbcL, ndhF, ndhF-rpl32, psbA-trnH, rbcL, trnK*. **3.4.2** Phylogenetic hypothesis of AGT1. **3.4.3** Phylogenetic hypothesis of GAI1. **3.4.4** Phylogenetic hypothesis of LFYB. **3.4.5** Phylogenetic hypothesis of PHYA. **3.4.6** Phylogenetic hypothesis of SQD1.

Appendix 3.4.1 Bayesian phylogenetic hypothesis of the concatenated chloroplast sequences i.e. *atpB-rbcL, ndhF, ndhF-rpl32, psbA-trnH, rbcL, trnK*.

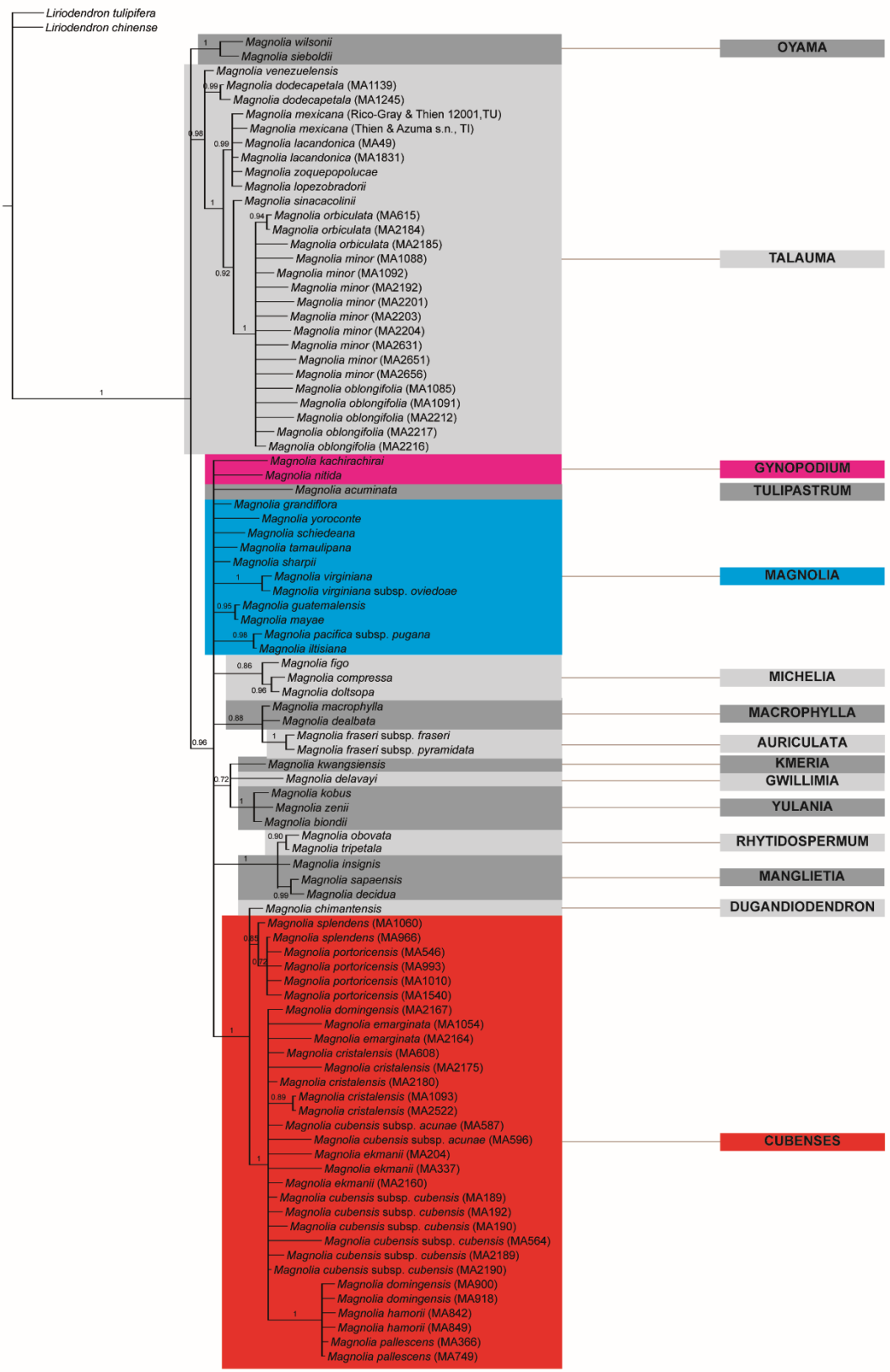


Appendix 3.4.2 Bayesian phylogenetic hypothesis of AGT1.

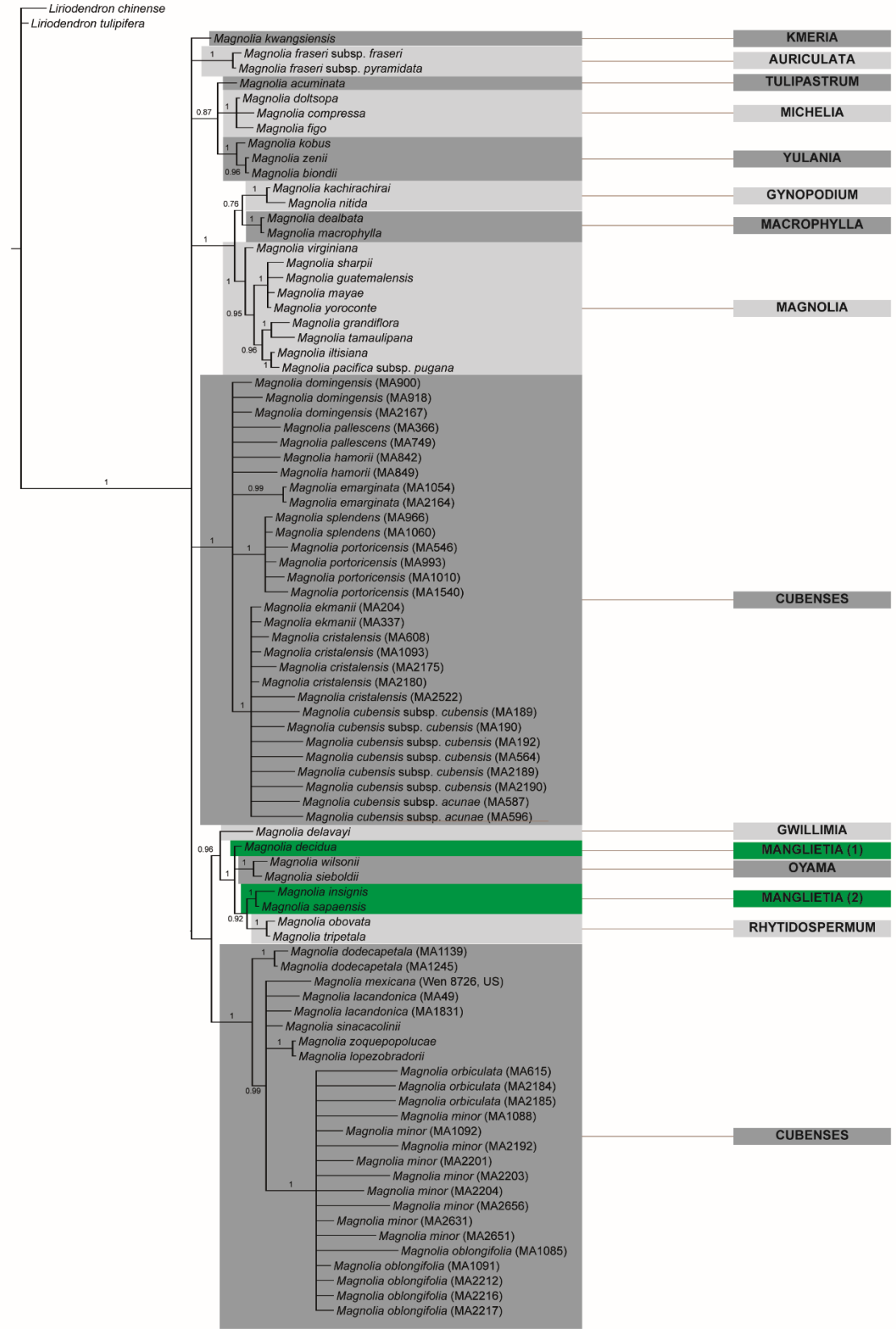


Appendix 3.4.3 Bayesian phylogenetic hypothesis of GAI1.

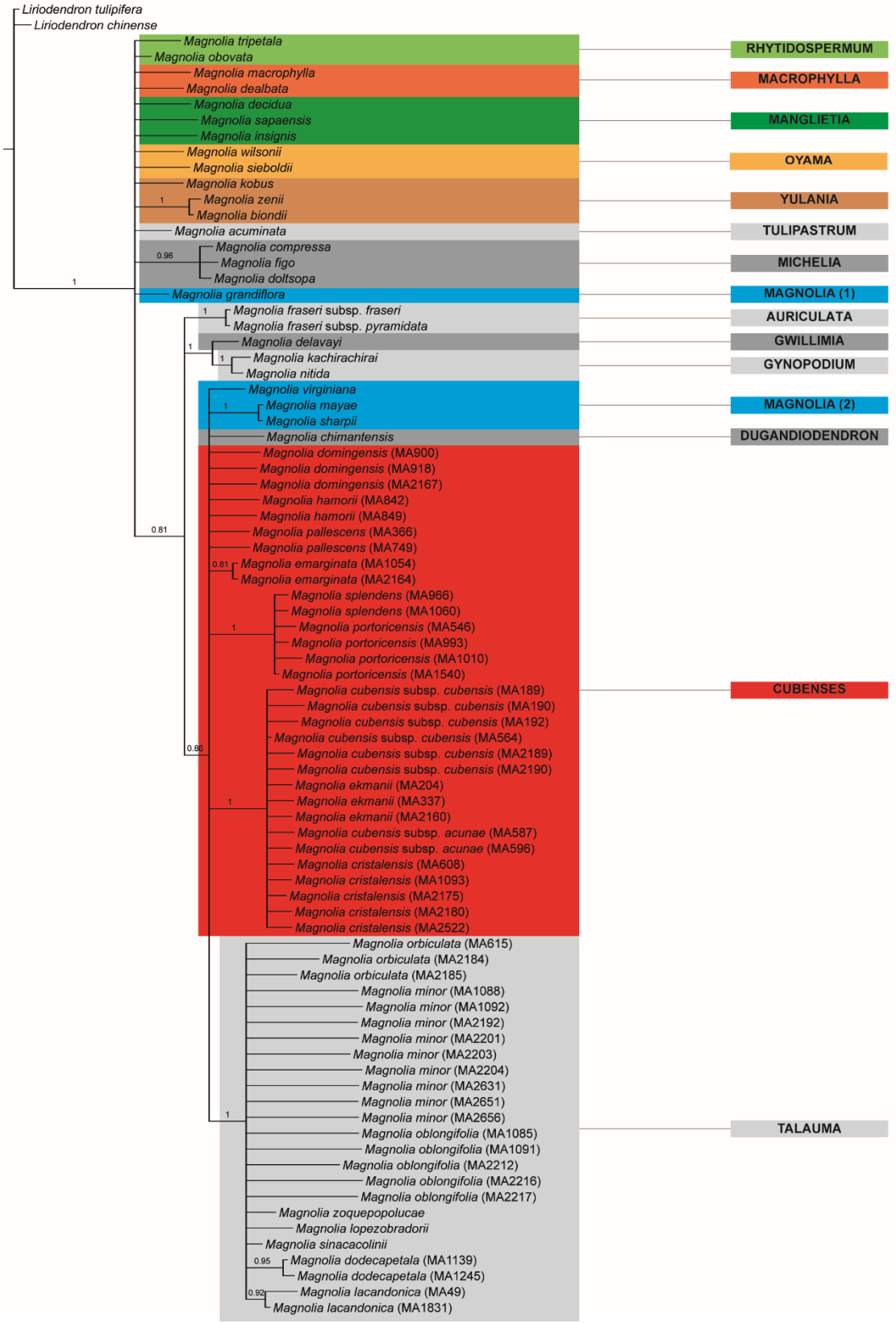
Appendix 3.4.4 Bayesian phylogenetic hypothesis of LFYB.



Appendix 3.4.5 Bayesian phylogenetic hypothesis of PHYA.



Appendix 3.4.6 Bayesian phylogenetic hypothesis of SQD1.



Appendix 3.5 The pairwise distance matrix for each nuclear gene (i.e. AGT1, GAI1, LEAFY, PHYA, SQD1), the concatenated chloroplast alignment and each separate chloroplast alignment (i.e. *atpB-rbcL*, *ndhF-rpL32*, *psbA-trnH*, *ndhF*, *rbcL*, *trnK*). Ambiguous sites and gaps (in their full length) are counted as a difference between two sequences. Magnoliaceae sequences are named according to their classification, unique lab number and species epitheton as listed in Appendix 3.1.

The file could not be converted to Word and/or PDF-format due to the size of the tables. However, the file can be temporarily consulted on: https://drive.google.com/open?id=1NZUfG0GsWG9tb50kHyJ_Eg3xwI9_8IuX; and will be published on Dryad together with the corresponding publication: **Veltjen E.**, Testé E., Palmarola Bejerano A., Asselman P., Hernández Rodríguez M., González Torres L. R., Chatrou L.W., Goetghebeur P., Larridon I., Samain M.-S. (submitted 9 december 2019) The evolutionary history of the Caribbean Magnolias (Magnoliaceae): testing species delimitations and biogeographical hypotheses using molecular data. Molecular Phylogenetics and Evolution.

Appendix 3.6 Partitioning schemes for all six Magnoliaceae alignments; used for gene and (calibrated) species phylogenetic tree reconstruction in the Bayesian framework.

1.CHLOROPLAST, concatenated dataset

1.1.Chloroplast concatenated subsets entered in PartitionFinder

1-789:	*atpB-rbcL*
790-1994:	*ndhF-rpl32*
1995-2441:	*psbA-trnH*
2442-4545\3:	*ndhF* partitioned according to codon position, start: position 1
2442-4545\3:	*ndhF* partitioned according to codon position, start: position 2
2444-4545\3:	*ndhF* partitioned according to codon position, start: position 3
4546-5913\3:	*rbcL* partitioned according to codon position, start: position 1
4547-5913\3:	*rbcL* partitioned according to codon position, start: position 2
4548-5913\3:	*rbcL* partitioned according to codon position, start: position 3
5914-6597:	*trnK* intron prior *matK*
6598-8121\3:	*trnK*, *matK,* partitioned according to codon position, start: position 1
6599-8121\3:	*trnK*, *matK,* partitioned according to codon position, start: position 2
6600-8121\3:	*trnK*, *matK,* partitioned according to codon position, start: position 3
8122-8351:	*trnK* intron after *matK*
8352-8409:	gaps coded with SeqState (Modified Complex Indel Coding (MCIC))

1.2.Chloroplast concatenated nexus-file defined charsets

```
charset Subset1 = 2443-4545\3 1-789 5914-6597;
charset Subset2 = 790-1994;
charset Subset3 = 1995-2441;
charset Subset4 = 6600-8121\3 2442-4545\3;
charset Subset5 = 2444-4545\3;
charset Subset6 = 4546-5913\3;
charset Subset7 = 4547-5913\3;
charset Subset8 = 4548-5913\3;
charset Subset9 = 6598-8121\3;
charset Subset10 = 6599-8121\3;
charset Subset11 = 8122-8351;
charset Subset12 = 8352-8409;
```

2.AGT1

2.1.AGT1 subsets entered in PartitionFinder

1-198\3:	AGT1, partitioned according to codon position, start: position 1
2-198\3:	AGT1, partitioned according to codon position, start: position 2
3-198\3:	AGT1, partitioned according to codon position, start: position 3
199-1126:	AGT1, intron
1127-1151:	gaps coded with SeqState (Modified Complex Indel Coding (MCIC))

2.2.AGT1 subsets nexus-file defined charsets

```
Charset Subset1 = 1-198\3 2-198\3;
Charset Subset2 = 3-198\3;
Charset Subset3 = 199-1126;
Charset Subset4 = 1127-1151;
```

3.GAI1

3.1.GAI1 subsets entered in PartitionFinder
1-968\3: GAI1, partitioned according to codon position, start: position 1
2-968\3: GAI1, partitioned according to codon position, start: position 2
3-968\3: GAI1, partitioned according to codon position, start: position 3
969: GAI1, gap coded with SeqState (Modified Complex Indel Coding (MCIC))

3.2.GAI1 subsets nexus-file defined charsets
Charset Subset1 = 1-968\3;
Charset Subset2 = 2-968\3;
Charset Subset3 = 3-968\3;
Charset Subset4 = 969;

4.LEAFY

4.1.LEAFY subsets entered in PartitionFinder
1-328\3: LEAFY, partitioned according to codon position, start: position 1
2-328\3: LEAFY, partitioned according to codon position, start: position 2
3-328\3: LEAFY, partitioned according to codon position, start: position 3
329-456: LEAFY, intron
457-463: LEAFY, gaps coded with SeqState (Modified Complex Indel Coding (MCIC))

4.2.LEAFY subsets nexus-file defined charsets
Charset Subset1 = 1-328\3 329-456;
Charset Subset2 = 3-328\3 2-328\3;
Charset Subset3 = 457-463;

5.PHYA

5.1.PHYA subsets entered in PartitionFinder
1-982\3: PHYA, partitioned according to codon position, start: position 1
2-982\3: PHYA, partitioned according to codon position, start: position 2
3-982\3: PHYA, partitioned according to codon position, start: position 3
983: PHYA, gap coded with SeqState (Modified Complex Indel Coding (MCIC))

5.2.PHYA subsets nexus-file defined charsets
Charset Subset1 = 1-982\3;
Charset Subset2 = 2-982\3;
Charset Subset3 = 3-982\3;
Charset Subset4 = 983;

6.SQD1

6.1.SQD1 subsets entered in PartitionFinder
1-502\3: SQD1, partitioned according to codon position, start: position 1
2-502\3: SQD1, partitioned according to codon position, start: position 2
3-502\3: SQD1, partitioned according to codon position, start: position 3

6.2.SQD1 subsets nexus-file defined charsets
Charset Subset1 = 1-502\3;
Charset Subset1 = 3-502\3 2-502\3;

Appendix 3.7 Output of the BioGeoBEARS analysis.

	LnL	numparams	d	e	j	AICc	AICc_wt
DEC	-32.69	2	0.0046	0.0017	0	69.91	0.0006
DEC+J	-24.6	3	1.0e-12	1.0e-12	0.027	56.29	0.52
DIVALIKE	-30.33	2	0.0061	1.0e-12	0	65.19	0.0061
DIVALIKE+J	-25	3	1.0e-12	1.0e-12	0.031	57.09	0.35
BAYAREALIKE	-40.37	2	0.0065	0.072 0	0	85.27	2.6e-07
BAYAREALIKE+J	-26.02	3	1.0e-07	1.0e-07	0.032	59.12	0.13

Appendix 4: SSR patterns of the Caribbean Magnolias

Appendix 4.1 Amplification tests. The 17 (sub)species are abbreviated according to Table 4.1. The results of the amplification tests are coded: **0** means no amplification; **1** means a single band on the agarose gel; **2** means multiple bands on the agarose gel. The sum of the number of (sub)species (S) for which 0, 1, and 2 are coded are given in **S0**, **S1** and **S2**, respectively. The sum of the number of markers (**M**) of which 0, 1, and 2 are coded are given in **M0**, **M1** and **M2,** respectively.

	ACU	CRI	CUB	DEA	DOD	DOM	EKM	HAM	LAC	MAY	MIN	OBL	ORB	PAL	POR	SPL	VIR	S0	S1	S2
MA39_023	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1	1	0	5	12	0
MA39_046	1	1	1	1	2	1	1	1	1	0	0	0	0	1	0	1	0	6	10	1
MA39_142	1	1	2	2	0	1	1	1	1	1	2	2	1	1	1	1	2	1	11	5
MA39_159	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	0	16	1
MA39_165	1	1	0	1	0	1	1	1	2	2	1	2	1	1	0	0	1	4	10	3
MA39_182	1	0	1	2	0	1	1	2	1	0	1	1	1	1	2	2	2	3	9	5
MA39_185	1	1	0	1	2	1	1	1	1	0	1	1	1	1	1	1	1	2	14	1
MA39_199	1	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	0	15	2
MA39_236	1	2	1	2	1	2	1	1	1	1	1	1	1	1	1	2	1	0	13	4
MA39_259	1	1	2	0	0	1	1	1	2	1	2	2	0	2	1	1	2	3	8	6
MA39_263	1	1	2	2	2	2	1	2	1	2	1	1	1	1	1	1	0	1	10	6
MA39_287	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	0	14	3
MA39_327	1	2	2	2	2	1	2	1	1	1	2	2	2	1	2	2	2	0	6	11
MA39_342	1	1	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1
MA39_348	1	1	1	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1
MA39_442	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	16	1
MA40_045	1	1	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1
MA40_072	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	2	1	0	14	3
MA40_136	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	16	0
MA40_175	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	16	1
MA40_223	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	15	0
MA40_282	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	15	0

MA41_076	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	2	15	0
MA41_215	1	2	1	1	1	1	1	1	0	1	2	2	1	1	1	1	2	1	12	4
MA41_264	1	2	1	1	1	2	1	1	1	1	1	2	1	2	1	1	2	0	12	5
MA41_373	1	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	0	15	2
MA42_001	1	1	1	0	1	1	1	1	0	0	2	2	2	1	1	1	2	3	10	4
MA42_028	1	2	1	1	2	2	1	2	1	1	1	1	1	2	2	2	2	0	9	8
MA42_059	1	1	0	2	1	1	1	1	2	2	1	1	1	1	1	1	1	1	13	3
MA42_063	1	1	1	0	1	1	1	1	0	0	4	4	4	1	1	1	1	3	11	0
MA42_072	1	1	1	2	2	1	1	1	2	1	1	1	1	1	1	1	1	0	14	3
MA42_077	1	1	1	2	2	1	1	1	1	2	2	2	1	1	1	1	1	0	12	5
MA42_083	1	1	1	1	2	1	1	1	2	1	1	0	0	2	2	2	1	2	10	5
MA42_087	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	16	1
MA42_102	1	1	0	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	15	1
MA42_126	2	1	1	2	1	1	1	1	2	2	0	0	2	1	1	1	2	2	9	6
MA42_147	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	4	13	0
MA42_166	1	2	0	1	2	1	1	1	0	1	1	2	1	2	1	1	2	2	10	5
MA42_185	2	1	1	2	2	2	1	1	2	4	2	2	1	2	1	2	1	0	7	9
MA42_197	1	2	1	1	1	2	2	2	0	2	2	0	2	1	2	2	2	2	5	10
MA42_202	1	2	1	1	2	1	1	1	2	2	1	2	1	2	1	2	2	0	9	8
MA42_203	1	1	1	2	2	1	1	1	2	0	2	2	0	1	1	1	2	2	9	6
MA42_231	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	17	0
MA42_241	1	1	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1
MA42_247	1	1	0	1	0	1	1	1	2	1	1	2	1	1	1	1	2	2	12	3
MA42_253	1	1	1	1	1	2	1	2	1	1	1	1	2	1	2	2	1	0	12	5
MA42_255	1	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	0	15	2
MA42_265	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	17	0
MA42_274	1	2	1	1	1	2	2	2	1	1	2	2	2	2	2	2	2	0	6	11
MA42_279	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	17	0
MA42_293	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	1	0	16
MA42_296	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	1	1	0	15	2
MA42_333	1	1	2	1	2	1	1	0	0	0	0	1	0	1	1	1	1	5	10	2
MA42_334	2	2	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	14	2

MA42_372	1	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	0	15	2
MA42_397	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	0	15	2
MA42_413	1	1	1	2	1	1	1	1	2	1	0	0	0	1	1	1	1	3	12	2
MA42_421	1	1	0	1	0	1	1	1	1	1	1	2	1	1	1	1	1	2	14	1
MA42_471	1	2	1	0	1	2	1	1	0	0	1	1	2	1	1	1	1	3	11	3
MA42_472	1	1	1	2	1	1	1	1	2	2	1	0	1	1	1	1	1	1	13	3
MA42_481	1	1	0	1	1	1	1	1	1	1	2	0	0	1	1	1	1	3	13	1
MA42_491	1	1	1	0	1	1	1	0	0	0	0	1	0	1	1	0	1	7	10	0
MA42_495	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	17	0
M0	0	1	3	6	6	0	0	3	10	10	6	9	9	0	2	2	3			
M1	59	49	56	38	43	53	59	53	39	43	44	35	44	54	52	49	44			
M2	4	13	4	19	14	10	4	7	14	9	12	18	9	9	9	12	16			

Appendix 4.2 Polymorphism tests. The 10 taxa are abbreviated according to Table 4.1. The results of the polymorphism tests are coded: **A**: polymorphic and unambiguous; **B**: monomorphic genotyped for all 20 individuals; **C**: monomorphic genotyped for 4 or 8 individuals; **D**: ambiguous to score; **E**: not amplifying (well) in the PCR. The sum of the number of (sub)species (**S**) for which A, B, C, D and E are coded are given in **SA**, **SB**, **SC**, **SD** and **SE**, respectively. The sum of the number of markers (**M**) of which A, B, C, D and E are coded are given in **MA**, **MB**, **MC**, **MD** and **ME**, respectively. Some marker × species combinations were not submitted to fragment analyses due to no amplification or double amplification products in the amplification tests (0 and 2, respectively, in Appendix 4.1). The number of (sub)species submitted to fragment analyses per marker is given in **SF** (maximum = 10). The number of markers tested in fragment analyses per (sub)species is given in **MF** (maximum = 63). The markers employed in the taxon-datasets are those coded with an A for the taxon at hand. The ten markers employed in the *Cubenses*-normalised-dataset (dataset 3) are the markers labelled with an asterisk.

	ACU	CUB	DOD	DOM	EKM	HAM	LAC	PAL	POR	SPL	SA	SB	SC	SD	SE	SF	SA+B+C
MA39_023	A	A	A	A	A	A	A	A	A	A	10	0	0	0	0	10	10
MA39_046	B	A		E	C	E	D	E	E	E	1	1	1	1	5	9	3
MA39_142	C	C	C	C	C	C	A	C	A	C	2	0	8	0	0	10	10
MA39_159	E	E	A	E	D	E	A	C	E	E	2	0	1	1	6	10	3
MA39_165	C	C		A	C	C	D	D	E	C	1	0	5	2	1	9	6
MA39_182	E	E	A	E	D	C	A	E	E		2	0	1	1	5	9	3
MA39_185*	A	A	A	A	A	A	A	A	A	A	10	0	0	0	0	10	10
MA39_199	A	A	A	A	C	A	C	A	A	C	7	0	3	0	0	10	10
MA39_236	E	E	D	E	A	A	A	E	A	E	4	0	0	1	5	10	4
MA39_259	C	C	A	C	A	E	A	A	C	C	4	0	5	0	1	10	9
MA39_263	A	B			A	C	C	C	C	C	2	1	5	0	0	8	8
MA39_287	C	C	A	C	C	C	A	C	C	C	2	0	8	0	0	10	10
MA39_327	E	E		E		E	A	E			1	0	0	0	5	6	1
MA39_342	E	E		E	E	E	A	E	E	E	1	0	0	0	8	9	1
MA39_348	E	E	E	E	E	E	A	E	A	A	3	0	0	0	7	10	3
MA39_442	A	A	A	C	C	C	A	C	B	C	4	1	5	0	0	10	10
MA40_045	A	A	B	D	B	A	B	A	A	D	5	3	0	2	0	10	8
MA40_072	C	C	E	D	A	C	A	D			2	0	3	2	1	8	5
MA40_136	C	C	A	C	C	C	C	C	C	A	2	0	8	0	0	10	10
MA40_175	C	C	B	C	C	C	C	C	C	A	1	1	8	0	0	10	10
MA40_223	C	C	C	D	C	A	C	C	C	A	2	0	7	1	0	10	9

MA40_282*	A	A	A	A	A	A	A	A	A	A	10	0	0	0	0	10	10
MA41_076	A	A	D	E	A	E	A	C	A	A	6	0	1	1	2	10	7
MA41_215	D	D		D	A	A		D	A	D	3	0	0	5	0	8	3
MA41_264	A	A	D		A	E	D		E	E	3	0	0	2	3	8	3
MA41_373*	A	A	B	A	A	A	A	A	A	A	9	1	0	0	0	10	10
MA42_001*	A	A	E	A	A	A	E	A	A	A	8	0	0	0	2	10	8
MA42_028	A	A	E		A		A				4	0	0	0	1	5	4
MA42_059	C	C	E	A	C	C	E	A	C	C	2	0	6	0	2	10	8
MA42_063	A	A	E	C	E	C	E	D	A	A	4	0	2	1	3	10	6
MA42_072	C	C	A	C	C	C	D	C	C	E	1	0	7	1	1	10	8
MA42_077*	B	A	A	A	A	A	E	A	A	A	8	1	0	0	1	10	9
MA42_083	A	A	D	C	A	A	D	A	E	D	5	0	1	3	1	10	6
MA42_087	A	A	C	D	A	E	C	E	A	E	4	0	2	1	3	10	6
MA42_102	D	D		D	A	A	D	D	A	A	4	0	0	5	0	9	4
MA42_126	A	D	E	A	B	A	E	E	A	A	5	1	0	1	3	10	6
MA42_147	D	D	E	C	C	C	D	C	A	A	2	0	4	3	1	10	6
MA42_166	A	A	D	A	D	D			D	D	3	0	0	5	0	8	3
MA42_185			D		D	D	E		A		1	0	0	3	1	5	1
MA42_197	D	A						D			1	0	0	2	0	3	1
MA42_202	A	D	D	D	A	D	D	D	D		2	0	0	7	0	9	2
MA42_203*	A	A	E	A	A	A	E	A	A	A	8	0	0	0	2	10	8
MA42_231*	A	A	A	A	A	A	A	A	A	A	10	0	0	0	0	10	10
MA42_241	A	A	C	D	E	A	C	A	D	A	5	0	2	2	1	10	7
MA42_247	A	A	E	D	A	D	C	D	D	D	3	0	1	5	1	10	4
MA42_253	C	C	C		A		E	D			1	0	3	1	1	6	4
MA42_255*	A	A	A	A	A	A	A	A	A	A	10	0	0	0	0	10	10
MA42_265	A	B	D	C	C	C	C	C	C	C	1	1	7	1	0	10	9
MA42_274	A	A	A				A				4	0	0	0	0	4	4
MA42_279	A	D	E	E	D	C	E	D	E	E	1	0	1	3	5	10	2
MA42_293				A	C	D	E	A	E	D	2	0	1	2	2	7	3
MA42_296	A	A	D	D	D	A	E	D	D	D	3	0	0	6	1	10	3
MA42_333	D	D	A	D	D	D		D	D	E	1	0	0	7	1	9	1

MA42_334	D	D	D	A	D	D	C	D	D	E	1	0	1	7	1	10	2
MA42_372	D	D	A	D	D	D	D	D	C	C	1	0	2	7	0	10	3
MA42_397*	A	A	A	A	A	A	D	A	A	A	9	0	0	1	0	10	9
MA42_413	B	A	E	D	A	A	E	D	A	A	5	1	0	2	2	10	6
MA42_421*	A	A	A	A	A	B	A	A	A	A	9	1	0	0	0	10	10
MA42_471	A	A	A	D	B	A	A	A	A	A	8	1	0	1	0	10	9
MA42_472	A	A	E	A	A	A	E	A	A	A	8	0	0	0	2	10	8
MA42_481	A	A	E	A	A	A	E	E	A	A	7	0	0	0	3	10	7
MA42_491	D	D	E	D	A	D	E	E	E		1	0	0	4	4	9	1
MA42_495	D	D	A	A	A	D	A	E	A	C	5	0	1	3	1	10	6
MA	**32**	**31**	**21**	**21**	**30**	**24**	**23**	**20**	**29**	**25**							
MB	3	2	3	0	3	1	1	0	1	0							
MC	11	11	5	11	14	15	10	12	10	11							
MD	9	11	10	15	9	10	10	15	7	7							
ME	6	6	15	9	4	9	15	11	10	10							
MF	61	61	54	56	60	59	59	58	57	53							
MA+MB+MC	46	44	29	32	47	40	34	32	40	36							

Appendix 4.3 Microsatellite primer information. Primers that scored polymorphic, unambiguous and with no null alleles for at least one of the ten tested species are listed. Primers with the locus name starting with **MA39** are developed on *Magnolia lacandonica*, **MA40** on *M. mayae*, **MA41** on *M. dealbata* and **MA42** on *M. cubensis* subsp. *acunae*. All SSR primers run with an annealing temperature (T_a) of 60°C. Size range of all the amplicons for the specific primer pair are given applicable to all the species on which the primer pair was tested to be valuable. The original size of the fragment on which the primer pair is developed is given between square brackets. The highlighted primers have an error reported in one of the duplicated genotypes. **NT**: not tested.

Locus Name	Primer sequences (5'-3')	Repeat motif	Size range [size] (bp)	Error rate (%) [#errors/#tests]	GenBank Accession Number
MA39_023	F: ATCACGCATCTGCACAGACA R: GGACAACGAACGTCTGGCTA	(AG)7	118–198 [97]	0% [0/120]	MH923371
MA39_046	F: CCATCCAGAGCACGAGTGTT R: CACACGGAAACTCCAGACCA	(AG)18	132–134 [137]	0% [0/4]	MH923372
MA39_142	F: ATGTGGCCTACGTTGCTCAA R: GGATCTCAGACCCATCGTGC	(TAA)10	207–221 [190]	0% [0/48]	MH923373
MA39_159	F: ATCAGGAGTGTAACGCCACC R: GGCGAGCTCGTTAGATCCTC	(TC)16	146–178 [139]	0% [0/67]	MH923374
MA39_165	F: AATGTAGTGGGTCCGGCTTC R: CCAAACCATGTGCGTCCTTG	(TC)18	197–199 [181]	0% [0/12]	MH923375
MA39_182	F: CTACACGGGTGAAGCCTACC R: GGCCGTAATCAGAGTCCACC	(TC)12	144–148 [129]	0% [0/33]	MH923376
MA39_185	F: CGGGTGTTGTAGATGACGCT R: AAGACACGGAATGGGACGAG	(AG)15	231–358 [209]	0% [0/107]	MH923377
MA39_199	F: CGCCCACATCTACCTCTTCG R: TCCAGGAGTTTCTGTGCACC	(GGA)5	193–222 [187]	0% [0/45]	MH923378
MA39_236	F: GGCAGAAGCAAGGAAGAGGA R: GAATCAAACCGCAGCTCGAC	(GA)19	163–194 [153]	0% [0/52]	MH923379
MA39_259	F: TGATAGAGTGGGATGGCGGA R: TGCTGCTTTGAGGCCTGTTA	(CT)11	105–179 [96]	0% [0/98]	MH923380
MA39_263	F: GTAGCCATGTGGGTCTGTCC R: AGTTGGTAGGGCACATGTCC	(CT)13	148–158 [126]	0% [0/12]	MH923381
MA39_287	F: CCTCGAGCATCACCACCTTC R: GGTGGACCCTACACATGTGG	(AG)16	142–176 [129]	0% [0/68]	MH923382
MA39_327	F: CCCATTCGCAATCTTACGCC R: TGGTTTCAATGCGAGACGGT	(CT)15	136–172 [117]	3.33% [2/60]	MH923383

MA39_342	F: TCCCTTCAGTCTTCACACGC R: AAAGGAGCGTTGAGTGGTGG	(TC)14	164–208 [146]	0% [0/24]	MH923384
MA39_348	F: GTAGAGCTCCCATGCCTCAC R: GGGCTGTCTACTGGATGGAC	(TC)17	150–180 [119]	0% [0/28]	MH923385
MA39_442	F: AGTCGATCCTCTTGCTGCAC R: GAGGGAGCATCGGCCATTAC	(AAG)8	133–145 [109]	0% [0/53]	MH923386
MA40_045	F: TTGTGGGCCAAGCTCGATAG R: ATTGTGGCATGTACCTCGCA	(TC)13	246–292 [231]	1.10% [1/91]	MH923387
MA40_072	F: ATCCGATTCCCATTCGGACG R: CTGCCGGAGAAGAGAACGAG	(CT)14	117–137 [111]	3.85% [1/26]	MH923388
MA40_136	F: CTGGGCATTGCAGAGTAGCT R: CATCCCAGCAGTTACGACGA	(GCC)6	116–128 [108]	0% [0/21]	MH923389
MA40_175	F: CGTTCTGCGCGATCAATCTC R: GCATCCGAATCCCAGCTACA	(GCT)6	105–111 [91]	0% [0/14]	MH923390
MA40_223	F: TTCAGTGGCTGGAGCTTCAG R: GGAGCATCTTGGCCTTTGGA	(GAT)5	116–132 [93]	0% [0/21]	MH923391
MA40_282	F: TCTCTTTCCCTCCGTCCTCC R: TCTTCCGGCTTCATGTCGTC	(GA)15	128–166 [116]	0% [0/66]	MH923392
MA41_076	F: AACAACGCTGGGTGATGGAA R: TGGAGTTGACGCCTCTAGGA	(GA)26	169–209 [176]	1.59% [1/63]	MH923393
MA41_215	F: TTCAGCCAACTGGAATCCGG R: GTGCCTTGAAATGAGCTGGC	(AG)18	219–237 [207]	0% [0/43]	MH923394
MA41_264	F: AACAGCCTTTGGGAAGTGCA R: CAGCCATTCCGCTTCCCTTA	(GA)15	236–245 [173]	0% [0/28]	MH923395
MA41_373	F: GCGCCCAATCAGAACACAAC R: GGGAAGAGCTTCTTTCGCCA	(CT)16	166–207 [165]	0% [0/94]	MH923396
MA42_001	F: ATCCGACCCAACATGGTGAC R: AGCCGAGTCTGAGCTGAGTA	(TC)11	144–169 [130]	0% [0/82]	MH923397
MA42_028	F: GGATCGTCTTCCGCCATTCT R: TTCCGTACGATGCTCCCATG	(CT)33	129–147 [151]	0% [0/31]	MH923398
MA42_059	F: AGGGACTCGGCATCTATGGA R: GAGTCGACTCAGCAACTCCC	(AG)8	246–248 [217]	0% [0/25]	MH923399
MA42_063	F: ATAGCAACAACGTAGCCGGT R: TGGCGAGGTCCCTCTACTAC	(GA)14	218–250 [203]	0% [0/35]	MH923400
MA42_072	F: CCCACCTAGGTTTCCAGTGC R: TGCGTTCGAAAGGCACAATG	(CA)5	269–273 [245]	0% [0/8]	MH923401

MA42_077	F: GAGACATGGAACCCACACGT R: CTGGTGGTCTAGCCGATCTG	(AG)8	234–284 [211]	0% [0/99]	MH923402
MA42_083	F: GTCTTCCACGGGAGCAAGAG R: CGAGTTGGACCCAGTGAGTC	(GAA)17	101–145 [120]	0% [0/47]	MH923403
MA42_087	F: TAAGTCAGAACCCAGCTGGC R: GGCGAATCGGGACCCTTTAA	(GA)17	179–204 [157]	0% [0/18]	MH923404
MA42_102	F: CTGTCTCAGCGTCTCACTCC R: AGACGAAGGGAGGGAAGGAG	(CT)21	89–116 [90]	0% [0/43]	MH923405
MA42_126	F: CACATCGTCCGTCCAGACAT R: TCGCCTAGCCAATAGTCTGC	(AT)9	126–135 [103]	0% [0/46]	MH923406
MA42_147	F: AAATCACGGTCGGGATTCGA R: GGGCATGAGCTGTGGATCTT	(CT)8	251–263 [222]	0% [0/33]	MH923407
MA42_166	F: CTCTTGGCCGATGGAGATGG R: GGACGTGGGAAGCATCTCTG	(TC)13	126–142 [122]	0% [0/25]	MH923408
MA42_185	F: CTGCTGGACGGTCTGGATTG R: TCGAGCTGTCCATCATCACG	(AG)11	120–153 [90]	0% [0/14]	MH923409
MA42_197	F: GGCTAGCCGACTTAACCTGA R: CGTCAAGTCTGAGTCGGGTC	(TC)25	185–205 [184]	NT	MH923410
MA42_202	F: AGGGAGGGCTCATAGTGGTG R: CGGACAGTGGTGTGGTTCAT	(CT)11	188–220 [122]	0% [0/16]	MH923411
MA42_203	F: TGAAGAACACAGGCCATGGA R: GAGAGGTGCTTCACGGGTAG	(TC)16	105–136 [102]	1% [1/100]	MH923412
MA42_231	F: GGGTGCGAAATGTGCATCAA R: GGGCCAGTGAGCATTAGAGC	(AG)14	152–194 [131]	0% [1/77]	MH923413
MA42_241	F: GGGTACCCTATGGTCCAACC R: GTCCGACTAAGGCCCATTGT	(CA)11	108–114 [92]	0% [0/30]	MH923414
MA42_247	F: AGGTGGGCAATCATACAAGGG R: AGGGCCCATAGTACAGGGTT	(AG)24	120–154 [112]	0% [0/23]	MH923415
MA42_253	F: GACGGACTTAGAGCATGGGT R: GCTTGAATTTGTGGTGGCCC	(TC)36	154–178 [182]	0% [0/8]	MH923416
MA42_255	F: ACGTGGGTCGAGGATCAAGT R: GGACCCACCTCCAACAGATC	(AG)14	144–174 [137]	1.89% [2/106]	MH923417
MA42_265	F: CGCACACCAAAGCTGCATT R: CGGCTACTTCCCAAGGGATG	(AAG)12	251–254 [238]	0% [0/10]	MH923418
MA42_274	F: CAGCCATTCCTTGAGATGGGT R: GCCGAAACGATCTCTTCCCT	(GA)18	161–209 [154]	0% [0/48]	MH923419

MA42_279	F: AGACAGTCCAGTAGGGTGGA R: GAGCTCCTCCAATCTCCACC	(AG)17	142–150 [142]	0% [0/5]	MH923420
MA42_293	F: TGCAACTGAGACGAGTTGGG R: GGTACGGACTAGGGTACAGGT	(GA)16	120–124 [109]	0% [0/24]	MH923421
MA42_296	F: TTGACAGTCTGGCAAGGTGG R: GAGGGCTCATAGTGGTGGC	(AG)15	166–180 [144]	0% [0/17]	MH923422
MA42_333	F: GGAGTCAAGCGACAACTCCA R: GTGTGCATGTGGATAAGCCA	(GA)33	225–283 [257]	0% [0/8]	MH923423
MA42_334	F: TGCAGATGGTGGCAATGCTT R: GGTCAAGTTTACACCGCGGA	(TCA)10	154–172 [143]	0% [0/14]	MH923424
MA42_372	F: ATCCGAACTCGACTGTGACT R: CCTACCCAAGTCAGCCCATC	(TC)20	145–217 [141]	0% [0/7]	MH923425
MA42_397	F: TAGTAGCAGGGTCCCTCCTC R: TCCATTCATTAGGGTGGGCA	(TC)20	100–163 [98]	0% [0/9]	MH923426
MA42_413	F: GCCGAGTGCAAGCCATAAGG R: TGCACCTAAGCTCCACAGTC	(GA)9	127–153 [103]	0% [0/45]	MH923427
MA42_421	F: GACAGCAGACCTGACCGATT R: GACCAGTGCATCCCATCAAA	(TC)10	298–390 [280]	0% [0/69]	MH923428
MA42_471	F: TGATGAAGAGCCCAGATCGTC R: TGGCCTTGTTCTCCATACGT	(GA)16	163–230 [153]	0% [0/134]	MH923429
MA42_472	F: AGAGTTACACATGCAAACCCG R: TGATGTTGTTGCTCGGCTGA	(AG)17	157–205 [140]	0% [0/97]	MH923430
MA42_481	F: CGATCTGAGTCCGCAAGAGT R: GACGCAGAAATCTCAGCAAGA	(TC)15	212–238 [197]	0% [0/62]	MH923431
MA42_491	F: TGGAAGAGTCAACCACACTGG R: ACTGTAATGGACCAACAGCCA	(CT)27	103–123 [108]	0% [0/8]	MH923432
MA42_495	F: TGCATCTCCTCATCCTCCCA R: ACGCCATTCAATTACCTACGG	(GA)26	92–152 [97]	0% [0/51]	MH923433

Appendix 4.4 Population statistics given per (sub)species, marker and location. **N**: (mean) number of genotyped individuals, consistent individuals with no peaks: *****. **A**: mean number of alleles. $\mathbf{H_O}$: observed heterozygosity. $\mathbf{H_E}$: expected heterozygosity. $\mathbf{F_{IS}}$: inbreeding coefficient, significant deviations from Hardy-Weinberg Proportions (HWP): ***** (p = 0.05) and ****** (p = 0.05, Bonferroni corrected), M: monomorphic. $\mathbf{A_0}$: estimated null allele frequency, locus-population combinations recognised by MICRO-CHECKER: *****. Statistics averaged per population: "Pop (number of SSR markers)". **[1, 2]**: excluded markers. **SD** = Standard Deviation. **a** *Magnolia cubensis* subsp. *acunae*: 32 polymorphic SSR markers. **b** *Magnolia cubensis* subsp. *cubensis*: 31 polymorphic SSR markers. **c** *Magnolia dodecapetala*: 21 polymorphic SSR markers. **d** *Magnolia domingensis*: 21 polymorphic SSR markers. **e** *Magnolia ekmanii*: 30 polymorphic SSR markers. **f** *Magnolia hamorii*: 24 polymorphic SSR markers. **g** *Magnolia lacandonica*: 23 polymorphic SSR markers. **h** *Magnolia pallescens*: 20 polymorphic SSR markers. **i** *Magnolia portoricensis*: 29 polymorphic SSR markers. **j** *Magnolia splendens*: 25 polymorphic SSR markers.

Appendix4.4a *Magnolia cubensis* subsp. *acunae*: 32 polymorphic SSR markers.

Population	TOP					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	4	0.500	0.596	0.186	0.069
MA39_185	20	10	0.750	0.816	0.107*	0.004
MA39_199	18	3	0.500	0.440	-0.109	0
MA39_263	20	2	0.450	0.469	0.066	0.013
MA39_442	20	2	0.300	0.255	-0.152	0
MA40_045	20	8	0.700	0.806	0.157	0.068
MA40_282	20	9	0.850	0.835	0.008	0
MA41_076	20	2	0.050	0.049	0	0
MA41_264	20	4	0.750	0.724	-0.011	0
MA41_373	20	7	0.800	0.770	-0.013	0
MA42_001	20	5	0.600	0.693	0.159	0.068
MA42_028[1]	20	6	0.300	0.518	0.441**	0.161*
MA42_063	19	11	0.895	0.855	-0.02	0
MA42_083	20	8	0.800	0.643	-0.221	0
MA42_087	20	7	0.750	0.743	0.016	0.016
MA42_126	20	3	0.350	0.366	0.07	0.016
MA42_166	20	4	0.600	0.469	-0.256	0
MA42_202	20	11	0.750	0.643	-0.142	0
MA42_203	20	6	0.850	0.793	-0.047	0
MA42_231	20	3	0.350	0.486	0.304	0.092
MA42_241	20	3	0.550	0.595	0.101	0.009
MA42_247	20	9	0.900	0.823	-0.069	0
MA42_255	20	5	0.550	0.646	0.174	0.048
MA42_265	20	2	0.100	0.095	-0.027	0
MA42_274	20	5	0.550	0.445	-0.212	0
MA42_279	20	5	0.600	0.623	0.062	0
MA42_296	20	7	0.700	0.649	-0.053	0
MA42_397	20	5	0.550	0.654	0.184*	0
MA42_421	20	8	0.800	0.779	-0.002	0
MA42_471	19	3	0.526	0.543	0.058	0
MA42_472	20	5	0.500	0.586	0.172	0.030
MA42_481	20	3	0.500	0.445	-0.098	0
Pop (31[1])	19.871	5.452	0.594	0.591	0.021	
SD (31[1])	0.077	0.493	0.038	0.037		

Appendix 4.4b *Magnolia cubensis* subsp. *cubensis*: 31 polymorphic SSR markers.

Population	PIC					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	4	0.550	0.629	0.150	0.048
MA39_046	20	2	0.100	0.095	-0.027	0
MA39_185	20	9	0.750	0.758	0.036	0
MA39_199	19	2	0.211	0.188	-0.091	0
MA39_442	20	2	0.100	0.095	-0.027	0
MA40_045	20	9	0.800	0.809	0.037	0
MA40_282	20	6	0.650	0.605	-0.049	0
MA41_076	20	3	0.500	0.454	-0.077	0
MA41_264	20	4	0.850	0.588	-0.426*	0
MA41_373	20	7	0.600	0.578	-0.013	0
MA42_001	20	5	0.700	0.709	0.038	0
MA42_028[1]	19*	7	0.474	0.785	0.419**	0.236*
MA42_063	20	4	0.650	0.611	-0.038	0
MA42_077	20	3	0.300	0.329	0.113	0.029
MA42_083	20	9	0.750	0.790	0.076	0.020
MA42_087[2]	20	5	0.400	0.553	0.300*	0.074
MA42_166	20	4	0.600	0.614	0.048	0
MA42_197	20	11	0.850	0.829	0.000	0.020
MA42_203	20	8	0.750	0.808	0.097	0.027
MA42_231	20	3	0.400	0.505	0.232	0.094
MA42_241	20	2	0.550	0.439	-0.229	0
MA42_247	20	11	0.850	0.866	0.044	0
MA42_255[2]	20	7	0.550	0.740	0.281*	0.067
MA42_274	20	7	0.700	0.710	0.040	0
MA42_296	20	6	0.600	0.719	0.190	0.051
MA42_397	20	11	0.850	0.891	0.072	0.032
MA42_413	20	3	0.400	0.366	-0.067	0
MA42_421	20	7	0.700	0.818	0.169	0.054
MA42_471	20	5	0.550	0.648	0.176	0.067
MA42_472	20	8	0.900	0.834	-0.054	0
MA42_481	20	8	0.750	0.825	0.116	0.023
Pop (30[1])	19.967	5.833	0.597	0.613	0.052	
SD (30[1])	0.033	0.521	0.040	0.041		
Pop (28[1,2])	19.964	5.821	0.606	0.611	0.034	
SD (28[1,2])	0.036	0.556	0.042	0.043		

Appendix 4.4c *Magnolia dodecapetala*: 21 polymorphic SSR markers.

Population	MART						GUA					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	11	0.850	0.831	0.003	0	20	2	0.250	0.219	-0.118	0
MA39_159	20	2	0.350	0.399	0.147	0.040	20	3	0.100	0.096	-0.013	0
MA39_182	20	2	0.350	0.399	0.147	0.040	20	2	0.300	0.375	0.224	0.064
MA39_185	19	8	0.789	0.803	0.044	0.010	20	8	0.700	0.810	0.161	0.068
MA39_199[1]	20	2	0.150	0.139	-0.056	0	19*	3	0.053	0.445	0.888**	0.352*
MA39_259[2]	20	2	0.050	0.049	0	0	20	2	0.000	0.095	1.000*	0.149*
MA39_287[2]	20	10	0.650	0.834	0.245*	0.090*	20	9	0.700	0.778	0.125	0.015
MA39_442	20	2	0.050	0.049	0	0	20	1	0.000	0.000	M	0
MA40_136	20	1	0.000	0.000	M	0	20	3	0.500	0.535	0.091	0.014
MA40_282[2]	20	8	0.450	0.724	0.400**	0.147*	20	10	0.750	0.838	0.130*	0.022
MA42_072	20	2	0.250	0.219	-0.118	0	19	3	0.263	0.342	0.256	0.088
MA42_077[2]	20	13	0.500	0.825	0.415**	0.182*	20	13	0.950	0.895	-0.036	0
MA42_231	20	12	0.850	0.863	0.040	0	20	8	0.750	0.821	0.112	0.058
MA42_255	20	6	0.900	0.710	-0.244	0	20	10	0.850	0.860	0.037	0
MA42_274	20	14	0.900	0.910	0.037	0	20	15	0.850	0.878	0.057*	0.042
MA42_333	20	7	0.650	0.766	0.177	0.032	20	18	0.950	0.909	-0.020	0
MA42_372[1]	19	15	0.526	0.909	0.443**	0.245*	20	8	0.800	0.784	0.005	0.005
MA42_397[1]	19	3	0.211	0.652	0.692**	0.326*	20	3	0.300	0.516	0.440	0.139*
MA42_421	20	1	0.000	0.000	M	0	20	2	0.100	0.095	-0.027	0
MA42_471	20	18	0.900	0.910	0.037	0	20	13	0.800	0.865	0.101	0.037
MA42_495	20	2	0.100	0.095	-0.027	0	20	16	0.850	0.881	0.061	0.003
Pop (21)	19.857	6.714	0.451	0.528	0.170*		19.905	7.238	0.515	0.573	0.127*	
SD (21)	0.078	1.179	0.072	0.078			0.066	1.173	0.075	0.071		
Pop (18[1])	19.944	6.722	0.477	0.521	0.110*		19.944	7.667	0.537	0.572	0.087	
SD (18[1])	0.056	1.252	0.081	0.085			0.056	1.326	0.081	0.082		
Pop (14[1,2])	19.929	6.286	0.496	0.497	0.028		19.929	7.429	0.519	0.549	0.081	
SD (14[1,2])	0.071	1.488	0.099	0.098			0.071	1.606	0.090	0.093		

Appendix 4.4d *Magnolia domingensis:* 21 polymorphic SSR markers. Null alleles: MA39_199. Pairs of loci in linkage disequilibrium: 43/210 pairwise tests with a p-value lower than 0.05, of which two pairs: MA39_199 × MA42_421 (indicated with A1) and MA42_231 × MA42_472 (indicated with A2) remained significant after sequential Bonferroni corrections. There were 210 pairwise tests, 10.5 [6, 16] expected to test false positive when p = 0.05. When considering the two populations separately, 15/210 and 41/210 pairwise tests were significant for BAR and ROD, respectively.

Population	BAR						ROD					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	4	0.600	0.621	0.06	0.004	20	4	0.800	0.666	-0.176*	0
MA39_165	20	1	0.000	0.000	M	0	20	2	0.250	0.439	0.451	0.138
MA39_185	20	5	0.850	0.759	-0.095	0	20	4	0.750	0.528	-0.4	0
MA39_199[1(A1)]	20	4	0.350	0.539	0.373*	0.126*	20	3	0.350	0.301	-0.137	0
MA40_282	20	6	1.000	0.794	-0.236	0	20	6	0.850	0.729	-0.141	0
MA41_373	20	6	0.700	0.679	-0.006	0.014	20	5	0.800	0.746	-0.046	0
MA42_001	20	5	0.750	0.694	-0.056	0	20	2	0.250	0.219	-0.118	0
MA42_059	20	2	0.550	0.399	-0.357	0	20	1	0.000	0.000	M	0
MA42_077	20	3	0.500	0.486	-0.003	0	20	2	0.350	0.489	0.307	0.094
MA42_126	20	2	0.500	0.455	-0.073	0	20	2	0.600	0.480	-0.226	0
MA42_166	20	3	0.400	0.486	0.202	0.049	20	3	0.150	0.141	-0.036	0
MA42_203	20	5	0.800	0.609	-0.291	0	20	3	0.550	0.514	-0.045	0
MA42_231[A2]	20	5	0.750	0.754	0.031	0	20	5	0.650	0.715	0.116	0.018
MA42_255	20	4	0.850	0.675	-0.235	0	20	4	0.550	0.581	0.079	0.017
MA42_293	20	2	0.600	0.455	-0.295	0	20	2	0.050	0.049	0	0
MA42_334	20	2	0.400	0.480	0.191	0	20	2	0.250	0.219	-0.118	0
MA42_397	20	11	0.850	0.835	0.008	0.055	20	5	0.800	0.668	-0.174*	0
MA42_421[A1]	20	4	0.450	0.446	0.017	0	20	2	0.450	0.499	0.123	0
MA42_472[1(A2)]	20	10	0.750	0.828	0.119	0.026	20	8	0.950	0.793	-0.174	0
MA42_481	20	8	0.850	0.768	-0.082	0	20	6	0.750	0.826	0.118	0.035
MA42_495	19	3	0.474	0.492	0.064	0.016	20	4	0.700	0.648	-0.056	0
Pop (19^1)	19.947	4.263	0.625	0.573	-0.065		20	3.368	0.503	0.482	-0.018	
SD (19^1)	0.053	0.551	0.054	0.045			0	0.352	0.064	0.056		

Appendix 4.4e *Magnolia ekmanii*: 30 polymorphic SSR markers.

Population	GRA						MAN					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023[1]	20	4	0.200	0.596	0.679**	0.240*	19	3	0.474	0.566	0.190	0.125
MA39_185	20	2	0.150	0.219	0.337	0.085	20	5	0.700	0.731	0.068	0
MA39_236	20	7	0.700	0.693	0.015*	0	20	8	0.750	0.686	-0.067	0
MA39_259	20	2	0.200	0.180	-0.086	0	20	2	0.500	0.495	0.016	0
MA39_263	20	1	0.000	0.000	M	0	20	2	0.500	0.420	-0.166	0
MA40_072	20	2	0.050	0.049	0	0	20	2	0.550	0.499	-0.077	0
MA40_282	20	4	0.750	0.714	-0.025	0	20	3	0.450	0.454	0.034	0
MA41_076	20	7	0.650	0.695	0.090	0	20	3	0.250	0.366	0.340	0.103
MA41_215	20	1	0.000	0.000	M	0	20	2	0.400	0.420	0.073	0.016
MA41_264	20	2	0.300	0.320	0.088	0.020	20	2	0.100	0.180	0.465	0.106
MA41_373	20	5	0.550	0.546	0.019	0	20	1	0.000	0.000	M	0
MA42_001	20	4	0.700	0.580	-0.182	0	20	3	0.550	0.540	0.007	0
MA42_028	20	1	0.000	0.000	M	0	20	2	0.350	0.349	0.022	0
MA42_077	20	3	0.550	0.526	-0.020	0	20	2	0.300	0.255	-0.152	0
MA42_083	20	3	0.300	0.261	-0.123	0	20	2	0.250	0.219	-0.118	0
MA42_087[1]	20	5	0.350	0.585	0.423*	0.119*	20	4	0.600	0.616	0.052	0
MA42_102	20	5	0.750	0.700	-0.046	0	20	6	0.850	0.800	-0.037	0
MA42_202	20	12	0.950	0.893	-0.039	0	20	9	1.000	0.774	-0.269	0
MA42_203	20	4	0.650	0.656	0.035	0	20	3	0.550	0.526	-0.020	0
MA42_231	20	3	0.100	0.096	-0.013	0	20	1	0.000	0.000	M	0
MA42_247	20	8	0.950	0.798	-0.166	0	20	5	0.400	0.433	0.101	0.003
MA42_253	20	6	0.600	0.614	0.048	0	20	6	0.700	0.756	0.100	0.022
MA42_255	20	3	0.100	0.096	-0.013	0	20	3	0.500	0.576	0.157	0.059
MA42_397	20	9	0.900	0.826	-0.064	0	19	10	1.000	0.778	-0.260	0
MA42_413	20	4	0.500	0.516	0.057	0	20	2	0.050	0.049	0	0
MA42_421	20	6	0.750	0.695	-0.054	0	20	3	0.600	0.629	0.071	0.008
MA42_472	20	4	0.300	0.270	-0.086	0	20	4	0.300	0.269	-0.091	0
MA42_481	20	4	0.550	0.569	0.059	0	20	2	0.100	0.095	-0.027	0
MA42_491	20	6	0.650	0.665	0.048	0	20	5	0.650	0.681	0.071	0
MA42_495	20	9	0.850	0.820	-0.011	0	19	8	0.947	0.838	-0.104	0
Pop (28[1])	20	4.536	0.482	0.464	-0.013		19.929	3.786	0.475	0.458	-0.012	
SD (28[1])	0	0.516	0.059	0.055			0.050	0.470	0.055	0.048		

Appendix 4.4f *Magnolia hamorii*: 24 polymorphic SSR markers.

Population	COR						CAC					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	2	0.300	0.480	0.397	0.124	20	2	0.450	0.349	-0.267	0
MA39_185	20	6	0.650	0.753	0.161	0.051	20	6	0.550	0.733	0.273	0.091
MA39_199	20	2	0.250	0.219	-0.118	0	20	3	0.400	0.516	0.249	0.072
MA39_236	20	5	0.750	0.706	-0.036	0	20	7	0.850	0.771	-0.077*	0
MA40_045	20	12	0.900	0.878	0	0.004	20	11	0.950	0.883	-0.051	0
MA40_223[1]	20	2	0.250	0.489	0.508*	0.162*	20	2	0.500	0.375	-0.31	0
MA40_282	20	13	0.800	0.898	0.134	0.051	20	14	0.900	0.851	-0.032	0
MA41_215	20	5	0.900	0.769	-0.146	0	20	6	0.800	0.755	-0.034	0
MA41_373	20	8	0.850	0.824	-0.006	0	20	8	0.800	0.830	0.062	0
MA42_001	20	3	0.450	0.511	0.145	0.035	20	3	0.500	0.564	0.138	0.077
MA42_077	20	2	0.550	0.489	-0.1	0	20	2	0.450	0.469	0.066	0.013
MA42_083	20	5	0.750	0.691	-0.059	0	20	5	0.700	0.689	0.009	0
MA42_102	20	9	0.900	0.861	-0.019	0	20	8	0.800	0.813	0.041	0
MA42_126	20	4	0.550	0.579	0.075	0	20	2	0.600	0.480	-0.226	0
MA42_203	20	6	0.700	0.786	0.135	0.041	20	6	0.700	0.705	0.033	0.003
MA42_231	20	6	0.850	0.775	-0.071	0	20	7	0.850	0.776	-0.07	0
MA42_241	20	2	0.750	0.499	-0.484	0	20	2	0.300	0.375	0.224	0.064
MA42_255	20	7	0.850	0.823	-0.008	0	20	9	0.800	0.835	0.067*	0.031
MA42_296	20	8	0.850	0.760	-0.093	0	20	5	0.600	0.750	0.224	0.089
MA42_397	20	10	0.800	0.825	0.056	0	20	9	0.800	0.844	0.077	0.001
MA42_413[1]	20	8	0.600	0.798	0.272*	0.114*	20	6	0.500	0.711	0.320	0.116
MA42_471	20	7	0.850	0.804	-0.032	0	20	6	0.900	0.803	-0.096	0
MA42_472	20	16	0.950	0.905	-0.024	0	20	15	0.950	0.896	-0.034	0
MA42_481	20	9	0.700	0.833	0.184	0.089	20	9	0.900	0.796	-0.105	0
Pop (22[1])	20	6.682	0.723	0.712	0.011		20	6.591	0.707	0.704	0.021	
SD (22[1])	0	0.804	0.041	0.037			0	0.783	0.042	0.036		

Appendix 4.4g *Magnolia lacandonica*: 23 polymorphic SSR markers. Pairs of loci in linkage disequilibrium (LD): there were 36/231 pairwise tests with a p-value lower than 0.05. Two pairs: MA39_185 × MA39_442 (A1) and MA41_373 × MA42_028 (A2) remained significant after sequential Bonferroni corrections. Of the 231 pairwise tests, 12.65 [7, 19] were expected to test false positive when p = 0.05. When the populations were considered separately, the high amount of LD remains: 27/231 for LAC and 33/231 for YAJ.

Population	LAC						YAJ					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	1	0.000	0.000	M	0	20	2	0.300	0.320	0.088	0.020
MA39_142	20	2	0.300	0.255	-0.152	0	20	2	0.300	0.255	-0.152	0
MA39_159	20	7	0.900	0.828	-0.062	0	20	4	0.600	0.509	-0.154	0
MA39_182[1]	19*	5	0.474	0.715	0.361*	0.227*	20	4	0.150	0.404	0.644*	0.192*
MA39_185[(A1)]	20	5	0.650	0.560	-0.136	0	20	5	0.750	0.664	-0.105*	0
MA39_236	20	7	0.800	0.825	0.056	0.030	20	7	1.000	0.768	-0.279	0
MA39_259	20	3	0.350	0.515	0.343	0.100	20	3	0.750	0.526	-0.404	0
MA39_287	20	4	0.800	0.678	-0.156	0	20	7	0.900	0.766	-0.15	0
MA39_327	20	4	0.800	0.739	-0.057	0	20	6	0.850	0.691	-0.205	0
MA39_342	20	7	0.900	0.814	-0.081	0	20	9	0.850	0.799	-0.039	0
MA39_348	20	5	0.700	0.701	0.027	0.017	20	4	0.650	0.646	0.02	0.016
MA39_442[1(A1)]	20	2	0.350	0.399	0.147	0.040	20	2	0.500	0.480	-0.016	0
MA40_072	20	3	0.250	0.335	0.278	0.059	20	6	0.950	0.770	-0.209	0
MA40_282	20	5	0.750	0.738	0.009	0	20	6	0.800	0.631	-0.243	0
MA41_076	20	8	0.800	0.749	-0.043	0	20	6	0.650	0.754	0.163	0.076
MA41_373[(A2)]	20	5	0.800	0.686	-0.141	0	20	5	0.850	0.771	-0.077	0
MA42_028[1(A2)]	20	6	0.650	0.710	0.110*	0	20	4	0.750	0.681	-0.075	0
MA42_231	20	5	0.800	0.725	-0.078	0	20	4	0.250	0.269	0.095	0
MA42_255	20	4	0.700	0.666	-0.025	0.016	20	4	0.600	0.486	-0.21	0
MA42_274	20	4	0.650	0.621	-0.021	0	20	5	0.850	0.703	-0.185	0
MA42_421	20	3	0.650	0.554	-0.149	0	20	4	0.850	0.676	-0.233	0
MA42_471	20	6	0.750	0.749	0.024	0	20	4	0.550	0.446	-0.208	0
MA42_495	20	2	0.400	0.320	-0.226	0	20	2	0.450	0.399	-0.103	0
Pop (20[1])	20	4.500	0.638	0.603	-0.032		20	4.750	0.688	0.592	-0.135	
SD (20[1])	0	0.420	0.055	0.049			0	0.410	0.050	0.040		

Appendix 4.4h *Magnolia pallescens*: 20 polymorphic SSR markers.

Population	SAL						MON					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023[1]	20	5	0.400	0.569	0.320*	0.130*	20	3	0.550	0.454	-0.188	0
MA39_185	20	6	0.700	0.813	0.164	0.065	20	5	0.650	0.641	0.012	0.031
MA39_199	20	2	0.450	0.399	-0.103	0	20	2	0.100	0.095	-0.027	0
MA39_259	20	2	0.100	0.095	-0.027	0	20	2	0.400	0.375	-0.041	0
MA40_045[2] (A1)	20	6	0.850	0.766	-0.084	0	20	5	0.500	0.644	0.180*	0.088
MA40_282	20	7	0.450	0.573	0.238	0.066	20	8	0.750	0.776	0.059	0
MA41_373	20	4	0.500	0.569	0.146	0.064	20	3	0.500	0.591	0.179	0.057
MA42_001	20	2	0.350	0.349	0.022	0	20	2	0.500	0.495	0.016	0
MA42_059	20	2	0.250	0.219	-0.118	0	20	1	0.000	0.000	M	0
MA42_077	20	2	0.200	0.255	0.240	0.063	20	2	0.100	0.095	-0.027	0
MA42_083[2]	20	3	0.150	0.226	0.360*	0.108	20	2	0.050	0.049	0	0
MA42_203	20	6	0.800	0.728	-0.074	0	20	5	0.650	0.630	-0.006	0
MA42_231	20	4	0.500	0.636	0.239	0.059	20	3	0.400	0.335	-0.169	0
MA42_241	20	1	0.000	0.000	M	0	20	2	0.250	0.289	0.159	0.042
MA42_255	20	5	0.900	0.774	-0.138	0	20	5	0.800	0.786	0.008	0
MA42_293	20	3	0.300	0.265	-0.107	0	20	2	0.450	0.499	0.123	0.033
MA42_397	20	12	0.950	0.874	-0.062	0	20	12	0.800	0.870	0.106	0.049
MA42_421	20	7	0.900	0.814	-0.081	0	20	7	0.650	0.719	0.121	0.042
MA42_471	20	9	0.900	0.853	-0.030	0	20	9	0.800	0.809	0.037	0
MA42_472[1] (A1)	20	7	0.750	0.725	-0.009	0	20	7	0.500	0.841	0.427**	0.186*
Pop (18[1])	20	4.611	0.514	0.511	0.021		20	4.278	0.464	0.483	0.066	
SD (18[1])	0	0.687	0.074	0.068			0	0.713	0.064	0.067		
Subset (16[1,2])	20	4.625	0.516	0.513	0.018		20	4.375	0.488	0.500	0.045	
SD (16[1,2])	0	0.763	0.077	0.072			0	0.790	0.066	0.069		

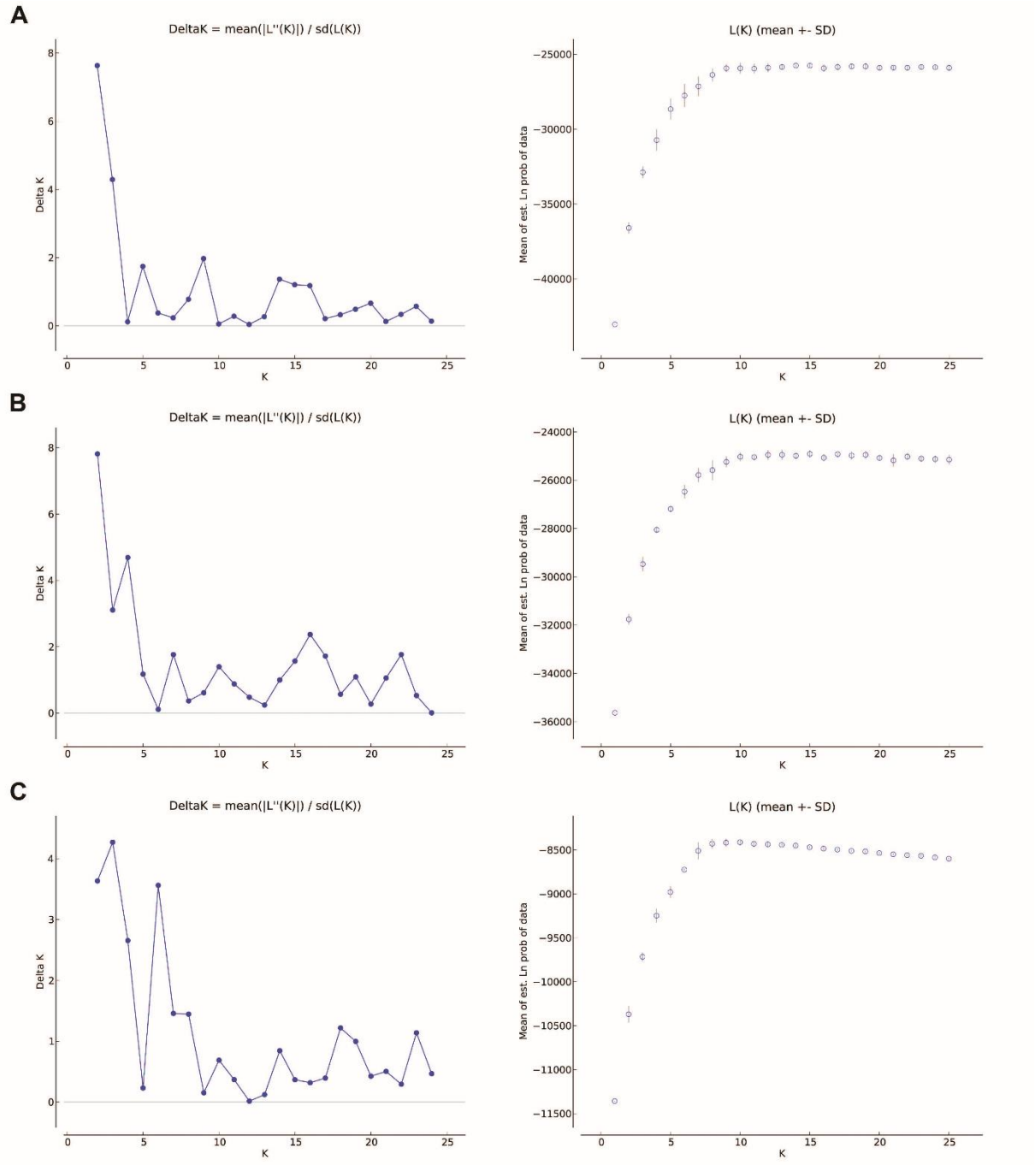
Appendix 4.4i *Magnolia portoricensis*: 29 polymorphic SSR markers.

Population	TOR						MARI					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	20	8	0.700	0.776	0.124	0.034	20	7	0.700	0.779	0.126	0.069
MA39_142	20	1	0.000	0.000	M	0	20	2	0.100	0.095	-0.027	0
MA39_185	20	14	0.700	0.868	0.218*	0.086*	20	7	0.700	0.755	0.098	0.045
MA39_199	20	2	0.050	0.139	0.655	0.128	20	1	0.000	0.000	M	0
MA39_236	20	6	0.750	0.791	0.078	0.012	20	3	0.400	0.340	-0.152	0
MA39_348	20	10	0.750	0.826	0.118	0.017	20	9	0.700	0.833	0.184	0.065
MA40_045	20	8	0.600	0.766	0.241	0.080	20	8	0.800	0.786	0.008	0.013
MA40_282	20	8	0.550	0.780	0.318*	0.152*	20	8	0.950	0.826	-0.125	0
MA41_076	20	3	0.100	0.096	-0.013	0	20	2	0.050	0.049	0.000	0
MA41_215	20	4	0.200	0.186	-0.048	0	20	2	0.250	0.219	-0.118	0
MA41_373	20	7	0.700	0.765	0.110	0	20	9	0.800	0.838	0.070	0.017
MA42_001	20	4	0.150	0.306	0.529*	0.159*	20	3	0.700	0.524	-0.314	0
MA42_063	20	8	0.750	0.801	0.089	0.009	19	10	0.684	0.828	0.200*	0.082
MA42_077	20	2	0.050	0.049	0.000	0	20	1	0.000	0.000	M	0
MA42_087	20	6	0.600	0.701	0.169	0.061	20	7	0.750	0.795	0.082	0.041
MA42_102	20	10	0.800	0.830	0.062	0.017	20	5	0.800	0.746	-0.046	0
MA42_126	20	3	0.200	0.445	0.568*	0.181*	20	4	0.550	0.638	0.162	0.036
MA42_147	20	3	0.600	0.476	-0.236	0	20	3	0.350	0.366	0.070	0.016
MA42_185	20	9	0.750	0.838	0.130	0.039	20	6	0.800	0.745	-0.048	0
MA42_203	20	4	0.400	0.516	0.249	0.072	20	3	0.350	0.301	-0.137	0
MA42_231[2]	20	5	0.450	0.795	0.455**	0.195*	20	5	0.900	0.753	-0.171	0
MA42_255	20	4	0.650	0.681	0.071	0.037	20	7	0.650	0.735	0.141	0.054
MA42_397	20	12	0.900	0.875	-0.003	0	20	12	0.850	0.876	0.056	0
MA42_413	20	4	0.350	0.584	0.422*	0.137*	20	2	0.100	0.095	-0.027	0
MA42_421	20	4	0.550	0.618	0.135	0.017	20	5	0.550	0.616	0.133	0.026
MA42_471	20	9	0.900	0.824	-0.067	0	20	9	0.800	0.800	0.026	0
MA42_472	20	8	0.700	0.789	0.138	0.045	20	4	0.550	0.684	0.220	0.073
MA42_481[1]	20	9	0.450	0.559	0.219*	0.064	19*	6	0.684	0.536	-0.251	0
MA42_495	20	10	0.800	0.863	0.098	0.037	20	6	1.000	0.761	-0.290	0
Pop (28[1])	20	6.286	0.525	0.607	0.160*		19.964	5.357	0.566	0.564	0.022	
SD (28[1])	0	0.623	0.053	0.053			0.036	0.564	0.057	0.057		

Appendix 4.4j *Magnolia splendens*: 25 polymorphic SSR markers.

Population	YUN					
SSR marker	**N**	**A**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023[1]	20	13	0.600	0.790	0.265**	0.127*
MA39_185	20	9	0.650	0.696	0.092	0
MA39_348	19	11	0.850	0.880	0.060	0.009
MA40_136	20	2	0.200	0.180	-0.086	0
MA40_175	20	2	0.250	0.399	0.395	0.117
MA40_223	20	2	0.100	0.095	-0.027	0
MA40_282	20	8	0.850	0.825	-0.005	0.020
MA41_076	20	4	0.550	0.690	0.227	0.076
MA41_373	20	7	0.800	0.795	0.019	0
MA42_001	20	7	0.750	0.779	0.063	0
MA42_063	20	6	0.800	0.766	-0.018	0
MA42_077	20	3	0.250	0.359	0.326	0.095
MA42_102	20	8	0.800	0.830	0.062	0
MA42_126	20	3	0.350	0.386	0.119	0.008
MA42_147	19	6	0.737	0.734	0.023	0
MA42_203	20	3	0.350	0.486	0.304	0.092
MA42_231	20	6	0.750	0.710	-0.031	0
MA42_241	20	3	0.450	0.421	-0.043	0
MA42_255	20	7	0.850	0.783	-0.061	0.035
MA42_397	20	10	0.650	0.808	0.220	0.073
MA42_413	20	4	0.650	0.631	-0.004	0
MA42_421	20	2	0.400	0.375	-0.041	0
MA42_471	20	4	0.700	0.528	-0.304*	0
MA42_472	20	7	0.600	0.695	0.162	0.017
MA42_481[1]	20	9	0.650	0.849	0.258	0.101*
Pop (23[1])	19.957	5.391	0.580	0.602	0.063	
SD (23[1])	0.043	0.572	0.049	0.046		

Appendix 4.5 STRUCTURE ΔK (Evanno et al., 2005) and mean likelihood plots. **A** dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **B** dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **C** dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk). **D** DR-dataset comprising the 120 individuals representing 6 populations and 3 species of the Dominican Republic for all the markers of which data was generated (See Appendix 4.2: categories A, B and C in the columns DOM, HAM and PAL); **D1**: analysis run with the independent allele model; **D2**: analysis run with the correlated allele model. **E** PR-dataset comprising 60 individuals representing three populations and two species of Puerto Rico for all the markers of which data was generated (See Appendix 4.2: categories A, B and C in the columns POR and SPL); **E1**: analysis run with the independent allele model; **E2**: analysis run with the correlated allele model. **F** *Magnolia cubensis*. **G** *M. dodecapetala*. **H** *M. domingensis*. **I** *M. ekmanii*. **J** *M. hamorii*. **K** *M. lacandonica*. **L** *M. pallescens*. **M** *M. portoricensis*. **N** *M. splendens*. **O1** *M. cubensis* subsp. *acunae*. **O2** *M. cubensis* subsp. *cubensis*. **P1** *M. dodecapetala*: GUA population. **P2** *M. dodecapetala*: MART population. **Q1** *M. domingensis*: BAR population. **Q2** *M. domingensis*: ROD population. **R1** *M. ekmanii*: GRA population. **R2** *M. ekmanii*: MAN population. **S1** *M. hamorii*: CAC population. **S2** *M. hamorii*: COR population. **T1** *M. lacandonica*: LAC population. **T2** *M. lacandonica*: YAJ population. **U1** *M. pallescens*: MON population. **U2** *M. pallescens*: SAL population. **V1** *M. portoricensis*: MARI population. **V2** *M. portoricensis*: MARI population.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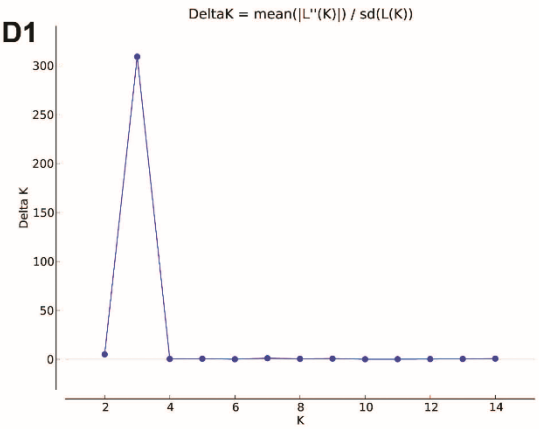
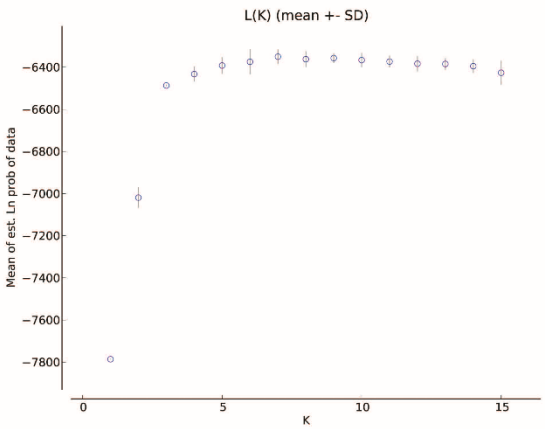
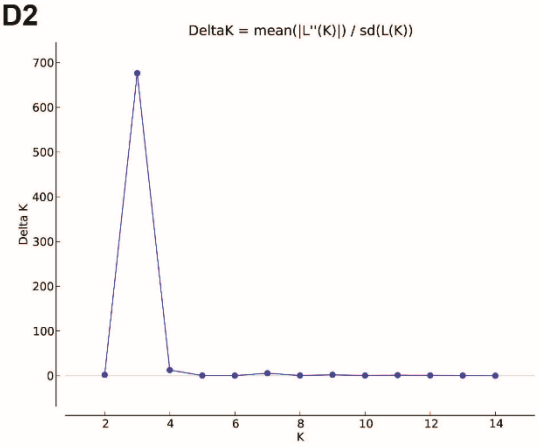
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
K
B
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
K
C
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
K

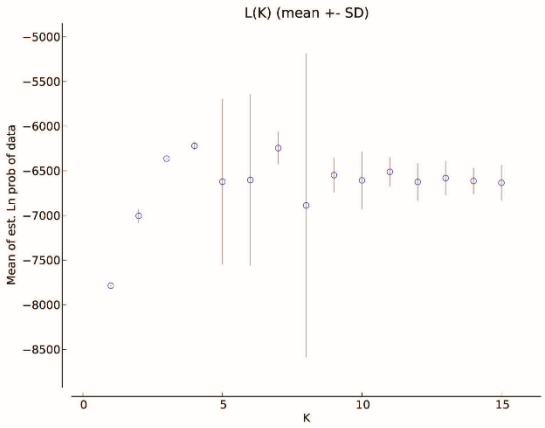
D1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

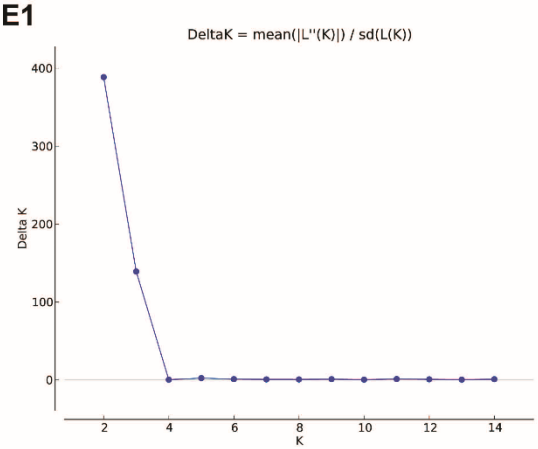

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

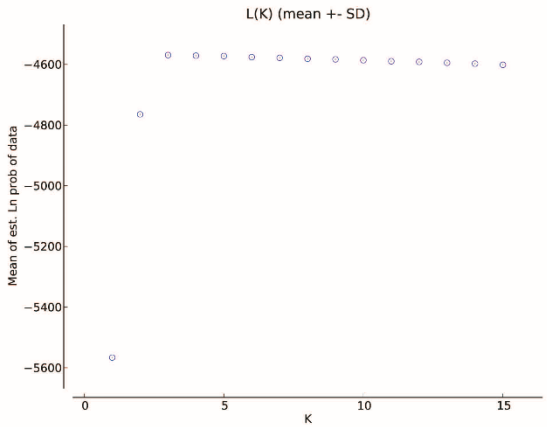
D2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

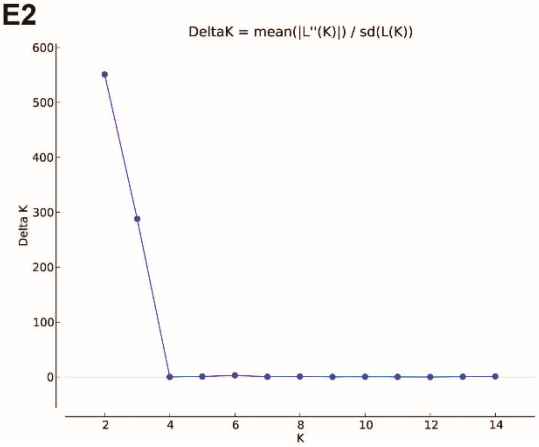

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

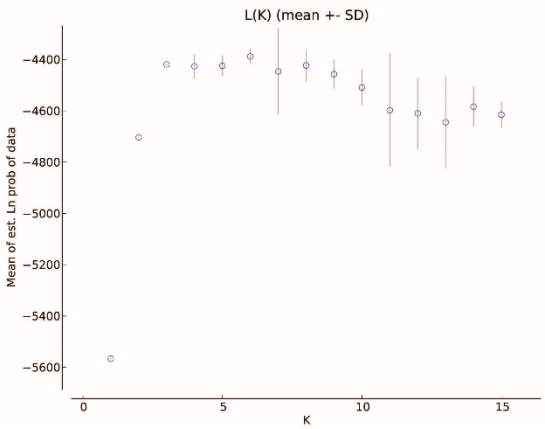
E1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

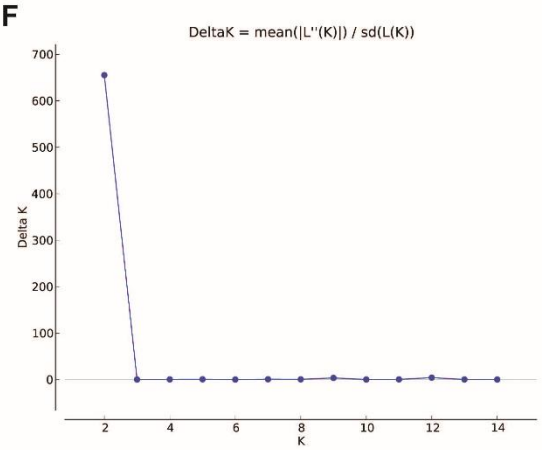

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

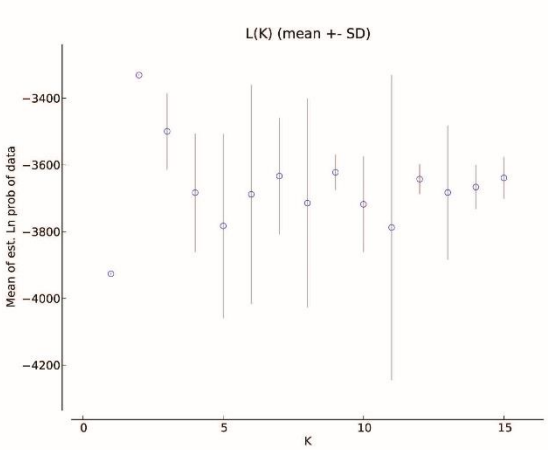
E2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

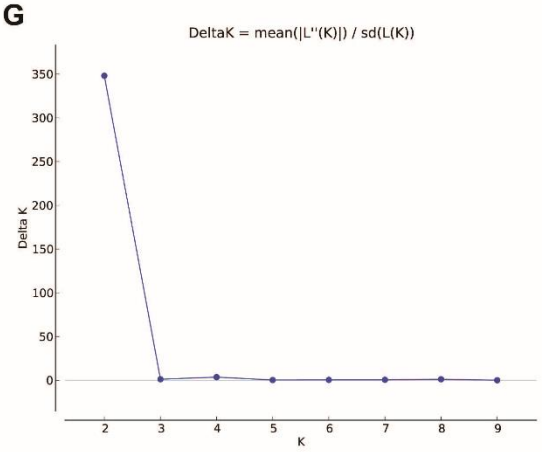

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

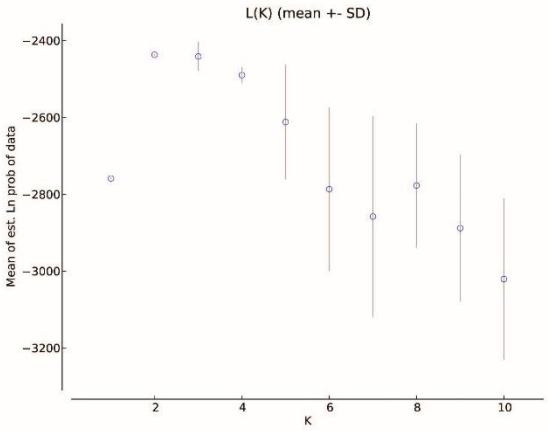
F


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

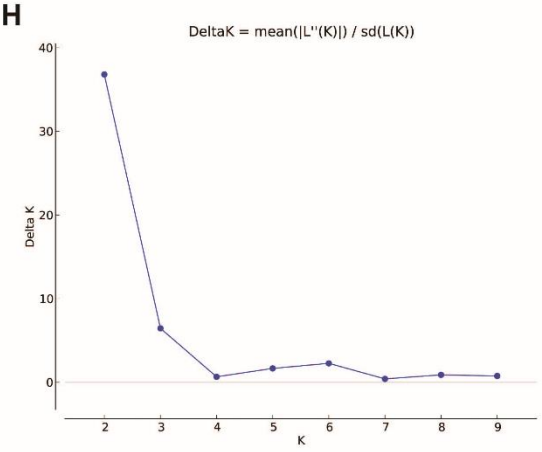

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

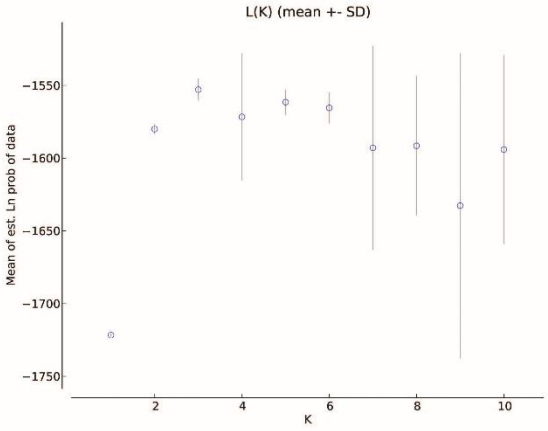
G


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

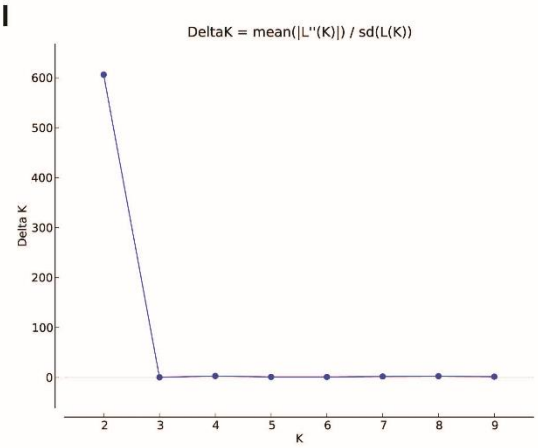

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

H


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

I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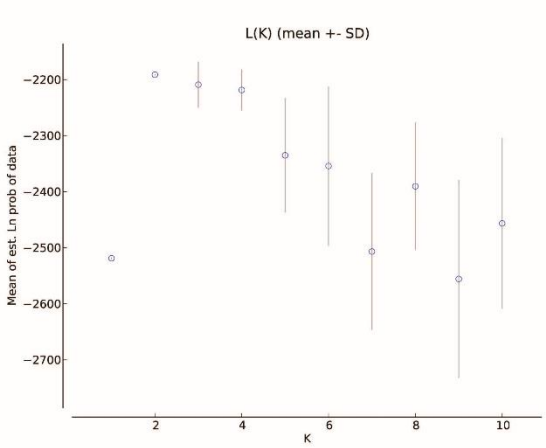
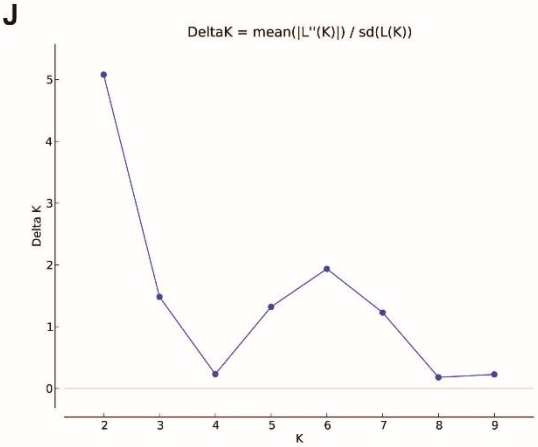
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

J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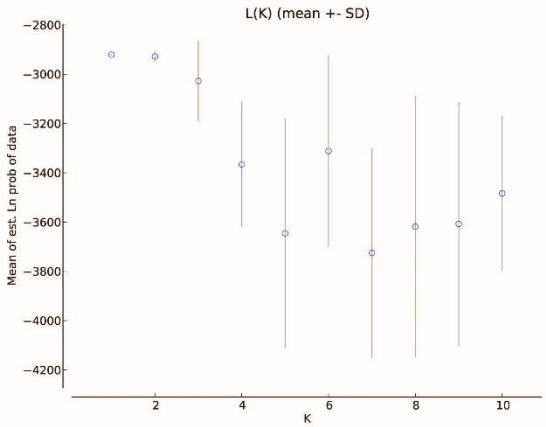
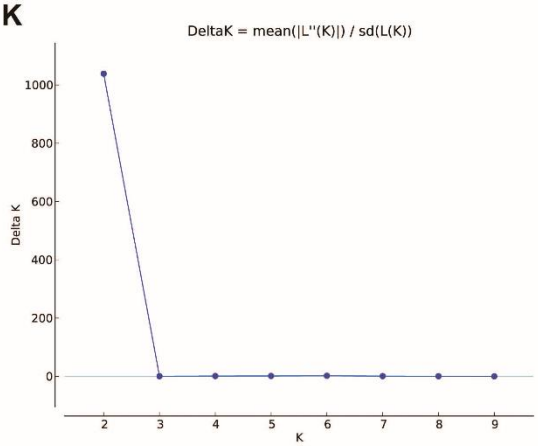
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

K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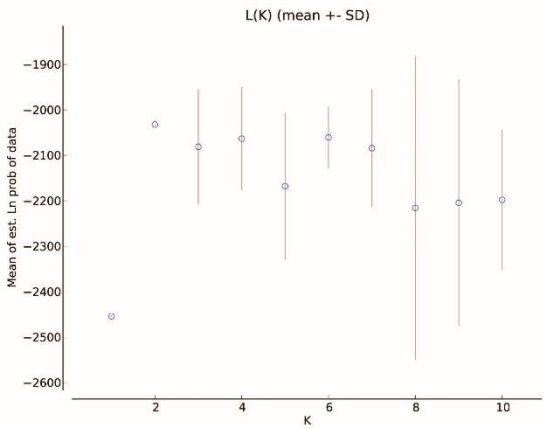
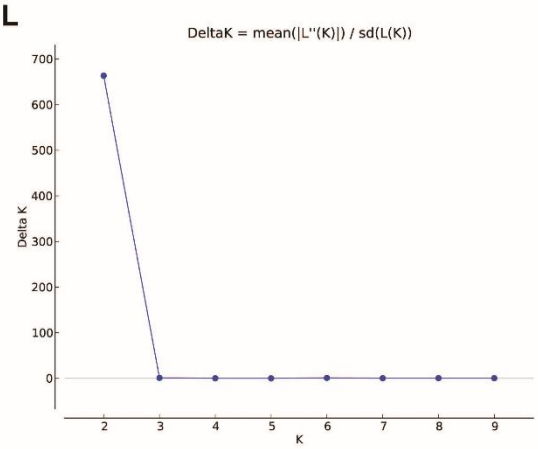
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

L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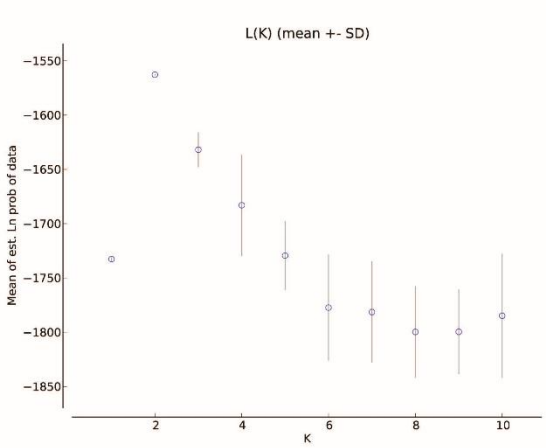
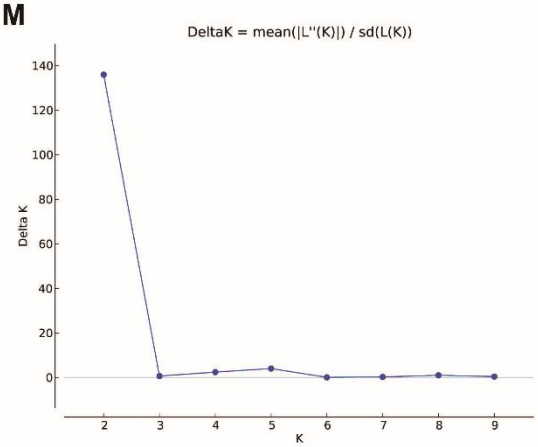
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

M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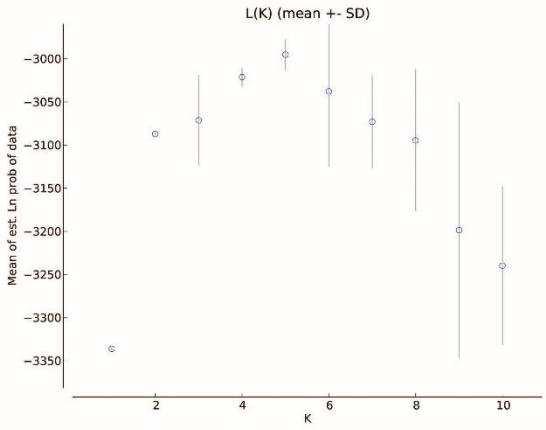
Delta K
K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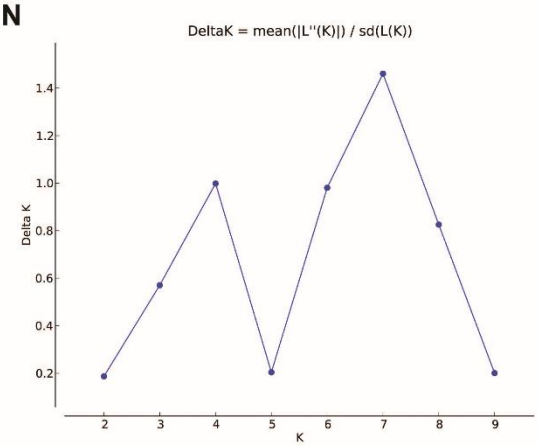
K

N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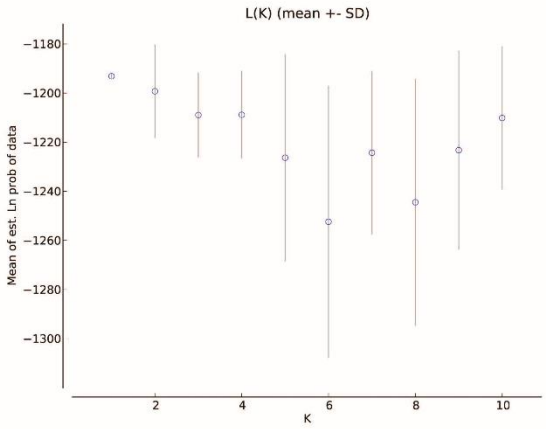
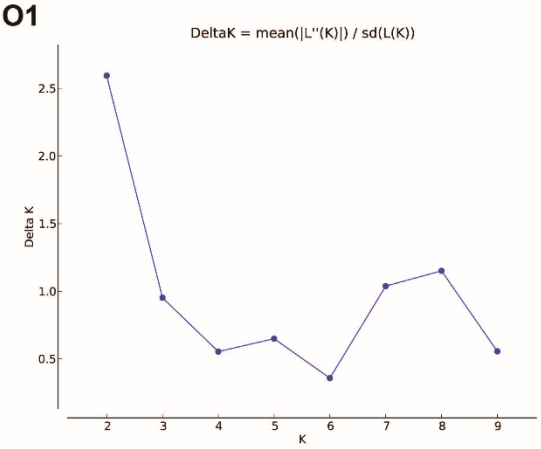
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

O1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Delta K
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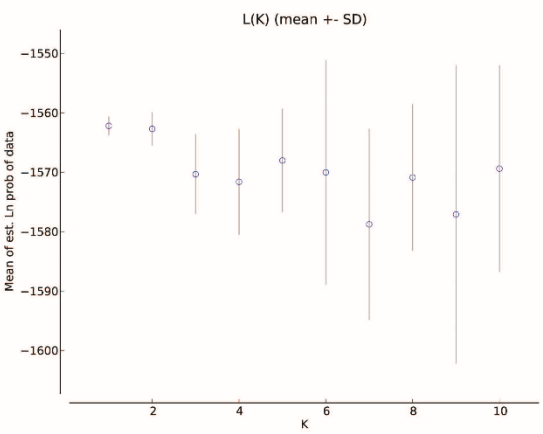
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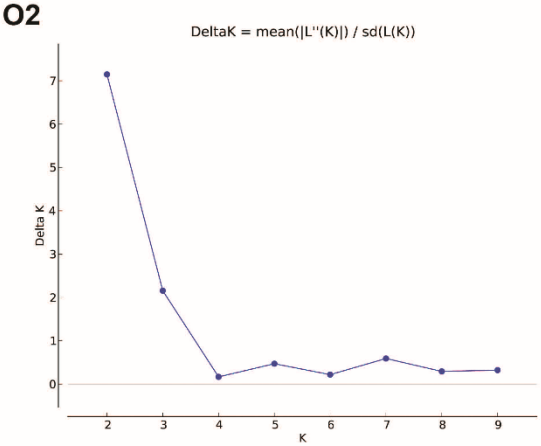
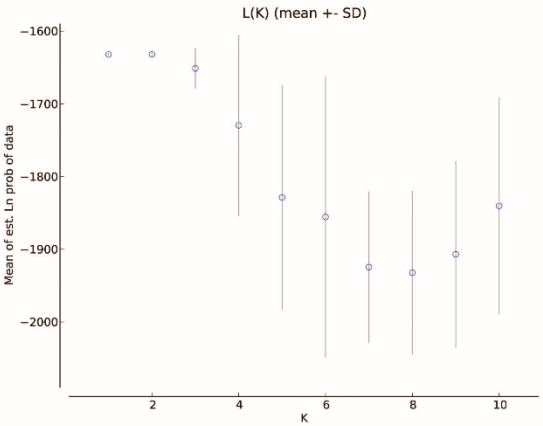
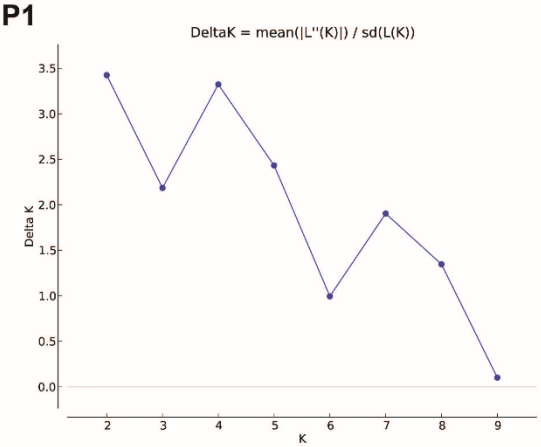
K

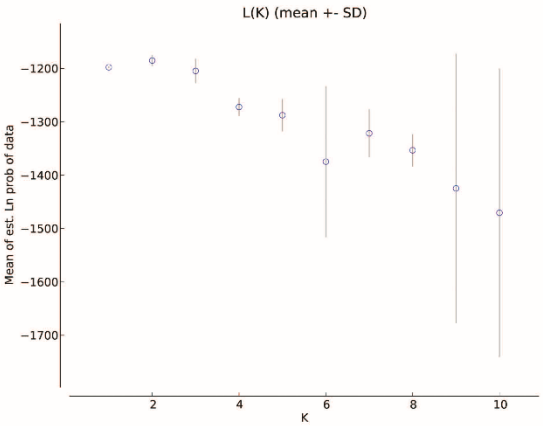
O2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

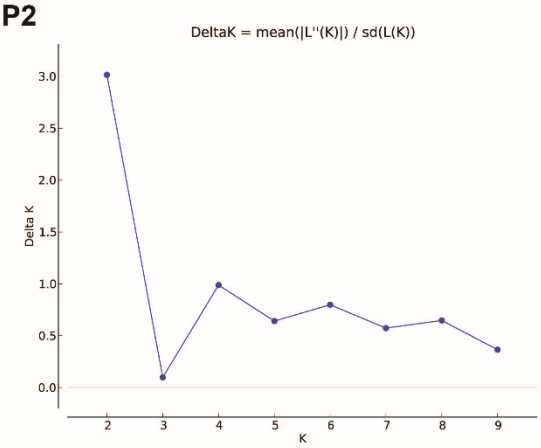

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

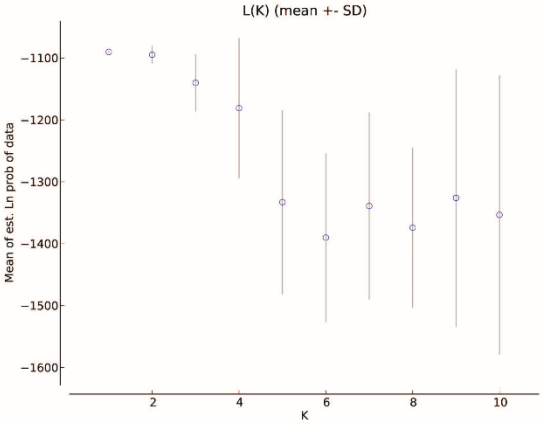
P1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

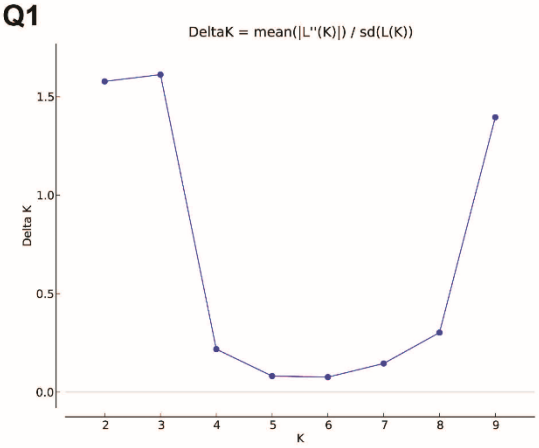

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

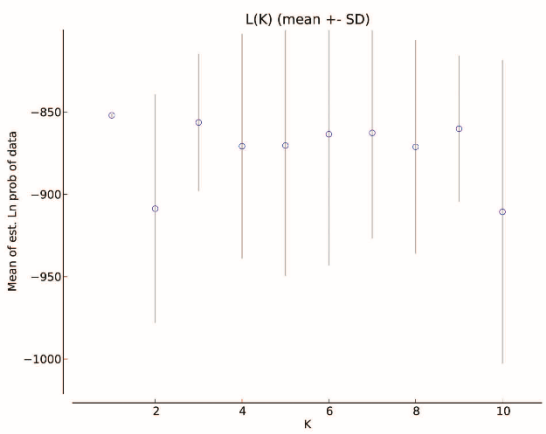
P2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

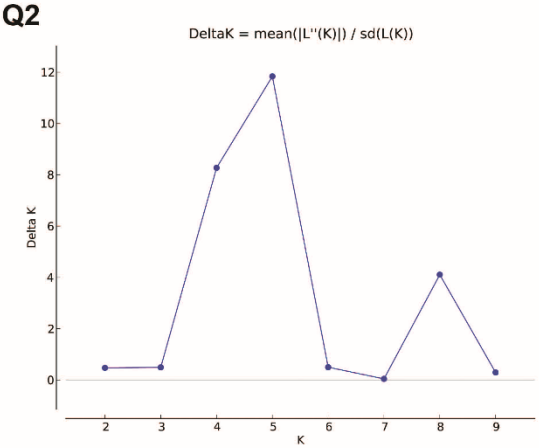

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

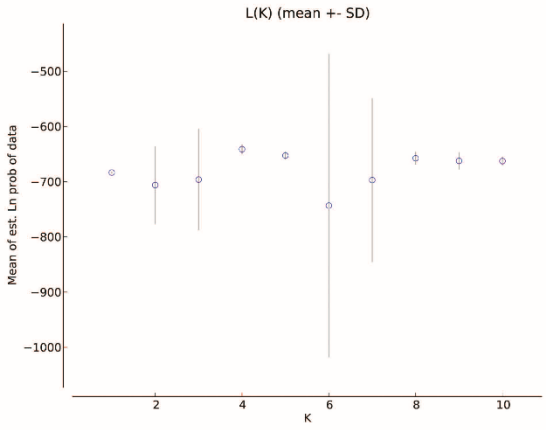
Q1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

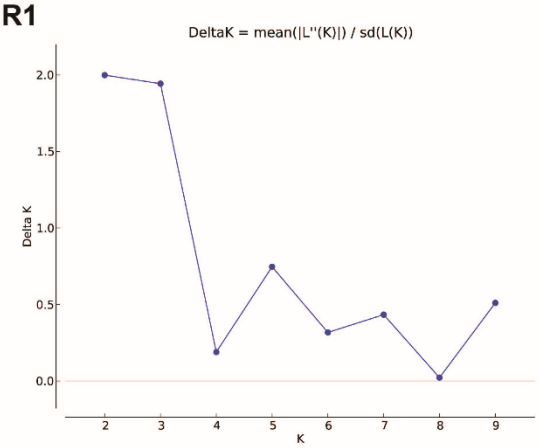

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

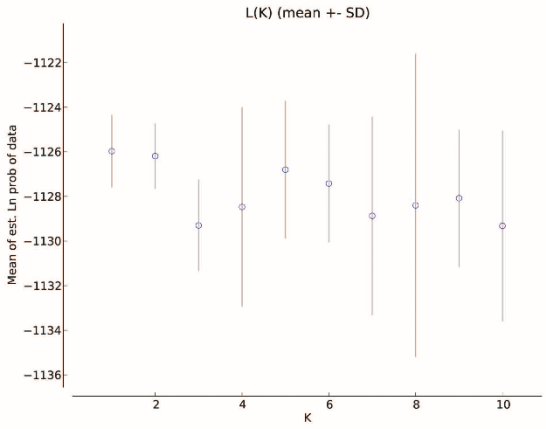
Q2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

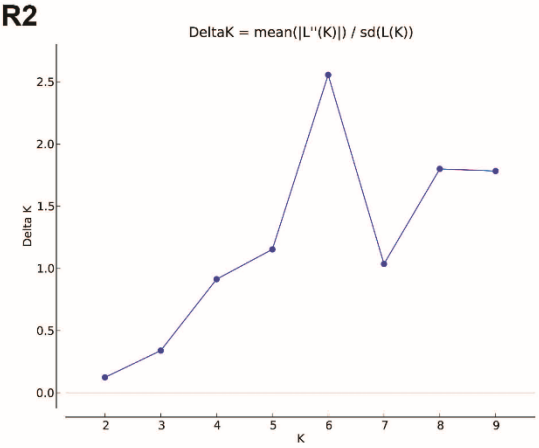

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

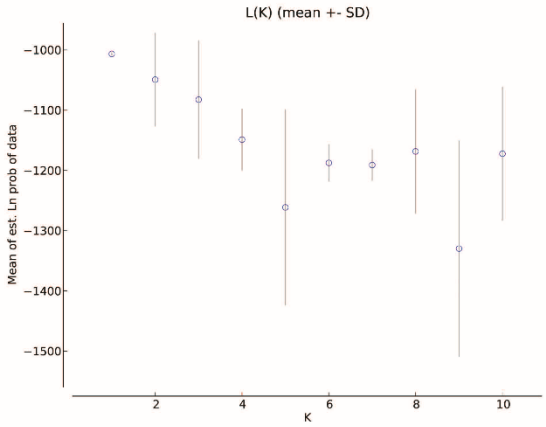
R1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

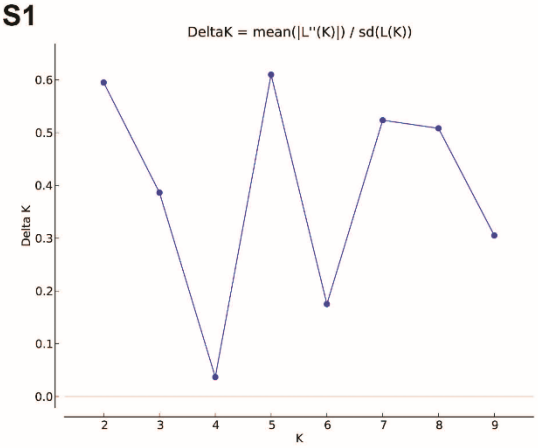

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

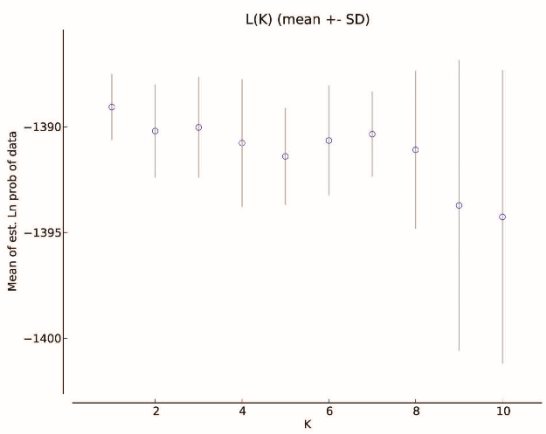
R2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

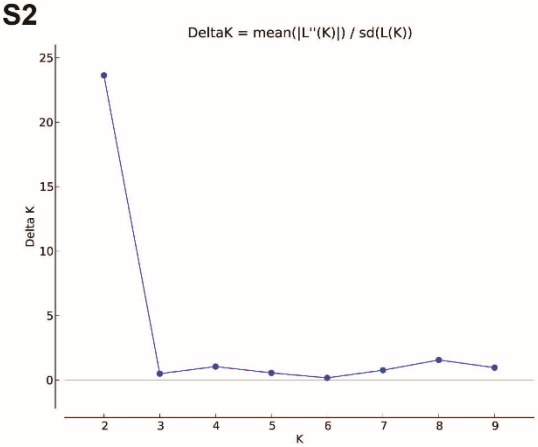

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

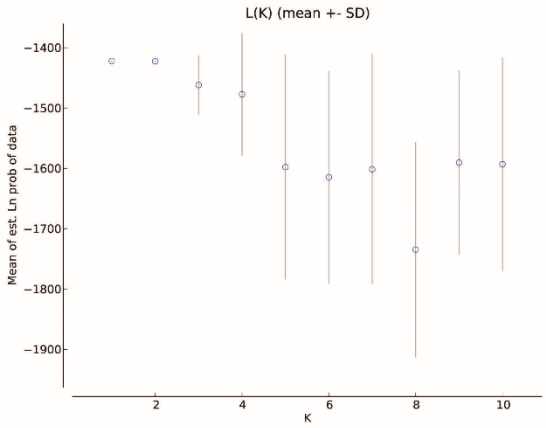
S1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

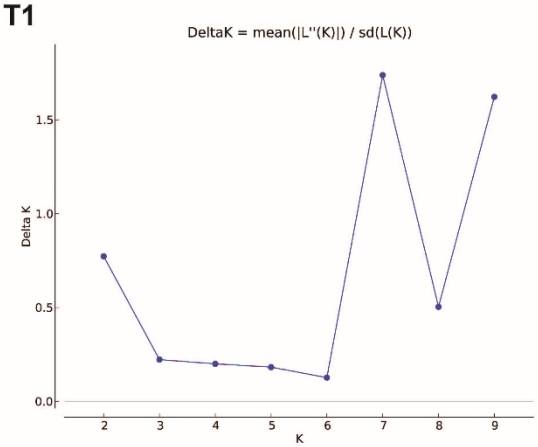

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

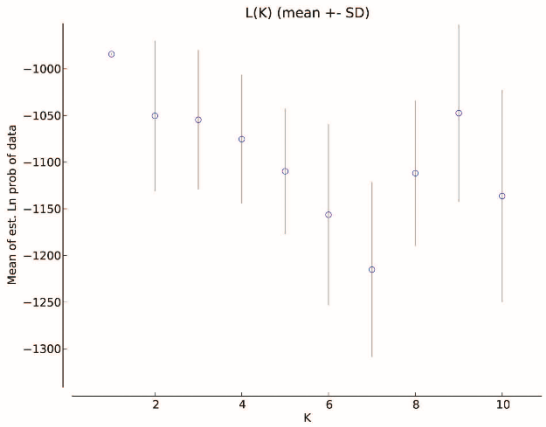
S2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

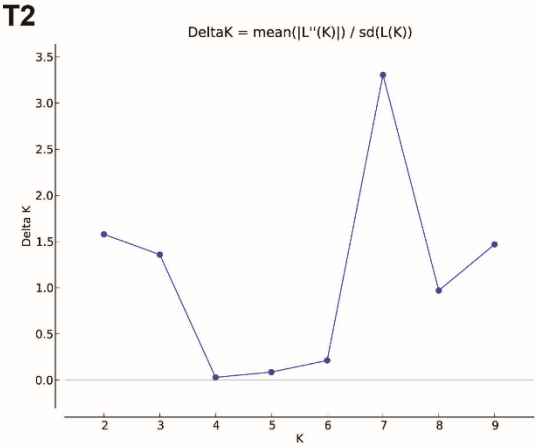

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

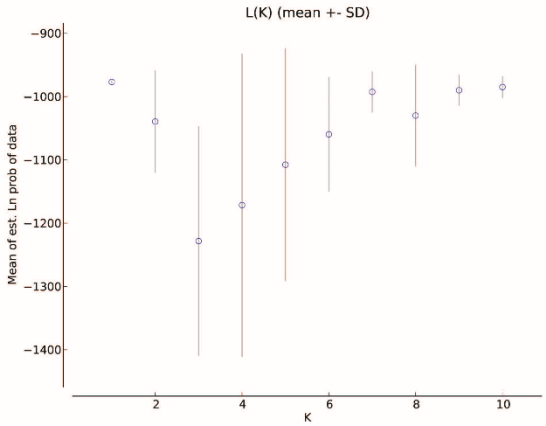
T1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

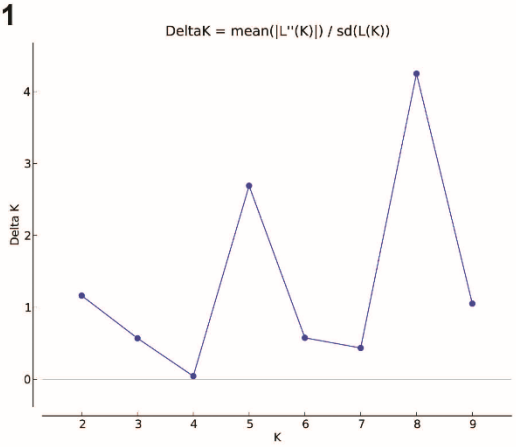

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

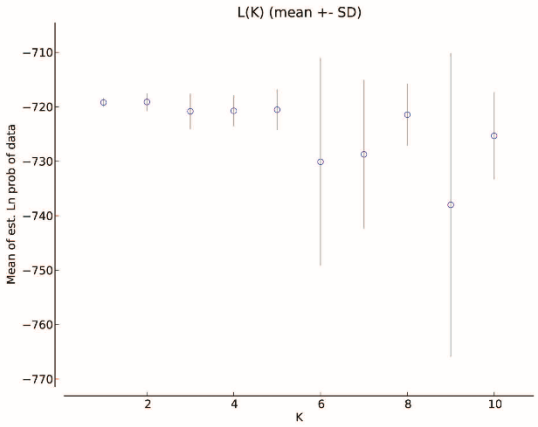
T2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

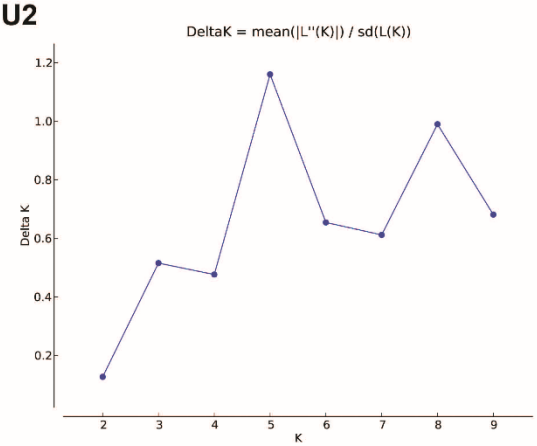

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

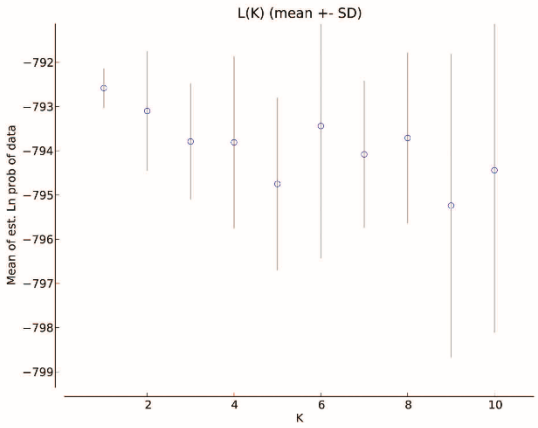
U1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

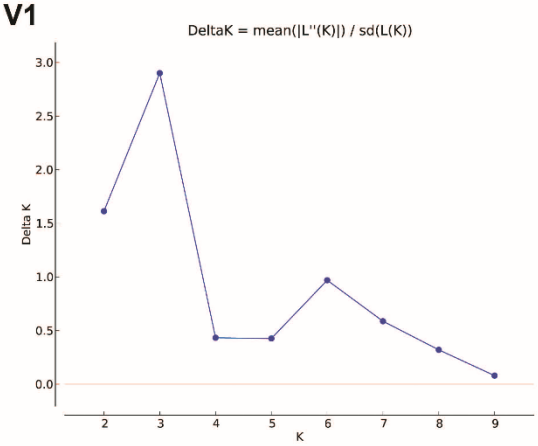

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

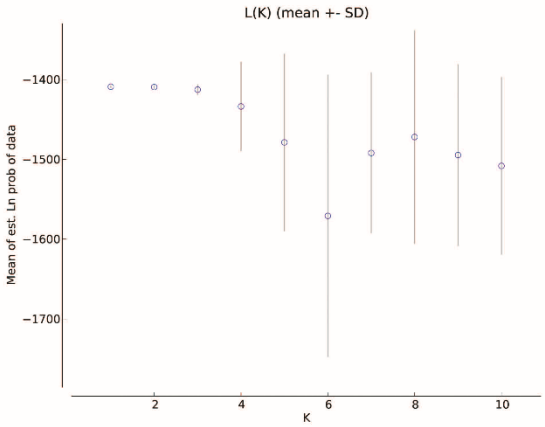
U2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

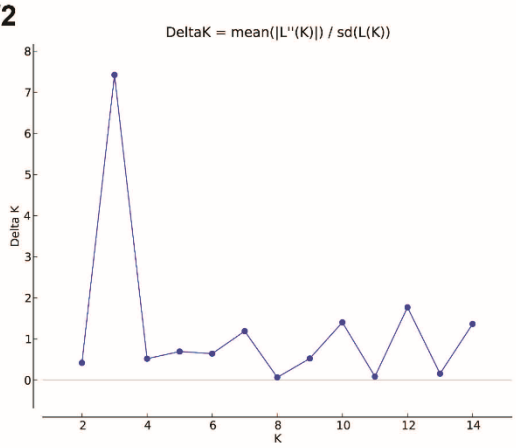

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

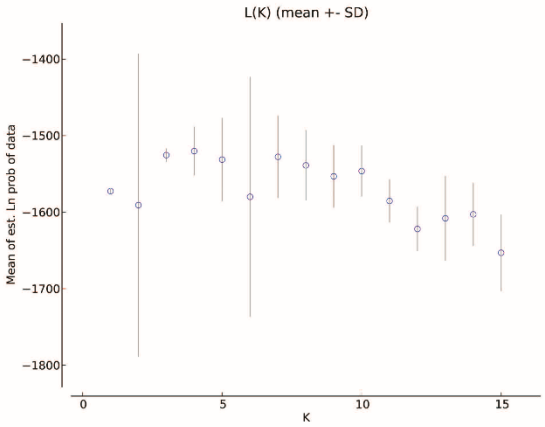
V1


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

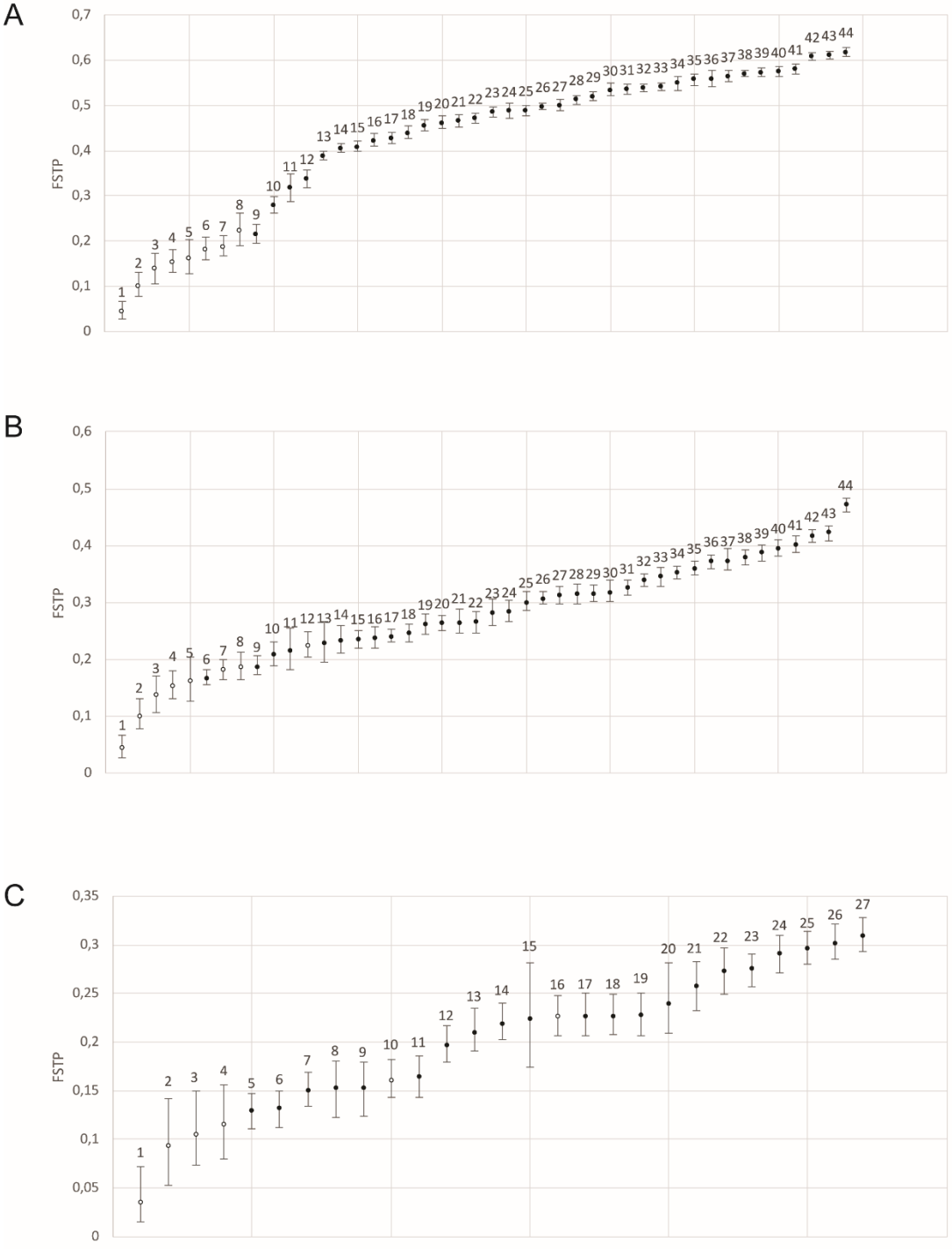

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

V2


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

Appendix 4.6A Confidence intervals of the pairwise F_{ST} values from Table 4.4. **FSTP** = pairwise F_{ST} (Weir and Cockerham, 1984). White data points represent infraspecific pairwise F_{ST} values. Black data points represent supraspecific pairwise F_{ST} values. **A** dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **B** dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **C** dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk).

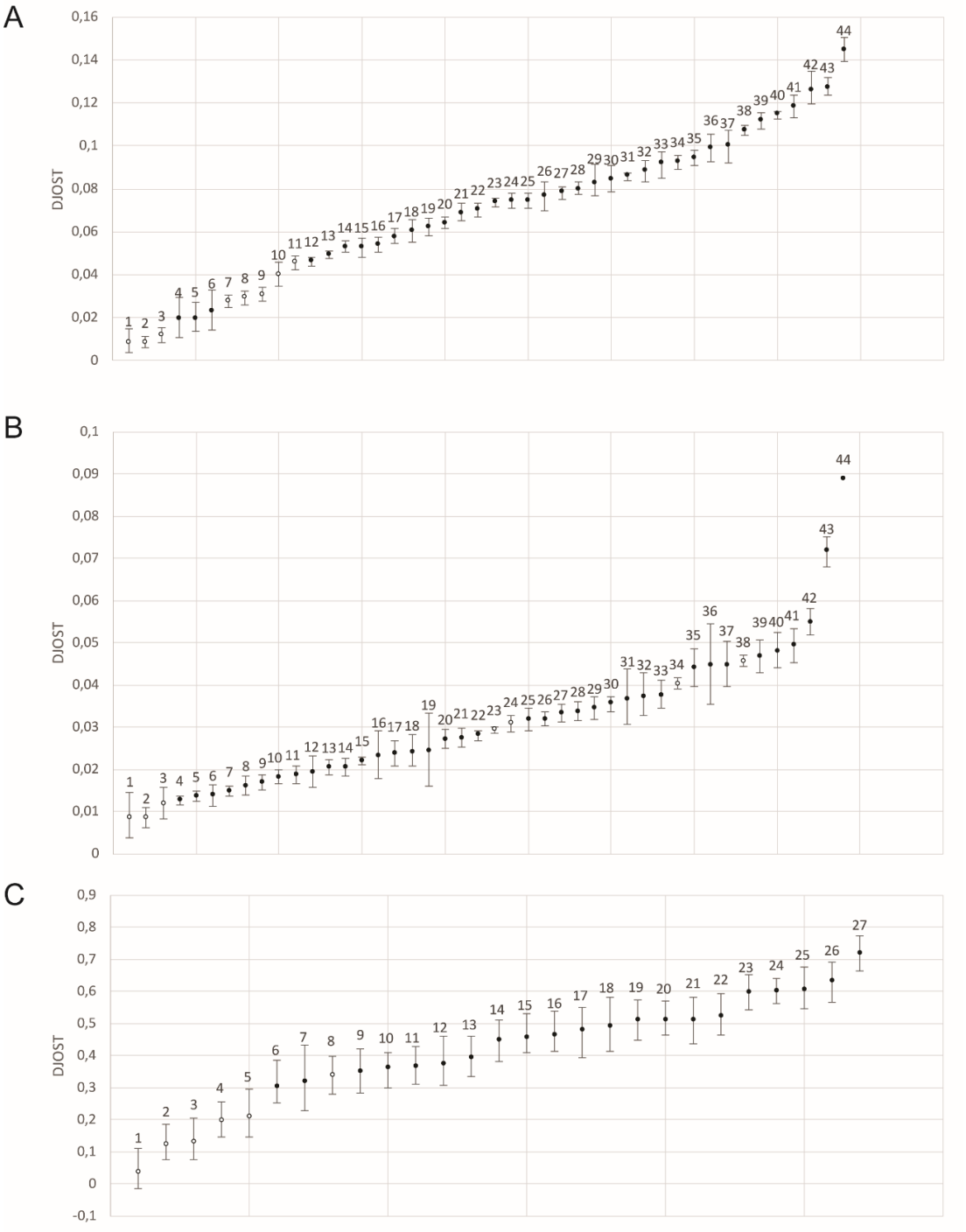


FSTP(A) Dataset 1								
	Comparison	Actual	Mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower	BC_Upper
1	**HAM vs. HAM**	**0.0444**	**0.0692**	**0.0444**	**0.0521**	**0.0911**	**0.0273**	**0.0663**
2	**POR vs. POR**	**0.1009**	**0.125**	**0.1009**	**0.1009**	**0.1551**	**0.0768**	**0.131**
3	**DOM vs. DOM**	**0.1383**	**0.1592**	**0.1383**	**0.1269**	**0.1927**	**0.1061**	**0.1719**
4	**CU vs. CU**	**0.1535**	**0.1758**	**0.1535**	**0.153**	**0.203**	**0.1307**	**0.1806**
5	**PAL vs. PAL**	**0.1627**	**0.1844**	**0.1627**	**0.1487**	**0.2249**	**0.1269**	**0.2032**
6	**DOD vs. DOD**	**0.1811**	**0.2048**	**0.1811**	**0.1836**	**0.2328**	**0.1599**	**0.2091**
7	**LAC vs. LAC**	**0.1854**	**0.2037**	**0.1854**	**0.1848**	**0.2288**	**0.1665**	**0.2105**
8	**EKM vs. EKM**	**0.2231**	**0.243**	**0.2231**	**0.2095**	**0.2823**	**0.1895**	**0.2624**
9	DOM vs. HAM	0.2158	0.2243	0.2158	0.2031	0.2457	0.1946	0.2372
10	HAM vs. PAL	0.2787	0.2901	0.2787	0.2741	0.3103	0.2627	0.2989
11	DOM vs. PAL	0.3179	0.3277	0.3179	0.2972	0.3575	0.2874	0.3477
12	POR vs. SPL	0.3378	0.3523	0.3378	0.333	0.3723	0.3185	0.3579
13	CU vs. HAM	0.3886	0.3973	0.3886	0.3879	0.4085	0.3791	0.3997
14	HAM vs. POR	0.4044	0.4126	0.4044	0.4043	0.4238	0.3962	0.4156
15	CU vs. POR	0.4085	0.4171	0.4085	0.4078	0.4296	0.3992	0.421
16	DOM vs. POR	0.4215	0.4285	0.4215	0.4159	0.4451	0.4088	0.438
17	CU vs. DOM	0.4281	0.4361	0.4281	0.4247	0.4479	0.4167	0.4399
18	CU vs. SPL	0.4373	0.4501	0.4373	0.4387	0.4677	0.4259	0.4549
19	CU vs. EKM	0.4548	0.4624	0.4548	0.4511	0.475	0.4435	0.4674
20	HAM vs. SPL	0.4612	0.473	0.4612	0.4611	0.4879	0.4493	0.4761
21	CU vs. PAL	0.4655	0.4742	0.4655	0.4608	0.4877	0.4522	0.4791
22	DOD vs. LAC	0.4712	0.4778	0.4712	0.4662	0.4895	0.4597	0.4829
23	DOM vs. EKM	0.4856	0.4915	0.4856	0.4805	0.5031	0.4746	0.4972
24	DOM vs. SPL	0.487	0.4965	0.487	0.4816	0.5135	0.4721	0.504
25	DOD vs. POR	0.4886	0.4956	0.4886	0.4849	0.5077	0.4778	0.5006
26	EKM vs. HAM	0.4973	0.5038	0.4973	0.4976	0.5124	0.4911	0.5059
27	DOD vs. DOM	0.4989	0.5056	0.4989	0.4949	0.5202	0.4881	0.5134
28	CU vs. DOD	0.5126	0.5193	0.5126	0.5089	0.5286	0.5023	0.522
29	DOD vs. HAM	0.5198	0.5263	0.5198	0.5163	0.5376	0.5098	0.5311
30	PAL vs. POR	0.5337	0.541	0.5337	0.5304	0.556	0.5231	0.5487
31	EKM vs. POR	0.5354	0.5419	0.5354	0.5313	0.5544	0.5249	0.5479
32	CU vs. LAC	0.5385	0.5448	0.5385	0.5375	0.5534	0.5312	0.547
33	LAC vs. POR	0.5407	0.5471	0.5407	0.5404	0.5548	0.5341	0.5484
34	PAL vs. SPL	0.5494	0.5599	0.5494	0.5435	0.5753	0.5329	0.5648
35	DOD vs. PAL	0.5573	0.5644	0.5573	0.5509	0.576	0.5437	0.5688
36	DOD vs. SPL	0.5594	0.5685	0.5594	0.5515	0.5858	0.5424	0.5767
37	EKM vs. SPL	0.5635	0.5729	0.5635	0.5623	0.5868	0.5528	0.5773
38	HAM vs. LAC	0.5699	0.5754	0.5699	0.5679	0.5838	0.5624	0.5782
39	DOM vs. LAC	0.5733	0.5782	0.5733	0.5692	0.5886	0.5643	0.5836
40	EKM vs. PAL	0.5738	0.5806	0.5738	0.5709	0.5928	0.5642	0.5861
41	LAC vs. SPL	0.58	0.5872	0.58	0.5768	0.5979	0.5696	0.5906
42	LAC vs. PAL	0.6071	0.6132	0.6071	0.6051	0.6217	0.599	0.6156
43	EKM vs. LAC	0.6111	0.616	0.6111	0.6088	0.6241	0.604	0.6193
44	DOD vs. EKM	0.618	0.6235	0.618	0.6126	0.6345	0.6072	0.629

FSTP(B) Dataset 2								
	Comparison	Actual	Mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower	BC_Upper
1	**HAM vs. HAM**	**0.0444**	**0.0692**	**0.0444**	**0.0521**	**0.0911**	**0.0273**	**0.0663**
2	**POR vs. POR**	**0.1009**	**0.125**	**0.1009**	**0.1009**	**0.1551**	**0.0768**	**0.131**
3	**DOM vs. DOM**	**0.1383**	**0.1592**	**0.1383**	**0.1269**	**0.1927**	**0.1061**	**0.1719**
4	**CU vs. CU**	**0.1535**	**0.1758**	**0.1535**	**0.153**	**0.203**	**0.1307**	**0.1806**
5	**PAL vs. PAL**	**0.1627**	**0.1844**	**0.1627**	**0.1487**	**0.2249**	**0.1269**	**0.2032**
6	DOM vs. HAM	0.1662	0.1753	0.1662	0.1574	0.1939	0.1483	0.1848
7	**DOD vs. DOD**	**0.1811**	**0.2048**	**0.1811**	**0.1836**	**0.2328**	**0.1599**	**0.2091**
8	**LAC vs. LAC**	**0.1854**	**0.2037**	**0.1854**	**0.1848**	**0.2288**	**0.1665**	**0.2105**
9	CU vs. HAM	0.1874	0.199	0.1874	0.1876	0.2139	0.176	0.2023
10	HAM vs. SPL	0.2082	0.2217	0.2082	0.2068	0.2395	0.1934	0.2261
11	HAM vs. PAL	0.2158	0.2261	0.2158	0.2075	0.2488	0.1973	0.2385
12	**EKM vs. EKM**	**0.2231**	**0.243**	**0.2231**	**0.2095**	**0.2823**	**0.1895**	**0.2624**
13	DOM vs. PAL	0.2295	0.2393	0.2295	0.2048	0.2752	0.195	0.2654
14	POR vs. SPL	0.2331	0.2481	0.2331	0.2258	0.275	0.2108	0.26
15	DOM vs. POR	0.2362	0.2476	0.2362	0.2316	0.263	0.2202	0.2516
16	DOM vs. SPL	0.2373	0.2495	0.2373	0.2311	0.2703	0.2189	0.2581
17	HAM vs. POR	0.2401	0.2509	0.2401	0.2405	0.2632	0.2297	0.2523
18	CU vs. POR	0.2457	0.2573	0.2457	0.2424	0.2726	0.2308	0.2609
19	CU vs. DOM	0.2616	0.2723	0.2616	0.2557	0.29	0.2451	0.2794
20	DOD vs. DOM	0.2639	0.2746	0.2639	0.2614	0.2891	0.2507	0.2783
21	CU vs. SPL	0.2641	0.2778	0.2641	0.2601	0.3011	0.2464	0.2874
22	LAC vs. SPL	0.2657	0.2786	0.2657	0.2599	0.2976	0.247	0.2847
23	PAL vs. SPL	0.2823	0.2959	0.2823	0.2735	0.3188	0.2599	0.3052
24	LAC vs. PAL	0.2833	0.2925	0.2833	0.2756	0.3139	0.2664	0.3047
25	CU vs. PAL	0.3003	0.3099	0.3003	0.2952	0.328	0.2856	0.3184
26	HAM vs. LAC	0.3072	0.3162	0.3072	0.3059	0.3279	0.2969	0.3188
27	PAL vs. POR	0.3137	0.3239	0.3137	0.3076	0.3389	0.2974	0.3287
28	CU vs. LAC	0.3159	0.3272	0.3159	0.3092	0.3436	0.298	0.3323
29	LAC vs. POR	0.316	0.3257	0.316	0.3106	0.3408	0.3008	0.3311
30	DOM vs. LAC	0.3183	0.3271	0.3183	0.31	0.3486	0.3013	0.3398
31	EKM vs. HAM	0.3251	0.3339	0.3251	0.3219	0.3484	0.3131	0.3396
32	DOD vs. HAM	0.3386	0.3478	0.3386	0.3381	0.3591	0.3289	0.3499
33	DOD vs. PAL	0.3458	0.3553	0.3458	0.338	0.3713	0.3284	0.3617
34	DOD vs. POR	0.3524	0.3619	0.3524	0.351	0.3738	0.3415	0.3642
35	CU vs. DOD	0.3604	0.3699	0.3604	0.3587	0.3821	0.3493	0.3726
36	DOD vs. LAC	0.3726	0.3817	0.3726	0.3691	0.3929	0.36	0.3837
37	DOD vs. SPL	0.3732	0.3848	0.3732	0.3676	0.4067	0.3561	0.3952
38	DOM vs. EKM	0.3795	0.3865	0.3795	0.3728	0.4	0.3658	0.3929
39	CU vs. EKM	0.3873	0.397	0.3873	0.3814	0.4119	0.3717	0.4023
40	EKM vs. POR	0.3958	0.4055	0.3958	0.39	0.4193	0.3804	0.4097
41	EKM vs. SPL	0.4019	0.4135	0.4019	0.4003	0.4296	0.3887	0.418
42	EKM vs. PAL	0.4163	0.4246	0.4163	0.4135	0.4372	0.4052	0.4289
43	EKM vs. LAC	0.4226	0.4313	0.4226	0.4167	0.4439	0.408	0.4352
44	DOD vs. EKM	0.4719	0.4798	0.4719	0.4678	0.4923	0.4599	0.4844

FSTP (C) Dataset 3								
	Comparison	Actual	Mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower	BC_Upper
1	**HAM vs. HAM**	**0.0347**	**0.0316**	**0.0184**	**0.0209**	**0.0517**	**0.0147**	**0.0718**
2	**DOM vs. DOM**	**0.0934**	**0.0595**	**0.0483**	**0.0375**	**0.0875**	**0.0532**	**0.1421**
3	**POR vs. POR**	**0.1047**	**0.0713**	**0.0565**	**0.0537**	**0.0967**	**0.0731**	**0.1492**
4	**PAL vs. PAL**	**0.1151**	**0.0761**	**0.0617**	**0.056**	**0.1003**	**0.0803**	**0.1559**
5	CU vs. HAM	0.1296	0.1431	0.1296	0.1263	0.1651	0.1128	0.1516
6	DOM vs. HAM	0.1317	0.1443	0.1317	0.1254	0.1664	0.1128	0.1538
7	HAM vs. PAL	0.1496	0.1613	0.1496	0.1429	0.1789	0.1312	0.1672
8	CU vs. PAL	0.152	0.1646	0.152	0.1454	0.1831	0.1328	0.1705
9	CU vs. POR	0.1524	0.1644	0.1524	0.1491	0.1838	0.1371	0.1719
10	**CU vs. CU**	**0.1602**	**0.0316**	**0.0184**	**0.0209**	**0.0517**	**0.1306**	**0.1884**
11	DOM vs. PAL	0.1643	0.176	0.1643	0.147	0.2031	0.1353	0.1915
12	CU vs. DOM	0.1956	0.209	0.1956	0.1873	0.2304	0.1739	0.217
13	PAL vs. POR	0.2097	0.2206	0.2097	0.2044	0.2424	0.1936	0.2315
14	HAM vs. POR	0.2178	0.2277	0.2178	0.2088	0.2528	0.1989	0.2429
15	HAM vs. SPL	0.2232	0.2376	0.2232	0.222	0.2595	0.2075	0.245
16	**EKM vs. EKM**	**0.2256**	**0.1422**	**0.1269**	**0.1111**	**0.1809**	**0.1768**	**0.2834**
17	DOM vs. SPL	0.2259	0.2413	0.2259	0.2224	0.2654	0.2071	0.2501
18	DOM vs. POR	0.2261	0.2369	0.2261	0.2189	0.2603	0.208	0.2494
19	CU vs. SPL	0.2266	0.241	0.2266	0.2215	0.2652	0.207	0.2507
20	POR vs. SPL	0.2387	0.2524	0.2387	0.2225	0.2958	0.2088	0.2821
21	PAL vs. SPL	0.2573	0.2703	0.2573	0.2448	0.2955	0.2318	0.2824
22	CU vs. EKM	0.2722	0.2834	0.2722	0.2604	0.3077	0.2492	0.2964
23	EKM vs. HAM	0.2752	0.2864	0.2752	0.2686	0.302	0.2574	0.2908
24	EKM vs. SPL	0.2901	0.3038	0.2901	0.2849	0.3231	0.2712	0.3094
25	DOM vs. EKM	0.2961	0.306	0.2961	0.2904	0.3232	0.2805	0.3133
26	EKM vs. PAL	0.301	0.311	0.301	0.2958	0.3315	0.2858	0.3215
27	EKM vs. POR	0.308	0.3181	0.308	0.3027	0.3378	0.2926	0.3276

Appendix 4.6B Confidence intervals of the pairwise D_{JOST} (Jost, 2008) from Table 4.4. White data points represent infraspecific pairwise D_{JOST} values. Black data points represent supraspecific pairwise D_{JOST} values. **A** dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **B** dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **C** dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.2: marker names indicated with an asterisk).

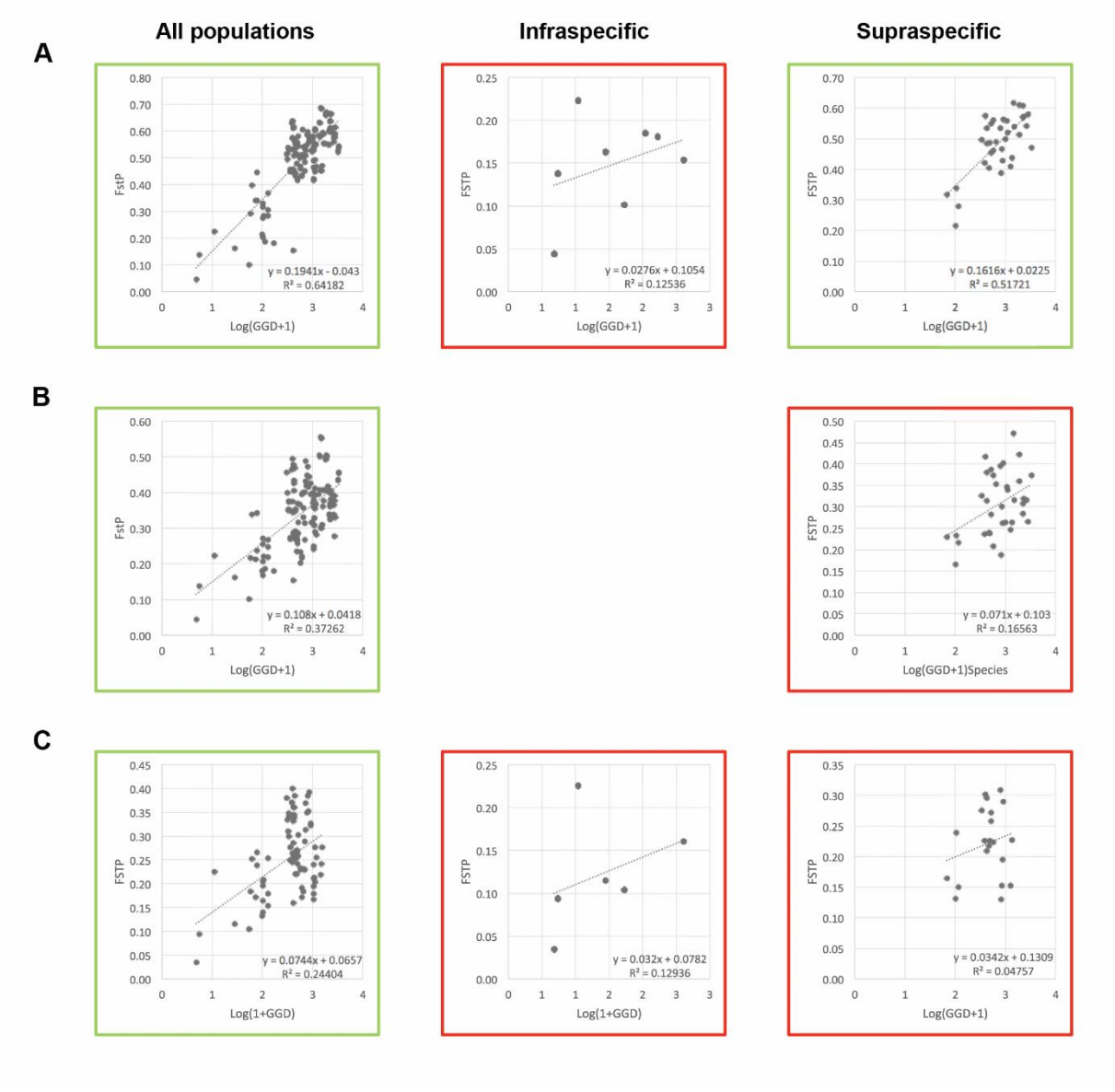


DJOST(A) Dataset 1								
	Comparison	Actual	Mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower	BC_Upper
1	**HAM vs. HAM**	**0.0085**	**0.0149**	**0.0085**	**0.0103**	**0.021**	**0.0039**	**0.0147**
2	**PAL vs. PAL**	**0.0085**	**0.0102**	**0.0085**	**0.0078**	**0.0127**	**0.0062**	**0.0111**
3	**DOM vs. DOM**	**0.0118**	**0.0128**	**0.0118**	**0.0092**	**0.0167**	**0.0083**	**0.0157**
4	DOM vs. PAL	0.0196	0.02	0.0196	0.0173	0.0229	0.0169	0.0225
5	DOM vs. HAM	0.0198	0.0205	0.0198	0.017	0.0236	0.0163	0.023
6	HAM vs. PAL	0.023	0.0241	0.023	0.021	0.027	0.0199	0.026
7	**DOD vs. DOD**	**0.0275**	**0.0314**	**0.0275**	**0.0261**	**0.0371**	**0.0222**	**0.0332**
8	**LAC vs. LAC**	**0.0294**	**0.031**	**0.0294**	**0.0278**	**0.0343**	**0.0262**	**0.0327**
9	**POR vs. POR**	**0.0309**	**0.0365**	**0.0309**	**0.0277**	**0.0466**	**0.0221**	**0.041**
10	**EKM vs. POR**	**0.0403**	**0.0415**	**0.0403**	**0.0353**	**0.0486**	**0.0341**	**0.0474**
11	**CU vs. CU**	**0.0456**	**0.0505**	**0.0456**	**0.0418**	**0.0605**	**0.0369**	**0.0556**
12	DOD vs. DOM	0.0464	0.0473	0.0464	0.0451	0.0494	0.0441	0.0484
13	DOM vs. LAC	0.0493	0.0495	0.0493	0.0478	0.0511	0.0476	0.0509
14	DOM vs. EKM	0.0529	0.0535	0.0529	0.0512	0.0566	0.0505	0.0559
15	DOM vs. SPL	0.0529	0.0543	0.0529	0.0497	0.0585	0.0483	0.0571
16	DOM vs. POR	0.0539	0.0546	0.0539	0.0513	0.0581	0.0505	0.0573
17	DOD vs. LAC	0.0578	0.0584	0.0578	0.0555	0.0625	0.0549	0.0619
18	PAL vs. POR	0.0604	0.0629	0.0604	0.0577	0.0683	0.0552	0.0659
19	CU vs. PAL	0.062	0.0638	0.062	0.0599	0.0681	0.0581	0.0663
20	DOD vs. PAL	0.064	0.0651	0.064	0.0626	0.0677	0.0616	0.0667
21	CU vs. DOM	0.0688	0.0698	0.0688	0.066	0.0742	0.065	0.0733
22	DOD vs. HAM	0.0704	0.072	0.0704	0.0688	0.0749	0.0672	0.0733
23	LAC vs. PAL	0.0738	0.0739	0.0738	0.0717	0.0759	0.0716	0.0758
24	DOD vs. POR	0.0744	0.0764	0.0744	0.0729	0.0802	0.071	0.0783
25	DOD vs. SPL	0.0746	0.0764	0.0746	0.0727	0.08	0.0709	0.0781
26	POR vs. SPL	0.077	0.0816	0.077	0.0743	0.088	0.0697	0.0834
27	HAM vs. LAC	0.0784	0.079	0.0784	0.0758	0.0814	0.0752	0.0808
28	EKM vs. PAL	0.08	0.0807	0.08	0.0781	0.0838	0.0773	0.0831
29	HAM vs. SPL	0.0827	0.0887	0.0827	0.0826	0.0974	0.0766	0.0913
30	PAL vs. SPL	0.0845	0.0862	0.0845	0.0806	0.0924	0.0788	0.0907
31	DOD vs. EKM	0.086	0.0862	0.086	0.0841	0.0877	0.084	0.0876
32	CU vs. POR	0.0884	0.0911	0.0884	0.0859	0.0962	0.0832	0.0935
33	CU vs. SPL	0.092	0.0952	0.092	0.0884	0.1003	0.0852	0.0971
34	CU vs. DOD	0.0927	0.0936	0.0927	0.0903	0.0963	0.0893	0.0953
35	LAC vs. SPL	0.0946	0.0951	0.0946	0.0916	0.0985	0.0911	0.0981
36	HAM vs. POR	0.099	0.1013	0.099	0.095	0.1078	0.0928	0.1055
37	CU vs. HAM	0.1	0.1034	0.1	0.0955	0.1106	0.092	0.1072
38	EKM vs. LAC	0.1075	0.108	0.1075	0.1055	0.1102	0.1049	0.1097
39	LAC vs. POR	0.1122	0.1131	0.1122	0.109	0.1164	0.1081	0.1156
40	CU vs. LAC	0.1147	0.1148	0.1147	0.1125	0.1163	0.1125	0.1162
41	EKM vs. HAM	0.1184	0.1192	0.1184	0.1141	0.1248	0.1133	0.1239
42	EKM vs. SPL	0.1258	0.1285	0.1258	0.1224	0.1375	0.1197	0.1348
43	CU vs. EKM	0.1274	0.1278	0.1274	0.1239	0.1323	0.1234	0.1319
44	EKM vs. POR	0.1447	0.146	0.1447	0.1408	0.1517	0.1395	0.1505

DJOST(B) Dataset 2								
	Comparison	Actual	Mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower	BC_Upper
1	**HAM vs. HAM**	**0.0085**	**0.0148**	**0.0085**	**0.0099**	**0.0208**	**0.0037**	**0.0146**
2	**PAL vs. PAL**	**0.0085**	**0.0101**	**0.0085**	**0.0078**	**0.0126**	**0.0062**	**0.0111**
3	**DOM vs. DOM**	**0.0118**	**0.0126**	**0.0118**	**0.0091**	**0.0164**	**0.0084**	**0.0157**
4	DOM vs. LAC	0.0127	0.0127	0.0127	0.012	0.0136	0.012	0.0135
5	DOM vs. PAL	0.0136	0.0138	0.0136	0.0117	0.0158	0.0115	0.0156
6	DOM vs. SPL	0.0139	0.0144	0.0139	0.0132	0.0159	0.0127	0.0154
7	DOD vs. DOM	0.0148	0.0153	0.0148	0.014	0.0167	0.0135	0.0162
8	DOM vs. HAM	0.0162	0.0166	0.0162	0.0138	0.0195	0.0134	0.0191
9	LAC vs. PAL	0.0169	0.0169	0.0169	0.0157	0.0178	0.0157	0.0178
10	LAC vs. SPL	0.0182	0.0186	0.0182	0.0174	0.0199	0.0171	0.0196
11	HAM vs. PAL	0.0188	0.0195	0.0188	0.017	0.0219	0.0163	0.0212
12	HAM vs. LAC	0.0192	0.0194	0.0192	0.0181	0.0206	0.018	0.0204
13	PAL vs. POR	0.0205	0.0218	0.0205	0.0197	0.0241	0.0184	0.0228
14	DOD vs. PAL	0.0206	0.0212	0.0206	0.0196	0.023	0.0189	0.0223
15	DOD vs. SPL	0.0221	0.023	0.0221	0.0215	0.0248	0.0206	0.0239
16	PAL vs. SPL	0.0233	0.0238	0.0233	0.0217	0.0258	0.0213	0.0254
17	HAM vs. SPL	0.0239	0.0266	0.0239	0.0232	0.0304	0.0205	0.0278
18	DOD vs. HAM	0.0242	0.025	0.0242	0.0233	0.0269	0.0224	0.026
19	DOM vs. POR	0.0243	0.0249	0.0243	0.0225	0.0268	0.022	0.0263
20	CU vs. LAC	0.0272	0.0273	0.0272	0.0262	0.0282	0.0261	0.0281
21	DOD vs. DOD	0.0275	0.0312	0.0275	0.0258	0.0371	0.022	0.0334
22	CU vs. PAL	0.0283	0.0296	0.0283	0.0264	0.0323	0.0252	0.0311
23	**LAC vs. LAC**	**0.0294**	**0.031**	**0.0294**	**0.0277**	**0.035**	**0.0261**	**0.0334**
24	**POR vs. POR**	**0.0309**	**0.0363**	**0.0309**	**0.0281**	**0.0455**	**0.0227**	**0.0401**
25	DOM vs. EKM	0.0319	0.0323	0.0319	0.0299	0.0346	0.0296	0.0343
26	DOD vs. POR	0.032	0.0335	0.032	0.0314	0.0359	0.0299	0.0344
27	DOD vs. EKM	0.0334	0.0335	0.0334	0.0319	0.0344	0.0319	0.0343
28	CU vs. DOM	0.0338	0.0342	0.0338	0.0316	0.0369	0.0311	0.0364
29	EKM vs. LAC	0.0345	0.035	0.0345	0.0333	0.0368	0.0328	0.0363
30	EKM vs. PAL	0.0357	0.0361	0.0357	0.034	0.0383	0.0336	0.0379
31	DOD vs. LAC	0.0366	0.0372	0.0366	0.035	0.0396	0.0344	0.039
32	LAC vs. POR	0.0374	0.0378	0.0374	0.0352	0.0405	0.0348	0.0401
33	CU vs. DOD	0.0377	0.0384	0.0377	0.0366	0.0402	0.0358	0.0394
34	**EKM vs. EKM**	**0.0403**	**0.0413**	**0.0403**	**0.0354**	**0.0484**	**0.0344**	**0.0474**
35	CU vs. SPL	0.0441	0.0455	0.0441	0.0408	0.0511	0.0394	0.0497
36	EKM vs. SPL	0.0446	0.0459	0.0446	0.0427	0.0493	0.0414	0.048
37	CU vs. HAM	0.0447	0.0469	0.0447	0.0425	0.0515	0.0403	0.0493
38	**CU vs. CU**	**0.0456**	**0.0505**	**0.0456**	**0.0415**	**0.0606**	**0.0366**	**0.0557**
39	POR vs. SPL	0.0467	0.0499	0.0467	0.0448	0.0555	0.0416	0.0523
40	CU vs. POR	0.048	0.05	0.048	0.0461	0.0539	0.0441	0.0519
41	HAM vs. POR	0.0495	0.0515	0.0495	0.0475	0.056	0.0456	0.0541
42	EKM vs. HAM	0.0549	0.0557	0.0549	0.0515	0.0596	0.0507	0.0588
43	EKM vs. POR	0.072	0.0731	0.072	0.07	0.0763	0.0689	0.0752
44	CU vs. EKM	0.0889	0.0891	0.0889	0.0851	0.0924	0.0848	0.0921

DJOST(C) Dataset 3								
	Comparison	Actual	Mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower	BC_Upper
1	**HAM vs. HAM**	**0.0366**	**0.0929**	**0.0366**	**0.0442**	**0.1684**	**-0.0121**	**0.112**
2	**PAL vs. PAL**	**0.1236**	**0.1638**	**0.1236**	**0.1183**	**0.2246**	**0.078**	**0.1844**
3	**DOM vs. DOM**	**0.13**	**0.1451**	**0.13**	**0.0917**	**0.2209**	**0.0767**	**0.2058**
4	**EKM vs. EKM**	**0.1975**	**0.2049**	**0.1975**	**0.1558**	**0.2625**	**0.1483**	**0.255**
5	**POR vs. POR**	**0.2112**	**0.2382**	**0.2112**	**0.1751**	**0.3221**	**0.148**	**0.295**
6	DOM vs. HAM	0.3051	0.3192	0.3051	0.2619	0.3775	0.2478	0.3634
7	CU vs. HAM	0.3195	0.3467	0.3195	0.2949	0.4262	0.2677	0.399
8	**CU vs. CU**	**0.3387**	**0.3582**	**0.3387**	**0.2681**	**0.4702**	**0.2486**	**0.4507**
9	DOM vs. PAL	0.3526	0.3612	0.3526	0.2924	0.4285	0.2839	0.42
10	HAM vs. PAL	0.3631	0.376	0.3631	0.3117	0.4232	0.2988	0.4103
11	CU vs. PAL	0.3647	0.3962	0.3647	0.342	0.4624	0.3105	0.4309
12	HAM vs. SPL	0.3755	0.4134	0.3755	0.3476	0.5	0.3097	0.462
13	PAL vs. POR	0.3947	0.4307	0.3947	0.3725	0.4975	0.3365	0.4616
14	CU vs. POR	0.4473	0.4615	0.4473	0.3952	0.5263	0.381	0.5121
15	DOM vs. SPL	0.4558	0.479	0.4558	0.4335	0.553	0.4103	0.5298
16	EKM vs. SPL	0.4665	0.4848	0.4665	0.4321	0.5559	0.4138	0.5376
17	POR vs. SPL	0.4807	0.5112	0.4807	0.4264	0.5807	0.3959	0.5502
18	HAM vs. POR	0.4939	0.51	0.4939	0.429	0.5983	0.4128	0.5822
19	DOM vs. EKM	0.5114	0.5184	0.5114	0.4575	0.582	0.4505	0.5749
20	EKM vs. HAM	0.5119	0.5223	0.5119	0.4739	0.5805	0.4635	0.5701
21	CU vs. DOM	0.5133	0.5377	0.5133	0.4598	0.6072	0.4354	0.5827
22	DOM vs. POR	0.525	0.5405	0.525	0.4798	0.6101	0.4644	0.5946
23	EKM vs. POR	0.5993	0.6163	0.5993	0.5591	0.6703	0.5422	0.6533
24	EKM vs. PAL	0.6005	0.6054	0.6005	0.5672	0.6472	0.5623	0.6423
25	CU vs. SPL	0.6073	0.6288	0.6073	0.5671	0.6991	0.5455	0.6775
26	PAL vs. SPL	0.6314	0.639	0.6314	0.5759	0.6999	0.5683	0.6923
27	CU vs. EKM	0.7209	0.7246	0.7209	0.6698	0.7793	0.6661	0.7756

Appendix 4.7 Mantel tests. **GGD** = Geographic Distance, **FSTP** = pairwise F_{ST} (Weir and Cockerham, 1984). **A** dataset 1 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, including the assumed monomorphic data (See Appendix 4.2: categories A, B and C). **B** dataset 2 which comprises 340 individuals representing 17 populations, genotyped for all 63 microsatellite markers where possible, excluding the assumed monomorphic data (See Appendix 4.2: categories A and B). **C** dataset 3 which comprises 260 individuals representing 13 populations of the 8 taxa of section *Talauma* subsection *Cubenses* (See Table 4.1: Class. = TAS), genotyped for 10 microsatellite markers (See Appendix 4.1: marker names indicated with an asterisk).



Appendix 5: SSR study of *Magnolia cubensis* subsp. *acunae*

Appendix 5.1 Summary statistics given in adults and juveniles of *Magnolia cubensis* subsp. *acunae* in the subpopulations of 'Topes de Collantes' (TC) and 'Lomas de Banao' (LB) for all markers. A: mean number of alleles. H_O: observed heterozygosity. H_E: expected heterozygosity. F_{IS}: inbreeding coefficient, significant deviations from HWP: *p<0,005 Bonferroni corrected probability were considered statistically significant. NI: non-informative comparison because it is a monomorphic locus or presents low values of Ho and He. TC: Topes de Collantes.

Population	Locus	A	A_R	H_O	H_E	F_{IS}
TC (Adults) N=39	MA39_333	3	2.358	0.351	0.333	-0.042
	MA41_264	9	7.020	0.718	0.858	0.176*
	MA41_076	2	1.205	0.026	0.025	NI
	MA42_255	6	4.228	0.513	0.695	0.174
	MA42_274	6	3.783	0.564	0.512	-0.088
	MA42_083	9	4.807	0.711	0.618	-0.137
	MA40_045	9	5.985	0.684	0.758	0.111
	MA42_166	4	3.048	0.541	0.449	-0.191
	MA42_063	11	7.710	0.895	0.853	-0.035
	MA42_279	5	3.104	0.385	0.575	0.342*
	MA42_265	2	1.889	0.179	0.204	0.134
TC (Juveniles) N=19	MA39_333	2	1.999	0.111	0.401	0.736*
	MA41_264	4	3.285	0.444	0.591	0.275
	MA41_076	2	1.698	0.111	0.105	-0.030
	MA42_255	4	3.896	0.842	0.708	-0.164
	MA42_274	2	1.727	0.091	0.087	NI
	MA42_083	3	2.619	0.316	0.314	0.023
	MA40_045	6	4.963	0.833	0.738	-0.102
	MA42_166	3	2.874	0.474	0.421	-0.098
	MA42_063	8	6.070	0.737	0.759	0.056
	MA42_279	3	2.972	0.474	0.586	0.217*
	MA42_265	2	1.998	0.353	0.360	0.049
LB (Adults) N=9	MA39_333	2	2.000	0.222	0.346	0.407
	MA41_264	3	2.993	0.333	0.438	0.294
	MA41_076	1	1.000	0.000	0.000	NI
	MA42_255	3	3.000	0.625	0.617	0.054
	MA42_274	2	2.000	0.000	0.219	1.000
	MA42_083	6	5.765	0.778	0.722	-0.018
	MA40_045	4	3.882	0.444	0.599	0.312
	MA42_166	2	1.993	0.222	0.198	-0.067
	MA42_063	5	4.765	0.667	0.525	-0.215
	MA42_279	3	2.889	0.444	0.512	0.189
	MA42_265	2	2.000	0.222	0.346	0.407

Appendix 6: SSR study & biogeography of *M. dodecapetala*

Appendix 6.1 Summary statistics given for the five island populations (SV, SL, M, D, G) and the four subpopulations (VER, HER+TOS; SYN+SYM, TPA+TPM+SYL) found with STRUCTURE for *Magnolia dodecapetala* in the Lesser Antilles. **D(19)**: Dataset with 19 SSR markers i.e. the full dataset. **D(15)**: Dataset with 15 SSR markers i.e. the conservative dataset 1. **D(7)**: Dataset with 7 SSR markers i.e. the conservative dataset 2. $\mathbf{N_G}$: (mean) number of genotyped individuals. **A**: (mean) number of alleles. $\mathbf{A_R}$: allelic richness (rarefaction to 28 individuals) – not given, nor calculated with inclusion of, the subpopulations. $\mathbf{A_P}$: (mean) number of private alleles – not given, nor calculated with inclusion of, the subpopulations. $\mathbf{H_O}$: (mean) observed heterozygosity. $\mathbf{H_E}$: (mean) expected heterozygosity. $\mathbf{F_{IS}}$: inbreeding coefficient, significant deviations from Hardy-Weinberg proportions (HWP): ***** ($p = 0.05$) and ****** ($p = 0.05$ Bonferroni corrected), $\mathbf{A_0}$: estimated null allele frequency by ML-NullFreq (* = $p > 0.05$), recognised by MICROCHECKER: ******. **Mean**: mean of statistic averaged over the markers (sum of the values, divided by the number of samples). **SE**: Standard Error of the mean (standard deviation, divided by the square root of the number of samples). **P**: percentage of polymorphic loci (%). **NA**: Not Available.

POPULATION: SV (Saint Vincent)										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	28	1	1	0	0.000	0.000	NA	0
MA39_159	X		28	1	1	0	0.000	0.000	NA	0
MA39_182	X	X	28	1	1	0	0.000	0.000	NA	0
MA39_185	X		28	2	2	0	0.036	0.270	0.872**	0.219**
MA39_191	X		28	4	4	0	0.464	0.721	0.372**	0.160**
MA39_199	X		28	1	1	0	0.000	0.000	NA	0
MA39_259	X	X	28	2	2	0	0.036	0.035	0.000	0
MA39_287			28	4	4	0	0.214	0.411	0.492*	0.166**
MA39_442	X	X	28	1	1	0	0.000	0.000	NA	0
MA40_136	X	X	28	1	1	0	0.000	0.000	NA	0
MA40_282			28	5	5	2	0.464	0.638	0.289	0.104**
MA42_072	X		28	1	1	0	0.000	0.000	NA	0
MA42_231			28	3	3	0	0.036	0.322	0.893**	0.245**
MA42_255	X		28	1	1	0	0.000	0.000	NA	0
MA42_274	X	X	28	1	1	0	0.000	0.000	NA	0
MA42_333	X		28	1	1	0	0.000	0.000	NA	0
MA42_421	X	X	28	1	1	0	0.000	0.000	NA	0
MA42_471			28	4	4	0	0.071	0.462	0.850**	0.290**
MA42_495	X		28	1	1	0	0.000	0.000	NA	0
$P_{D(19)}$ = 36.84	**$P_{D(15)}$** = 20.00	**$P_{D(7)}$** = 14.29								
Mean D(19)	NA	NA	28.000	1.895	1.895	0.105	0.389	0.440	0.551**	NA
SE D(19)	NA	NA	0.000	0.314	0.314	0.105	0.034	0.056	NA	NA
Mean D(15)	NA	NA	28.000	1.333	1.333	0	0.036	0.068	0.492*	NA
SE D(15)	NA	NA	0.000	0.211	0.211	0	0.031	0.050	NA	NA
Mean D(7)	NA	NA	28.000	1.143	1.143	0	0.005	0.005	0	NA
SE D(7)	NA	NA	0.000	0.143	0.143	0	0.005	0.005	NA	NA

POPULATION: SL (Saint Lucia)										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	29	2	2	1	0.310	0.307	0.008	0
MA39_159	X		28	3	3	0	0.500	0.487	-0.009	0
MA39_182	X	X	29	2	1.966	0	0.034	0.034	0.000	0
MA39_185	X		29	10	9.930	2	0.793	0.826	0.058	0.041
MA39_191	X		29	8	8	0	0.897	0.820	-0.076	0
MA39_199	X		29	2	2	1	0.379	0.441	0.156	0.046
MA39_259	X	X	29	3	2.966	2	0.586	0.463	-0.249	0
MA39_287			29	10	10	0	0.828	0.843	0.036	0
MA39_442	X	X	29	2	2	1	0.345	0.328	-0.033	0
MA40_136	X	X	29	1	1	0	0.000	0.000	NA	0
MA40_282			29	12	11.930	0	0.862	0.883	0.041	0
MA42_072	X		29	2	2	0	0.034	0.098	0.659	0.111*
MA42_231			29	8	8	0	0.793	0.847	0.081**	0.004
MA42_255	X		29	9	8.965	1	0.828	0.823	0.012	0
MA42_274	X	X	29	13	12.793	0	0.862	0.784	-0.082	0
MA42_333	X		29	9	8.931	0	0.793	0.835	0.068	0.021
MA42_421	X	X	29	1	1	0	0.000	0.000	NA	0
MA42_471			29	11	10.964	0	0.862	0.871	0.028	0.006
MA42_495	X		29	2	2	0	0.103	0.098	-0.037	0
$P_{D(19)}$ = 89.47	**$P_{D(15)}$** = 86.67	**$P_{D(7)}$** = 71.43								
Mean D(19)	NA	NA	28.947	5.789	5.760	0.421	0.516	0.515	0.015	NA
SE D(19)	NA	NA	0.053	0.984	0.977	0.159	0.080	0.079	NA	NA
Mean D(15)	NA	NA	28.933	4.600	4.570	0.533	0.431	0.423	-0.001	NA
SE D(15)	NA	NA	0.067	1.027	1.016	0.192	0.089	0.085	NA	NA
Mean D(7)	NA	NA	29.000	3.429	3.389	0.571	0.305	0.274	-0.098	NA
SE D(7)	NA	NA	0.000	1.616	1.588	0.297	0.124	0.110	NA	NA

POPULATION: M (Martinique)										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	49	3	2.571	1	0.245	0.248	0.021	0.003
MA39_159	X		49	2	2	1	0.306	0.359	0.158	0.047
MA39_182	X	X	49	2	2	0	0.449	0.425	-0.047	0
MA39_185	X		49	9	8.709	2	0.837	0.822	-0.007	0
MA39_191	X		49	15	12.411	4	0.776	0.865	0.113	0.035
MA39_199	X		49	3	2.559	1	0.122	0.116	-0.045	0
MA39_259	X	X	49	2	1.571	1	0.020	0.020	0.000	0
MA39_287			49	12	10.828	3	0.673	0.855	0.222**	0.094**
MA39_442	X	X	49	3	2.743	1	0.102	0.098	-0.030	0
MA40_136	X	X	49	1	1	0	0.000	0.000	NA	0
MA40_282			49	9	8.282	2	0.531	0.772	0.322**	0.120**
MA42_072	X		49	3	2.571	0	0.204	0.185	-0.092	0
MA42_231			49	17	15.259	5	0.816	0.905	0.108	0.047*
MA42_255	X		49	7	6.378	1	0.755	0.726	-0.030	0
MA42_274	X	X	49	21	17.860	1	0.918	0.911	0.002	0
MA42_333	X		49	8	6.926	0	0.735	0.733	0.007	0
MA42_421	X	X	49	1	1	0	0.000	0.000	NA	0
MA42_471			49	24	20.204	2	0.959	0.928	-0.024	0
MA42_495	X		49	2	1.969	0	0.082	0.078	-0.032	0
$P_{D(19)}$ = 89.47	**$P_{D(15)}$** = 86.67	**$P_{D(7)}$** = 71.43								
Mean D(19)	NA	NA	49.000	7.579	6.676	1.316	0.449	0.476	0.067*	NA
SE D(19)	NA	NA	0.000	1.634	1.390	0.325	0.080	0.085	NA	NA
Mean D(15)	NA	NA	49.000	5.467	4.818	0.867	0.370	0.372	0.017	NA
SE D(15)	NA	NA	0.000	1.489	1.259	0.274	0.088	0.089	NA	NA
Mean D(7)	NA	NA	49.000	4.714	4.106	0.571	0.248	0.243	-0.009	NA
SE D(7)	NA	NA	0.000	2.732	2.307	0.202	0.128	0.126	NA	NA

POPULATION: D (Dominica)										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	48	2	1.931	0	0.063	0.061	-0.022	0
MA39_159	X		48	2	2	0	0.313	0.489	0.371*	0.120**
MA39_182	X	X	48	1	1	0	0.000	0.000	NA	0
MA39_185	X		48	10	8.441	1	0.604	0.717	0.168*	0.063*
MA39_191	X		48	11	9.935	1	0.771	0.736	-0.037	0
MA39_199	X		48	2	1.998	0	0.104	0.135	0.239	0.056*
MA39_259	X	X	48	2	1.829	0	0.042	0.041	-0.011	0
MA39_287			48	10	8.824	2	0.729	0.828	0.129*	0.063
MA39_442	X	X	48	3	3	2	0.333	0.659	0.502**	0.195**
MA40_136	X	X	47	1	1	0	0.000	0.000	NA	0.120
MA40_282			48	4	3.989	3	0.063	0.620	0.901**	0.346**
MA42_072	X		48	2	2	0	0.500	0.486	-0.018	0
MA42_231			48	13	11.215	1	0.833	0.821	-0.004	0
MA42_255	X		48	11	10.165	1	0.688	0.823	0.175**	0.087**
MA42_274	X	X	48	20	16.368	5	0.833	0.887	0.071	0.011
MA42_333	X		47	18	14.557	4	0.574	0.841	0.327**	0.162**
MA42_421	X	X	48	1	1	0	0.000	0.000	NA	0
MA42_471			45	21	18.435	6	0.889	0.930	0.056	0.011
MA42_495	X		47	9	8.305	2	0.596	0.772	0.238**	0.121**
$P_{D(19)}$ = 84.21%	**$P_{D(15)}$** = 80.00	**$P_{D(7)}$** = 57.14								
Mean D(19)	NA	NA	47.684	7.526	6.631	1.474	0.418	0.518	0.204**	NA
SE D(19)	NA	NA	0.172	1.557	1.299	0.421	0.076	0.081	NA	NA
Mean D(15)	NA	NA	47.800	6.333	5.569	1.067	0.361	0.443	0.195**	NA
SE D(15)	NA	NA	0.107	1.658	1.359	0.408	0.080	0.093	NA	NA
Mean D(7)	NA	NA	47.857	4.286	3.733	1.000	0.182	0.235	0.239*	NA
SE D(7)	NA	NA	0.143	2.634	2.124	0.724	0.118	0.141	NA	NA

POPULATION: G (Guadeloupe)										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	41	1	1.000	0	0.000	0.000	NA	0
MA39_159	X		41	3	2.674	1	0.122	0.116	-0.042	0
MA39_182	X	X	41	2	2.000	1	0.439	0.497	0.129	0.039
MA39_185	X		41	8	7.802	2	0.732	0.780	0.075	0.029
MA39_191	X		41	2	2.000	1	0.390	0.343	-0.127	0
MA39_199	X		40	3	3.000	1	0.175	0.387	0.556**	0.226**
MA39_259	X	X	41	2	2.000	0	0.122	0.195	0.387	0.095*
MA39_287			41	9	8.576	1	0.732	0.813	0.112	0.021
MA39_442	X	X	41	2	1.683	0	0.024	0.024	0.000	0
MA40_136	X	X	41	3	2.971	2	0.463	0.495	0.077	0.017
MA40_282			41	12	10.792	0	0.756	0.830	0.102*	0.015
MA42_072	X		41	3	2.683	1	0.293	0.449	0.360*	0.109**
MA42_231			41	8	7.366	0	0.756	0.822	0.092	0.051*
MA42_255	X		41	10	9.550	0	0.878	0.840	-0.033	0
MA42_274	X	X	41	19	16.944	2	0.878	0.891	0.027	0.028
MA42_333	X		41	21	19.466	2	0.878	0.921	0.059	0.022
MA42_421	X	X	41	2	1.902	1	0.049	0.048	-0.013	0
MA42_471			41	15	13.272	2	0.756	0.874	0.147*	0.056**
MA42_495	X		41	19	16.637	14	0.951	0.897	-0.048	0
$P_{D(19)}$ = 94.74%	**$P_{D(15)}$** = 93.33	**$P_{D(7)}$** = 85.71								
Mean D(19)	NA	NA	40.947	7.579	6.964	1.632	0.494	0.538	0.093*	NA
SE D(19)	NA	NA	0.053	1.536	1.374	0.710	0.078	0.078	NA	NA
Mean D(15)	NA	NA	40.933	6.667	6.154	1.867	0.426	0.459	0.083*	NA
SE D(15)	NA	NA	0.067	1.848	1.660	0.888	0.091	0.088	NA	NA
Mean D(7)	NA	NA	41.000	4.429	4.071	0.857	0.282	0.307	0.094	NA
SE D(7)	NA	NA	0.000	2.438	2.157	0.340	0.123	0.126	NA	NA

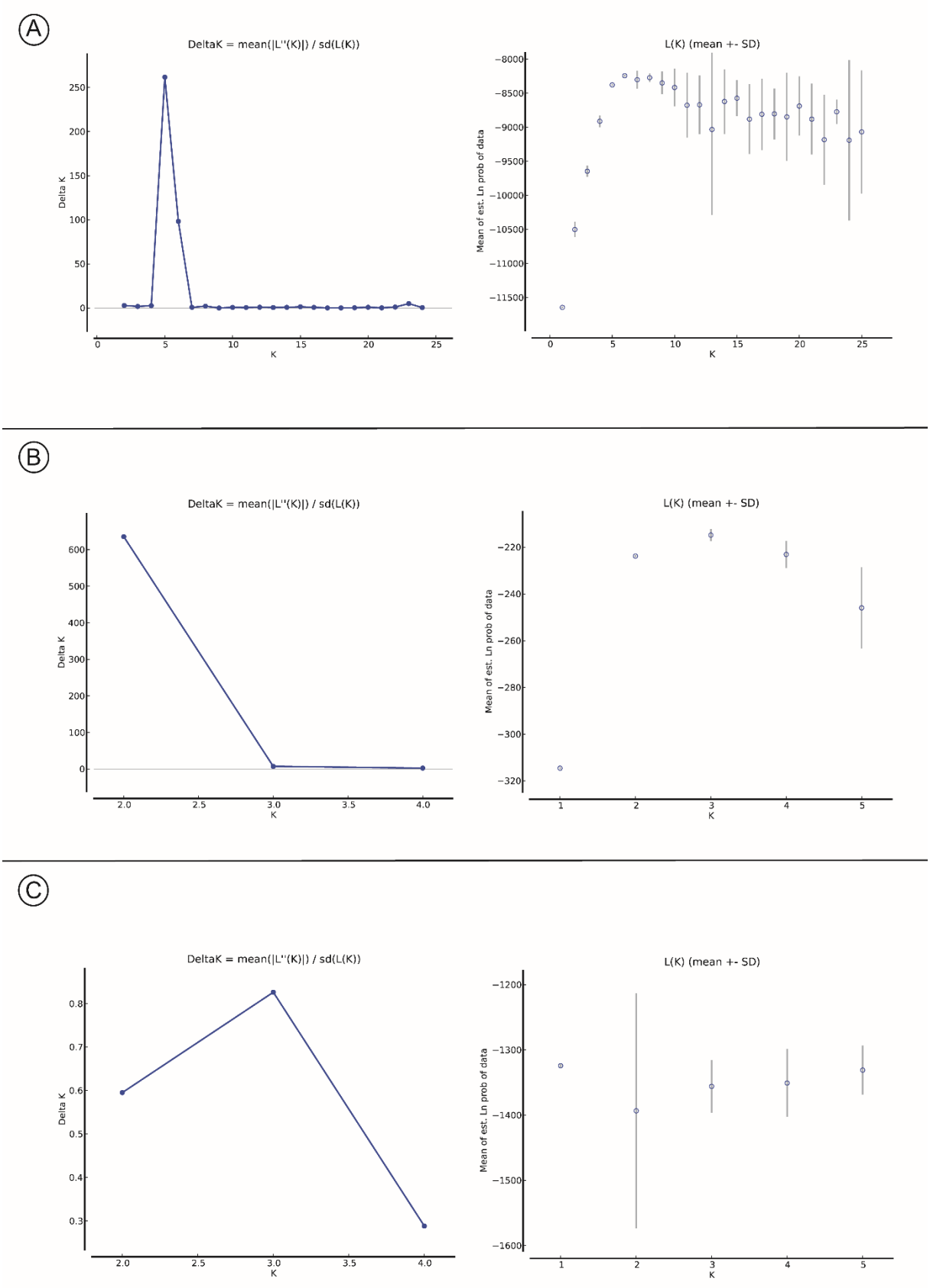
SUBPOPULATION: SV (Saint Vincent) – VER (Vermont)										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	5	1	NA	NA	0	0	NA	0
MA39_159	X	X	5	1	NA	NA	0	0	NA	0
MA39_182	X		5	1	NA	NA	0	0	NA	0
MA39_185	X		5	2	NA	NA	0.200	0.180	0	0
MA39_191	X		5	2	NA	NA	0.200	0.180	0	0
MA39_199			5	1	NA	NA	0	0	NA	0
MA39_259	X		5	1	NA	NA	0	0	NA	0
MA39_287	X	X	5	1	NA	NA	0	0	NA	0
MA39_442	X	X	5	1	NA	NA	0	0	NA	0
MA40_136	X	X	5	1	NA	NA	0	0	NA	0
MA40_282			5	1	NA	NA	0	0	NA	0
MA42_072	X		5	1	NA	NA	0	0	NA	0
MA42_231			5	1	NA	NA	0	0	NA	0
MA42_255	X		5	1	NA	NA	0	0	NA	0
MA42_274	X	X	5	1	NA	NA	0	0	NA	0
MA42_333	X		5	1	NA	NA	0	0	NA	0
MA42_421	X	X	5	1	NA	NA	0	0	NA	0
MA42_471			5	2	NA	NA	0	0.480	1*	0.327*
MA42_495	X		5	1	NA	NA	0	0	NA	0
$P_{D(19)}$ = 15.79	**$P_{D(15)}$** = 13.33	**$P_{D(7)}$** = 0								
Mean D(19)	NA	NA	5	1.158	NA	NA	0.021	0.044	0.600	NA
SE D(19)	NA	NA	0	0.086	NA	NA	0.014	0.027	NA	NA
Mean D(15)	NA	NA	5	1.133	NA	NA	0.027	0.024	0	NA
SE D(15)	NA	NA	0	0.091	NA	NA	0.018	0.016	NA	NA
Mean D(7)	NA	NA	5	1	NA	NA	0	0	NA	NA
SE D(7)	NA	NA	0	0	NA	NA	0	0	NA	NA

SUBPOPULATION: SV (Saint Vincent) – HER + TOS										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	23	1	NA	NA	0	0	NA	0
MA39_159	X	X	23	1	NA	NA	0	0	NA	0
MA39_182	X		23	1	NA	NA	0	0	NA	0
MA39_185	X		23	1	NA	NA	0	0	NA	0
MA39_191	X		23	4	NA	NA	0.522	0.674	0.247*	0.109*
MA39_199			23	1	NA	NA	0	0	NA	0
MA39_259	X		23	2	NA	NA	0.043	0.043	0	0
MA39_287	X	X	23	4	NA	NA	0.261	0.476	0.470*	0.167**
MA39_442	X	X	23	1	NA	NA	0	0	NA	0
MA40_136	X	X	23	1	NA	NA	0	0	NA	0
MA40_282			23	5	NA	NA	0.565	0.710	0.225	0.084*
MA42_072	X		23	1	NA	NA	0.000	0.000	NA	0
MA42_231			23	2	NA	NA	0.043	0.043	0	0
MA42_255	X		23	1	NA	NA	0	0	NA	0
MA42_274	X	X	23	1	NA	NA	0	0	NA	0
MA42_333	X		23	1	NA	NA	0	0	NA	0
MA42_421	X	X	23	1	NA	NA	0	0	NA	0
MA42_471			23	2	NA	NA	0.087	0.227	0.630*	0.152**
MA42_495	X		23	1	NA	NA	0	0	NA	0
$P_{D(19)}$ = 31.58	**$P_{D(15)}$** = 13.33	**$P_{D(7)}$** = 14.29								
Mean D(19)	NA	NA	23	1.684	NA	NA	0.080	0.114	0.319*	NA
SE D(19)	NA	NA	0	0.287	NA	NA	0.040	0.054	NA	NA
Mean D(15)	NA	NA	23	1.267	NA	NA	0.038	0.048	0.232	NA
SE D(15)	NA	NA	0	0.206	NA	NA	0.035	0.045	NA	NA
Mean D(7)	NA	NA	23	1.143	NA	NA	0.006	0.006	0	NA
SE D(7)	NA	NA	0	0.143	NA	NA	0.006	0.006	NA	NA

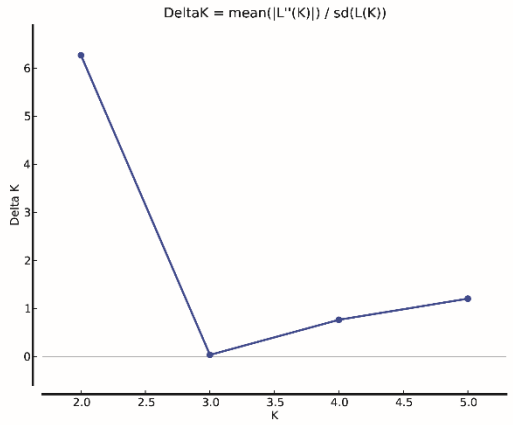
SUBPOPULATION: D (Dominica) – SYN + SYM										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	15	1	NA	NA	0.000	0.000	0	0
MA39_159	X	X	15	2	NA	NA	0.267	0.231	0.592*	0.185*
MA39_182	X		15	1	NA	NA	0.000	0.000	NA	0
MA39_185	X		15	8	NA	NA	0.933	0.778	-0.153	0
MA39_191	X		15	8	NA	NA	0.800	0.791	0.023	0.010
MA39_199			15	1	NA	NA	0.000	0.000	NA	0
MA39_259	X		15	1	NA	NA	0.000	0.000	NA	0
MA39_287	X	X	15	7	NA	NA	0.733	0.791	0.072	0.041
MA39_442	X	X	15	3	NA	NA	0.067	0.500	0.451	0.111*
MA40_136	X	X	14	1	NA	NA	0.000	0.000	NA	0.165*
MA40_282			15	4	NA	NA	0.067	0.704	0.744**	0.310**
MA42_072	X		15	2	NA	NA	0.400	0.391	0.349	0.100*
MA42_231			15	10	NA	NA	0.867	0.829	0.043	0
MA42_255	X		15	9	NA	NA	0.667	0.864	0.335**	0.145**
MA42_274	X	X	15	14	NA	NA	0.933	0.893	-0.055	0
MA42_333	X		14	11	NA	NA	0.643	0.824	0.329*	0.154**
MA42_421	X	X	15	1	NA	NA	0.000	0.000	NA	0
MA42_471			14	11	NA	NA	0.857	0.839	0.156	0.042
MA42_495	X		14	3	NA	NA	0.357	0.538	0.253	0.118*
$P_{D(19)}$ = 68.42	**$P_{D(15)}$** = 60.00	**$P_{D(7)}$** = 28.57								
Mean D(19)	NA	NA	14.789	5.158	NA	NA	0.399	0.472	0.203*	NA
SE D(19)	NA	NA	0.096	0.995	NA	NA	0.087	0.085	NA	NA
Mean D(15)	NA	NA	14.800	4.400	NA	NA	0.338	0.387	0.181*	NA
SE D(15)	NA	NA	0.107	1.129	NA	NA	0.095	0.096	NA	NA
Mean D(7)	NA	NA	14.857	3.143	NA	NA	0.143	0.199	0.045	NA
SE D(7)	NA	NA	0.143	1.831	NA	NA	0.132	0.135	NA	NA

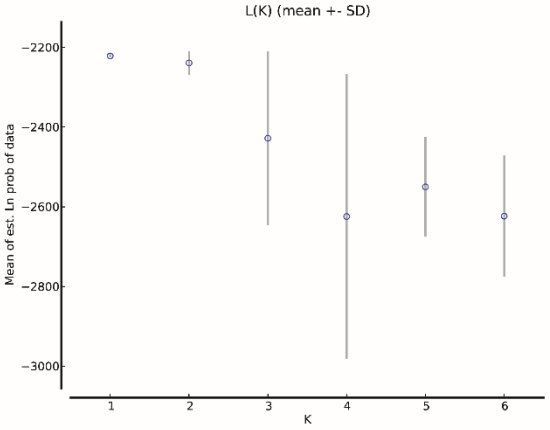
SUBPOPULATION: D (Dominica) – TPA + TPM + SYL										
D(19)	**D(15)**	**D(7)**	**N_G**	**A**	**A_R**	**A_P**	**H_O**	**H_E**	**F_{IS}**	**A_0**
MA39_023	X	X	33	2	NA	NA	0.091	0.087	-0.016	0
MA39_159	X	X	33	2	NA	NA	0.333	0.493	0.281	0.089*
MA39_182	X		33	1	NA	NA	0	0	NA	0
MA39_185	X		33	7	NA	NA	0.455	0.661	0.245	0.079*
MA39_191	X		33	8	NA	NA	0.758	0.679	-0.117	0
MA39_333			33	2	NA	NA	0.152	0.190	0.216	0.055*
MA39_259	X		33	2	NA	NA	0.061	0.059	-0.016	0
MA39_287	X	X	33	8	NA	NA	0.727	0.815	0.147*	0.066*
MA39_442	X	X	33	3	NA	NA	0.455	0.657	0.330*	0.114**
MA40_136	X	X	33	1	NA	NA	0	0	NA	0
MA40_282			33	4	NA	NA	0.061	0.564	1**	0.345**
MA42_072	X		33	2	NA	NA	0.545	0.500	-0.199	0
MA42_231			33	11	NA	NA	0.818	0.798	-0.052	0
MA42_255	X		33	8	NA	NA	0.697	0.758	-0.011	0.004
MA42_274	X	X	33	14	NA	NA	0.788	0.871	0.110	0.034
MA42_333	X		33	13	NA	NA	0.545	0.800	0.230**	0.070**
MA42_421	X	X	33	1	NA	NA	0	0	NA	0
MA42_471			31	19	NA	NA	0.903	0.929	0.006	0
MA42_495	X		33	9	NA	NA	0.697	0.824	0.170	0.077**
$P_{D(19)}$ = 84.21	**$P_{D(15)}$** = 80.00	**$P_{D(7)}$** = 57.14								
Mean D(19)	NA	NA	32.895	6.158	NA	NA	0.426	0.510	0.143**	NA
SE D(19)	NA	NA	0.105	1.205	NA	NA	0.074	0.078	NA	NA
Mean D(15)	NA	NA	33	5.000	NA	NA	0.372	0.439	0.119*	NA
SE D(15)	NA	NA	0	1.155	NA	NA	0.077	0.088	NA	NA
Mean D(7)	NA	NA	33	3.429	NA	NA	0.199	0.239	0.188	NA
SE D(7)	NA	NA	0	1.784	NA	NA	0.116	0.138	NA	NA

Appendix 6.2 ΔK and L(K) plots per run STRUCTURE analysis on *Magnolia dodecapetala* from the Lesser Antilles. **A**: complete D(19) dataset (195 individuals). **B**: Saint Vincent D(19) dataset. **C**: Saint Lucia D(19) dataset. **D**: Martinique D(19) dataset. **E**: Dominica D(19) dataset. **F**: Guadeloupe D(19) dataset. **G**: complete D(15) dataset. **H**: Saint Vincent D(15) dataset. **I**: Saint Lucia D(15) dataset. **J**: Martinique D(15) dataset. **K**: Dominica D(15) dataset. **L**: Guadeloupe D(15) dataset. **M**: complete D(7) dataset. **N**: Saint Vincent D(7) dataset. **O**: Saint Lucia D(7) dataset. **P**: Martinique D(7) dataset. **Q**: Dominica D(7) dataset. **R**: Guadeloupe D(7) dataset. **D(19)**: individuals genotyped for all 19 SSR markers. **D(15)**: individuals genotyped for 15/19 SSR markers. **D(7)**: individuals genotyped for 7/19 SSR markers.

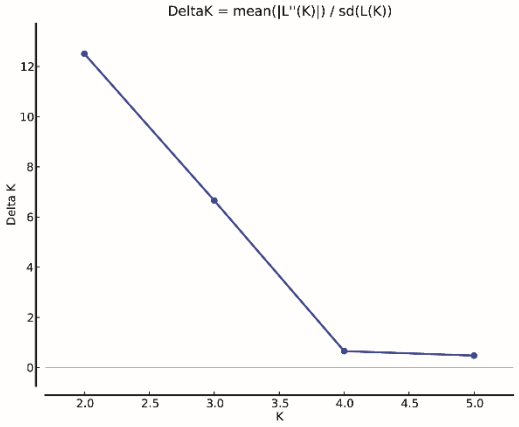

A
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
K
B
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
K
C
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
K

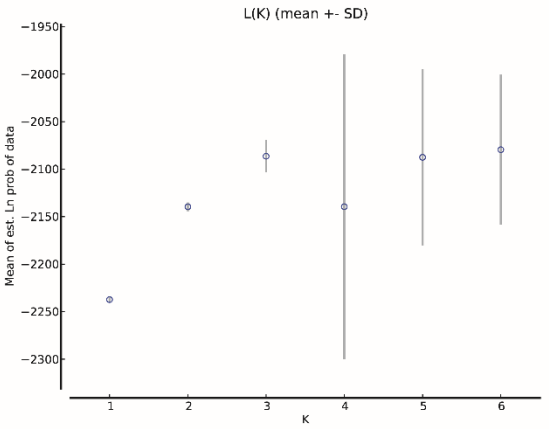
D


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

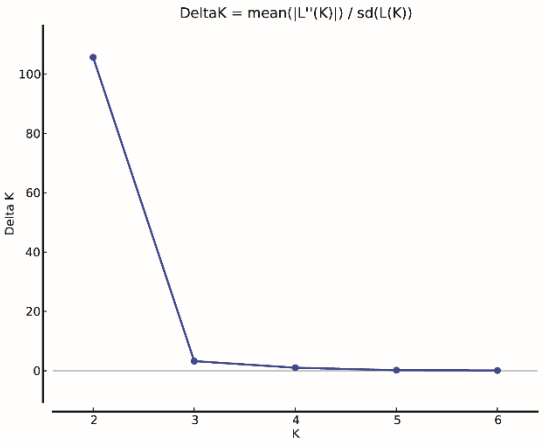

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

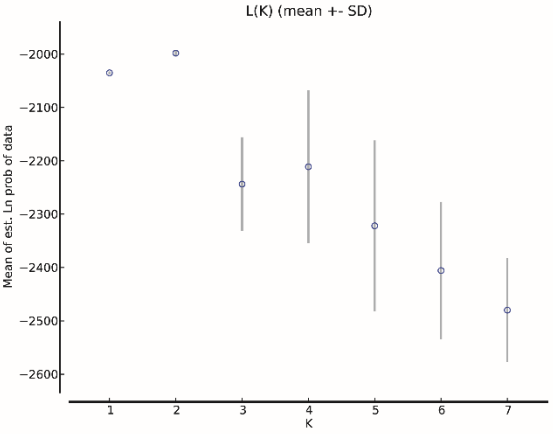
E


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

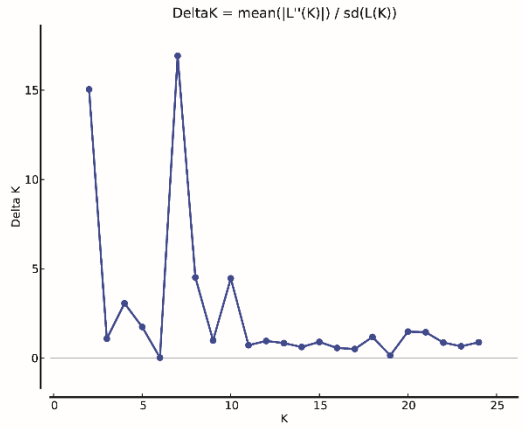

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

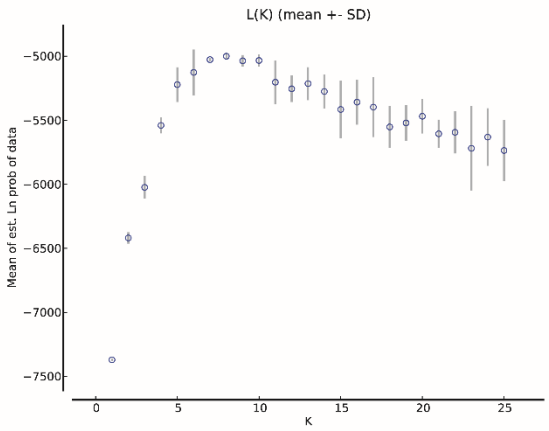
F


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

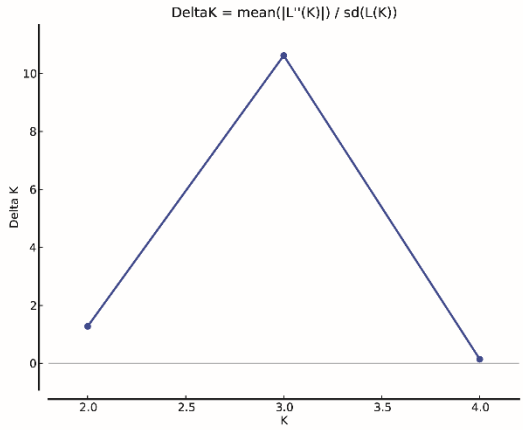

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

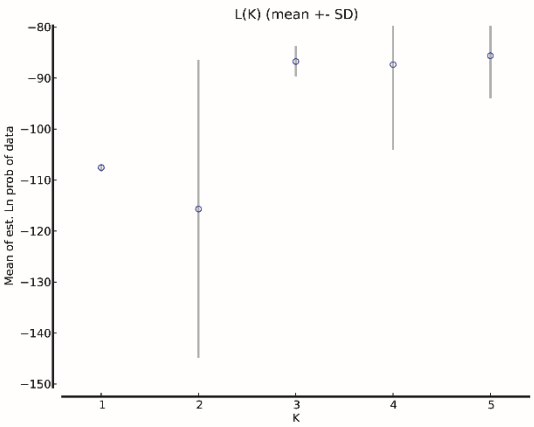
G


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

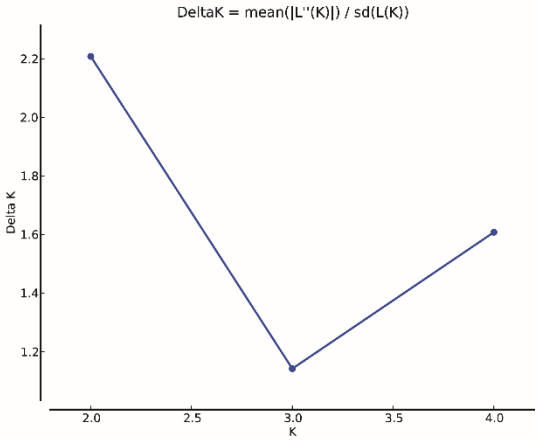

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

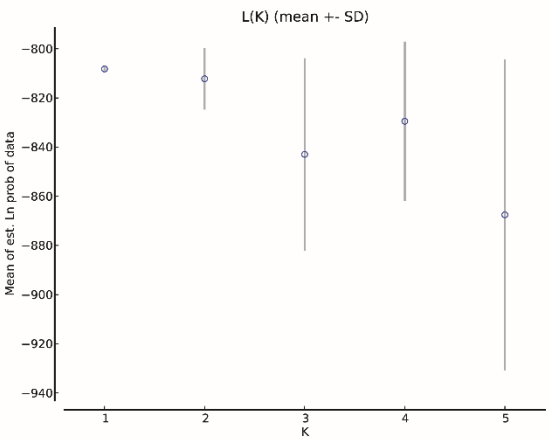
H


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

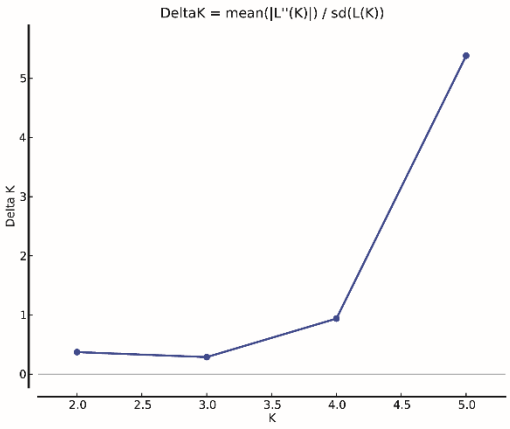

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

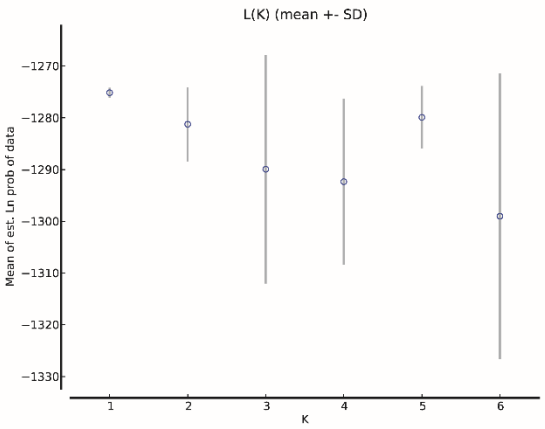
I


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

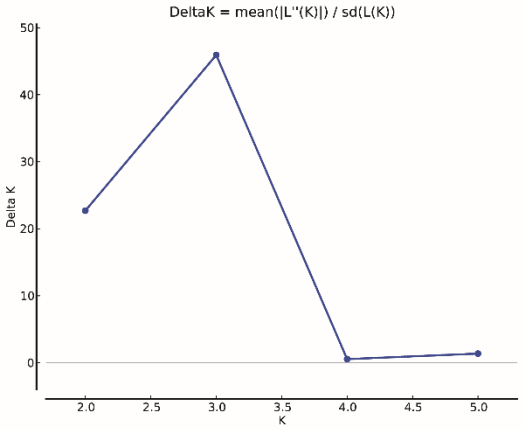

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

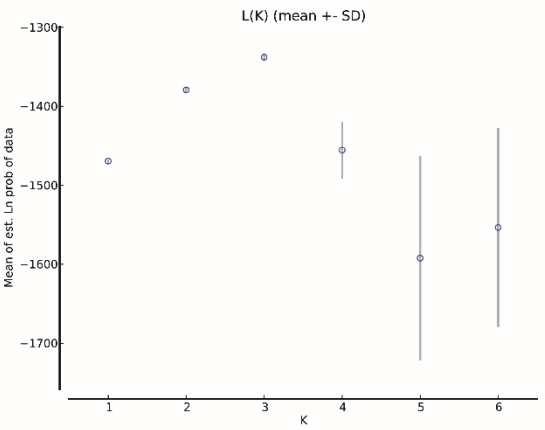
J


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

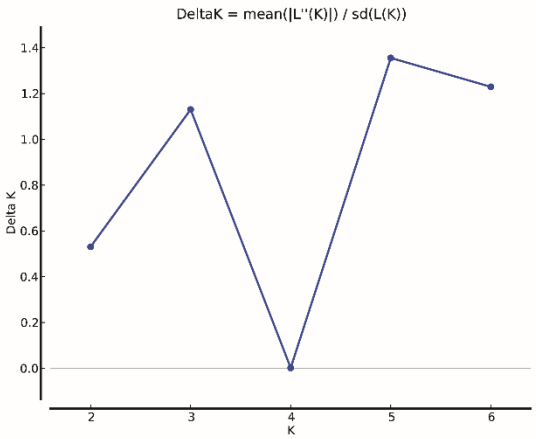

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

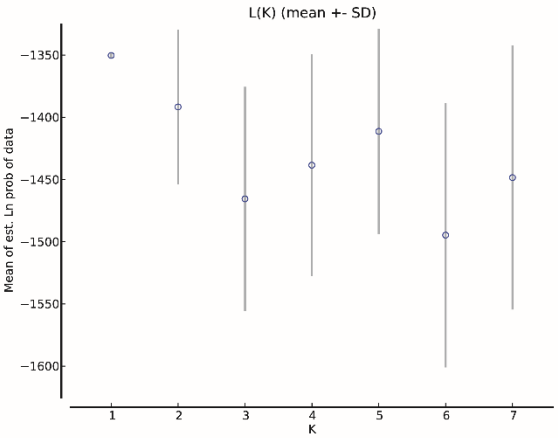
K


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

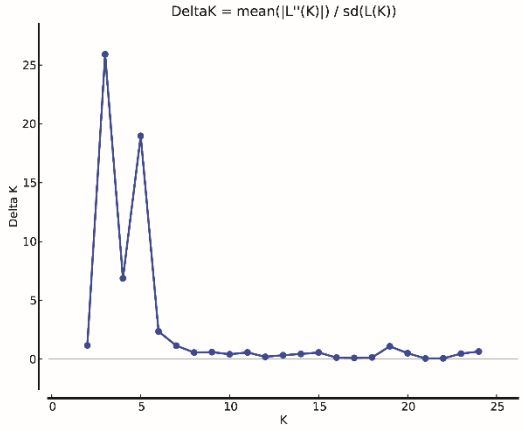

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

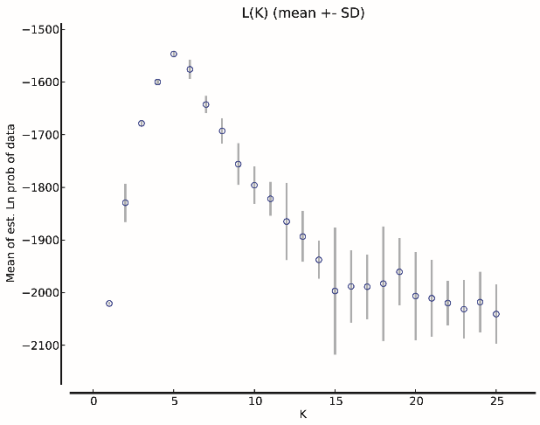
L


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

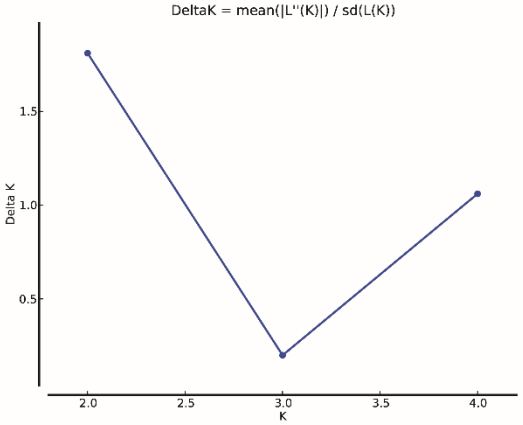

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

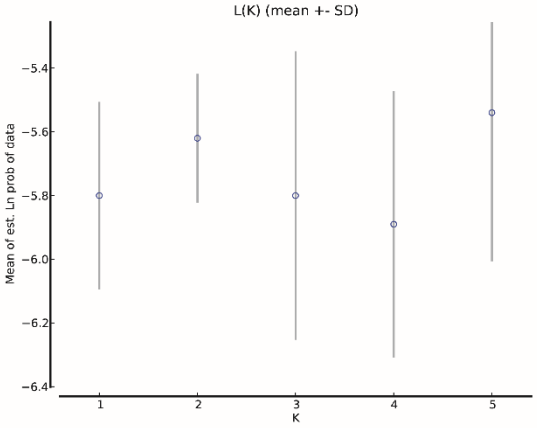
M


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

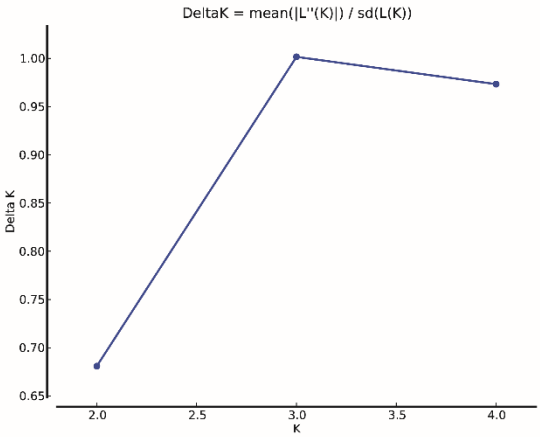

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

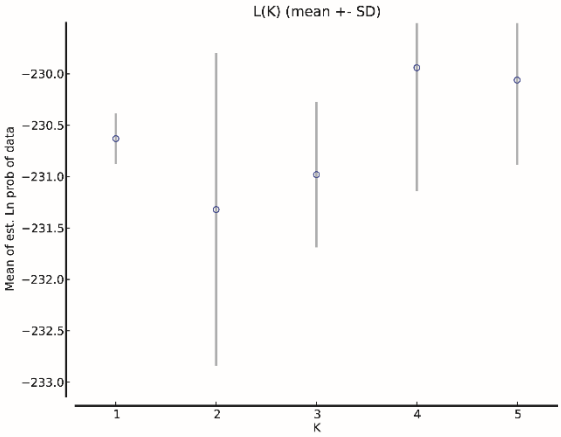
N


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

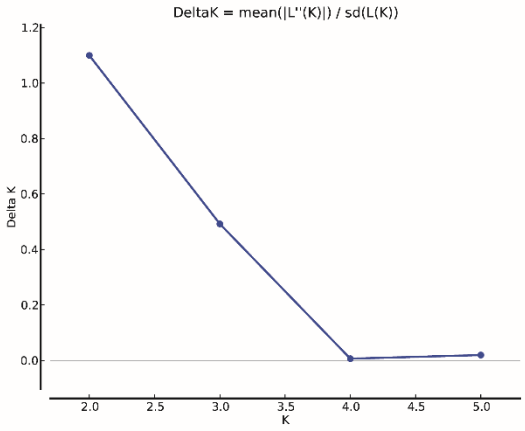

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

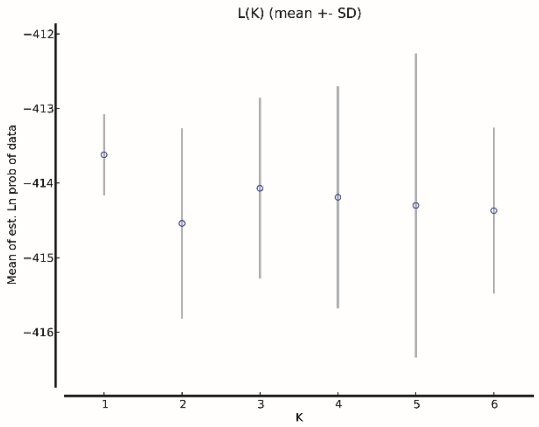
O


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

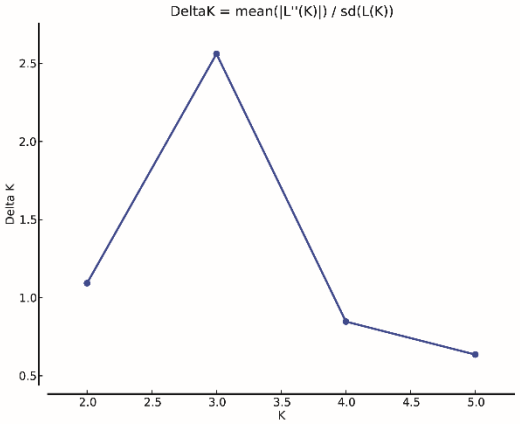

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

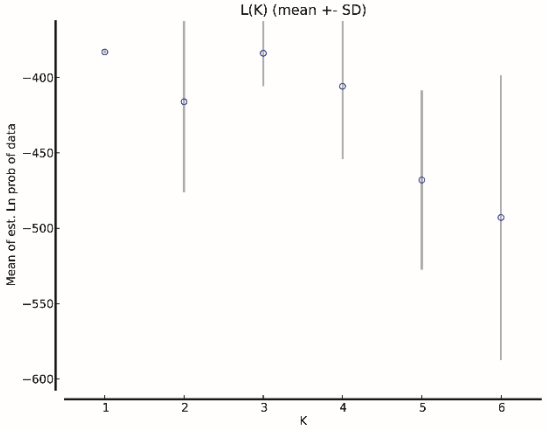
Ⓟ


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

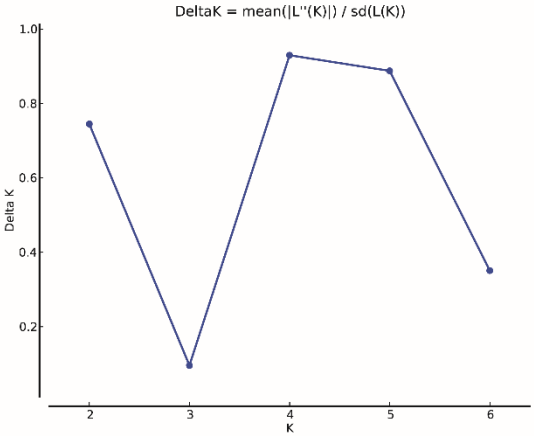

L(K) (mean +- SD)
Mean of est. Ln prob of data
K

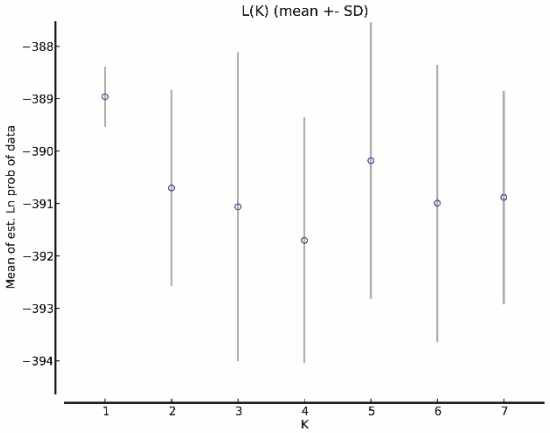
Ⓠ


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

Ⓡ


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
K

Appendix 6.3 DAPC analyses on *Magnolia dodecapetala* from the Lesser Antilles. **Top figure**: DAPC analysis on dataset D(15). Number of PCAs retained: 40. Number of DA eigenvalues: 4. **Bottom figure**: DAPC analysis on dataset D(7). Number of PCAs retained: 30. Number of DA eigenvalues: 4.

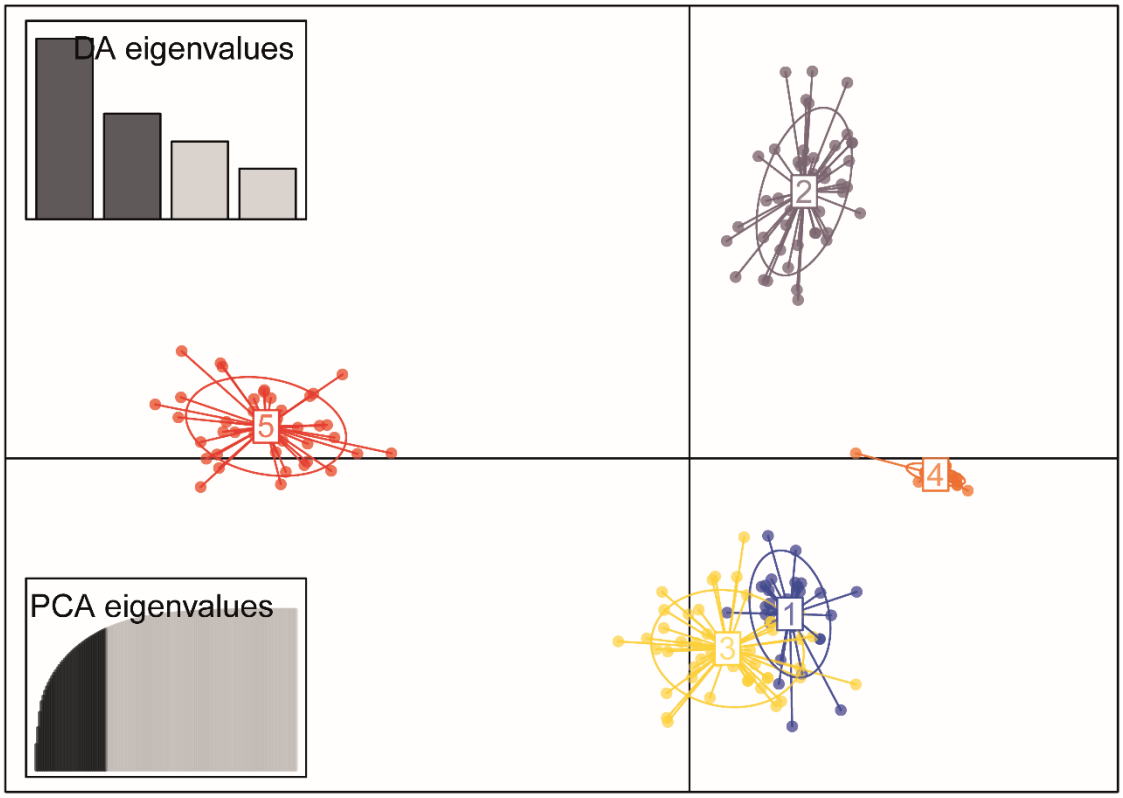
Populations found by the find.clusters function for D(15):

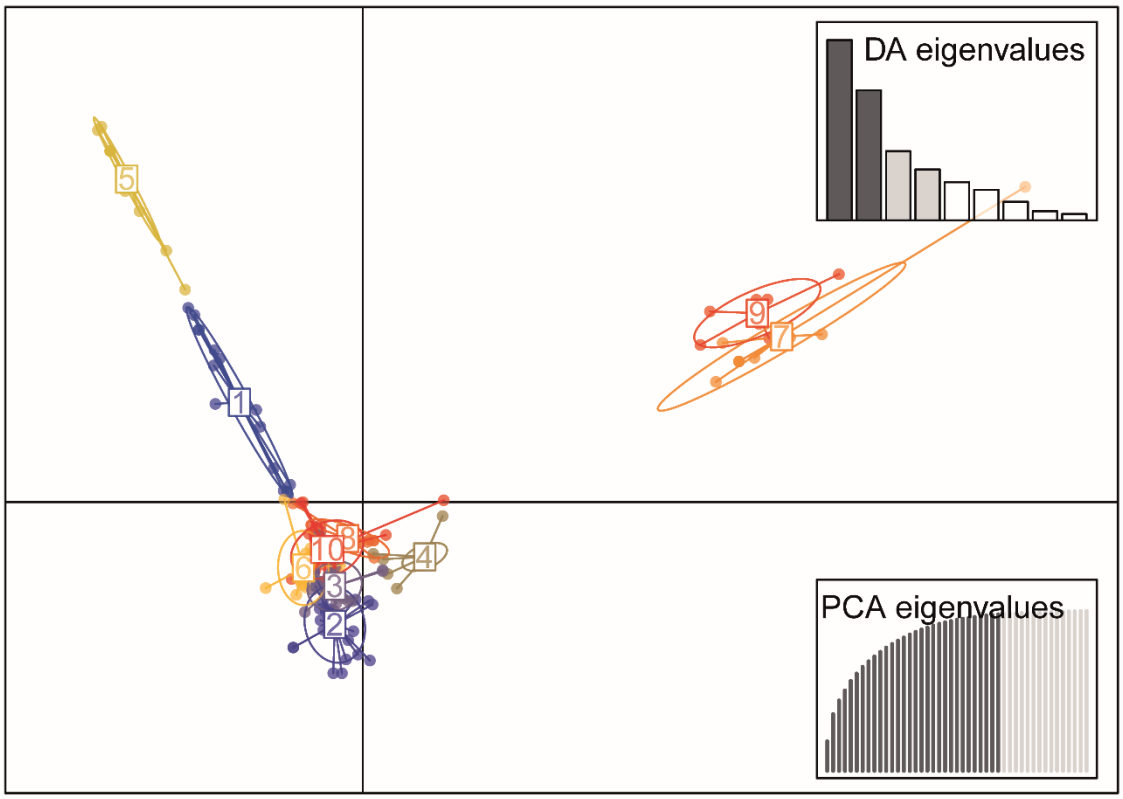
	1	2	3	4	5
MA1130	0	0	28	0	0
MA1344	0	29	0	0	0
MA1191	0	0	1	0	48
MA1244	48	0	0	0	0
MA1289	0	0	0	41	0

Populations found by the find.clusters function for D(7):

	1	2	3	4	5	6	7	8	9	10
MA1130	0	0	0	28	0	0	0	0	0	0
MA1344	0	0	0	3	0	0	11	5	7	3
MA1191	0	26	0	2	0	0	0	5	0	16
MA1244	17	0	0	1	16	0	0	1	0	13
MA1289	0	0	24	0	0	12	0	0	0	5

MA1130: SV: Saint Vincent
MA1344: SL: Saint Lucia
MA1191: M: Martinique
MA1244: D: Dominica
MA1289: G: Guadeloupe

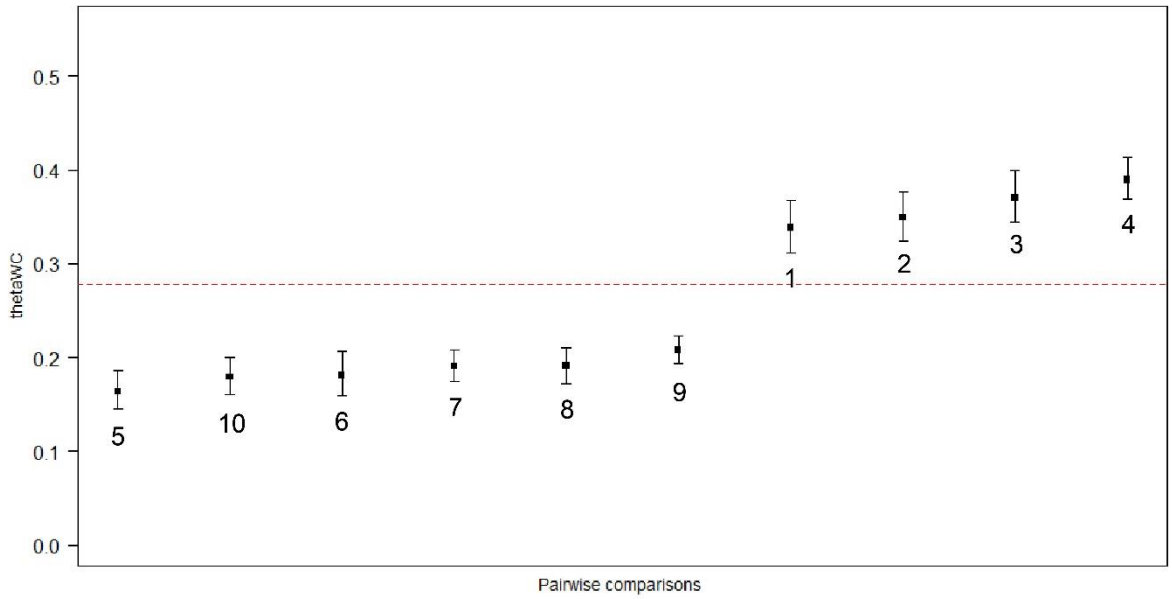

DA eigenvalues
2
5
4
PCA eigenvalues
1
3


DA eigenvalues
5
9
7
1
10
8
6
4
3
2
PCA eigenvalues

Appendix 6.4 F_{ST} values (Weir and Cockerham, 1984), G_{ST} (Nei, 1973; Nei and Chesser, 1983) and D_{JOST} (Jost, 2008) calculated for the *Magnolia dodecapetala* populations of the Lesser Antilles. **SV**: Saint Vincent. **SL**: Saint Lucia. **M**: Martinique. **D**: Dominica. **G**: Guadeloupe. **D(19)**: dataset comprising all 19 SSR markers. **D(15)**: dataset comprising 15 SSR markers. **D(7):** dataset comprising 7 SSR markers. **CI** = Confidence Interval. **BC** = Bias Corrected.

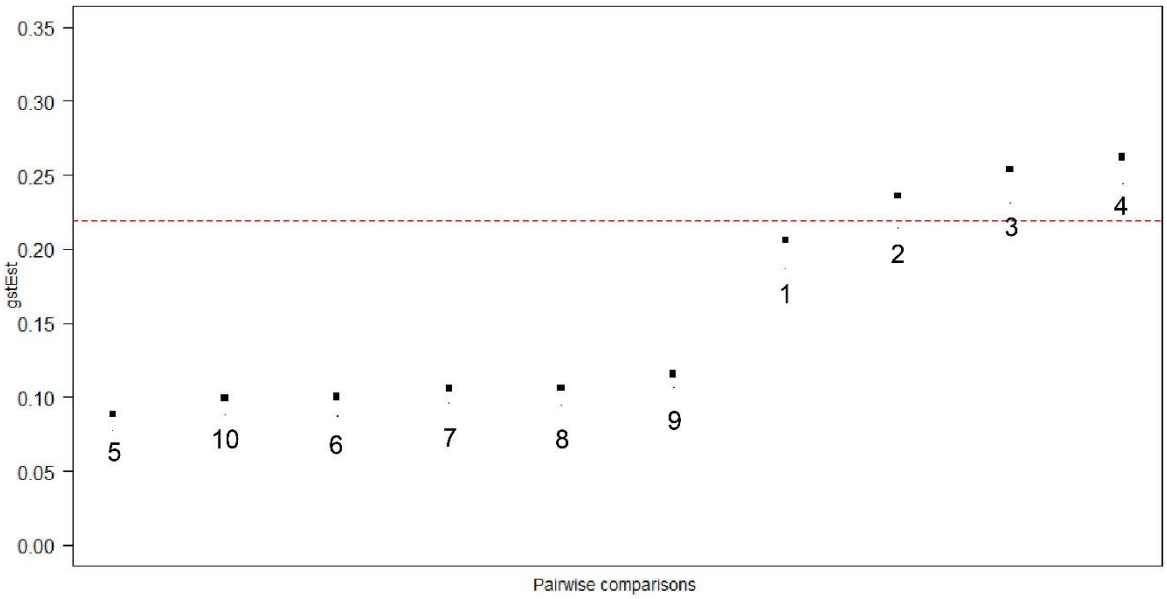
Appendix 6.4.A D(19) dataset, F_{ST} values (Weir and Cockerham, 1984).

thetaWC							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.3381	0.3518	0.3381	0.3247	0.3818	0.3110	0.3681
SV vs. M (2)	0.3492	0.3577	0.3492	0.3332	0.3852	0.3247	0.3768
SV vs. D (3)	0.3702	0.3792	0.3702	0.3535	0.4079	0.3444	0.3989
SV vs. G (4)	0.3895	0.3989	0.3895	0.3784	0.4224	0.3689	0.4129
SL vs. M (5)	0.1635	0.1755	0.1635	0.1569	0.1978	0.1449	0.1858
SL vs. D (6)	0.1810	0.1934	0.1810	0.1716	0.2193	0.1592	0.2069
SL vs. G (7)	0.1903	0.2030	0.1903	0.1871	0.2210	0.1744	0.2083
M vs. D (8)	0.1910	0.2003	0.1910	0.1818	0.2200	0.1725	0.2107
M vs. G (9)	0.2077	0.2171	0.2077	0.2031	0.2321	0.1936	0.2226
D vs. G (10)	0.1794	0.1898	0.1794	0.1707	0.2106	0.1603	0.2001



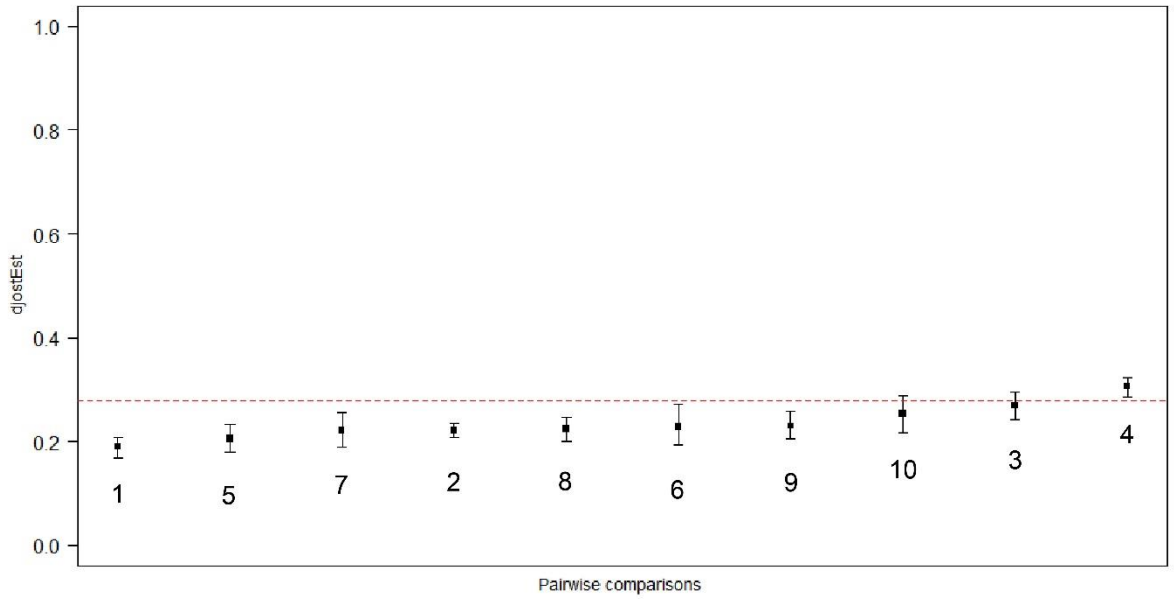
Appendix 6.4.B D(19) dataset, G_{ST} (Nei, 1973; Nei and Chesser, 1983).

gstEst							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.2061	0.2161	0.2061	0.1966	0.2386	0.1866	0.2286
SV vs. M (2)	0.2359	0.2429	0.2359	0.2213	0.2667	0.2142	0.2597
SV vs. D (3)	0.2535	0.2611	0.2535	0.2387	0.2868	0.2311	0.2792
SV vs. G (4)	0.2623	0.2702	0.2623	0.2523	0.2913	0.2444	0.2834
SL vs. M (5)	0.0884	0.0955	0.0884	0.0844	0.1093	0.0773	0.1021
SL vs. D (6)	0.1004	0.1080	0.1004	0.0946	0.1246	0.0870	0.1170
SL vs. G (7)	0.1059	0.1137	0.1059	0.1039	0.1251	0.0962	0.1173
M vs. D (8)	0.1062	0.1119	0.1062	0.1006	0.1241	0.0949	0.1184
M vs. G (9)	0.1157	0.1216	0.1157	0.1129	0.1308	0.1070	0.1249
D vs. G (10)	0.0993	0.1056	0.0993	0.0941	0.1182	0.0878	0.1119



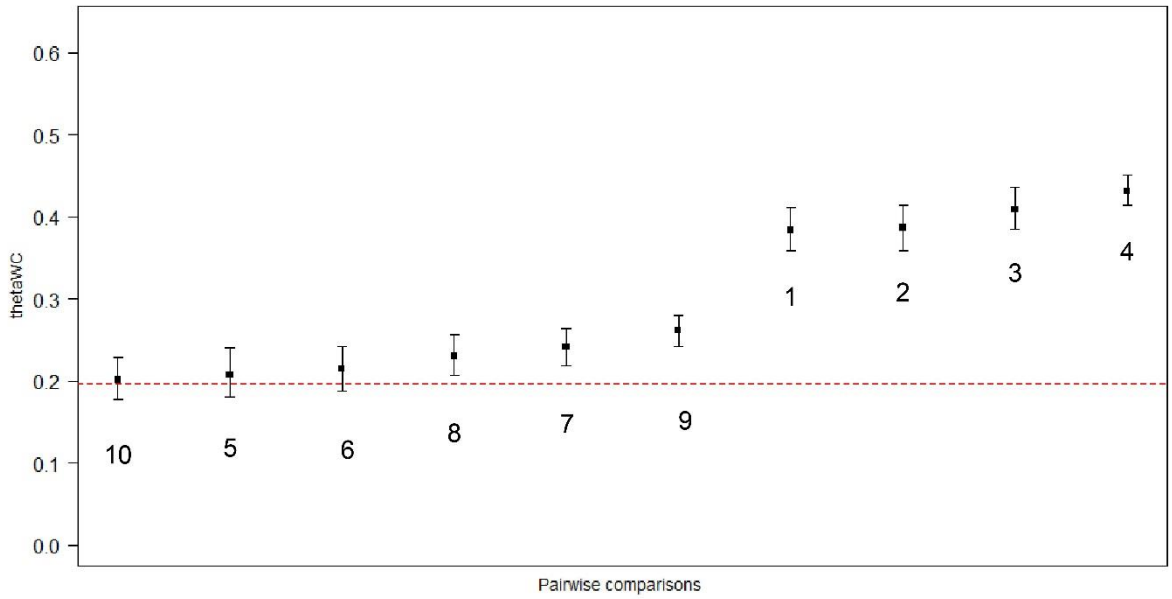
Appendix 6.4.C D(19) dataset, D_{JOST} (Jost, 2008).

djostEst							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.1893	0.193	0.1893	0.1727	0.2114	0.1690	0.2077
SV vs. M (2)	0.2210	0.2217	0.2210	0.2089	0.2348	0.2082	0.2341
SV vs. D (3)	0.2683	0.2699	0.2683	0.2437	0.2972	0.2421	0.2956
SV vs. G (4)	0.3048	0.3063	0.3048	0.2870	0.3246	0.2855	0.3231
SL vs. M (5)	0.2051	0.2133	0.2051	0.1873	0.2409	0.1790	0.2327
SL vs. D (6)	0.2282	0.2392	0.2282	0.2039	0.2824	0.1929	0.2714
SL vs. G (7)	0.2207	0.2377	0.2207	0.2048	0.2730	0.1878	0.2561
M vs. D (8)	0.2232	0.2324	0.2232	0.2085	0.2567	0.1993	0.2475
M vs. G (9)	0.2297	0.2426	0.2297	0.2173	0.2705	0.2043	0.2576
D vs. G (10)	0.2534	0.2611	0.2534	0.2233	0.2961	0.2156	0.2884



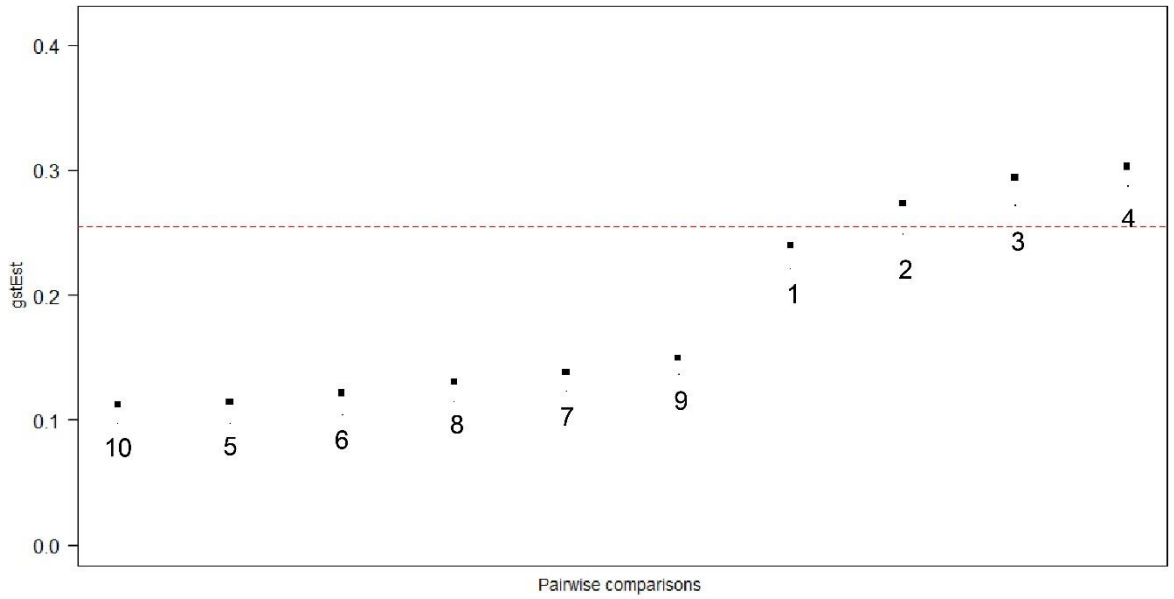
Appendix 6.4.D D(15) dataset, F_{ST} values (Weir and Cockerham, 1984).

thetaWC							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.3834	0.3946	0.3834	0.3702	0.4231	0.3591	0.4119
SV vs. M (2)	0.3866	0.3928	0.3866	0.3652	0.4202	0.359	0.414
SV vs. D (3)	0.4092	0.4172	0.4092	0.393	0.4438	0.385	0.4359
SV vs. G (4)	0.4312	0.439	0.4312	0.4217	0.4585	0.4138	0.4506
SL vs. M (5)	0.2081	0.2185	0.2081	0.1912	0.2503	0.1808	0.2399
SL vs. D (6)	0.2147	0.2262	0.2147	0.1996	0.254	0.1881	0.2425
SL vs. G (7)	0.241	0.2522	0.241	0.23	0.2757	0.2189	0.2645
M vs. D (8)	0.2303	0.2391	0.2303	0.215	0.2654	0.2062	0.2566
M vs. G (9)	0.2618	0.2704	0.2618	0.2513	0.289	0.2427	0.2804
D vs. G (10)	0.2009	0.2119	0.2009	0.1885	0.2392	0.1775	0.2282



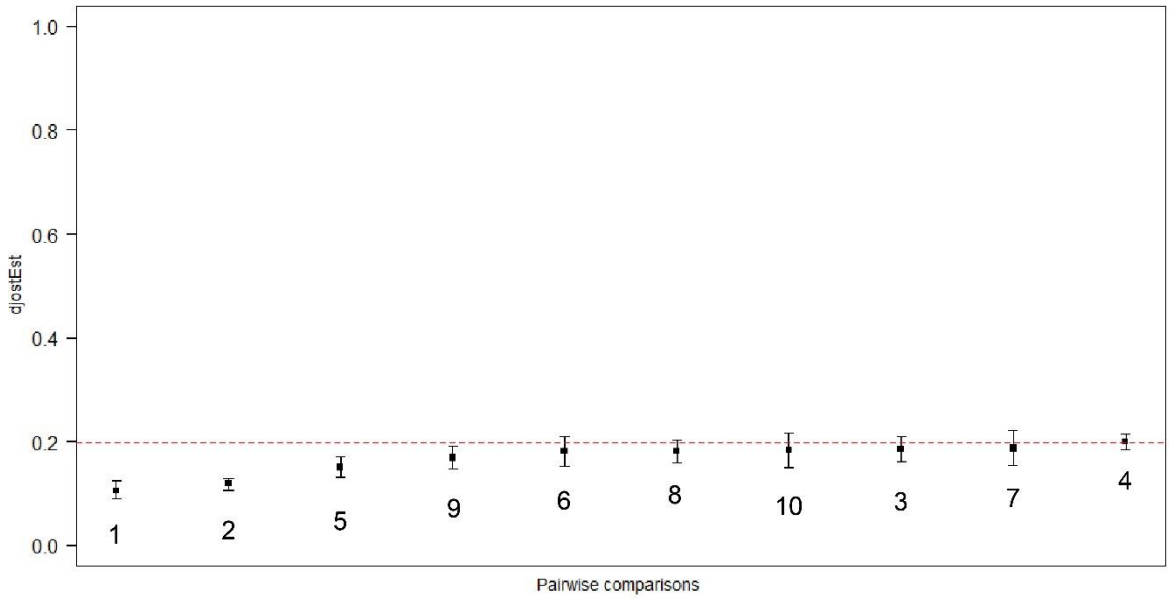
Appendix 6.4.E D(15) dataset, G_{ST} (Nei, 1973; Nei and Chesser, 1983).

gstEst							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.2398	0.2484	0.2398	0.2296	0.2708	0.221	0.2622
SV vs. M (2)	0.2734	0.2788	0.2734	0.2549	0.3027	0.2495	0.2973
SV vs. D (3)	0.294	0.301	0.294	0.2791	0.3252	0.2721	0.3182
SV vs. G (4)	0.3028	0.3097	0.3028	0.2942	0.3276	0.2873	0.3207
SL vs. M (5)	0.1143	0.1208	0.1143	0.1038	0.141	0.0973	0.1345
SL vs. D (6)	0.1216	0.1289	0.1216	0.112	0.1471	0.1046	0.1397
SL vs. G (7)	0.1381	0.1454	0.1381	0.131	0.1613	0.1237	0.154
M vs. D (8)	0.1305	0.1362	0.1305	0.1208	0.1533	0.1152	0.1477
M vs. G (9)	0.1496	0.1553	0.1496	0.1428	0.1678	0.1371	0.1622
D vs. G (10)	0.1123	0.1192	0.1123	0.1048	0.1362	0.098	0.1293



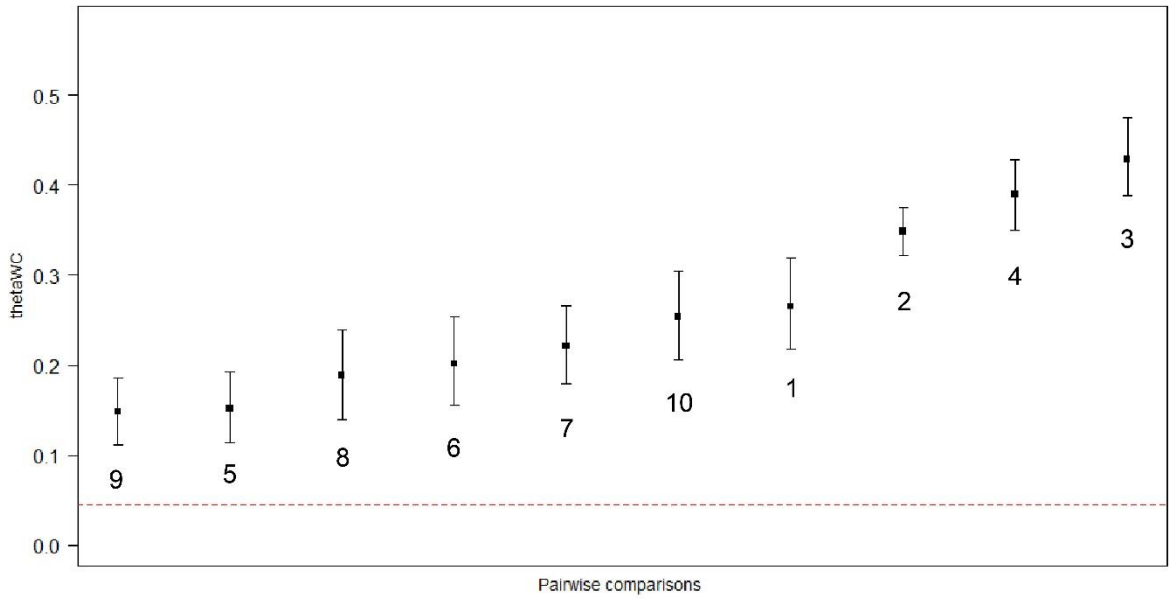
Appendix 6.4.F D(15) dataset, D_{JOST} (Jost, 2008).

djostEst							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.1047	0.1082	0.1047	0.0921	0.1279	0.0886	0.1244
SV vs. M (2)	0.118	0.1185	0.118	0.1068	0.1292	0.1063	0.1287
SV vs. D (3)	0.186	0.1877	0.186	0.163	0.2119	0.1613	0.2103
SV vs. G (4)	0.1989	0.2004	0.1989	0.1866	0.2164	0.1851	0.2149
SL vs. M (5)	0.1494	0.1521	0.1494	0.1339	0.1725	0.1313	0.1698
SL vs. D (6)	0.1804	0.186	0.1804	0.1574	0.2162	0.1518	0.2105
SL vs. G (7)	0.1863	0.1955	0.1863	0.1627	0.2309	0.1535	0.2217
M vs. D (8)	0.1814	0.1845	0.1814	0.1618	0.2063	0.1587	0.2033
M vs. G (9)	0.168	0.1781	0.168	0.1563	0.2013	0.1462	0.1912
D vs. G (10)	0.1834	0.1903	0.1834	0.1555	0.2244	0.1486	0.2174



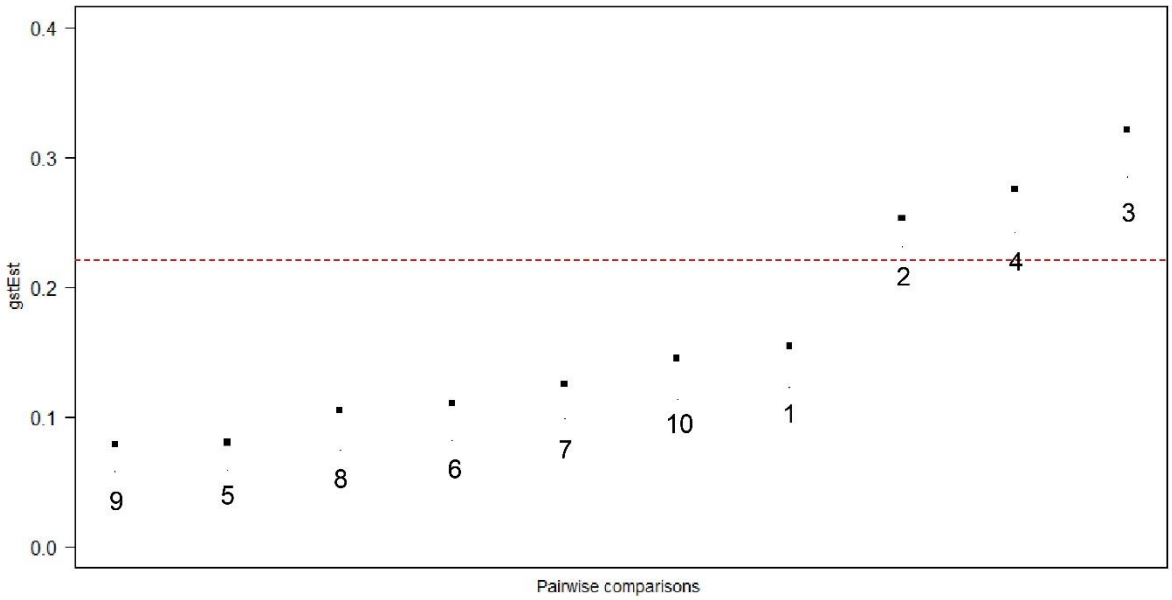
Appendix 6.4.G D(7) dataset, F_{ST} values (Weir and Cockerham, 1984).

thetaWC							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.2656	0.2741	0.2656	0.2269	0.3272	0.2183	0.3186
SV vs. M (2)	0.3482	0.3541	0.3482	0.3282	0.3814	0.3224	0.3756
SV vs. D (3)	0.4285	0.4348	0.4285	0.3942	0.4813	0.3879	0.4749
SV vs. G (4)	0.3896	0.3967	0.3896	0.3563	0.4353	0.3493	0.4282
SL vs. M (5)	0.1514	0.1619	0.1514	0.1248	0.2026	0.1143	0.1921
SL vs. D (6)	0.2014	0.2131	0.2014	0.1672	0.2649	0.1555	0.2533
SL vs. G (7)	0.221	0.2315	0.221	0.1896	0.2762	0.179	0.2656
M vs. D (8)	0.1886	0.1971	0.1886	0.1478	0.2478	0.1393	0.2393
M vs. G (9)	0.1478	0.1576	0.1478	0.121	0.1963	0.1112	0.1865
D vs. G (10)	0.2539	0.2634	0.2539	0.215	0.3144	0.2055	0.305



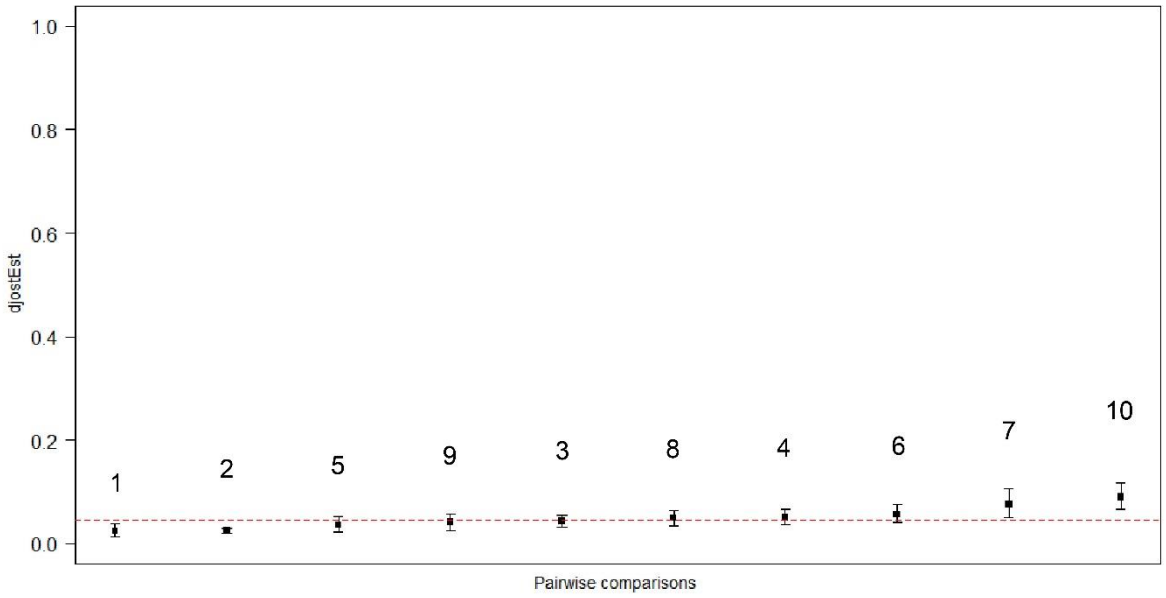
Appendix 6.4.H D(7) dataset, G_{ST} (Nei, 1973; Nei and Chesser, 1983).

gstEst							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.1545	0.1604	0.1545	0.1288	0.1969	0.1229	0.191
SV vs. M (2)	0.2529	0.2577	0.2529	0.2362	0.2818	0.2313	0.2769
SV vs. D (3)	0.3217	0.3275	0.3217	0.2904	0.3692	0.2846	0.3634
SV vs. G (4)	0.2753	0.2813	0.2753	0.2482	0.3152	0.2421	0.3091
SL vs. M (5)	0.0803	0.0865	0.0803	0.0657	0.1101	0.0595	0.1039
SL vs. D (6)	0.1106	0.1179	0.1106	0.0898	0.1503	0.0824	0.143
SL vs. G (7)	0.1254	0.1323	0.1254	0.1061	0.1612	0.0992	0.1543
M vs. D (8)	0.1047	0.1101	0.1047	0.0803	0.1421	0.0749	0.1367
M vs. G (9)	0.0792	0.085	0.0792	0.0642	0.1078	0.0584	0.102
D vs. G (10)	0.1452	0.1515	0.1452	0.1204	0.1858	0.114	0.1794

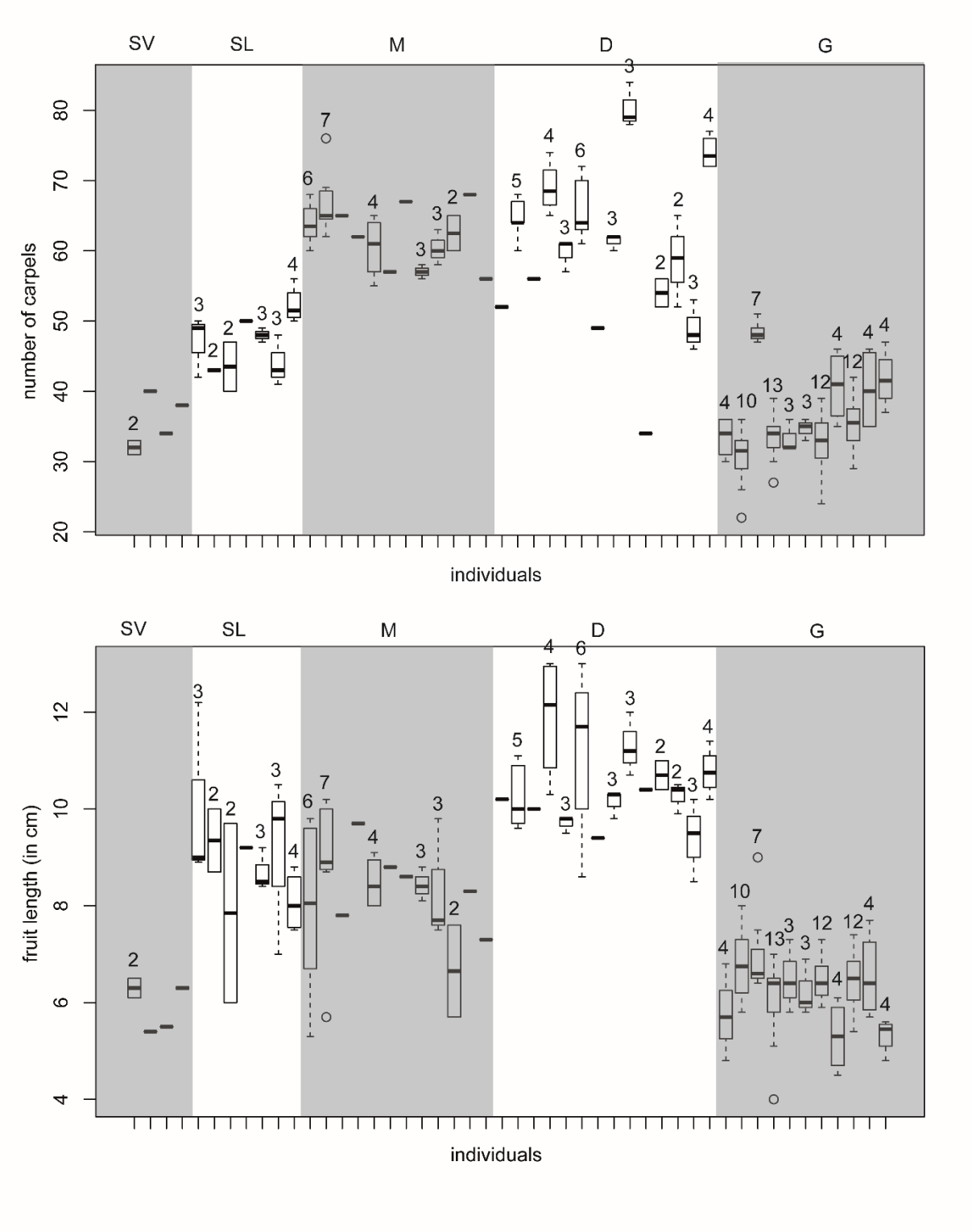


Appendix 6.4.I D(7) dataset, D_{JOST} (Jost, 2008).

djostEst							
comparison	actual	mean	BC_mean	Lower_95%CI	Upper_95%CI	BC_Lower_95%CI	BC_Upper_95%CI
SV vs. SL (1)	0.0237	0.0248	0.0237	0.0141	0.0395	0.0131	0.0384
SV vs. M (2)	0.0244	0.0248	0.0244	0.0202	0.0307	0.0197	0.0303
SV vs. D (3)	0.0429	0.0431	0.0429	0.0322	0.054	0.032	0.0537
SV vs. G (4)	0.05	0.051	0.05	0.0373	0.0666	0.0363	0.0656
SL vs. M (5)	0.0358	0.038	0.0358	0.0256	0.0542	0.0234	0.052
SL vs. D (6)	0.0566	0.0583	0.0566	0.0417	0.0772	0.0401	0.0755
SL vs. G (7)	0.0756	0.0781	0.0756	0.0534	0.1083	0.0509	0.1058
M vs. D (8)	0.0485	0.0495	0.0485	0.0355	0.0646	0.0344	0.0635
M vs. G (9)	0.0407	0.0445	0.0407	0.0282	0.0615	0.0244	0.0576
D vs. G (10)	0.0894	0.0903	0.0894	0.0664	0.117	0.0655	0.1162



Appendix 6.5 Individual morphological variation for the number of carpels (top) and fruit length (bottom) in *Magnolia dodecapetala* from the Lesser Antilles. **SV**: Saint Vincent population. **SL**: Saint Lucia population. **M**: Martinique population. **D**: Dominica population. **G**: Guadeloupe population. Each entry on the x-axis represents one individual. Barplots indicate the variation found in one individual, whereby the number above the barplot indicates the number of fruits available for that individual.



Appendix X: Curriculum vitae of Emily Veltjen

PERSONALIA

Last name:	VELTJEN
First name:	EMILY
Nationality:	Belgian
Birthplace and -date:	Ghent, 05 June 1991
Address:	Machelenstraat 101, 9800 Deinze, Belgium
E-mail:	emily.veltjen@ugent.be; emily_veltjen@hotmail.com
Telephone:	+32498542190

WORK EXPERIENCE

2018–today: **Research assistant and lab technician, Ghent Botanical Garden, Ghent University**
Project: Magnolias of the Caribbean and Mesoamerica: tracing the evolutionary and biogeographic history of the Caribbean and Mesoamerican Magnolia species; Applying conservation genetic studies on a selection of these species to inform and undertake specific conservation actions.
Responsibilities: lab management, financial management, student supervision, planning and executing botanical expeditions for data collection, executing phylogenetic and conservation genetic analyses, writing scientific manuscripts, (co-)writing of (sub)projects.

HIGHER EDUCATION

2014–2020: **PhD candidate, Systematic and Evolutionary Botany Lab (formerly Research Group Spermatophytes), Department of Biology, Ghent University**
Project: The Caribbean Magnolia species (Magnoliaceae): assessment of the genetic diversity and the underlying evolutionary history.
BOF PhD fellowship grant 01D24114
Courses taken at the Doctoral School:
2020 Fostering responsible conduct of research
2020 Introduction to the HPC of Ghent University
2018 UCT Spanish 2
2018 UGent Advanced Academic English: Writing Skills
2017 UGent Module 1: Werken met PMGE systeem
2017 Kew Tropical Plant Identification Course
2015 EMBO Practical course on Computational Evolution
2015 BSBB Course tree climbing 1
2015 UCT Intensive Spanish for beginners - Ahora
2014 UGent Conservation genetics
2014 UGent Biodiversity patterns in space and time

2012–2014: **MSc in Biology, Ghent University (*magna cum laude*).**
Minor: Research
Majors: Biodiversity and evolution
Thesis: The evolution of a male dimorphism in a dwarf spider: unravelling the genomic basis and the importance of female choice.
+ Award of Francine Ronsse (UGent, 2014).
+ Kets Award (Zoo Antwerp, 2014).

2009–2012: **BSc in Biology, Ghent University (*magna cum laude*).** Thesis: Experimental research on the reproduction of the cryptic species of the *Litoditis marina* species complex.

PUBLICATIONS in SCI listed journals

Veltjen E., Asselman P., Goetghebeur P., Samain M.-S., Larridon I. (submitted 2020) An integrative approach to understanding the diversity of *Magnolia dodecapetala* (Magnoliaceae: *Talauma* subsect. *Talauma*) in the Lesser Antilles. Frontiers in Plant Science. Impact factor 2018: 4.106.

Veltjen E., Testé E., Palmarola Bejerano A., Asselman P., Hernández Rodríguez M., González Torres L. R., Chatrou L.W., Goetghebeur P., Larridon I., Samain M.-S. (submitted 2019) The evolutionary history of the Caribbean Magnolias (Magnoliaceae): testing species delimitations and biogeographical hypotheses using molecular data. Molecular Phylogenetics and Evolution. Impact factor 2018: 3.992.

Hernández M., Palmarola A., **Veltjen E.**, Asselman P., Testé, E., Larridon I., Samain M.-S., González-Torres L. R. (2020) Population structure and genetic diversity of *Magnolia cubensis* subsp. *acunae* (Magnoliaceae): Effects of habitat fragmentation and implications for conservation. Oryx: 1–9. https://doi.org/10.1017/S003060531900053X. Impact factor 2018: 2.801.

Veltjen E., Asselman P., Hernández Rodríguez M., Testé Lozano E., Palmarola Bejerano A., González Torres L.R., Goetghebeur P., Larridon I. & Samain M.S. (2019) Genetic patterns in Neotropical Magnolias (Magnoliaceae) using *de novo* developed microsatellite markers. Heredity 122: 485–500. https://doi.org/10.1038/s41437-018-0151-5. Impact factor 2018: 3.179.

Larridon I., **Veltjen E.**, Semmouri, I., Asselman P., Guerrero P.C., Duarte M., Walter H.E., Cisternas M.A. & Samain M.S. (2018) Investigating taxon boundaries and extinction risk in endemic Chilean cacti (*Copiapoa* subsection *Cinerei*, Cactaceae) using chloroplast DNA sequences, microsatellite data and 3D mapping. Kew Bulletin 73: 55. https://doi.org/10.1007/s12225-018-9780-3. Impact Factor 2018: 0.680.

Veltjen E., Browning J., Goetghebeur P., Larridon I. (2015) *Bulbostylis albidostricta* (Abildgaardieae, Cyperaceae): a new sedge species from Angola. Phytotaxa 201(3): 221–226. http://dx.doi.org/10.11646/phytotaxa.201.3.6. Impact Factor 2015: 1.087.

PUBLICATIONS, other

Veltjen E. (2018) The Magnolias of the Caribbean: adventures in Hispaniola. Magnolia: The Journal of the Magnolia Society International 53(103): 8–12.

Veltjen E. (2015) Magnolia's van de Caraïben. De Vrienden van de Plantentuin Gent 34(4): 195–211.

SYMPOSIA

Veltjen E., Vázquez García, A., Palmarola Bejerano A., Serna González, M., Asselman P., Hernández Rodríguez M., Testé Lozano, E., González Torres L.R., Neill, D., Goetghebeur P., Kim, S., Figlar, R., I., Samain M.S., Larridon (2018) Uniting morphological, taxonomical and field expertise with sequence data: phylogenomcs of Neotropical magnolias (Magnoliaceae). XII Congreso Latinoamericano de Botánica, 21-28 Octubre 2018, Quito, Ecuador (poster presentation).

Veltjen E., Palmarola Bejerano A., Asselman P., Larridon, I., Claeys, K., Leroux, O., Hernández Rodríguez M., Testé Lozano, E., González Torres L.R., Goetghebeur P., Samain M.S., (2018) The genetic diversity of the Caribbean Magnolias. XII Congreso Latinoamericano de Botánica, 21-28 Octubre 2018, Quito, Ecuador. Botánica en Latinoamérica Realidad y Desarrollo virtual: Memorias. p.99 (oral presentation).

Veltjen E., Larridon I., Samain M.S., Dugardin C., Goetghebeur P., Torres Santana C. (2017) Genetically targeted *ex situ* collections and *in situ* reintroductions. 6GBGC: 6th Global Botanic Gardens Congress, Book of Abstracts. p. 136 (oral presentation).

Veltjen E., Palmarola Bejerano A., Asselman P., Hernández Rodríguez M., González Torres L.R., Larridon I., Samain M.S., Goetghebeur P. (2016) Conserving the *Magnolia* diversity of the Caribbean: progress and prospects. Family Magnoliaceae, 3rd International Symposium, Abstracts. p. 20 (oral presentation).

Samain M.S., González Torres L.R., Martínez Salas E.M., Oldfield S., **Veltjen E.**, Asselman P., Larridon I., Goetghebeur P. (2016) Towards conservation of Magnolia section *Talauma* in the Caribbean and Mesoamerica. Family Magnoliaceae, 3rd International Symposium, Abstracts. p. 21 (oral presentation).

Veltjen E., Asselman P., Larridon I., Samain M.S., Goetghebeur P. (2016) First steps towards conservation of the Caribbean Magnoliaceae. AMPEE3: 3rd annual meeting on plant ecology and evolution. p. 16-17 (poster presentation).

Veltjen E., Hendrickx F. (2014) The evolution of a male dimorphism in a dwarf spider: unravelling the genomic basis and the importance of female choice. Zoology symposium 2014. (oral presentation: invited speaker Kets Award 2014).

BOTANICAL EXPEDITIONS

COLOMBIA	29th of June until 20th of July 2019 (22 days)
ECUADOR	8th of October until 28th of October 2018 (21 days)
PUERTO RICO	10th of August until 10th of October 2016 (62 days)
LESSER ANTILLES	15th of June until 15th of July 2016 (31 days)
PUERTO RICO	12th of May until 20th of May 2015 (9 days)
DOMINICAN REPUBLIC	28th of April until 11th of May 2015 (14 days)
HAITI	14th of April until 27th of April 2015 (14 days)

WORKSHOPS

2018	Title: “Conservation genetics and phylogenetic analysis of Magnoliaceae: Workshop III”. Location: Quito, Ecuador. Participant institutes: INECOL, Havana National Botanical Garden, Ghent University. Role: attendance.
2017	Title: “Conservation genetics and phylogenetic analysis of Magnoliaceae: Workshop II”. Location: Ghent, Belgium. Participant institutes: INECOL, Havana National Botanical Garden, Ghent University. Role: co-hosting the workshop: practical organization.
2015	Title: “Conservation genetics and phylogenetic analysis of Magnoliaceae: Workshop I” Location: Pátzcuaro, Mexico. Participant institutes: INECOL, Havana National Botanical Garden, Ghent University. Role: organizer of the sessions “phylogeny” and “conservation genetics” + co-organizer for the laboratory sessions.

STUDENT SUPERVISION

2020-2021	Claerhout Tim (Ghent University, Belgium), Master thesis: “Conservation of Magnolias from the Dominican Republic”.

2019-2020 Aldaba Nuñez Fabian (Instituto de Ecología, A.C., México), Master thesis: "Sistemática, diversidad genética y conservación de *Magnolia* en Veracruz, México". International visitor from 16/11/2019 until 31/01/2020.

2018-2019 Hernández Rodríguez Majela (University of Havana, Cuba), PhD on the Caribbean *Cubenses* Magnolia. International visitor from 15/11/2018 until 15/01/2019.

Testé Lozano Ernesto (Botanical Garden of Havana, Cuba), PhD on the Caribbean *Talauma* Magnolias. International visitor from 15/11/2018 until 15/02/2019.

2017-2018 van Kleinwee Catharina (Ghent University, Belgium), Master thesis: "In the name of *Sansevieria* (Asparagaceae) – An integrative study on identification and classification of the *Sansevieria* diversity"

2016-2017 Claeys Koen (Ghent University, Belgium), Bachelor thesis: "Plantengenomen onder de loep: het bepalen van de ploïdiegraad met flowcytometrie en chromosoomtellingen"

Habraken Joos (Ghent University, Belgium), Integrated Biodiversity Research Project: "The columnar cactus genus Eulychnia from the Atacama Desert"

Merkx Ruud (Ghent University, Belgium), Master thesis: "Contributing to the conservation of some Mexican Magnolias: an SSR marker analysis"

van Kleinwee Catharina (Ghent University, Belgium), Integrated Biodiversity Research Project: "Phylogenetic Relationships within the genus *Sansevieria*"

Verschaete Severine (Ghent University, Belgium), Bachelor thesis: "Mystery plants, DNA barcoding in de Plantentuin Universiteit Gent"

LANGUAGES

Dutch: Native speaker.

English: Professional level: during the MSc in Biology and most of the third year of the BSc in Biology, courses, lectures and student seminars were all in English.

French: Basic knowledge from secondary school.

Spanish: Basic knowledge from two beginner courses at the UCT (Ghent University Language Centre) and usage during fieldwork.

REFERENCES

Prof. Dr. Marie-Stéphanie Samain
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Visiting professor: Ghent University, Belgium.

Prof. Dr. Isabel Larridon
B.A. Krukoff Curator of African Botany, Identification and Naming department (Africa & Madagascar), Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK. i.larridon@kew.org
Visiting professor: Ghent University, Belgium.

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