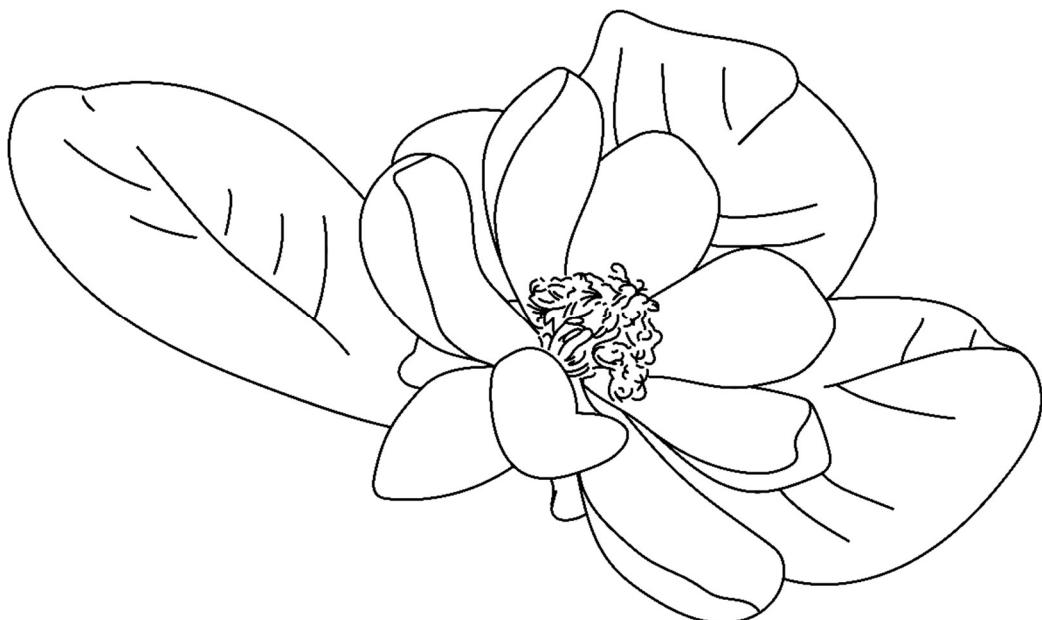


# An integrative study of species distribution modelling and conservation genetics: *Magnolia* in Hispaniola



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## Objectives and Research Hypotheses

In this master's dissertation, we aim to deliver insights regarding the distribution and genetic health (i.e. the amalgamation between genetic diversity, gene flow and geographic extent of a species and/or population) of four endangered *Magnolia* species (Magnoliaceae) from the island of Hispaniola. We strive to formulate concrete suggestions for each species' future conservation efforts. Using molecular data, we investigate their genetic diversity by employing conservation genetic analyses. Furthermore, using species distribution modelling, a popular modelling framework in conservation biology, we predict the species' potential distribution and habitat suitability across Hispaniola. Seven null hypotheses (**H<sub>x</sub>**) across three objectives were tested:

- 1) To predict suitable *Magnolia* habitat for conservation efforts and to guide explorations for new populations.  
**H<sub>1</sub>**: Suitable *Magnolia* habitat is only present in the mountain ranges in which they reside.  
**H<sub>2</sub>**: IUCN Red List Status is correlated with the amount of suitable *Magnolia* habitat in the vicinity.
- 2) To assess the population structure to infer conservation units.  
**H<sub>3</sub>**: Genetic structuring of the Magnolias of the Dominican Republic follows morphological species delineation as described by Howard (1948).  
**H<sub>4</sub>**: Each sampled locality represents one genetic cluster, as found for the majority of *Magnolia* species in Veltjen et al. (2019).
- 3) To quantify genetic diversity, enabling us to evaluate the genetic health of the populations.  
**H<sub>5</sub>**: Given the small population sizes, their endemic and (Critically) Endangered status, the *Magnolia* populations of the Dominican Republic have inbreeding coefficients that significantly differ from zero.  
**H<sub>6</sub>**: Genetic diversity is correlated with the IUCN Red List Status.  
**H<sub>7</sub>**: Genetic diversity is correlated with the amount of suitable *Magnolia* habitat in the vicinity.

## Executive Summaries (EN/NL/ES)

### Summary

On the island of Hispaniola, five endemic species of *Magnolia* occur, all of which are threatened with extinction: *Magnolia domingensis*, *M. ekmanii*, *M. emarginata*, *M. hamorii* and *M. pallescens*. The most prevalent threats include habitat fragmentation, illegal logging, land conversion for agriculture and livestock use, forest fires and little natural regeneration. Current knowledge regarding the distribution and genetic health (i.e. the amalgamation between genetic diversity, gene flow and geographic extent of a species and/or population) of the Magnolias of Hispaniola is extremely scarce, hampering targeted conservation actions. To define conservation units and their respective threats, information on the distribution of the focal species is essential. Species distribution modelling (SDM) is a popular modelling framework that allows us to predict the Magnolias' potential distribution and quantify its habitat suitability in Hispaniola. Furthermore, genetic information on the focal species enables conservation practitioners to answer key questions regarding their management. One preliminary study already reported high genetic structuring, yet little inbreeding for four of the five *Magnolia* species in Hispaniola. The objective of this study is to deliver data on the potential distribution and the genetic health of the Magnolias of Hispaniola to state concrete guidelines for effective conservation management. More specifically, we predicted suitable *Magnolia* habitat for conservation efforts and to guide explorations for new populations using SDM. Furthermore, applying conservation genetics, we assessed the genetic structure and quantified genetic diversity parameters. This allowed us to make statements about the genetic health of the populations. By integrating these results, we inferred conservation units and proposed conservation recommendations that strive to ensure a sustainable future for the Magnolias of Hispaniola. Firstly, using SDM, we analysed 21 variables describing climate and landscape features and fitted eight modelling algorithms in an ensemble framework to predict the potential distribution for the Magnolias of Hispaniola. These predictions were summarized in three final distribution maps. The distribution of potential habitats was restricted to the various mountain ranges on Hispaniola. Secondly, 16 microsatellite markers were employed to test the genetic structure and degree of inbreeding for 417 individuals across three of the five species: *M. domingensis*, *M. hamorii* and *M. pallescens*. Diversity statistics were calculated for each of those three species' populations and localities. Analyses for genetic structure showed strong species integrity and moderate genetic differentiation between population pairs of *M. domingensis* and *M. pallescens*. Consequently, five populations across three species were defined. For *M. pallescens*, significant inbreeding was retrieved in the population Ébano Verde; and the localities "Entrance" and "Montellano." The population Loma Rodríguez (*M. domingensis*) had the lowest genetic diversity. Combining all results, we designated each species as one conservation unit. *Magnolia domingensis* is highlighted as it contains the lowest genetic diversity, the least number of (known) individuals and the lowest number of sampling localities. We conclude that the three genetically investigated Magnolias consist of five populations in total, with pronounced genetic structuring found only in *M. pallescens*. Ample genetic diversity is present with minimal inbreeding. Much potential *Magnolia* habitat in Hispaniola is unexplored, which might contain yet undiscovered *Magnolia* individuals. For conservation management, we propose concrete species-specific actions in terms of exploration, protection and reinforcement. The main recommendation includes protection and/or instatement of habitat corridors between populations of *M. domingensis* and *M. ekmanii*, whereby the SDM results will serve as a guideline for spatial prioritization.

## Samenvatting

Op het eiland Hispaniola komen vijf endemische soorten *Magnolia* voor, die allen met uitsterven bedreigd zijn: *Magnolia domingensis*, *M. ekmanii*, *M. emarginata*, *M. hamorii* and *M. pallescens*. De belangrijkste bedreigingen zijn habitat fragmentatie, illegale houtkap, land conversie in functie van landbouw en veeteelt, bosbrand en een tekort aan natuurlijke verjonging. De huidige kennis omtrent de verspreiding en genetische toestand van de Magnolias van Hispaniola is zeer beperkt, wat gerichte conservatie praktijken belemmert. Om conservatie eenheden en hun respectievelijke bedreigingen te bepalen is informatie over de verspreiding van de doelsoorten essentieel. "Species distribution modelling" (SDM) is een populaire modelleertechniek die ons toelaat om de potentiële verspreiding en de geschiktheid van het habitat van de *Magnolia* soorten in Hispaniola te voorspellen. Bovendien laat de genetische informatie toe om essentiële vragen omtrent het beheer van de doelsoorten te beantwoorden. Eén preliminaire studie rapporteerde reeds een uitgesproken genetische structuur, maar weinig intelt voor vier van de vijf *Magnolia* soorten in Hispaniola. Deze studie doelt op het leveren van data over de potentiële verspreiding en de genetische toestand van de Magnolias van Hispaniola. Zo kunnen we concrete richtlijnen opstellen voor toekomstige, doelgerichte beheersmaatregelen. Specifiek hebben we geschikt *Magnolia* habitat voorspeld voor toekomstige conservatie praktijken en expedities die zullen zoeken naar nieuwe populaties. Bovendien, met behulp van conservatie genetica, hebben we de genetische structuur beoordeeld en parameters van genetische diversiteit berekend. Dit liet ons toe om uitspraken te doen over de genetische toestand van de populaties. Door deze resultaten te integreren, hebben we conservatie eenheden bepaald en aanbevelingen gedaan inzake conservatie beheer dat streeft naar een duurzame toekomst voor de Magnolias van Hispaniola. Ten eerste, met behulp van SDM analyseerden we 21 variabelen met betrekking tot klimaat en landschapseigenschappen om er vervolgens acht modellen op toe te passen en zo de potentiële verspreiding van de Magnolias van Hispaniola te voorspellen. Deze voorspellingen werden samengevat in drie finale verspreidingskaarten. De verspreiding van het geschikte habitat was beperkt tot de diverse bergketens op Hispaniola. Ten tweede, met behulp van 16 microsatelliet merkers, testten we de genetische structuur en de mate van intelt voor 417 individuen over drie van de vijf soorten: *M. domingensis*, *M. hamorii* and *M. pallescens*. Diversiteitsindices werden berekend voor elke populatie en locatie van de drie soorten. Analyses met betrekking tot de genetische structuur toonden een sterke soortintegriteit en middelmatige genetische differentiatie tussen populatie paren van *M. domingensis* en *M. pallescens*. Daarbij werden vijf populaties verspreid over drie soorten vastgesteld. Voor *M. pallescens* werd significant intelt gevonden voor de populatie Ébano Verde en de locaties "Entrance" en "Montellano." De populatie Loma Rodríguez (*M. domingensis*) vertoonde de laagste genetische diversiteit. Door alle resultaten te integreren werd elke soort als één conservatie eenheid aangewezen. *Magnolia domingensis* werd aangewezen als het meest kwetsbaar aangezien het de laagste genetische diversiteit vertoont en het laagste aantal (gekende) individuen en locaties heeft. We concluderen dat de drie *Magnolia* soorten die genetisch bestudeerd zijn in totaal uit vijf populaties bestaan, waarvan enkel *M. pallescens* een uitgesproken genetische structuur vertoont. Verder is nog genoeg genetische diversiteit aanwezig en is intelt slechts minimaal aanwezig. Veel potentieel geschikt *Magnolia* habitat in Hispaniola is nog steeds niet geëxplooreerd. Hier zouden nog onontdekte *Magnolia* individuen aanwezig kunnen zijn. Voor conservatie beheer stellen we soortspecifieke maatregelen voor op het gebied van exploratie, bescherming en versterking. De voornaamste aanbevelingen omvatten het beschermen en/of oprichten van habitat corridors tussen populaties van *M. domingensis* en *M. ekmanii*, waarbij de SDM-resultaten kunnen dienen als een richtlijn voor het stellen van ruimtelijke prioriteiten.

## Resumen

Se encuentran cinco especies endémicas de *Magnolia* en la isla La Española, todas en peligro de extinción: *Magnolia domingensis*, *M. ekmanii*, *M. emarginata*, *M. hamorii* y *M. pallescens*. Las amenazas más frecuentes incluyen fragmentación del hábitat, tala ilegal, conversión de tierras para uso agrícola y ganadero, incendios forestales y poca regeneración natural. El conocimiento actual sobre la distribución y la salud genética de las Magnolias de Hispaniola es extremadamente escaso, lo cual dificulta las acciones de conservación específicas. Para definir las unidades de conservación y sus respectivas amenazas, la información sobre la distribución de las especies focales es fundamental. El modelado de distribución de especies (MDE) es un marco de modelado popular que nos permite predecir la distribución potencial de las Magnolias y cuantificar la idoneidad de su hábitat en La Española. Además, la información genética sobre las especies focales permite a los profesionales de la conservación responder preguntas clave sobre su gestión. Un estudio preliminar ya informó una alta estructuración genética, pero poca endogamia para cuatro de las cinco especies de *Magnolia* en La Española. El objetivo de este estudio es presentar datos sobre la distribución potencial y la salud genética de las Magnolias de La Española para establecer pautas concretas para una gestión de conservación eficaz. Más específicamente, predijimos un hábitat de *Magnolia* adecuado para los esfuerzos de conservación y para guiar las exploraciones de nuevas poblaciones utilizando MDE. Además, aplicando la genética de la conservación, evaluamos la estructura genética y cuantificamos los parámetros de diversidad genética. Esto nos permitió presentar información sobre la salud genética de las poblaciones. Al integrar estos resultados, inferimos unidades de conservación y propusimos recomendaciones de conservación con el fin de asegurar un futuro sostenible para las Magnolias de La Española. En primer lugar, utilizando MDE, analizamos 21 variables que describen las características del clima y el paisaje y ajustamos ocho algoritmos de modelado en un marco de conjunto para predecir la distribución potencial de las Magnolias de La Española. Estas predicciones se resumieron en tres mapas de distribución finales. La distribución de hábitats potenciales se restringió a las diversas cadenas montañosas de La Española. En segundo lugar, se emplearon 16 marcadores de microsatélites para probar la estructura genética y el grado de endogamia de 417 individuos en tres de las cinco especies: *M. domingensis*, *M. hamorii* y *M. pallescens*. Se calcularon estadísticas de diversidad para cada una de las poblaciones y localidades de esas tres especies. Los análisis de la estructura genética mostraron una fuerte integridad de las especies y una diferenciación genética moderada entre los pares de poblaciones de *M. domingensis* y *M. pallescens*. En consecuencia, se definieron cinco poblaciones de tres especies. Para *M. pallescens*, se obtuvo una endogamia significativa en la población Ébano Verde; y las localidades "Entrance" y "Montellano". La población Loma Rodríguez (*M. domingensis*) tuvo la menor diversidad genética. Combinando todos los resultados, designamos a cada especie como una unidad de conservación. *Magnolia domingensis* se destaca porque contiene la menor diversidad genética, el menor número de individuos (conocidos) y el menor número de localidades de muestreo. Concluimos que las tres Magnolias investigadas genéticamente consisten en cinco poblaciones en total, con una estructura genética pronunciada encontrada solo en *M. pallescens*. Existe una amplia diversidad genética con una mínima consanguinidad. Gran parte del hábitat potencial de *Magnolia* en La Española está inexplorado, lo cual podría contener individuos de *Magnolia* aún no descubiertos. Para la gestión de la conservación, proponemos acciones concretas para especies específicas en términos de exploración, protección y refuerzo. La recomendación principal incluye la protección y/o establecimiento de corredores de hábitat entre las poblaciones de *M. domingensis* y *M. ekmanii*, por lo que los resultados del MDE servirán como guía para la priorización espacial.

# An integrative study of species distribution modelling and conservation genetics: *Magnolia* in Hispaniola

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**Abstract** On the island of Hispaniola, five endemic species of *Magnolia* occur, all of which are threatened with extinction. Little is known about their distribution and genetic health, hampering targeted conservation actions. One preliminary study already reported high genetic structuring, but little inbreeding. The objective of this study is to deliver data on the potential distribution and the genetic health of the Magnolias of Hispaniola to state concrete guidelines for effective conservation management. Using species distribution modelling (SDM), we analyzed 21 variables describing cli-

mate and landscape features to predict the potential distribution of the Magnolias of Hispaniola. Furthermore, 16 microsatellite markers were employed to test the genetic structure and degree of inbreeding for 417 individuals across three of the five species. The distribution of potential habitats was restricted to the various mountain ranges on Hispaniola. Analyses for genetic structure showed strong species integrity and moderate genetic differentiation between population pairs among two species. We conclude that the Magnolias of Hispaniola consist of five populations with ample genetic

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diversity and only minimal inbreeding. Moreover, much potential habitat is unexplored, which might contain new *Magnolia* individuals. For conservation management, we propose concrete species-specific actions in terms of exploration, protection, and reinforcement. The main recommendation includes protection and/or instatement of habitat corridors between populations of *M. domingensis* and *M. ekmanii*, whereby these SDM results will serve as a guideline for spatial prioritization.

**Resumen** Se encuentran cinco Magnolias endémicas en la isla La Española, todas en peligro de extinción. Se sabe poco sobre su distribución y salud genética, dificultando las acciones de conservación específicas. Un estudio preliminar reportó una estructura genética alta y poca endogamia. El objetivo de este estudio es presentar datos sobre distribución potencial y salud genética de las Magnolias de La Española para establecer pautas concretas para gestión de conservación eficaz. Utilizando modelos de distribución de especies (MDE), analizamos 21 variables que describen las características del clima y el paisaje para predecir su distribución potencial. Además, se emplearon 16 marcadores de microsatélites para pro-

bar la estructura genética y el grado de endogamia de 417 individuos en tres de las cinco especies. La distribución de hábitats potenciales se restringió a las cadenas montañosas de La Española. Los análisis de estructura genética mostraron una fuerte integridad de las especies y una diferenciación genética moderada entre pares de poblaciones entre dos especies. Concluimos que las tres *Magnolias* genéticamente investigadas consisten en cinco poblaciones en total, con una amplia diversidad genética y una mínima consanguinidad. Además, gran parte del hábitat potencial está inexplicado, pudiendo contener individuos de *Magnolia* aún no descubiertos. Para la gestión de la conservación, proponemos acciones concretas para especies específicas en términos de exploración, protección y refuerzo. La recomendación principal incluye protección y/o establecimiento de corredores de hábitat entre las poblaciones de *M. domingensis* y *M. ekmanii*, por lo que estos resultados del MDE servirán como guía para la priorización espacial.

**Keywords** Species distribution modelling · conservation genetics · Magnoliaceae · Hispaniola · microsatellites · genetic diversity

## Introduction

Tropical montane cloud forests (henceforth TMCFs) are a rare, but extremely biodiverse habitat. Although TMCFs only comprises 0.26% of the Earth's surface, they are home to a disproportionate amount of species (Bubb et al. 2004). This habitat is characterized by heavy rainfall, high species endemism and low resilience to disturbance (Hamilton et al. 1995). Despite its importance as a vital source of water and its high concentrations of biodiversity, TMCFs face similar threats as other tropical habitats: habitat fragmentation, climate change, illegal logging, etc. (Bubb et al. 2004). Since the 1993 international TMCF symposium (Hamilton et al. 1995), many challenges still need to be overcome to guarantee sustainable conservation of TMCFs.

One island that comprises this rare habitat is Hispaniola (Fig. 1), the second largest island in the Caribbean sea. It is divided in two nations, Haiti and the Dominican Republic (henceforth DR). While the former is infamous for being one of the most deforested countries on earth, with less than 1% of its original primary forests remaining (Hedges et al. 2018), the latter likewise suffers from forest loss and degradation (Sangermano et al. 2015a). Nonetheless, Hispaniola remains a biodiversity hotspot due to its high species endemism, housing about 6000 endemic plant species (Maunder et al. 2008; Fajardo



**Fig. 1.** Tropical montane cloud forest, the typical habitat of *Magnolia* in the Caribbean. Photograph: Emily Veltjen.

et al. 2016; Cano-Ortiz et al. 2016). Five of these are endemic and threatened *Magnolia* species, *M. emarginata*, *M. ekmanii*, *M. domingensis*, *M. pallescens* and *M. hamorii* (Castillo 2016), consisting of only a few populations each (Fig. 2). Consequently, the *Magnolias* of Hispaniola are in great risk of extinction with one of the most prevalent factors being habitat loss due to timber exploitation, land conversion and other destructive activities (Castillo et al. 2018; Veltjen et al. 2019). As these species reside in TMCFs, their survival is intricately linked to the preservation of this habitat. Moreover, *Magnolia* is an eye-catching genus due to their beautiful flowers, aromatic leaves and ornamental use, which gives it great potential as an umbrella species for conservation (Roberge and Angelstam 2004). Hence, conservation of these species warrants the conservation of their habitat.

To employ effective conservation strategies, several sources of information are essential: threats, taxonomy, conservation units, the species biology, geographical extent, and the underlying genetic diversity (Kramer and Havens 2009; IUCN 2017). Current knowledge regarding the distribution and genetic diversity of the *Magnolias* of Hispaniola is extremely scarce (Cires et al. 2013; Castillo et al. 2018). We aim to resolve this issue by integrating two research fields, species distribution modelling (SDM) and conservation genetics, in forthcoming

conservation strategies. This integration allows us to identify and set priorities for management of these species, to improve future decision making and to ensure a sustainable future as such.

A first problem arises when considering the geographic extent of the species. This information is only scarcely available for the Magnolias of Hispaniola (Castillo et al. 2018; Veltjen et al. 2019), which is exemplar of the Wallacean shortfall (i.e. the incomplete knowledge of species distributions) in tropical countries (Urbina-Cardona et al. 2019). However, to define conservation units and their threats, this information is essential. Species distribution modelling is a popular modelling framework which is increasingly used to address questions in conservation biology, ecology and evolution (Guisan and Thuiller 2005; Guillera-Arroita et al. 2015). It allows us to predict the (potential) species distribution and the habitat suitability in Hispaniola, based on species occurrence data and environmental information. These insights set the scene for future explorations to potentially find new populations and provides a guideline for spatial prioritisation of conservation actions.

Other than a species' geographical extent, its genetic information enables us to solve key questions regarding management of threatened species. Conservation genetics aims to reduce the risk of extinction in endangered species by quantifying and investigating their genetic diversity (Frankham et al. 2015). It strives to safeguard species as dynamic entities with sufficient evolutionary potential over the long term. A preliminary study of Veltjen et al. (2019) assessed the population structure and genetic health (i.e. the amalgamation between genetic diversity, gene flow and geographic extent of a species) for all Caribbean *Magnolia* species, including four out of the five species from Hispaniola. *Magnolia emarginata* was not assessed as no individuals of this Haitian species were found. The authors reported little inbreeding, yet high genetic structuring. As this study included only twenty individuals per population, which is only a fraction of the known number of populations and individuals of the *Magnolia* diversity in Hispaniola, we aim to study the genetic diversity of *M. pallescens*, *M. domingensis* and *M. hamorii* in depth. With this information, we can propose concrete conservation actions in terms of exploration, protection and reinforcement to minimize future inbreeding with the goal of maximizing the species' genetic diversity over the long term. By doing so, we strive to maintain healthy populations with enough evolvability to make them flexible and/or resistant to future disturbances.

Here, we aim to resolve urgent questions regarding the distribution and genetic health of the

Magnolias of Hispaniola with the intention to make concrete suggestions for each species' future conservation efforts. We specifically wish to (1) predict suitable *Magnolia* habitat for conservation efforts and to guide explorations for new populations using SDM. Furthermore, using conservation genetics, we will (2) assess the population structure to infer conservation units and (3) quantify genetic diversity, enabling us to make statements about the genetic health of the populations. As a result, the proposed conservation actions strive to save these species from extinction.

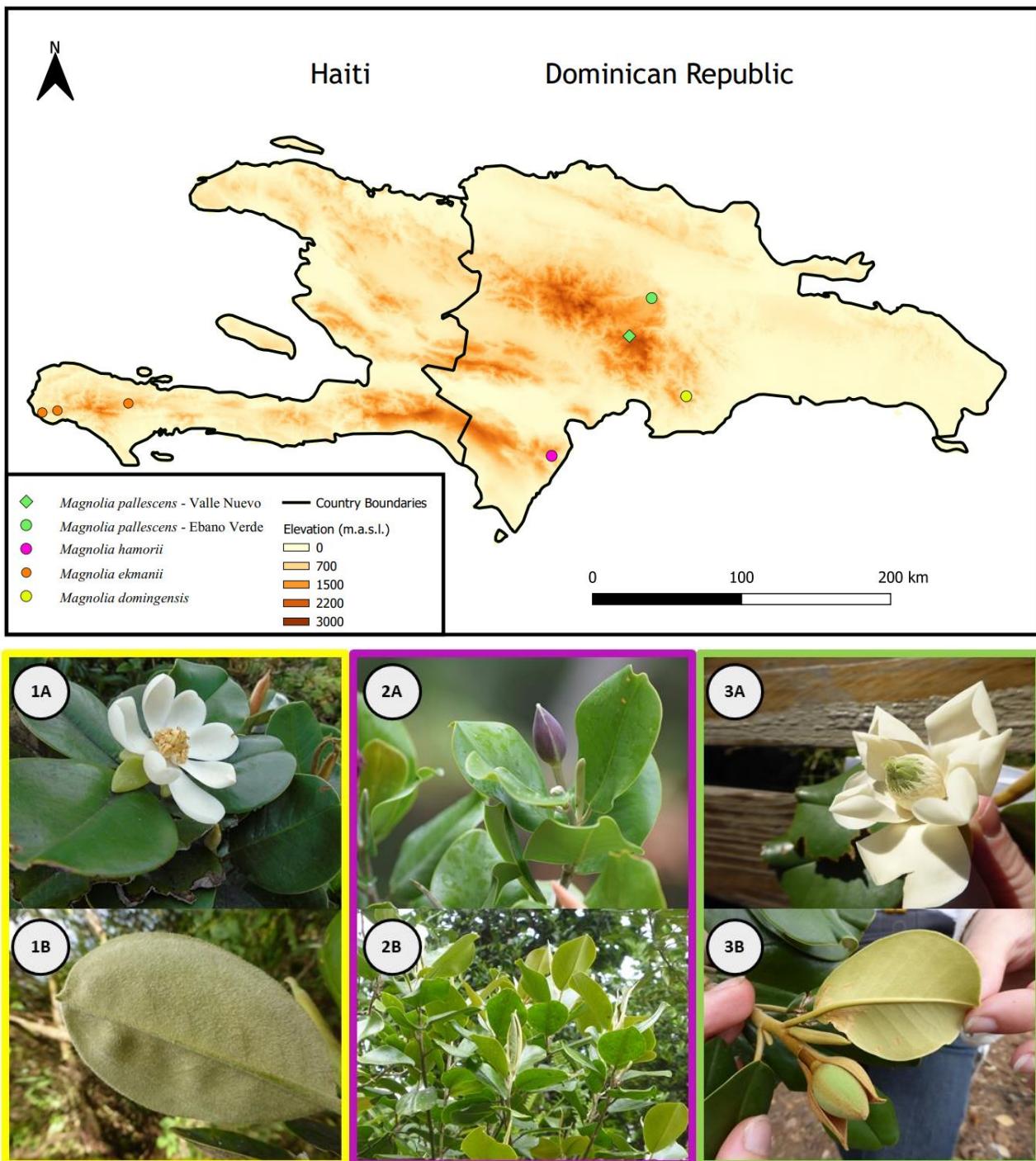
## Materials and Methods

### Species Distribution Modelling

SDMs are capable of generating predictions of species' potential distributions. It consists of two main steps: data preparation and model fitting.

Data preparation allows the data to be pre-processed. First, occurrence data need to be rarefied over a predefined rarefying distance to minimize spatial autocorrelation. Available occurrence data are often not gathered using systematic surveying methods and do not represent a random sampling across a species' range (Phillips et al. 2009). Occurrence data are thus often biased in terms of sampling effort. Without rarefaction, predictions will be overestimated in environments that are similar to the more intensely sampled areas. Second, species absences somehow need to be incorporated in SDM algorithms, which are typically only rarely available (Zurell 2020). To tackle this lack of reliable absence data, "pseudo-absences" (also referred to as background data) are generated from a predefined background area (Barbet-Massin et al. 2012). The number of pseudoabsences to choose, relative to the number of presences (i.e. the prevalence ratio), has led to much discussion, but mainly depends on the SDM algorithm (Liu et al. 2019). Therefore, we tested different pseudo-absence selection strategies in accordance with Lobo et al. (2010).

Model fitting follows after data preparation. As algorithms have different underlying assumptions and extrapolate to the environment in a different manner, it has been proposed to combine predictions across multiple modelling methods. This is called ensemble modelling and enhances predictive performances (e.g. Hao et al. (2020)). We built and evaluated the performance of the modelling algorithms by cross-validating the data. This model fitting and evaluation strategy divides the data in two subsets: a training set, which trains the respective model, and a test set, which tests its predictive accuracy. Finally, an ensemble of SDM models that answer to a predefined minimum predictive accuracy was used to obtain final pre-



**Fig. 2.** Map visualizing the five *Magnolia* species from Hispaniola with additional morphological illustration. Base layer depicts elevation as meters above sea level (m.a.s.l.). Classification follows Figlar and Nootboom (2004). **1.** *Magnolia domingensis*. A. Flower in male phase. B. Villose leaves. **2.** *Magnolia hamorii*. A. Closed flower bud. B. Leaves and evidence of conduplicate leaf prefiliation. **3.** *Magnolia pallescens*. A. Immature flower with underdeveloped reproductive structures. B. Elliptic leaf with abaxial sericeous pubescence. Photographs: 1A & 2A – Ramon Elías Castillo Torres; 1B, 2B, 3A, 3B – Emily Veltjen.

dictions of a species habitat suitability and potential geographic distribution across the study area.

Despite our focus on Magnolias from the Dominican Republic, SDM was executed on Hispaniola as a whole (i.e. including Haiti) as delineating study areas based on ecologically meaningless criteria such as country borders is likely to result in spurious predictions of habitat suitability (Bystriakova et al. 2012).

#### **Occurrence Data and Predictor Variables**

Occurrence data of four of the five species from Hispaniola were obtained from Veltjen (2020), Castillo et al. (2018), herbarium material (Online Resource 1) and pers. comm. of Joel Timyan (Société Audubon Haiti). Occurrence data were retained if they had a spatial resolution of  $\leq 1$ " or  $\sim 0.0083333^{\circ}$ . No occurrence data of *M. emarginata* were taken into account, because there are no recent nor precise enough locality data. Similarly, no occurrence data of *M. domingensis* from Haiti were included. This led to a presence-only dataset of 635 occurrences. This dataset was subsequently rarefied with a rarefying distance of 1 km, reducing it to 30 independent occurrences. After rarefying the dataset, too few occurrences remained to perform SDM on each species individually. Instead, we opted for an explicit 'genus-level' approach and performed SDM on all remaining independent occurrences.

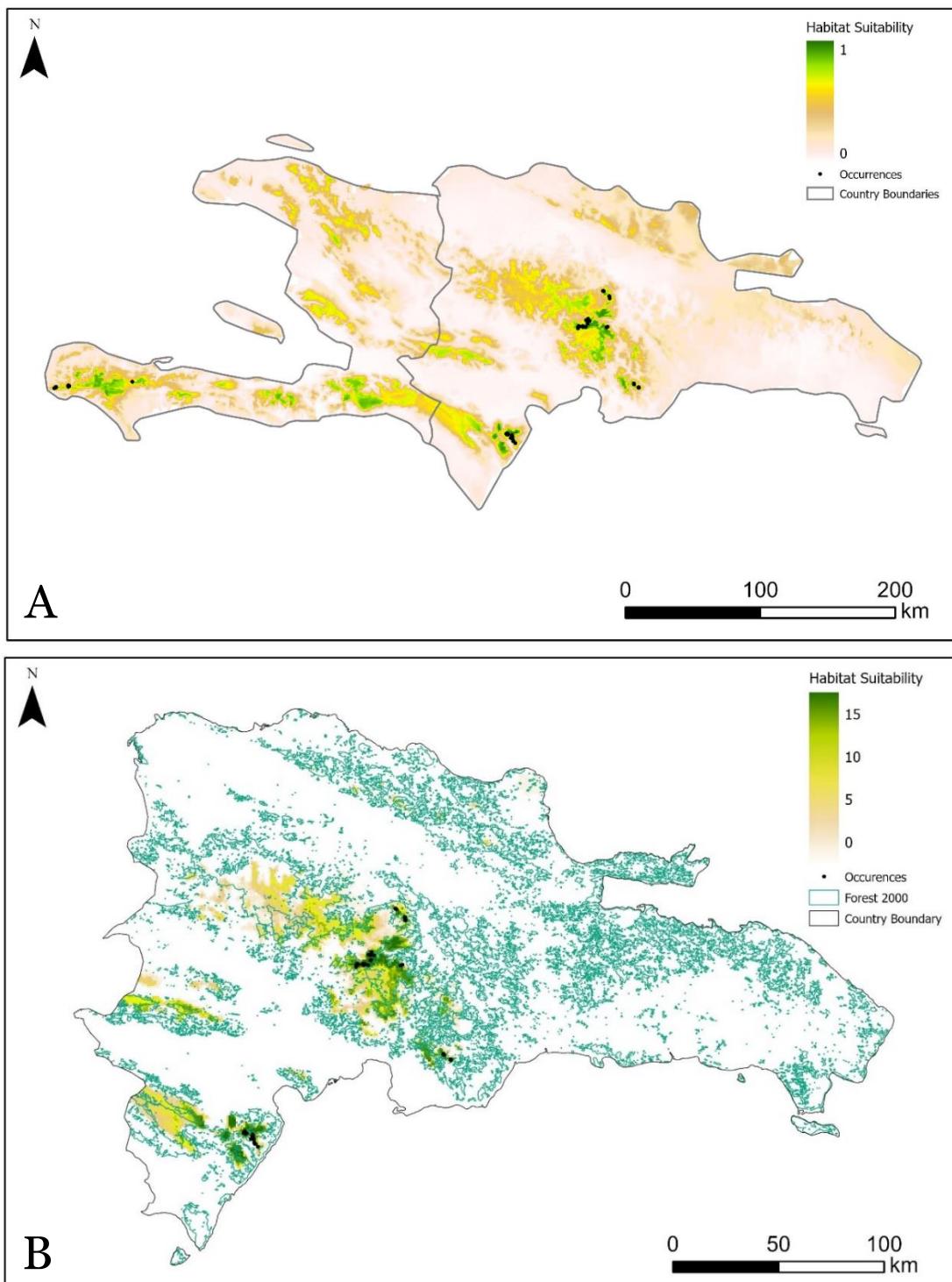
Predictor variables were obtained from ENVIREM (Title and Bemmels 2018), CHELSA (Karger et al. 2017) and SEDAC (Venter et al. 2016, 2018). The included variables comprise environmental variables, describing temperature, precipitation, aridity, evapotranspiration and topography, and one variable describing cumulative human pressures on the environment. To identify strongly correlated variables, we used the "raster.cor.matrix" and "raster.cor.plot" function in the R package ENMTools (Warren et al. 2010). The Pearson correlation coefficient "r" was used to determine which variables were most strongly correlated ( $|r| > 0.7$ ). Of the correlated variables, we selected the most ecophysiological meaningful ones in accordance to Mod et al. (2016) (see Online Resource 4). All variables were obtained with a spatial resolution of  $1 \text{ km}^2$  or  $\sim 0.0083333^{\circ}$ .

#### **Model Fitting and Evaluation**

The different pseudo-absence selection strategies explored here resulted in 15 ensemble model predictions depicting the suitability of Hispaniola for the focal *Magnolia* spp. Each ensemble model consisted of eight different modelling algorithms, as implemented in the ensemble modelling framework of the package "sdm" (Naimi and Araújo 2016) in R

(R Core Team 2020): two regression-based models (Generalised Linear Model (GLM) & Multivariate Adaptive Regression Splines (MARS)), five machine-learning models (Classification and Regression Trees (CART), Boosted Regression Trees (GBM), Maximum Entropy (MaxEnt), Random Forest (RF) & Support Vector Machine (SVM)) and one likelihood-based model (Maxlike). Each SDM algorithm ran with default settings (Naimi and Araújo 2016). Pseudo-absences were sampled at random in background areas defined as either concentric zones around the presence data (using four different radii (i.e. 10 km, 25 km, 50 km & 75 km)) or across the whole of Hispaniola (i.e. no radius restriction) *sensu* VanDerWal et al. (2009). Pseudo-absences were not selected from pixels containing *Magnolia* presences. Pixels correspond with the spatial resolution of the predictor variables (i.e.  $1 \text{ km}^2$ ). Three prevalence ratios were used to sample pseudo-absences: neutral prevalence (1:1), twice the number of presences (1:2) and five times as much (1:5). Prevalence ratios were kept low to account for a low number of (independent) *Magnolia* occurrences, as proposed by Liu et al. (2019). This led to a total of 15 ( $5 \times 3$ ) predictions of *Magnolia* habitat suitability (on a scale of 0 to 1), which were combined into ensemble predictions. Each individual model was calibrated using a 10-fold cross-validation with 80-20% random split of the presence data to serve as training data for each replicate. Model evaluation is given by the True Skill Statistic (TSS) since it is considered the most optimal measure for the performance of predictive models (Allouche et al. 2006). TSS values range from -1 to +1, with values of zero or less indicating a performance that is no better than random, while a value of +1 suggests a perfect model capacity to discriminate between suitable and unsuitable areas. Interpretation of the TSS values follow Allouche et al. (2006). For each of the 15 ensemble models, only "good performing" algorithms, characterized by a TSS-value of  $> 0.7$ , were kept and ensemble habitat suitabilities were obtained using simple averaging of the good performing algorithm outcomes.

Finally, to obtain a single prediction of likely *Magnolia* occurrence across Hispaniola, three different methods were used to summarize the 15 ensemble model outcomes above. First, the 15 *Magnolia* habitat suitability predictions were transformed from continuous suitabilities (0 to 1) to discrete predicted presences versus absences, using the habitat suitability threshold maximizing model TSS as cut-off and setting the suitability for pixels below this threshold to zero. A first final distribution map was then obtained through "vote counting" pixels in every presence-absence prediction, thus



**Fig. 3.** Ensemble-based distribution map for the *Magnolia* species from Hispaniola and the Dominican Republic, respectively. **A.** ensemble-based distribution map for Hispaniola using weighted averaging. Habitat suitability ranges from zero to one, representing the weighted mean TSS value for each pixel. **B.** ensemble-based distribution map for the Dominican Republic using “vote counting.” Habitat suitability ranges from zero to fifteen, representing the number of ensemble analyses that labelled a pixel as suitable (i.e. has a TSS-value > 0.7). Forest 2000 represents the forest cover in the year 2000 (Sangermano et al. 2015).

creating a gradient from 0 (area was never suitable) to 15 (area was always indicated as suitable). Secondly, we used a weighted averaging procedure, combining the 15 habitat suitabilities into a single habitat suitability map, weighing individual models by their TSS values (better models contribute more strongly to the final prediction).

Thirdly, a similar standard “unweighted” averaging was performed. These latter two methods resulted in a continuous prediction of habitat suitability between 0 and 1. These three final maps were further edited to include occurrence data. An additional ensemble distribution map, specifically for the DR, was established from the “vote counting” distribution map of Hispaniola (Fig. 3B). Here, we added an additional layer of forest cover from the year 2000, obtained from Sangermano et al. (2015).

## Conservation Genetics

### Plant Material and DNA Extraction

Populations of *Magnolia domingensis*, *M. hamorii* and *M. pallescens* in the DR were sampled in 2015 and 2021 by haphazard sampling (Ward and Jasieniuk 2009; Veltjen et al. 2019). In total 417 individuals were genotyped at 12 distinct localities. For the localities sampled in 2015, one herbarium voucher is listed as a morphological voucher. Sample information can be found in Table 1 and a map of the respective sampling locations is given in Fig. 2. Samples were validated using the identification key from Veltjen (2020).

DNA isolation was performed on dried leaf tissue according to a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle 1987), with MagAttract Suspension G solution (Qiagen, Germantown, USA) mediated cleaning (Larridon et al. 2015). A Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, USA) and Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) were used to assess DNA quantity and quality control, respectively.

### ***Microsatellite Testing and Genotypification***

Twenty-five microsatellite or Simple Sequence Repeats (SSR) markers from Veltjen et al. (2019) were selected, based on the SSR marker being polymorphic in at least one of the three *Magnolia* species from the DR. From each population of every species, four individuals were selected to re-evaluate the performance of these microsatellite markers (Online Resource 2). The four individuals were chosen to include as much variation as possible on a spatial scale and to have 260/230 and 260/280 optical density (OD) ratios approaching 2. Performance testing was done using simplex PCRs (i.e. one microsatellite marker per PCR) with a three-primer PCR (Vartia et al. 2014). A reaction contained 2 x QIA Multiplex PCR Master Mix (Qiagen), 5ng/µL DNA, 0.025 µM for each forward primer, 0.1 µM for each reverse primer and 0.1 µM for each specified dye. The selected primer pairs were labelled with a

<b>Species</b>	<b>ID</b>	<b>Locality</b>	<b>Loc. ID</b>	<b>RL</b>	<b>Herbarium Reference</b>
<i>M. domingensis</i>	DOM	Loma Barbacoa	BAR	CR	Veltjen et al. 2015-011 (GENT, JBSD)
		Loma Rodríguez	ROD		Veltjen et al. 2015-012 (GENT, HAJB, JBSD)
<i>M. hamorii</i>	HAM	Cachote	CAC	E	Veltjen et al. 2015-010 (GENT, JBSD)
		Cortico	COR		Veltjen et al. 2015-009 (GENT, HAJB, JBSD, K)
		Laguneta	LAG		/
		Tonobán	TON		/
<i>M. pallescens</i>	PAL	Casabito	CAS	E	Veltjen et al. 2015-005 (GENT, JBSD, HAJB)
		Entrance	ENT		Veltjen et al. 2015-006 (GENT, JBSD, HAJB, IEB, K)
		Camino Guayabal	GUA		/
		Loma de la Sal	SAL		Veltjen et al. 2015-004 (GENT, JBSD)
		Montellano	MON		Veltjen et al. 2015-007 (GENT, JBSD)
		Rancho Guaraguao	RAN		/

**Table 1.** Sample information of the three *Magnolia* species from the Dominican Republic. **Species:** taxonomy according to Howard (1948). **ID:** three letter code to uniquely identify a species. **Loc. ID:** three letter code to uniquely identify a locality. **RL:** Red List status following Rivers et al. (2016); CR: Critically Endangered; E: Endangered. Herbarium acronyms follow the Index Herbariorum (Thiers, [continuously updated]). Sampling was executed in April-May 2015 and January–February 2021.

fluorescent dye FAM, NED, PET and VIC, which were linked to their respective universal tail T3, Hill, Neo and M13. PCRs ran on a volume of 5 $\mu$ L under the following conditions: an initial activation step of 15 min at 95°C, followed by 35 cycles of 30 sec at 94°C, 90 sec at 57°C and 90 sec at 72°C; and a final extension for 10 min at 72°C. An ABI 3730XL fragment analyser (Thermo Fisher Scientific) with a GeneScanTM 500 LIZ™ ladder (Thermo Fisher Scientific) was used to separate and visualise the PCR products. The results of the simplex tests that ran on 24 test individuals were analysed in Geneious v.8.1.9 (<http://www.geneious.com>, Kearse et al. 2012) using the microsatellite plugin. When a SSR marker could unambiguously be scored for all tested species and populations, and was polymorphic for at least one individual, the microsatellite marker was included in the subsequent multiplex design and final genotyping. Multiplex pools were designed using Multiplex Manager (Holleley and Geerts 2009). PCR conditions and peak calling occurred cfr. the simplex testing above. In total, four multiplex pools allowed unambiguous genotypification of the test-individuals, whereafter we genotyped the full dataset of 417 individuals. In total we evaluated 25 SSR markers of which 17 were considered qualitative and unambiguous.

To account for human and technical flaws such as genotyping errors and null alleles, the final microsatellite dataset was subjected to MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004) and ML-NullFreq (Kalinowski and Taper 2006). Null alleles are alleles that do not produce a functional end-product, or have a mutation in the primer region which may inhibit PCR amplification (Frankham et al. 2015). Null alleles may lead to preferential amplification of short alleles (i.e. large allele dropout) or slippage during PCR amplification (Gagneux et al. 1997). Therefore, null alleles can obscure genotype frequencies and its derived population statistics. MICROCHECKER and ML-NullFreq ran for 1000 and 100 000 repetitions, respectively. No markers were removed.

To assure random sampling of the genome, we examined the dataset for the presence of linkage disequilibrium (LD) using the program GENEPOP v.4.7.5 (Raymond and Rousset 1995; Rousset 2008). GENEPOP ran with the dememorization number set to 10 000, with 1000 batches and with 50 000 iterations per batch. Following Waples (2015), both uncorrected and (sequential Bonferroni) corrected p-values were considered. One marker, MA40\_045, was discarded, bringing the final number of markers to 16 (see Online Resource 3).

### Data Analyses

In STRUCTURE v.2.3.4 (Pritchard et al. 2000), analyses were done using six datasets: one with all three focal species, three datasets for each species and one dataset for each genetic cluster of PAL as a result of the first analysis (see further). STRUCTURE ran under the following conditions: 100 000 MCMC replicates after an initial burn-in of 100 000, correlated allelic frequencies and presuming the presence of an admixture model. The number of groups (K) was set from one to fifteen for the general dataset (all species included), from one to ten for the species-specific datasets and from one to five for the genetic clusters from PAL. Each value of K was run ten times for each dataset. A visualization of the results was obtained from Structure Harvester Web v.0.6.94 (Earl and vonHoldt 2012). The optimal K-value was selected based on the  $\Delta K$  statistic, following Evanno et al. (2005), and the mean maximum likelihood (Mean LnK or  $\bar{LnK}$ ). Mean LnK proved valuable as  $\Delta K$  cannot detect single clusters. Visualisation of the STRUCTURE barplots was done with DISTRUCT v.1.1 (Rosenberg 2004). DAPC (Discriminant Analysis of Principal Components) analyses were executed in R using the package “adegenet” (Jombart and Ahmed 2011) on the same datasets as the STRUCTURE analyses. Using the “find.cluster” function, we retained the maximum number of PCs and selected groups on the basis of the Bayesian Information Criterion (BIC). The number of Principal Component Analysis (PCA) eigenvalues was determined using 1000 cross-validation replicates. The PCA of the DAPC was set according to the lowest Mean Squared Error (MSE). All discriminant functions (i.e. DA eigenvalues) were kept. Furthermore, pairwise  $F_{ST}$  (Weir and Cockerham 1984),  $G_{ST}$  (Nei and Chesson 1983),  $G'_{ST}$  (Hedrick 2005) and Jost's D ( $D_J$ ; Jost 2008) values and their respective confidence intervals were calculated for each population and locality (Table 4, Online Resource 8) using the R package diveRsity (Keenan et al. 2013). Interpretation of the  $F_{ST}$ -values follows Hartl and Clark (1997).

Diversity statistics were calculated for each species, population, and locality (Table 2). Genetic diversity was estimated by quantifying the following statistics in GenAlEx 6.503 (Peakall and Smouse 2006, 2012): number of alleles per locus (A), number of private alleles ( $A_P$ ), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ) and the absolute number of polymorphic loci (P). Moreover, allelic richness ( $A_R$ ) and the inbreeding coefficient ( $F_{IS}$ ) was calculated in FSTAT v.2.9.4 (Goudet 2000).  $A_R$  was calculated for the population and localities based on a minimum sample size of 6, 14 and 36 individuals. To quantify if  $F_{IS}$  significantly differed from zero, deviations from Hardy-Weinberg proportions

ID	No	A	AR-6	AR-14	AR-36	APP (APA)	HO	HE	FIS	P
<b>DOM</b>										
<b>BAR</b>	36	5.250	3.585	4.412	5.250	25 (4)	0.627	0.587	-0.054	16
<b>ROD</b>	50	4.188	2.972	3.473	3.990	8 (2)	0.538	0.524	-0.016	16
<b>HAM</b>										
<b>BAH</b>	118	8.063	/	/	6.859	46 (-)	0.579	0.605	0.047	15
CAC	52	7.063	4.027	5.318	6.608	11 (7)	0.575	0.575	0.011	13
COR	52	6.625	4.129	5.378	6.329	8 (5)	0.578	0.590	0.029	14
LAG	7	3.750	3.565	/	/	1 (1)	0.616	0.540	-0.064	14
TON	7	4.375	4.153	/	/	4 (3)	0.588	0.574	0.053	14
<b>PAL</b>										
<b>EBV</b>	80	5.813	/	/	5.375	28 (7)	0.546	0.562	0.035*	15
CAS	40	5.125	3.389	4.231	5.039	4 (1)	0.545	0.541	0.004	15
SAL	40	5.438	3.686	4.635	5.376	4 (2)	0.547	0.548	0.016	15
<b>VAL</b>	133	6.250	/	/	5.367	21 (6)	0.527	0.552	0.048	16
ENT	61	5.188	3.349	4.188	4.951	4 (1)	0.520	0.529	0.025*	16
GUA	15	4.063	3.135	4.252	/	2 (1)	0.518	0.482	-0.038	15
MON	41	5.188	3.433	4.003	5.073	3 (3)	0.527	0.537	0.030*	15
RAN	16	4.500	3.496	4.402	/	1 (0)	0.564	0.554	0.015	16

**Table 2.** Population and locality statistics for the Magnolias of the Dominican Republic. **ID.** Three letter code to uniquely identify a species, population, or locality, following Table 1. **No.** Number of sampled individuals. **A.** Average allelic diversity. **AR-x.** Allelic richness based on a minimum sample size of 6, 14 and 36 individuals. **AP.** Number of private alleles between all localities (**APA**) or between populations only (**App**). **HO.** Average observed heterozygosity. **HE.** Average expected heterozygosity. **Fis.** Inbreeding coefficient. Significant ( $p = 0.05$ ) deviations from Hardy-Weinberg proportions are indicated with an asterisk (\*). **P.** Number of polymorphic loci.

(HWP) were tested in GENEPOL with 2-tailed exact tests for every marker x population combination. If possible, a complete enumeration was executed (Louis and Dempster 1987). Otherwise, MCMC chains were employed with 200 batches and 50 000 iteration (Guo and Thompson 1992).

## Results

### Species Distribution Modelling

Out of 39 variables, 21 were uncorrelated and considered ecophysiological meaningful and were retained for the subsequent analyses (Online Resource 4). During model evaluation, no ensemble models were discarded. Model performance for each of the 15 ensemble predictions can be found in Table 3. An upward trend of TSS-values is visible for increasing pseudo-absence sampling distance as well as for a higher prevalence ratio. For each of the three methods to summarize the 15 ensemble models (vote counting, weighted and unweighted averaging), maps were made to visualize the habitat suitabilities across Hispaniola and the DR. These maps can be found in Fig. 3 and Online Resource 5 (A-B). Regions of high habitat suitability for the Magnolias of Hispaniola seem to co-occur with the numerous mountain ranges on the islands (Fig 1, Fig.2A).

### Microsatellite Markers

Twenty-five microsatellite markers were tested in simplex and multiplex. Seventeen markers were polymorphic for at least one individual and allowed unambiguous genotyping. Subsequently, MICROCHECKER labelled four markers with potential null alleles: MA40\_045, MA42\_001, MA42\_059, MA42\_126 and MA42\_472. However, they were only highlighted in MON. ML-NullFreq indicated high values ( $>0.1$ ) for MA42\_001 and MA42\_059 in locality MON and for MA42\_203, MA42\_241 and MA42\_397 in locality TON. GENEPOL revealed that one marker, MA40\_045, showed significant LD with three markers after sequential Bonferroni correction: MA42\_231 in ROD, MA40\_282 in BAR and finally MA42\_472 in ROD, SAL, and MON. Hence, MA40\_045 was discarded to guarantee independent sampling of the genome with respect to other microsatellite markers, bringing the final number of markers to a total of 16 (see Online Resource 3).

### Population Structure and Genetic Diversity

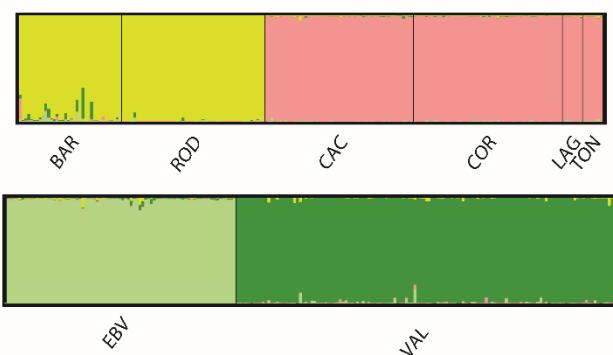
$\Delta K$  and mean LnK plots for all four STRUCTURE-analyses are presented in Online Resource 6. The optimal K derived from the  $\Delta K$  and mean LnK plots were similar for each analysis except for HAM. For the STRUCTURE analysis of the entire dataset

	Pseudo-absence sampling distance					
	10 km	25 km	50 km	75 km	Random	
Prevalence ratio	<b>1:1</b>	0.830 ± 0.051	0.897 ± 0.053	0.917 ± 0.039	0.950 ± 0.036	0.977 ± 0.022
	<b>1:2</b>	0.800 ± 0.069	0.953 ± 0.028	0.943 ± 0.052	0.917 ± 0.032	0.950 ± 0.036
	<b>1:5</b>	0.957 ± 0.035	0.950 ± 0.048	0.940 ± 0.041	0.993 ± 0.014	0.993 ± 0.021

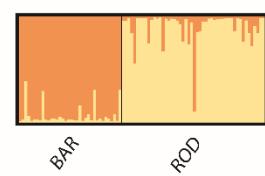
**Table 3.** Model performance, indicated by TSS-values, for the 15 ensemble analyses. TSS-values and their respective standard deviation are rounded to three decimals.

encompassing all species, the optimal K is four. In the species datasets the optimal K for DOM, HAM and PAL is two. The mean LnK plots also depict 2 as the best value for K, except for HAM. Here, the likelihood for K = 1 is not that much lower than K=2 and we consider K=1 to be the optimal number of genetic clusters since  $\Delta K$  is unable to consider only one cluster. Representative replicate barplots for the optimal K groups are portrayed in Fig. 4. Based on these results, five populations were defined: BAR, ROD, BAH, SAL and VAL. Populations retained their names from Veltjen et al. (2019) if locations resulted in an identical population, otherwise a unique population ID was designated. Fig. 5 visualizes the DAPC analysis on all individuals, showing four genetic clusters in accordance with the STRUCTURE analysis on all species (Fig. 4A). The first axis shows a clear distinction between BAH and the three

(A)



(B)



**Fig. 4.** STRUCTURE barplots for the Magnolias of the Dominican Republic and *Magnolia domingensis* specifically. Optimal K-values are given based on mean LnK. **A.** Dataset comprising all three species, K = 4. **B.** *Magnolia domingensis*, K = 2.

remaining populations, which are subsequently separated by the second axis. The results from the other DAPC analyses can be found in Online Resource 7. Multiple, unambiguous genetic clusters were obtained for the full dataset and for PAL. Similar to the STRUCTURE-analysis, DOM showed an intermediate result. Pairwise  $F_{ST}$ ,  $G_{ST}$ ,  $G'_{ST}$  and Jost's D ( $D_J$ ) values can be found in Table 4 and Online Resource 8. Values between populations range from 0.081 to 0.271 for  $F_{ST}$ , from 0.042 to 0.158 for  $G_{ST}$ , from 0.149 to 0.550 for  $G'_{ST}$  and from 0.081 to 0.378 for  $D_J$ . Confidence intervals (CIs) of the pairwise indices can be found in Online Resource 9.

Diversity statistics for each population, locality and microsatellite marker are compiled in Online Resource 10. The mean values for the individual populations and localities can be found in Table 2. One population and two localities, namely EBV, ENT and MON, showed significant deviations from HWP for seven, four and one out of 16 markers, respectively, which resulted in an  $F_{IS}$  that was significantly deviating from zero.

## Discussion

### Evaluation of Species Distribution Modelling and Microsatellites

The ensemble SDM predicted the habitat of *Magnolia* across Hispaniola with supposedly great accuracy. This is visible in Table 3, which indicates the model performance across prevalence ratios and pseudo-absence sampling distance. These values range from 0.800 to 0.993, which are promising results as values greater than 0.7 are indicative for good predictive models (Allouche et al. 2006). Furthermore, all 66 additional occurrences from the sampling effort in 2021 fell within highly suitable predicted areas. Similarly, herbarium occurrence data that was deemed too unprecise for the SDM analyses, were often situated in highly suitable habitats (green to dark green areas in Fig. 3A). This validation of predictive power of the SDMs has to be nuanced since all new sampled locations were in relatively close proximity to known populations from

<b>Genetic differentiation statistics</b>					
Statistic	Pop	BAR	ROD	BAH	SAL
F <sub>ST</sub>	ROD		0.081		
G <sub>ST</sub>			0.042		
G' <sub>ST</sub>			0.149		
D <sub>J</sub>			0.081		
F <sub>ST</sub>	BAH	0.208	0.257		
G <sub>ST</sub>		0.117	0.151		
G' <sub>ST</sub>		0.468	0.550		
D <sub>J</sub>		0.372	0.378		
F <sub>ST</sub>	SAL	0.215	0.271	0.219	
G <sub>ST</sub>		0.120	0.158	0.124	
G' <sub>ST</sub>		0.450	0.540	0.475	
D <sub>J</sub>		0.268	0.328	0.369	
F <sub>ST</sub>	VAL	0.216	0.264	0.218	0.147
G <sub>ST</sub>		0.119	0.153	0.122	0.079
G' <sub>ST</sub>		0.439	0.515	0.460	0.280
D <sub>J</sub>		0.281	0.302	0.352	0.154

►Table 4. Pairwise fixation and allelic differentiation statistics ( $F_{ST}$ ,  $G_{ST}$ ,  $G'_{ST}$  &  $D_J$ ) for every population combination.

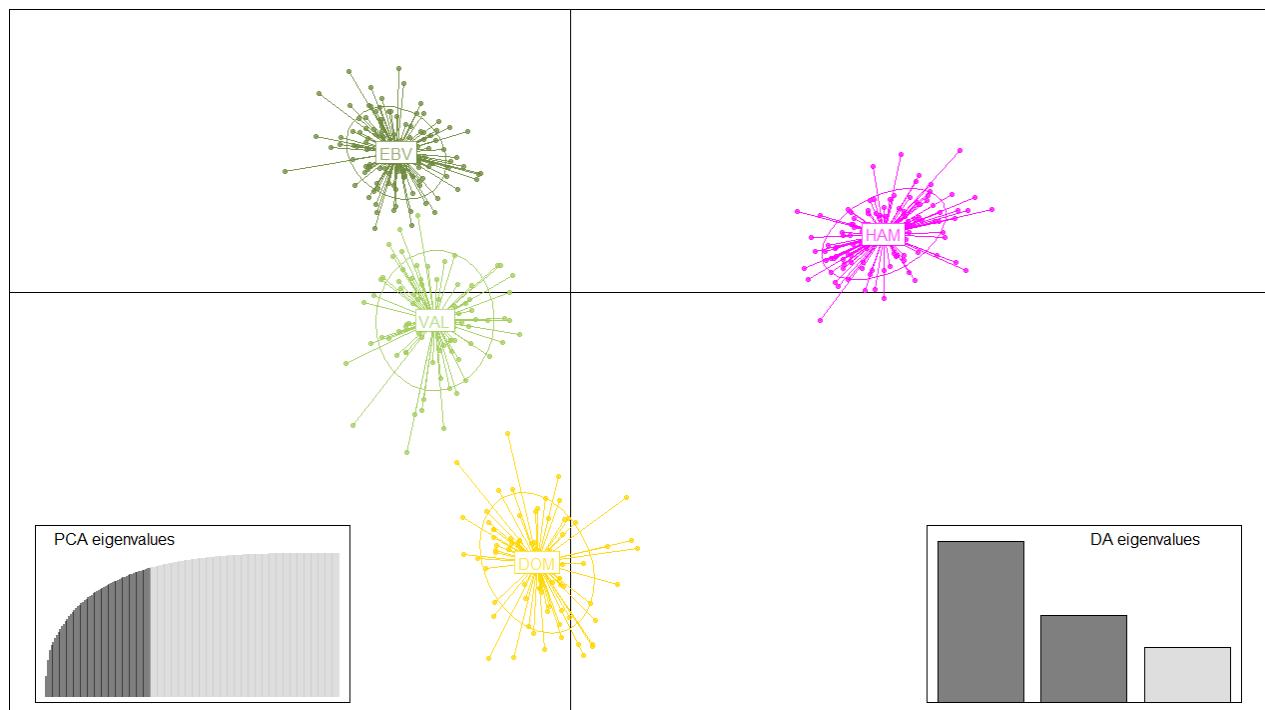


Fig. 5. DAPC (Discriminant Analysis of Principal Components) plots for the Magnolias of the Dominican Republic. Names follow the species ID (if  $K = 1$ ) or population ID (if  $K > 1$ ), depending on the number of clusters ( $K$ ). Four clusters are visible across three species: DOM, HAM, EBV and VAL. The latter two belong to PAL.

the DR. Moreover, high TSS values may indicate model overfitting as model evaluation metrics are both a function of prediction accuracy as well as modelling conditions (Woodcock 1976; Wunderlich et al. 2019).

During microsatellite testing, a downward trend of qualitative, usable markers was observed (Hodel et al. 2016). Starting with 25 markers from Veltjen et al. (2019) which ought to be polymorphic, 17 proved to be qualitative and unambiguous after genotyping. Subsequently, tests on null alleles indicated several markers to contain potential null alleles in MON and TON (Table 1). However, as TON is a locality with a small sample size, we do not consider these results as a true indication for null alleles. Similarly, as null alleles were only found in MON and in no other locality, no markers were removed from the subsequent downstream analyses. The presence of LD between the locus pairs MA40\_045 – MA40\_282, MA40\_045 – MA42\_231 and MA40\_045 – MA42\_472, led to the subsequent removal of marker MA40\_045. This reduced the final number of microsatellite markers to 16. Interestingly, due to an increment in sample size compared to Veltjen et al. (2019), we no longer see LD for population ROD. In Veltjen et al. (2019), this was attributed to a recent bottleneck and a subsequent lack of random mating to restore linked loci (Slatkin 2008). Now, we can elucidate that this was a random sampling error, but see Waples (2015).

### **Guiding Explorations Using the Modelled Suitable Habitat**

Mountain ranges with the most promising areas of detecting new *Magnolia* populations in the DR are the Cordillera Central, the Sierra de Bahoruco and the Sierra de Neiba. Possible remnant populations could be found in the Cordillera Septentrional and the Sierra Martín García, an isolated extension of the Sierra de Neiba. In the DR, areas with high habitat suitability mainly reside in areas of the Forest 2000 layer (Fig. 3B). For Haiti, areas of interest reside in the mountain ranges Massif de la Hotte and Massif de la Selle, the Haitian counterpart of the Sierra de Bahoruco. To a lesser degree, possible areas of interest can be found in Massif du Nord, Montagnes Noires and Chaîne des Matheux (Fig. 3A). In Haiti, expeditions to Massif de la Selle have did not find any *Magnolia* species (Pers. comm. Joel Timyan, Société Audubon Haiti). Due to an extensive history of deforestation and the absence of dark green patches of suitable habitat in northern Haiti (Fig. 3A), new populations in mountain ranges aside from Massif de la Hotte and Massif de la Selle are deemed unlikely (Hedges et al. 2018). Knowledge about the potential habitat provides policy makers and on-the-

ground conservationists with a guideline for spatial prioritisation of conservation efforts (Villero et al. 2017). Concretely, it can serve as the basis for reinforcement efforts and the designation of habitat corridors; and allows to search for the true or full extent of populations more efficiently (Williams et al. 2009). Habitat corridors have successfully been applied in Baruah et al. (2019), Adhikari et al. (2012) and Liu et al. (2018).

### **Defining Conservation Units**

By integrating the results of the SDM (Fig. 3, Online Resource 5) and conservation genetic analyses (Fig. 4 – 5, Table 2 & 4, Online Resource 6 – 9), we propose to treat the five genetic *Magnolia* populations of the DR (Fig. 4) as three conservation units (CU), following the morphospecies as described by Howard (1984) and confirmed with recent *in situ* observations (Castillo et al. 2018; Veltjen 2020). Conservation or management units are groups of individuals that are clustered together to contain the genetic diversity necessary to ensure evolvability in the light of changing environments and local adaptation (Weckworth et al. 2018). In the following three paragraphs we deliver the argumentation species-by species to treat them as one CU each.

According to the STRUCTURE and DAPC analysis (Fig. 4 – 5, Online Resource 7D - F), *M. pallescens* is divided in two populations: a northern population (EBV) in Ébano Verde Scientific Reserve and a southern population (VAL) in Valle Nuevo National Park (Fig. 3B). Furthermore, there are no recent observations of *Magnolia* in the suitable habitat patch between EBV and VAL (Fig. 2, Fig. 3B). Hence, we see distinct spatial and genetic grouping. Three herbarium vouchers (Garcia\_1002, Garcia\_1184 and Veloz\_3237) do imply a historical distribution of *M. pallescens* in which the two populations were connected. These vouchers were excluded from the SDM analysis. The division of populations is reinforced by the pairwise genetic differentiation statistics from Table 4. Between EBV and VAL, we see an  $F_{ST}$ -value of 0.147, which is considered moderate genetic differentiation following Hartl and Clark (1997). Furthermore, a value of 0.154 for  $D_J$  suggests sufficient genetic differentiation to be considered independent populations, especially when compared to  $D_J$ -values between population of different species, ranging from 0.268 to 0.378. When we compare infraspecific  $F_{ST}$ - and  $D_J$ -values with other studies on island tree endemic populations, we find similar results. For example,  $F_{ST} = 0.000 – 0.229$  for *Pinus caribaea* var. *bahamensis*, an endemic variety from the Bahaman archipelago (Sanchez et al. 2014). In terms of genetic diversity, the population summary statistics for EBV

and VAL indicate average genetic health when compared with other *Magnolia* populations from the DR (Table 2). Notably, EBV is the only population to exhibit a significant, but low,  $F_{IS}$ -value. Despite the apparent genetic substructure of the species, we propose to treat *M. pallescens* as one CU due to the significant  $F_{IS}$ -value of population EBV and localities ENT and MON (population VAL); and the potential historical connection between the populations.

For *M. domingensis*, it is less straightforward to state the number of (genetic) populations, with both arguments in favor of one and two populations. Although the STRUCTURE (Fig. 4A) and DAPC (Fig. 5) analyses on the full dataset depict *M. domingensis* individuals to be one genetic cluster, the species-specific analyses (Fig. 4B & 5, Online Resource 7B) retrieve two with a few migrants/relict trees in each of the localities. Considering the habitat suitability of the area, connecting the two populations, there is no strong indication for any spatial schism. The two populations are connected by a continuous mountain ridge and only a minor reduction in habitat suitability of the connecting pixels is visible. This raises the question whether *M. domingensis* effectively consists of two populations or whether this distinction is artificial. Taking into account the genetic differentiation indices (Table 4), we see  $F_{ST}$ - and  $D_J$ -values between BAR and ROD ( $F_{ST}$ : 0.081;  $D_J$ : 0.081) that reside in the same category (moderate genetic differentiation; Hartl and Clark 1997) as the values between EBV and VAL ( $F_{ST}$ : 0.147;  $D_J$ : 0.154), but in the minimum as opposed to the maximum values of the range for that category (i.e. 0.05 – 0.15). These intraspecific values are comparable with a study on *Metrosideros* species from Hawaii ( $F_{ST}$  = 0.09 – 0.12; Harbaugh et al. 2009). If we look at the indices between localities within the populations of Magnolia in the DR (Online Resource 8), values range from 0.023 to 0.078 for  $F_{ST}$  and from 0.011 to 0.074 for  $D_J$ . As these maximum values closely approximate the values between BAR and ROD, we are inclined to conclude that BAR and ROD should be considered localities within one population. However, these maxima are derived from values between localities with a small number of sampled individuals (GUA, LAG, RAN and TON; Table 1 – 2) that potentially split one large continuous population. Considering only populations with  $N > 39$ , the maximum range of  $F_{ST}$  and  $D_J$  drops down to 0.049 and 0.068, respectively. The high number of private alleles ( $A_{PP}$ ; Table 2) in population BAR compared to ROD confirm this apparent differentiation. To summarize, the STRUCTURE (Fig. 4B) and DAPC (Online Resource 7B) analyses on the species-specific dataset indicate two populations, while these analyses on the full dataset (Fig. 4A, Fig. 5) and the SDM map (Fig. 3)

only predict one. Additionally, the genetic differentiation statistics indicate intermediate values (Table 4) and there is a notable number of private alleles that sets these localities apart (Table 2). Although the signal is not as strong as the *M. pallescens* populations, there is enough genetic evidence to state that we have two recently differentiated populations. The genetic diversity of BAR is average across the Magnolias of the DR (Table 2). In contrast, ROD exhibits the worst genetic health among the three studied species, with the lowest values for any A-statistic. However, both populations have low and non-significant  $F_{IS}$ -values. As ROD is genetically degraded, genetic substructuring is not pronounced and the localities are linked by suitable habitat, we consider *M. domingensis* to be one CU. This approach allows genetic rescue effects between these populations to negate a further decline in genetic diversity (Ingvarsson 2001; Whiteley et al. 2014).

By integrating the genetic structuring results of *M. ekmanii* from Veltjen et al. (2019) with the SDM analyses (Fig. 3A), we see a similar issue as the spatial segregation of *M. domingensis*. The species is split in two populations, Morne Grand Bois (GRA) and Morne Mansinte (MAN), evidenced by a STRUCTURE and DAPC analysis. Based on our SDM results (Fig. 3A), this disjunction is unexpected considering that both populations are connected by a stretch of mountains with highly suitable potential habitat. Further sampling efforts in the area ought to resolve this issue. Considering its genetic substructure and troublesome genetic health, we treat *M. ekmanii* as one CU. Noteworthy, new occurrences of *M. ekmanii* were found relatively recently in the region of Bois Pangnol (easternmost point on Fig. 2), an isolated location given the geographic distance between other Magnolias in Hispaniola. As these were not sampled for any genetic analysis, we cannot make inferences with regard to their genetic structure or health. We recommend including this new locality in future conservation genetic studies.

*M. hamorii* only has one population, BAH, situated in the Natural Protected Landscape Miguel Domingo Fuerte. It is the most differentiated from any other population from the DR with respect to  $D_J$  (Table 4). When considering the DAPC plot (Fig. 5) and the ensemble-based distribution map (Fig. 3), *M. hamorii* is situated the furthest from any other population from the DR, both in terms of kilometres and genetic distance. This is also visible in the genetic differentiation indices of Table 4. Many of the genetic diversity indices ( $A$ ,  $H_E$ ) of *M. hamorii* are the highest among the Magnolias of the DR (Table 2). The high number of private alleles ( $A_{PP}$ ) further shows that *M. hamorii* is very distinct from

the other Magnolias of the DR. Its  $F_{IS}$ -value of 0.047 is the highest among all populations, but non-significant and unproblematic as such. We consider *M. hamorii* as one CU since the sampled localities have one continuous modelled suitable habitat (Fig. 3B), they show low genetic differentiation (Table 4) and hence, there is no detectable genetic substructure (Fig. 4A & 5).

#### Priorities Based on Genetic Diversity

Because no non-threatened, related *Magnolia* species were included in this research, we can only make statements about the genetic diversity being healthy, low or high when comparing the localities and populations with each other (Spielman et al. 2004; Väli et al. 2008). Comparisons with diversity statistics generated in other studies can be made but should be treated with caution. We will discuss these statistics in view of the previously instated population structure, the habitat availability and the species' Red List assessment of Rivers et al. (2016).

Solely comparing the population statistics for the five populations, we do not find dramatic results in terms of genetic health. In general, only three significant  $F_{IS}$ -values are found across all populations and localities: population EBV and two localities within population VAL (ENT and MON). Even so, these values are relatively low compared to other *Magnolia* species (e.g. *M. nuevoleonensis* – population SPE and PE:  $F_{IS} = 0.583^*$  and  $0.749^*$  (Chávez-Cortázar et al. 2021); *M. stellata* – population Asahidani:  $F_{IS} = 0.233^*$  (Tamaki et al. 2016)). Both populations, EBV and VAL, have average values for all population statistics, except for a significant deviation from HWP of the  $F_{IS}$ -value for EBV. Peculiarly, EBV has an average number of individuals compared to other DR *Magnolia* species (Table 2), yet it is the only population with a significant  $F_{IS}$ -value. Interestingly, no significant  $F_{IS}$ -values are found for the two localities in EBV. One potential explanation could be that a Wahlund-effect was artificially created by merging the two localities (De Meeûs 2018). The Wahlund effect reduces heterozygosity in a population due to population substructuring (Wahlund 1928). The opposite situation as EBV is visible in VAL. We attribute this to unrepresentative sampling across a small spatial scale. When comparing these results with Veltjen et al. (2019), no drastic differences are visible, aside from the significant  $F_{IS}$ -value for EBV. *Magnolia domingensis*, shows the highest  $H_o$  of all populations in BAR, but no other statistic stands out. ROD, however, has the lowest A-,  $A_R$ - and  $A_P$ -values of all. Despite this,  $F_{IS}$ -values remain low and non-significant for both populations. We see that low values for the A statistics in ROD could explain the higher values of genetic differentiation (Table 4) and

the STRUCTURE and DAPC results (Fig. 4, Online Resource 7B). *Magnolia hamorii* with its sole population BAH has the highest reported values for A,  $A_R$ ,  $A_P$  and  $H_E$ . The pattern of limited gene flow, leading to high genetic structuring, and little inbreeding is similar to other studies such as Aldaba Núñez et al. (2021) and Veltjen et al. (2019). Inbreeding is expected to be minimal as the flowers of *Magnolia* are protogynous (Thien 1974) and promote outcrossing (Tamaki et al. 2009), but not absent as geitonogamy has been reported in other *Magnolia* species (Bernhardt and Thien 1987; Hirayama et al. 2005; Tamaki et al. 2008). The strong inbreeding depression in the early life stages of trees ensures that adult plants result solely from outcrossing (Sorensen 1999), increasing outcrossing rates and maintaining genetic diversity (Petit and Hampe 2006). High genetic diversity is desirable as their sessile lifestyle allows evolution of locally adapted ecotypes. Additionally, genetic diversity is "stored" in their great longevity, allowing genetic variants to circulate a long time within the populations (Alberto et al. 2013). This allows for potential reinforcement of genetically degraded populations (Aitken et al. 2008).

Unexpected results are found when comparing the species' genetic diversity with their habitat availability and Red List assessment category of Rivers et al. (2016). *Magnolia domingensis* is Critically Endangered (CR; Wheeler 2015), has the worst genetic health of the Magnolias of the DR and has the least amount of suitable *Magnolia* habitat in its near vicinity (Fig. 3B), being restricted to the Padre Luis Quinn National Park. *Magnolia hamorii* is Endangered (E; Global Tree Specialist Group 2014), has more suitable habitat in its vicinity than *M. domingensis*, and despite it being reduced to one population, has the highest genetic diversity statistics. *Magnolia pallescens*, Endangered (E; Global Tree Specialist Group 2014b) with the most available suitable habitat of all the magnolias of Hispaniola by far, has only a mediocre genetic health. When we consider the population statistics for *M. ekmanii* (CR; Wheeler and Rivers 2020) of Veltjen et al. (2019) together with the SDM analysis (Fig. 3A), *M. ekmanii* has a relatively large potential habitat in Massif de la Hotte, exceeding those of *M. domingensis* and *M. hamorii*, but also the worst population statistics among the Magnolias of Hispaniola. We do not see a clear correlation between available (potential) habitat and Red List category or genetic diversity, nor between the latter two. This is surprising as geographic range was the main criteria for *M. hamorii* and *M. pallescens* to be labelled as "Endangered." This mismatch has already been reported in publications such as Vellend and Geber (2005) and Rivers et al. (2014).

### Conservation Recommendations

To ensure a sustainable future for the Magnolias of Hispaniola, we propose the following conservation strategy. We prioritize *M. domingensis* and *M. ekmanii* for conservation. *Magnolia ekmanii* because its low genetic diversity was demonstrated in Veltjen et al. (2019) and because its distribution is situated in a region susceptible to deforestation (Hedges et al. 2018). Similarly, *M. domingensis* is prioritized because of the degrading genetic health of population ROD and since both populations are distributed in an isolated extension of the Cordillera Central. *Magnolia pallescens* was not prioritized because the inbreeding in EBV can easily be reduced with propagation efforts and because it has sufficient suitable *Magnolia* habitat in its vicinity (Fig. 3B). For the Magnolias of Hispaniola, the most effective conservation actions that we propose are exploration, protection, and reinforcement.

Exploration aims to further elucidate the contemporary distribution of the Magnolias of Hispaniola using Fig. 3 as a guideline. We propose the following strategy to search for new *Magnolia* individuals/populations, with decreasing priority. Initially, the highly suitable areas between the populations of *M. domingensis*, *M. pallescens* and *M. ekmanii* should be explored, followed by nearby highly suitable, mountainous areas in Massif de la Hotte, Sierra de Bahoruco and Cordillera Central. Subsequently, search for remnant populations of *M. emarginata* and/or *M. domingensis* in Massif du Nord and Montagnes Noires. Finally, areas with no prior indication of *Magnolia* populations such as Sierra de Neiba, Sierra Martín García and Chaîne des Matheux should be further explored. Newfound individuals may lead to the formation or expansion of (new) protected areas (i.e. National Parks and Scientific Reserves) as these charismatic trees are likely candidates to serve as flagship and/or umbrella species (Veltjen 2020).

Protection aims to conserve and expand the species' habitat, TMCFs. Given that an adequate amount of genetic diversity is available within the conservation units, the focus should lie on the expansion of protected areas. Ideally, protected areas should strive to connect forest fragments in the landscape and the different populations as such. These habitat corridors (Christie and Knowles 2015)

are especially important for the populations of *M. ekmanii* and *M. domingensis*, should no natural corridor exist. When successful, gene flow between populations should increase and enhance genetic diversity. For *M. hamorii*, protection of habitat is a crucial step in ensuring its survival.

Although protection is considered the most long-term important goal, it often takes time to implement. Hence, we propose to immediately apply reinforcement to bridge this temporal gap. Reinforcement aims to preserve and enhance genetic diversity and population sizes. Our interpretation of “reinforcement” follows Seddon et al. (2014): “The release of an organism into an existing population of conspecifics to enhance population viability.” Since gene flow between populations is limited, illustrated by the lower genetic health of ROD and a significant inbreeding value in EBV, we propose to execute reinforcement efforts between populations (Brichieri-Colombi and Moehrenschlager 2016). Here, populations BAR and VAL will function as source populations of genetic diversity and ROD and EBV as their respective receptive sink populations. The benefits of minimizing loss of genetic diversity due to inbreeding and fragmentation outweighs the undoing of potential local adaptations. We encourage conservation practitioners to raise seedlings in nurseries and plant them close/within the sink population. Moreover, new populations could be established in suitable *Magnolia* habitat in the vicinity of known populations. Preferably, in an area secure from large scale threats. Nurseries should avoid hybridization and potential subsequent outbreeding depression (Keller et al. 2000; Tamaki et al. 2017) at any cost. In the case of *M. pallescens*, we propose the suitable area in between the existing populations (Fig. 3B). Propagation efforts in nurseries are ongoing for the Magnolias of the DR, with mixed results (Castillo et al. 2018). We emphasize the importance of collecting seeds from multiple localities and from as many different trees as possible across a large spatial scale. This ensures representative subsampling of the genetic diversity within the conservation unit (Oldfield and Newton 2012). To ensure success, a monitoring programme is to be set up to keep track of the survival rates of the seedlings and mature trees. This allows a quick and swift respond to new threats (Godefroid et al. 2011).

## Conclusions

The SDM analyses suggests that the distribution of potential habitats is restricted to the various mountain ranges on Hispaniola. We indicate the mountains of the Montagnes Noires as a priority area to search for the potentially extinct *M. emarginata* and *M. domingensis* in Haiti. The Sierra de Neiba is the most promising area, without prior known *Magnolia* occurrences, to search for new populations in the Dominican Republic. Highly suitable habitat is present between populations of *M. domingensis*, *M. ekmanii* and *M. pallescens*. These patches are highlighted for future reinforcement efforts should no natural habitat corridor exist. The Magnolias of the DR consist of five genetic populations, representing three species. We recommend each species to be treated as its own conservation units (CU). The pattern of little inbreeding for Caribbean *Magnolia* species seems to persist in our sampling. Population Ébano Verde and localities Entrance and Montellano exhibit minimal, but significant, inbreeding. Out of the three DR *Magnolia* species, *M. domingensis* is highlighted as it contained the lowest genetic diversity, the least number of (known) individuals and the lowest number of sampling localities. For conservation management, we propose to focus on three actions: exploration, protection, and reinforcement. The main recommendation includes protection and/or instatement of habitat corridors between populations of *M. domingensis* and *M. ekmanii*, whereby the SDM results will serve as a guideline for spatial prioritization.

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### Conflict of interest/Competing interests

The authors hereby declare no conflict of interests.

### Availability of data and material

The authors confirm that the supporting data of these findings are available within the article and its supplementary materials.

### Code availability

Not applicable.

### Authors' contributions

Conceptualization: Emily Veltjen, Marie-Stéphanie Samain; Methodology: Diederik Strubbe, Emily Veltjen; Formal analysis and investigation: Tim Claerhout; Writing – original draft preparation: Tim Claerhout; Writing – review and editing: Diederik Strubbe, Emily Veltjen, Lars Chatrou, Marie-Stéphanie Samain, Ramón Elías Castillo Torres, Tim Claerhout; Funding acquisition: Emily Veltjen, Marie-Stéphanie Samain; Resources: Emily Veltjen, Ramón Elías Castillo Torres; Supervision: Diederik Strubbe, Emily Veltjen, Lars Chatrou.

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Not applicable.

### Consent to participate

All authors hereby consent to participate.

### Consent for publication

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**Online Resource 1** Herbarium material employed as occurrence data for species distribution modelling analyses with their respective latitudinal and longitudinal positions as decimal degrees. Herbarium acronyms follow Thiers ([continuously updated]).

Thiers B, (continuously updated). Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/>

SPECIES	ACRONYM	COLLECTOR(S)	HERBARIUM N°	DATE OF COLLECTION	LATITUDE	LONGITUDE
<i>Magnolia domingensis</i> Urb.	JBSD	Veloz A. & Castillo R. E.	3117	9/05/2004	18.43361111	-70.3075
<i>Magnolia ekmanii</i> Urb.	FLAS	J. C. Timyan	39	7/06/2013	18.37339	-74.29497
<i>Magnolia hamorii</i> Howard	US	Acevedo-Rdgz. P. et al.	13814	20/05/2004	18.10755556	-71.19186111
<i>Magnolia hamorii</i> Howard	JBSD	Clase T. et al.	8416	1/03/2014	18.11980556	-71.22669444
<i>Magnolia hamorii</i> Howard	JBSD	Clase T. et al.	8034	30/07/2013	18.110115	-71.220421
<i>Magnolia hamorii</i> Howard	JBSD	Clase T. et al.	3541	4/04/2003	18.067638	-71.182667
<i>Magnolia hamorii</i> Howard	JBSD	Judd W. S. et al.	8101	31/05/2006	18.11072222	-71.22691667
<i>Magnolia hamorii</i> Howard	JBSD	Skean J. D. et al.	4304	31/05/2006	18.11497222	-71.23155556
<i>Magnolia pallescens</i> Urb. & Ekman	JBSD	Clase T. & Encarnacion W.	6767	3/03/2011	18.836189	-70.682663
<i>Magnolia pallescens</i> Urb. & Ekman	JBSD	Clase T. et al.	6415	23/09/2010	18.8405	-70.72572222
<i>Magnolia pallescens</i> Urb. & Ekman	JBSD	Peguero B. et al.	727	10/06/1998	18.836517	-70.535853
<i>Magnolia pallescens</i> Urb. & Ekman	JBSD	Peguero B. et al.	735	11/06/1998	18.839738	-70.527617

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**Online Resource 2** Sample information regarding the 24 test individuals used for SSR marker testing.

**Ref. N°:** reference number to uniquely identify an individual. **OD (260/230):** ratio of absorbance (i.e. Optical Density ratio) at 260 and 230 nm as a proxy for DNA purity. A ratio between 2.0 and 2.2 is considered pure. **OD (260/280):** ratio of absorbance at 260 and 280 nm as a proxy for DNA purity. A ratio of ~1.8 is considered pure. **ID:** three letter code to uniquely identify a species. **Loc. ID:** three letter code to uniquely identify locality of origin.

REF. N°	OD (260/230)	OD (260/280)	ID	LOC. ID
<b>MA354</b>	2.02	2.09	PAL	CAS
<b>MA437</b>	1.96	2.04	PAL	CAS
<b>MA468</b>	1.99	1.98	PAL	SAL
<b>MA499</b>	2.02	2.13	PAL	SAL
<b>MA716</b>	2.05	1.76	PAL	ENT
<b>MA743</b>	2.09	1.97	PAL	ENT
<b>MA763</b>	2.02	1.86	PAL	MON
<b>MA780</b>	2.05	1.99	PAL	MON
<b>MA795</b>	2.05	1.77	HAM	COR
<b>MA809</b>	1.97	1.71	HAM	COR
<b>MA822</b>	2.05	1.89	HAM	COR
<b>MA839</b>	1.97	2.04	HAM	COR
<b>MA849</b>	2.07	1.88	HAM	CAC
<b>MA862</b>	2.09	1.99	HAM	CAC
<b>MA869</b>	1.99	2.01	HAM	CAC
<b>MA882</b>	2.06	1.93	HAM	CAC
<b>MA891</b>	2.06	1.91	DOM	BAR
<b>MA903</b>	2.03	1.91	DOM	BAR
<b>MA904</b>	2.01	1.88	DOM	BAR
<b>MA906</b>	2.05	1.93	DOM	BAR
<b>MA908</b>	2.05	1.95	DOM	ROD
<b>MA922</b>	2.04	1.99	DOM	ROD
<b>MA931</b>	2.04	1.96	DOM	ROD
<b>MA949</b>	2.05	1.94	DOM	ROD

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**Online Resource 3** Total set of 25 microsatellite markers that were evaluated for their performance and for which primer information is given. This resulted in a final set of 16 markers. Primers were developed on *Magnolia laecondonica* (MA39), *M. mayae* (MA40), *M. dealbata* (MA41) and *M. cubensis* subsp. *acunae* (MA42). Markers indicated with an asterisk (\*) were removed due to non-random allelic associations (Linkage Disequilibrium; LD). Markers were obtained from Veltjen et al. (2019).

LOCUS NAME	PRIMER SEQUENCE (5'-3')	DYE	REPEAT MOTIF	GENBANK ACCESSION NUMBER	FINAL MARKER
MA39_023	F: ATCACGCATCTGCACAGACA R: GGACAACGAACGTCTGGCTA	FAM	(AG)7	MH923371	☒
MA39_165	F: AATGTAGTGGTCCGGCTTC R: CCAAACCATGTGCGTCCTTG	PET	(TC)18	MH923375	☒
MA39_185	F: CGGGTGGTAGATGACGCT R: AAGACACGGAATGGGACGAG	PET	(AG)15	MH923377	☒
MA39_199	F: CGCCCACATCTACCTCTCG R: TCCAGGAGTTCTGTGCACC	FAM	(GGA)5	MH923378	☐
MA39_259	F: TGATAGAGTGGATGGCGGA R: TGCTGCTTGAGGCCTGTTA	VIC	(CT)11	MH923380	☐
MA40_045*	F: TTGTGGCCAAGCTGATAG R: ATTGTGGCATGTACCTCGCA	VIC	(TC)13	MH923387	☐
MA40_223	F: TTCAGTGGCTGGAGCTTCAG R: GGAGCATCTGGCCTTGGA	VIC	(GAT)5	MH923391	☐
MA40_282	F: TCTTTCCCTCCGTCTCC R: TCTTCCGGCTTCATGTCGTC	FAM	(GA)15	MH923392	☒
MA41_373	F: GCGCCAATCAGAACACAAC R: GGGAAAGAGCTTCTTCGCCA	NED	(CT)16	MH923396	☒
MA42_001	F: ATCCGACCCAACATGGTGAC R: AGCCGAGCTGAGCTGAGTA	PET	(TC)11	MH923397	☒

<b>MA42_059</b>	F: AGGGACTCGGCATCTATGGA R: GAGTCGACTCAGCAACTCCC	PET	(AG)8	MH923399	<input checked="" type="checkbox"/>
<b>MA42_077</b>	F: GAGACATGGAACCCACACGT R: CTGGTGGCTAGCCGATCTG	FAM	(AG)8	MH923402	<input type="checkbox"/>
<b>MA42_083</b>	F: GTCTTCCACGGGAGCAAGAG R: CGAGTTGGACCCAGTGAGTC	VIC	(GAA)17	MH923403	<input type="checkbox"/>
<b>MA42_126</b>	F: CACATCGTCCGTCCAGACAT R: TCGCCTAGCCAATAGTCTGC	FAM	(AT)9	MH923406	<input checked="" type="checkbox"/>
<b>MA42_203</b>	F: TGAAGAACACAGGCCATGGA R: GAGAGGTGCTTCACGGGTAG	FAM	(TC)16	MH923412	<input checked="" type="checkbox"/>
<b>MA42_231</b>	F: GGGTGCAGAATGTGCATCAA R: GGGCCAGTGAGCATTAGAGC	VIC	(AG)14	MH923413	<input checked="" type="checkbox"/>
<b>MA42_241</b>	F: GGGTACCCATTGGTCCAACC R: GTCCGACTAAGGCCATTGT	NED	(CA)11	MH923414	<input checked="" type="checkbox"/>
<b>MA42_255</b>	F: ACGTGGGTCGAGGATCAAGT R: GGACCCACCTCCAACAGATC	PET	(AG)14	MH923417	<input checked="" type="checkbox"/>
<b>MA42_293</b>	F: TGCAACTGAGACGAGTTGGG R: GGTACGGACTAGGGTACAGGT	NED	(GA)16	MH923421	<input checked="" type="checkbox"/>
<b>MA42_397</b>	F: TAGTAGCAGGGTCCCTCCTC R: TCCATTCTTACAGGGTGGCA	NED	(TC)20	MH923426	<input checked="" type="checkbox"/>
<b>MA42_421</b>	F: GACAGCAGACCTGACCGATT R: GACCAAGTGCATCCCATCAAA	VIC	(TC)10	MH923428	<input checked="" type="checkbox"/>
<b>MA42_471</b>	F: TGATGAAGAGCCCAGATCGTC R: TGGCCTTGTCTCCATACGT	FAM, VIC	(GA)16	MH923429	<input type="checkbox"/>
<b>MA42_472</b>	F: AGAGTTACACATGCAAACCCG R: TGATGTTGCTCGGCTGA	PET	(AG)17	MH923430	<input checked="" type="checkbox"/>
<b>MA42_481</b>	F: CGATCTGAGTCCGCAAGAGT R: GACGCAGAAATCTCAGCAAGA	NED	(TC)15	MH923431	<input type="checkbox"/>
<b>MA42_495</b>	F: TGCATTCCTCATCCTCCCA R: ACGCCATTCAATTACCTACGG	PET	(GA)26	MH923433	<input type="checkbox"/>

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**Online Resource 4** Final set of predictor variables included in the species distribution modelling analyses of the genus *Magnolia* in Hispaniola. Sources: **A:** Karger et al. (2017); **B:** Title and Bemmels (2018); **C:** Venter et al. (2016, 2018).

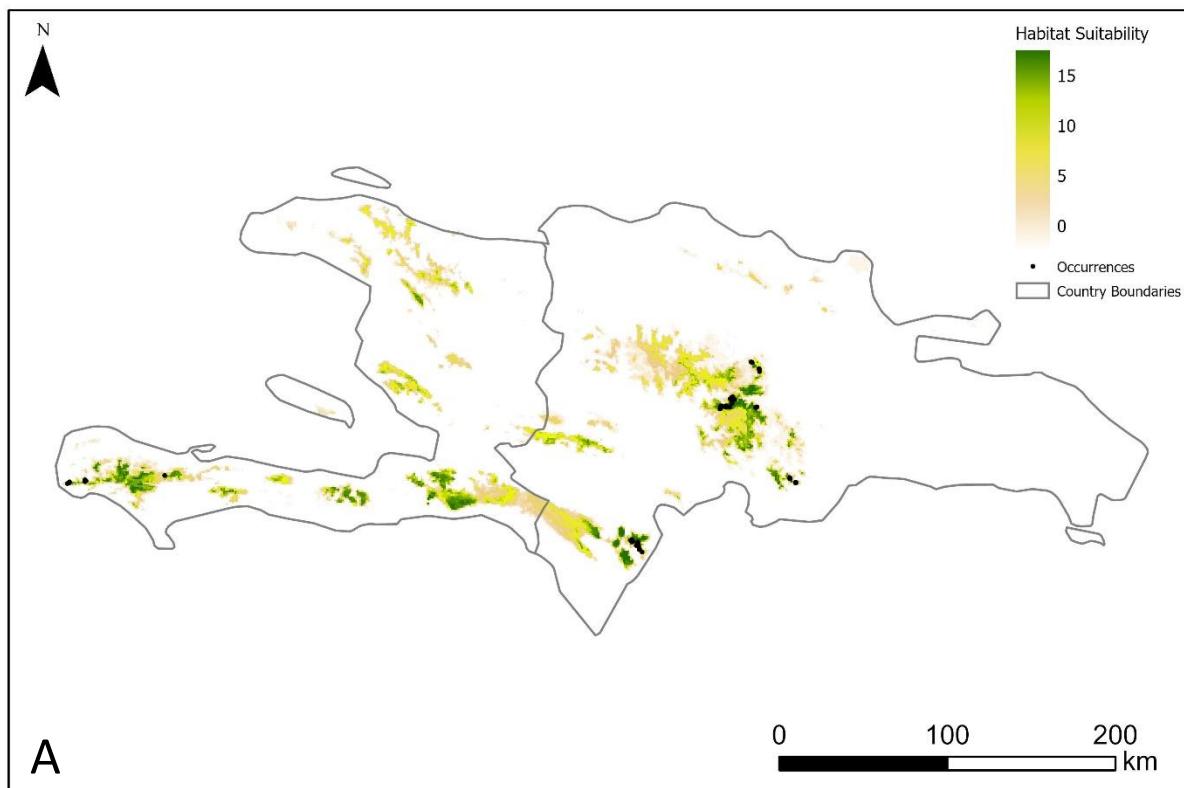
N°	VARIABLE	DESCRIPTION	UNIT	SOURCE
1	Bio1	Annual mean temperature.	°C*10	A
2	Bio2	Mean diurnal range.	°C	A
3	Bio3	Isothermality.	-	A
4	Bio4	Temperature seasonality.	SD	A
5	Bio8	Mean temperature of wettest quarter.	°C*10	A
6	Bio12	Annual precipitation.	mm/year	A
7	Bio15	Precipitation seasonality.	Coefficient of variation	A
8	Bio16	Precipitation of wettest quarter.	mm/quarter	A
9	Bio18	Precipitation of warmest quarter.	mm/quarter	A
10	AnnualPET	Annual potential evapotranspiration: a measure of the ability of the atmosphere to remove water through evapotranspiration processes, given unlimited moisture.	mm/year	B
11	AridityIndexT	Thornthwaite aridity index: Index of the degree of water deficit below water need.	-	B
12	ClimaticMoisture Index	A metric of relative wetness and aridity.	-	B
13	Continentiality	Average temp. of warmest month – average temp. of coldest month.	°C	B
14	EmbergerQ	Emberger's pluviothermic quotient: a metric that was designed to differentiate among Mediterranean type climates.	-	B
15	MonthCountBy Temp10	Count of the number of months with mean temp. greater than 10°C.	months	B
16	PETColdestQuarter	Mean monthly PET of coldest quarter.	mm/month	B
17	PETDriestQuarter	Mean monthly PET of driest quarter.	mm/month	B
18	PETWettestQuarter	Mean monthly PET of wettest quarter.	mm/month	B
19	Tri	Terrain roughness index.	-	B
20	TopoWet	SAGA-GIS topographic wetness index.	-	B
21	Footprint	Quantitative measure of human alteration of terrestrial environments in 2009, based on human population size, land use and infrastructure.	-	C

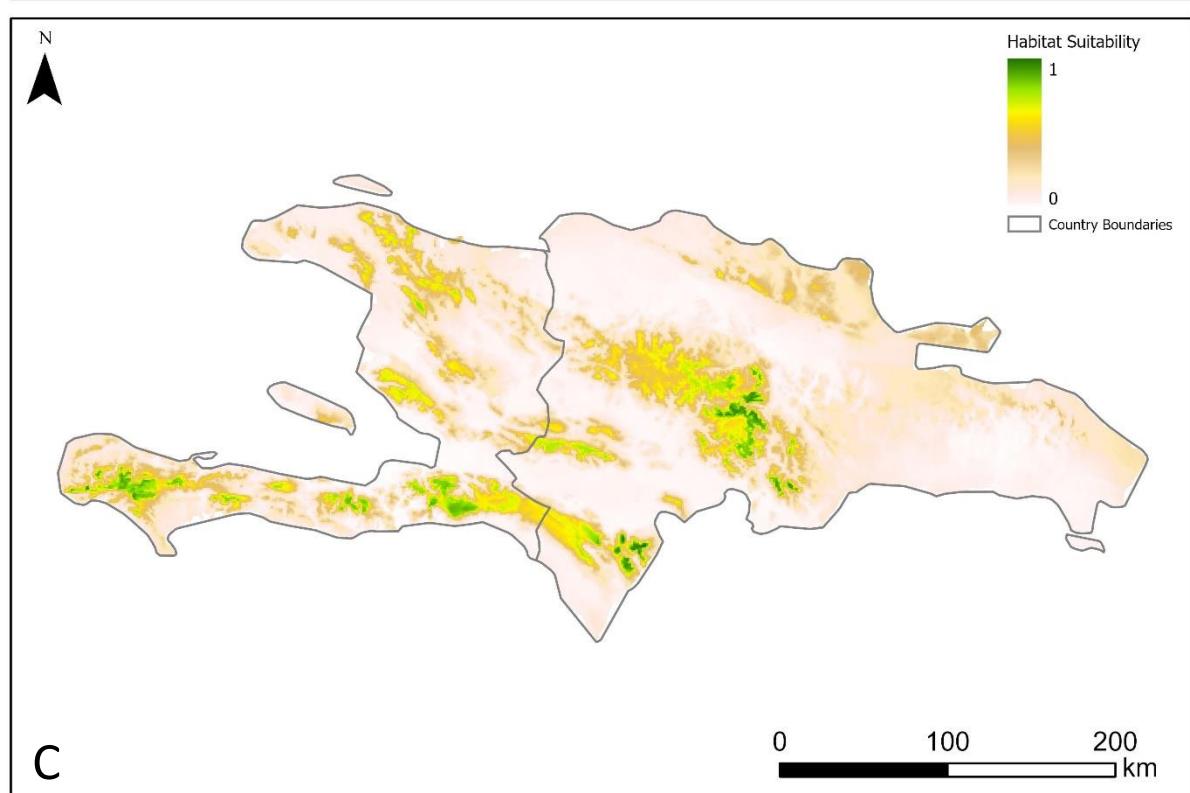
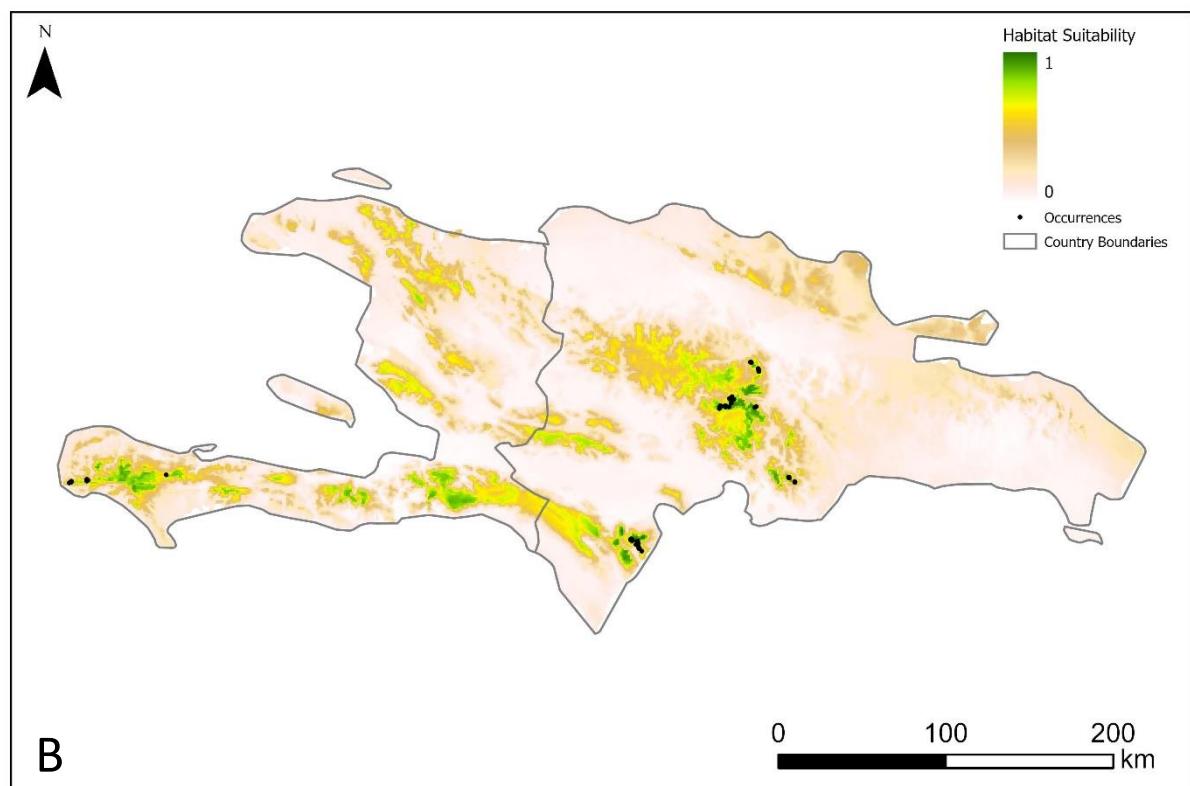
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**Online Resource 5** Maps visualizing the potential distribution and habitat suitability for the Magnolias of Hispaniola. **A.** Ensemble-based distribution map for using “vote counting.” Habitat suitability ranges from zero to fifteen, indicating the number of ensemble analyses that labelled a pixel as suitable (i.e. TSS > 0.7). **B.** Ensemble-based distribution map for using “unweighted averaging.” Habitat suitability ranges from zero to one, indicating the average TSS-value of a pixel. **C.** The same map as **B**, but without plotted occurrences.





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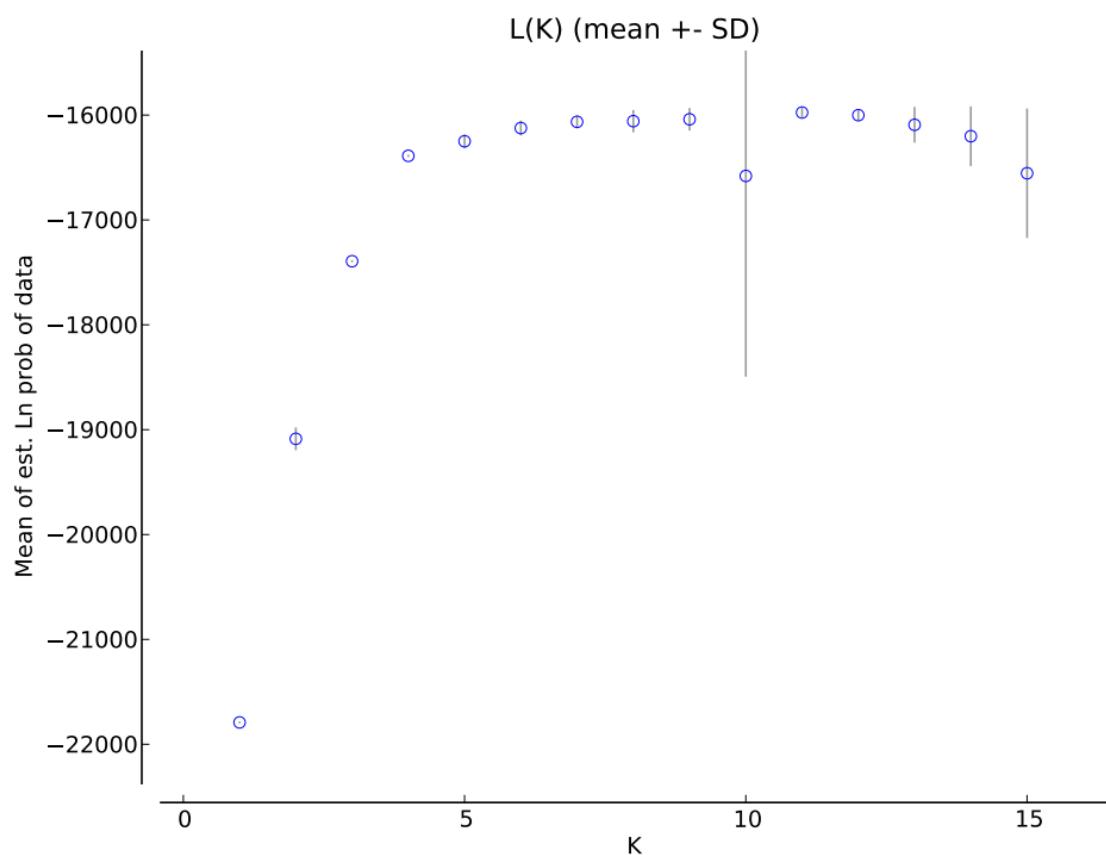
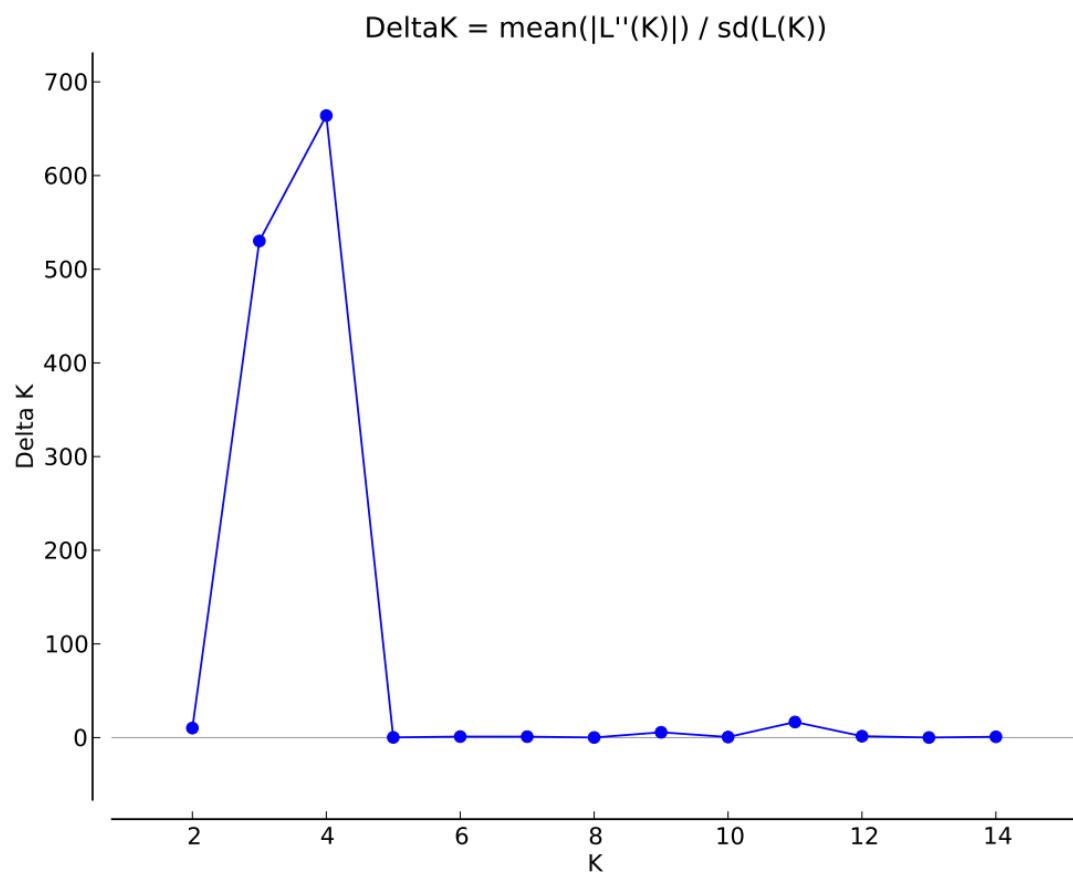
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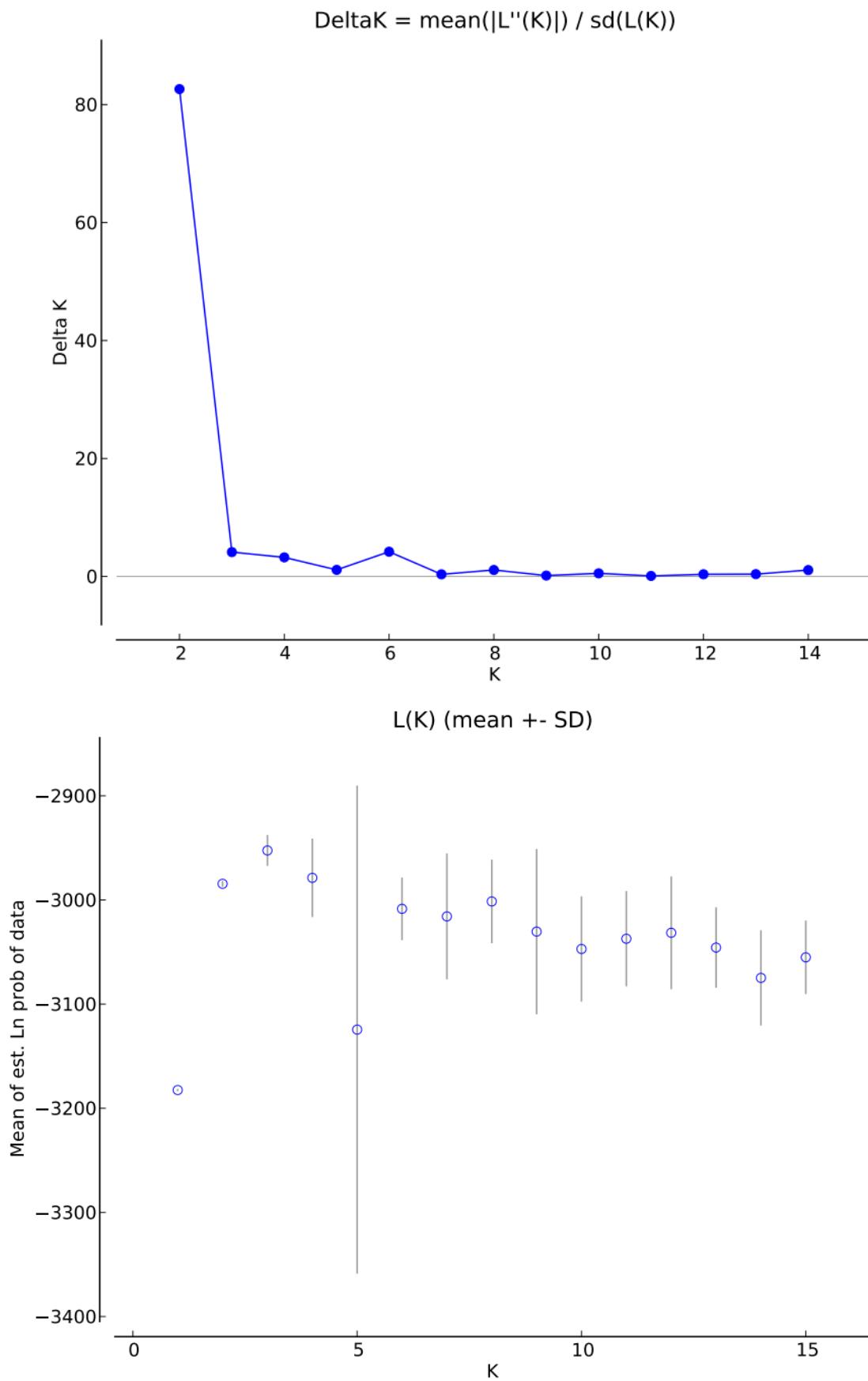
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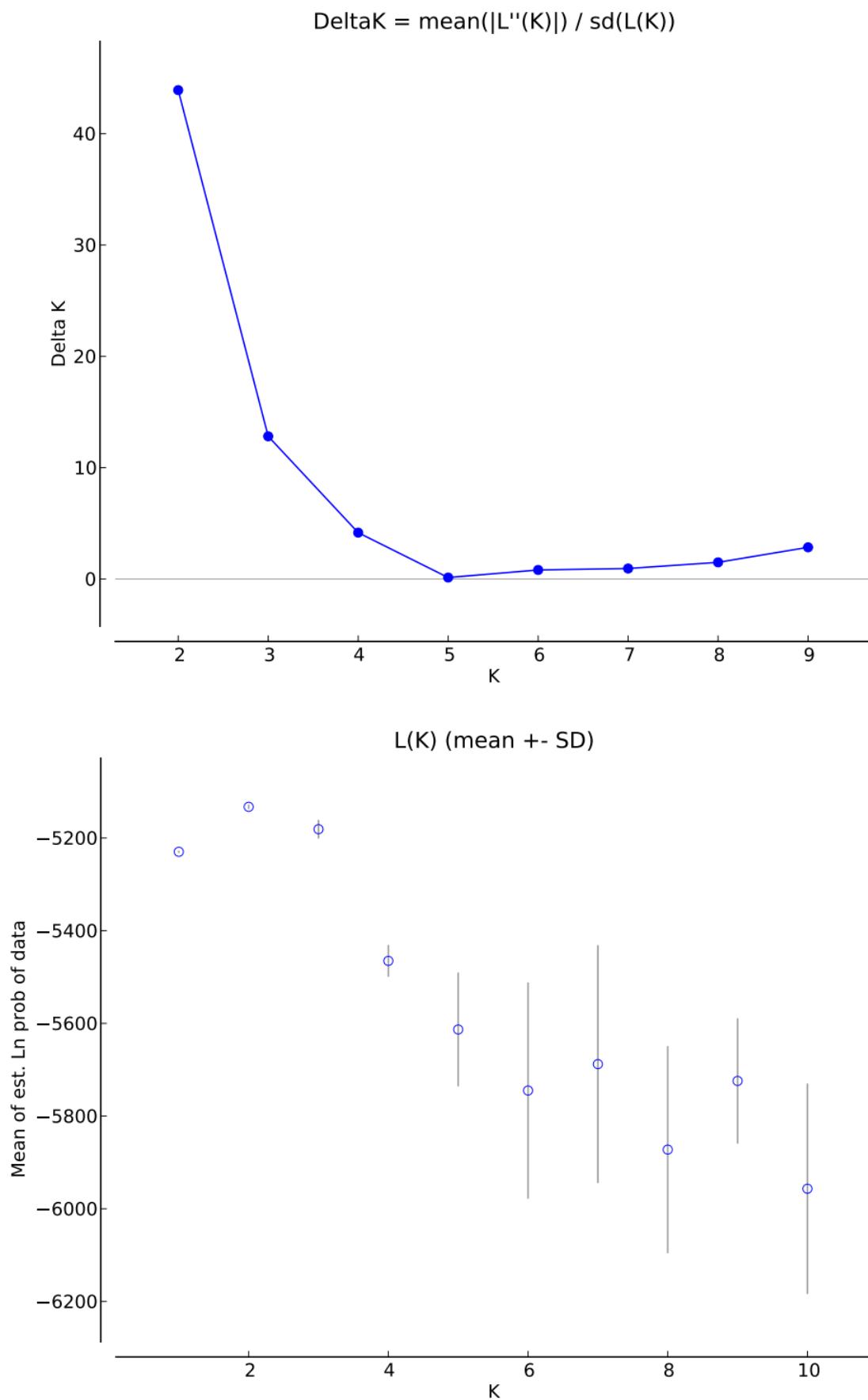
**Online Resource 6** STRUCTURE  $\Delta K$  (Evanno et al. 2005) and mean LnK plots for all four analyses.

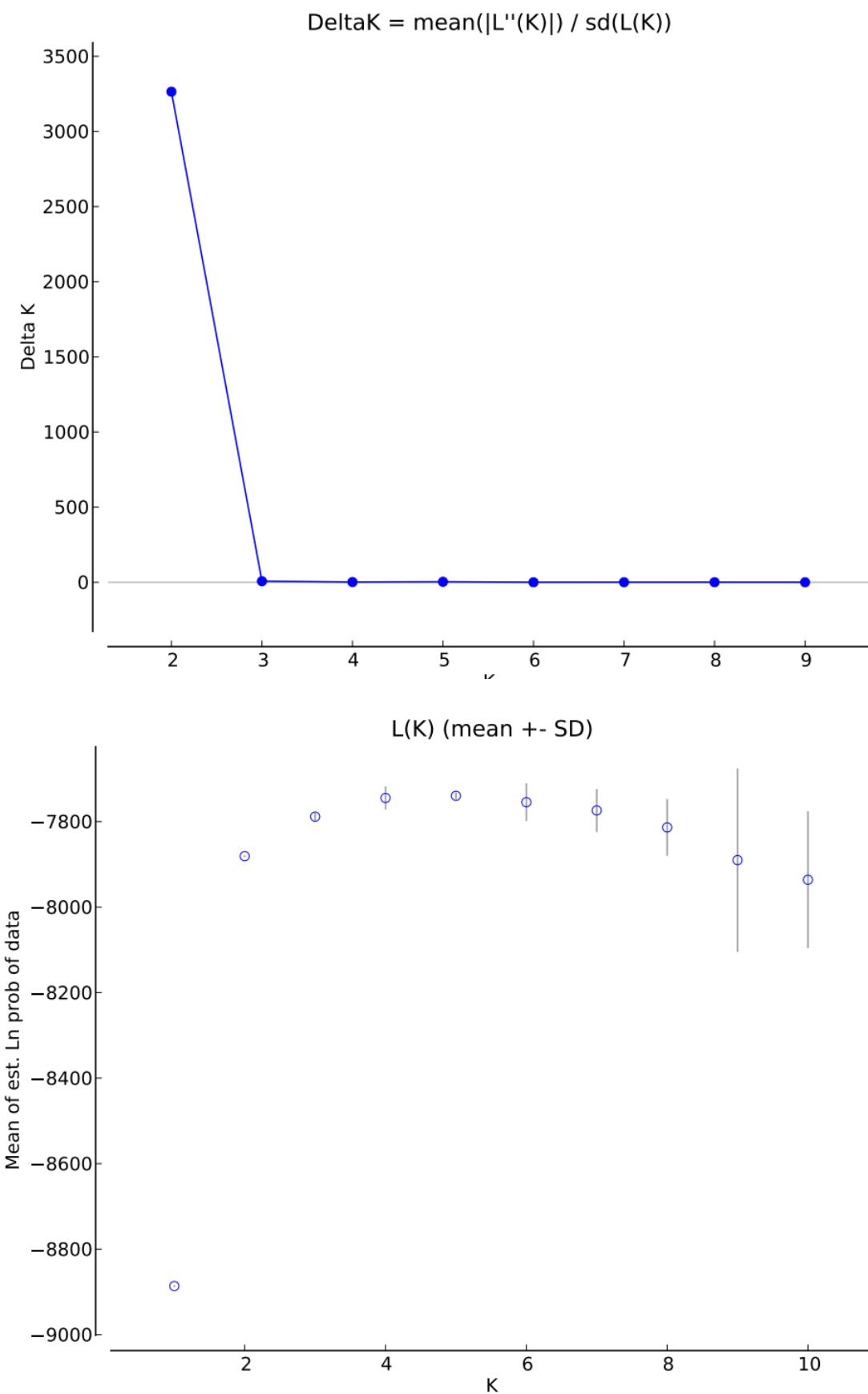
Individuals are genotyped for 16 SSR microsatellite markers. **A.** Dataset comprising all 417 individuals of every species. **B.** *Magnolia domingensis*. **C.** *Magnolia hamorii*. **D.** *Magnolia pallescens*.

**A. *Magnolia spp.* from the Dominican Republic.**



**B. *Magnolia domingensis*.**

**C. *Magnolia hamorii*.**

**D. *Magnolia pallescens*.**

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**Online Resource 7** DAPC analyses results. Tables show the distribution of individuals across X genetic clusters. Between brackets: number of Principal Component Axes retained, based on the lowest mean squared error. **LOC/POP**: three letter code to uniquely identify locality or population, following Table 2.

**A. Full dataset (60)**

LOC   CLUSTERS	1	2	3	4
BAR	0	0	0	36
ROD	0	0	0	50
CAC	0	52	0	0
COR	0	52	0	0
LAG	0	7	0	0
TON	0	7	0	0
CAS	0	0	40	0
SAL	0	0	40	0
ENT	61	0	0	0
MON	41	0	0	0
GUA	15	0	0	0
RAN	16	0	0	0

**B. *Magnolia domingensis* (10)**

LOC   CLUSTERS	1	2
BAR	34	2
ROD	6	44

**C. *Magnolia hamorii* (10)**

LOC   CLUSTERS	1	2
CAC	46	6
COR	18	34
LAG	5	2
TON	2	5

**D. *Magnolia pallescens* (10)**

POP   CLUSTERS	1	2
EBV	80	0
VAL	0	133

**E. Ébano Verde (10)**

LOC   CLUSTERS	1	2
CAS	37	3
SAL	8	32

**F. Valle Nuevo (10)**

LOC   CLUSTERS	1	2
ENT	39	22
MON	14	27
GUA	12	3
RAN	1	15

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**Online Resource 8** Pairwise fixation and allelic differentiation statistics (FST, GST, G'ST & DJ) for every locality combination. Locality names follow Table 1.

Statistic	Locality	BAR	ROD	CAC	COR	LAG	TON	CAS	SAL	ENT	MON	GUA	RAN
<b>FST</b>	ROD	0.081											
<b>GST</b>		0.042											
<b>G'ST</b>		0.149											
<b>DJ</b>		0.081											
<b>FST</b>	CAC	0.221	0.263										
<b>GST</b>		0.124	0.152										
<b>G'ST</b>		0.477	0.529										
<b>DJ</b>		0.364	0.335										
<b>FST</b>	COR	0.225	0.291	0.050									
<b>GST</b>		0.127	0.171	0.026									
<b>G'ST</b>		0.500	0.609	0.099									
<b>DJ</b>		0.392	0.435	0.069									
<b>FST</b>	LAG	0.212	0.261	0.023	0.069								
<b>GST</b>		0.118	0.144	0.012	0.037								
<b>G'ST</b>		0.457	0.504	0.045	0.141								
<b>DJ</b>		0.328	0.297	0.011	0.074								
<b>FST</b>	TON	0.204	0.272	0.054	0.033	0.047							
<b>GST</b>		0.111	0.149	0.027	0.017	0.024							
<b>G'ST</b>		0.453	0.546	0.107	0.070	0.095							
<b>DJ</b>		0.318	0.358	0.052	0.035	0.015							

<b>FST</b>	CAS	0.216	0.266	0.238	0.232	0.234	0.226					
<b>GST</b>		0.120	0.153	0.136	0.132	0.130	0.122					
<b>G'ST</b>		0.441	0.511	0.487	0.483	0.466	0.461					
<b>DJ</b>		0.257	0.290	0.360	0.369	0.343	0.358					
<b>FST</b>	SAL	0.232	0.299	0.256	0.242	0.256	0.234	0.049				
<b>GST</b>		0.131	0.175	0.147	0.139	0.145	0.128	0.025				
<b>G'ST</b>		0.485	0.589	0.535	0.514	0.526	0.489	0.087				
<b>DJ</b>		0.316	0.393	0.406	0.400	0.416	0.382	0.041				
<b>FST</b>	ENT	0.234	0.289	0.246	0.252	0.262	0.253	0.170	0.143			
<b>GST</b>		0.131	0.169	0.140	0.144	0.146	0.137	0.093	0.077			
<b>G'ST</b>		0.469	0.551	0.491	0.516	0.513	0.508	0.310	0.260			
<b>DJ</b>		0.327	0.344	0.366	0.392	0.367	0.365	0.168	0.128			
<b>FST</b>	MON	0.213	0.264	0.225	0.233	0.237	0.230	0.179	0.154	0.035		
<b>GST</b>		0.119	0.152	0.127	0.133	0.132	0.125	0.098	0.083	0.018		
<b>G'ST</b>		0.432	0.504	0.454	0.484	0.469	0.469	0.334	0.287	0.060		
<b>DJ</b>		0.238	0.261	0.345	0.361	0.341	0.349	0.186	0.145	0.023		
<b>FST</b>	GUA	0.244	0.312	0.259	0.263	0.283	0.265	0.211	0.178	0.039	0.067	
<b>GST</b>		0.143	0.187	0.154	0.158	0.159	0.147	0.120	0.100	0.020	0.036	
<b>G'ST</b>		0.491	0.584	0.517	0.541	0.534	0.518	0.384	0.324	0.064	0.114	
<b>DJ</b>		0.294	0.344	0.360	0.404	0.358	0.381	0.219	0.168	0.024	0.048	
<b>FST</b>	RAN	0.195	0.229	0.202	0.227	0.211	0.211	0.213	0.196	0.055	0.034	0.078
<b>GST</b>		0.108	0.127	0.113	0.129	0.117	0.116	0.118	0.108	0.028	0.017	0.041
<b>G'ST</b>		0.413	0.437	0.420	0.492	0.436	0.458	0.419	0.388	0.097	0.061	0.135
<b>DJ</b>		0.250	0.222	0.321	0.388	0.295	0.339	0.280	0.235	0.031	0.020	0.047

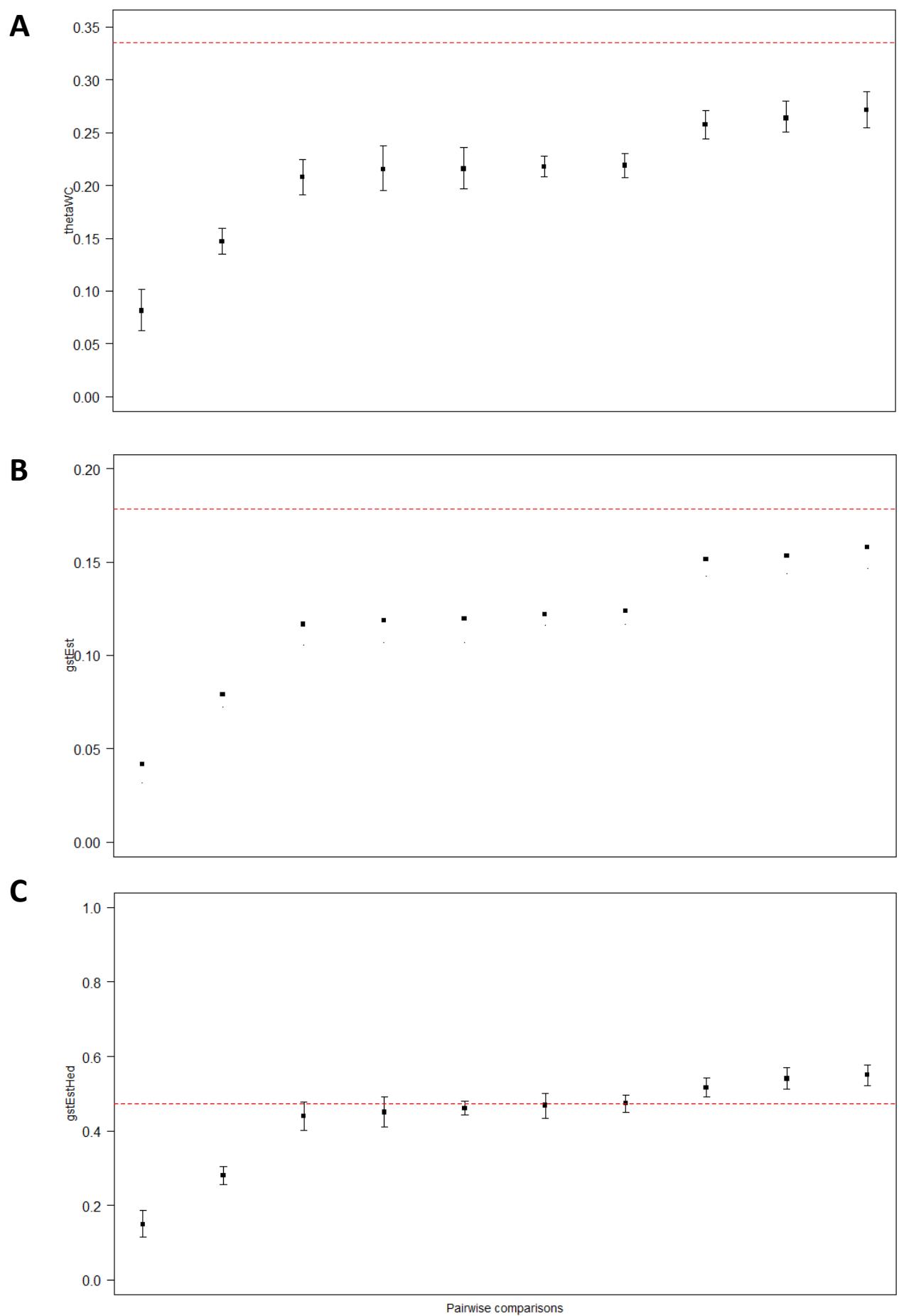
**Article title:** An integrative study of species distribution modelling and conservation genetics: *Magnolia* in Hispaniola.

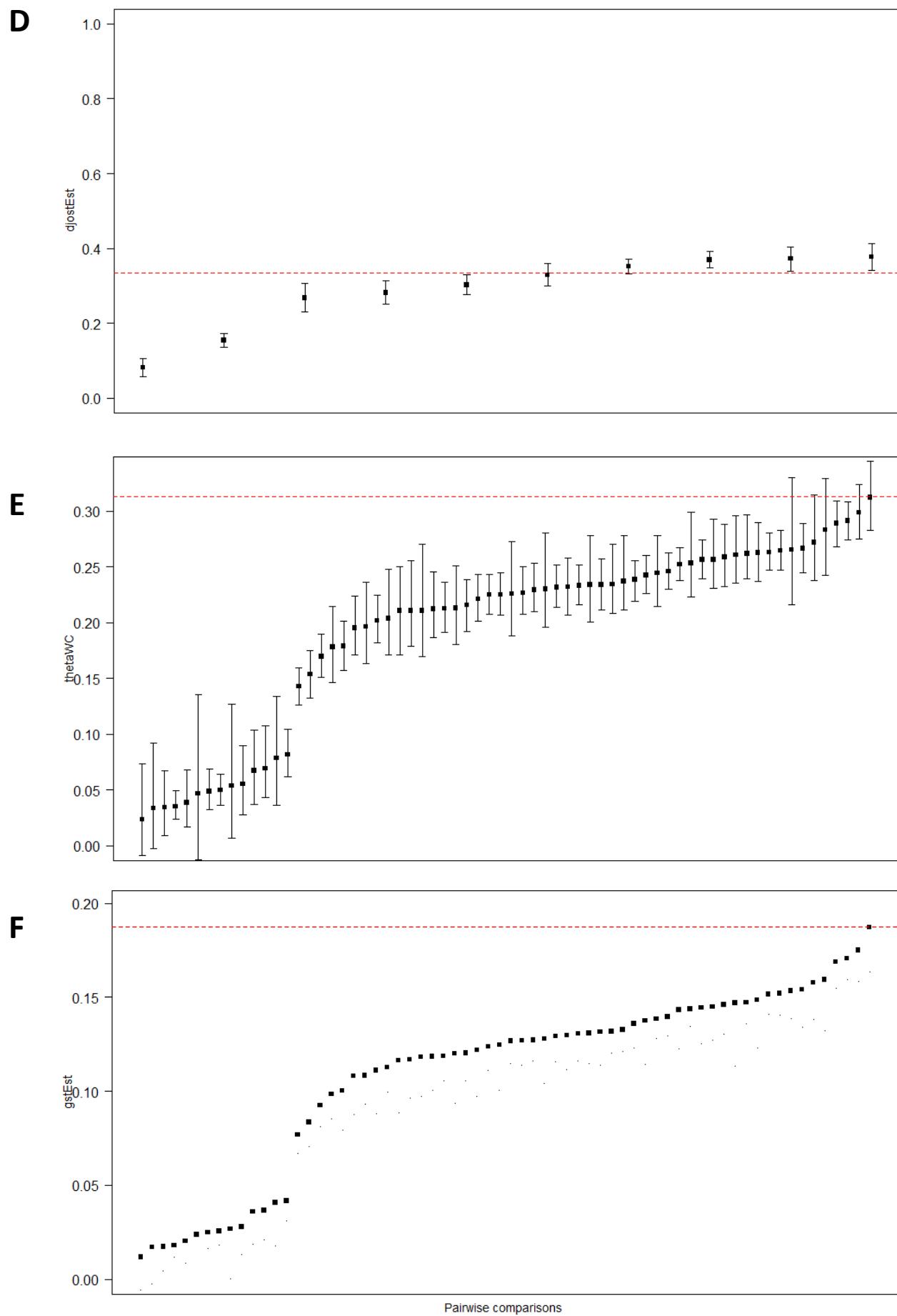
**Author names:** Tim Claerhout, Emily Veltjen, Diederik Strubbe, Ramón Elías Castillo Torres, Marie-Stéphanie Samain, Lars Chatrou

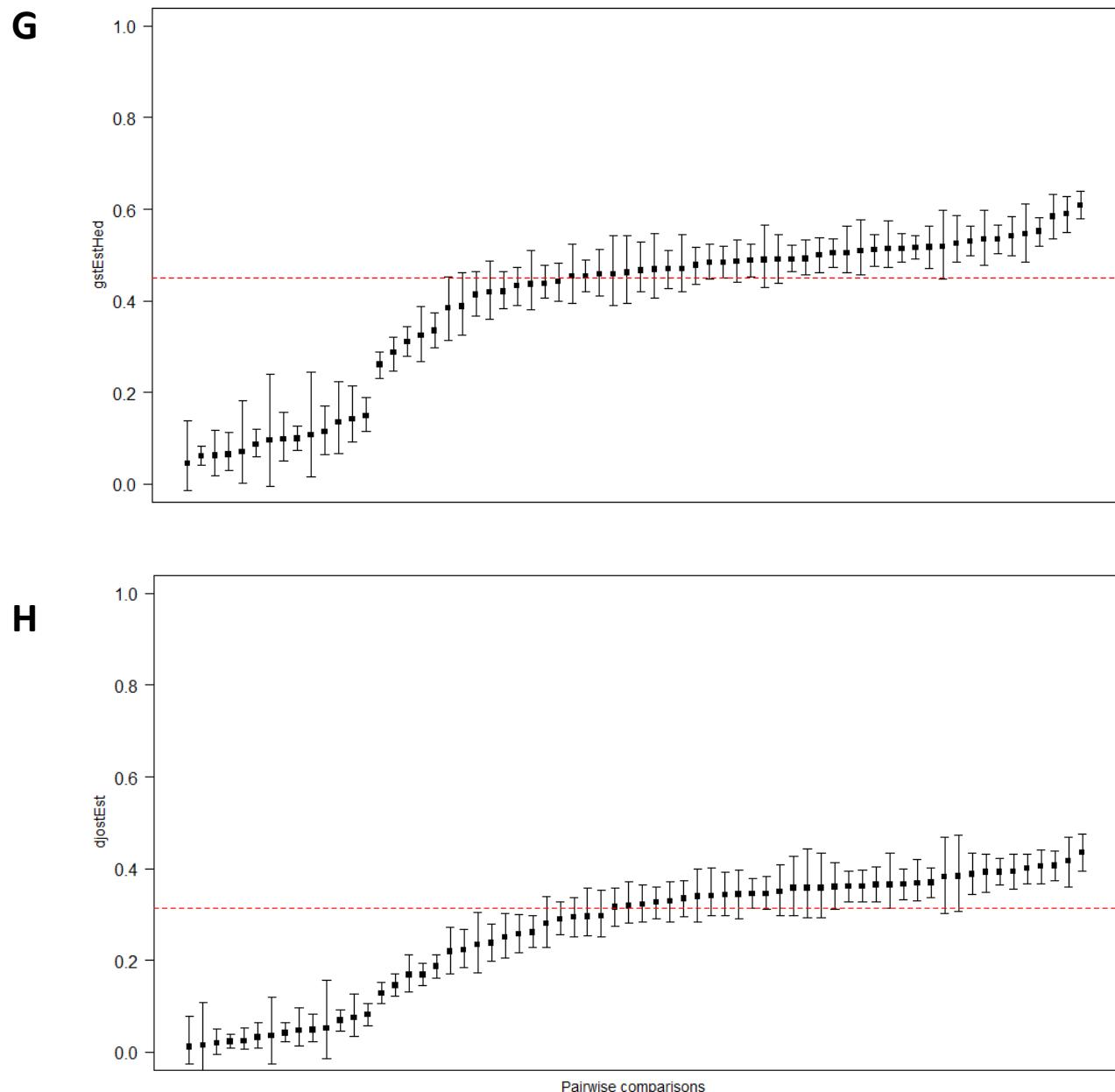
**Corresponding author, affiliation and e-mail address:** Tim Claerhout; Systematics and Evolutionary Botany lab, Research Group Spermatophytes, Department of Biology; Tim.Claerhout@UGent.be

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**Online Resource 9** 95% Confidence Intervals (CIs) of the pairwise fixation and allelic differentiation indices between populations (**A – D**) and between localities (**E – H**) from Table 4. Red dotted lines represent the global statistic value. **A.** Pairwise population  $F_{ST}$  (Weir and Cockerham 1984). **B.** Pairwise population  $G_{ST}$  (Nei and Chesser 1983). **C.** Pairwise population  $G'_{ST}$  (Hedrick 2005). **D.** Pairwise population Jost's D ( $D_J$ ; Jost 2008). **E – H:** same indices as **A – D**, respectively, but between localities.







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**Online Resource 10** Population and locality statistics for the Magnolias of the Dominican Republic across every SSR microsatellite marker. **Population/Locality.** Species, population/locality name and three letter code to uniquely identify a species, population or locality, following Table 1. **N<sub>0</sub>.** Number of sampled individuals. **A.** Average allelic diversity. **A<sub>R-X</sub>.** Allelic richness based on a minimum sample size of 6, 14 and 36 individuals. **A<sub>P</sub>.** Number of private alleles between all localities (**A<sub>PA</sub>**) or between populations only (**A<sub>PP</sub>**). **H<sub>O</sub>.** Average observed heterozygosity. **H<sub>E</sub>.** Average expected heterozygosity. **F<sub>IS</sub>.** Inbreeding coefficient. Significant ( $p = 0.05$ ) deviations from Hardy-Weinberg proportions are indicated with an asterix (\*). Monomorphic loci are indicated by "M." **P.** Number of polymorphic loci. **Pop:** Population statistics for each population and locality as a whole. **SE:** Standard error.

Population	SSR marker	Summary statistics						
		N	A	A <sub>R-36</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>S</sub>
<i>Magnolia domingensis</i> Loma Barbacoa (BAR)	MA39_023	36	4	4.000	1 (1)	0.583	0.568	-0.014
	MA39_165	36	2	2.000	0 (0)	0.028	0.027	0.000
	MA39_185	36	5	5.000	0 (0)	0.806	0.752	-0.057
	MA40_282	36	8	8.000	2 (0)	0.972	0.808	-0.190
	MA41_373	36	7	7.000	3 (0)	0.694	0.705	0.029
	MA42_001	36	5	5.000	3 (2)	0.806	0.672	-0.186
	MA42_059	36	2	2.000	0 (0)	0.389	0.346	-0.111
	MA42_126	36	2	2.000	0 (0)	0.472	0.497	0.063
	MA42_203	36	5	5.000	2 (0)	0.750	0.585	-0.268*
	MA42_231	36	5	5.000	1 (0)	0.722	0.752	0.053
	MA42_241	36	3	3.000	1 (1)	0.472	0.421	-0.108
	MA42_255	36	4	4.000	0 (0)	0.722	0.680	-0.048
	MA42_293	36	2	2.000	0 (0)	0.472	0.389	-0.202
	MA42_397	36	15	15.000	7 (0)	0.889	0.861	-0.018
	MA42_421	36	5	5.000	3 (0)	0.500	0.495	0.004
<i>Magnolia domingensis</i> Loma Rodríguez (ROD)	MA42_472	36	10	10.000	2 (0)	0.750	0.834	0.114*
	Pop	36	5.250	5.250	25 (4)	0.627	0.587	-0.054
	SE	0	0.864	0.864		0.058	0.055	
	MA39_023	50	4	3.720	1 (0)	0.700	0.670	-0.035
	MA39_165	50	2	2.000	0 (0)	0.360	0.403	0.117
	MA39_185	50	5	4.420	0 (0)	0.660	0.542	-0.209
	MA40_282	50	7	6.718	1 (0)	0.820	0.757	-0.074
	MA41_373	50	6	5.715	2 (1)	0.760	0.740	-0.017
	MA42_001	50	2	2.000	0 (0)	0.240	0.269	0.117
	MA42_059	50	2	1.924	0 (0)	0.040	0.039	-0.010
	MA42_126	50	2	2.000	0 (0)	0.460	0.455	-0.001
	MA42_203	50	3	3.000	0 (0)	0.660	0.615	-0.062
	MA42_231	50	6	5.720	2 (0)	0.660	0.733	0.110
	MA42_241	50	3	2.720	1 (1)	0.460	0.399	-0.142
	MA42_255	50	4	3.980	0 (0)	0.600	0.621	0.045
	MA42_293	50	2	2.000	0 (0)	0.100	0.13	0.241
	MA42_397	50	8	7.362	0 (0)	0.800	0.746	-0.062
	MA42_421	50	2	2.000	0 (0)	0.420	0.466	0.109
	MA42_472	50	9	8.567	1 (0)	0.860	0.792	-0.076
	Pop	50	4.188	3.990	8 (2)	0.538	0.524	-0.016
	SE	0	0.593	0.550		0.063	0.058	

Population	SSR marker	Summary statistics						
		N	A	A <sub>R-36</sub>	A <sub>PA</sub>	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia hamorii</i> Bahoruco (BAH)  N <sub>0</sub> = 118 P = 15	MA39_023	118	2	2.000	0	0.432	0.474	0.092
	MA39_165	118	1	1.000	0	0.000	0.000	M
	MA39_185	118	7	5.896	1	0.729	0.744	0.025
	MA40_282	118	17	14.959	7	0.839	0.886	0.057
	MA41_373	117	11	9.467	2	0.778	0.850	0.089
	MA42_001	118	4	3.304	1	0.568	0.569	0.007
	MA42_059	118	2	1.891	1	0.051	0.050	-0.022
	MA42_126	118	5	4.178	2	0.568	0.555	-0.018*
	MA42_203	118	9	8.062	2	0.712	0.823	0.139*
	MA42_231	118	8	7.179	3	0.788	0.781	-0.005
	MA42_241	118	2	2.000	0	0.466	0.481	0.035
	MA42_255	118	10	8.767	5	0.831	0.833	0.007
	MA42_293	118	11	9.205	6	0.788	0.830	0.055
	MA42_397	118	13	11.193	2	0.831	0.872	0.052
	MA42_421	118	2	1.518	1	0.017	0.017	-0.004
	MA42_472	118	25	19.130	13	0.873	0.916	0.051
	Pop	118	8.063	6.859	46	0.579	0.605	0.047
	SE	0	1.629	1.304		0.077	0.081	

Population	SSR marker	Summary statistics						
		N	A	A <sub>R-36</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia pallescens</i> Valle Nuevo (VAL)  N <sub>0</sub> = 133 P = 16	MA39_023	132	4	3.797	1 (0)	0.371	0.359	-0.031
	MA39_165	131	2	2.000	0 (0)	0.321	0.345	0.074
	MA39_185	133	9	6.891	0 (1)	0.624	0.599	-0.038
	MA40_282	131	9	7.073	3 (1)	0.748	0.784	0.050
	MA41_373	131	5	4.095	1 (0)	0.649	0.662	0.024*
	MA42_001	131	3	2.475	0 (0)	0.389	0.489	0.208
	MA42_059	129	3	2.662	0 (1)	0.031	0.090	0.656
	MA42_126	133	4	3.501	2 (0)	0.128	0.156	0.182
	MA42_203	133	9	7.486	4 (2)	0.692	0.723	0.047
	MA42_231	133	4	3.999	1 (0)	0.594	0.527	-0.123
	MA42_241	133	2	2.000	0 (0)	0.361	0.358	-0.006
	MA42_255	133	7	6.746	0 (0)	0.759	0.808	0.064
	MA42_293	131	4	3.001	1 (0)	0.466	0.517	0.102
	MA42_397	129	15	12.538	4 (1)	0.837	0.889	0.063
	MA42_421	131	7	5.821	4 (2)	0.740	0.705	-0.047
	MA42_472	133	13	11.790	0 (0)	0.729	0.820	0.114
<b>Pop</b>		131.688	6.250	5.367	21 (6)	0.527	0.552	0.048
<b>SE</b>		0.35	0.968	0.811		0.060	0.060	
<i>Magnolia pallescens</i> Ebano Verde (EBV)  N <sub>0</sub> = 80 P = 15	MA39_023	80	5	4.996	0 (1)	0.525	0.545	0.043
	MA39_165	80	2	2.000	0 (0)	0.325	0.289	-0.119
	MA39_185	80	7	6.402	2 (0)	0.775	0.795	0.031
	MA40_282	80	9	7.189	3 (0)	0.500	0.556	0.107
	MA41_373	80	4	3.450	2 (0)	0.488	0.572	0.154
	MA42_001	80	2	2.000	1 (0)	0.425	0.420	-0.006*
	MA42_059	80	2	2.000	1 (0)	0.288	0.312	0.086*
	MA42_126	80	6	5.348	0 (2)	0.538	0.529	-0.010*
	MA42_203	80	8	7.865	5 (2)	0.763	0.737	-0.028*
	MA42_231	80	5	4.908	0 (0)	0.625	0.635	0.021
	MA42_241	80	1	1.000	1 (0)	0.000	0.000	M
	MA42_255	80	5	5.000	2 (0)	0.788	0.776	-0.008*
	MA42_293	80	3	3.000	2 (0)	0.425	0.443	0.048
	MA42_397	80	17	14.828	2 (0)	0.838	0.900	0.076*
	MA42_421	79	9	8.676	2 (2)	0.696	0.726	0.047
	MA42_472	80	8	7.346	5 (0)	0.738	0.756	0.031*
<b>Pop</b>		79.938	5.813	5.375	28 (7)	0.546	0.562	0.035*
<b>SE</b>		0.063	0.993	0.861		0.056	0.058	

Locality	SSR marker	Summary statistics						
		N	A	A <sub>R-6</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia domingensis</i> Loma Barbacoa (BAR)	MA39_023	36	4	3.051	1 (1)	0.583	0.568	-0.014
	MA39_165	36	2	1.167	0 (0)	0.028	0.027	0.000
	MA39_185	36	5	4.340	0 (0)	0.806	0.752	-0.057
	MA40_282	36	8	5.257	2 (0)	0.972	0.808	-0.190
	MA41_373	36	7	4.495	3 (0)	0.694	0.705	0.029
	MA42_001	36	5	3.778	3 (2)	0.806	0.672	-0.186
	MA42_059	36	2	1.964	0 (0)	0.389	0.346	-0.111
	MA42_126	36	2	2.000	0 (0)	0.472	0.497	0.063
	MA42_203	36	5	3.247	2 (0)	0.750	0.585	-0.268*
	MA42_231	36	5	4.431	1 (0)	0.722	0.752	0.053
	MA42_241	36	3	2.153	1 (1)	0.472	0.421	-0.108
	MA42_255	36	4	3.657	0 (0)	0.722	0.680	-0.048
	MA42_293	36	2	1.983	0 (0)	0.472	0.389	-0.202
	MA42_397	36	15	6.937	7 (0)	0.889	0.861	-0.018
	MA42_421	36	5	2.768	3 (0)	0.500	0.495	0.004
	MA42_472	36	10	6.125	2 (0)	0.750	0.834	0.114*
	Pop	36	5.250	3.585	25 (4)	0.627	0.587	-0.054
	SE	0	0.864	0.405		0.058	0.055	
<i>Magnolia domingensis</i> Loma Rodríguez (ROD)	MA39_023	50	4	3.100	1 (0)	0.700	0.670	-0.035
	MA39_165	50	2	1.985	0 (0)	0.360	0.403	0.117
	MA39_185	50	5	2.560	0 (0)	0.660	0.542	-0.209
	MA40_282	50	7	4.703	1 (0)	0.820	0.757	-0.074
	MA41_373	50	6	4.178	2 (1)	0.760	0.740	-0.017
	MA42_001	50	2	1.892	0 (0)	0.240	0.269	0.117
	MA42_059	50	2	1.227	0 (0)	0.040	0.039	-0.010
	MA42_126	50	2	1.996	0 (0)	0.460	0.455	-0.001
	MA42_203	50	3	2.903	0 (0)	0.660	0.615	-0.062
	MA42_231	50	6	4.269	2 (0)	0.660	0.733	0.110
	MA42_241	50	3	2.099	1 (1)	0.460	0.399	-0.142
	MA42_255	50	4	3.214	0 (0)	0.600	0.621	0.045
	MA42_293	50	2	1.603	0 (0)	0.100	0.13	0.241
	MA42_397	50	8	4.478	0 (0)	0.800	0.746	-0.062
	MA42_421	50	2	1.997	0 (0)	0.420	0.466	0.109
	MA42_472	50	9	5.352	1 (0)	0.860	0.792	-0.076
	Pop	50	4.188	2.972	8 (2)	0.538	0.524	-0.016
	SE	0	0.593	0.316		0.063	0.058	

Locality	SSR marker	Summary statistics						
		N	A	A <sub>R-6</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>S</sub>
<i>Magnolia hamorii</i> Cachote (CAC)  N <sub>0</sub> = 52 P = 13	MA39_023	52	2	1.982	0 (0)	0.462	0.393	-0.163
	MA39_165	52	1	1.000	0 (0)	0.000	0.000	M
	MA39_185	52	7	3.874	1 (0)	0.673	0.713	0.065
	MA40_282	52	16	6.775	2 (2)	0.827	0.851	0.038
	MA41_373	52	10	6.203	2 (2)	0.769	0.833	0.086
	MA42_001	52	4	2.702	1 (0)	0.596	0.565	-0.046
	MA42_059	52	1	1.000	0 (0)	0.000	0.000	M
	MA42_126	52	4	2.579	0 (0)	0.654	0.539	-0.205
	MA42_203	52	8	4.818	0 (0)	0.712	0.719	0.021
	MA42_231	52	7	4.982	0 (0)	0.788	0.791	0.013
	MA42_241	52	2	1.987	0 (0)	0.423	0.411	-0.021
	MA42_255	52	9	5.649	0 (0)	0.827	0.819	0.000
	MA42_293	52	11	5.744	3 (2)	0.750	0.819	0.094*
	MA42_397	52	10	6.389	0 (0)	0.885	0.859	-0.020
	MA42_421	52	1	1.000	0 (0)	0.000	0.000	M
	MA42_472	52	20	7.743	2 (1)	0.827	0.893	0.084
	<b>Pop</b>	52	7.063	4.027	11 (7)	0.575	0.575	0.011
	<b>SE</b>	0	1.392	0.572		0.078	0.081	
<i>Magnolia hamorii</i> Cortico (COR)  N <sub>0</sub> = 52 P = 14	MA39_023	52	2	2.000	0 (0)	0.385	0.499	0.239
	MA39_165	52	1	1.000	0 (0)	0.000	0.000	M
	MA39_185	52	6	4.090	0 (0)	0.769	0.722	-0.055
	MA40_282	52	14	7.388	1 (1)	0.827	0.889	0.080
	MA41_373	52	9	5.818	1 (0)	0.808	0.823	0.028
	MA42_001	52	3	2.734	0 (0)	0.500	0.511	0.032
	MA42_059	52	2	1.392	0 (0)	0.077	0.074	-0.030
	MA42_126	52	4	2.593	0 (0)	0.481	0.473	-0.008
	MA42_203	52	8	5.424	0 (0)	0.731	0.816	0.114*
	MA42_231	52	7	4.859	1 (0)	0.750	0.743	0.000
	MA42_241	52	2	2.000	0 (0)	0.500	0.499	0.008
	MA42_255	52	9	5.552	1 (1)	0.846	0.831	-0.009
	MA42_293	52	8	5.844	0 (0)	0.808	0.820	0.025
	MA42_397	52	12	6.363	2 (1)	0.846	0.832	-0.007
	MA42_421	52	1	1.000	0 (0)	0.000	0.000	M
	MA42_472	52	18	8.011	2 (2)	0.923	0.903	-0.013
	<b>Pop</b>	52	6.625	4.129	8 (5)	0.578	0.590	0.029
	<b>SE</b>	0	1.251	0.582		0.079	0.079	

Locality	SSR marker	Summary statistics						
		N	A	A <sub>R-6</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia hamorii</i> Laguneta (LAG)  N <sub>0</sub> = 7 P = 14	MA39_023	7	2	2.000	0 (0)	0.571	0.408	-0.333
	MA39_165	7	1	1.000	0 (0)	0.000	0.000	M
	MA39_185	7	3	2.989	0 (0)	0.857	0.602	-0.358
	MA40_282	7	10	8.978	0 (0)	0.857	0.878	0.100
	MA41_373	7	3	3.000	0 (0)	0.571	0.663	0.213
	MA42_001	7	2	2.000	0 (0)	0.714	0.459	-0.500
	MA42_059	7	2	1.989	0 (0)	0.286	0.245	-0.091
	MA42_126	7	4	3.714	0 (0)	0.571	0.622	0.158
	MA42_203	7	5	4.703	1 (1)	0.714	0.704	0.063
	MA42_231	7	5	4.835	0 (0)	1.000	0.724	-0.313
	MA42_241	7	2	2.000	0 (0)	0.714	0.500	-0.364
	MA42_255	7	4	3.857	0 (0)	0.714	0.694	0.048
	MA42_293	7	4	3.857	0 (0)	0.857	0.694	-0.161
	MA42_397	7	4	3.846	0 (0)	0.571	0.643	0.186
	MA42_421	7	1	1.000	0 (0)	0.000	0.000	M
	MA42_472	7	8	7.264	0 (0)	0.857	0.806	0.014
	Pop	7	3.750	3.565	1 (1)	0.616	0.540	-0.064
	SE	0	0.609	0.539		0.073	0.065	
<i>Magnolia hamorii</i> Tonobán (TON)  N <sub>0</sub> = 7 P = 14	MA39_023	7	2	2.000	0 (0)	0.429	0.459	0.143
	MA39_165	7	1	1.000	0 (0)	0.000	0.000	M
	MA39_185	7	4	4.000	0 (0)	0.714	0.745	0.118
	MA40_282	7	10	8.978	0 (0)	1.000	0.878	-0.063
	MA41_373	6	6	6.000	0 (0)	0.833	0.750	-0.020
	MA42_001	7	3	2.857	0 (0)	0.714	0.541	-0.250
	MA42_059	7	1	1.000	0 (0)	0.000	0.000	M
	MA42_126	7	4	3.714	1 (0)	0.571	0.531	0
	MA42_203	7	5	4.835	0 (0)	0.571	0.745	0.304
	MA42_231	7	5	4.703	0 (0)	0.857	0.704	-0.143
	MA42_241	7	2	2.000	0 (0)	0.286	0.490	0.478
	MA42_255	7	6	5.560	0 (0)	0.857	0.735	-0.091
	MA42_293	7	5	4.714	0 (0)	0.857	0.735	-0.091
	MA42_397	7	6	5.692	0 (0)	0.571	0.776	0.333
	MA42_421	7	2	1.989	1 (1)	0.286	0.245	-0.091
	MA42_472	7	8	7.407	2 (2)	0.857	0.847	0.065
	Pop	6.938	4.375	4.153	4 (3)	0.588	0.574	0.053
	SE	0.063	0.632	0.572		0.078	0.069	

Locality	SSR marker	Summary statistics						
		N	A	A <sub>R-6</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia pallescens</i> Casabito (CAS)  N <sub>0</sub> = 40 P = 15	MA39_023	40	5	3.334	0 (0)	0.525	0.480	-0.081
	MA39_165	40	2	1.933	0 (0)	0.325	0.305	-0.054
	MA39_185	40	6	4.487	0 (0)	0.800	0.751	-0.052
	MA40_282	40	7	3.538	1 (0)	0.525	0.534	0.030
	MA41_373	40	3	2.631	0 (0)	0.475	0.535	0.125
	MA42_001	40	2	1.997	0 (0)	0.425	0.462	0.093
	MA42_059	40	2	1.977	0 (0)	0.350	0.375	0.079
	MA42_126	40	5	3.095	0 (0)	0.550	0.529	-0.028
	MA42_203	40	7	4.522	1 (1)	0.775	0.744	-0.029
	MA42_231	40	5	3.616	1 (0)	0.650	0.552	-0.165
	MA42_241	40	1	1.000	0 (0)	0.000	0.000	M
	MA42_255	40	5	4.406	0 (0)	0.725	0.746	0.041
	MA42_293	40	3	2.730	0 (0)	0.475	0.524	0.106
	MA42_397	40	13	6.653	1 (0)	0.800	0.848	0.069
	MA42_421	40	8	3.282	0 (0)	0.475	0.476	0.014
	MA42_472	40	8	5.022	0 (0)	0.850	0.788	-0.065
	Pop	40	5.125	3.389	4 (1)	0.545	0.541	0.004
	SE	0	0.769	0.352		0.055	0.053	
<i>Magnolia pallescens</i> Entrance (ENT)  N <sub>0</sub> = 61 P = 16	MA39_023	61	4	2.143	0 (0)	0.230	0.224	-0.016
	MA39_165	61	2	1.952	0 (0)	0.295	0.335	0.128
	MA39_185	61	6	2.912	0 (0)	0.574	0.489	-0.166
	MA40_282	61	5	4.236	0 (0)	0.754	0.723	-0.035
	MA41_373	61	3	2.965	0 (0)	0.672	0.648	-0.030
	MA42_001	61	2	1.995	0 (0)	0.426	0.451	0.064
	MA42_059	59	3	1.865	1 (1)	0.051	0.173	0.710*
	MA42_126	61	4	1.784	0 (0)	0.131	0.140	0.071
	MA42_203	61	8	4.477	1 (0)	0.672	0.723	0.079*
	MA42_231	61	4	3.466	0 (0)	0.705	0.603	-0.162
	MA42_241	61	2	1.945	0 (0)	0.377	0.326	-0.149
	MA42_255	61	6	4.438	0 (0)	0.639	0.756	0.162*
	MA42_293	61	4	2.282	1 (0)	0.426	0.487	0.134
	MA42_397	58	13	6.791	0 (0)	0.776	0.866	0.113*
	MA42_421	61	4	3.730	0 (0)	0.738	0.682	-0.074
	MA42_472	61	13	6.598	1 (0)	0.852	0.836	-0.011
	Pop	60.688	5.188	3.349	4 (1)	0.520	0.529	0.025*
	SE	0.218	0.862	0.404		0.062	0.059	

Locality	SSR marker	Summary statistics						
		N	A	A <sub>R-6</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia pallescens</i> Camino Guayabal (GUA)	MA39_023	14	4	2.540	0 (0)	0.286	0.258	-0.072
	MA39_165	14	2	1.911	0 (0)	0.286	0.245	-0.130
	MA39_185	15	6	3.985	1 (1)	0.667	0.527	-0.233
	MA40_282	14	3	2.827	0 (0)	0.500	0.559	0.142
	MA41_373	14	4	3.51	1 (0)	0.714	0.630	-0.097
	MA42_001	14	2	1.996	0 (0)	0.286	0.408	0.333
	MA42_059	14	1	1.000	0 (0)	0.000	0.000	M
	MA42_126	15	3	2.384	0 (0)	0.400	0.407	0.051
	MA42_203	15	6	3.939	0 (0)	0.667	0.558	-0.162
	MA42_231	15	4	3.185	0 (0)	0.533	0.436	-0.191
	MA42_241	15	2	1.984	0 (0)	0.467	0.358	-0.273
	MA42_255	15	5	4.571	0 (0)	0.733	0.753	0.061
	MA42_293	14	2	2.000	0 (0)	0.357	0.497	0.316
	MA42_397	15	10	5.831	0 (0)	0.867	0.764	-0.100
	MA42_421	14	4	3.806	0 (0)	0.857	0.712	-0.169
	MA42_472	15	7	4.695	0 (0)	0.667	0.609	-0.061
	<b>Pop</b>	14.500	4.063	3.135	2 (1)	0.518	0.482	-0.038
	<b>SE</b>	0.129	0.581	0.321		0.060	0.051	
<i>Magnolia pallescens</i> Loma de la Sal (SAL)	MA39_023	40	5	3.902	0 (0)	0.525	0.597	0.133
	MA39_165	40	2	1.900	0 (0)	0.325	0.272	-0.182
	MA39_185	40	7	5.018	0 (0)	0.750	0.798	0.072
	MA40_282	40	8	3.551	1 (0)	0.475	0.572	0.182
	MA41_373	40	4	3.039	0 (0)	0.500	0.582	0.154*
	MA42_001	40	2	1.971	0 (0)	0.425	0.362	-0.161
	MA42_059	40	2	1.854	0 (0)	0.225	0.237	0.064
	MA42_126	40	6	3.583	1 (1)	0.525	0.515	-0.007
	MA42_203	40	6	4.375	1 (0)	0.750	0.669	-0.108
	MA42_231	40	4	3.388	0 (0)	0.600	0.650	0.089
	MA42_241	40	1	1.000	0 (0)	0.000	0.000	M
	MA42_255	40	5	4.634	0 (0)	0.850	0.786	-0.069
	MA42_293	40	3	2.269	0 (0)	0.375	0.316	-0.175
	MA42_397	40	16	7.795	0 (0)	0.875	0.901	0.041
	MA42_421	39	9	6.299	1 (1)	0.923	0.851	-0.071
	MA42_472	40	7	4.400	0 (0)	0.625	0.667	0.076
	<b>Pop</b>	39.938	5.438	3.686	4 (2)	0.547	0.548	0.016
	<b>SE</b>	0.063	0.917	0.441		0.062	0.063	

Locality	SSR marker	Summary statistics						
		N	A	A <sub>R-6</sub>	A <sub>PP</sub> (A <sub>PA</sub> )	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>
<i>Magnolia pallescens</i> Montellano (MON)  N <sub>0</sub> = 41 P = 15	MA39_023	41	4	2.925	0 (0)	0.561	0.505	-0.099
	MA39_165	41	2	1.960	0 (0)	0.341	0.343	0.016
	MA39_185	41	7	3.924	0 (0)	0.707	0.667	-0.047
	MA40_282	41	9	5.269	1 (1)	0.805	0.792	-0.003
	MA41_373	41	4	3.236	0 (0)	0.610	0.662	0.090
	MA42_001	41	2	2.000	0 (0)	0.390	0.493	0.220
	MA42_059	41	1	1.000	0 (0)	0.000	0.000	M
	MA42_126	41	3	1.293	0 (0)	0.049	0.048	-0.006
	MA42_203	41	7	4.191	1 (1)	0.707	0.690	-0.012
	MA42_231	41	4	2.607	0 (0)	0.390	0.336	-0.150
	MA42_241	41	2	1.928	0 (0)	0.268	0.299	0.115
	MA42_255	41	6	4.720	0 (0)	0.854	0.790	-0.068
	MA42_293	41	3	2.146	0 (0)	0.610	0.511	-0.181
	MA42_397	41	13	7.131	0 (0)	0.902	0.884	-0.009
	MA42_421	41	7	4.601	1 (1)	0.634	0.726	0.139
	MA42_472	41	9	5.993	0 (0)	0.610	0.843	0.288*
	<b>Pop</b>	41	5.188	3.433	3 (3)	0.527	0.537	0.030*
	<b>SE</b>	0	0.823	0.443		0.067	0.068	
<i>Magnolia pallescens</i> Rancho Guaraguao (RAN)  N <sub>0</sub> = 16 P = 16	MA39_023	16	4	2.949	0 (0)	0.500	0.443	-0.096
	MA39_165	15	2	1.999	0 (0)	0.400	0.444	0.134
	MA39_185	16	5	3.366	0 (0)	0.563	0.604	0.100
	MA40_282	15	4	3.776	0 (0)	0.800	0.704	-0.102
	MA41_373	15	4	3.288	0 (0)	0.600	0.629	0.080
	MA42_001	15	3	2.645	1 (0)	0.333	0.504	0.369*
	MA42_059	15	2	1.400	0 (0)	0.067	0.064	0.000
	MA42_126	16	3	1.992	0 (0)	0.063	0.174	0.659*
	MA42_203	16	7	5.468	0 (0)	0.750	0.789	0.082
	MA42_231	16	4	3.551	0 (0)	0.750	0.631	-0.158
	MA42_241	16	2	2.000	0 (0)	0.438	0.498	0.153
	MA42_255	16	6	5.000	0 (0)	1.000	0.785	-0.244
	MA42_293	15	3	2.369	0 (0)	0.333	0.371	0.136
	MA42_397	15	10	7.155	0 (0)	0.867	0.864	0.032
	MA42_421	15	5	3.598	0 (0)	0.933	0.627	-0.463*
	MA42_472	16	8	5.389	0 (0)	0.625	0.727	0.171
	<b>Pop</b>	15.500	4.500	3.496	1 (0)	0.564	0.554	0.015
	<b>SE</b>	0.129	0.570	0.391		0.071	0.055	