UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Use of molecular markers to describe the invasion history of *Cedrela odorata* L. in Galapagos, Ecuador

Martina Isabel Albuja Quintana

Biología

Trabajo de fin de carrera presentado como requisito
para la obtención del título de
Biología, concentración en Biología Molecular y Microbiología

Quito, 21 de mayo de 2021

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

HOJA DE CALIFICACIÓN
DE TRABAJO DE FIN DE CARRERA

Use of molecular markers to describe the invasion history of *Cedrela odorata* L. in Galapagos, Ecuador

Martina Isabel Albuja Quintana

Nombre del profesor, Título académico	**Gonzalo Rivas-Torres, PhD.**
Nombre del profesor, Título académico	**María de Lourdes Torres, PhD.**

Quito, 21 de mayo de 2021

© DERECHOS DE AUTOR

Nombres y apellidos: Martina Isabel Albuja Quintana

Código: 00200798

Cédula de identidad: 1724373905

Lugar y fecha: Quito, 21 de mayo de 2021

ACLARACIÓN PARA PUBLICACIÓN

Nota: El presente trabajo, en su totalidad o cualquiera de sus partes, no debe ser considerado como una publicación, incluso a pesar de estar disponible sin restricciones a través de un repositorio institucional. Esta declaración se alinea con las prácticas y recomendaciones presentadas por el Committee on Publication Ethics COPE descritas por Barbour et al. (2017) Discussion document on best practice for issues around theses publishing, disponible en http://bit.ly/COPETheses.

UNPUBLISHED DOCUMENT

Note: The following capstone project is available through Universidad San Francisco de Quito USFQ institutional repository. Nonetheless, this project – in whole or in part – should not be considered a publication. This statement follows the recommendations presented by the Committee on Publication Ethics COPE described by Barbour et al. (2017) Discussion document on best practice for issues around theses publishing available on http://bit.ly/COPETheses.

AGRADECIMIENTOS

A mi familia, especialmente a mis padres y hermana, por el apoyo, confianza, guía y cariño. Por ser mi inspiración, mi pilar y por siempre creer en mí. Sin ellos nada de esto hubiera sido posible.

A mis amigos con los que viví momentos inolvidables, a los que estuvieron conmigo desde el primer día y siempre me ayudaron cuando lo necesité.

A Gonzalo Rivas-Torres, por darme la oportunidad de participar en este proyecto, por apoyarme y por la mentoría recibida. A María de Lourdes Torres, por brindarme la oportunidad de realizar el proyecto en conjunto con el laboratorio y guiarme a lo largo del proyecto. Al Laboratorio de Biotecnología Vegetal, por las enseñanzas, consejos y apoyo constante. En especial a Estefanía Rojas y a María Paula Erazo, por realizar los métodos del laboratorio del proyecto, por guiarme en la realización de los análisis y por la ayuda y consejos invaluables. Además, quiero extender mi agradecimiento a María Paula Erazo por realizar los análisis del cuarto objetivo específico de este proyecto.

A todos mis profesores, por los conocimientos y pasión impartidos en estos 4 años, en especial a Nelson Miranda, por las oportunidades y apoyo recibido a lo largo de mi tiempo en la universidad.

Al Dr. Stephen Cavers, del UK Centre for Ecology & Hydrology, por facilitar el acceso muy amablemente a los datos de su investigación que fueron incluidos como parte de los análisis.

A Walter Palacios, Janeth Santiana, Génesis Vinueza, Pacarina Asadobay y a todos los colaboradores que hicieron posible la realización de esta investigación.

Finalmente, quisiera agradecer al Colegio de Ciencias Biológicas y Ambientales de la USFQ por concedernos los fondos necesarios para realizar este proyecto y al Ministerio del Ambiente y Parque Nacional Galápagos por otorgarnos los permisos para la investigación.

RESUMEN

Cedrela odorata L. (Meliaceae) es un árbol semideciduo neotropical, ampliamente cotizado por su madera. En Galápagos, es considerada una especie invasora. Se conoce que fue introducida en los años 40 a la zona agrícola de Santa Cruz, encontrándose ahora en las cuatro islas habitadas del archipiélago. Entender la historia de invasión de esta especie podría ayudar a desarrollar planes de manejo que permitan controlar su propagación e impactos esperados. Por ello, el objetivo de esta investigación fue establecer la diversidad genética y estructura poblacional de *C. odorata* en Galápagos y esclarecer el origen y número de veces que ingresó al archipiélago. Para esto, se extrajo ADN de hojas de 40 individuos de Galápagos y 32 muestras de Ecuador Continental y amplificó 9 microsatélites homólogos. Los resultados revelaron una heterocigosidad esperada global de 0.55; valor menor a lo reportado para continente, pero mayor a otras especies de plantas invasoras insulares, sugiriendo múltiples eventos de introducción. Los valores medios a altos de diferenciación genética permitieron preliminarmente evidenciar que existe estructura a esta escala entre las islas. Por otro lado, los PCoA indicaron que *C. odorata* de Galápagos es genéticamente más parecida a las muestras de la Costa del Ecuador mientras que, usando matrices de microsatélites para otras regiones del neotrópico; no se encontró una asociación genética clara entre las poblaciones de otras regiones con respecto a las de Galápagos. En conclusión, la diversidad genética de *C. odorata* en Galápagos es moderadamente alta con aparente estructura y diferenciación existente entre las islas. Asimismo, este estudio permite interpretar preliminarmente que, es posible que este árbol haya ingresado desde la Costa del Ecuador de una subpoblación nativa, ya que inicialmente, no se encontró evidencia de una posible introducción secundaria. Sin embargo, se recomienda a futuro como complemento a este estudio, aumentar el número de muestras de la Costa y realizar análisis a nivel genómico para esclarecer estos resultados.

Palabras clave: *Cedrela odorata*, Galápagos, diversidad genética, historia de invasión, especie invasora, microsatélites, estructura poblacional, Cedro.

ABSTRACT

Cedrela odorata L. (Meliaceae) is a semi-deciduous neotropical tree, widely valued for its timber. In Galapagos, it is considered an invasive species. Historical records mention that it was introduced in the 1940s to the agricultural area of Santa Cruz and can now be found on the four inhabited islands of the archipelago. Understanding the invasion history of this species could help in the development of management plans to control its propagation and expected impacts. Thus, the objective of this investigation was to establish the genetic diversity and population structure of *C. odorata* in Galapagos and to elucidate the origin and number of introduction events. For this, leaves of 40 individuals collected in 4 islands of Galapagos and 32 samples from mainland Ecuador were used to extract DNA and amplify 9 homologous microsatellite loci. The results revealed a global expected heterozygosity of 0.55; lower than that reported for the mainland, but higher than other invasive insular plant species, suggesting multiple introductory events. The moderate to high values of genetic differentiation provide preliminary evidence of structure at this scale between the islands. Furthermore, the PCoA indicated that the *C. odorata* in Galapagos are genetically more similar to the samples from the Coast of Ecuador while, using microsatellite matrices for other neotropical regions where this species has been reported; found no clear genetic association between the populations of other regions and those of Galapagos. In conclusion, the genetic diversity of *C. odorata* in Galapagos is moderately high with apparent structure and differentiation between the islands. Likewise, this study allows us to preliminary interpret that it is possible that this tree was introduced from the Coast of Ecuador from a native subpopulation since initially, no evidence of a possible secondary introduction was found. However, in the future and to complement this study, the analysis of more samples from the Coast and further genomic analyses are recommended to clarify these results.

Key words: *Cedrela odorata*, Galapagos, genetic diversity, invasion history, invasive species, microsatellites, population structure, Spanish Cedar.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Invasive plant species are introduced species that successfully establish and disperse outside their native distribution (Diagne et al., 2021). They are also considered to have a significant negative effect on biodiversity, ecosystems (Jahodová et al., 2007; Tobin, 2018; Vilà & Weiner, 2004), and the economy with around $26.8 billion dollars invested every year worldwide in management and control plans (Diagne et al., 2021). Their success and impact usually depends on their ecology, adaptability and features of the invaded environment (Shi & Ma, 2006; Vilà & Weiner, 2004). In general, these species are known to disturb and change ecosystem structure and function, community interactions, resource availability and to outcompete native organisms (Pyšek & Richardson, 2010; Rentería et al., 2012).

These same ripple effects have been seen and studied in invasive plant species in the Galapagos islands (Rentería et al., 2012; Urquía et al., 2019) where a higher number of introduced plant species (~891 spp.) have been reported in relation to the native plants (~599 spp.); 15% of which are now considered invasive (Rivas-Torres et al., 2018). They pose an even greater threat than in mainland environments because insular ecosystems are intrinsically more sensitive to biological invasions. This happens because of their low species richness, simple trophic composition and high habitat availability compared to these mainland systems (Pearson, 2009).

Cedrela odorata L., an important source of timber in the Galapagos islands (Gardener et al., 2013), is now considered the second most invasive tree species in the archipelago because of the great extensions of land it occupies (Rivas-Torres, personal communication, 2021). It not only outcompetes endemic tree species like *Scalesia* (Laso et al., 2020), but transforms Galapagos' ecosystems (Rivas-Torres et al., 2018) and easily propagates and colonizes new areas with its light winged seeds (Mauchamp, 1997). Despite that its ecology, effect and impacts have been widely studied (Gardener et al., 2013; Laso et al., 2020;

Rentería & Buddenhagen, 2006; Rivas-Torres et al., 2018), little is known about its invasion history and source of origin (Lundh, 2006; Vinueza, 2020); information that could be useful for designing effective management plans to control its spread and expected impact.

Studies have mentioned that identifying or inferring the source of origin as well as history and introduction events of an invasive plant species, is important for developing effective control and management plans (Lawson Handley et al., 2011; Le Roux & Wieczorek, 2008; Roderick & Navajas, 2003); sometimes even considered the first step when characterizing an invasion (Fieldsend et al., 2021). This kind of information is especially relevant for biological control approaches that seek to pinpoint natural enemies that could potentially be used as biocontrol agents in the introduced range (Prentis et al., 2009). Many believe that the less harmful for the native biota, most effective and host specific natural enemies are those that originate from the locality from where the invaded population was originally introduced from (Le Roux & Wieczorek, 2008; Roderick & Navajas, 2003). Although biocontrol agents can sometimes be effective in various localities, this is not always the case. Failed strategies have been reported in places that used the same natural enemies that were effective in others (Shea et al., 2005). This can happen because some biological control agents can differentiate between (Muller Scharer et al., 2004) and sometimes prefer a variant or genotype over another (Le Roux & Wieczorek, 2008). This can be the case for introduced species that genetically change due to the effect of factors like founder effects or the conditions of the new environment (Muller Scharer et al., 2004).

Molecular markers and genetic techniques can be useful tools for describing biological invasions (Bossdorf et al., 2005). They can help infer the source of origin (Roderick & Navajas, 2003), routes of invasion (Lawson Handley et al., 2011; Roderick & Navajas, 2003), genetic diversity of the invasive population (Bossdorf et al., 2005; Le Roux & Wieczorek, 2008) and presence of single or multiple genetic lineages (Fieldsend et al.,

2021; Roderick & Navajas, 2003). These tools can be particularly useful for species that lack enough information about their history of invasion in historical records (Le Roux & Wieczorek, 2008) or to clarify and complement documented data (Lawson Handley et al., 2011).

Microsatellites are one of the most widely used markers in studies about genetic diversity and population structure (Huamán, 2014), mainly because of their high variability and mutation rates (Le Roux & Wieczorek, 2008). These markers are short noncoding sequences of DNA tandemly repeated (Huamán, 2014) whose alleles vary in the number of repetitions of the motif or repeat unit (Guichoux et al., 2011). They are highly informative markers for population genetic studies because they are codominant (Hernández et al., 2008), not influenced by selective pressures and are abundant and distributed along the genome (Huamán, 2014; Le Roux & Wieczorek, 2008). They also present high numbers of alleles per locus (Huamán, 2014) and are flanked by conserved regions that facilitate their amplification (Le Roux & Wieczorek, 2008). This has led to them being commonly employed to infer the origin, invasion routes, dispersal and genetic diversity of introduced or invasive species (Roderick & Navajas, 2003),

Using this background information, the main goal of this investigation is to provide, using molecular markers, preliminary and relevant evidence about the invasion history of *Cedrela odorata* L. in Galapagos. Specifically, the objectives of this study were **(1)** to establish and describe the structure, connectivity and genetic diversity of *C. odorata* in the archipelago; **(2)** to elucidate the region of Ecuador from where *C. odorata* was introduced to the Galapagos Islands through the use of molecular tools; **(3)** to infer the number of times that *C. odorata* was introduced to the Galapagos Islands through the analysis of the genetic variation of its populations; and, **(4)** to establish a preliminary analysis of the genetic affinity

of the populations of *C. odorata* in Galapagos within the native Neotropical distribution (Continental Ecuador and other regions) of this species.

2. STUDY AREA

Galapagos is an archipelago of volcanic islands located 1000 km west of the Pacific Coast of Ecuador (Rivas-Torres & Rivas, 2018). It is made up of 13 major islands, 5 minor islands and 216 islets and rocks (DPNG, 2014) of which only Santa Cruz, San Cristóbal, Isabela and Floreana are inhabited (Laso et al., 2020). The Galapagos National Park occupies 97% of the archipelago while human settlements and agricultural and livestock fields found primarily in the highlands, cover the remaining 3% (Gardener et al., 2010). The highlands of the islands are characterized by temperatures between 16 and 20ºC, 85 to 93% humidity and a greater diversity of plants than the lowlands (Laso et al., 2020). In Galapagos, 40% of all vascular plants are endemic (Rivas-Torres et al., 2018) with a higher proportion of endemic species found within the highlands of the islands (Johnson & Raven, 1973). Further, Galapagos presents certain seasonality throughout the year with a dry season between January and May and a wet season between June and December (Itow, 1992). Galapagos' ecosystems are spatially distributed from the coast where deciduous forests dominate, followed from lowlands to highlands by evergreen forests and shrublands, seasonal evergreen forests and humid tallgrasses composed primarily of herbs and ferns that are located in the mountain tops of the islands (Laso et al., 2020). Notably, invasive plant species are mostly located in the highlands of the archipelago where agriculture and anthropogenic activities have deteriorated the native vegetation (Mauchamp & Atkinson, 2009). They now cover 2.2% of the Galapagos National Park (Rivas-Torres et al., 2018) and 28.5% of the non – protected areas (Laso et al., 2020).

Mainland Ecuador, on the other hand, is located on the northwest coast of South America in the equator (Borchsenius, 1997) between the Pacific Ocean, Colombia and Peru

(Mestanza-Ramón et al., 2019). Its geography, topography and climate vary along three distinctive natural regions (Borchsenius, 1997) due to the presence of the Andes (Moreno et al., 2018) and the influence of the Humboldt and Panama currents (Muriel, 2008). The Coast stretches from the lowlands west of the Andes (Borchsenius, 1997) to the Pacific Ocean reaching altitudes of up to 1200 m (Moreno et al., 2018). It consists of both dry (south) and humid (north) areas due to the effect of the marine currents (Muriel, 2008). The Andes or Highland region consists of mountains and volcanoes that reach up to 5000 m (Borchsenius, 1997). They are distributed along the western and eastern Andes mountain range with environmental conditions and biodiversity varying along the altitudinal gradient (Moreno et al., 2018). The Amazon stretches from the lowlands east of the Andes and is part of the western region of the Amazon Basin (Borchsenius, 1997). It covers 50% of the country´s territory (Muriel, 2008) and receives on average ~ 2800 mm of rain per year (Laraque et al., 2007). In general, the Amazon has higher plant diversity than the Coast but lower endemism (Muriel, 2008).

3. STUDY SPECIES

Cedrela odorata L. (Meliaceae, Order Sapindales), commonly known as Spanish or Cuban Cedar (Rivas-Torres & Rivas, 2018), is a semideciduous, fast growing canopy tree (CITES, 2007; Laso et al., 2020) that can reach heights of up to 30 to 40 m (Cintron, 1990). This trait and its winged seeds dispersed by wind are usually associated to successful invaders (Rivas-Torres et al., 2018). In some places of its distribution, it loses its leaves during the dry season and has adaptations like deciduous leaves, scaled protected buds and fruit maturation during this season that allows it to live in these conditions (Cavers et al., 2013). *C. odorata* is considered one of the most valuable Neotropical timber species in the world due to its aroma, durability and insect and rot resistance (CITES, 2007), making it vulnerable to illegal logging. It was listed under CITES Appendix III in 2001 and upgraded to Appendix II in 2019 (Finch et al., 2020). Considered Vulnerable by the IUCN, its main threats include unsustainable wood extraction, deforestation and the loss and fragmentation of its habitat (Mark & Rivers, 2017).

As a Neotropical species (26ºN - 28ºS) (CITES, 2007), its distribution ranges from Mexico throughout Central America and the Caribbean to the northern part of Argentina in places of up to 1500 m (Cavers et al., 2013). In Ecuador, *C. odorata* is distributed along the Coast and Amazon region because populations from the Andes previously classified as this species, are currently being described as a new one. This was reported in the study by Asadobay (2019) were clear genetic differentiation was found between the population of the Andes and the rest of the regions.

In Galapagos, *C. odorata* is considered an invasive species. It was introduced to the agricultural area of Santa Cruz in the 1940s for its timber and is now considered one of the

islands' most important sources of timber (Lundh, 2006). It is distributed along the four inhabited islands of the archipelago between 120 and 700 m and is mostly found around agricultural areas (Laso et al., 2020). This species usually covers entire forests that were previously dominated by endemic species (Rivas-Torres & Rivas, 2018); resulting in a lower diversity of native plants and a community structure different from that observed in forests dominated by species like *Scalesia* (Rivas-Torres et al., 2018).

4. METHODOLOGY

4.1. Sampling

The leaf samples used in this study were collected by Vinueza (2020) in Galapagos and Asadobay (2019) in mainland Ecuador. Fresh leaves of *C. odorata* were collected from 40 individuals from 4 populations in the Galapagos islands (10 from each population) between February and March of 2018. Sampled individuals (at least 20 m apart) were taken from the high and lowlands of the islands and from agricultural lands and border zones to have a more representative set of samples (see Appendix A & Figure 1). From mainland Ecuador, 32 individuals were sampled from 7 provinces along the Coast, Andes and Amazon regions between May and June of 2018 (see Appendix A & Figure 1). A map of the distribution of the samples was drawn in ArcGis Pro Desktop v. 2.7 (Esri, 2021).

For each sampled individual, 2 to 4 young leaves were collected and placed in sealed bags with silica gel for transport to the laboratory (Laboratorio de Biotecnología Vegetal at USFQ) for posterior analysis. All sampled individuals also have an herbarium voucher stored at QUSF under Gonzalo Rivas – Torres' collection and have been taxonomically verified by Walter Palacios, expert taxonomist of this genus in Ecuador. It is important to note that based on morphological features, not all samples from mainland Ecuador were taxonomically identified as *C. odorata* since the descriptions of new species within the *C. odorata* complex are currently underway in Ecuador (W. Palacios, personal communication, 2020) (see Appendix A); however, they were included in some of the analyses for comparison and control reasons. All sampling of fresh leaves for genetic analyses and herbarium vouchers were made under the permits 025-2018-IC-FLO, MAE-DNB-2018-0106, PC-18-19 and

MAE-DNB-2016-0041 issued by the Ministry of the Environment and the Galapagos National Park.

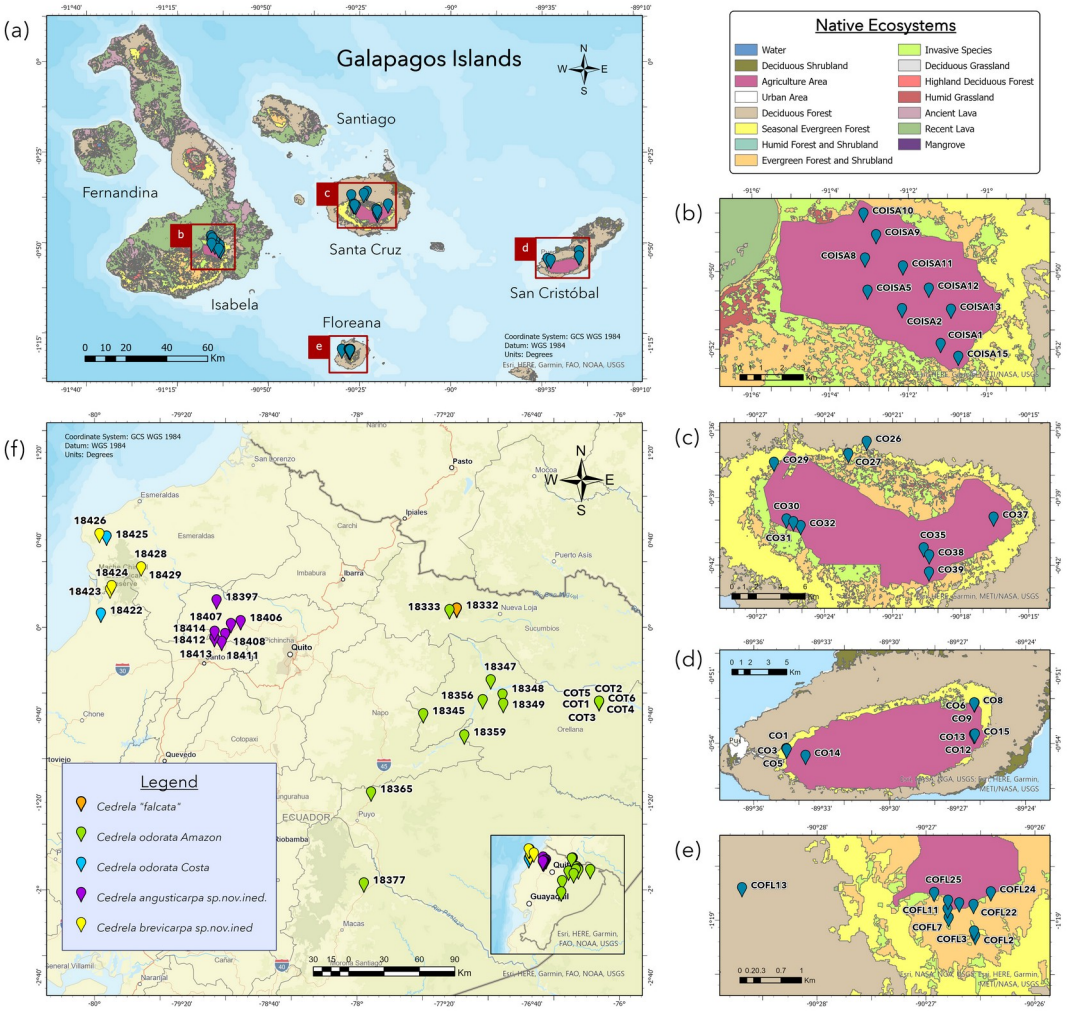

Figure 1. Sampling locations of a) 40 individuals of *C. odorata* in Galapagos distributed among b) Isabela, c) San Cristóbal, d) Santa Cruz and e) Floreana, and f) 32 individuals of *Cedrela spp.* from mainland Ecuador.

4.2. DNA Extraction and Quantification

DNA was extracted from 20g of leaf tissue for every sampled individual following the protocol for recalcitrant plants described by Rezadoost et al. (2016) with modifications, described by Asadobay (2019), to ensure better quality and concentration of DNA and to avoid amplification errors.

Leaf tissue was macerated in a mortar with liquid nitrogen and then transferred to a 1.5 ml tube where 400 µl of Buffer 1 and 0.1% (w/v) PVP were added. The solution was then placed in a vortex for 20s and transferred to a heat block incubator (60ºC) for 30 min. 400 µl of chloroform: isoamyl alcohol (24:1, v/v) were added before shaking vigorously for 2 min and centrifuging (10000 rpm) for 15 min. 300 µl of supernatant were transferred to a tube, and ½ volume of Buffer 2 was added before placing the solution on a heat block incubator (40ºC) for 15 min. ½ volume of 4M NaCl was added, stirred and placed in ice for 5 min before adding 2 volumes of cold isopropanol and letting it rest for 1h at -20ºC. The solution was then centrifuged (12000 rpm) for 20 minutes, the supernatant removed, and the pellet rinsed with 75% ethanol. This solution was then centrifuged (10000 rpm) for 5 min and the pellet removed in a laminar flow cabinet, dissolved in 30 µl of PCR water and transferred to a heat block incubator (70ºC) for 10 min. Samples were stored at -20ºC (Asadobay, 2019).

The quality and quantity of extracted DNA was assessed using a NanoDrop 2000 (Thermo Fisher Scientific, 2009). Samples without contaminants (phenols, guanidine residues) were expected to retrieve values between 2 – 2.2 for the 260/230 nm ratio and 1.8 - 2.0 for the 260/280 nm ratio (Desjardins & Conklin, 2010). An agarose gel (1%) electrophoresis was also used to evaluate the integrity of the extracted DNA (Asadobay, 2019; Vinueza, 2020).

4.3. Microsatellite Amplification

Nine homologous microsatellite loci for *C. odorata* (c) were selected for polymerase chain reaction (PCR) amplification (Hernández et al., 2008). The nine SSR regions were amplified with fluorophore-labeled *forward* primers and a standardized protocol described by Asadobay (2019). The PCR master mix used for each reaction contained: 21.02 µl of PCR water, 3 µl of 10X PCR Buffer (final concentration 1X), 1.5 µl of $MgCl_2$ 50 mM (final concentration 2.5 mM), 0.6 µl of dNTPs 10mM (final concentration 0.2 mM), 0.6 µl of

primer *forward* 10 µM (final concentration 0.2 µM), 0.6 µl of primer *reverse* 10 µM (final concentration 0.2 µM), 0.2 µl of Platinum Taq DNA Polymerase 1U per reaction, 0.48 µl of BSA 1 mg/ml (final concentration 0.016 mg/ml) and 2 µl of DNA per sample. To ensure amplification success, the final concentration of BSA was increased to 1 mg/ml for primers Ced18 and Ced61a and for Galapagos samples only, the final concentration of $MgCl_2$ was increased to 3 mM for primer Ced61a (see Appendix B).

The Polymerase Chain Reactions (PCR) were performed using the thermocycler program defined by Hernández et al. (2008) with an initial denaturation of 1 min at 94ºC followed by 30 to 40 cycles depending on the primer (see Appendix C), of denaturation for 1 min at 94ºC, annealing for 1 min at 55ºC, extension for 1 min at 72ºC and a final extension of 5 min at 72ºC. Some variations were also made for annealing temperatures for the amplification of 6 primers of some Galapagos samples (see Appendix C). PCR products were visualized on 1.5% agarose gel electrophoresis (35 min, 100V) and stored in refrigeration. All laboratory methods were performed by Laboratorio de Biotecnología Vegetal at USFQ directed by María de Lourdes Torres and led by Estefanía Rojas and María Paula Erazo.

4.4. Microsatellite Genotyping

PCR products were transferred to MicroAmp™ Optical 96-Well Reaction Plates and sent off to Macrogen South Korea for genotyping. The genotype of each individual was determined through capillary electrophoresis in an ABI 3130 Genetic Analyzer (ThermoFisher Scientific, USA) using 500LIZ as a size standard. Results were sent back as *.fsa* documents and analyzed in GeneMarker® software (SoftGenetics LLC, 2012) to produce an allele matrix for all individuals sampled.

Microsatellite data for 6 loci (Ced44, Ced41, Ced61a, Ced65, Ced95, Ced131) was also provided by Dr. Stephen Cavers for 528 individuals sampled along *C. odorata*

neotropical distribution (Cavers et al., 2013). This data was cross calibrated to avoid inconsistencies when combining two different microsatellite datasets and some individuals were eliminated due to missing data.

4.5. Data Analyses

4.5.1. Structure, connectivity, and genetic diversity of *C. odorata* in Galapagos.

4.5.1.1. Genetic diversity estimation.

Genetic diversity parameters were calculated for each of the 9 SSRs used in this study to evaluate their resolution power (Lemopoulos et al., 2019). Number of alleles (Na), expected heterozygosity (He) and observed heterozygosity (Ho) per locus were estimated on RStudio v. 4.0.3 with the *summary(genind)* function of the *adegenet* v. 2.1.3 package (Jombart et al., 2020). Deviations from Hardy Weinberg equilibrium were calculated for each locus on Genepop v. 4.7 (Raymond & Rousset, 1995). The probability test was performed under the default Markov Chain parameters (Dememorization number: 1000, Number of batches: 100, Number of iterations per batch: 1000). Further, FreeNa (Chapuis & Estoup, 2007) was used to estimate null allele frequency (No) per locus, considering the expectation maximization (EM) algorithm. Given the fact that null alleles can bias population structure analysis and genetic diversity statistics (Dakin & Avise, 2004), corrected F_{ST} values were compared to uncorrected values using the excluding null alleles (ENA) method with a paired t-test ($\alpha = 0.05$) on Social Science Statistics (Stangroom, 2021).

Further, the four sampled islands in the Galapagos were considered as separate populations in the analyses, and genetic diversity indicators per population were estimated on RStudio v. 4.0.3. Number of alleles (Na) were calculated with the *summary(genind)* function and expected heterozygosity (He) with the *Hs* function of the *adegenet* v. 2.1.3 package (Jombart et al., 2020) while observed heterozygosity (Ho) and inbreeding coefficients (F_{IS})

were estimated with the *basic.stats* function of the *hierfstat* v. 0.5-7 package (Goudet et al., 2020). The *private_alleles* function of the *poppr* v. 2.9.0 package (Kamvar et al., 2021) was used to estimate the number of private alleles (Npa) per island while allelic richness (Rs) per island was calculated using the *allel.rich* function of the *PopGenReport* v. 3.0.4 package (Gruber & Adamack, 2019). Global expected and observed heterozygosity and inbreeding coefficient were also estimated.

4.5.1.2. Population structure determination.

An analysis of molecular variance (AMOVA) was conducted in Arlequin v. 3.5.2.2 (Weir & Cockerham, 1984) to evaluate the level of genetic differentiation found between and within the islands. A model was used that partitioned the genetic variation in two hierarchal levels: between and within populations. The significance was estimated using 10,000 permutations. Pairwise fixation indexes (F_{ST}) were also calculated in RStudio v. 4.0.3 with the *pairwise.fstb* function of the PopGenReport v. 3.0.4 package (Gruber & Adamack, 2019). A Mantel test (23 permutations) with the *mantel* function of the vegan v. 2.5-7 package (Oksanen et al., 2020) was performed to assess a possible correlation between genetic and geographic distances of the individual islands. Further, a Principal Coordinate Analysis (PCoA) was performed to evaluate population structure of the populations of *C. odorata* in Galapagos with the *pcoa* function of the ape v. 5.4-1 package (Paradis et al., 2020) and plotted with the *ggplot* function of the ggplot2 v. 3.3.3 package (Wickham et al., 2020). In order to determine the directional gene flow and its relative magnitude among the islands analyzed in this study, the R-function *divMigrate* (Sundqvist et al., 2016) implemented in the R-package diveRsity v. 1.9.9 (Keenan, 2017) was used.

Population structure was inferred through a Bayesian individual-based clustering approach using STRUCTURE v. 2.3.4 (Pritchard et al., 2010). An admixture model was applied, and islands were defined as putative populations a priori. Potential K values were

evaluated between 1 and 10 with 10 independent iterations per K, 100,000 step-burn in periods and 1,000,000 Markov Chain Monte Carlo (MCMC) steps. The optimal K value was defined using the Evanno method (Evanno et al., 2005) implemented in Structure Harvester v. 0.6.94 (Earl & vonHoldt, 2012), and an overall graph of the 10 iterations for each K was generated in CLUMPAK (Kopelman et al., 2015). The final STRUCTURE plot was obtained using the Distruct Software (Rosenberg, 2004). All graphs generated in the analyses were then edited in Adobe Illustrator.

4.5.2. Invasion history: inferring the origin and number of times *C. odorata* was introduced to the Galapagos Islands.

4.5.2.1. Origin of introduction.

A Principal Coordinate Analysis (PCoA) was performed to infer from which region of mainland Ecuador *C. odorata* was introduced to the Galapagos Islands. The same methods described previously were used (see Data Analyses – Population structure determination), but additional individuals from mainland Ecuador were added to complement the data. Only individuals identified as *C. odorata* were included (see Appendix A & Sampling).

The Bayesian clustering approach of STRUCTURE v. 2.3.4 (Pritchard et al., 2010) was also used to identify similarities between the genetic lineages of the populations of Galapagos and those from mainland Ecuador. The same parameters described for the samples of Galapagos (see Data Analyses – Population structure determination) were also used for this analysis. Further, pairwise fixation indexes (F_{ST}) were also calculated between the populations of Galapagos and mainland Ecuador.

Additionally, all available samples of mainland Ecuador, divided by species (see Appendix A & Sampling), were used in another Principal Coordinate Analysis (PCoA). This was done to compare and corroborate the population of introduction since *C. odorata* is

considered a cryptic species (Cavers et al., 2013), with new ones currently being described in Ecuador (W. Palacios, personal communication, 2020).

4.5.2.2. Introduction events.

To infer the possible routes of invasion and number of introduction events of *C. odorata* in the Galapagos islands, Approximate Bayesian Computation (ABC) analyses were performed on DIYABC (v2.1.0) (J.-M. Cornuet et al., 2014). This software calculates the posterior probabilities of proposed scenarios of introduction, comparing simulated and observed summary statistics of each scenario through a logistic regression (J.-M. Cornuet et al., 2014) to infer the most likely one based on available data (Hirsch et al., 2021). A total of 35 different scenarios were proposed and run along three stages. In the first stage, only individuals sampled in Galapagos were used and the population of origin was specified as having an unknown effective population size (Ne). In the second and third stages, only 15 of the 35 considered scenarios for the first stage were analyzed and samples from mainland Ecuador were included as the population of origin.

In more detail, the second stage included all available samples of *C. odorata* from mainland Ecuador (Amazon + Coast); while in the third stage only those belonging to the Coastal region were included. Scenarios were built based on literature review (Astudillo, 2018; Domínguez, 2016; Guézou et al., 2010; Lundh, 2006) and previous experience (Rivas-Torres, personal communication, 2021), testing scenarios with one to multiple independent introductions to the various islands. Bottleneck events were considered after each introduction and admixture origins were also tested in multiple scenarios for the four islands. 1,000,000 simulations were run for each scenario following a stepwise mutation model (SMM) and using mean number of alleles, mean genic diversity and F_{ST} values as summary statistics. All scenarios were plotted taking into account effective population sizes for the four islands and mainland origin ($N_{e/1}$, N_2, N_3, N_4, N_5), post – bottleneck population sizes (N_{2b}, N_{3b},

N_{4b}, N_{5b}), times of divergence between populations (t_1, t_2, t_3, t_4) and duration of bottleneck events ($t_{1\text{-}db}$, $t_{2\text{-}db}$, $t_{3\text{-}db}$, $t_{4\text{-}db}$). A logistic regression estimate was used to calculate the posterior probabilities of each scenario. All graphs obtained in the analyses were then edited in Adobe Illustrator.

Further, the software BOTLLENECK (v.1.2.02) (J. M. Cornuet & Luikart, 1996) was used to test the possibility of occurrence of bottleneck events in the four islands to better understand the introduction history of *C. odorata* in Galapagos and to complement the Approximate Bayesian Computation (ABC) analyses. Stepwise mutation models (SMM) and Two-phase models (TPM) were used in a Wilcoxon Sign – Rank test to calculate the probability of heterozygosity excess and/or deficiency in the populations.

4.5.3. Preliminary analysis: genetic affinity of Galapagos' populations within the native Neotropical distribution of this species.

To inquire into and examine the possibility of a secondary introduction from Central America or the Caribbean for *C. odorata* in Galapagos, a preliminary analysis was conducted to test genetic similarity between the populations of Galapagos, mainland Ecuador and other neotropical regions within the native distribution of this species. The microsatellite data provided by Dr. Stephen Cavers (see Microsatellite Genotyping) was used to perform Principal Coordinate Analysis (PCoA) by regions using the same methods described previously (see Data Analyses – Population structure determination). The regions defined a priori included North, Central and South America, the Caribbean and Ecuador (Appendix D) for the individuals provided by Dr. Cavers, and Galapagos and mainland Ecuador for the individuals used in this study. These analyses were performed by María Paula Erazo from Laboratorio de Biotecnología Vegetal at USFQ.

5. RESULTS

5.1. Structure, connectivity, and genetic diversity of *C. odorata* in Galapagos

5.1.1. Genetic diversity estimation.

All nine homologous microsatellites tested were polymorphic with an average of 3.9 alleles per locus, ranging from 2 to 7 alleles. Observed heterozygosity (Ho) per locus varied from 0.07 (Ced18) to 0.65 (Ced61a) while expected heterozygosity (He) per locus from 0.32 (Ced131) to 0.74 (Ced95/ Ced61a), making Ced95 and Ced61a (He = 0.74) the most informative SSRs. Null allele frequency ranged up to 0.28 (Ced18), but no significant differences were found between uncorrected and corrected FST values (p = 0.47, t = 0.068), and only 3 (Ced131, Ced65, Ced54) of the 9 microsatellites analyzed showed significant deviations from Hardy Weinberg Equilibrium (Table 1).

Table 1. Genetic diversity estimates for nine microsatellites used for *C. odorata* in Galapagos.

	Na	He	Ho	No	F_{ST}^{A}	F_{ST}^{B}	**HWD**
Ced131	2	0.32	0.40	0.00	0.30	0.29	0.31
Ced18	3	0.49	0.07	0.28	-0.07	-0.05	0.00
Ced2	4	0.51	0.22	0.19	0.35	0.35	0.00
Ced65	2	0.48	0.40	0.06	0.29	0.29	0.33
Ced95	5	0.74	0.35	0.22	0.24	0.20	0.00
Ced44	4	0.70	0.42	0.16	0.36	0.33	0.00
Ced41	7	0.44	0.22	0.14	0.12	0.13	0.00
Ced54	3	0.51	0.48	0.02	-0.02	-0.02	0.61
Ced61a	5	0.74	0.65	0.06	0.13	0.12	0.00

Na, number of alleles; **He**, expected heterozygosity; **Ho**, observed heterozygosity; **No**, null allele frequency; $\mathbf{F_{ST}^{A}}$, uncorrected; $\mathbf{F_{ST}^{B}}$, corrected for the effect of null alleles; **HWD**, deviation from Hardy Weinberg Equilibrium p value.

The number of alleles (Na) found for each island ranged from 21 (Isabela) to 29 (Santa Cruz). Private alleles represented 11% of all reported alleles, varying from 1 in Isabela to 5 in Santa Cruz. Expected heterozygosity (He) was higher than observed heterozygosity (Ho) in all four islands. Observed heterozygosity (Ho) ranged from 0.23 (San Cristóbal) to 0.53 (Santa Cruz), with an overall value of 0.36 while expected heterozygosity (He) ranged from 0.35 (San Cristóbal) to 0.55 (Santa Cruz), with a global value of 0.55. Santa Cruz presented the highest genetic diversity values (He = 0.55, Ho = 0.53, Rs = 3.15). Further, the degree of inbreeding analyzed through the inbreeding coefficient F_{IS}, ranged from 0.07 (Santa Cruz) to 0.36 (San Cristóbal) in the individual islands but presented an overall global value of 0.23 (Table 2), suggesting possible inbreeding within the islands (Mangaravite et al., 2019).

Table 2. Genetic diversity parameters estimated for *C. odorata* populations of Galapagos.

	N	Na	He	Ho	F_{IS}	Npa	**Rs**
Santa Cruz	10	29	0.55	0.53	0.07	5	3.15
Isabela	10	21	0.40	0.30	0.28	1	2.29
San Cristóbal	10	22	0.35	0.23	0.36	2	2.32
Floreana	10	23	0.45	0.37	0.22	2	2.54

N, number of individuals; **Na**, number of alleles; **He**, expected heterozygosity; **Ho**, observed heterozygosity; $\mathbf{F_{IS}}$, inbreeding coefficient; **Npa**, number of private alleles; **Rs**, allelic richness.

5.1.2. Population structure determination

The Analysis of Molecular Variance (AMOVA) demonstrated that 20.70% of the reported variation was observed between populations and 79.30% within populations (Table 3), suggesting connectivity and low genetic differentiation between the islands (Li et al., 2012).

Table 3. Analysis of Molecular Variance (AMOVA) for *C. odorata* populations of Galapagos

Source of variation	% of variation	**p- value**
Between populations	20.70	0.000
Within populations	79.30	0.000

However, the moderate (F_{ST} = 0.05 - 0.15) to great (F_{ST} = 0.15 - 0.25) genetic differentiation (Hartl & Clark, 1997) found within the reported pairwise F_{ST} distances (range: 0.079 - 0.191) (Table 4) between populations suggested certain degree of differentiation among the islands (Ottewell et al., 2016). Floreana appeared to be the most divergent of the islands since the genetic distances between Floreana and Isabela (F_{ST} = 0.191) and Floreana and San Cristóbal (F_{ST} = 0.185) were higher than that reported for the other islands. Meanwhile, Isabela and San Cristóbal (F_{ST} = 0.079) presented the lowest genetic differentiation. This low degree of differentiation did not coincide with the geographic distances between islands since no significant correlation was found between genetic and geographical distances of the islands in the Mantel test performed (p = 0.833, r^2 = -0.37).

Table 4. Pairwise F_{ST} distances between the islands where *C. odorata* is distributed in Galapagos.

	Santa Cruz	San Cristóbal	Isabela	**Floreana**
Santa Cruz	-	0.134	0.119	0.126
San Cristóbal	0.134	-	0.079	0.185
Isabela	0.119	0.079	-	0.191
Floreana	0.126	0.185	0.191	-

*****As reference, Wright (1978) has suggested that F_{ST} values between 0 - 0.05 represent little genetic differentiation, 0.05 - 0.15 moderate genetic differentiation, 0.15 - 0.25 great genetic differentiation, and >0.25 very great genetic differentiation (Hartl & Clark, 1997).

The same patterns reported for the pairwise F_{ST} distances (Table 4) coincided with the Principal Coordinate Analysis (PCoA). Two – dimensional plots (Figure 2) were obtained for the first three principal coordinates. The sum of the three principal coordinates accounted for 49.1% of variation of the data (PCoA1 = 20.74%, PCoA2 = 15.97%, PCoA3 = 12.39%). The

analysis distinguished four genetic clusters for each of the four islands that were defined a priori as separate populations. No clear differentiation between San Cristóbal and Isabela was observed in either of the three principal coordinates. However, the first principal coordinate clearly separated Floreana and Santa Cruz from the rest of the islands while the third (Figure 2b) segregated Floreana from Santa Cruz.

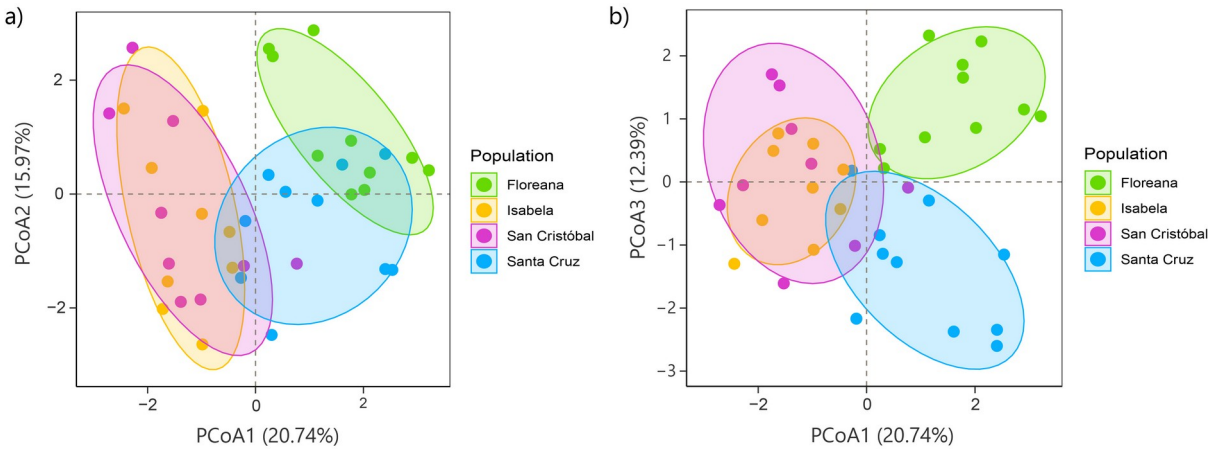

Figure 2. Principal Coordinate Analysis (PCoA) of 40 individuals of *C. odorata* sampled in Floreana (green), Isabela (orange), San Cristóbal (purple) and Santa Cruz (blue) using 9 SSR markers. The sum of the first three principal coordinates represents 49.1% of the variation of the data. No clear differentiation is found between San Cristóbal and Isabela, but Floreana and Santa Cruz are separated from the rest of the islands.

Further, the Bayesian inference analysis of population structure (Figure 3a) suggested the presence of two possible clusters (K = 2) based on the highest ΔK (68.98) (see Appendix E), obtained using the Evanno method (Evanno et al., 2005). One cluster (blue) was predominantly present in Isabela and San Cristóbal while the other (green) in Floreana. Santa Cruz was characterized by a combination of both clusters. Notably, when the number of possible genetic clusters was increased to K = 4 (ΔK = 49.48), similar patterns were observed (Figure 3b). Isabela and San Cristóbal shared cluster 2 (blue) and 3 (grey) while Floreana (cluster 4) and Santa Cruz (cluster 1) displayed predominantly their own clusters. In general,

these same patterns coincided with those observed for the Pairwise F_{ST} values (Table 4) and Principal Coordinate Analysis (Figure 2).

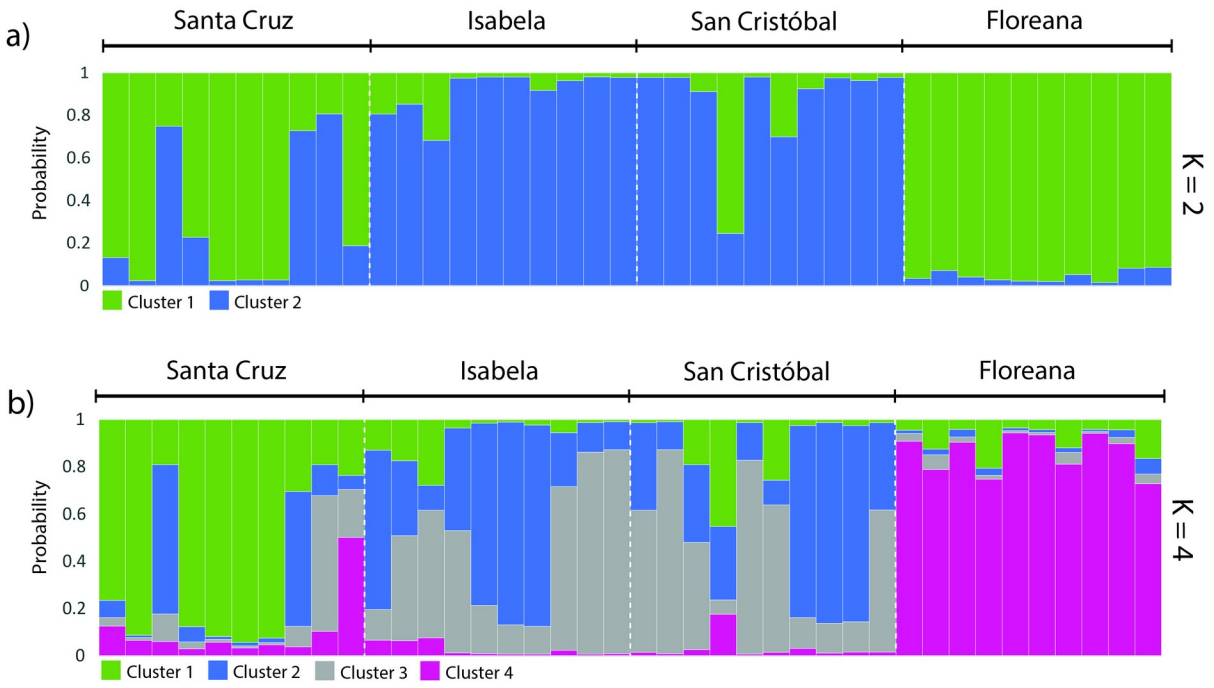

Figure 3. Bayesian analysis of the population structure of *C. odorata* in Galapagos under the Admixture model. Results are presented for **a)** the optimum K value, K = 2 (ΔK = 68.98) and **b)** K = 4 (ΔK = 49.48) to analyze substructure. K values indicate the number of genetic clusters represented in different colors.

The relative migration network (Figure 4) revealed differences in gene flow rate between the four islands, but the same patterns described by the PCoA (Figure 2), STRUCTURE (Figure 3) and F_{ST} distances (Table 4) were observed: Isabela and San Cristóbal presented the greatest genetic similarity (clustered in closer proximity). A bidirectional symmetric gene flow was found between all islands; however, the highest migration rate was reported between San Cristóbal and Isabela and the lowest between Isabela/ San Cristóbal and Floreana. Santa Cruz was characterized by moderate rates of bidirectional gene flow with the other islands.

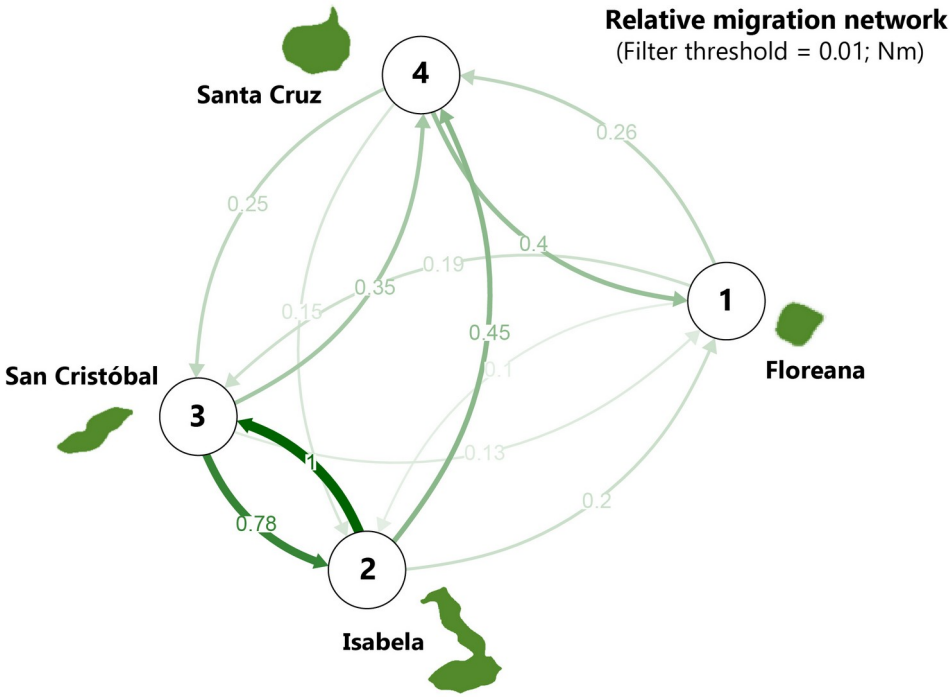

Figure 4. Directional gene flow and relative migration network among the four islands of Galapagos where *C. odorata* is distributed. The distance between points is proportional to the genetic differentiation between the islands while the arrows indicate directionality and the numbers gene flow rate (0 – 1). The color shade and line thickness are proportional to the relative strength of the gene flow (Sundqvist et al., 2016).

5.2. Invasion history: inferring the origin and number of times *C. odorata* was introduced to the Galapagos Islands

5.2.1. Origin of introduction.

To infer from what region of mainland Ecuador *C. odorata* was introduced to the Galapagos islands, a Principal Coordinate Analysis (PCoA) was performed. Two – dimensional plots (Figure 5) were obtained for the first three principal coordinates. The sum of the three principal coordinates accounted for 43.26% of the variation of the data (PCoA1 = 26.43%, PCoA2 = 9.21%, PCoA3 = 7.62%). The analysis distinguished six genetic clusters for each of the four islands and two regions of mainland Ecuador that were defined a priori as separate populations. The first principal coordinate clearly separated the Amazon (dark blue) from the rest of the regions while the two samples from the Coast (dark red) clustered close

to the populations of Galapagos: suggesting a possible introduction from the Coast of Ecuador. Additionally, the analysis of the first and third component (Figure 5b) proposed a greater similarity between the Coast and Santa Cruz than with the other three islands.

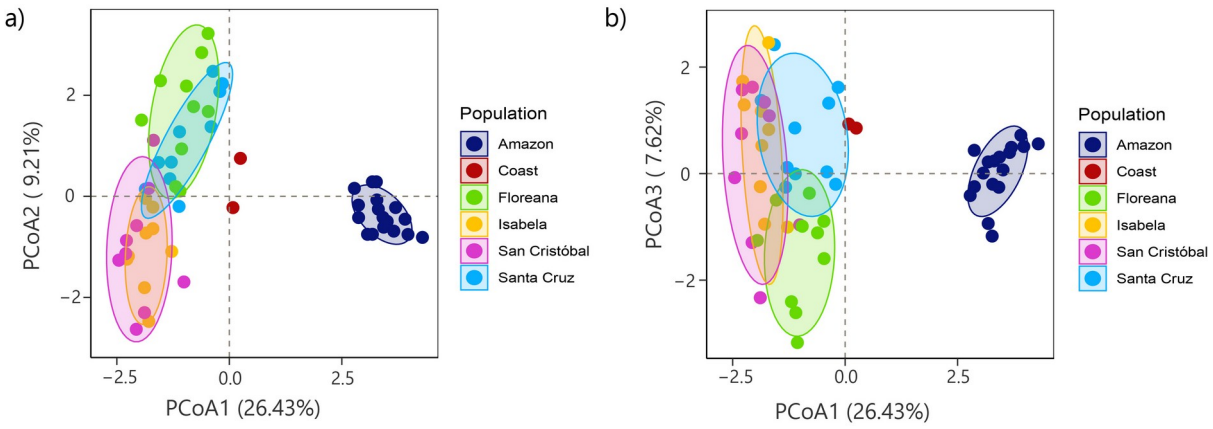

Figure 5. Principal Coordinate Analysis (PCoA) of 40 individuals of *C. odorata* sampled in Floreana (green), Isabela (orange), San Cristóbal (purple) and Santa Cruz (light blue), and 19 sampled in the Coast (dark red) and Amazon (dark blue) region of mainland Ecuador using 9 SSR markers. The sum of the three first principal coordinates represents 43.26% of the variation of the data. The Amazon (dark blue) samples are clearly separated from the rest of the regions while the two samples from the Coast (red) cluster closer together to the samples of Galapagos.

The results of the Bayesian inference analysis of population structure (Figure 6) coincided with those reported in the Principal Coordinate Analysis (Figure 5). The presence of two possible clusters (K = 2) was suggested based on the highest ΔK (204.95) (see Appendix F), obtained using the Evanno method (Evanno et al., 2005). One cluster (blue) was predominantly present in the mainland regions of Ecuador while the other in the Galapagos Islands (green). However, the cluster reported for Galapagos (green) was also present in the Coast. Additionally, cluster 2 (blue) was also slightly observed in San Cristóbal and Santa Cruz.

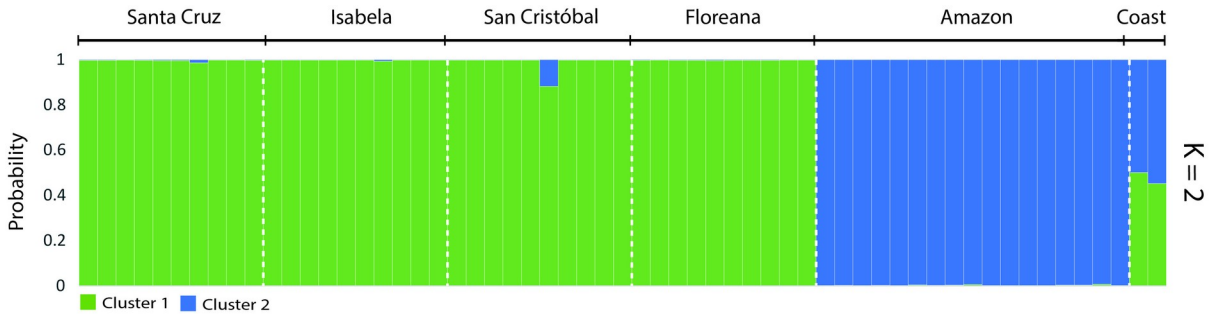

Figure 6. Bayesian analysis of population structure of *C. odorata* in Galapagos and mainland Ecuador (Coast + Amazon) under the Admixture model. Results are presented for the optimum K value: K = 2 (ΔK = 204.95). K values indicate the number of genetic clusters represented in different colors.

Further, estimated pairwise F_{ST} distances suggested great (F_{ST} = 0.15 - 0.25) genetic differentiation (Hartl & Clark, 1997) between mainland Ecuador (Amazon + Coast) and the four islands of Galapagos (Table 5). Isabela and the mainland region presented the highest genetic differentiation while Santa Cruz and mainland Ecuador were genetically the most similar, pattern that coincided with that observed in the Principal Coordinate Analysis (Figure 5b). Additionally, Floreana reported the second lowest pairwise F_{ST} distance with the mainland region followed closely behind by San Cristóbal.

Table 5. Pairwise F_{ST} distances between the islands where *C. odorata* is distributed in Galapagos and mainland Ecuador.

	Santa Cruz	**San Cristóbal**	**Isabela**	**Floreana**
Mainland	0.156	0.205	0.219	0.192

*As reference, Wright (1978) has suggested that F_{ST} values between 0 - 0.05 represent little genetic differentiation, 0.05 - 0.15 moderate genetic differentiation, 0.15 - 0.25 great genetic differentiation, and >0.25 very great genetic differentiation (Hartl & Clark, 1997).

Since *C. odorata* is a cryptic species, a second Principal Coordinate Analysis (PCoA) was performed with all available samples for mainland Ecuador. These samples included three additional species to verify that the species of origin of the populations of Galapagos was in fact *C. odorata*. Two – dimensional plots (Figure 7) were obtained for the first three

principal coordinates. The sum of the three principal coordinates accounted for 44.68% of the variation of the data (PCoA1 = 24.55%, PCoA2 = 12.28%, PCoA3 = 7.85%). The analysis distinguished six genetic clusters for three populations of *C. odorata* and three species currently being described that were defined a priori as separate populations. As observed in the previous PCoA (Figure 5), the *C. odorata* from Galapagos were only genetically similar to the samples of this species from the Coast. The first and second principal coordinates clearly separated the Galapagos and Coast groups from the other species and the *C. odorata* from the Amazon. Notably, when analyzing the first and second principal coordinates, the *C. angusticarpa* sp.nov.ined. (W. Palacios et al.) formed a visibly separate cluster while the third principal coordinate, isolated the *C. brevicarpa* sp.nov.ined. (W. Palacios et al.) from the rest of the groups. All this information suggests a possible introduction from a subpopulation of *C. odorata* from the Coast of Ecuador.

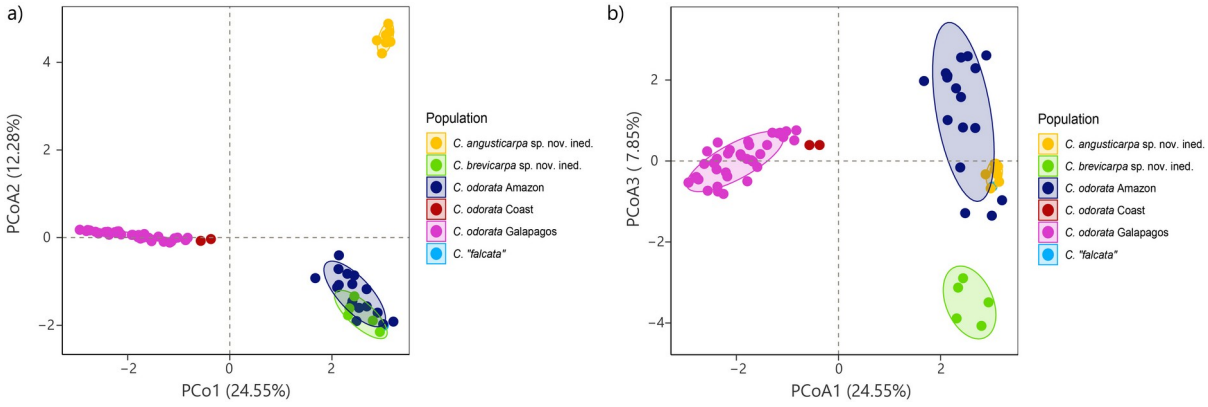

Figure 7. Principal Coordinate Analysis (PCoA) of 40 individuals of *C. odorata* sampled in Galapagos (purple), 2 sampled in the Coast (dark red), 17 in the Amazon (dark blue) and 13 belonging to other species of *Cedrela spp*. from mainland Ecuador using 9 SSR markers. The sum of the three first principal coordinates represents 44.68% of the variation of the data. The populations from Galapagos are only genetically similar to the *C. odorata* from the Coast. **(a)** *C. angusticarpa* sp.nov.ined. and **(b)** *C. brevicarpa* sp.nov.ined. are clearly separated as their own clusters.

5.2.2. Introduction events.

Since multiple introductions seem to be common within invasive plant species (Bossdorf et al., 2005; Wilson et al., 2009), Approximate Bayesian Computation (ABC) analyses were performed to infer the number of introduction events and possible routes of invasion of *C. odorata* in Galapagos. A total of 35 different scenarios were tested using only samples from Galapagos (Stage 1). The two best supported scenarios (Figure 8) with the highest posterior probabilities (8a. 0.8415 and 8b. 0.6750) suggested at least two independent introduction events from the population of origin in the mainland. In more detail, scenario one (Figure 8a) suggested initial introductions to Santa Cruz and Floreana and subsequent introductions from Floreana to San Cristóbal and then to Isabela. Scenario two (Figure 8b), on the other hand, proposed independent introduction events to Santa Cruz and San Cristóbal from the population of origin in the mainland with a subsequent introduction to Isabela from San Cristóbal. The population of Floreana was a result of two introduction events: one from the mainland and one from Santa Cruz.

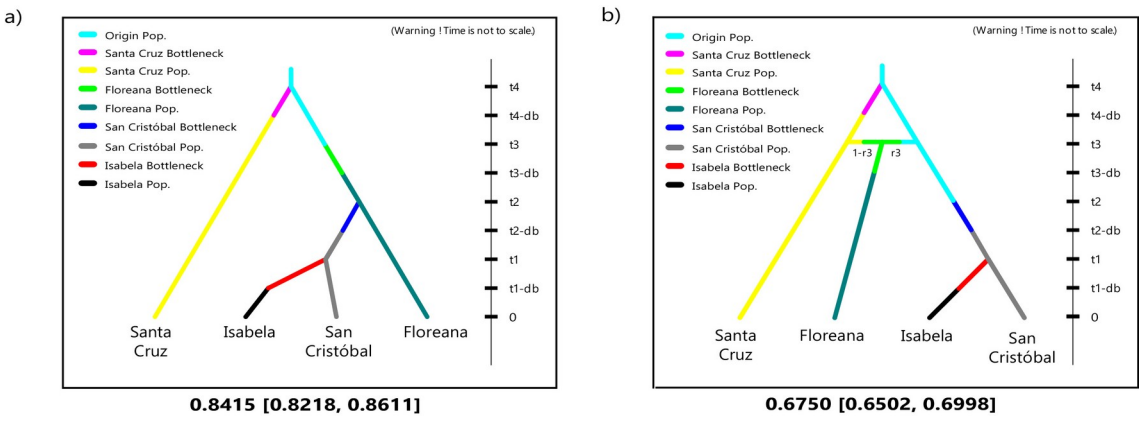

Figure 8. Introduction history of *C. odorata* in Galapagos estimated through Approximate Bayesian Computation (ABC) analyses using 40 individuals sampled in Santa Cruz, Isabela, San Cristóbal and Floreana. Origin population defined as having an unknown effective population size. Results are presented for the two scenarios with the highest posterior probabilities. **Note:** All colors represent effective population sizes during bottleneck events or present populations. Times of divergence between populations (t1, t2, t3, t4) and duration of bottleneck events (t1-db, t2-db, t3-db, t4-db) are also represented.

However, when sampled individuals from the Coast and Amazon (stage 2) were specified as the population of origin (Figure 9), two different scenarios were found as having the highest posterior probabilities (9a. 0.9988 and 9b. 0.9981). The best supported scenario (Figure 9a) suggested initial introductions to Santa Cruz and San Cristóbal and subsequent introductions to Floreana from Santa Cruz and to Isabela from San Cristóbal. The other scenario (Figure 9b) instead proposed a single initial introduction to San Cristóbal and subsequent introductions to the other three islands.

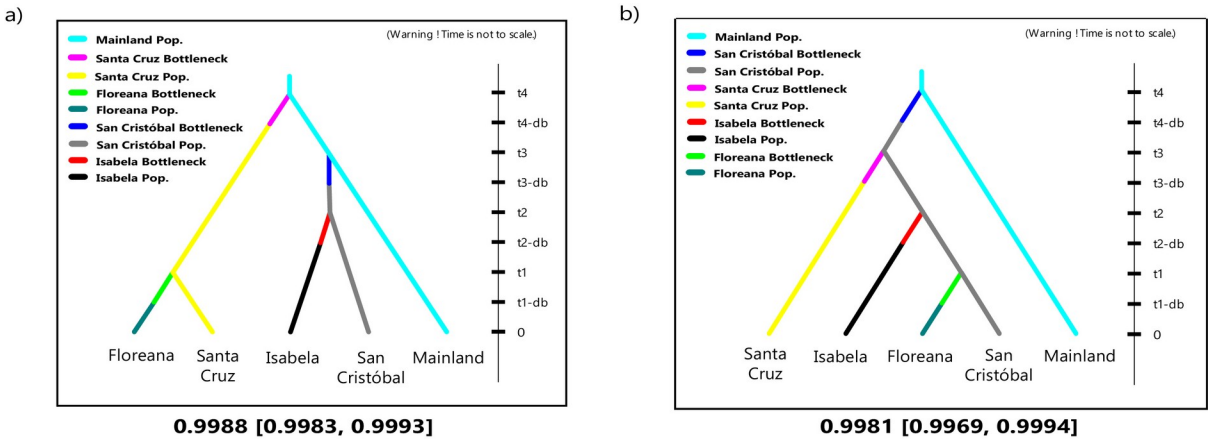

Figure 9. Introduction history of *C. odorata* in Galapagos estimated through Approximate Bayesian Computation (ABC) analyses using 40 individuals sampled in Santa Cruz, Isabela, San Cristóbal and Floreana, and 19 individuals from the Coast and Amazon region. Results are presented for the two scenarios with the highest posterior probabilities. **Note:** All colors represent effective population sizes during bottleneck events or present populations. Times of divergence between populations (t1, t2, t3, t4) and duration of bottleneck events (t1-db, t2-db, t3-db, t4-db) are also represented.

Additionally, when only samples from the Coast (Stage 3) were used and specified as the population of origin, the scenario (Figure 10) with the highest posterior probability (0.8764) coincided with the best supported scenario reported for the Coast and Amazon samples (Figure 9a). This scenario as described previously, suggested initial introductions to Santa Cruz and San Cristóbal with subsequent introductions to Floreana from Santa Cruz and to Isabela from San Cristóbal.

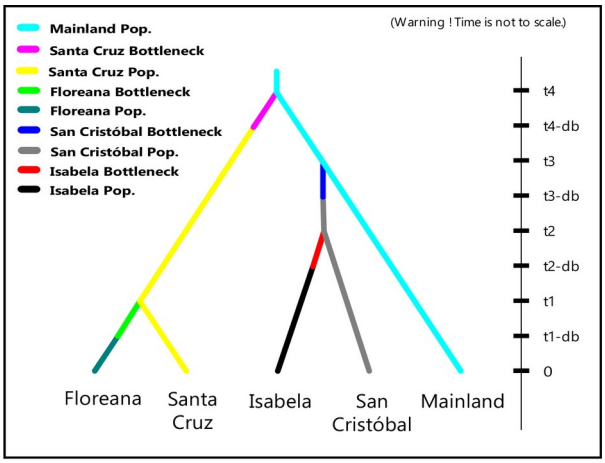

Figure 10. Introduction history of *C. odorata* in Galapagos estimated through Approximate Bayesian Computation (ABC) analyses using 40 individuals sampled in Santa Cruz, Isabela, San Cristóbal and Floreana, and 2 individuals from the Coast from mainland Ecuador. Results are presented for the best supported scenario. **Note:** All colors represent effective population sizes during bottleneck events or present populations. Times of divergence between populations (t1, t2, t3, t4) and duration of bottleneck events (t1-db, t2-db, t3-db, t4-db) are also represented.

To complement the Approximate Bayesian Computation (ABC) analyses, the occurrence of bottlenecks events was tested through a Wilcoxon sign rank test. Two mutations models were considered. However, significant evidence ($p < 0.05$) (J. M. Cornuet & Luikart, 1996) of the presence of bottleneck events was only found under the TPM model (Table 6). In more detail, heterozygosity deficiency or excess was observed for Santa Cruz and Floreana while significant evidence for heterozygosity excess or population expansion was found for Santa Cruz, Isabela and Floreana, suggesting at least two to three recent bottleneck events in the individual islands. Heterozygosity excess is usually considered as evidence of bottleneck events since after a bottleneck event, allelic richness diminishes faster than observed heterozygosity (Piry et al., 1999).

Table 6. Wilcoxon sign rank test estimates for the possibility of occurrence of past genetic bottleneck events in the four islands where *C. odorata* can be found in the Galapagos.

	SMM Model				TPM Model			
	Santa Cruz	**Isabela**	**San Cristóbal**	**Floreana**	**Santa Cruz**	**Isabela**	**San Cristóbal**	**Floreana**
H deficiency	0.898	0.844	0.320	0.936	0.995	0.973	0.727	0.995
H excess	0.125	0.191	0.727	0.082	0.007*	0.037*	0.320	0.007*
H deficiency or excess	0.250	0.383	0.641	0.164	0.014*	0.074	0.641	0.014*

*p values < 0.05 indicate possible genetic bottleneck events (BOTTLENECK software)

5.3. Preliminary analysis: genetic affinity of Galapagos' populations within the native Neotropical distribution of this species

Historical reports have proposed a possible secondary introduction for *C. odorata* of Galapagos from a subpopulation of the Coast of Ecuador that was originally brought from Central America or Cuba (Lamb, 1968; W. Palacios, personal communication, 2020). To preliminary test this hypothesis a series of five Principal Coordinate Analyses (PCoAs) (Figure 11) were performed with the microsatellite data provided by Cavers et al. (2013). Each Principal Coordinate Analysis compared the samples used in this study from Galapagos and mainland Ecuador (Figure 1, Appendix A) with the data provided by Cavers et al. (2013) divided by regions (Appendix D). For all regions, two – dimensional plots were obtained for the first two principal coordinates. Overall, the *C. odorata* of Galapagos were only genetically similar to the samples used in this study from the Coast (C), as was noted previously in Figure 5. However, no clear genetic association was observed with any of the populations from the other regions of the Neotropics, suggesting a possible native origin for the subpopulation of the Coast of Ecuador from where *C. odorata* was introduced to the Galapagos.

In more detail, the first principal coordinate of the first PCoA (Figure 11a) separated Galapagos and North America; however, a few individuals from Escarcega (E) and Zona Maya (ZM) were found within Galapagos' ellipsis. Galapagos and Central America (Figure

11b) were also differentiated with a few individuals from Costa Rica (CR) slightly closer to the samples from Galapagos. Further, the PCoA that included Galapagos, mainland Ecuador and the Caribbean (Figure 11c) clearly separated the Caribbean from Galapagos, refuting a possible introduction to mainland Ecuador from Cuba and later to Galapagos. The same patterns of divergence were also observed with the samples of South America (Figure 11d), where the two first principal coordinates clearly isolated the Galapagos and Coast (C) individuals from the rest of the groups. Finally, the PCoA (Figure 11e) that included samples from Galapagos, mainland Ecuador and those used by Cavers et al. (2013) for Ecuador segregated the Galapagos and Coast individuals used in this study from the ones used by Cavers.

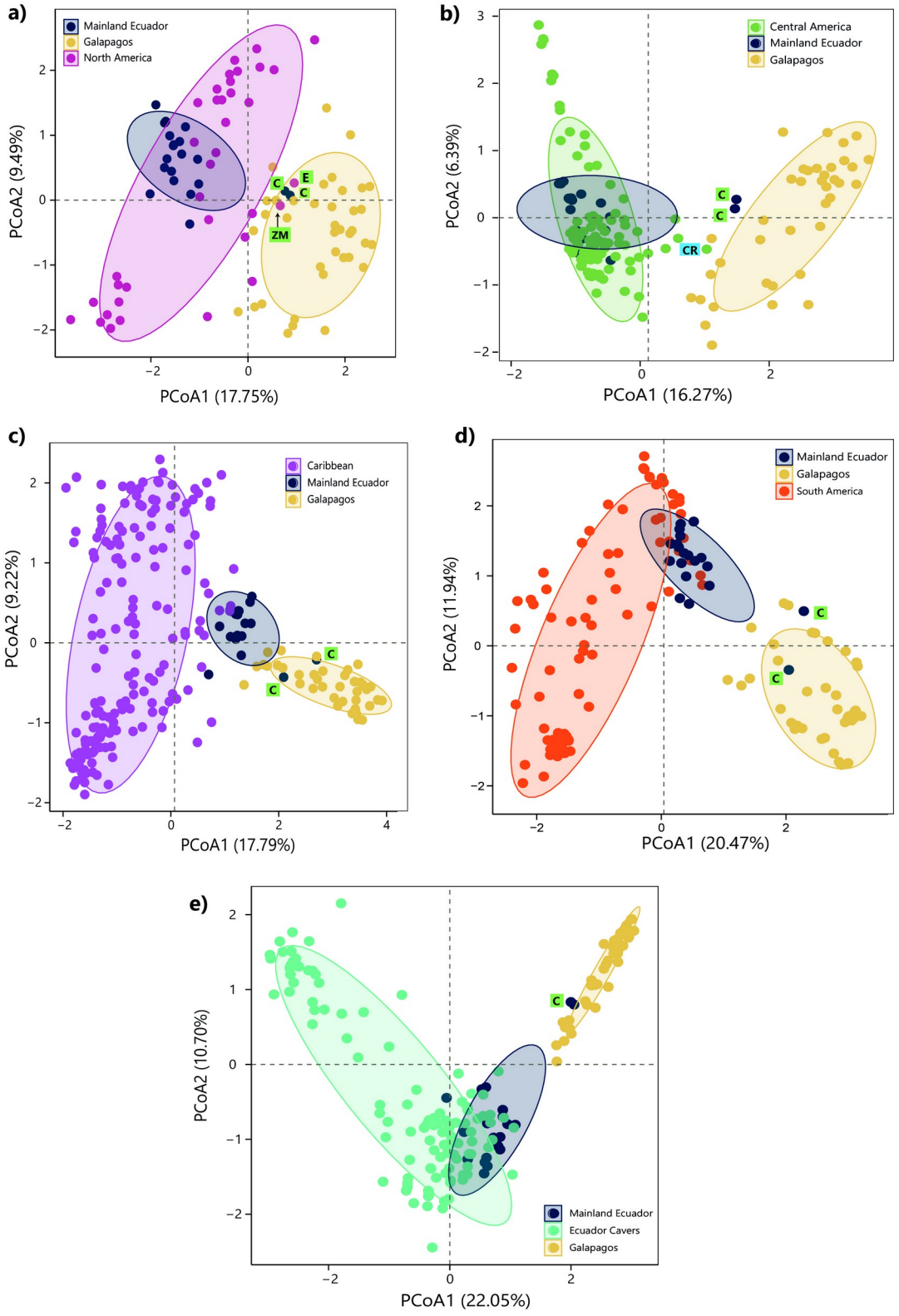

Figure 11. Principal Coordinate Analysis (PCoA) using 6 SSR markers of individuals of *C. odorata* sampled in Galapagos (yellow), mainland Ecuador (dark blue) and data provided by Cavers et al. (2013) from **a)** North America, **b)** Central America, **c)** Caribbean, **D)** South America and **e)** Ecuador. The first two principal coordinates were considered. **Note:** PCoA performed by María Paula Erazo from Laboratorio de Biotecnología Vegetal at USFQ.

6. DISCUSSION

6.1. Structure, connectivity, and genetic diversity of *C. odorata* in Galapagos

6.1.1. Genetic diversity estimation.

As reported by Hernández et al. (2008), the nine microsatellites used in this study were polymorphic, defined as having more than one allele per locus (Tomás et al., 2000), with 2 to 7 alleles reported for each locus (Table 1). Allelic diversity and observed heterozygosity (Ho = 0.07 – 0.65) were lower than that found for 487 samples of *C. odorata* from Mesoamerica (14 – 30 alleles, Ho = 0.61 - 0.88) (Hernández et al., 2008). This could be attributed to the comparatively small sample size used in this study and bottleneck events common to introduced species that reduced the expected polymorphism and diversity of each locus (Selkoe & Toonen, 2006).

The global expected heterozygosity (He = 0.55) found in this study for the populations of *C. odorata* in Galapagos, was low compared to the genetic diversity reported for this species within its native range in studies conducted in Ecuador (He = 0.81) (Asadobay, 2019), Mesoamerica (He = 0.869) (Hernández et al., 2008) and Bolivia (He = 0.83 - 0.89) (Paredes-Villanueva et al., 2019). Moreover, other studies analyzing the genetic diversity of species within the genus: *C. fissilis* in Brazil (He = 0.83) (Mangaravite et al., 2019) and *C. balansae* in Argentina (He = 0.64) (Soldati et al., 2013), also reported higher heterozygosity values when compared to the populations of Galapagos. This reduced level of genetic diversity found within the introduced range (Downie, 2002) might be a consequence of the founder effects (Lawson Handley et al., 2011; Urquía et al., 2019) and severe bottlenecks (Kelly et al., 2006; Roux et al., 2008) that populations suffer during the introduction phase (Lawson Handley et al., 2011). Such pattern could be consequence of the small fraction of genetic diversity (Urquía et al., 2019) and genotypes (Dlugosch & Parker, 2008; Roux et al., 2008) introduced from the source population because of founder effects.

However, the reported expected heterozygosity (He = 0.55) for *C. odorata* in Galapagos is much higher than the genetic diversity exhibited by other invasive plant species in insular ecosystems like *Psidium guajava* (He = 0.356) in Galapagos (Urquía et al., 2019) and *Cortaderia selloana* (He = 0.095) and *Cortaderia jubata* (He = 0.061) in New Zealand (Houliston & Goeke, 2017). This high genetic diversity has been commonly associated to multiple introduction events (Lawson Handley et al., 2011; Thompson et al., 2016) or high propagule pressure (Kang et al., 2007; Kelly et al., 2006). This has been reported for other invasive tree species like *Paraserianthes lophantha subspecies lophantha* in Hawaii whose high genetic diversity (He = 0.60) was found to be the result of large colonizing populations (Thompson et al., 2016). This can help introduced populations overcome founder effects (Lawson Handley et al., 2011), bottlenecks (Kelly et al., 2006) and adapt to the new environment (Lawson Handley et al., 2011). As multiple introductions can increase the genetic diversity of a population, the high genetic diversity found in the populations of *C. odorata* in Galapagos could be explained by more than one introduction event into the archipelago.

Additionally, low (~0.25) to moderate (~0.60) levels of genetic diversity (Lesher Gordillo et al., 2018; Trujillo-Sierra et al., 2013) were found for the populations of the individual islands (Table 2). Notably, Santa Cruz presented the highest genetic diversity values (He = 0.55, Ho = 0.53, Rs = 3.15) of the four islands. This could indicate that Santa Cruz was one of the islands where *C. odorata* was first introduced to the archipelago since higher genetic diversity values have been associated to older introduced populations (Dlugosch & Parker, 2008). This is consequence of genetic diversity levels rising as populations recover from founder effects and bottleneck events (Dlugosch & Parker, 2008). Alternatively, multiple introduction events could have happened within the same island from the source population (Urquía et al., 2019). This has been suggested by historical records that

mention that *C. odorata* was introduced at least two times to Santa Cruz by Danish Consul Pedro Holst and Captain Castro, both early settlers (Lundh, 2006).

Further, though high genetic diversity was detected in the archipelago, possible inbreeding within the islands was also suggested since the global inbreeding coefficient found (FIS = 0.23) was higher than the significant levels of inbreeding (FIS = 0.178 – 0.223) reported for *Swetenia humilis* (Novick et al., 2003), another Meliaceae tree species; and for *C. fissilis* (FIS = 0.14), a species within the same genus (Mangaravite et al., 2019). This is expected for invasive species since founder effects and bottleneck events usually lead to nonrandom mating (Le Roux et al., 2010). However, inbreeding levels for *C. odorata* in Galapagos were lower than those reported for other insular invasive plant species like *Miconia calvescens* in the Pacific Islands (FIS = 0.27) and *Senecio madagascariensis* in Hawaii (FIS = 0.398) and Maui (FIS = 0.296) (Le Roux et al., 2010). This could be explained by *Cedrela* high self-incompatibility and predominantly outcrossing reproduction (Soldati et al., 2013).

6.1.2. Population structure determination.

The Analysis of Molecular Variance (AMOVA) (Table 3) revealed higher genetic variation within populations (79.30%) than between populations (20.70%) for *C. odorata* in Galapagos. This suggests low differentiation and connectivity and gene flow between the islands (Lesher Gordillo et al., 2018). This same pattern has been reported for other species of *Cedrela*, like *C. fissilis* in Brazil with 82.5% of genetic variation found within populations (Mangaravite et al., 2019) and *C. odorata* and *C. fissilis* in the Upper Parana River with 84.93% of genetic diversity found within populations (Huamán, 2014). This is expected for species of *Cedrela* since outcrossing, long-lived woody plant species usually maintain higher intrapopulation than interpopulation variation (Cavers et al., 2003; Hamrick et al., 1992; Hamrick & Godt, 1996). This is due to their small population densities and heights that

usually lead to greater gene flow since they allow effective pollen and seeds to be dispersed over longer distances (Hamrick & Godt, 1996). Furthermore, the high genetic variation found within populations coincides with that found for reed canary grass (Lavergne & Molofsky, 2007) and *Bromus tectorum* (Novak & Mack, 1993) were high interpopulation variation was consequence of repeated introductions to North America, suggesting once again, the possibility of multiple introduction events for *C. odorata* in Galapagos.

The relative migration network analysis (Figure 4) further favored the possibility of gene flow between the islands, especially between Isabela and San Cristóbal. This connectivity probably occurs by human mediated dispersal since *C. odorata* is the main source of timber for the inhabitants of Galapagos (G. Rivas-Torres & Adams, 2018). Still, it is also possible, that dispersion is occurring naturally through its long distance wind dispersed seeds (Lesher Gordillo et al., 2018; Paredes-Villanueva et al., 2019) since studies have reported that populations of *Cedrela* found within 180 to 250 km of each other are still within potential breeding distance (Cavers et al., 2003; Soldati et al., 2013). This could suggest that gene flow could even be happening between San Cristobal and Isabela, found 209 km apart. Nevertheless, the lack of evidence of island to island dispersion reports for this species in Galapagos, supports that human mediated dispersal is the more probable cause (Gaudeul et al., 2011), though more studies are needed to evaluate this particularity.

Despite the low genetic variation found between populations suggests gene flow and connectivity between the islands, the moderate (F_{ST} = 0.05 - 0.15) to great (F_{ST} = 0.15 - 0.25) genetic differentiation (Hartl & Clark, 1997) found within the reported pairwise F_{ST} distances (0.079 - 0.191) (Table 4) proposes that certain differentiation and structure exists between the islands. Similar values have been reported for other introduced plant species like *Cytisus scoparius* in Chile (0.017 – 0.152) (Kang et al., 2007) and *M. calvescens* in the Pacific

Islands (0.009 – 0.197) (Roux et al., 2008) where both gene flow and genetic structure were suggested for the introduced populations.

This was also evident in the Principal Coordinate Analysis (Figure 2) and STRUCTURE analysis (Figure 3) since both, San Cristóbal and Isabela, appear to be part of the same genetic cluster; Floreana and Santa Cruz were genetically differentiated from the two with Santa Cruz being represented by a combination of both genetic clusters (Figure 3). This could further suggest that gene flow is occurring between the populations of San Cristóbal and Isabela. Alternatively, it is also possible that either one of the two islands, San Cristóbal or Isabela, is the population of origin of the other. This has been observed for *M. calvescens,* were low differentiation between the introduced populations of the Society Islands was consequence of introduction events from Tahiti to the other islands (Roux et al., 2008).

Additionally, the differentiation and structure found between the islands, especially for the populations of Floreana and Santa Cruz, is probably consequence of multiple introduction events from mainland Ecuador to Galapagos. Genetic drift (Gaudeul et al., 2011), environmental conditions (Dlugosch & Parker, 2008) and geographical barriers (Cavers et al., 2003) like the ocean, tend to differentiate populations after their establishment (Gaudeul et al., 2011). However, relatively little time has passed since the introduction of *C. odorata* to Galapagos (1940s) (Lundh, 2006) for processes of adaptation to have occurred (Urquía et al., 2019). In general, the contrast between low genetic variation (Table 3) and moderate to great genetic differentiation (Table 4) and structure (Figure 3) observed between the islands, could be explained by multiple introduction events (Gaudeul et al., 2011) that came from the same place of origin (Roux et al., 2008).

6.2. Invasion history: inferring the origin and number of times *C. odorata* was introduced to the Galapagos Islands

6.2.1. Origin of introduction.

Understanding the origin or source population of an invasive plant species is considered a useful step when designing effective management plans. For example, to identify natural enemies that could potentially be used as biocontrol agents (Lawson Handley et al., 2011). The Principal Coordinate Analysis (Figure 5) performed in this study suggests that *C. odorata* in Galapagos could have been introduced from the Coast of Ecuador since these were the only samples from mainland Ecuador used that were genetically similar to those in the archipelago. Additionally, in the Bayesian population structure (Figure 6) analysis, part of the genetic cluster reported for Galapagos (Green) was also present in the samples from the Coast (Blue).

In spite of the lack of knowledge regarding the introduction history of this species to the Galapagos, historical records about the colonization of the archipelago mention that until the 1970s, ships were the only mean of transportation into the islands (Gordillo, 2000). Visits were scarce. However, most of these vessels traveled from and to Guayaquil in the Coast of Ecuador (Gordillo, 2000; Vera, 1941). This higher connectivity between Galapagos and the Coast compared to other regions from mainland Ecuador could have driven the introduction of *C. odorata* from this region since it was easier for people of the Coast to travel to the archipelago.

Additionally, historical records mention that Dr Pedro Holst, Danish consul in Guayaquil during the 1940s, was one of the people who prompted the introduction of *C. odorata* seeds to the Galapagos (Lundh, 2006), further endorsing the possibility of a Coastal origin for this tree. However, it is also important to mention that only two samples of *C. odorata* from the

Coast were included in this study, so further analyses with more samples are still necessary to confirm these results.

6.2.2. Introduction events.

Most of the proposed scenarios with the highest posterior probabilities obtained in the ABC analyses (Figures 8, 9, 10), suggest at least two independent introductions to different islands from the source of origin for *C. odorata* in Galapagos; coinciding and reinforcing the results of the other analyses performed in this study. This is expected since multiple introduction events appear to be more common than single introductions for invasive plant species (Kang et al., 2007), as has been suggested for *Phalaris arundinacea* (Lavergne & Molofsky, 2007) and *Alliaria petiolate* (Durka et al., 2005) in North America.

Though little is known through historical records about the routes of invasion and introduction events of *C. odorata* in Galapagos, accounts about the colonization history of the islands can be useful to complement the results found in the population genetics of this species in the archipelago. Most of the best supported scenarios obtained through the Approximate Bayesian Computation (ABC) analyses (Figures 8, 9a, 10**)** suggest that Santa Cruz could have been one of the islands to receive initial introductions from the mainland. This is endorsed by the moderately high genetic diversity reported for the population of Santa Cruz (Table 2), and the possibility of recent bottleneck events due to the heterozygosity excess evidenced in the analysis (Table 6). This is mentioned by Lundh (2006), who reports at least two introduction events to Santa Cruz around the 1940s led separately by Danish consul Dr. Pedro Holst and Captain Castro. However, Santa Cruz was the last island to be colonized in the 1920s (Tye et al., 2002). Isabela (1897), San Cristóbal (1866) and Floreana (1832) (Gordillo, 2000) received settlers and developed agriculture and plantations years

before (Quiroga, 2013). During these times of colonization, *C. odorata* could have been introduced to the other islands.

Additionally, four of the five (Figures 8b, 9a, 9b, 10) best supported scenarios also suggest an introduction event to San Cristóbal from mainland Ecuador. No historical records mention this event. However, San Cristóbal was the second island to be colonized and to establish plantations, bringing in plant species from mainland Ecuador (Quiroga, 2013); one of which could have been *C. odorata*. Additionally, until the 1970s, ships were the only way into the archipelago (Gordillo, 2000; Vera, 1941) with San Cristóbal as the main point of entry (Quiroga, 2013) before it was moved to Santa Cruz (Ospina, 2001). As San Cristóbal received people, letters and goods from mainland Ecuador (Quiroga, 2013), it is possible that seeds from plant species like *C. odorata*, were introduced during this time even before they reached Santa Cruz.

Furthermore, the history of colonization of the islands could explain why Isabela was not suggested as a point of entry in any of the scenarios analyzed. Specifically, it could help clarify the genetic similarity (Figure 2) and implied gene flow (Table 4) observed between *C. odorata* of San Cristóbal and Isabela. Based on the order of establishment of settlers in the islands, it is possible that seeds of *C. odorata* were introduced to Isabela from the introduced population of San Cristóbal since settlers colonized Isabela and brought in agriculture around 30 years later after San Cristóbal was established (Gordillo, 2000). This could also explain why no bottleneck events were implied for San Cristóbal (Table 6) since constant gene flow between these islands could make it difficult for heterozygosity – excess based tests to detect them (Hagenblad et al., 2015; Urquía et al., 2019).

On the other hand, the proposed introduction to Floreana from the mainland observed in one of the scenarios (Figure 8a) and sustained with how genetically differentiated its population appears to be (Figures 2, 3), could be consequence of introduction events that

happened when Floreana was first established. Floreana was not only the first island to be colonized (Quiroga, 2013) but started introducing plant species since agriculture began at the time of colonization (Tye et al., 2002).

6.3. Preliminary analysis: genetic affinity of Galapagos' populations within the native Neotropical distribution of this species

Invasive plant species are not always introduced from their native distribution but can originate from a successful introduced population of the species, a phenomenon usually referred to as a bridgehead event or secondary introductions (Lawson Handley et al., 2011; Lombaert et al., 2010). Secondary introductions have been reported for other invasive plant species like *Senecio madagascariensis* that was introduced to Hawaii from Australia and not from its native distribution in Madagascar (Le Roux et al., 2010), and *M. calvescens* that was introduced to Hawaii from Tahiti and not from its neotropical native distribution (Roux et al., 2008). Based on morphological similarities (W. Palacios, personal communication, 2020) and reports about past introductions of *C. odorata* seeds from Cuba to the Coast of Ecuador (G. Galloway, personal communication, 2021), it was hypothesized that the populations of this tree in Galapagos could have been introduced from a subpopulation in the Coast which in turn originated from Central America or Cuba. Historical records of the 1950s, even mention the presence of large plantations of *C. odorata* of Cuban origin in Finca "La Mina" near Guayaquil (Lamb, 1968), the port from where ships traveled to and from Galapagos during the 1900´s (Gordillo, 2000; Vera, 1941), further reinforcing this hypothesis. Additionally, secondary introductions have been reported for exotic populations with high levels of genetic diversity (Kolbe et al., 2004), as is observed for the populations of *C. odorata* in Galapagos. However, and as shown in the Principal Coordinate Analyses performed here and using the allele matrix provided by Cavers et al. (2013), no clear genetic association was found

between the populations of *C. odorata* of Galapagos and any of the populations from other regions of its neotropical distribution (Figure 11). This could suggest a possible native origin for the subpopulation of the Coast of Ecuador from where *C. odorata* was introduced to the archipelago and preliminary reject the possibility of a secondary introduction.

Nevertheless, it is important to mention that only six microsatellite loci were used in this analysis and that microsatellites are not always the best markers to study long term population history because of their high mutation rates (Guichoux et al., 2011) that can sometimes lead to homoplasy (Morin et al., 2004). Additionally, it has been suggested that morphologically, the *C. odorata* of Galapagos are more similar to the ones from Central America than to the native ones from Ecuador (W. Palacios, personal communication, 2020), so more studies are still needed to corroborate these results.

7. CONCLUSIONS

The populations of *C. odorata* in Galapagos present moderately high genetic diversity compared to other invasive insular plant species, suggesting multiple introduction events into the archipelago. The differentiation and structure found between the populations with only San Cristóbal and Isabela appearing to be part of one same genetic cluster, could imply that the introduction history of *C. odorata* is more complex than originally thought. This kind of information should be taken into consideration when developing management plans and biocontrol measures.

Based on genetic association and available data, it is possible to preliminary conclude that *C. odorata* could have been introduced to the Galapagos Islands from the Coast of Ecuador. Furthermore, results presented here support the likelihood of multiple introduction events with Santa Cruz, San Cristóbal and Floreana as possible entry points; events that could have happened sometime during the archipelago's colonization history. However, it is important to mention that more studies are needed and that analyses with more samples from the Coast are currently underway to clarify these results.

Results also allow to assess that no genetic association was found between the populations of Galapagos and those from other regions of this species' distribution (outside from Coastal Ecuador), suggesting a possible native origin for the subpopulation of the Ecuadorian Coast, from where *C. odorata* was introduced to the archipelago. However, the possibility of a secondary introduction should not yet be discarded since these were

preliminary analyses that still require further examination. Finally, these results represent a valuable source of baseline information that could be useful for the future management and proper control of one of the most invasive tree species in the Galapagos Islands.

8. REFERENCES

Asadobay, P. (2019). *Diversidad y conectividad genética del cedro (Cedrela odorata, Meliaceae) en Ecuador continental*. http://repositorio.usfq.edu.ec/handle/23000/8844

Astudillo, F. J. (2018). Environmental and historical archaeology of the Galápagos islands: Archaeobotany of Hacienda El Progreso, 1870–1920. *Vegetation History and Archaeobotany*, *27*(5), 737–751. https://doi.org/10.1007/s00334-018-0668-9

Borchsenius, F. (1997). Patterns of plant species endemism in Ecuador. *Biodiversity & Conservation*, *6*(3), 379–399. https://doi.org/10.1023/A:1018312724137

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, *144*(1), 1–11. https://doi.org/10.1007/s00442-005-0070-z

Cavers, S., Navarro, C., & Lowe, A. (2003). A combination of molecular markers identifies evolutionarily significant units in *Cedrela odorata* L. (Meliaceae) in Costa Rica. *Conservation Genetics*, *4*, 571–580. https://doi.org/10.1023/A:1025692321860

Cavers, S., Telford, A., Cruz, F. A., Castañeda, A. J. P., Valencia, R., Navarro, C., Buonamici, A., Lowe, A. J., & Vendramin, G. G. (2013). Cryptic species and phylogeographical structure in the tree *Cedrela odorata* L. throughout the Neotropics. *Journal of Biogeography*, *40*(4), 732–746. https://doi.org/10.1111/jbi.12086

Chapuis, M.-P., & Estoup, A. (2007). Microsatellite Null Alleles and Estimation of Population Differentiation. *Molecular Biology and Evolution*, *24*(3), 621–631. https://doi.org/10.1093/molbev/msl191

Cintron, B. (1990). *Cedrela odorata* L. Cedro hembra, Spanish cedar. In *Agriculture Handbook* (Vol. 2, pp. 250–257). U.S. Department of Agriculture.

CITES. (2007). *Examen de las propuestas de enmienda a los Apéndices I y II*. Convención sobre el comercio internacional de especies amenazadas de fauna y flora silvestres. https://cites.org/sites/default/files/esp/cop/14/prop/S14-P33.pdf

Cornuet, J. M., & Luikart, G. (1996). Description and Power Analysis of Two Tests for Detecting Recent Population Bottlenecks from Allele Frequency Data. *Genetics*, *144*(4), 2001–2014. https://doi.org/10.1093/genetics/144.4.2001

Cornuet, J.-M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.-M., & Estoup, A. (2014). DIYABC v2.0: A software to make approximate Bayesian computation inferences about population history using single nucleotide

polymorphism, DNA sequence and microsatellite data. *Bioinformatics*, *30*(8), 1187–1189. https://doi.org/10.1093/bioinformatics/btt763

Dakin, E. E., & Avise, J. C. (2004). Microsatellite null alleles in parentage analysis. *Heredity*, *93*(5), 504–509. https://doi.org/10.1038/sj.hdy.6800545

Desjardins, P., & Conklin, D. (2010). NanoDrop Microvolume Quantitation of Nucleic Acids. *JoVE (Journal of Visualized Experiments)*, *45*, e2565. https://doi.org/10.3791/2565

Diagne, C., Leroy, B., Vaissière, A.-C., Gozlan, R. E., Roiz, D., Jarić, I., Salles, J.-M., Bradshaw, C. J. A., & Courchamp, F. (2021). High and rising economic costs of biological invasions worldwide. *Nature*. https://doi.org/10.1038/s41586-021-03405-6

Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, *17*(1), 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Domínguez, N. (2016). *Manejo de Cedrela odorata en Galápagos y su influencia en el ensamblaje de comunidades de plantas nativas y no nativas en los primeros estadios de sucesión*. 59.

Downie, D. (2002). Locating the sources of an invasive pest using a mtDNA gene genealogy. *Molecular Ecology*, *11*, 2013–2026. https://doi.org/10.1046/j.1365-294X.2002.01584.x

DPNG. (2014). *Plan de Manejo de las Áreas Protegidas de Galápagos para el buen vivir*. Dirección del Parque Nacional Galápagos. http://www.galapagos.gob.ec/wp-content/uploads/downloads/2016/07/DPNG_Plan_de_Manejo_2014.pdf

Durka, W., Bossdorf, O., Prati, D., & Auge, H. (2005). Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. *Molecular Ecology*, *14*(6), 1697–1706. https://doi.org/10.1111/j.1365-294X.2005.02521.x

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fieldsend, T. W., Krysko, K. L., Sharp, P., & Collins, T. M. (2021). Provenance and genetic diversity of the non-native geckos *Phelsuma grandis* Gray 1870 and *Gekko gecko* (Linnaeus 1758) in southern Florida, USA. *Biological Invasions*. https://doi.org/10.1007/s10530-021-02463-1

Finch, K. N., Cronn, R. C., Ayala Richter, M. C., Blanc-Jolivet, C., Correa Guerrero, M. C., De Stefano Beltrán, L., García-Dávila, C. R., Honorio Coronado, E. N., Palacios-

Ramos, S., Paredes-Villanueva, K., & Jones, F. A. (2020). Predicting the geographic origin of Spanish Cedar (*Cedrela odorata* L.) based on DNA variation. *Conservation Genetics*, *21*(4), 625–639. https://doi.org/10.1007/s10592-020-01282-6

Galloway, G. (2021). *Cedrela odorata Cuba* [Com.pers.].

Gardener, M. R., Atkinson, R., & Rentería, J. L. (2010). Eradications and People: Lessons from the Plant Eradication Program in Galapagos. *Restoration Ecology*, *18*(1), 20–29. https://doi.org/10.1111/j.1526-100X.2009.00614.x

Gardener, M., Trueman, M., Buddenhagen, C., Heleno, R., Jäger, H., Atkinson, R., & Tye, A. (2013). *A Pragmatic Approach to the Management of Plant Invasions in Galapagos* (pp. 349–374). https://doi.org/10.1007/978-94-007-7750-7_16

Gaudeul, M., Giraud, T., Kiss, L., & Shykoff, J. A. (2011). Nuclear and Chloroplast Microsatellites Show Multiple Introductions in the Worldwide Invasion History of Common Ragweed, *Ambrosia artemisiifolia. PLOS ONE*, *6*(3), e17658. https://doi.org/10.1371/journal.pone.0017658

Gordillo, J. (2000). Relatos de 44 años en Galápagos. *Centro Cultural Abya Yala Del Ecuador*. https://digitalrepository.unm.edu/abya_yala/184

Goudet, J., Jombart, T., Kamvar, Z. N., Archer, E., & Hardy, O. (2020). *hierfstat: Estimation and Tests of Hierarchical F-Statistics* (0.5-7) [Computer software]. https://CRAN.R-project.org/package=hierfstat

Gruber, B., & Adamack, A. (2019). *PopGenReport: A Simple Framework to Analyse Population and Landscape Genetic Data* (3.0.4) [Computer software]. https://CRAN.R-project.org/package=PopGenReport

Guézou, A., Trueman, M., Buddenhagen, C. E., Chamorro, S., Guerrero, A. M., Pozo, P., & Atkinson, R. (2010). An Extensive Alien Plant Inventory from the Inhabited Areas of Galapagos. *PLOS ONE*, *5*(4), e10276. https://doi.org/10.1371/journal.pone.0010276

Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., Lepoittevin, C., Malausa, T., Revardel, E., Salin, F., & Petit, R. J. (2011). Current trends in microsatellite genotyping. *Molecular Ecology Resources*, *11*(4), 591–611. https://doi.org/10.1111/j.1755-0998.2011.03014.x

Hagenblad, J., Hülskötter, J., Acharya, K. P., Brunet, J., Chabrerie, O., Cousins, S. A. O., Dar, P. A., Diekmann, M., De Frenne, P., Hermy, M., Jamoneau, A., Kolb, A., Lemke, I., Plue, J., Reshi, Z. A., & Graae, B. J. (2015). Low genetic diversity despite multiple introductions of the invasive plant species *Impatiens glandulifera* in Europe. *BMC Genetics*, *16*(1), 103. https://doi.org/10.1186/s12863-015-0242-8

Hamrick, J., Godt, M., & Sherman-Broyles, S. (1992). Factors Influencing Levels of Genetic Diversity in Woody Plant Species. *New Forests*, *6*, 95–124. https://doi.org/10.1007/978-94-011-2815-5_7

Hamrick, J. L., & Godt, M. J. W. (1996). Effects of Life History Traits on Genetic Diversity in Plant Species. *Philosophical Transactions: Biological Sciences*, *351*(1345), 1291–1298.

Hartl, D. L., & Clark, A. G. (1997). *Principles of Population Genetics, Third Edition* (Third). Sinauer Associates Inc.

Hernández, G., Buonamici, A., Walker, K., Vendramin, G. G., Navarro, C., & Cavers, S. (2008). Isolation and characterization of microsatellite markers for *Cedrela odorata* L. (Meliaceae), a high value neotropical tree. *Conservation Genetics*, *9*(2), 457–459. https://doi.org/10.1007/s10592-007-9334-y

Hirsch, H., Richardson, D. M., Pauchard, A., & Roux, J. J. L. (2021). Genetic analyses reveal complex introduction histories for the invasive tree *Acacia dealbata* Link around the world. *Diversity and Distributions*, *27*(2), 360–376. https://doi.org/10.1111/ddi.13186

Houliston, G. J., & Goeke, D. F. (2017). *Cortaderia spp.* In New Zealand: Patterns of genetic variation in two widespread invasive species. *New Zealand Journal of Ecology*, *41*(1), 6.

Huamán, A. (2014). *Structure and genetic diversity of Cedrela (Meliaceae) on the upper Parana River*. 44. (Doctoral dissertation, Tesis de Maestría).

Itow, S. (1992). Altitudinal change in plant endemism and species turn-over and diversity on Isla Santa Cruz, the Galapagos Island. *Pacific Science: A Quarterly Devoted to the Biological and Physical Sciences of the Pacific Region*, *46*(2), 251–268.

Jahodová, Š., Trybush, S., Pyšek, P., Wade, M., & Karp, A. (2007). Invasive species of Heracleum in Europe: An insight into genetic relationships and invasion history. *Diversity and Distributions*, *13*(1), 99–114. https://doi.org/10.1111/j.1366-9516.2006.00305.x

Johnson, M. P., & Raven, P. H. (1973). Species Number and Endemism: The Galápagos Archipelago Revisited. *Science*, *179*(4076), 893–895.

Jombart, T., Kamvar, Z. N., Collins, C., Lustrik, R., Beugin, M.-P., Knaus, B. J., Solymos, P., Mikryukov, V., Schliep, K., Maié, T., Morkovsky, L., Ahmed, I., Cori, A., Calboli, F., Ewing, R. J., Michaud, F., DeCamp, R., & Courtiol, A. (2020). *adegenet: Exploratory Analysis of Genetic and Genomic Data* (2.1.3) [Computer software]. https://CRAN.R-project.org/package=adegenet

Kamvar, Z. N., Tabima, J. F., Everhart, S. E., Brooks, J. C., Krueger-Hadfield, S. A., Sotka, E., Knaus, B. J., Meirmans, P. G., Chevalier, F. D., Folarin, D., & Grünwald, N. J. (2021). *poppr: Genetic Analysis of Populations with Mixed Reproduction* (2.9.1) [Computer software]. https://CRAN.R-project.org/package=poppr

Kang, M., Buckley, Y. M., & Lowe, A. J. (2007). Testing the role of genetic factors across multiple independent invasions of the shrub Scotch broom (*Cytisus scoparius*). *Molecular Ecology*, *16*(22), 4662–4673. https://doi.org/10.1111/j.1365-294X.2007.03536.x

Keenan, K. (2017). *diveRsity: A Comprehensive, General Purpose Population Genetics Analysis Package* (1.9.90) [Computer software]. https://CRAN.R-project.org/package=diveRsity

Kelly, D. W., Muirhead, J. R., Heath, D. D., & Macisaac, H. J. (2006). Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. *Molecular Ecology*, *15*(12), 3641–3653. https://doi.org/10.1111/j.1365-294X.2006.03012.x

Kolbe, J., Glor, R., Schettino, L., Lara, A., Larson, A., & Losos, J. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, *431*, 177–181. https://doi.org/10.1038/nature02807

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Lamb, A. F. A. (1968). *Fast growing timber trees of the lowland tropics*. Commonwealth Forestry Institute, University of Oxford. https://ora.ox.ac.uk/objects/uuid:392739ea-7be6-48cf-b427-42711a7be588

Laraque, A., Ronchail, J., Cochonneau, G., Pombosa, R., & Guyot, J. L. (2007). Heterogeneous Distribution of Rainfall and Discharge Regimes in the Ecuadorian Amazon Basin. *Journal of Hydrometeorology*, *8*(6), 1364–1381. https://doi.org/10.1175/2007JHM784.1

Laso, F. J., Benítez, F. L., Rivas-Torres, G., Sampedro, C., & Arce-Nazario, J. (2020). Land Cover Classification of Complex Agroecosystems in the Non-Protected Highlands of the Galapagos Islands. *Remote Sensing*, *12*(1), 65. https://doi.org/10.3390/rs12010065

Lavergne, S., & Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences*, *104*(10), 3883–3888. https://doi.org/10.1073/pnas.0607324104

Lawson Handley, L.-J., Estoup, A., Evans, D. M., Thomas, C. E., Lombaert, E., Facon, B., Aebi, A., & Roy, H. E. (2011). Ecological genetics of invasive alien species. *BioControl*, *56*(4), 409–428. https://doi.org/10.1007/s10526-011-9386-2

Le Roux, J. J., Wieczorek, A. M., Tran, C. T., & Vorsino, A. E. (2010). Disentangling the dynamics of invasive fireweed (*Senecio madagascariensis* Poir. Species complex) in the Hawaiian Islands. *Biological Invasions*, *12*(7), 2251–2264. https://doi.org/10.1007/s10530-009-9635-9

Le Roux, J., & Wieczorek, A. (2008). Molecular systematics and population genetics of biological invasions: Towards a better understanding of invasive species management. *Annals of Applied Biology*, *154*, 1–17. https://doi.org/10.1111/j.1744-7348.2008.00280.x

Lemopoulos, A., Prokkola, J. M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., Koljonen, M.-L., Koskiniemi, J., & Vainikka, A. (2019). Comparing RADseq and microsatellites for estimating genetic diversity and relatedness—Implications for brown trout conservation. *Ecology and Evolution*, *9*(4), 2106–2120. https://doi.org/10.1002/ece3.4905

Lesher Gordillo, J. M., Martínez Sánchez, J. L., Orduña Rodas, P., Hernández Carreta, A. I., Lesher Gordillo, J. M., Martínez Sánchez, J. L., Orduña Rodas, P., & Hernández

Carreta, A. I. (2018). Diversidad genética del cedro rojo (*Cedrela odorata*) en el estado de Tabasco, México. *Bosque (Valdivia)*, *39*(3), 411–417. https://doi.org/10.4067/S0717-92002018000300411

Li, J., Li, H., Jakobsson, M., Li, S., Sjödin, P., & Lascoux, M. (2012). Joint analysis of demography and selection in population genetics: Where do we stand and where could we go? *Molecular Ecology*, *21*(1), 28–44. https://doi.org/10.1111/j.1365-294X.2011.05308.x

Lombaert, E., Guillemaud, T., Cornuet, J.-M., Malausa, T., Facon, B., & Estoup, A. (2010). Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird. *PLOS ONE*, *5*(3), e9743. https://doi.org/10.1371/journal.pone.0009743

Lundh, J. P. (2006). Farm area and cultivated plants on Santa Cruz, 1932-1965, with remarks on other parts of Galapagos. Galapagos Research, 64, 12-25.

Mangaravite, É., Silveira, T. C. da, Huamán-Mera, A., Oliveira, L. O. de, Muellner-Riehl, A. N., & Schnitzler, J. (2019). Genetic diversity of *Cedrela fissilis* (Meliaceae) in the Brazilian Atlantic Forest reveals a complex phylogeographic history driven by Quaternary climatic fluctuations. *Journal of Systematics and Evolution*, *57*(6), 655–669. https://doi.org/10.1111/jse.12545

Mark, J., & Rivers, M. C. (2017). *IUCN Red List of Threatened Species: Cedrela odorata*. IUCN Red List of Threatened Species. https://www.iucnredlist.org/en

Mauchamp, A. (1997). Threats from Alien Plant Species in the Galápagos Islands. *Conservation Biology*, *11*(1), 260–263.

Mauchamp, A., & Atkinson, R. (2009). *Rapid, recent and irreversible habitat loss: Scalesia forest on the Galapagos Islands* (Galapagos Report 2009 - 2010, pp. 108–112). Charles Darwin Foundation. /paper/Rapid-%2C-recent-and-irreversible-habitat-loss-%3A-on/1017f9f72592833c9d9ad87973f2429b371019fa

Mestanza-Ramón, C., Capa, M. S., Saavedra, H. F., & Paredes, J. R. (2019). Integrated Coastal Zone Management in Continental Ecuador and Galapagos Islands: Challenges and Opportunities in a Changing Tourism and Economic Context. *Sustainability*, *11*(22), 6386. https://doi.org/10.3390/su11226386

Moreno, J., Bernal, G., & Espinosa, J. (2018). Introduction. In J. Espinosa, J. Moreno, & G. Bernal (Eds.), *The Soils of Ecuador* (pp. 1–25). Springer International Publishing. https://doi.org/10.1007/978-3-319-25319-0_1

Morin, P. A., Luikart, G., & Wayne, R. K. (2004). *SNPs in ecology, evolution and conservation. Trends in ecology & evolution, 19(4), 208-216.*

Muller Scharer, H., Schaffner, U. & Steinger, T. (2004). Evolution in invasive plants: Implications for biological control. *Trends in Ecology & Evolution*, *19*(8), 417–422. https://doi.org/10.1016/j.tree.2004.05.010

Muriel, P. (2008). La diversidad de ecosistemas en el Ecuador. In *Enciclopedia de las Plantas Útiles del Ecuador* (pp. 28–38). Herbario QCA & Herbario AAU.

https://www.ecotec.edu.ec/documentacion/investigaciones/docentes_y_directivos/articulos/5453_TRECALDE_00098.pdf

Novak, S. J., & Mack, R. N. (1993). Genetic variation in *Bromus tectorum* (Poaceae): Comparison between native and introduced populations. *Heredity*, *71*(2), 167–176. https://doi.org/10.1038/hdy.1993.121

Novick, R. R., Dick, C. w., Lemes, M. R., Navarro, C., Caccone, A., & Bermingham, E. (2003). Genetic structure of Mesoamerican populations of Big-leaf mahogany (*Swietenia macrophylla*) inferred from microsatellite analysis. *Molecular Ecology*, *12*(11), 2885–2893. https://doi.org/10.1046/j.1365-294X.2003.01951.x

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *vegan: Community Ecology Package* (2.5-7) [Computer software]. https://CRAN.R-project.org/package=vegan

Ospina, P. (2001). Migraciones, actores e identidades en Galápagos. *Informe final del concurso: Culturas e identidades en América Latina y el Caribe. Programa Regional de Becas CLACSO.*

Ottewell, K. M., Bickerton, D. C., Byrne, M., & Lowe, A. J. (2016). Bridging the gap: A genetic assessment framework for population-level threatened plant conservation prioritization and decision-making. *Diversity and Distributions*, *22*(2), 174–188. https://doi.org/10.1111/ddi.12387

Palacios, W. (2020). *Cedrela odorata* [Personal Communication].

Paradis, E., Blomberg, S., Bolker [aut, B., cph, Brown, J., Claramunt, S., Claude, J., Cuong, H. S., Desper, R., Didier, G., Durand, B., Dutheil, J., Ewing, R. J., Gascuel, O., Guillerme, T., Heibl, C., Ives, A., Jones, B., Krah [aut, F., … Vienne, D. de. (2020). *ape: Analyses of Phylogenetics and Evolution* (5.4-1) [Computer software]. https://CRAN.R-project.org/package=ape

Paredes-Villanueva, K., de Groot, G. A., Laros, I., Bovenschen, J., Bongers, F., & Zuidema, P. A. (2019). Genetic differences among *Cedrela odorata* sites in Bolivia provide limited potential for fine-scale timber tracing. *Tree Genetics & Genomes*, *15*(3), 33. https://doi.org/10.1007/s11295-019-1339-4

Pearson, D. E. (2009). *Biological invasions on oceanic islands: Implications for island ecosystems and avifauna*. 14.

Piry, S., Luikart, G., & Cornuet, J.-M. (1999). Computer note. BOTTLENECK: A computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity*, *90*(4), 502–503. https://doi.org/10.1093/jhered/90.4.502

Prentis, P. J., Sigg, D. P., Raghu, S., Dhileepan, K., Pavasovic, A., & Lowe, A. J. (2009). Understanding invasion history: Genetic structure and diversity of two globally invasive plants and implications for their management. *Diversity and Distributions*, *15*(5), 822–830. https://doi.org/10.1111/j.1472-4642.2009.00592.x

Pritchard, J. K., Wen, X., & Falush, D. (2010). *Documentation for structure software: Version 2.3*. 39.

Pyšek, P., & Richardson, D. M. (2010). Invasive Species, Environmental Change and Management, and Health. *Annual Review of Environment and Resources*, *35*(1), 25–55. https://doi.org/10.1146/annurev-environ-033009-095548

Quiroga, D. (2013). Changing Views of the Galapagos. In S. J. Walsh & C. F. Mena (Eds.), *Science and Conservation in the Galapagos Islands* (Vol. 1, pp. 23–48). Springer New York. https://doi.org/10.1007/978-1-4614-5794-7_2

Raymond, M., & Rousset, F. (1995). GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. *Journal of Heredity*, *86*(3), 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rentería, Jorge L, & Buddenhagen, C. (2006). Invasive plants in the *Scalesia pedunculata* forest at los Gemelos, Santa Cruz, Galapagos. *Galapagos Research,* 64, 31-35.

Rentería, Jorge Luis, Gardener, M. R., Panetta, F. D., Atkinson, R., & Crawley, M. J. (2012). Possible Impacts of the Invasive Plant *Rubus niveus* on the Native Vegetation of the Scalesia Forest in the Galapagos Islands. *PLOS ONE*, *7*(10), e48106. https://doi.org/10.1371/journal.pone.0048106

Rezadoost, M. H., Kordrostami, M., & Kumleh, H. H. (2016). An efficient protocol for isolation of inhibitor-free nucleic acids even from recalcitrant plants. *3 Biotech*, *6*(1), Article 1. https://doi.org/10.1007/s13205-016-0375-0

Rivas-Torres, G. (2021). *Cedrela odorata in Galapagos* [Personal Communication].

Rivas-Torres, G., & Adams, D. (2018). *A Conceptual Framework for the Management of a Highly Valued Invasive Tree in the Galapagos Islands* (pp. 193–217). https://doi.org/10.1007/978-3-319-67177-2_11

Rivas-Torres, G. F., Benítez, F. L., Rueda, D., Sevilla, C., & Mena, C. F. (2018). A methodology for mapping native and invasive vegetation coverage in archipelagos: An example from the Galápagos Islands. *Progress in Physical Geography: Earth and Environment*, *42*(1), 83–111. https://doi.org/10.1177/0309133317752278

Rivas-Torres, G., Luke Flory, S., & Loiselle, B. (2018). Plant community composition and structural characteristics of an invaded forest in the Galápagos. *Biodiversity and Conservation*, *27*(2), 329–344. https://doi.org/10.1007/s10531-017-1437-2

Rivas-Torres, G., & Rivas, M. G. (2018). Allelopathic Impacts of the Invasive Tree *Cedrela odorata* L. (Meliaceae, Sapindales = Magnoliidae) in the Galapagos Flora. In M. de L. Torres & C. F. Mena (Eds.), *Understanding Invasive Species in the Galapagos Islands: From the Molecular to the Landscape* (pp. 77–93). Springer International Publishing. https://doi.org/10.1007/978-3-319-67177-2_6

Roderick, G., & Navajas, M. (2003). Genes in new environments: Genetics and evolution in biological control. Nat Rev Gen 4: 889-899. *Nature Reviews. Genetics*, *4*, 889–899. https://doi.org/10.1038/nrg1201

Rosenberg, N. A. (2004). distruct: A program for the graphical display of population structure. *Molecular Ecology Notes*, *4*(1), 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Roux, J. J. L., Wieczorek, A. M., & Meyer, J.-Y. (2008). Genetic diversity and structure of the invasive tree *Miconia calvescens* in Pacific islands. *Diversity and Distributions*, *14*(6), 935–948. https://doi.org/10.1111/j.1472-4642.2008.00504.x

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters*, *9*(5), 615–629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Shea, K., Kelly, D., Sheppard, A. W., & Woodburn, T. L. (2005). Context-Dependent Biological Control of an Invasive Thistle. *Ecology*, *86*(12), 3174–3181.

Shi, G., & Ma, C. (2006). Biological characteristics of alien plants successful invasion. *Ying Yong Sheng Tai Xue Bao = The Journal of Applied Ecology*, *17*(4), 727–732.

SoftGenetics LLC. (2012). *SoftGenetics—Software PowerTools for Genetic Analysis*. SoftGenetics. https://softgenetics.com/GeneMarker.php

Soldati, M. C., Fornes, L., Van Zonneveld, M., Thomas, E., & Zelener, N. (2013). An assessment of the genetic diversity of *Cedrela balansae* C. DC. (Meliaceae) in Northwestern Argentina by means of combined use of SSR and AFLP molecular markers. *Biochemical Systematics and Ecology*, *47*, 45–55. https://doi.org/10.1016/j.bse.2012.10.011

Stangroom, J. (2021). *T-Test Calculator for 2 Independent Means*. Social Science Statistics. https://www.socscistatistics.com/tests/studentttest/default2.aspx

Sundqvist, L., Keenan, K., Zackrisson, M., Prodöhl, P., & Kleinhans, D. (2016). Directional genetic differentiation and relative migration. *Ecology and Evolution*, *6*(11), 3461–3475. https://doi.org/10.1002/ece3.2096

Thermo Fisher Scientific. (2009). *NanoDrop 2000/2000c Spectrophotometer V1.0 User Manual*. Thermo Fisher Scientific Inc. https://assets.thermofisher.com/TFS-Assets/CAD/manuals/NanoDrop-2000-User-Manual-EN.pdf

Thompson, G. D., Richardson, D. M., Wilson, J. R. U., Bellstedt, D. U., & Le Roux, J. J. (2016). Genetic diversity and structure of the globally invasive tree, *Paraserianthes lophantha subspecies lophantha*, suggest an introduction history characterised by varying propagule pressure. *Tree Genetics & Genomes*, *12*(2), 27. https://doi.org/10.1007/s11295-016-0984-0

Tobin, P. C. (2018). Managing invasive species. *F1000Research*, *7*. https://doi.org/10.12688/f1000research.15414.1

Tomás, M. A., Carrera, A. D., & Poverene, M. (2000). Is there any genetic differentiation among populations of *Piptochaetium napostaense* (Speg.) Hack (Poaceae) with different grazing histories? *Plant Ecology*, *147*(2), 227–235. https://doi.org/10.1023/A:1009856732262

Trujillo-Sierra, J. E., Delgado-Valerio, P., Ramírez-Morillo, I., Rebolledo-Camacho, V., & Pérez-Nasser, N. (2013). Variación genética en poblaciones mexicanas de *Swietenia macrophylla* King, una especie tropical en expansión geográfica reciente. *Botanical Sciences*, *91*(3), 307–317.

Tye, A., Soria, M., & Gardener, M. (2002). A strategy for Galapagos weeds. *Turning the tide: the eradication of invasive species,* 336-341.

Urquía, D., Gutierrez, B., Pozo, G., Pozo, M. J., Espín, A., & Torres, M. de L. (2019). *Psidium guajava* in the Galapagos Islands: Population genetics and history of an invasive species. *PLOS ONE*, *14*(3), e0203737. https://doi.org/10.1371/journal.pone.0203737

Vera, A. (1941). *Mito y Destino de Galápagos*. Imp. de Portugal. http://repositorio.casadelacultura.gob.ec/bitstream/34000/19299/2/FBNCCE-Vera-4466-PUBCOM.pdf

Vilà, M., & Weiner, J. (2004). Are Invasive Plant Species Better Competitors Than Native Plant Species?: Evidence from Pair-Wise Experiments. *Oikos*, *105*(2), 229–238.

Vinueza, G. (2020). *Diversidad genética y conectividad del cedro (Cedrela odorata, Meliaceae), árbol invasor en las Islas Galápagos*. http://repositorio.usfq.edu.ec/handle/23000/8734

Weir, B., & Cockerham, C. (1984). Weir BS, Cockerham CC.. Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38: 1358-1370. *Evolution*, *38*, 1358–1370. https://doi.org/10.2307/2408641

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., & RStudio. (2020). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics* (3.3.3) [Computer software]. https://CRAN.R-project.org/package=ggplot2

Wilson, J. R. U., Dormontt, E. E., Prentis, P. J., Lowe, A. J., & Richardson, D. M. (2009). Something in the way you move: Dispersal pathways affect invasion success. *Trends in Ecology & Evolution*, *24*(3), 136–144. https://doi.org/10.1016/j.tree.2008.10.007

9. APPENDICES

APPENDIX A: INFORMATION ABOUT THE SAMPLED INDIVIDUALS USED IN THIS STUDY IN THE GALAPAGOS ISLANDS (VINUEZA, 2020) AND MAINLAND ECUADOR (ASADOBAY, 2019)

ID	Species	Locality	Latitude	Longitude	Collector	Altitude (m.a.s.l)	**Voucher**
CO26	*Cedrela odorata*	Galapagos Santa Cruz	-0.61532	-90.36913	G. Vinueza (2020)	571	QUSF
CO39	*Cedrela odorata*	Galapagos Santa Cruz	-0.71205	-90.32325	G. Vinueza (2020)	137	QUSF
CO29	*Cedrela odorata*	Galapagos Santa Cruz	-0.63088	-90.43728	G. Vinueza (2020)	394	QUSF
CO30	*Cedrela odorata*	Galapagos Santa Cruz	-0.67282	-90.42805	G. Vinueza (2020)	246	QUSF
CO37	*Cedrela odorata*	Galapagos Santa Cruz	-0.67140	-90.27580	G. Vinueza (2020)	270	QUSF
CO27	*Cedrela odorata*	Galapagos Santa Cruz	-0.62443	-90.38260	G. Vinueza (2020)	615	QUSF
CO32	*Cedrela odorata*	Galapagos Santa Cruz	-0.67777	-90.41762	G. Vinueza (2020)	233	QUSF
CO31	*Cedrela odorata*	Galapagos Santa Cruz	-0.67482	-90.42302	G. Vinueza (2020)	250	QUSF
CO35	*Cedrela odorata*	Galapagos Santa Cruz	-0.69412	-90.32717	G. Vinueza (2020)	195	QUSF
CO38	*Cedrela odorata*	Galapagos Santa Cruz	-0.69937	-90.32312	G. Vinueza (2020)	184	QUSF
COISA 12	*Cedrela odorata*	Galapagos Isabela	-0.84457	-91.02462	G. Vinueza (2020)	358	QUSF
COISA 15	*Cedrela odorata*	Galapagos Isabela	-0.87375	-91.01208	G. Vinueza (2020)	178	QUSF
COISA 13	*Cedrela odorata*	Galapagos Isabela	-0.85358	-91.01517	G. Vinueza (2020)	275	QUSF
COISA 10	*Cedrela odorata*	Galapagos Isabela	-0.81240	-91.05247	G. Vinueza (2020)	541	QUSF
COISA 11	*Cedrela odorata*	Galapagos Isabela	-0.83503	-91.03565	G. Vinueza (2020)	428	QUSF
COISA 2	*Cedrela odorata*	Galapagos Isabela	-0.85333	-91.03600	G. Vinueza (2020)	390	QUSF
COISA 8	*Cedrela odorata*	Galapagos Isabela	-0.83165	-91.05180	G. Vinueza (2020)	527	QUSF
COISA 9	*Cedrela odorata*	Galapagos Isabela	-0.82163	-91.04710	G. Vinueza (2020)	498	QUSF
COISA 1	*Cedrela odorata*	Galapagos Isabela	-0.86825	-91.01958	G. Vinueza (2020)	240	QUSF
COISA 5	*Cedrela odorata*	Galapagos Isabela	-0.84545	-91.05072	G. Vinueza (2020)	489	QUSF
CO13	*Cedrela*	Galapagos	-0.90027	-89.43775	G. Vinueza	237	QUSF

	odorata	San Cristóbal			(2020)		
CO12	*Cedrela odorata*	Galapagos San Cristóbal	-0.90035	-89.43792	G. Vinueza (2020)	238	QUSF
CO3	*Cedrela odorata*	Galapagos San Cristóbal	-0.91095	-89.57610	G. Vinueza (2020)	192	QUSF
CO1	*Cedrela odorata*	Galapagos San Cristóbal	-0.91092	-89.57597	G. Vinueza (2020)	193	QUSF
CO6	*Cedrela odorata*	Galapagos San Cristóbal	-0.87702	-89.43752	G. Vinueza (2020)	324	QUSF
CO5	*Cedrela odorata*	Galapagos San Cristóbal	-0.91132	-89.57570	G. Vinueza (2020)	187	QUSF
CO8	*Cedrela odorata*	Galapagos San Cristóbal	-0.87693	-89.43775	G. Vinueza (2020)	326	QUSF
CO9	*Cedrela odorata*	Galapagos San Cristóbal	-0.87690	-89.43777	G. Vinueza (2020)	327	QUSF
CO15	*Cedrela odorata*	Galapagos San Cristóbal	-0.90005	-89.43725	G. Vinueza (2020)	232	QUSF
CO14	*Cedrela odorata*	Galapagos San Cristóbal	-0.91597	-89.56182	G. Vinueza (2020)	238	QUSF
COFL 13	*Cedrela odorata*	Galapagos Floreana	-1.31312	-90.47817	G. Vinueza (2020)	334	QUSF
COFL 24	*Cedrela odorata*	Galapagos Floreana	-1.31372	-90.44008	G. Vinueza (2020)	326	QUSF
COFL2	*Cedrela odorata*	Galapagos Floreana	-1.32045	-90.44247	G. Vinueza (2020)	300	QUSF
COFL7	*Cedrela odorata*	Galapagos Floreana	-1.31770	-90.44653	G. Vinueza (2020)	326	QUSF
COFL 11	*Cedrela odorata*	Galapagos Floreana	-1.31635	-90.44670	G. Vinueza (2020)	326	QUSF
COFL 25	*Cedrela odorata*	Galapagos Floreana	-1.31382	-90.44875	G. Vinueza (2020)	346	QUSF
COFL3	*Cedrela odorata*	Galapagos Floreana	-1.31975	-90.44265	G. Vinueza (2020)	306	QUSF
COFL 20	*Cedrela odorata*	Galapagos Floreana	-1.31542	-90.44492	G. Vinueza (2020)	333	QUSF
COFL 17	*Cedrela odorata*	Galapagos Floreana	-1.31502	-90.44663	G. Vinueza (2020)	332	QUSF
COFL 22	*Cedrela odorata*	Galapagos Floreana	-1.31567	-90.44273	G. Vinueza (2020)	312	QUSF
18332	*Cedrela "falcata"*	Ecuador Amazon	0.07380	-77.23960	P. Asadobay (2019)	389	QUSF
18333	*Cedrela odorata*	Ecuador Amazon	0.06230	-77.29490	P. Asadobay (2019)	470	QUSF
18345	*Cedrela odorata*	Ecuador Amazon	-0.73057	-77.49470	P. Asadobay (2019)	688	QUSF
18347	*Cedrela odorata*	Ecuador Amazon	-0.47193	-76.98070	P. Asadobay (2019)	239	QUSF
18348	*Cedrela odorata*	Ecuador Amazon	-0.58117	-76.89100	P. Asadobay (2019)	258	QUSF

18349	*Cedrela odorata*	Ecuador Amazon	-0.65239	-76.88510	P. Asadobay (2019)	276	QUSF
18356	*Cedrela odorata*	Ecuador Amazon	-0.62365	-77.04200	P. Asadobay (2019)	333	QUSF
18359	*Cedrela odorata*	Ecuador Amazon	-0.89229	-77.18190	P. Asadobay (2019)	352	QUSF
18365	*Cedrela odorata*	Ecuador Amazon	-1.32650	-77.89230	P. Asadobay (2019)	1012	QUSF
18377	*Cedrela odorata*	Ecuador Amazon	-2.02000	-77.94720	P. Asadobay (2019)	975	QUSF
COT1	*Cedrela odorata*	Ecuador Amazon	-0.63898	-76.155133	P. Asadobay (2019)	215	QUSF
COT2	*Cedrela odorata*	Ecuador Amazon	-0.63897	-76.155117	P. Asadobay (2019)	211	QUSF
COT3	*Cedrela odorata*	Ecuador Amazon	-0.63907	-76.15505	P. Asadobay (2019)	210	QUSF
COT4	*Cedrela odorata*	Ecuador Amazon	-0.6349	-76.154967	P. Asadobay (2019)	208	QUSF
COT5	*Cedrela odorata*	Ecuador Amazon	-0.63472	-76.155367	P. Asadobay (2019)	229	QUSF
COT6	*Cedrela odorata*	Ecuador Amazon	-0.63467	-76.155383	P. Asadobay (2019)	230	QUSF
COT7	*Cedrela odorata*	Ecuador Amazon	-0.63475	-76.155317	P. Asadobay (2019)	231	QUSF
COT8	*Cedrela odorata*	Ecuador Amazon	-0.63475	-76.155317	P. Asadobay (2019)	230	QUSF
18422	*Cedrela odorata*	Ecuador Coast	0.03584	-79.95113	P. Asadobay (2019)	261	QUSF
18423	**Cedrela brevicarpa*	Ecuador Coast	0.21922	-79.88104	P. Asadobay (2019)	10	QUSF
18424	**Cedrela brevicarpa*	Ecuador Coast	0.24578	-79.86882	P. Asadobay (2019)	6	QUSF
18425	*Cedrela odorata*	Ecuador Coast	0.62291	-79.90772	P. Asadobay (2019)	18	QUSF
18426	**Cedrela brevicarpa*	Ecuador Coast	0.64255	-79.96195	P. Asadobay (2019)	18	QUSF
18428	**Cedrela brevicarpa*	Ecuador Coast	0.38845	-79.64418	P. Asadobay (2019)	333	QUSF
18429	**Cedrela brevicarpa*	Ecuador Coast	0.38845	-79.64418	P. Asadobay (2019)	333	QUSF
18406	**Cedrela angusticarpa*	Ecuador Andes	-0.02140	-78.88610	P. Asadobay (2019)	1191	QUSF
18407	**Cedrela angusticarpa*	Ecuador Andes	-0.04340	-78.96030	P. Asadobay (2019)	857	QUSF
18408	**Cedrela angusticarpa*	Ecuador Andes	-0.11930	-79.00430	P. Asadobay (2019)	698	QUSF
18411	**Cedrela angusticarpa*	Ecuador Andes	-0.17900	-79.03060	P. Asadobay (2019)	751	QUSF
18412	**Cedrela angusticarpa*	Ecuador Andes	-0.14955	-79.08610	P. Asadobay (2019)	581	QUSF
18413	**Cedrela angusticarpa*	Ecuador Andes	-0.14958	-79.08560	P. Asadobay (2019)	587	QUSF
18414	**Cedrela angusticarpa*	Ecuador Andes	-0.10248	-79.08448	P. Asadobay (2019)	614	QUSF

* W. Palacios et al., sp.nov.ined.

APPENDIX B: FINAL CONCENTRATIONS OF BSA (MG/ML) AND $MGCL_2$ (MM) USED IN THE AMPLIFICATION OF NINE MICROSATELLITE LOCI FOR *C. ODORATA* OF GALAPAGOS AND MAINLAND ECUADOR (MODIFICATIONS ARE SPECIFIED)

Primer	BSA (mg/ml)	**$MgCl_2$ (Mm)**
Ced2	0.016	2.5
Ced18	1	2.5
Ced131	0.016	2.5
Ced65	0.016	2.5
Ced95	0.016	2.5
Ced44	0.016	2.5
Ced54	0.016	2.5
Ced41	0.016	2.5
Ced61a	1	3*

*Modification applied only for Galapagos samples.

APPENDIX C: ANNEALING TEMPERATURES AND THERMOCYCLER CYCLES USED IN THE AMPLIFICATION OF NINE MICROSATELLITE LOCI FOR *C. ODORATA* OF GALAPAGOS AND MAINLAND ECUADOR (MODIFICATIONS ARE SPECIFIED)

Primer	Annealing Temperature (ºC)	**Thermocycler Cycles**
Ced2	51*	30 (40*)
Ced18	55	35
Ced131	55	30
Ced65	55	30
Ced95	53*	35 (40*)
Ced44	51/53*	30 (40*)
Ced54	53*	30 (40*)
Ced41	53*	30 (40*)
Ced61a	51	35 - 40

*Modifications applied only for some samples of Galapagos.

APPENDIX D: DETAILS ABOUT THE REGIONS DEFINED FOR SAMPLES OF *C. ODORATA* PROVIDED BY CAVERS ET AL. (2013) USED TO PERFORM PRINCIPAL COORDINATE ANALYSES

Region	Countries	# of sampled individuals
North America	Mexico (Escarcega, Zona Maya, Guadalupe, unidentified)	36
Central America	Costa Rica	37
	Guatemala	25
	Honduras	14
	Nicaragua	4
	Panama	12
Caribbean	Cuba	174
South America	Brazil	31
	Colombia	5
	French Guyana	7
	Peru	51
Ecuador	Ecuador	126

APPENDIX E: ESTIMATION OF THE OPTIMAL K VALUE BASED ON THE ANALYSIS IN STRUCTURE OF INDIVIDUALS SAMPLED IN FOUR ISLANDS OF THE GALAPAGOS

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	**|Ln''(K)|**	**Delta K**
1	10	-713.74	0.1647	-	-	-
2	10	-643.23	0.6429	70.51	44.33	68.94
3	10	-617.05	1.0298	26.18	9.12	8.86
4	10	-599.99	1.7298	17.06	85.59	49.48
5	10	-668.52	6.7201	-68.53	67.63	10.06
6	10	-669.42	3.6043	-0.90	8.49	2.36
7	10	-678.81	3.0881	-9.39	5.26	1.70
8	10	-682.94	8.5534	-4.13	3.05	0.36
9	10	-690.12	8.9662	-7.18	5.72	0.64
10	10	-703.02	5.3233	-12.90	-	-

K, number of analyzed clusters; **Reps**, number of iterations; **Mean LnP(K)**, mean of the natural logarithm of the likelihood per K value; **Stdev LnP(K)**, standard deviation of the natural logarithm of the likelihood per K value; **Ln'(K)**, rate of change of the likelihood distribution; **|Ln''(K)|**, absolute value of the 2nd order rate of change of the likelihood distribution; **Delta K**, rate of change in the ln probability of data between successive K values.

APPENDIX F: ESTIMATION OF THE OPTIMAL K VALUE BASED ON THE ANALYSIS IN STRUCTURE OF INDIVIDUALS SAMPLED IN FOUR ISLANDS OF THE GALAPAGOS AND TWO REGIONS OF MAINLAND ECUADOR

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	**|Ln''(K)|**	**Delta K**
1	10	-1919.48	0.3458	-	-	-
2	10	-1456.42	1.9246	463.06	394.44	204.95
3	10	-1387.80	27.342	68.62	20.10	0.74
4	10	-1339.28	42.518	48.52	40.45	0.95
5	10	-1331.21	136.67	8.07	86.20	0.63
6	10	-1409.34	262.69	-78.13	215.63	0.82
7	10	-1271.84	65.215	137.50	255.60	3.91
8	10	-1389.94	87.176	-118.10	122.69	1.41
9	10	-1385.35	186.07	4.59	4.35	0.02
10	10	-1376.41	111.50	8.94	-	-

K, number of analyzed clusters; **Reps**, number of iterations; **Mean LnP(K)**, mean of the natural logarithm of the likelihood per K value; **Stdev LnP(K)**, standard deviation of the natural logarithm of the likelihood per K value; **Ln'(K)**, rate of change of the likelihood distribution; **|Ln''(K)|**, absolute

value of the 2nd order rate of change of the likelihood distribution; **Delta K**, rate of change in the ln probability of data between successive K values.