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Genetic Diversity and Conservation of Pseudophoenix (Arecaceae) in Hispaniola

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GENETIC DIVERSITY AND CONSERVATION OF *PSEUDOPHOENIX*

(ARECACEAE) IN HISPANIOLA

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To: Dean Kenneth G. Furton
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This thesis, written by Rosa Rodriguez, and entitled Genetic Diversity and Conservation of Pseudophoenix (Arecaceae) in Hispaniola, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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ABSTRACT OF THE THESIS

CONSERVATION AND GENETIC DIVERSITY OF PSEUDOPHOENIX

(ARECACEAE) IN HISPANIOLA

by

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Florida International University, 2014

Miami, Florida

Professor Javier Francisco-Ortega, Major Professor

The Caribbean genus *Pseudophoenix* (Arecaceae) has its center of taxonomic diversity in Hispaniola (Haiti and the Dominican Republic). Three species (*P. ekmanii*, *P. lediniana*, and *P. vinifera*) are restricted to this island. In this thesis I investigated the population genetic diversity and structure of *Pseudophoenix* using ten microsatellite loci. Results showed homozygote excess and high inbreeding coefficients in all populations across all polymorphic loci. Overall, there was high differentiation among populations. Results from the Bayesian and Neighbor Joining cluster analyses identified groups that were consistence with currently accepted species delimitation. We included the only known population of an undescribed morph from the Dominican Republic that has been suggested to represent a new species. Results from the cluster analyses suggested that this putative species is closely related to *P. sargentii* from Turk and Caicos. Our study provided insights pertinent to the conservation genetics and management of this genus in Hispaniola.

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CONCEPTUAL FRAMEWORK

The Caribbean Island Biodiversity Hotspot (Fig. 1.1) is one of the world hotspots with the highest probability for extinction of endemic plants and vertebrates as a consequence of the negative impact of deforestation (Brooks et al. 2002; Mittermeier et al. 2004). In addition, Shi et al. (2005) indicated that the Caribbean Hotspot is one of the four hotspots with the highest pressure from human activities. Therefore, it is not surprising that 39% of the Caribbean palms are threatened by extinction (Zona et al. 2007).

Pseudophoenix H. Wendl. ex Sarg. (Arecaceae) is the sole genus in the tribe Cyclospathe O. F. Cook, subfamily Ceroxyloideae Drude. As such, it is an isolated lineage with no close relatives within the palm family (Dransfield et al. 2008). The four currently recognized species (Zona 2002) occur in the Lesser Antilles (only Dominica), Greater Antilles [Cuba, Navassa Island (USA territory), Hispaniola, and Puerto Rico (island of Mona)], southern Florida, USA (a few islets in Biscayne National Park), the Bahamas, Turk and Caicos Islands, and the Yucatan Peninsula (both Mexico and Belize). The species generally occur in seasonally dry forest or coastal scrub (Zona 2002).

The genus was the subject of a modern taxonomic revision by Zona (2002), and his revision provides the taxonomy that is followed herein. The island of Hispaniola is the center of highest taxonomic diversity with three endemic species (i.e., *P. ekmanii* Burret, *P. lediniana* Read*, and *P. vinifera* (Mart.) Becc.) and one native, *P. sargentii* H. Wendl. ex Sargent. *Pseudophoenix sargentii* is widespread within the area where the genus is found, although it is rare in Hispaniola, Florida, Puerto Rico, and Cuba (Zona

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2002). Zona (2002) noted a single specimen of *Pseudophoenix* from northern Dominican Republic that he was unable to assign to species on the basis of comparative morphology. The single specimen was a tantalizing suggestion that additional morphological diversity exists in the Dominican Republic. The unique morph (thereafter *Pseudophoenix* sp. nova) has an unusual ecology, as it is the only species of the genus to thrive on serpentine soils, which are soils characterized by high nickel and magnesium content.

Population genetic studies are widely accepted as providing important perspectives for conservation, allowing biologists to assess the genetic diversity of species and to establish conservation management strategies (Allendorf and Luikart 2007; Höglund 2009). Conservation genetic studies follow the assumption that habitat changes can influence both extinction risks and three population genetic components: (1) genetic drift, because of stochastic changes in allele frequencies; (2) gene flow, because of the disruption of migration barriers; and (3) the frequency of alleles with adaptive value (Frankham et al. 2004). Therefore, a major paradigm in conservation genetics is that habitat degradation results in loss of genetic variation (Young et al. 1996), as measured by population genetic parameters such as allelic richness and heterozygosity levels (e.g., Van Treuren et al. 1991; Young et al. 1999). Inbreeding increases homozygosity and, as a consequence, the frequency of homozygotes exposing deleterious alleles (e.g., Raijmann et al. 1994). Moreover, when the habitat is fragmented, populations lose their connectivity, and gene flow is interrupted (e.g., Hickerson and Wolf 1998). In addition, fitness – the capability of an individual to survive and reproduce during its life – is expected to decline when population size decreases because of the negative effects of genetic drift and inbreeding (Reed and Frankham 2003). When genetic diversity

decreases many alleles with adaptive value are likely to be lost because of stochastic events (Reed and Frankham 2003; Leimu et al. 2006). As a consequence, it has been postulated that loss of genetic diversity is detrimental to the ability of populations to adapt, making them more susceptible to environmental changes (Palstra and Ruzzante 2008).

Although population genetic models clearly show the negative relationship between habitat fragmentation and genetic diversity (Herron and Freeman 2013), the extent to which habitat fragmentation always results in reduced population genetic diversity is still controversial. For instance, Honnay and Jacquemyn (2007) found a positive correlation between genetic diversity and population size among different studies for 52 plant species. However, other studies have found negative relationships between these two factors. Greimler and Dobes (2000) described this relationship for *Gentianella austriaca* (A. Kern. & Jos. Kern.) Holub (Gentianaceae). They compared the genetic diversity of populations of different sizes and found that in some cases smaller populations have higher genetic diversity than larger ones.

Because conservation genetic studies can provide important instruments for conservation, they are clearly applicable for organisms found in Biodiversity Hotspots. The Caribbean Island Biodiversity Hotspot has a high number of endemic plant species and therefore many plant conservation challenges (Maunder et al. 2008). As noted by Geiger et al. (2014), few population genetic studies have been conducted in these islands. Eight of these studies have used nuclear DNA microsatellite data (SSRs) to document the genetic structure and diversity of Caribbean plants. They focused on *Ipomoea microdactyla* Griseb. (Convolvulaceae) (Geiger et al. 2014), *Pinus caribaea* Morelet var.

bahamensis (Griseb.) W. H. Barrett & Golfari (Pinaceae) (Sanchez et al. 2014),

Pterocarpus officinalis Jacq. (Fabaceae) (Muller et al. 2009), *Pseudophoenix ekmanii*

(Namoff et al. 2011), *Zamia lucayana* Britton (Zamiaceae) (Calonje et al. 2013)*,* and *Z.*

pumila L. (Meerow et al. 2012).

The principal objective of the present thesis was to investigate the population

genetic structure of the four species of *Pseudophoenix* that occur in Hispaniola.

Additionally, we aimed to explore its relationship with patterns of geographical

distribution and morphological differentiation as understood by the currently accepted

taxonomy (Zona 2009). The three main working hypotheses are:

H1: Patterns of genetic diversity will reflect the taxonomy proposed by Zona

(2002). Therefore, populations belonging to the same species will form discrete and

distinct clusters.

H2: Within each species, genetic distances within populations will be related to

geographical distances, i.e., geographically close populations will be genetically close as

well.

H3: A positive correlation will exist between population size and genetic

diversity. Therefore, fragmented or reduced populations found in unprotected areas with

high human impact will have lower levels of genetic diversity.

This thesis is presented in three chapters. Chapter 1 provides a general

introduction to the genus *Pseudophoenix* and a conceptual framework of the importance

of population genetic studies to address conservation biology projects. The second

chapter, "Conservation and genetics of two critically endangered Hispaniolan palms –

genetic erosion of P*seudophoenix lediniana* and high genetic diversity *P. ekmanii*" has

been accepted for publication in the peer-reviewed journal *Plant Systematics and Evolution*. It addresses main conservation biology issues of *P. lediniana*. The species is the most Critically Endangered (sensu IUCN 2013) species of the genus (approximately 70 plants without recruitment are known in a single population in southern Haiti). The second chapter is primarily derived from exhaustive field work to determine distribution patterns and conservation threats to the taxon. Also, a comparison between *P. lediniana* and *P. ekmanii* of levels of genetic variation based on microsatellite (SSR) data is provided. The latter species is also Critically Endangered but it is officially protected in a national park, where at least four populations are known. Previous demographic estimates suggest that these four populations harbor approximately 3,800 adult individuals. For the second chapter population genetic statistical data pertinent to *P. ekmanii* came from a previous conservation genetic study published by Namoff (2011). The third chapter provides a full genetic diversity study for all the species of *Pseudophoenix* occurring in Hispaniola. Additionally, three populations of *P. sargentii* (found in Puerto Rico [Mona Island], Dominica and the Turk and Caicos) were included in work in chapter three. They represent outliers that provide a sampling framework to improve understanding of the genetic relationships among the species and populations from Hispaniola.

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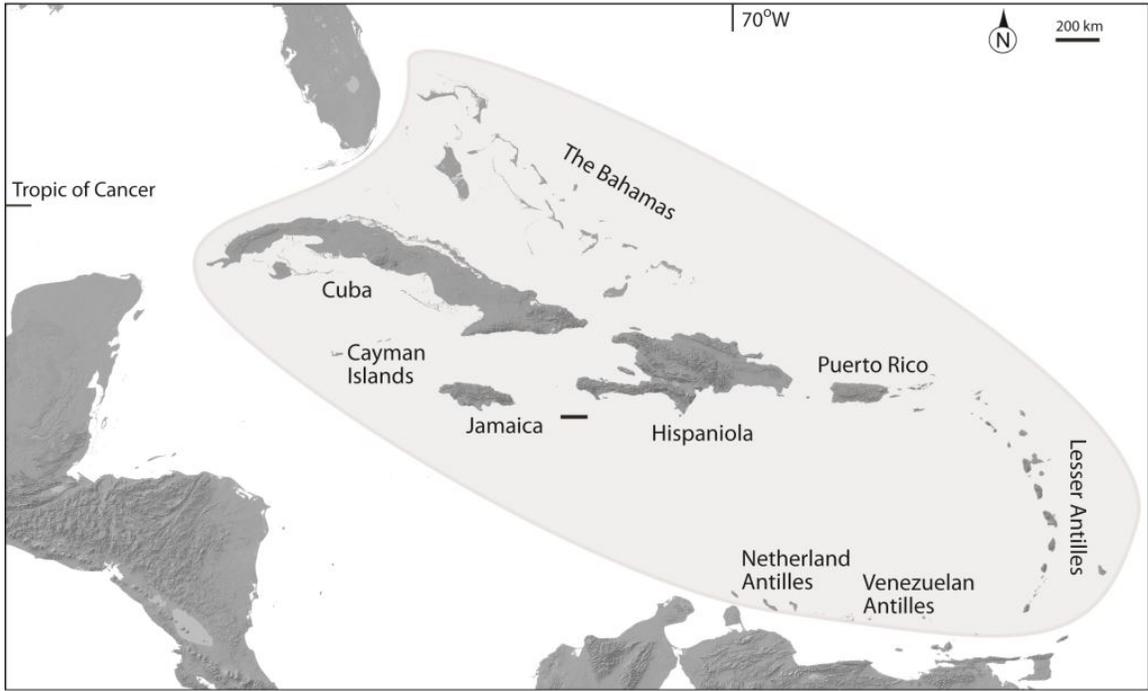
Fig 1.1. The Caribbean Island Biodiversity Hotspot.

CHAPTER II. CONSERVATION AND GENETICS OF TWO CRITICALLY
ENDANGERED HISPANIOLAN PALMS – GENETIC EROSION OF
PSEUDOPHOENIX LEDINIANA IN CONTRAST TO *P. EKMANII*

The flora and fauna of Haiti, part of the Caribbean Island Biodiversity Hotspot,

are recognized as a global priority for conservation (Smith et al. 2004; Maunder et al.

2008), characterized by both high levels of endemism and high levels of threat. Currently

only 2% of the land is covered with natural forest compared with a 60% coverage in the

1920s (Fox 2012). The high rate of deforestation has resulted in massive soil erosion and

associated declines in ecosystem services and biodiversity (Paryski et al. 1989; Paskett

and Philoctete 1990; Sergile and Woods 2001). On the basis of preliminary studies led by

the National Botanic Garden (Dominican Republic), Haiti is estimated to have over 467

endemic species of angiosperms (Clase and Peguero 2006). The flora of the country has

not been comprehensively assessed using IUCN Categories of Threat (IUCN 2013) and

the conservation status of many of the endemics is unknown.

In the last six years a consortium of Caribbean and US scientists has been

studying the conservation status of the Caribbean flora (Francisco-Ortega et al. 2007;

Maunder et al. 2008; Oleas et al. 2013; Carey et al. in press), using molecular techniques

to address conservation questions (e.g., Meerow and Nakamura 2007; Namoff et al. 2011;

Meerow et al. 2012; Calonje et al. 2013; Geiger et al. 2014). The consortium has also

conducted molecular phylogenetic research relevant to the taxonomy and management

strategies of Caribbean Endangered and Critically Endangered plant species (reviewed in

Oleas et al. 2013).

Palms illustrate many of the conservation challenges facing island-endemic plants

(Johnson 1996; Heywood 2011) of the Caribbean and in particular those of Haiti (Zona et

al. 2007). It is estimated that 58% of palm species are restricted to islands (Morici 2004).

Island endemic palms have suffered a long history of anthropogenic decline. There is

good evidence that human activities have been responsible for most of the extinctions of

palms of the Pacific Islands during the late Quaternary (Prebble and Dowe 2008;

Cañellas-Boltà et al. 2013). Major conservation threats for island palms include invasive

species (Strahm 1996; Fleischmann et al. 2005; Meyer and Butaud 2009), unsustainable

use (Read 1988; Ratsirarson et al. 1996; Rueger and von Wallmenich 1996; Morrison et

al. 2012), habitat clearance (Dowe et al. 1997; Maunder et al. 2002; Shapcott et al. 2007;

Manohara et al. 2010), and potentially the impact of climatic change (Shapcott et al.

2012a). A variety of approaches have been developed to recover threatened island palm

populations including ecological restoration and species reintroduction (Maschinski and

Duquesnel 2006), ex situ conservation (Namoff et al. 2010b), sustainable harvesting (Rist

et al. 2010; Francisco-Ortega and Zona 2013), development of species recovery plans

(Morrison et al. 2012), and invasive alien species control (Auld et al. 2010). The

government of the Dominican Republic has established a reserve ("Monumento Natural

Los Cacheos") near the border with Haiti to provide official protection to *Pseudophoenix*

vinifera (Mart.) Becc. (Arecaceae) (Sectorial Law Number 202–04 for Protected Areas),

specifically to prevent sap tapping, horticulture poaching, and to protect the palm's

natural habitat (Congreso Nacional de la República Dominicana 2004).

Approximately seven species of palms are endemic to Hispaniola with three of

these found only in Haiti (i.e., *Attalea crassispatha* (Mart.) Burret, *Copernicia ekmanii*

Burret, and *Pseudophoenix lediniana* Read) (Henderson et al. 1990; 1995). Henderson et

al. (1990) presented a review of the conservation status of Haitian palms, in large part

derived from fieldwork performed between 1988 and 1989. He reported that the endemic *Copernicia ekmanii* (Endangered sensu IUCN (2013)) was restricted to a single site with only one surviving individual. Subsequent extensive fieldwork organized by Fairchild Tropical Botanic Garden botanists in 1996 showed that the species, although locally restricted to coastal areas of northern Haiti, is more abundant with at least 1,200 individuals recorded within its limited distribution range (Timyan et al. 1997; Johnson 1998).

The second Haitian endemic palm, *Attalea crassispatha*, (Critically Endangered sensu IUCN (2013)), is confined to two areas in the south of the country. Field surveys performed by Timyan and Reep (1994) in the early 1990s indicated that, although the distribution range of the species is relatively large (11 sites) there were only 25 individuals known in the wild. The 1990s survey led to ex situ conservation initiatives. Currently a total of ten individuals are cultivated at Montgomery Botanical Center, 35 at Fairchild Tropical Botanic Garden and 14 at the Tropical Research and Education Center of the University of Florida. While there are more individuals in cultivation than in the wild, the level of founder representation in the ex situ populations is unknown.

Three out of the four known species of *Pseudophoenix* are endemic to Hispaniola. *Pseudophoenix ekmanii* Burret (Critically Endangered) is a restricted to southwestern Dominican Republic, (Namoff et al. 2011). *Pseudophoenix lediniana* (Critically Endangered) is confined to southern Haiti. *Pseudophoenix vinifera* (Vulnerable sensu IUCN (2013)) is found both in Haiti and the Dominican Republic (Zona 2002). The fourth, *P. sargentii*, is not globally threatened, with a relatively wide distribution range in the Caribbean Basin (the Bahamas, Dominica, Florida Keys, Greater Antilles, and

Yucatan Peninsula). However, many populations of the species are small and threatened on offshore islands (Zanoni and Buck 1999; Zona 2002; Maschinski and Duquesnel 2006; Santiago-Valentín et al. 2012). Both *P. ekmanii* and *P. vinifera* appear to be threatened by the unsustainable harvesting of sap in the Dominican Republic to make a non-alcoholic beverage known as "Mabí de Cacheo", few of the individuals survive the tapping process (Francisco-Ortega and Zona 2013).

Pseudophoenix lediniana, previously reported a wild population size of about 30 individuals, and is known from one site in southern Haiti, near Jacmel in the Province of Ouest (Henderson et al. 1990) where it is restricted to the slopes of the Rivière Lavange near the epicenter of the 2010 earthquake. From these previous accounts the species was known to grow on unstable mostly inaccessible steep cliffs and that the individuals of this palm were difficult to reach.

The present study was initiated to confirm that *P. lediniana* still occurred in the wild, with the objectives of mapping its current distribution area and to prepare conservation proposals. A second objective was to obtain DNA samples for a conservation genetic study to determine the levels of genetic diversity of this species when compared with the congeneric Dominican Republic endemic *P. ekmanii* (Namoff et al. 2011).

MATERIALS AND METHODS

COLLECTING SITE AND DEMOGRAPHIC STUDIES

Between November 2012 and July 2013 three of the authors (AV, BJ, and WC) made three visits to the area where the species was previously known to occur in the

Province of Ouest. Other localities near the reported population were visited but additional individuals of *P. lediniana* were not located.

During fieldwork several difficulties were faced as most of the individuals were growing on inaccessible sites with unstable substrates; therefore, exhaustive surveys were conducted only in one third of the area where the species occurs. For the rest of the population, the demographic estimates were derived from observations performed with binoculars by two of the team members independently. The number of individuals were counted for adults (i.e., reproductively mature, generally taller than 2 m), juveniles (not yet reproductively mature, generally less than 2 m), and seedlings (plants with no visible trunk, usually with only two emerging small leaves). The number of individuals that were flowering or fruiting was also recorded. The GPS points of plant localities were recorded.

MICROSATELLITE DNA ANALYSIS

Seven DNA microsatellite loci (i.e., pse2.1, pse3.33, pse3.6, pse5.2, pse5.5, pse5.6, and pse7.26) were used to investigate the patterns of genetic diversity of *P. lediniana*. These were the same variable markers that were utilized in a previous conservation genetic study of *P. ekmanii* (Namoff et al. 2011). The DNA was isolated from leaf fragments (fast-dried in silica gel) following Namoff et al. (2010a, 2011). Procedures for PCR amplification and visualization of microsatellite fragments (SSRs) also follow Namoff et al. (2010a, 2011). Fragments were separated using an ABI 3130XL Genetic Analyzer (Applied Biosystems) in the DNA core facility of Florida International University (FIU). Subsequently, alleles were visualized and scored using Peak-Scanner (Applied Biosystems). A total of 21 individuals were sampled encompassing approximately one third of the known wild individuals (see further details pertinent to the

special distribution of individuals below). The program Micro-Checker (Van Oosterhout et al. 2004) was used to evaluate the presence of null alleles and allelic dropouts, employing 10,000 randomizations. Descriptive statistics were obtained with GenAlEx v. 6.501 (Peakall and Smouse 2006, 2012). Tests for Hardy–Weinberg equilibrium (HWE) and the U test (Rousset and Raymond 1995) for heterozygote excess or deficiency were run with GenePop v. 4.0 (Raymond and Rousset 1995; Rousset 2008) using 10,000 Markov chain Monte Carlo iterations (Guo and Thompson 1992). Linkage disequilibrium (LD) was tested for each population with ARLEQUIN v. 3.5 (Excoffier et al. 2005) using a likelihood ratio test (Slatkin and Excoffier 1996). A Markov chain Monte Carlo method was applied with 100,000 iterations and a burn-in of 10,000. Inbreeding coefficients (Fis) were calculated for each population using FSTAT (Goudet 1995). The significance of deviations of Fis from 0 was accessed by permutation tests (1000 permutations with 0.05 alpha level for Bonferroni correction). These calculations were also performed with FSTAT. Principal coordinate analysis (PCO) among all the individuals included in the study was computed with GenAlEx that uses the algorithm developed by Orloci (1978), after conversion of the individual-by-individual genetic distance matrix, as defined by Smouse and Peakall (1999), to a covariance matrix and data standardization. The Bayesian clustering program STRUCTURE v.2.3.3 (Pritchard et al. 2000) was used to estimate the underlying genetic structure among the two population sites. The K-values of 1–5 were simulated across 20 replicate runs of 1,000,000 iterations after a burn-in of 100,000. The ΔK method of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl 2011) was used for determining the 'true' value of K across the samples. After the likely level of K was estimated, a consensus Q-matrix from the 20

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runs was constructed using the program CLUMPP (Jakobsson and Rosenberg 2007) for visualization with DISTRUCT (Rosenberg 2004).

RESULTS

FIELD STATUS

Field studies verified the presence of *P. lediniana* in the area where the species was originally described (Fig. 2.1). Only 71 adults and 2 juveniles were recorded, with no seedlings observed during our flied campaigns. Just a single tree produced fruits in 2013 (Fig. 2.1). In 2012 the dry remains of a fruiting branch was also found in a single tree. The population of *P. lediniana* is highly fragmented and is composed of six small clusters (thereafter sites). Only two of these sites had more than ten individuals and sampling for the genetic conservation study only targeted these two fragments (Fig. 2.2). In the other four sites, individuals were growing on inaccessible cliffs and DNA samples were unobtainable. The six sites extend along a ~3 km stretch of a stream and are on private land. Currently around 20 families live in the area. On the basis of conversations with farmers from this area, during the rainy season this stream carries considerable amount of water and landslides are relatively common in the area. The palm does not have a specific name nor does it have any ethnobotanical use. Locally, this type of palm is referred to by the generic name of "Palmis Maron" and is shared with the royal palm (*Roystonea borinquena* O.F. Cook). The vegetation in this area is degraded lowland tropical broadleaved gallery forest growing on limestone cliffs [dominant plant species were: *Acacia macracantha* Willd. (Fabaceae)*, Bursera simaruba* (L.) Sarg. (Burseraceae), *Eugenia* sp. (Myrtaceae), *Haematoxylon campechianum* L. (Fabaceae)*, Lantana camara* L. (Verbenaceae)*, Panicum maximum* Jacq. (Poaceae), *Rondeletia* sp. (Rubiaceae), and

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other widespread Caribbean low-elevation limestone species]. Despite not being reachable by road, most of the accessible hardwood trees and shrubs showed signs of being regularly cut (for charcoal production). The area was extensively disturbed, and local farmers reported that they burn the forest to plant subsistence staple crops such as sorghum, running beans, corn, cassava, and sweet potato. In July 2013 seeds were collected from the only reproductive individual for cultivation and there are currently 20 seedlings growing in the Botanic Garden of Cayes (Haiti).

CONSERVATION GENETICS

Only four of the seven loci were polymorphic (Table 2.1). The average number of alleles per locus was 2, and Site 2 had three private alleles that were not found in the other site. Three multilocus genotypes where shared among different individuals. Four plants from Site 1 have identical multilocus genotypes, the same pattern was observed in two plants from Site 2. A single multilocus genotype was shared by two individuals from Site 1 and one from Site 2. The global analyses showed that the population deviated significantly from HWE because of heterozygote deficit. The population had two loci that were not in HWE and it is highly inbred with a significantly positive Fis value of 0.508.

The program Micro-Checker did not detect allelic dropout, but there was general excess of homozygotes for those loci–population combinations that were not in HWE (see above), suggesting that null alleles might be present for these loci. Although these loci may account for null alleles, the high number of homozygotes found in those loci could well be the outcome of genetic drift and inbreeding in this highly fragmented population with such a reduced number of individuals.

The first and second axes of the PCO analysis accounted for approximately 67% of the genetic variation. The PCO scatter diagram showed most of the individuals from Site 2 with positive values along the second component. However, the diagram showed that individuals from the two collecting sites overlapped without a clear separation of two groups determined by site provenance (Fig. 2.3). The STRUCTURE analyses did not support the two collecting sites as two different clusters (Fig. 2.3).

DISCUSSION

DISTRIBUTION PATTERNS AND CONSERVATION THREATS

The results confirm that among the species of the genus, *P. lediniana* has the most restricted distribution and the lowest number of individuals in the wild. No other species of the genus is restricted to a single population. While both *P. ekmanii* and *P. lediniana* are Critically Endangered they have different conservation threats, distribution patterns, and protection status. *Pseudophoenix ekmanii* is currently protected in the National Park of Jaragua and a recent survey revealed that, although this species has a limited distribution range, some of its populations have between 330 and almost 2,500 individuals (Table 2.1; Namoff et al. 2011). With few exceptions the populations of *P. ekmanii* studied were composed of seedlings, juvenile, and adult individuals, even though, Namoff et al. (2011) found that in most of the locations individuals were tapped to produce "Mabí de Cacheo" (see above). *Pseudophoenix lediniana* is not harvested for making beverages but is restricted to a single and non-regenerating population with very few individuals. Its habitat is under more intense pressure because of massive forest clearance in a location without any official protection.

Extensive field work conducted in the rest of the Dominican Republic with *P. vinifera* (Rodríguez-Peña 2013) shows the detrimental effect of a combination of massive sap extraction, removal of individuals for the horticultural trade, habitat destruction, and lack of official habitat protection. Rodríguez-Peña (2013) reported that populations of *P. vinifera* near human settlements with active agricultural activities tend to have distribution patterns similar to that of *P. lediniana,* i.e., highly fragmented populations with few individuals. These populations are found in highly degraded habitats and are under strong human utilization pressure. The palm trees are overexploited for their sap, and in many instances individual trees were removed to be planted in tourist and residential developments.

CONSERVATION GENETIC AND MANAGEMENT IMPLICATIONS

Palms are one of the most emblematic plant groups on islands (see above); however, few studies use DNA microsatellite data to address population/conservation genetic questions with them (i.e., Namoff et al. 2011; Shapcott et al. 2007, 2012a,b). The work of Shapcott et al. (2007) combined SSR and Amplified Fragment Length Polymorphism (AFLP) data. These studies focused on Critically Endangered or Endangered species (sensu IUCN (2013)) from the Dominican Republic [*P. ekmanii* (Namoff et al. 2011)], Madagascar [*Beccariophoenix madagascariensis* Jum. & H. Perrier (Shapcott et al. 2007), and *Lemurophoenix halleuxii* J.Dransf. and *Voanioala gerardii* J.Dransf. (Shapcott et al. 2012b)], and Lord Howe Island [*Lepidorrhachis mooreana* (F. Muell.) O.F. Cook (Shapcott et al. 2012a)].

From a conservation genetic perspective, it has been suggested that a reduction of population size has two major effects on the genotype and allele frequencies of

populations (DeSalle and Amato 2009). Alleles with higher frequency will be fixed

through genetic drift (Allendorf and Luikart 2007) resulting in fewer polymorphic loci. In

addition, proportion of homozygous individuals will increase through inbreeding, which

results in a deficit of heterozygotes and reduced fitness (Höglund 2009). The few

population genetic studies of threatened palms endemic to islands support these two

conclusions. With the exception of *Lemurophoenix halleuxii*, populations of these

threatened species were highly inbred with mean allelic fixation index values higher than

0.2. Significance tests (not reported for *Lepidorrhachis mooreana*) showed deviation of

these populations from HWE with the mean observed heterozygosity lower than the mean

expected heterozygosity in all comparisons. Among these studies, *P. ekmanii* and

Voanioala gerardii (Shapcott et al. 2012b) were the only species that had all polymorphic

loci. In the two other species the proportion of polymorphic loci ranged between 67%

(for *Lepidorrhachis mooreana* (Shapcott et al. 2012a) and 70% (for *Lemurophoenix*

halleuxii (Shapcott et al. 2012b)).

The data for *P. lediniana* are in concordance with these conservation genetic

expectations. Although the sampling included approximately one third of the individuals

of the population of *P. lediniana*; robust conclusions pertinent to genetic diversity of *P.*

lediniana cannot be inferred on the basis of only four polymorphic loci. However, these

results provide some interesting insights pertinent to conservation genetics when they are

compared with those from *P. ekmanii* (Namoff et al. 2010a). The latter species has

multiple populations, with larger number of individuals, and the seven SSR loci were

polymorphic in all the collecting sites. Therefore no alleles have been fixed in the

populations of *P. ekmanii* (Namoff et al. 2010a). In contrast the Haitian species is

restricted to a single population, with few individuals and three of the loci are

monomorphic. Inbreeding coefficient values (0.508 in *P. lediniana* vs a maximum score

of 0.3 in *P. ekmanii*) support higher levels of inbreeding in the former than in the latter.

These results for *P. lediniana* are likely the consequence of a drastic decline in the

population size of this species. The decline in population size resented in both genetic

drift and inbreeding resulting in three loci with fixed alleles.

The wild population of the point endemic, *P. lediniana*, is at high risk of

extinction, with a tiny population surviving in an unprotected and privately owned

habitat, no regeneration has been observed and the habitat is subject to continued

degradation by charcoal harvesting and subsistence agriculture. The current conservation

status of *P. lediniana* reflects a situation that is being faced by other Caribbean Island

threatened endemics in which lack of official habitat protection and management plans

together with massive habitat clearance are major challenges for plant conservation

(Maunder et al. 2008, 2011; Torres-Santana et al. 2010; Carey, in press).

Genetic erosion coupled with habitat degradation will be the critical factors to

determine the viability of the species in the wild. The following steps to conserve this

species are proposed. Priority should be given to the management of the wild population

and to the protection of the surviving mature trees. A community conservation agreement

with local farmers whereby incentives would be provided to protect the lone population

could further the protection of this population. Further research is needed to understand

the regeneration ecology of the species, for long-term conservation it is essential to

establish a viable and regenerating wild population. Because of the unstable

topographical conditions of the site where *P. lediniana* occurs, locating alternative areas

on public land for species reintroduction is recommended. Equally important is an ex situ

program as per the recommendations outlined by Maunder et al. (2001). While *P.*

lediniana is currently being cultivated at the Botanic Garden of Cayes there is an urgent

need to expand the founder representation in the cultivated stocks. Botanists from the

Botanic Gaden of Cayes are making regular visits to the only wild population of *P.*

lediniana in order to follow the phenology of this species, to outreach the local

community, to determine seed predation patterns, and to collect germplasm to increase

the current genetic stock for ex situ conservation.

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Table 2.1 *Pseudophoenix lediniana* and *P. ekmanii* population genetic statistics. Data for *P. ekmanii* from Namoff et al. (2011)

Species and locality[a]	P	n_p	A	H_o	H_e	n_{ds}	F_{is}	N_{ig}	LDL
P. lediniana									
Site 1 (9/25)	57	0	1.6	0.032	0.153	1	0.814***	2	0
Site 2 (12/30)	57	3	2	0.226	0.310	2	0.309***	2	17
Population (21/73)	57	na	2	0.143	0.280	2	0.508***	4	17
P. ekmanii									
Popul. 1 (25/329)	100	1	2.8	0.288	0.451	5	0.300***	3	19
Popul. 2 (31/550)	100	3	4.9	0.442	0.666	5	0.211***	0	9
Popul. 3 (22/496)	100	7	5.4	0.528	0.591	4	0.255***	0	9
Popul. 4 (26/2475)	100	3	4	0.459	0.580	5	0.214***	0	9

Population genetic statistics are coded as follows: P, percentage of polymorphic loci; np, number of private alleles; A, average number of alleles per locus; Ho, observed heterozygosity; He, expected heterozygosity; nds, number of loci that deviate significantly from HWE ($P < 0.05$); Fis, inbreeding coefficient; Nig, number of identical genotype pairs; LDL, percentage of paired loci showing linkage disequilibrium. [a] Number of sampled individuals/total number of individuals are given inside the parenthesis. ***Values deviate significantly from 0 ($P < 0.05$)
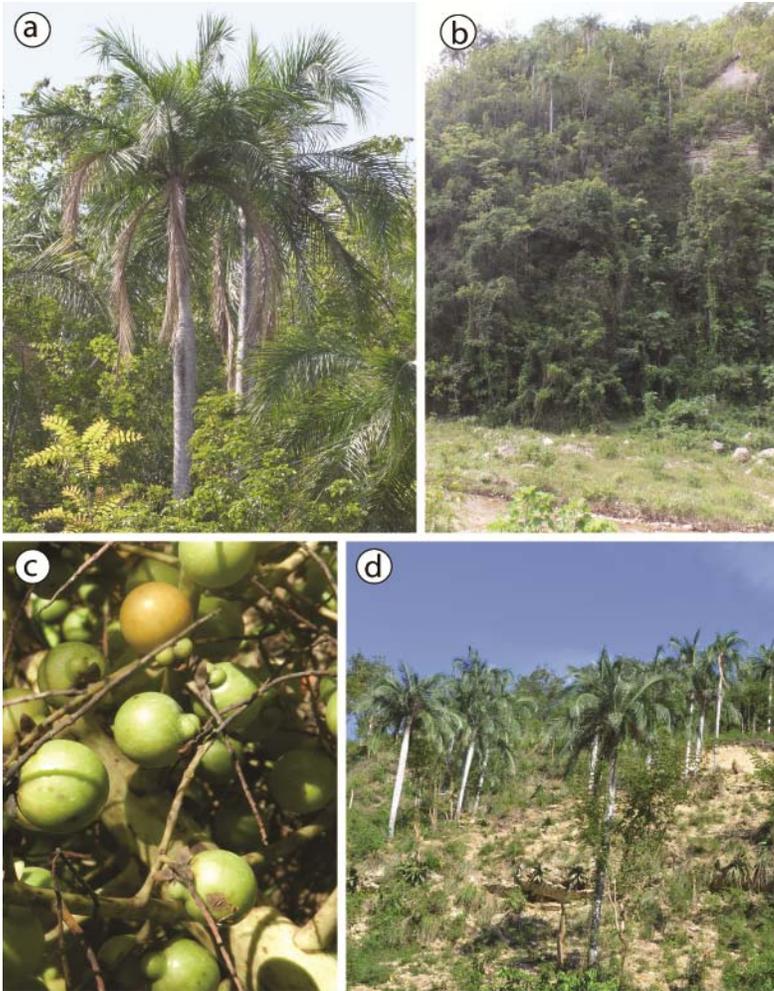
Fig. 2.1 *Pseudophoenix lediniana*: (a) Group of adult individuals (Site 2 for DNA sampling), (b) View of the habitat, notice the palms growing on the upper portion of the cliff and the stream on the bottom of the image (Site 2 for DNA sampling), (c) immature fruits, (d) view of the habitat showing the steep cliffs and lose substrate where the species grows (Site 1 for DNA sampling).
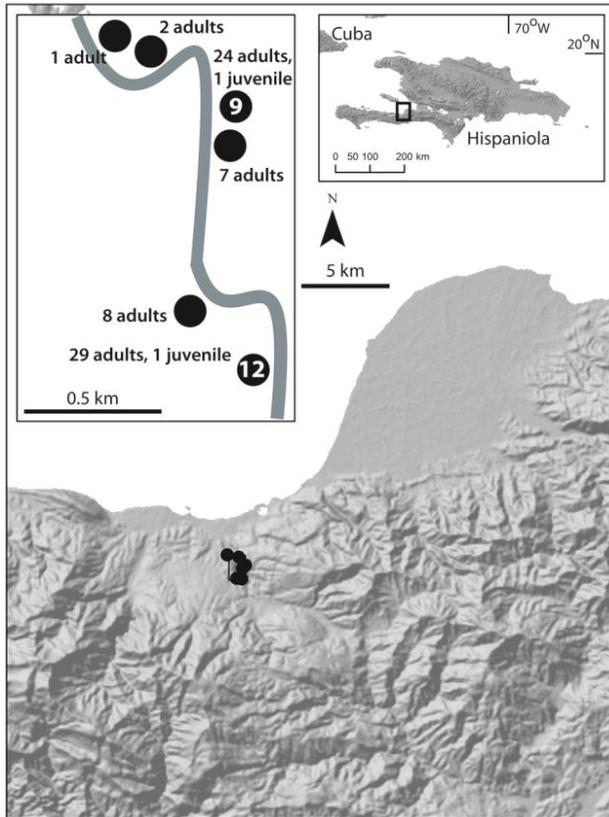
Fig. 2.2 Distribution of *Pseudophoenix lediniana* on Haiti, showing the spatial fragmented distribution of plants along the cliffs of a stream. Stream is shown in dark grey color. This is the only known population of this Haitian endemic palm. Each dot represents a site where plants were recorded. Numbers of adults and juveniles individuals found at in each site are indicated. The two sites were 9 (Site 1) and 12 (Site 2) plants were sampled for the genetic diversity study are also shown.
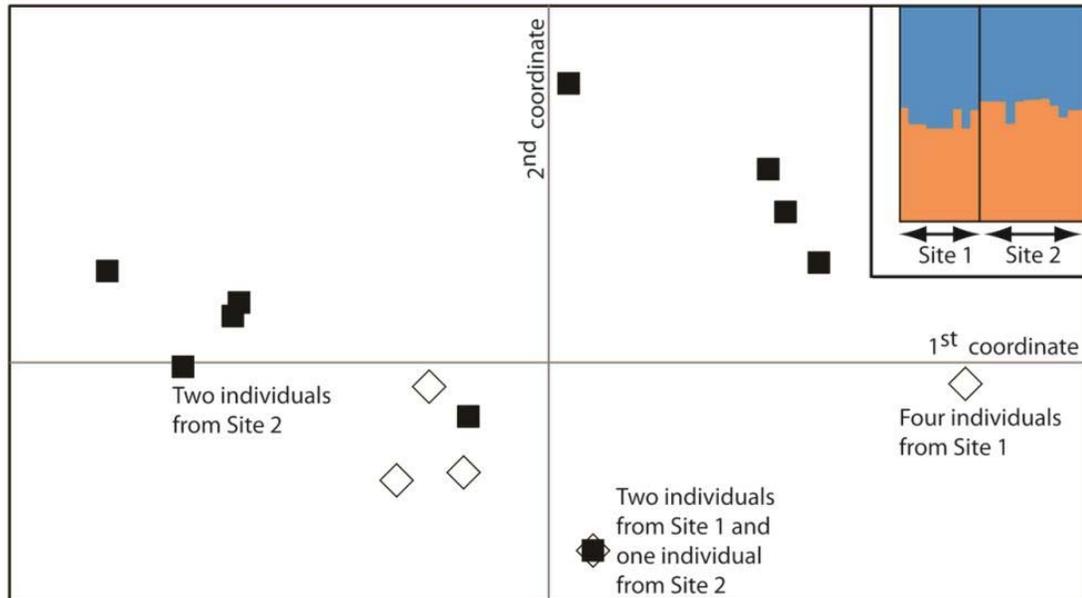
Fig. 2.3 Principal coordinate and STRUCTURE (K = 2) analyses of DNA microsatellite data for the only known population of *Pseudophoenix lediniana*. The scatter diagram shows PCO values along the first two coordinates. Each point represents a single individual, except for the three indicated points that are for multiple individuals with the same multilocus genotype. Individuals for Site 2 are coded with filled symbols. Unfilled symbols refer to individuals from Site 1. Insert in upper right corner shows results yielded by STRUCTURE. Color and box sizes indicate the cluster type of each individual and the number of plants sampled per site. The vertical lines indicate the probability that each individual belongs to an inferred cluster.

CHAPTER III. GENETIC DIVERSITY AND DIFFERENTIATION OF
PSEUDOPHOENIX (ARECACEAE) IN HISPANIOLA

Hispaniola is the second largest island of the Caribbean Island Biodiversity

Hotspot, and within this region it ranks second in plant endemism (Acevedo and Strong

2008). More than 4,000 species occur in Hispaniola, and over 41% of them are endemic

(Acevedo and Strong 2012). In the Dominican Republic, more than 90% of the endemic

plant species are threatened (Peguero and Jimenez 2011). The major factors affecting

biodiversity conservation in Hispaniola are deforestation, unsustainable use of natural

resources, urban development, and expansion of agricultural areas (Ottenwalder 1989;

Paryski et al. 1989; Anonymous 1995; Bolay 1997).

Compared with other plant families (e,g,, Asteraceae, Rubiaceae, Orchidaceae),

the Arecaceae do not have many endemic species in the Caribbean Islands. However,

palms from this biodiversity hotspot provide one of the best examples for

biogeographical disjunctions within the tropics. The palm subfamily Ceroxyloideae has

only eight genera, and they show a discontinuous distribution between the Caribbean

(*Pseudophoenix* H.Wendl. ex Sarg.), South America (*Ammandra* O.F.Cook, *Aphandra*

Barfod, *Ceroxylon* Bonpl. ex DC, *Juania* Drude, and *Phytelephas* Ruiz & Pav.),

Madagascar and the Comoros Islands (*Ravenea* C.D. Bouche), and Australia (*Oraniopsis*

J. Dransf., A. K. Irvine & N. W. Uhl) (Dransfield et al. 2008).

Compared with other palm subfamilies, the Ceroxyloideae appear to have a

relatively recent origin, as this subfamily shared a common ancestor with its sister clade

(the Arecoideae) approximately 52 MYA (Couvreur et al. 2011). *Pseudophoenix* is the

only member of a lineage that is sister to the rest of the Ceroxyloideae. The lineage

branched out approximately 40 MYA during the Eocene (Couvreur et al, 2011). Because of the complex geological history of the Caribbean Islands, with several episodes of transgression and subsidence, most of the West Indian biota is younger than the middle Eocene (Iturralde-Vinent and MacPhee 1999); therefore, the presence of *Pseudophoenix* in the Caribbean Islands fits well the palaeogeographical data available for the region.

Individuals of *Pseudophoenix* have pinnate leaves and a trunk shaped like a bottle; this morphological feature is more prominent in *P. ekmanii* and *P. vinifera* than in *P. sargentii* and *P. lediniana.* Inflorescences bear perfect flowers but produce a few staminate flowers at the end of the inflorescence (Zona 2002). Although field observations suggest that the genus is insect pollinated, *Pseudophoenix* flowers seem to be self-compatible, since isolated individuals growing in gardens produce viable seeds (Namoff et al. 2011).

Species of *Pseudophoenix* grow predominantly in dry forests over limestone soils (Zona 2002). In contrast, the rest of the species of the Ceroxyloideae occur mostly in tropical rain forests (Couvreu et al. 2011). The latest taxonomic treatment of the genus was published by Zona (2002). Hispaniola is the center of highest taxonomic diversity, as all four species of *Pseudophoenix* occur on this island (Figs. 3.1–3.2).

Pseudophoenix lediniana (Fig. 3.2) occurs in a single highly fragmented population close to the epicenter of the earthquake that struck Haiti in 2010. The habitat where it grows is highly disturbed. The species thrives on cliffs that are subject to frequent landslides during the rainy season. The site is usually burned to cultivate staple crops, and its woody species are regularly used to produce charcoal. The locality is not part of any protected area.

Pseudophoenix ekmanii (Fig. 3.1) is restricted to southwestern Dominican Republic, specifically on Barahona Peninsula and Beata Island, where it is protected in the National Park of Jaragua. Despite its threatened conservation status, the species has large populations, and in a single site at "Sabana de Algodón," Namoff et al. (2011) reported over 2,400 individuals. The species was the subject of a recent population genetic study (Namoff et al. 2011), which showed strong evidence for genetic drift, inbreeding, and moderate gene flow among populations. These genetic population patterns were suggested to be the result of habitat fragmentation by human activities, unsustainable use of this species (see below), illegal extraction of palms for the horticulture trade, and destruction of adult individuals in order to gain access to nests of the Hispaniolan parrot, which is harvested for the exotic pet trade (Namoff et al. 2011).

Pseudophoenix sargentii (Fig. 3.2) is the species of the genus with the widest distribution in the Caribbean. It is found in Florida (Biscayne National Park), Puerto Rico (Isla de Mona), Cuba, Navassa Island, Mexico (Yucatan), Belize, Lesser Antilles (Dominica), Haiti (Gonâve Island), and Dominican Republic (Saona Island and "Playa Palmilla", both in the "Parque Nacional del Este"), Turks and Caicos Islands, and the Bahamas. It usually occurs in coastal shores of dry forests although in Mexico and Belize it is also found inland. In some areas, populations of the species have been harvested for horticultural purposes. Good examples of this practice are the Florida populations in Long and Sands Keys, where the species is extinct (Lippincott 1992; Maschinski and Duquesnel 2006). Some populations in the Dominican Republic have been also been removed for tourism development and the horticultural trade.

Pseudophoenix vinifera (Figs. 3.1–3.2) has a wide distribution in the western part of Hispaniola. It grows in dry lowland forests, but its distribution in Haiti is poorly known. The species has two core distribution areas in the Dominican Republic. The first one is in the southern sector of the country (Populations 5–8, see below, Fig. 3.1), located mostly on the lowlands between the Sierra de Neiba and the Sierra de Baoruco (along the "Hoya de Enriquillo") and between the Cordillera Central and the Caribbean Sea (Population 9, see below, Fig. 3.3). The second core area is on the northern part of the country and occupies the lowlands that separate the Cordillera Septentrional and the Cordillera Central predominantly along "Valle del Cibao" (Populations 10 and 11, see below, Fig. 3.3). During interglacial periods from the Miocene (Cibao) and Pleistocene (Enriquillo), these two valleys were largely below sea level (Mann et al. 1984; McNeill et al. 2012). The government of the Dominican Republic has established one reserve ("Monumento Natural Los Cacheos") near the border with Haiti to provide official protection to *P. vinifera* (Sectorial Law Number 202–04 for Protected Areas), specifically to prevent sap tapping and horticulture poaching, and to protect the palm's natural habitat (Congreso Nacional de la República Dominicana 2004). The species also occurs in "Monumento Nacional Las Caobas" and in the "Reserva Biológica Loma Charco Azul."

Zona (2002) indicated that plants of *Pseudophoenix* found in northwestern Dominican Republic (hereafter: *Pseudophoenix* sp. nova, Fig. 3.1) are morphologically distinct and might represent a new species. However, these observations were made on a single herbarium specimen. Zona (2002) decided not to provide any formal taxonomic description. The putative new taxon is highly threatened, and during field studies I could locate only a single population with approximately 34 individuals (Table 3.1, see below).

Interestingly, this population occurs on serpentine soils; therefore, it is the only taxon in this genus to thrive in this unique soil environment characterized by a high content of nickel and magnesium (Lewis et al. 2006).

In the Dominican Republic, *P. ekmanii* and *P. vinifera* are used locally to prepare a sweet drink called "Mabí de Cacheo." Sap from juvenile trees is extracted to make this beverage; once they are tapped, the individual palms usually die (Francisco-Ortega and Zona 2013). The use of *Pseudophoenix* to prepare "Mabí de Cacheo" is one of the main reasons for the decline of these two species in the Dominican Republic (Namoff et al. 2011). *Pseudophoenix lediniana* does not have any known ethnobotanical use in Haiti; however, this species is highly threatened because of deforestation and habitat fragmentation (Henderson et al. 1990).

Two *Pseudophoenix* species are Critically Endangered (*P. ekmanii* and *P. lediniana*) sensu IUCN (2013). Both species have limited distribution areas. *Pseudophoenix ekmanii* is negatively affected by the unsustainable use of its sap and by illegal hunters who cut down palms in order to collect individuals of the endemic Hispaniolan parrot (*Amazona ventralis*) for the pet market. The crown of *P. ekmanii* is one of the nesting sites for this threatened and emblematic bird.

Microsatellites or Simple Sequence Repeats (SSRs) are molecular markers commonly used for population-level studies because they are codominant, biparentally inherited, and generally exhibit high levels of allelic diversity (Chase et al. 1996; Powell et al. 1996). These molecular markers can help to understand the biological features and the evolutionary history of a particular taxon (Fernandez-Silva et al. 2013). Recent examples of how SSRs have had a direct application for Caribbean endemic plants were

reported for *Ipomoea* L. (Geiger et al. 2014), *Pinus* L. (Pinaceae) (Sanchez et al. 2014),

Pseudophoenix (Namoff et al. 2011), *Pterocarpus* Jacq. (Fabaceae) (Muller et al. 2009),

and *Zamia* L. (Meerow et al. 2012; Calonje et al. 2013). In these studies microsatellites

have provided phytogeographical insights (Meerow et al. 2012; Geiger et al. 2014;

Sanchez et al, 2014), have helped to define conservation management units for Critically

Endangered species (Calonje et al. 2013), have revealed high levels of inbreeding in

threatened species (Namoff et al. 2011), and have demonstrated limited gene flow among

populations (Muller et al. 2009).

In this chapter I present the results, on the basis of SSR data, for a population

genetic study of the species of *Pseudophoenix* that occur in Hispaniola. The primary goal

was to use these molecular markers to determine if the taxonomic differentiation reported

within this genus is also revealed by the molecular data. In addition SSRs were used to

investigate the genetic structure and overall levels of genetic variation found in

populations of this genus from Hispaniola.

MATERIALS AND METHODS

STUDY SITES

The study focused on localities from the Dominican Republic (14 populations);

however, samples from the only known population of the Haitian endemic *P. ledinina*

were also collected (Table 3.1, Fig. 3.3). The sampled sites represent the whole

distribution area of the genus in the Dominican Republic. Three study sites were located

in the north (populations 10 and 11 for *P. vinifera*, and population 12 for *P.* sp. nova),

eight in the southwest (populations 2–4 for *P. ekmanii* and 5–10 for *P. vinifera*) and two

in the southeast (populations 14–15 for *P. sargentii*). For *P. vinifera,* I sampled, in

protected areas (populations 5 from "Monumento Nacional Las Caobas, and 7 from Reserva Biológica Loma Charco Azul), unprotected localities where the species still has large numbers of individuals (populations 6 and 10), and unprotected sites that have been highly influenced by human activities (the remaining populations 8, 9 and 11). I could not sample in "Monumento Nacional Los Cacheos", as the best stands of *P. vinifera* from this protected area were in remote areas that were difficult to access; however, the "Monumento Nacional Las Caobas" is adjacent to "Monumento Nacional Los Cacheos", and the present study included one population from this nature reserve. Samples of *P. ekmanii* were obtained from Fairchild Tropical Botanic Garden's DNA bank. These samples were previously used for a population genetic study focusing on this species (Namoff et al. 2011); however, the previous work was used seven SSR loci. For my study, I was able to obtain data for three additional loci (see below). Although the focus of my research was Hispaniola, samples of *P. sargentii* from Dominica, Turks and Caicos Islands, and Puerto Rico were also examined (Table 3.1, Fig. 3.3). These additional samples provided a wider biogeographical framework for the project. In total the population genetic study had samples from 18 populations (Table 3.1, Fig. 3.3).

DNA AND DEMOGRAPHIC SAMPLING

For the molecular studies I collected plant material from at least 25 adult plants per population whenever it was possible. However, for some populations, I obtained fewer than 25 samples because population size was small (i.e., population 1 of *P. lediniana* and the two populations of *P. sargentii* from Mona Island) or some DNA isolations had a very low yield (i.e., population 14). The number of sampled plants ranged between 12 (Mona Island) and 46 (population 3 of *P. ekmanii*).

Demographic inventories were performed to quantify the number of individuals within three plant classes: (1) seedlings (plants with fewer than 3 leaves); (2) juveniles (plants smaller than 1.5 m in height); and (3) adults (plants greater than 1.5 m in height). For the sampling performed in the Dominican Republic, the number of individuals that were tapped to prepare "Mabi de Cacheo" were quantified; tapped individuals have a man-made hole in the trunk right below the crown. Demographic studies for Mona Island included all the individuals found in these populations (Santiago Valentín et al. 2012). Demographic data for the only known population of *P. lediniana* were mostly obtained by observations performed with binoculars by two of the field team members. It was not possible to obtain actual censuses for all the area covered by this population, as most of its fragments were on inaccessible cliffs. Demographic data for the remaining populations were taken from initial censuses that covered approximately 10% of each of the visited sites. These data were subsequently extrapolated to the whole population area; therefore, they represent approximate estimates of the actual population.

DNA ISOLATIONS AND POLYMERASE CHAIN REACTION

Leaf samples were fast-dried in Drierite (W. A. Hammond Drierite Co. Ltd) and then used for DNA isolation with DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Liquid nitrogen was used to disrupt the leaf tissues. Ten microsatellite loci (pse2.1, pse 3.11, pse3.33, pse3.34, pse3.6, pse5.2, pse5.4, pse5.5, pse5.6, and pse7.26), originally developed for *P. sargentii* by Namoff et al. (2010), were used as molecular markers for our study. For *P. ekmanii,* I was unable to recover PCR products for locus pse3.34. Therefore, subsequent data analyses that either combined all 18 populations or targeted the three populations of *P. ekmanii* were used only nine loci;

the rest of the data analyses included data for the ten loci. The PCR conditions and amplification procedures followed the protocol described by Namoff et al. (2010). Samples were run on an ABI 3130XL Genetic Analyzer (Applied Biosystems) in the DNA core facility of Florida International University (FIU). Alleles were visualized and scored using Peak Scanner V1.0 (Applied Biosystems).

DATA ANALYSES

Tests for genotyping errors, null alleles, stuttering, and large allele dropout were conducted with Micro-Checker version 2.2.3 (Van Oosterhout 2004). The program GENALEX 6 v. 6.501 (Peakall and Smouse 2006, 2012) was used to quantify the number of private alleles and number of identical shared multilocus genotypes. The average number of alleles per locus, percent of polymorphic loci, observed heterozygosity, expected heterozygosity, and the percentage of paired loci showing linkage disequilibrium in each population were calculated with ARLEQUIN v. 3.5 (Excoffier et al. 2005). Tests for the number of loci that deviated from Hardy-Weinberg equilibrium (HWE) and the U test (Rousset and Raymond 1995) for heterozygote deficiency were run with GenePop v. 4.0 (Raymond and Rousset 1995; Rousset 2008) using 10,000 Markov chain Monte Carlo iterations (Guo and Thompson 1992) for each population. Inbreeding coefficients (F_{is}) were calculated for each population using FSTAT v. 1.2 (Goudet 1995).

Analysis of molecular variance (AMOVA) among populations (using Euclidean squared distance matrix and with P values obtained after 1000 replicates) was obtained with ARLEQUIN. Values for the diversity measure D_{est} (Jost 2008) were obtained with SMOGD (Crawford 2010). The diversity index has been suggested to provide better estimates for population differentiation than the G_{st} (Nei

1973) or F_{st} (Jost 2008; Heller and Siegismund 2009) indexes. To have estimations of levels of gene flow among populations, the pairwise number of migrants (N_m) per generation between populations were computed with the program ARLEQUIN.

Pairwise genetic distances among populations were computed with POPULATIONS v. 1.2.30 (Langella 1999) using Chord distance (Cavalli-Sforza and Edwards 1967). The resulting inter-population pairwise genetic distances were then used to construct a Neighbor-joining (NJ) tree. Bootstrap analysis was carried out for the obtained network with POPULATIONS and was based on 10,000 permutations. The NJ tree was plotted using FigTree v. 1.4.0 (Rambaut 2012).

Principal coordinate analysis (PCO) among all the individuals from the 18 populations was computed with GENALEX. The analysis was used the algorithm developed by Orloci (1978), after conversion of the individual-by-individual genetic distance matrix, as defined by Smouse and Peakall (1999), to covariance matrix and data standardization.

The program STRUCTURE v.2.3.3 (Pritchard et al. 2000) was used to reveal the genetic structure among populations. K values from 1 to 19 were simulated across 20 replicate runs of 1,000,000 iterations after a burn-in of 100,000. The $\mathit{\Delta}$k method of Evanno et al. (2005), as found in STRUCTURE HARVESTER (Earl and vonHoldt 2012), was used to determine the 'true' value of K across samples. Once the likely level of K was estimated, a consensus Q-matrix from the 20 runs was constructed using CLUMPP (Jakobsson and Rosenberg 2007). Final results were visualized with DISTRUCT (Rosenberg 2004). Six different data sets were analyzed with the Bayesian clustering algorithm. The first one included data for all the individuals from the 18

populations but only had data for nine loci (locus pse3.34 was excluded, see above). The second cluster analysis was also performed for only nine loci, and it included all individuals from the three populations of *P. ekmanii*. All ten loci were included in the four remaining data sets, and they were for: (1) the seven populations of *P. vinifera*; (2) the six populations of *P. sargentii*; (3) the six populations of *P. sargentii* together with the only population of *P.* sp. nova; and (4) the seven populations of *P. vinifera* plus the only known population of *P. lediniana*. Bayesian clustering analyses (3) and (4) were conducted because the NJ network and the PCO scatter diagram (see below) showed a close relationship between *P. lediniana* and *P. vinifera* and between *P. sargentii* and *P.* sp. nova.

To investigate whether a correlation exists between genetic and geographical distances, Mantel's tests of matrix correspondence (Mantel 1967) were conducted with GENALEX. Pairwise Nei's (1972) standard genetic distances among populations were used for these comparisons and they were computed with POPULATIONS. Three different sets of populations were analyzed, and they were for *P. ekmanii*, *P. sargentii*, and *P. vinifera*. Statistical significance for correlations was tested with 1000 random mutations and a 95% confidence interval (Smouse et al. 1986; Smouse and Long 1992).

The program GENALEX was used to investigate spatial genetic structure (SGS). Tests were performed for all the populations except for those of *P. sargentii* from Mona Island and of *P. lediniana*. Geographical coordinates for individuals sampled on these sites were not available. The study was based on autocorrelation analysis using the multilocus genetic correlation coefficient r (Smouse and Peakall 1999).

RESULTS

DEMOGRAPHIC STUDIES

Demographic data for *P. ekmanii* were presented by Namoff el al. (2011) and

therefore are not reported here. We analyzed 454 individual within 18 populations (Table

3.1). *Pseudophoenix lediniana* was the species with the fewest number of individuals (73

plants in its only known population). The majority of the individuals of this species were

adults (71), and no seedlings were found (Table 3.1). *Pseudophoenix sargentii* had the

widest distribution for the genus. For this species the population from Turks and Caicos

had the highest number of individuals (325). Populations from three of the studied sites

(those from Mona Island and Dominica) had fewer than 71 individuals and showed either

no recruitment or very low numbers of juveniles and seedlings. We could not count

seedlings for Population 14 (mainland Dominican Republic) because there were three

other species of palm growing in the same area, and we were not able to discriminate

among seedlings from these different palm species. The two largest populations of *P.

vinifera* were located in protected areas (Population 7 at "Reserva Biológica Loma

Charco Azul" with an estimate of 910 individuals and Population 5 at "Monumento

Nacional Las Caobas" with 610 individuals). Interestingly, we could not locate any

seedlings in Population 6, despite this being the population of *P. vinifera* with the third

largest number of individuals (460). The only known site of *P*. sp. nova (Population 12)

had the third lowest number of individuals (34) among all the populations of

Pseudophoenix included in this study. Contrary to original expectations, in none of the

populations individuals that appeared to have been tapped to produce Mabí de Cacheo

were located

GENETIC VARIABILITY

The final data matrix included genetic information for 454 individuals and had 1.7% of missing data. Population 4 (*P. ekmanii* from Isla Beata) and Population 14 (*P. sargentii* from the Dominican Republic) had the highest proportion of missing data (9.6% and 4.7%, respectively). Loci pse5.2 (3.7%) and pse7.26 (4.8%) had the highest percentage of missing data. Allele sizes ranged from 129 (locus pse3.34) to 479 bp (locus pse5.2). The total number of alleles across all populations and loci was 243 with a mean population value of 13.5 alleles.

The nine loci studied for *P. ekmanii* were polymorphic for all localities, except for the Isla Beata population, which exhibited only seven polymorphic loci. All loci amplified for *P. lediniana* (Population 1), but just four loci were polymorphic for this Haitian species. All loci were polymorphic for all the populations of *P. sargentii* from the Dominican Republic (Populations 14 and 15). However, just eight loci were polymorphic for the two *P. sargentii* sites from Mona Island (Populations 16 and 17) and Dominica (Population 18). Most loci were polymorphic for all populations of *P. vinifera* (Populations 5–11) with the exception of Population 9, which had nine polymorphic loci. (Table 3.2). The only population of *P.* sp. nova did not have any monomorphic loci. Population 13 (*P. sargentii* site from Turks and Caicos) had the largest number of alleles per locus (A = 8.4), while the only population of *P. lediniana* had the lowest value (A= 1.7, Table 3.2). We found 59 private alleles with an average value of n_p = 3.3 per population. Population 13 had the largest number of private alleles (n_p = 9) and Population 18 (*P. sargentii* site from Dominica) was the only locality that did not have private alleles (Table 3.2).

The U-tests showed that all populations departed significantly from HWE and displayed heterozygote deficiency (mean H_o value across all populations of 0.28 vs mean H_e value across all populations of 0.52, Table 3.2). Four of the populations that did not have monomorphic loci had more than seven loci that deviated significantly from HWI (Population 3 of *P. ekmanii*, Population 8 of *P. vinifera*, the only population of *P*. sp. nova, and Population 8 of *P. sargentii*) (Table 3.2). The six populations with monomorphic loci showed that at least 75% of their polymorphic loci deviated significantly from HWE. The average inbreeding coefficient value among all populations was 0.43. The two highest values for this coefficient were found in Population 16 (*P. sargentii* from Mona Island, $F_{is} = 0.67$) and Population 9 (*P. vinifera* from Bahía de Ocoa, $F_{is} = 0.63$, Table 3.2). The two lowest F_{is} values were exhibited by *P. sargentii* (Population 13 from Turks and Caicos ($F_{is} = 0.34$) and Population 15 from Saona Island ($F_{is} = 0.37$)).

Identical multilocus genotypes were detected only in Populations 1 (*P. lediniana*, $N_{ig} = 6$), 4 (*P. ekmanii*, $N_{ig} = 1$), and 9 (*P. sargentii*, $N_{ig} = 1$). All populations had at least four pairs of loci in linkage disequilibrium (Table 3.2). Populations 4 (*P. ekmanii*), 9 (*P. vinifera*), 12 (*P*. sp. nova), and 14 (*P. sargentii*) had the largest percentage of paired loci in linkage disequilibrium (LDL=33%). The three sampling sites with the lowest proportion of paired loci in linkage disequilibrium were found in *P. lediniana* (Population 1), *P. vinifera* (Population 10), and *P. sargentii* (Population 17) (LDL = 4% in these three populations).

No evidence of large allele dropout was found in any locus; however, the MICROCHECKER output indicated that there was general homozygote excess

suggesting the presence of null alleles. Although these loci may have null alleles, the high proportion of homozygotes detected in this study could also be the consequence of stochastic genetic processes and high levels of autogamy. In addition, no locus had excess of homozygotes in all populations. This provided additional support to interpret the MICOCHECKER results as evidence for true homozygote excess instead of presence of null alleles.

There were no clear relationships between population size and levels of genetic diversity. For example the two smallest sampled populations (those from Mona Island) had only 2.1 average alleles per locus and both displayed a low H_o value of 0.15 (Population 16) and 0.2 (Population 17). However, the results also showed notable exceptions to these initial predictions. For instance the population of *P. sargentii* from Turks and Caicos (325 individuals) displayed the highest H_o value (0.44) and the highest number of alleles per locus (8.4) among all the sampled sites; however, this population did not have the highest number of individuals. Another exception was provided by Population 2 (*P. ekmanii*), which had the second largest number of individuals (2,475) but exhibited a low average number of alleles per locus (3.7) and a low H_o value (0.22). Likewise, the very small population of *P.* sp. nova (34 individuals) had the second highest number of alleles per locus (6.3) and a relatively high H_o value of 0.36. Similar patterns were detected in two small populations of *P. vinifera,* Population 8 with 53 individuals and Population 11 with 76 individuals, which displayed high H_o values of 0.31 and 0.34, respectively. Indeed, none of the populations with more than 400 individuals had H_o scores higher than 0.3.

GENETIC DIFFERENTIATION AMONG POPULATIONS

The mean value for the diversity measure of Jost (2008) across all populations was 0.75, suggesting high differentiation among populations (Table 3.3). However, there was a trend for D_{est} values to be much larger among populations from different species than among conspecific populations. The highest D_{est} value was 0.98 and it was found between three populations of *P. ekmanii* and *P. vinifera* (Populations 2 and 5, Populations 2 and 7, and Populations 4 and 7). The lowest differentiation was found between Populations 14 and 15 of *P. sargentii* from the Dominican Republic ($D_{est} = 0.05$).

Average N_m values were 0.99 for all the samples. In most pairwise comparisons the number of migrants per generation was much higher between conspecific populations than among populations from different species (Table 3.3). The highest N_m values were obtained among conspecific populations located in close geographical proximity. The two highest of these values were between Populations 14 and 15 of *P. sargentii* from the Dominican Republic ($N_m = 8.55$) and Populations 2 and 3 of *P. ekmanii* ($N_m = 2.94$). Interestingly, the third highest value for this migration index was between the population of *P.* sp. nova and the population of *P. sargentii* from Turks and Caicos ($N_m = 2.69$).

The AMOVA analyses indicated that 24% of the genetic variation is found among species. These analyses also showed that 22% of the variation was found among populations within species and 54% of the variation within populations.

The result of the Mantel tests revealed that genetic and geographic distance among populations were correlated both for *P. sargentii* (r=0.82, *P*=0.02) and for *P. vinifera* (r=0.69, *P*=0.003).

POPULATION STRUCTURE

The first and second coordinates of the PCO explained only 23.51% of the genetic variation. *Pseudophoenix vinifera* and *P. lediniana* samples tended to have high positive values along the first PCO axis, and they grouped together (Fig. 3.4). In contrast, those individuals belonging to the three populations of *P. ekmanii* exhibited low scores along this first axis and formed a distinct cluster. Samples of *P. sargentii* and *P.* sp. nova displayed low values along the second coordinate and clustered together.

The NJ network recovered four groups that were concordant with the current taxonomy (Fig. 3.5). Each of these groups corresponds to one of the four species currently recognized with the genus. Interestingly, the only known site of *P.* sp. nova was part of the group that had the populations of *P. sargentii* and it was closely related to the population from Turks and Caicos.

The ΔK method of Evanno et al. (2005) suggested a "true value" of $K = 11$ clusters across all 18 populations of *Pseudophoenix* (Fig. 3.6). The results obtained using the Bayesian clustering analyses were consistent with species delimitation; therefore, populations shared clusters within species but not among species. Populations of *P. lediniana*, *P. ekmanii*, and *P.* sp. nova grouped into one different cluster each. The six populations of *P. sargentii* were assigned to three different clusters. Individuals of this species from Dominica mostly exhibited the first of these three clusters. Turks and Caicos samples were largely assigned to the second cluster. Finally those individuals of *P. sargentii* from Mona Island, Saona, and the main island of Hispaniola mostly were allocated to the third cluster. The seven populations of *P. vinifera* were distributed into five clusters. Samples from Populations 5, 7, and 8 primarily belonged to one different

cluster each, and little admixture was detected among them. These three clusters were very rarely found in the four remaining populations of *P. vinifera*. Samples from the two localities of this species from northern Dominican Republic (Populations 10 and 11) were predominantly assigned to another cluster that was not present in the other populations. Finally individuals from the two sites from southern Dominican Republic (Populations 6 and 9) mostly belonged to a cluster that was very rarely detected in the remaining populations.

When the Bayesian clustering analysis was run individually for each species, similar results were found only for *P. sargentii* (optimal $K = 3$, Fig. 3.6b) and *P. vinifera* (optimal $K = 5$, Fig. 3.6d). Association patterns between the samples of these two species and their recovered clusters were very similar to those yielded by the 18 population analysis (compare Fig. 3.6a and Fig 3.6b for *P. sargentii* and compare Fig. 3.6a and Fig. 3.6d for *P. vinifera*).

The Evanno method identified an optimal $K = 3$ from the analysis of the data matrix that combined samples of *P. sargentii* and *P.* sp. nova (Fig. 3.6c). This analysis clearly supported a close genetic connection between the only known site of *P.* sp. nova and that of *P. sargentii* from Turks and Caicos. All members from these two populations were mostly assigned to a single cluster. Cluster membership for the remaining five populations of *P. sargentii* followed the pattern detected in the separate analysis that only targeted this species (Fig. 3.6b).

Results from the Bayesian clustering analysis of the *P. ekmanii* samples (optimal $K = 4$) (Fig. 3.6e) were different from those found after the analysis of the 18 populations (Fig. 3.6a). The global analysis suggested that all the individuals of this species belonged

to a single cluster. However, the separate analysis showed that samples from Populations 2 and 4 of P. *ekmanii* were mostly assigned to one different cluster each, although a few individuals of these sites exhibited admixture with other clusters (Fig. 3.6e). The two remaining clusters were primarily confined to Population 3, but they showed admixture mostly involving these two clusters.

The analysis performed for the single population of P. *lediniana* and the populations of P. *vinifera* suggested an optimal *K* value of 3 (data not shown). This separate analysis assigned all the individuals of *P. lediniana* to a single cluster that was not present in any of the samples of *P. vinifera*. The remaining two clusters were restricted to the populations of *P. vinifera.*

DISCUSSION

GENETIC DIVERSITY AND DIFFERENTIATION

There is a general assumption that smaller populations tend to harbor lower genetic diversity than larger ones (Oostermeijer et al. 2003). The results of this study did not concur with this prediction as there was not a clear association between population size and genetic diversity estimates.

These unexpected results might be the consequence of relatively recent habitat fragmentation or population size decline coupled with the life-cycle features of *Pseudophoenix*. Although no data concerning the environmental history of the targeted populations was gathered for this study, it is well known that since the 15th century, the Caribbean Islands, and particularly the Dominican Republic, have experienced extensive habitat fragmentation and forest clearance associated with rapid human developments (Sambrook et al 1999; Alscher 2011). Currently, the Caribbean Islands rank third in

human population density among the Biodiversity Hotspots (Cincotta et al. 2000),

and just between 2000 and 2012, the Dominican Republic experienced an average annual

human population growth of 1.45%, which is one of the highest rates for the Western

Hemisphere (Anonymous 2014). Individuals of *Pseudophoenix* have long life cycles with

an estimated reproductive age of 57 years (Duran 1995). It has been suggested that these

kinds of long-lived organisms will show the negative impact of genetic drift on genetic

diversity only after several hundreds of years because they have long generation times

and overlapping cohorts (Glémin et al. 2006; Duminil et al. 2007, 2009).

Overall, the analysis of population genetic structure showed high genetic

differentiation, high genetic variation within populations, and high inbreeding

coefficients. For each of the species, the Isolation-by-Distance analysis showed that as

geographical distance increases, genetic similarity decreases. This tendency was stronger

in *P. sargentii* probably because of the larger geographical distances among populations

in this species, which spreads across several islands. The D_{est} values supported higher

differentiation among species than within populations of the same species. Likewise, the

number of migrants (N_m) was low among species but high among populations of the

same species. These results suggested that gene-flow is more relevant within conspecific

populations than among populations belonging to different species. A notable exception

to this pattern was found for Populations 5 and 6 (*P. vinifera*). These two sites are only

14 km apart but display high D_{est} values (0.31) and low Nm (1.2) scores, suggesting

limited genetic differentiation between them.

In four pairwise comparisons, populations from different islands showed little

genetic differentiation. The two most relevant examples were found between the main

island of Hispaniola and the small islands of Beata (Population 2 vs Population 3 of *P. ekmanii*) and Saona (Population 14 vs Population 15 of *P. sargentii*). Pairwise D_{est} values for these two population comparisons were the lowest detected in the study. In addition, their N_m scores were among the highest in the analysis. The islands of Beata and Saona are 29 and 13 km away from the main island of Hispaniola, respectively. It is likely that both of them were connected to the current Hispaniola during the last glacial period (~12,000 years ago) because of the shallow waters (< 15 m) of the straits that separate them (UASD 2002a, 2002b). Gene-flow cannot be ruled as a mechanism to account for the little differentiation shown by these populations; however, it is plausible that the detected N_m and D_{est} values are the result of recent vicariance events that followed the rise of sea-level that separated these small islands from the current island of Hispaniola during the Holocene. Little inter-island genetic differentiation was also detected among (1) populations of *P. sargentii* from Hispaniola and those from Mona Island and (2) the population of *P. sargentii* from Turks and Caicos and that of *P.* sp. nova from northern Dominican Republic. Deep waters separate these two islands from Hispaniola, and thus they were not connected to the latter during the last glacial period. Therefore, over-water dispersal is the only biogeographical avenue to explain the high N_m and low D_{est} values exhibited in these two inter-island population comparisons.

The inbreeding coefficients across all the populations were high and significant. Contrary to the initial expectations, F_{is} values were highly positive even in those sites that had a high number of individuals. These results suggested that expected fixation of alleles across populations via (1) low genetic migration because of habitat fragmentation (see above) and (2) stochastic events associated with genetic drift of small populations are not

the only evolutionary mechanisms behind the genetic structure of the species. It is well known that small isolated populations are more likely to have high levels of inbreeding as a consequence of limited gene flow and a higher frequency of mating among relatives (Leimu et al. 2006; Herron and Freeman 2013); however, in this study both small and large populations show evidence of reduced genetic diversity and inbreeding.

An alternative explanation is that population genetic structure is influenced not only by genetic drift and migration but also by breeding systems and their associated patterns of reproductive biology (Young et al. 1996). Generic drift is a strong evolutionary force in small populations but almost insignificant in large populations (Ouborg et al. 2006), and its effect is greatly diminished in species with long generation times

According to Duminil et al. (2007), mating system is one of the major factors that influence the population genetic structure of any plant species. Breeding systems will have a strong effect on population genetic structure regardless of population size and age. It is expected that outcrossing species will show low genetic differentiation among populations, high within-population genetic diversity, and low levels of inbreeding, particularly in large populations (Hamrick and Godt, 1996). In contrast, it is expected that a rapid increase of homozygote frequencies across generations without changes in allele frequencies will occur for those species that have self-pollination as their primary reproductive strategy (Herron and Freeman 2013).

No studies have been conducted pertinent to the reproductive biology and breeding systems of *Pseudophoenix* (Barfod et al. 2011). However, isolated individuals of the genus have been found to produce seeds, suggesting that they are self-compatible

(Zona pers. comm.). The high F_{is} values found across all of the populations sampled for this study suggest that these casual observations from botanic gardens might confirm that self-pollination is an important feature of the reproductive biology of this genus and could explain the levels of inbreeding shown by the results.

Two other population genetic studies based on SSRs that mostly focused on plants from the Caribbean Islands have also reported low levels of genetic diversity and high F_{is} values (Muller et al. 2009; Geiger et al. 2014). One of these studies concerned populations of a *self-incompatible species* (i.e., Geiger et al. 2014). In this case, the authors attributed the reduced levels of genetic diversity and significantly high inbreeding coefficients to genetic drift and mating among relatives. This example involved species with reduced population size within a highly fragmented habitat. However, the second study (Muller et al. 2009) suggested that the high F_{is} values found in populations of *Pterocarpus officinalis* Jacq. (Fabaceae) could be attributed both to stochastic events related to genetic drift and to breeding systems that promote self-pollination.

TAXONOMIC IMPLICATIONS

Results from the Bayesian and Neighbor Joining cluster analyses were consistent with the current taxonomy of the genus as published by Zona (2002). Additionally, no indication of admixture between populations belonging to different species was found. The NJ tree showed that the vast majority of the populations grouped according to their current taxonomic assignment, with the exception of Population 12 (*P*. sp. nova), which formed a cluster with *P. sargentii* from Turks and Caicos (Fig. 3.5). This cluster was part of another group that included all of the populations of *P. sargentii* (Fig 3.5). Zona (2002) was unable to assign plants of *Pseudophoenix* from northern Dominican Republic

(i.e., *P.* sp. nova) to any particular taxon, indicating that further studies were needed to clarify the taxonomic placement of this morph. Zona's (2002) morphological studies showed that plants belonging to *P.* sp. nova have a combination of unique traits not found in any other species of the genus. They include three-sided calyxes and ovoid fruits (as in *P. vinifera*) and divaricating rachillae (as in *P. sargentii*). However, his conclusion was based on the study of a single herbarium specimen. A full taxonomic and morphological study of *Pseudophoenix* is beyond the scope of this thesis. However, systematic studies are in progress to clarify the placement of this enigmatic morph and determine its specific identity (Rodríguez-Peña and Zona in prep.).

The results of this thesis showed that microsatellites markers have taxonomic value and that they can be useful for species delimitation. SSRs are not believed to be good molecular markers for phylogenetic reconstructions, as there are uncertainties concerning their mutation model (Jarne and Lagoda 1996). In addition, the utility of microsatellite allele frequencies to obtain phylogenies has been questioned (Scribner and Pearce 2000).

Microsatellite markers have been widely used in palm population genetic studies (e.g., Kaneko et al. 2011; Nazareno et al. 2011; Abreu et al. 2012; Menezes et al. 2012; Ramos et al. 2012; Cibrián-Jaramillo et al. 2013), but these works have mostly focused on research pertinent to population genetic diversity, genetic conservation, and genetic structure. Two particular studies have used these markers to address taxonomic questions. The first one concerned *Phoenix atlantica* A.Chev., and it supported this Cape Verde Island endemic as a distinct species, clearly differentiated from *P. dactylifera* L. (Henderson et al. 2006). The second one was conducted by Bacon et al. (2012), who

analyzed microsatellites and DNA nucleotide sequence data to investigate species boundaries within *Pritchardia* in Hawaii. However, the authors failed to reach robust conclusions because of rampant interspecific hybridization.

CONSERVATION IMPLICATIONS

Not all the species and sites included in this study are located inside protected areas. For instance, all of the populations of *P. ekmanii* are protected in the National Park of Jaragua, whereas the only known population of *P. lediniana* is found on private land and is not the subject of any *in situ* conservation initiative (see further details pertinent to the conservation status of these two species in Chapter 2). Concerning *P. vinifera*, the populations from the northern area of the Dominican Republic are not protected, but two of the populations from southern Dominican Republic are located inside the nature reserves of "Monumento Natural Las Caobas" or of "Reserva Biológica Loma Charco Azul." The *P. sargentii* populations from Mona Island and the Dominican Republic are also found on protected areas. However, no *in situ* conservation actions have been developed for *P.* sp. nova and *P. sargentii* from the Dominican Republic and Turks and Caicos, respectively. Field observations found that all of the populations that are located outside nature reserves (except for Population 6 of *P. vinifera* on Jimaní) have a reduced number of individuals and are in areas with human disturbance in a highly fragmented habitat. The two populations of Mona Island are the only ones that are found inside a protected area that have severely reduced population sizes of 14 and 24 individuals.

Among the species of the genus, *P. lediniana* and *P.* sp. nova should have the highest priority for conservation because they are restricted to one population each and have a reduced number of individuals. The extremely reduced levels of genetic diversity

found in *P. lediniana* (see Chapter 2) stresses the importance to have *in situ* conservation

action plans for this species. Contrary to initial expectations, the only population of *P*. sp.

nova exhibited relatively high H_o values, had 100% polymorphic loci, and had the second

highest average number of alleles per locus among all the populations included in the

study.

The lack of clear relationships between levels of genetic diversity and population

size (see above) was also found when comparisons were made between protected and

unprotected sites. Contrary to what was anticipated, protected areas do not harbor most of

the genetic diversity of the genus. Possible reasons for these results are the recent

creation of these protected areas. Established in 1975, the "Parque Nacional del Este" is

the oldest of the protected areas of the Dominican Republic where *Pseudophonenix*

occurs (Hernández et al. 1990). The nature reserve of Mona Island was created in

1919 (Sastre de Jesús and Santiago-Valentín, 1996); however, this island has serious

problems with feral goats and pigs, both of which are extremely detrimental to the native

flora (Santiago-Valentín et al. 2012). In addition, even the protected areas often

have poor conservation enforcement and are under severe anthropogenic pressure by

local communities who exploit these forests (Powell and Inchaustegui 2009).

Overall the results support Isolation-by-Distance for the study sites (see above);

however, in many instances populations of *P. vinifera* that were geographically distant

were more similar than those that were geographically close. These unexpected patterns

for population similarities might well reflect past gene-flow routes and common

population ancestry that have been disrupted by habitat fragmentation. It is likely that

such a disruption has been enhanced by small population sizes and the breeding system of

Pseudophoenix, which appears to favor self-pollination. Therefore it is suggested that future conservation efforts should aim to maintain population connectivity and increase population size, particularly targeting those populations where low genetic diversity was detected.

Species delimitation is very important for conservation management (Frankham et al. 2002). Without a clear idea of what needs to be protected, it is almost impossible to prepare sound conservation action plans. The results of this thesis help delineate *Pseudophoenix* species boundaries and identify taxa for which conservation actions are required immediately.

CONCLUSION

Population genetic diversity in *Pseudophoenix* species was poorly related to the size of most populations, but these results are in concordance with recent habitat fragmentation coupled with the life history strategies of *Pseudophoenix*. Generally, genetic differentiation and within-population genetic variability were high. More study is needed in order to establish the influence of reproductive biology in shaping the genetic structure of *Pseudophoenix* populations. Microsatellite data support the current taxonomy of *Pseudophoenix*. Field observations suggest that this genus is very sensitive to human disturbance, and it is likely that habitat fragmentation together with negative human activities will have stronger conservation genetic consequences in the near future. In order to counteract these impacts, conservation action initiatives should be implemented as soon as possible.

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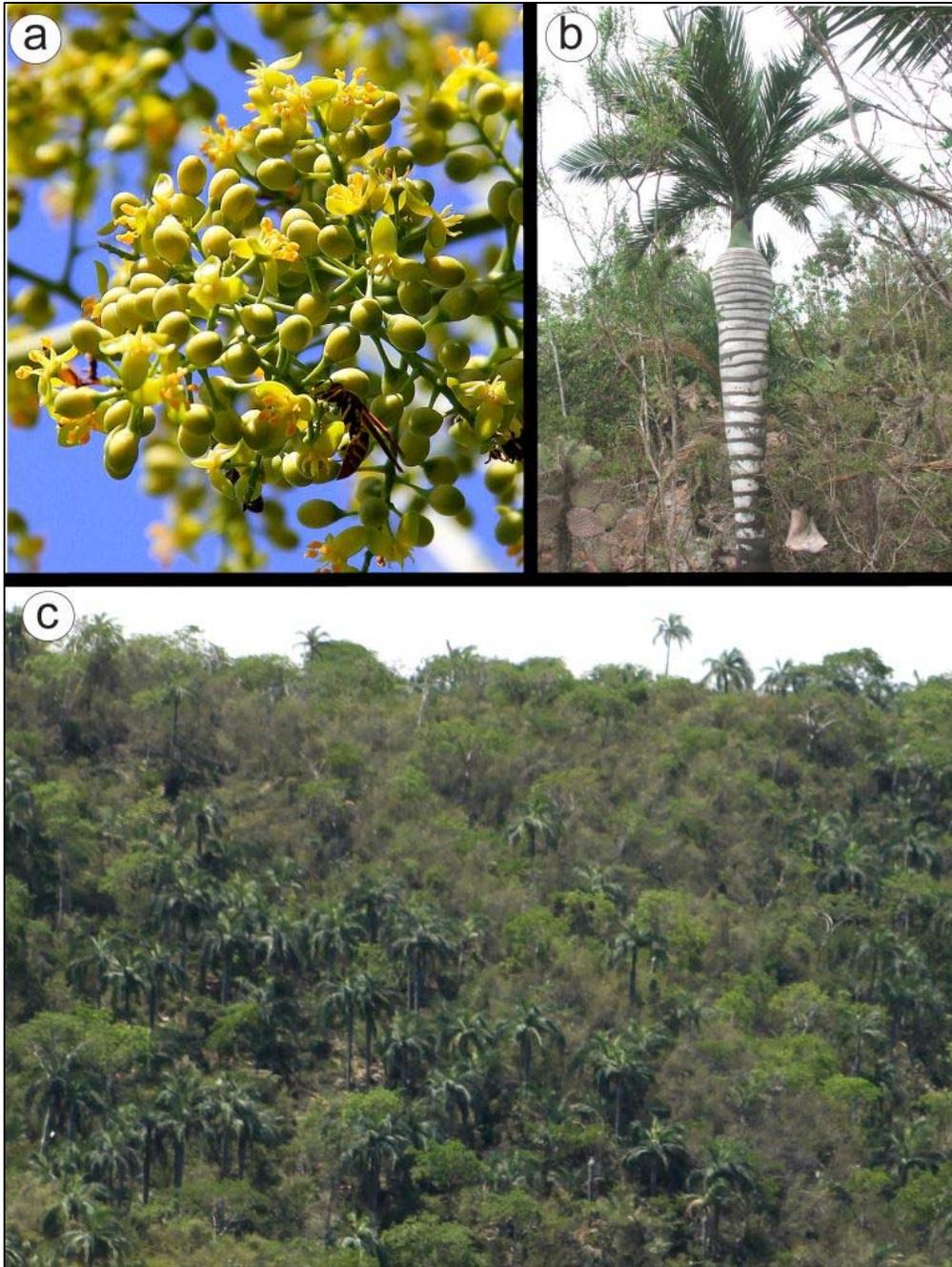
Fig 3.1. *Pseudophoenix* plants in Dominican Republic. a Inflorescence of *P.* sp. nova; b Adult individual of *P. ekmanii*; c Dense stand of *P. vinifera* in Jimaní. Images by Rosa Rodríguez.
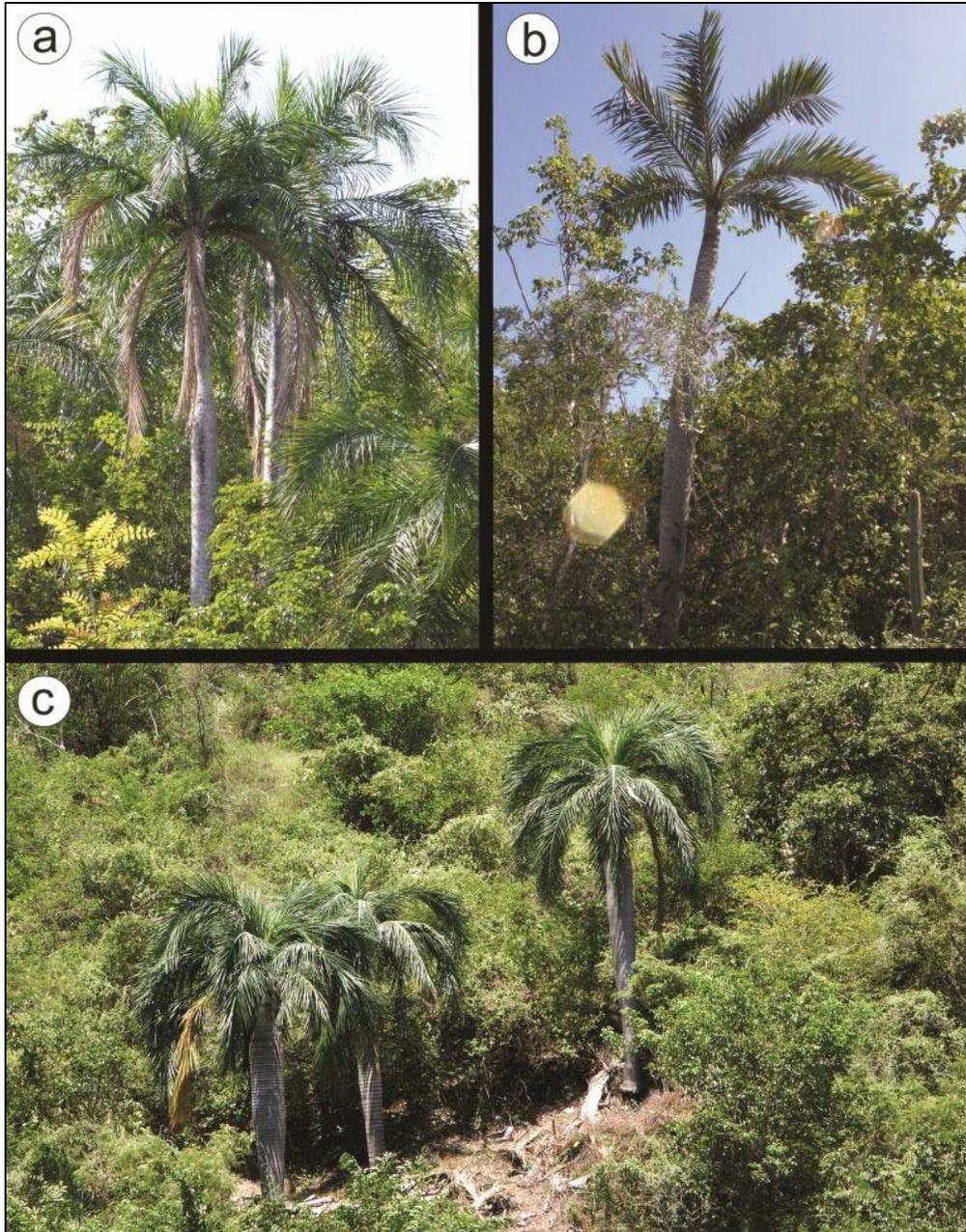
Fig 3.2. *Pseudophoenix* plants in Dominican Republic and Haiti. a Adult individual of *P. lediniana*, Haiti; b Adult individuals of *P. sargentii*, Dominican Republic; c Adult individuals of *P. vinifera*, Dominican Republic. Images by Rosa Rodríguez (a–b) and Scott Zona (c).
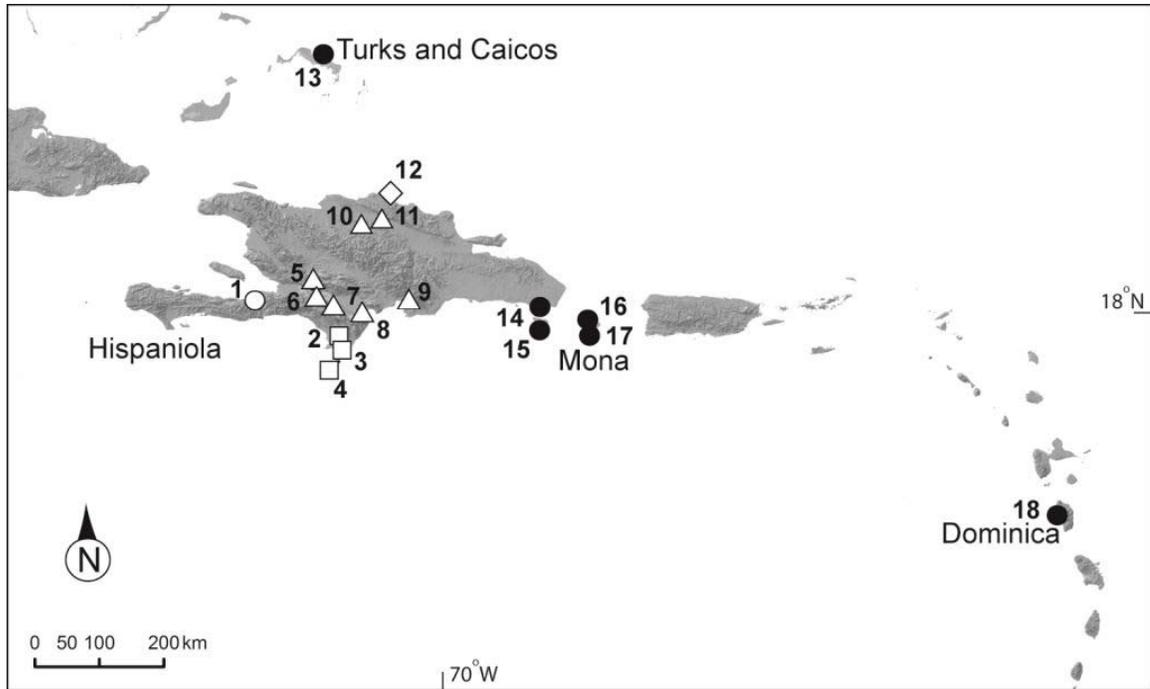
Fig. 3.3. Geographical distribution of the 18 populations of *Pseudophoenix* included in this study. ○ = *P. lediniana*, □ = *P. ekmanii*, △ = *P. vinifera*, ◇ = *P.* sp. Nova, ● = *P. sargentii*.
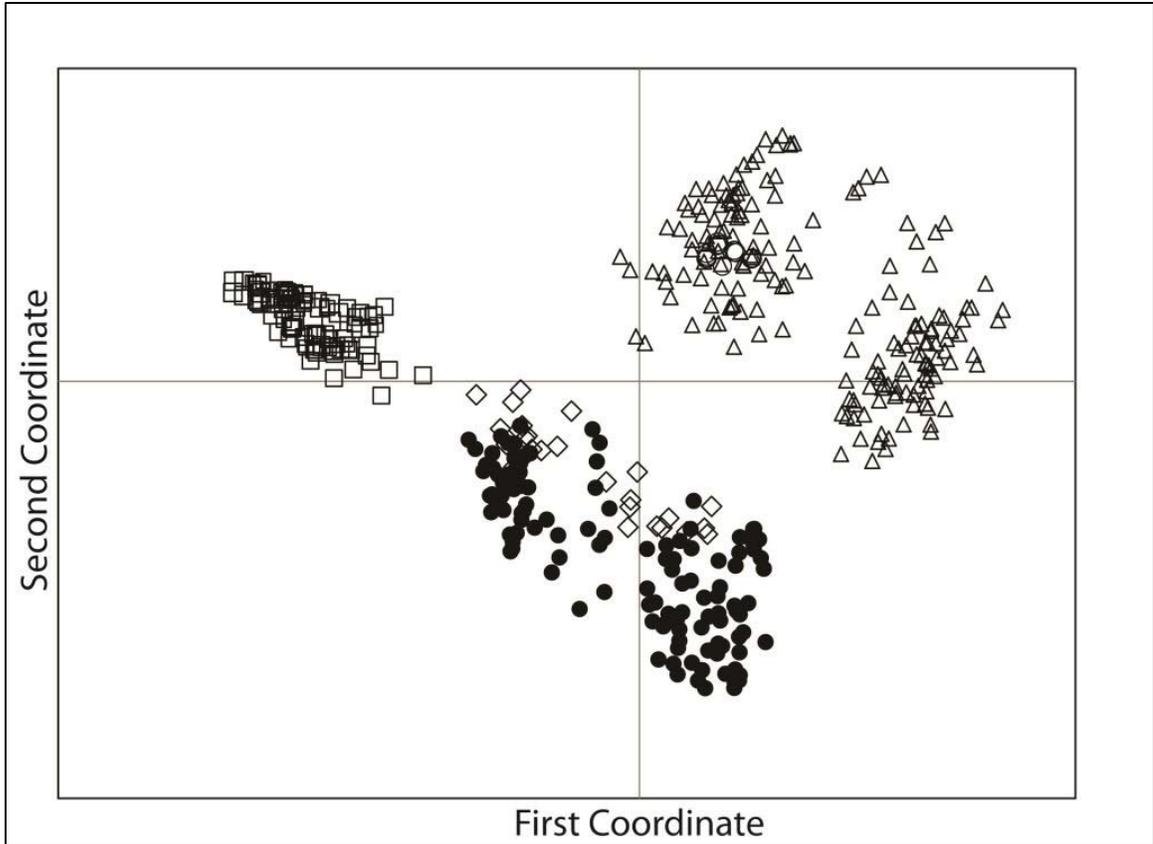
Fig. 3.4. Principal coordinate analysis scatter diagram along the two first coordinates. Based on nine microsatellite for 454 individuals belonging to 18 populations of *Pseudophoenix*. ○ = *P. lediniana*, □ = *P. ekmanii*, △ = *P. vinifera*, ◇ = *P.* sp. nova, ● = *P. sargentii*.
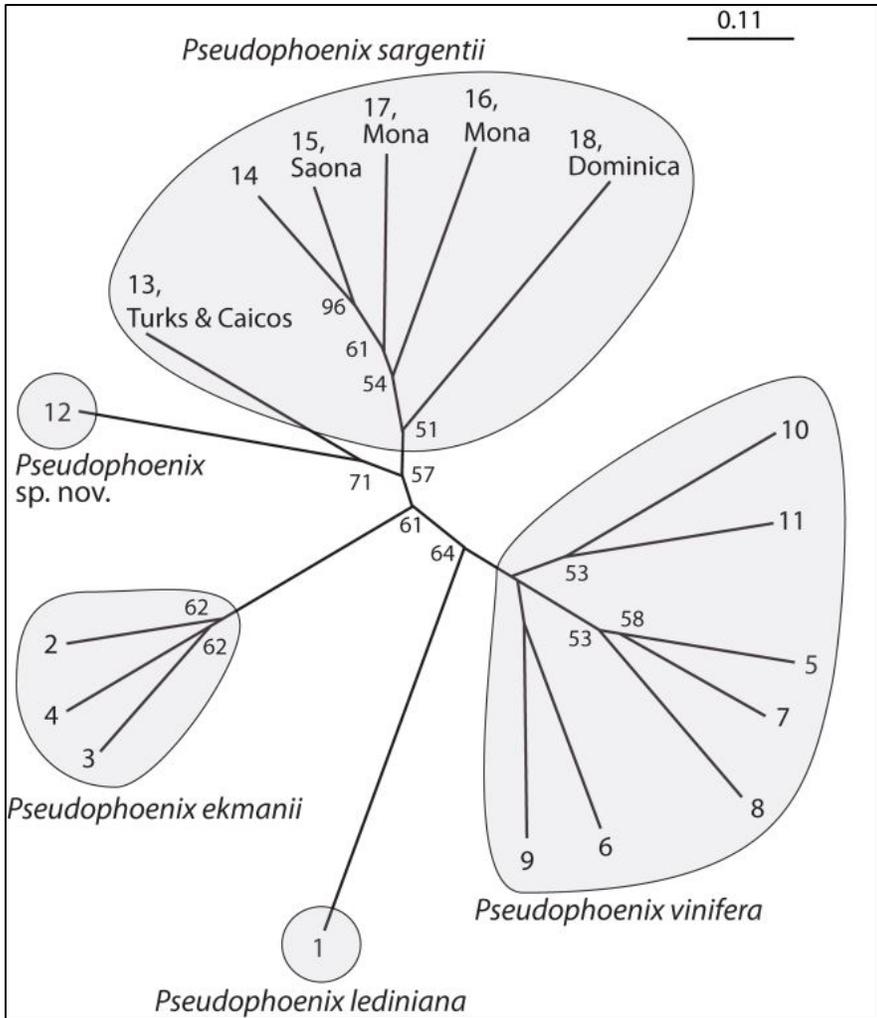
Fig. 3.5. Neighbor joining network (based on chord distance of Cavalli-Scorza and Edwards (1967). It shows the genetic relationships among 18 populations of *Pseudophoenix* from nine microsatellite loci. Branch lengths are proportional to distances and bootstrap supports for recovered clusters are also indicated. All populations are from Hispaniola except where indicated.
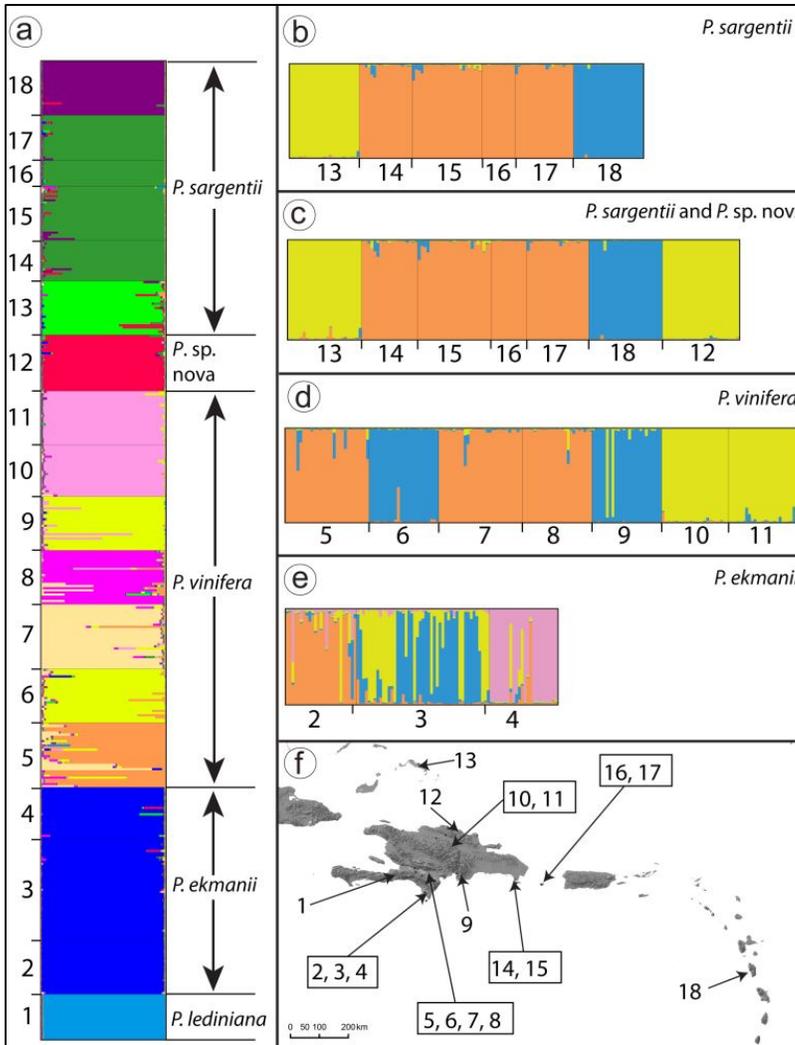
Fig. 3.6. STRUCTURE analyses for individuals from populations of *Pseudophoenix*. Color and box sizes indicate the cluster type of each individual and the number of plants sampled per population. The vertical lines indicate the probability that each individual belongs to an inferred cluster. a Analysis for the 18 populations included in the study. b–e Analyses of four separate data sets as indicated in each insert. f Geographical distribution of the 18 populations. STRUCTURE analyses for inserts a and e were based on 9 microsatellite loci, for the rest of analyses (inserts c and e) the data were generated by ten microsatellite loci.

Table 3.1. Demographic and geographical data of populations of *Pseudophoenix*.

Species and population number[a]	Estimate Number of Individuals[b]				Protected area
	Seedl.	Juven.	Adul.	Total	

P.lediniana | | | | | |
1 (Jacmel, HA)	0	2	71	73	None
P. ekmanii					
2 (Sabana del Algodón, DR)	105	1,550	820	2475	Parque Nacional Jaragua
3 (Trudillé, DR)	324	517	205	1046	Parque Nacional Jaragua
4 (Isla Beata, DR)	59	59	211	329	Parque Nacional Jaragua
P.vinifera					
5 (Martín Brunito, DR)	230	80	300	610	Monumento Natural Las Caobas
6 (Jimaní, DR)	0	160	300	461	None
7 (Loma Charco Azul, DR)	520	40	350	910	Reserva Biológica Loma Charco Azul
8 (Cabral, DR)	10	36	60	106	None
9 (Bahía de Ocoa, DR)	0	4	120	124	None
10 (Gurabo, DR)	300	20	150	470	None
11 (Esperanza, DR)	90	2	60	152	None
P. sp nova					
12 (Puerto Plata, DR)	5	20	9	34	None
P. sargentii					
13 (Montpeller Pond, TC)	150	125	50	325	None
14 (Palmilla, DR)	0	69	34	103	Parque Nacional del Este
15 (Isla Saona, DR)	100	100	37	237	Parque Nacional del Este
16 (Antena, Mona Island)	2	8	4	14	National Natural Landmark of Mona
17 (Uvero, Mona Island)	2	0	22	24	National Natural Landmark of Mona
18 (Heights of Mero, DO)	0	0	70	70	None

Table 3.1. Continued.

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[a]Localities and area of origin are given inside the parenthesis. Areas or origin are coded as HA= Haiti, DR = Dominican Republic, TC = Turks and Caicos Islands, DO = Dominica. [b]Demographic groups are coded as Seedl. = Seedlings, Juven. = Juveniles, Adul. = Adults. * Demographic information derived from Rodriguez (*in-press*). ** Demographic information derived from Namoff (2011).

Table 3.2. *Pseudophoenix* population genetic statistics. Data based on the analyses of 10 loci except for *P. ekmanii* for which 9 loci were studied.

Species and population[a]	Origin	P	n_p	A	H_o	H_e	n_{ds}	F_{is}	N_{ig}	LDL
P. lediniana										
1 (21)	Haiti	40	3	1.7	0.25	0.50	3	0.51	6	4
P. ekmanii										
2 (25)	Dominican Republic	100	4	3.7	0.22	0.47	7	0.55	0	8
3 (46)	Dominican Republic	100	5	5.1	0.29	0.53	8	0.45	0	25
4 (25)	Dominican Republic	77	3	2.6	0.20	0.44	6	0.54	1	33
P. vinifera										
5 (30)	Dominican Republic	100	2	5	0.29	0.51	7	0.43	0	24
6 (25)	Dominican Republic	100	2	4.7	0.26	0.50	5	0.49	0	24
7 (30)	Dominican Republic	100	2	4	0.30	0.49	5	0.39	0	6
8 (25)	Dominican Republic	100	4	4.6	0.31	0.50	8	0.40	0	6
9 (25)	Dominican Republic	90	2	3.2	0.17	0.45	7	0.63	1	33
10 (24)	Dominican Republic	100	3	3.5	0.29	0.50	5	0.42	0	4
11 (25)	Dominican Republic	100	2	4.8	0.34	0.55	6	0.38	0	29
P. sp. nova										
12 (26)	Dominican Republic	100	4	6.3	0.36	0.66	9	0.47	0	33
P. sargentii										
13 (25)	Turks and Caicos	100	9	8.4	0.44	0.66	8	0.34	0	31
14 (19)	Dominican Republic	100	5	4.4	0.40	0.64	6	0.38	0	33
15 (25)	Dominican Republic	100	4	4.8	0.40	0.62	7	0.37	0	24
16 (12)	Mona Island	80	3	2.1	0.15	0.43	6	0.67	0	6
17 (21)	Mona Island	80	3	2.1	0.20	0.40	6	0.48	0	4-
18 (25)	Dominica	80	0	2.8	0.22	0.45	6	0.52	0	11

Table 3.2. Continued.

Population genetic statistics are coded as follows: P, percentage of polymorphic loci; n_p, number of private alleles; A, average number of alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; n_{ds}, number of loci that deviate significantly from HWE ($P < 0.05$); F_{is}, inbreeding coefficient; N_{ig}, number of identical genotype pairs; LDL, percentage of paired loci showing linkage disequilibrium. [a]Number of sampled individuals are given inside the parenthesis. Notice that all F_{is} values deviate significantly from 0 ($P < 0.05$).

Table 3.3. Genetic differentiation and estimates of migration rate for populations of *Pseudophoenix*. Above diagonal = D$_{est}$ estimates. Below diagonal = N$_m$ values.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1		0.85	0.89	0.88	0.66	0.70	0.75	0.68	0.54	0.72	0.67	0.87	0.89	0.83	0.83	0.97	0.80	0.8
2	0.29		0.14	0.12	0.98	0.79	0.98	0.97	0.77	0.85	0.75	0.71	0.87	0.94	0.89	0.82	0.94	0.78
3	0.36	2.94		0.15	0.95	0.78	0.96	0.95	0.78	0.84	0.71	0.76	0.73	0.79	0.80	0.68	0.83	0.74
4	0.2	2.54	1.65		0.96	0.80	0.98	0.97	0.82	0.85	0.80	0.73	0.87	0.94	0.86	0.83	0.95	0.75
5	0.36	0.5	0.57	0.38		0.30	0.12	0.18	0.39	0.48	0.57	0.65	0.58	0.52	0.51	0.81	0.50	0.82
6	0.37	0.57	0.66	0.42	1.12		0.23	0.50	0.14	0.62	0.37	0.6	0.64	0.90	0.82	0.82	0.89	0.75
7	0.34	0.48	0.55	0.37	2.48	1.11		0.20	0.38	0.31	0.51	0.66	0.55	0.70	0.65	0.85	0.64	0.91
8	0.36	0.52	0.6	0.4	2.04	0.84	1.72		0.54	0.45	0.64	0.73	0.60	0.55	0.51	0.85	0.49	0.79
9	0.31	0.43	0.51	0.31	0.75	1.61	0.69	0.62		0.41	0.21	0.67	0.71	0.89	0.83	0.84	0.90	0.69
10	0.36	0.56	0.65	0.42	1.01	0.77	1.29	1.13	0.7		0.31	0.72	0.68	0.81	0.65	0.75	0.77	0.78
11	0.41	0.62	0.73	0.46	0.87	1.16	0.8	0.77	1.2	1.37		0.73	0.72	0.91	0.77	0.84	0.86	0.78
12	0.41	0.86	0.94	0.66	0.86	0.83	0.81	0.84	0.6	0.84	0.86		0.32	0.44	0.50	0.6	0.51	0.59
13	0.42	0.78	1.01	0.58	0.97	0.84	0.92	1.01	0.6	1	0.93	2.69		0.36	0.33	0.45	0.35	0.69
14	0.38	0.69	0.87	0.5	1.03	0.73	0.85	1	0.54	0.82	0.77	1.87	2.72		0.05	0.24	0.10	0.41
15	0.39	0.72	0.89	0.56	1.01	0.77	0.86	1.01	0.56	0.94	0.86	1.56	2.28	8.55		0.21	0.13	0.36
16	0.2	0.45	0.61	0.3	0.49	0.46	0.46	0.5	0.33	0.49	0.5	0.83	1.15	1.66	1.61		0.34	0.48
17	0.2	0.37	0.46	0.26	0.56	0.39	0.47	0.56	0.29	0.46	0.43	0.75	1.07	2.07	2.11	0.62		0.36
18	0.23	0.5	0.62	0.37	0.44	0.5	0.42	0.46	0.38	0.51	0.53	0.81	0.77	0.94	1.03	0.54	0.46	