The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2014, **176**, 469–485. With 4 figures

Genetic diversity and differentiation of *Pseudophoenix* (Arecaceae) in Hispaniola

ROSA A. RODRÍGUEZ-PEÑA[1,2,3]*, BRETT JESTROW[2], ALAN W. MEEROW[4], TEODORO CLASE[3], FRANCISCO JIMÉNEZ-RODRÍGUEZ[2], M. PATRICK GRIFFITH[5], EUGENIO SANTIAGO-VALENTÍN[6], JOSÉ A. SUSTACHE-SUSTACHE[7] and JAVIER FRANCISCO-ORTEGA FLS[1,2]

[1]*Department of Biological Sciences, College of Arts and Sciences, Florida International University, Miami, FL 33199, USA*
[2]*Kushlan Tropical Biology Institute, Fairchild Tropical Botanic Garden, Coral Gables, Miami, FL 33199, USA*
[3]*Jardín Botánico Nacional, Avenida República de Colombia, Santo Domingo, Dominican Republic*
[4]*USDA-ARS-SHRS, National Germplasm Repository, Miami, FL 33158, USA*
[5]*Montgomery Botanical Center, Coral Gables, Miami, FL 33156, USA*
[6]*Departamento de Biología, Universidad de Puerto Rico, Recinto de Río Piedras, PO Box 23360, San Juan, PR 00931, USA*
[7]*Departamento de Recursos Naturales y Ambientales, PO Box 366147, San Juan, PR 00936, USA*

Received 22 June 2014; revised 20 August 2014; accepted for publication 20 August 2014

Pseudophoenix ekmanii, *P. lediniana* and *P. vinifera* (Arecaceae) are endemic to Hispaniola. The more wide-ranging *P. sargentii* also occurs on the island. Population genetic diversity and structure of *Pseudophoenix* was investigated using ten microsatellite loci. The study focused on populations from Hispaniola, but also included samples from other Caribbean islands. Results showed homozygote excess and high inbreeding coefficients in all populations across all polymorphic loci. Populations were highly differentiated. Results from both Bayesian and neighbour-joining cluster analyses identified groups that were consistent with currently accepted species delimitations. We included the only known population of a possible undescribed taxon from the Dominican Republic. Results from the cluster analyses suggested that this putative taxon is closely related to *P. sargentii* from the Turks and Caicos Islands. There was no significant correlation between population size and observed heterozygosity. Contrary to what was anticipated, protected areas do not harbour most of the genetic diversity of the genus. The Haitian endemic *P. lediniana* should have the highest priority for conservation because it is restricted to a single population, it has a small number of individuals and it exhibited reduced levels of genetic diversity. The putative new taxon from the Dominican Republic has similar conservation concerns. Future conservation efforts should aim to maintain population connectivity and increase population size, particularly targeting populations with low genetic diversity. *Botanical Journal of the Linnean Society*, 2014, **176**, 469–485.

ADDITIONAL KEYWORDS: biodiversity hotspots – biogeography – Caribbean – palms – SSRs – taxonomy – threatened species – West Indies.

INTRODUCTION

Hispaniola is the second largest island of the Caribbean Island Biodiversity Hotspot, and in this region it ranks second in plant endemism (Acevedo-Rodríguez & Strong, 2008). Over 4000 species occur in Hispaniola, and it is estimated that *c*. 40% of these are endemic (Acevedo-Rodríguez & Strong, 2012). The latest conservation assessment for the Dominican Republic flora was undertaken by

*Corresponding author. E-mail: rosabotanic@gmail.com

Peguero & Jiménez (2011) who studied the conservation status of 639 endemic vascular plant taxa. This work showed that *c*. 90% of these taxa are threatened according to the IUCN (2013) Red List categories (Peguero & Jiménez, 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, Woods & Sergile, 1989; Bolay, 1997; Ministerio de Medio Ambiente y Recursos Naturales, 2011).

Compared with other plant families (e.g. Asteraceae, Rubiaceae, Orchidaceae), Arecaceae do not have many endemic species in the Caribbean Islands (Acevedo-Rodríguez & Strong, 2012). Palms from this biodiversity hotspot provide one of the best examples for biogeographical disjunctions in the tropics. Arecaceae subfamily Ceroxyloideae comprises 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). Ceroxyloideae shared a common ancestor with the sister clade Arecoideae *c*. 80 Mya (Baker & Couvreur, 2013). *Pseudophoenix* is the only member of a lineage that is sister to the rest of Ceroxyloideae. This lineage diverged *c*. 52 Mya during the Eocene (Baker & Couvreur, 2013). Because of the complex geological history of the Caribbean Islands, with several episodes of transgression and subsidence, it has been suggested that most of the West Indian biota is younger than the mid-Eocene (Iturralde-Vinent & MacPhee, 1999). Therefore, the presence of *Pseudophoenix* in the Caribbean Islands fits the palaeogeographical data available for the region.

Zona (2002) published the latest taxonomic treatment of the genus, reviewing the morphology, distribution and ecology of its species. Individuals of *Pseudophoenix* have pinnate leaves and a bottle-shaped trunk; this morphological feature is more prominent in *P. ekmanii* Burret and *P. vinifera* (Mart.) Becc. Inflorescences bear perfect flowers, but produce a few staminate flowers at the end of the inflorescence (Zona, 2002). *Pseudophoenix* species grow predominantly in dry forests on limestone soils (Zona, 2002). In contrast, the rest of the species of Ceroxyloideae occur mostly in tropical rain forests (Couvreur, Forest & Baker, 2011). Hispaniola is the centre of highest taxonomic diversity, as all four *Pseudophoenix* species occur on the island. Two *Pseudophoenix* species are Critically Endangered (*P. ekmanii* and *P. lediniana* Read) *sensu* IUCN (2013).

Pseudophoenix lediniana occurs in a single highly fragmented population with *c*. 73 individuals, and the habitat where it grows is highly disturbed. The species thrives on cliffs that are subject to frequent landslides during the rainy season. This site is usually burned to cultivate staple crops, and its woody species are regularly used to produce charcoal. This locality is not part of any protected area (Rodríguez-Peña *et al*., 2014).

Pseudophoenix ekmanii is restricted to the southwestern Dominican Republic, specifically on Barahona Peninsula and Beata Island, where it is protected in the Jaragua National Park. Despite its threatened conservation status, this species has large populations. In a single site at Sabana de Algodón, Namoff *et al*. (2011) reported > 2400 individuals in a recent population genetic study that showed strong evidence for genetic drift, inbreeding and moderate gene flow among populations. These patterns were attributed to habitat fragmentation by human activities, unsustainable ethnobotanical use of this species (sap extraction), illegal removal of palms for the horticulture trade and destruction of adult individuals to gain access to nests of the Hispaniolan parrot (*Amazona ventralis*), which is harvested for the exotic pet trade (Namoff *et al*., 2011).

Pseudophoenix sargentii H.Wendl. ex Sarg. is the species of the genus with the widest distribution in the Caribbean. It is found in Florida (Biscayne National Park), Puerto Rico (Mona Island), Cuba, Navassa Island, Mexico (Yucatan), Belize, Lesser Antilles (Dominica), Haiti (Gonâve Island), the 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 dry forests near coastal shores, although in Mexico and Belize it is also found inland (Zona, 2002). In some areas, populations of this species have been harvested for horticultural purposes, e.g. Florida populations on Long and Sands Keys, where this species is now extinct (Lippincott, 1992; Maschinski & Duquesnel, 2006). Some populations in the Dominican Republic have also been removed for tourism development and the horticultural trade.

Pseudophoenix vinifera has a wide distribution in western 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 is in the southern sector of the country (Populations 5–8, see below; Fig. 1), located mostly in the lowlands between the Sierra de Neiba and the Sierra de Bahoruco (along the Hoya de Enriquillo valley) and between the Cordillera Central and the Caribbean Sea (Population 9, see below; Fig. 1). The second core area is in the northern part of the country and occupies the lowlands that separate

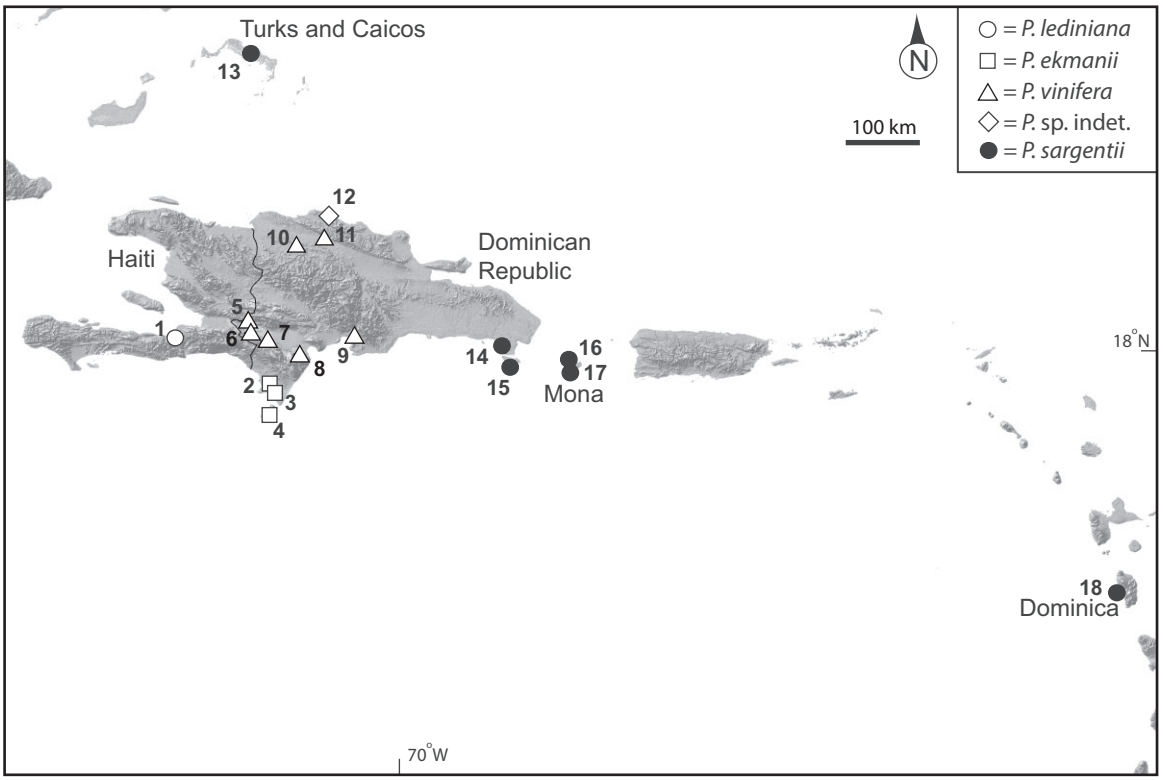

Figure 1. Geographical distribution of the 18 populations of *Pseudophoenix* included in this study.

the Cordillera Septentrional and the Cordillera Central, predominantly along Valle del Cibao (Populations 10 and 11, see below; Fig. 1). These two valleys were largely below sea level during interglacial periods from the Miocene (at Cibao) and Pleistocene (at Enriquillo) (Mann *et al*., 1984; McNeill *et al*., 2012). The government of the Dominican Republic has established one reserve (Monumento Natural Los Cacheos, province of Independencia) 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 (see below) and horticulture poaching, and to protect the palm's natural habitat (Congreso Nacional de la República Dominicana, 2004). The species also occurs in Monumento Natural Las Caobas and in the Reserva Biológica Loma Charco Azul, both also located in the province of Independencia.

Zona (2002) indicated that plants of *Pseudophoenix* found in the north-western Dominican Republic (hereafter *Pseudophoenix* sp. indet.) are morphologically distinct and might represent a novel taxon. This morph is highly threatened, and during field studies only a single population with *c*. 34 individuals was located at Puerto Plata (Population 12, Table 1). *Pseudophoenix* sp. indet. occurs on serpentinite soils; it is the only morphological variant in this genus to thrive in this unique soil environment characterized by a high content of heavy metals such as nickel and cobalt and high magnesium to calcium ratios (Brooks, 1987).

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 & Zona, 2013). The use of *Pseudophoenix* to prepare *mabí de cacheo* is one of the main reasons for the decline of these 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).

Microsatellites or simple sequence repeats (SSRs) are molecular markers commonly used for population-level studies because they are co-dominant, biparentally inherited and generally exhibit high levels of allelic diversity (Chase, Kesseli & Bawa, 1996; Powell, Machray & Provan, 1996). These molecular markers can help to understand the biological features and the evolutionary history of a particular taxon (Fernández-Silva *et al*., 2013). Recent examples of how SSRs have had a direct application for

Table 1. Demographic and geographical data of populations of *Pseudophoenix*

Species and population number[a]	Estimated no. of individuals				Protected area
	Seedlings	Juveniles	Adults	Total	
*P. lediniana**					
1 (Jacmel, HA)	0	2	71	73	None
P. ekmanii†					
2 (Sabana del Algodón, DR)	105	1550	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
Pseudophenix sp. indet.					
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

Areas of origin are coded as HA, Haiti; DR, Dominican Republic; TC, Turks and Caicos Islands; DO, Dominica.
[a]Localities and area of origin are given in parentheses.
*Demographic information derived from Rodriguez-Peña *et al*. (2014).
†Demographic information derived from Namoff *et al*. (2011).

Caribbean endemic plants were reported for *Ipomoea* L. (Geiger *et al*., 2014), *Pinus* L. (Pinaceae) (Sánchez *et al*., 2014), *Pseudophoenix* (Namoff *et al*., 2011; Rodríguez-Peña *et al*., 2014) and *Zamia* L. (Meerow *et al*., 2012; Calonje *et al*., 2013). In these studies, microsatellites have provided phytogeographic insights (Meerow *et al*., 2012; Geiger *et al*., 2014; Sánchez *et al*., 2014), have helped to define conservation management units for Critically Endangered species (Calonje *et al*., 2013), revealed high levels of inbreeding in threatened species (Namoff *et al*., 2011) and documented genetic erosion (Rodríguez-Peña *et al*., 2014).

In this study we present the results, based on SSR data, of a population genetic study of *Pseudophoenix* species that occur on Hispaniola. One of our goals was to determine if the taxonomic differentiation reported in this genus is also supported by molecular data. Microsatellites were also used to investigate the genetic structure and overall levels of genetic diversity found in populations of this genus from Hispaniola. In addition, our study had conservation implications as we explored relationships between population size, conservation status in protected areas and genetic diversity.

MATERIAL AND METHODS

Study sites and populations

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 included (Table 1, Fig. 1). 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*, Population 12 for *Pseudophoenix* sp. indet.), eight in the south-west (Populations 2–4 for *P. ekmanii*, 5–10 for *P. vinifera*) and two in the south-east (Populations 14–15 for *P. sargentii*). For *P. vinifera*, we sampled in protected areas (Population 5 from

Monumento Natural Las Caobas and Population 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 (Populations 8, 9 and 11). We could not sample in Monumento Nacional Los Cacheos as the best stands of *P. vinifera* from this protected area were in remote localities that were difficult to access; however, the Monumento Natural Las Caobas is adjacent to Monumento Nacional Los Cacheos and this study included one population from this nature reserve.

Samples of *P. ekmanii* and *P. lediniana* were obtained from the DNA bank of Florida International University (FIU) – Fairchild Tropical Botanic Garden. These samples were previously used for two population genetic studies focusing on these species (Namoff *et al*., 2011; Rodríguez-Peña *et al*., 2014); however, these previous works were based on seven SSR loci. For this study, we were able to obtain data for three additional loci (see below). Although the focus of this research was Hispaniola, samples of *P. sargentii* from Dominica, Turks and Caicos Islands and Puerto Rico were also examined (Table 1, Fig. 1). These additional samples provided a wider biogeographical framework for the project.

DNA and demographic sampling

For the molecular studies we collected plant material from at least 25 adult plants per population wherever this was possible. However, for some populations, we obtained < 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 low yield (i.e. Population 14). The number of sampled plants ranged between 12 (Population 16 from Mona Island) and 46 (Population 3 of *P. ekmanii*).

Demographic inventories were performed to quantify the number of individuals in three plant classes: (1) seedlings (plants with fewer than three leaves); (2) juveniles (plants < 1.5 m in height); and (3) adults (plants > 1.5 m in height). For the sampling performed in the Dominican Republic, the number of individuals that were tapped to prepare *mabí de cacheo* were recorded (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 *P. ekmanii* and *P. lediniana* were reported by Namoff *et al*. (2011) and Rodríguez-Peña *et al*. (2014), respectively. Demographic data for the remaining populations were based on initial censuses that covered *c*. 10% of each of the visited sites. These data were subsequently extrapolated to the whole population area; therefore, they represent estimates from the actual populations.

DNA extraction and amplification

Leaf samples were fast-dried in Drierite (W. A. Hammond Drierite Co. Ltd) and then used for DNA isolation with the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Liquid nitrogen was used to disrupt the leaf tissues. Ten microsatellite loci, originally developed for *P. sargentii* by Namoff *et al*. (2010), were used as molecular markers for our study. For *P. ekmanii*, we were 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 based on only nine loci. 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 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 *et al*., 2004). The program GENALEX 6 v. 6.501 (Peakall & Smouse, 2006, 2012) was used to quantify the number of private alleles (n_p) and number of identical genotype pairs (N_{ig}). The average number of alleles per locus (A), percentage of polymorphic loci (P), observed heterozygosity (H_o), expected heterozygosity (H_e) and the percentage of paired loci showing linkage disequilibrium (LDL) in each population were calculated with ARLEQUIN v. 3.5 (Excoffier, Laval & Schneider, 2005). Tests for the number of loci that deviated from Hardy–Weinberg equilibrium (HWE) and the U-test (Rousset & Raymond, 1995) for heterozygote deficiency were run with GenePop v. 4.0 (Raymond & Rousset, 1995; Rousset, 2008) using 10 000 Markov chain Monte Carlo iterations (Guo & Thompson, 1992) for each population. Inbreeding coefficients (F_{is}) were calculated for each population using FSTAT v. 1.2 (Goudet, 1995). We also tested for a correlation between population size and H_o values using the program Social Science Statistics (Social Science Statistics, 2014).

Analysis of molecular variance (AMOVA) among populations was obtained with ARLEQUIN. Values for the diversity measure D_{est} (Jost, 2008) were obtained with SMOGD (Crawford, 2010). This

diversity index has been suggested to provide better estimates for population differentiation than the G_{st} (Nei, 1973) or F_{st} (Jost, 2008; Heller & Siegismund, 2009) indexes. The pairwise number of migrants (N_m) per generation between populations was computed with the program ARLEQUIN as an estimate of levels of gene flow among populations.

Pairwise genetic distances among populations were computed with POPULATIONS v. 1.2.30 (Langella, 1999) using Chord distance (Cavalli-Sforza & Edwards, 1967). The resulting inter-population pairwise genetic distances were then used to construct a neighbour-joining (NJ) tree with bootstrapping (resampling loci) based on 10 000 permutations. The NJ tree was plotted using FigTree v. 1.4.0 (Rambaut, 2012).

The program STRUCTURE v.2.3.3 (Pritchard, Stephens & Donnelly, 2000) was used to estimate the genetic structure among populations. *K* values from 1 to 19 were simulated across 20 replicate runs of 1000 000 iterations after a burn-in of 100 000. The Δk method of Evanno, Regnaut & Goudet (2005), as implemented in STRUCTURE HARVESTER (Earl & 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 & Rosenberg, 2007). Final results were visualized with DISTRUCT (Rosenberg, 2004). Five different data sets were analysed with this 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 three remaining data sets, and they were for: (1) the seven populations of *P. vinifera*; (2) the six populations of *P. sargentii*; and (3) the six populations of *P. sargentii* and the only population of *Pseudophoenix* sp. indet. The last analysis was conducted because the NJ network (see below) showed a close relationship between *P. sargentii* and *Pseudophoenix* sp. indet.

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

RESULTS

Demographic studies

Demographic data for *P. ekmanii* and *P. lediniana* were presented by Namoff *et al*. (2011) and Rodríguez-Peña *et al*. (2014), respectively, and are not reported here. *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 < 71 individuals and showed either no recruitment or low numbers of juveniles and seedlings (Table 1). We could not count seedlings for Population 14 because there were three other species of palm growing in the same area, and we were not able to discriminate among seedlings of these 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 Natural Las Caobas with 610 individuals). 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 *Pseudophoenix* sp. indet. (Population 12) had the third lowest number of individuals (34) among all the populations of *Pseudophoenix* included in this study. Contrary to original expectations, there were no dead individuals in any of the populations that appeared to have been tapped to produce *mabí de cacheo*.

Genetic diversity

The final data matrix included genetic information for 454 individuals and had 1.7% 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 (Table 2). Six of the populations had no polymorphic loci. Just four loci were polymorphic for the Haitian endemic *P. lediniana* (Population 1). Population 13 (*P. sargentii* site from Turks and Caicos) had the largest number of alleles per locus ($A = 8.4$), and the only population of *P. lediniana* had the lowest value ($A = 1.7$, Table 2). We found 59 private alleles with an average value of $n_p = 3.3$ per population. Population 13 (*P. sargentii* locality on Turks and Caicos) had the largest number of private alleles ($n_p = 9$) and Population 18 (*P. sargentii* site

Table 2. *Pseudophoenix* population genetic statistics; data are based on the analyses of ten loci except for *P. ekmanii* for which nine 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
Pseudophoenix sp. indet.										
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

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 in parentheses.

from Dominica) was the only locality that had none (Table 2). Observed heterozygosity values ranged between 0.15 (*P. sargentii* population on Mona Island) and 0.44 (*P. sargentii* site on Turks and Caicos). There was a positive correlation between observed heterozygosity and population size; however, this relationship was not significant ($R^2 = 0.0212$, $P = 0.564$).

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 versus mean H_e value across all populations of 0.52, Table 2). 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 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 2). Populations 4 (*P. ekmanii*), 9 (*P. vinifera*), 12 (*Pseudophoenix* sp. indet.) 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 genetic drift and high levels of autogamy. As no locus had homozygote excess in all populations, we interpreted the MICOCHECKER results as evidence for true homozygote excess instead of presence of null alleles.

Genetic differentiation

The mean value for the diversity measure of Jost (2008) across all populations was 0.75, suggesting high differentiation among populations (Table 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. Highest N_m values were obtained among conspecific populations located in close geographical proximity (Table 3). The two highest 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* ($N_m = 2.94$). The third highest value for this migration index was between the population of *Pseudophoenix* sp. indet. and the population of *P. sargentii* from Turks and Caicos ($N_m = 2.69$).

The AMOVAs 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 geographical distance among populations were significantly correlated both for *P. sargentii* ($R^2 = 0.6714$, $P = 0.021$) and for *P. vinifera* ($R^2 = 0.694$, $P = 0.003$) (Fig. 2). Correlation for the three populations of *P. ekmanii* ($R^2 = 0.503$, $P = 0.334$) was not statistically significant; however, the analysis for this species had a small sample size, limited to only three pairwise comparisons.

Population structure

Global analyses of all populations

The NJ network recovered four groups that were concordant with the current taxonomy (Fig. 3). The only known site of *Pseudophoenix* sp. indet. was part of the group that included the populations of *P. sargentii*, and appeared 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. 4). The results were consistent with species delimitation; therefore, populations shared genetic clusters within species but not among species. Populations of *P. lediniana*, *P. ekmanii* and *Pseudophoenix* sp. indet. grouped into one cluster each. The six populations of *P. sargentii* were assigned to three different clusters. Individuals of this species from Dominica mostly comprised 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 were mostly placed in 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 cluster each, and little admixture was detected among them. These three clusters contributed little to the genotypes of 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, as was also the case for individuals from southern Dominican Republic (Populations 6 and 9).

Separate species analyses

When the Bayesian clustering analysis was run individually for each species, similar results were obtained only for *P. sargentii* (optimal $K = 3$, Fig. 4B) and *P. vinifera* (optimal $K = 5$, Fig. 4D). However, the Evanno method identified an optimal $K = 3$ from the analysis of the data matrix that combined samples of *P. sargentii* and *Pseudophoenix* sp. indet. (Fig. 4C). This analysis clearly supported a close genetic connection between *Pseudophoenix* sp. indet. and *P. sargentii* from Turks and Caicos. All members from these two species 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. 4B).

Results from the Bayesian clustering analysis of the *P. ekmanii* samples (optimal $K = 4$) (Fig. 4E) were different from those found in the analysis of the 18 populations (Fig. 4A). The separate analysis showed that samples from Populations 2 and 4 of *P. ekmanii* were mostly each assigned to a different cluster (Fig. 4E). The two remaining clusters were primarily confined to Population 3, but they included admixture between them.

DISCUSSION

Genetic diversity and differentiation

There is a general assumption that smaller populations tend to harbour lower genetic diversity than larger ones (Oostermeijer, Luijten & den Nijs, 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

Table 3. Genetic differentiation and estimates of migration rate for the 18 populations of *Pseudophoenix*

	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	0.80
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	

Above diagonal, D_{est} estimates; below diagonal, N_m values.

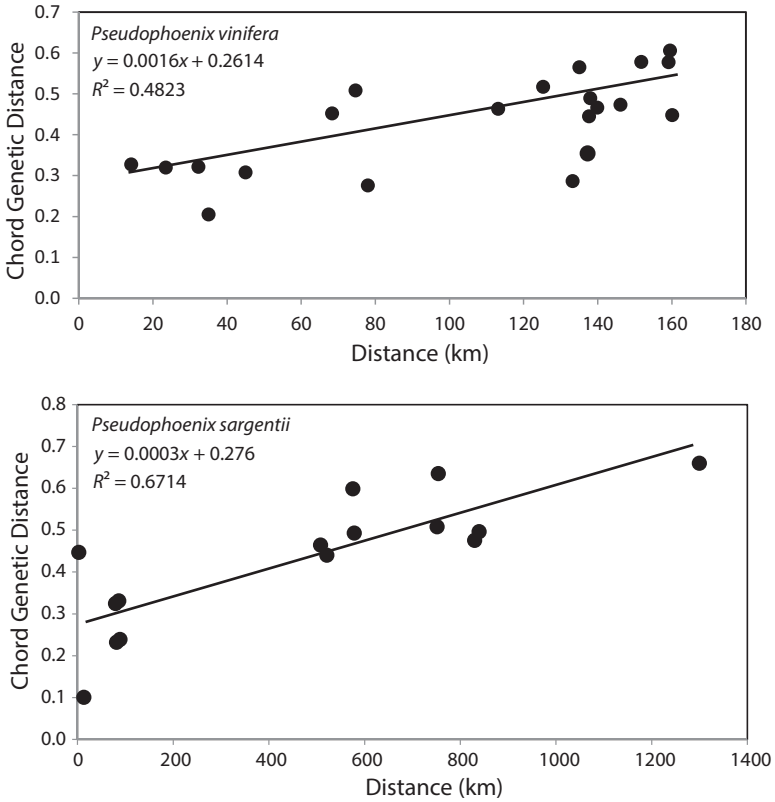

Figure 2. Relationship between pairwise geographical distances and chord genetic distances of Cavalli-Sforza & Edwards (1967) among populations of *Pseudophoenix vinifera* and *P. sargentii*.

features of *Pseudophoenix*. Although no data concerning the environmental history of the targeted populations were gathered for this study, it is well known that since the 15th century, the Caribbean Islands, particularly the Dominican Republic, have experienced extensive habitat fragmentation and forest clearance associated with rapid human developments Sambrook, Pigozzi & Thomas, 1999; Alscher, 2011). Currently, the Caribbean Islands rank third in human population density among the Biodiversity Hotspots (Cincotta, Wisnewski & Engelman, 2000). Between 1990 and 2010, the Dominican Republic experienced an average annual human population growth of 1.65%, which is one of the highest rates for the Western Hemisphere (Anonymous, 2013). Individuals of *Pseudophoenix* have long life cycles with an estimated reproductive age of 57 years (Durán, 1995). It has been suggested that long-lived organisms will show the negative impact of genetic drift on genetic diversity only after several hundred years because they have long generation times and overlapping cohorts (Amos & Balmford, 2001; Glémin, Bazin & Charlesworth, 2006; Duminil *et al*., 2007; Duminil, Hardy & Petit, 2009).

Overall, the analysis of population genetic structure supported high genetic differentiation, low genetic diversity within populations and high inbreeding coefficients. For *P. sargentii* and *P. vinifera*, 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, encompassing 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 in conspecific populations than among populations belonging to different species. These results indicate that there is limited interspecific hybridization in the genus.

In four pairwise comparisons, populations from different islands displayed little genetic differentiation. The two most relevant examples were found between the main island of Hispaniola and its small satellite 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

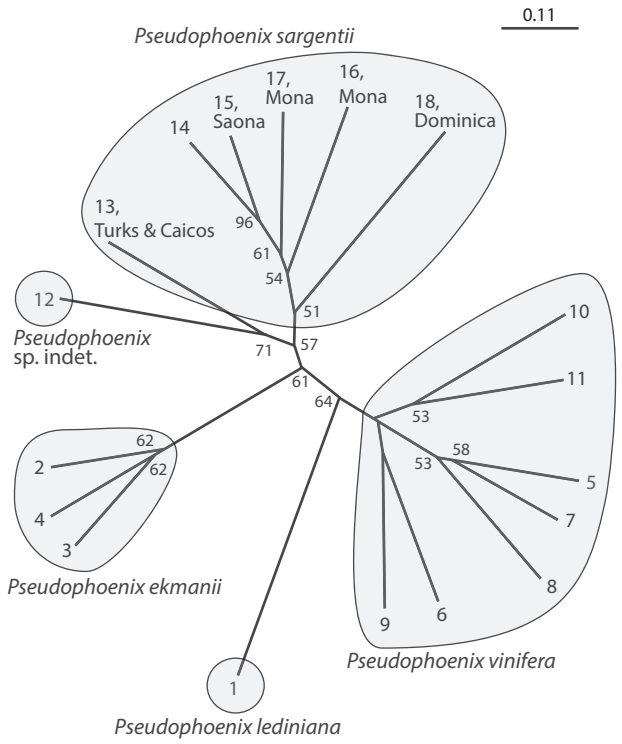

Figure 3. NJ network [based on chord distance of Cavalli-Sforza & Edwards (1967)]. It shows the genetic relationships among 18 populations of *Pseudophoenix* from nine microsatellite loci. Branch lengths are proportional to distances and bootstrap supports for recovered clusters are also indicated. All populations are from Hispaniola except where indicated.

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, b). High gene flow cannot be ruled out as an evolutionary mechanism to account for the little differentiation shown by these populations; however, more likely scenarios include recent vicariance events engendered by sea-level rise 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 *Pseudophoenix* sp. indet. from the 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. Hydrochoric dispersal could explain the high N_m and low D_{est} values exhibited in these two inter-island population comparisons. Between the Late Eocene and Mid-Miocene (33–16 Mya) there were land bridges between Puerto Rico and Hispaniola (Iturralde-Vinent & MacPhee, 1999). It could be argued that the low genetic differentiation detected between populations of *P. sargentii* from Puerto Rico and Hispaniola is the result of vicariance. However, we would expect that such an ancient vicariance disjunction would have resulted in much higher D_{est} scores among these distant populations. Therefore, the low genetic differentiation values between these sites appear to be the result of recent migration events.

The inbreeding coefficients across all the populations were high and significant. Contrary to initial expectations, F_{is} values were highly positive even in those sites that had a high number of individuals. It is well known that small isolated populations are more likely to have high levels of inbreeding as a consequence of drift, limited gene flow and a higher frequency of mating among relatives (Leimu *et al*., 2006; Herron & Freeman, 2012); however, in our study both small and large populations showed evidence of inbreeding and reduced genetic diversity. Several studies show 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, Boyle & Brown, 1996). Genetic drift is a strong evolutionary force in small populations, but almost insignificant in large populations (Ouborg, Vergeer & Mix, 2006), and its effect is greatly diminished in species with long generation times (Amos & Balmford, 2001). 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 (Herron & Freeman, 2012). It is expected that inbreeding species will show high genetic differentiation among populations, low within-population genetic diversity and high levels of inbreeding, even in large populations (Hamrick & Godt, 1996) such as we found in *Pseudophoenix*.

No studies have been conducted pertinent to the reproductive biology and breeding systems of *Pseudophoenix* (Barfod, Hagen & Borchsenius, 2011). In subfamily Ceroxyloideae, *Pseudophoenix* is the only genus with hermaphroditic flowers and therefore with the potential for self-fertilization (Dransfield *et al*., 2008). In addition, isolated individuals of the genus have been found to produce seeds, suggesting that they are self-compatible (S. 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 high levels of inbreeding shown by the results.

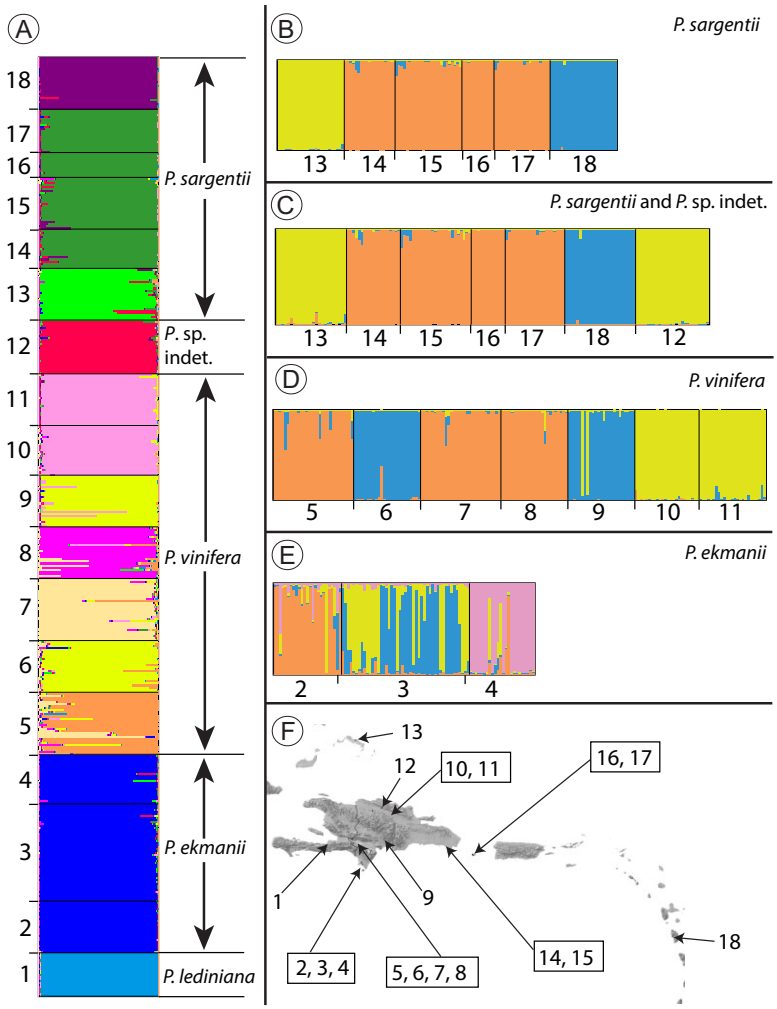

Figure 4. STRUCTURE analyses for individuals from populations of *Pseudophoenix*. Colour and box sizes indicate the cluster type of each individual and the number of plants sampled per population. The vertical lines indicate the probability that each individual belongs to an inferred cluster. A, analysis 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 A and E were based on nine microsatellite loci; for the remaining analyses (inserts B, C and D) the data were generated from ten microsatellite loci.

Another population genetic study of Caribbean Island plants based on SSRs also reported low levels of genetic diversity and high F_{is} values. This work concerned populations of *Ipomoea microdactyla* Griseb., a self-incompatible species (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 in a highly fragmented habitat.

Taxonomic implications

Results from the Bayesian and NJ 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. Population 12 (*Pseudophoenix* sp. indet.) formed a cluster with *P. sargentii* from Turks and Caicos (Fig. 3). This cluster was part of another group that included all of the populations of *P. sargentii* (Fig. 2).

Zona (2002) was unable to assign plants of *Pseudophoenix* from the northern Dominican Republic (i.e. *Pseudophoenix* sp. indet.) to any particular taxon, indicating that further studies were needed to clarify the taxonomic placement of this unique morph that is the only one growing on serpentinite soils. Zona's

(2002) morphological studies showed that plants belonging to *Pseudophoenix* sp. indet. 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 paper. However, systematic studies are in progress to clarify the placement of this enigmatic morph and determine its specific identity (R. A. Rodríguez-Peña & S. Zona, unpubl. observ).

Our study had a limited sampling of populations of *P. sargentii*, and therefore we cannot infer robust population genetic conclusions pertinent to this species and its relationships with *Pseudophoenix* sp. indet. Clearly, future studies should include samples *of P. sargentii* from Cuba, the rest of the Bahamas, the Yucatan Peninsula and Florida to gain a complete understanding of population genetic structure and relationships in the genus.

Our results indicated that microsatellite markers have taxonomic value and 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 & Lagoda, 1996).

Microsatellite markers have been widely used in palm population genetic studies (e.g. Kaneko, Kondo & Isagi, 2011; Nazareno, Zucchi & dos Reis, 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 results supported this Cape Verde endemic as a distinct species, clearly differentiated from *P. dactylifera* L. (Henderson, Billotte & Pintaud, 2006). The second was conducted by Bacon *et al*. (2012), who analysed microsatellites and DNA nucleotide sequence data to investigate species boundaries in *Pritchardia* Seem. & H.Wendl 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 Jaragua National Park, whereas the only known population of *P. lediniana* is found on private land and is not the subject of any *in situ* conservation initiative (Rodríguez-Peña *et al*., 2014). 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 *Pseudophoenix* sp. indet. 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 occur in areas with human disturbance in a highly fragmented habitat. The two populations on Mona Island are the only ones that thrive inside a protected area that have severely reduced population sizes of 14 and 24 individuals.

Among the species of the genus, *P. lediniana* and *Pseudophoenix* sp. indet. should have the highest priority for conservation because they are restricted to a single population each and have a small number of individuals. The extremely reduced levels of genetic diversity in the only population of *P. lediniana* (Rodríguez-Peña *et al*., 2014) stress the importance of having an *in situ* conservation action plan for this species. Contrary to initial expectations, the sole population of *Pseudophoenix* sp. indet. had relatively high H_o values, 100% polymorphic loci and the second highest average number of alleles per locus among all the populations included in the study. This case highlights the lack of association between genetic diversity estimates and population size found during our research.

The lack of clear relationships between levels of genetic diversity and population size (see above) was also detected when comparisons were made between protected and unprotected sites. Contrary to what was anticipated, protected areas do not harbour 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 *Pseudophoenix* occurs (Hernández, Bautista & Schubert, 1990). In addition, it has been indicated that some of the protected areas of the Dominican Republic often have poor conservation enforcement and are under severe anthropogenic pressure by local communities who exploit these forests (Powell & Inchaustegui, 2009). The nature reserve of Mona Island was created in 1919 (Sastre de Jesús & Santiago-Valentín, 1996); however, this island has serious problems with feral

goats and pigs, both of which are extremely detrimental to the native flora (Griffith *et al.*, 2012; Santiago-Valentín *et al.*, 2012).

Overall, the results support isolation-by-distance for the study sites (see above); however, in one case two populations of *P. vinifera* (at Jimaní and Cabral sites) that were geographically distant were more similar than those that were geographically close. The unexpected similarity patterns for these populations might well reflect past gene-flow routes and common population ancestry that have been disrupted by habitat fragmentation. 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 important for conservation management (Frankham, Briscoe & Ballou, 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 study reinforce species boundaries in *Pseudophoenix* and identify taxa for which conservation actions are required immediately.

ACKNOWLEDGEMENTS

We thank the Fulbright-LASPAU programme in the Dominican Republic for providing R.R.-P. with a grant to join the Master's degree program of FIU. Research was primarily supported by the Mohamed bin Zayed Species Conservation Fund (project numbers 11252872 and 12254927) and matching research funds from Fairchild Tropical Botanic Garden. Additional support was provided by the International Palm Society and the Montgomery Botanical Center. FIU provided a tuition waiver to R.R.-P. We thank the National Botanical Garden (Dominican Republic), for helping to organize field trips in the Dominican Republic. Dr Liz Harrison and Dr Nora Oleas helped with the data analyses. Mr Daniel Gann and Dr Paul Sharp provided help with the preparation of maps and DNA protocols, respectively. Professor Grenville Draper provided valuable insights pertinent to the geology of Hispaniola. Dr Andrew Henderson provided guidance on biogeography and systematics of Haitian palms. Our gratitude goes to Mr William Cinea (Haiti), Mr Arlington James (Dominica), Mr Bryan N. Manco (Turks and Caicos) and Mr Alberto Veloz (The Dominican Republic) for providing plant material and relevant information pertinent to the conservation status of populations from outside the Dominican Republic. Professor Jennifer Richards and Dr Scott Zona critically read the manuscript. This is contribution 280 from the Tropical Biology Program of FIU.

REFERENCES

Abreu AG, Priolli RHG, Azevedo-Filho JA, Nucci SM, Zucchi MI, Coelho RM, Colombo CA. 2012. The genetic structure and mating system of *Acrocomia aculeata* (Arecaceae). *Genetics and Molecular Biology* **35:** 119–121.

Acevedo-Rodríguez P, Strong MT, eds. 2012. Catalogue of seed plants of the West Indies. *Smithsonian Contributions to Botany* **98:** 1–1192.

Acevedo-Rodríguez P, Strong MT. 2008. Floristic richness and affinities in the West Indies. *Botanical Review* **74:** 5–36.

Alscher S. 2011. Environmental degradation and migration on *Hispaniola Island.* International Migration **49**(s1): e164–e188.

Amos W, Balmford A. 2001. When does conservation genetics matter? *Heredity* **87:** 257–265.

Anonymous. 2013. *World population prospects: the 2012 revision, highlights and advance tables.* Working Paper No. ESA/P/WP.228. New York: Department of Economic and Social Affairs, Population Division, The United Nations.

Bacon CD, McKenna MJ, Simmons MP, Wagner WL. 2012. Evaluating multiple criteria for species delimitation: an empirical example using Hawaiian palms (Arecaceae: *Pritchardia*). *BMC Evolutionary Biology* **12:** 23.

Baker WJ, Couvreur TLP. 2013. Global biogeography and diversification of palms sheds light on the evolution of tropical lineages. II. Diversification history and origin of regional assemblages. *Journal of Biogeography* **40:** 286–298.

Barfod AS, Hagen M, Borchsenius F. 2011. Twenty-five years of progress in understanding pollination mechanisms in palms (Arecaceae). *Annals of Botany* **108:** 1503–1516.

Bolay E. 1997. *The Dominican Republic. A country between rain forest and desert. Contributions to the ecology of a Caribbean island*. Weikersheim: Margraf Verlag.

Brooks RR. 1987. *Serpentine and its vegetation: a multidisciplinary approach*. London: Croom Helm.

Calonje M, Meerow AW, Knowles L, Knowles D, Griffith P, Nakamura K, Francisco-Ortega J. 2013. Cycad biodiversity in the Bahama Archipelago and conservation genetics of the Critically Endangered *Zamia lucayana* (Zamiaceae). *Oryx* **47:** 190–198.

Cavalli-Sforza LL, Edwards AWF. 1967. Phylogenetic analysis. Models and estimation procedures. *American Journal of Human Genetics* **19:** 233–257.

Chase M, Kesseli R, Bawa K. 1996. Microsatellite markers for population and conservation genetics of tropical trees. *American Journal of Botany* **83:** 51–57.

Cibrián-Jaramillo A, Hird A, Oleas N, Ma H, Meerow AW, Francisco-Ortega J, Griffith MP. 2013. What is the conservation value of a plant in a botanic garden? Using indicators to improve management of *ex situ* collections. *Botanical Review* **79:** 559–577.

Cincotta RP, Wisnewski J, Engelman R. 2000. Human population in the biodiversity hotspots. *Nature* **204:** 990–992.

Congreso Nacional de la República Dominicana. 2004. *Ley sectorial de áreas protegidas, No. 202–04*. Santo Domingo: Congreso Nacional de la República Dominicana.

Available at: http://www.wipo.int/wipolex/en/text.jsp?file_id=236193 Downloaded on 26 May 2014.

Couvreur TLP, Forest F, Baker WJ. 2011. Origin and global diversification patterns of tropical rain forests: inferences from a complete genus-level phylogeny of palms. *BMC Biology* **9:** 44.

Crawford NG. 2010. SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources* **10:** 556–557.

Dransfield J, Uhl NW, Asmussen CB, Baker JW, Harley MM, Lewis CE. 2008. *Genera palmarum: the evolution and classification of palms*. Kew: Kew Publishing.

Duminil J, Fineschi S, Arndt H, Jornado P, Salvini D, Vendramin GG, Petit RJ. 2007. Can population genetic structure be predicted from life-history traits? *American Naturalist* **169:** 662–672.

Duminil J, Hardy OJ, Petit RJ. 2009. Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evolutionary Biology* **9:** 177.

Durán R. 1995. *Pseudophoenix sargentii*: an endangered palm species. *Principes* **39:** 219–224.

Earl DA, Vonholdt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources* **4:** 359–361.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of cluster of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14:** 2611–2620.

Excoffier L, Laval G, Schneider S. 2005. ARLEQUIN. Version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1:** 47–50.

Fernández-Silva I, Whitney J, Wainwright B, Andrews KR, Ylitalo-Ward H, Bowen BW, Toonen RJ, Goetze E, Karl SA. 2013. Microsatellites for next-generation ecologists: a post-sequencing bioinformatics pipeline. *PLoS ONE* **8:** e55990.

Francisco-Ortega J, Zona S. 2013. Sweet sap from palms, a source of beverages, alcohol, vinegar, syrup, and sugar. *Vieraea* **41:** 91–113.

Frankham R, Briscoe DA, Ballou JD. 2002. *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

Geiger JH, Meerow AW, Lewis C, Oviedo R, Francisco-Ortega J. 2014. Genetic diversity and conservation of *Ipomoea microdactyla* (Convolvulaceae): an endemic vine from the Bahamas, Cuba, and southeastern Florida. *Plant Species Biology* **29:** 2–15.

Glémin S, Bazin E, Charlesworth D. 2006. Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings of the Royal Society B: Biological Sciences* **273:** 3011–3019.

Goudet J. 1995. FSTAT version 1.2: a computer program to calculate F-Statistics. *Journal of Heredity* **86:** 485–486.

Griffith MP, Sustache J, Francisco-Ortega J, Santiago-Valentin E. 2012. Cactus, succulent, and pachycaul flora of Mona Island. *Haseltonia* **18:** 56–66.

Guo SW, Thompson EA. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48:** 361–372.

Hamrick JL, Godt MJW. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **351:** 1291–1298.

Heller R, Siegismund HR. 2009. Relationships between three measures of genetic differentiation G_{st}, De_{st} and G'st: how wrong have we been? *Molecular Ecology* **18:** 2080–2083.

Henderson A, Aubry M, Timyan J, Balick M. 1990. Conservation status of Haitian palms. *Principes* **34:** 134–142.

Henderson S, Billotte N, Pintaud J-C. 2006. Genetic isolation of Cape Verde Island *Phoenix atlantica* (Arecaceae) revealed by microsatellite markers. *Conservation Genetics* **7:** 213–223.

Hernández C, Bautista E, Schubert A. 1990. Situación actual de la conservación de la biodiversidad. In: Departamento de Vida Silvestre, Secretaría de Estado de Agricultura, ed. *La diversidad biológica en la República Dominicana. Departamento de Vida Silvestre*. Santo Domingo: Secretaría de Estado de Agricultura, Deutscher Entwicklungsdienst, World Wildlife Fund, 151–202.

Herron JC, Freeman S. 2012. *Evolutionary analysis, 5th edn*. San Francisco: Benjamin Cummings.

Iturralde-Vinent MA, MacPhee RDE. 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History* **238:** 1–95.

IUCN. 2013. *The IUCN red list of threatened species. Version 2013.1*. Available at: http://www.iucnredlist.org/ Downloaded on 26 May 2014.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23:** 1801–1806.

Jarne P, Lagoda PJL. 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **10:** 424–429.

Jost L. 2008. G_{st} and its relatives do not measure differentiation. *Molecular Ecology* **17:** 4015–4026.

Kaneko S, Kondo T, Isagi Y. 2011. Development of microsatellite markers for the northern Australian endemic fan palm *Livistona rigida* (Arecaceae), with cross-amplification in the five related species. *Conservation Genetics Resources* **3:** 697–699.

Langella O. 1999. *POPULATIONS. Version 1.2.30. A population genetic software. CNRS, UPR9034*. Available at: http://bioinformatics.org/;tryphon/populations/ Downloaded on 30 January 2014.

Leimu R, Mutikainen P, Koricheva J, Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* **94:** 942–952.

Lippincott C. 1992. Restoring Sargent's cherry palm on the Florida Keys. *Fairchild Tropical Garden Bulletin* **47:** 12–21.

Mann P, Taylor FW, Burke K, Kulstad R. 1984. Subaerially exposed Holocene coral reef, Enriquillo Valley, Dominican Republic. *Geological Society of America Bulletin* **95:** 1084–1092.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27:** 209–220.

Maschinski J, Duquesnel J. 2006. Successful reintroductions of the endangered long-lived Sargent's cherry palm, *Pseudophoenix sargentii*, in the Florida Keys. *Biological Conservation* **134:** 122–129.

McNeill DF, Klaus JS, Budd AF, Lutz BP, Ishman SE. 2012. Late Neogene chronology and sequence stratigraphy of mixed carbonate-siliciclastic deposits of the Cibao Basin, Dominican Republic. *Geological Society of America Bulletin* **124:** 35–58.

Meerow AW, Francisco-Ortega J, Calonje M, Griffith MP, Ayala-Silva T, Stevenson DW, Nakamura K. 2012. *Zamia* (Cycadales: Zamiaceae) on Puerto Rico: asymmetric genetic differentiation and the hypothesis of multiple introductions. *American Journal of Botany* **99:** 1828–1839.

Menezes EV, Souto WF, Ciampi AY, Azevedo VC, Valério HM, Pimenta MA. 2012. Development and characterization of DNA microsatellite primers for buriti (*Mauritia flexuosa* L.f.). *Genetics and Molecular Research* **11:** 4058–4062.

Ministerio de Medio Ambiente y Recursos Naturales. 2011. *Estrategia nacional de conservación y uso sostenible de la biodiversidad y plan de acción 2011–2020* (*ENBPA*). Santo Domingo: Ministerio de Medio Ambiente y Recursos Naturales.

Namoff S, Francisco-Ortega J, Zona S, Lewis CE. 2010. Microsatellite markers developed for the Caribbean palm *Pseudophoenix sargentii* – two PCR-based methods. *Conservation Genetic Resources* **2:** 85–87.

Namoff S, Veloz A, Jiménez F, Rodríguez-Peña RA, Peguero B, Lewis C, Moynihan J, Abdo M, Maunder M, Meerow A, Von Wettberg E, Griffith P, Francisco-Ortega J. 2011. Sweet drinks are made of this: conservation genetics of an endemic palm species from the Dominican Republic. *Journal of Heredity* **102:** 1–10.

Nazareno AG, Zucchi MI, dos Reis MS. 2011. Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic rainforest of Brazil. *American Journal of Botany* **98:** e198–e200.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* **70:** 3321–3323.

Oostermeijer JGB, Luijten SH, den Nijs JCM. 2003. Integrating demographic and genetic approaches in plant conservation. *Biological Conservation* **113:** 389–398.

Ottenwalder JA. 1989. A summary of the conservation trends in the Dominican Republic. In: Woods CA, ed. *Biogeography of the West Indies: past, present, and future*. Gainesville: Florida Museum of Natural History, 845–850.

Ouborg NJ, Vergeer P, Mix C. 2006. The rough edges of the conservation genetics paradigm for plants. *Journal of Ecology* **94:** 1233–1248.

Paryski P, Woods CA, Sergile F. 1989. Conservation strategies and the preservation of biological diversity in Haiti. In: Woods CA, ed. *Biogeography of the West Indies. Past, present, and future*. Gainesville: Florida Museum of Natural History, 855–878.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288–295.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* **28:** 2537–2539.

Peguero B, Jiménez F. 2011. Inventario y estado de conservación de las plantas vasculares exclusivas de la Republica Dominicana. *Moscosoa* **17:** 29–57.

Powell R, Inchaustegui SJ. 2009. Conservation of the herpetofauna of the Dominican Republic. *Applied Herpetology* **6:** 103–122.

Powell W, Machray GC, Provan JA. 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* **1:** 215–222.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Rambaut A. 2012. *FigTree. Version 1.3.1*. Available at: http://tree.bio.ed.ac.uk/software/figtree Downloaded on 15 January 2014.

Ramos SL, de Macêdo JL, Lopes MT, Batista JS, Formiga KM, Da Silva PP, Saulo'Machado AC, Veasey EA. 2012. Microsatellite loci for tucumã of Amazonas (*Astrocaryum aculeatum*) and amplification in other Arecaceae. *American Journal of Botany* **99:** e508–e510.

Raymond M, Rousset F. 1995. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86:** 248–249.

Rodríguez-Peña R, Jestrow B, Cinea W, Veloz A, Jiménez-Rodríguez F, García R, Meerow AW, Griffith MP, Maunder M, Francisco-Ortega J. 2014. Conservation and genetics of two Critically Endangered Hispaniolan palms – genetic erosion of *Pseudophoenix lediniana* in contrast to *P. ekmanii*. *Plant Systematics and Evolution*. doi: 10.1007/s00606-014-1028-6

Rosenberg NA. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* **4:** 137–138.

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* **8:** 103–106.

Rousset F, Raymond M. 1995. Testing heterozygote excess and deficiency. *Genetics* **140:** 1413–1419.

Sambrook RA, Pigozzi BW, Thomas RN. 1999. Population pressure, deforestation, and land degradation. A case study from the Dominican Republic. *Professional Geographer* **51:** 25–40.

Sánchez M, Ingrouille MJ, Cowan RS, Hamilton MA, Fay MF. 2014. Spatial structure and genetic diversity of natural populations of the Caribbean pine, *Pinus caribaea*

var. *bahamensis* (Pinaceae), in the Bahaman archipelago. *Botanical Journal of the Linnean Society* **174:** 359–383.

Santiago-Valentín E, Sustache-Sustache J, Francisco-Ortega J, Cielo Figuerola C, Fumero-Cabán J, Griffith P. 2012. *Pseudophoenix sargentii* on Mona Island: conservation survey and a new discovery. *Palms* **56:** 78–90.

Sastre De Jesús I, Santiago-Valentín E. 1996. Botanical explorations of Puerto Rico by N. L. Britton and E. G. Britton: their significance in plant conservation, horticulture and education. *Brittonia* **48:** 322–336.

Smouse PE, Long JC. 1992. Matrix correlation analysis in anthropology and genetics. *American Journal of Physical Anthropology* **35:** 187–213.

Smouse PE, Long JC, Sokal RR. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Biology* **35:** 627–632.

Social Science Statistics. 2014. *Social Science Statistics*. Available at: http://www.socscistatistics.com/Default.aspx Downloaded on 5 May 2014.

UASD. 2002a. *Universidad Autónoma de Santo Domingo: mapa topográfico general: [Dominican Republic] [cartographic material]. Instituto Geográfico Universitario NE 19–5*. Santo Domingo: Universidad Autónoma de Santo Domingo.

UASD. 2002b. *Universidad Autónoma de Santo Domingo: mapa topográfico general [Dominican Republic] [cartographic material]. Instituto Geográfico Universitario NE 19–7*. Santo Domingo: Universidad Autónoma de Santo Domingo.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4:** 535–538.

Young A, Boyle T, Brown T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11:** 413–418.

Zona S. 2002. A revision of *Pseudophoenix*. *Palms* **46:** 19–37.