DIVERSITY, PHYLOGENY, AND EVOLUTION IN THE MONOCOTYLEDONS
EDITED BY SEBERG, PETERSEN, BARFOD & DAVIS
AARHUS UNIVERSITY PRESS, DENMARK, 2010

Species Delimitation in the Genus *Phoenix* (Arecaceae) Based on SSR Markers, with Emphasis on the Identity of the Date Palm (*Phoenix dactylifera*)

Jean-Christophe Pintaud,[1] Salwa Zehdi,[2] Thomas Couvreur,[1] Sasha Barrow[3], Sally Henderson,[4] Frédérique Aberlenc-Bertossi,[1] James Tregear,[1] and Norbert Billotte[5]

[1]IRD (Institut de Recherche pour le Développement), UMR DIA–PC, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France.

[2]Laboratoire de Génétique Moléculaire, Immunologie and Biotechnologie, Faculté des Sciences de Tunis, Campus Universitaire El Manar I, Tunis, Tunisia

[3]The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, U.K.

[4]Department of Botany, The Natural History Museum, London, SW7 5BD, UK

[5]CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), UMR 1096 Polymorphismes d'Intérêt Agronomique, TA 40/03 Avenue Agropolis, 34398 Montpellier Cedex 5, France

Author for correspondence (jean-christophe.pintaud@ird.fr)

Abstract—The genus *Phoenix* (Arecaceae, Coryphoideae, Phoeniceae) comprises 14 species widely distributed in the Old World subtropics and tropics west of Wallace's line. The identity of various taxa within the genus remains unclear, especially in the complex of cultivated, feral and wild populations of the date palm (*P. dactylifera*) and related species (*P. sylvestris*, *P. theophrasti*, *P. atlantica*, *P. canariensis*). In this study, we genotyped 17 Simple Sequence Repeats loci (SSR), namely 16 dinucleotide nuclear microsatellites and one dodecanucleotide plastid minisatellite, in 308 accessions of *Phoenix* representing 12 species, with emphasis on the date palm complex. Results revealed a high level of polymorphism with 18-41 alleles at the nuclear loci and five haplotypes at the minisatellite locus. Multi-loci fingerprints were informative since all individuals from the same species clustered together, supporting the existing taxonomy. In particular, *Phoenix dactylifera*, *P. theophrasti* and *P. sylvestris*, previously shown as comprising the '*P. dactylifera* clade', and the related species *P. atlantica* and *P. canariensis*,

appear highly distinct from each other, with few or no alleles in common. This indicates that the date palm was initially domesticated from wild populations of *Phoenix dactylifera* itself, with only secondary and localized genetic contributions from other species.

Keywords—Date palm, domestication, nuclear microsatellites, chloroplast minisatellite, taxonomy *Phoenix*.

The genus *Phoenix* L. includes 14 species (Govaerts and Dransfield 2005) of dioecious, pinnate-leaved palms, distributed in the Old World subtropics and tropics, from the Canary and Cape Verde islands in the Atlantic ocean, throughout Africa, Madagascar and Asia, reaching Sumatra, Taiwan and the Philippines in the East. The main centre of diversity of the genus spans from India to Indochina where eight species are found.

The genus is morphologically and phylogenetically isolated and forms the monogeneric tribe Phoeniceae within subfamily Coryphoideae (Asmussen et al. 2006; Dransfield et al. 2008b). At the molecular level, the genus *Phoenix* is highly divergent from all other palm genera and difficult to place phylogenetically (Asmussen and Chase 2001; Hahn 2002). In the most comprehensive phylogenetic survey of the Coryphoideae available to date (Dransfield et al. 2008a), it is placed as sister to Trachycarpeae with moderately high bootstrap support.

Within the genus, relatively little sequence variation is seen between species for the DNA regions commonly used in phylogenetic studies. Nuclear sequences of the intergenic spacer of 5S DNA units showed some variability at the interspecific level, however, alignment problems limited the phylogenetic resolution obtained (Barrow 1998, 1999). Plastid DNA sequences confirmed the monophyly of *Phoenix* with 100% bootstrap support but revealed almost no informative variation among three species studied (Dransfield et al. 2008a).

Morphological diversity is also narrow amongst the different species of *Phoenix*, with almost no reliable character available to distinguish closely related species such as *P. dactylifera* and *P. theophrasti* (Barrow 1998) or *P. atlantica* and *P. dactylifera* (Henderson et al. 2006), raising questions about their distinctiveness. Moreover, reproductive barriers appear to be very limited within the genus. Many fertile interspecific hybrids are known in various anthropogenic contexts where species are brought into proximity (Henderson et al. 2006). Little is known however about interspecific hybridization in areas of natural sympatry.

The low level of variation of morphological and DNA sequence data available means that it is necessary to use more polymorphic markers to unravel patterns of genetic differentiation within the genus *Phoenix*.

We chose to use multilocus microsatellite markers because they are among those genomic structures that show the highest mutation rates (Ellegren 2000) and are thus good candidate markers to distinguish between very closely related species. Billotte et al. (2004) showed that most microsatellite loci developed for

the date-palm (*Phoenix dactylifera*) are readily transferable to all other *Phoenix* species, indicating close interspecific affinities and allowing the use of these markers across the genus.

Microsatellite markers have been successfully used in the delimitation of closely related species in various groups of organisms (Primmer and Ellegren 1998; Zhu et al. 2000; Kretzer et al. 2003, Duminil et al. 2006), including another wild/cultivated palm species complex, namely that of the Neotropical *Bactris gasipaes* Kunth in Humb., Bonpl. and Kunth (Couvreur et al. 2006, 2007). Within *Phoenix*, a wide range of molecular markers have been used to study the agrobiodiversity of the date-palm, including flavonoids (Ouafi et al. 1988), isozymes (Benaceur et al. 1991, Salem et al. 2001), RFLP, PCR-RFLP (Cornicquel and Mercier 1996, Sakka et al. 2004), RAPD, ISSR, RAPD-ISSR or RAMPO (Sedra et al. 1998, Zehdi et al. 2002, 2004a, Rhouma et al. 2008), AFLP (Diaz et al. 2003; El-Assar et al. 2003, Elhoumaizi et al. 2006, Rhouma et al. 2007), and microsatellites or SSR (Zehdi et al. 2004b, 2006, Al-Ruqaishi et al. 2008, Elshibli and Korpelainen 2008, 2009, Ahmed and Al-Qaradawi 2009). Henderson et al. (2006) used microsatellites to distinguish *Phoenix atlantica* from *P. dactylifera*, *P. sylvestris* and *P. canariensis*. All four species were clearly separated in this study, with many species-specific alleles detected.

The origin and domestication history of the date palm (*Phoenix dactylifera*) remains obscure. The main problem is that it is extremely difficult to demonstrate whether this taxon truly exists in the wild, given that the plant has been cultivated for millennia within its putative natural area of distribution. Several authors mention putative wild stands of *Phoenix dactylifera*, but apparently natural populations can be abandoned cultivated stands or feral plants rather than truly wild ones (Zohary and Spiegel-Roy 1975, Zahran and Willis 1992, Zohary and Hopf 2000, Boulos 2005).

In a combined analysis of morphological and molecular data, Barrow (1998) identified a clade comprising the cultivated date palm *P. dactylifera* and the most closely related species *P. sylvestris* and *P. theophrasti*, and also *P. canariensis* in the analysis of morphological data alone. In addition to these species, *Phoenix atlantica* has been reported as allied to, or even doubtfully distinct from the cultivated date palm. *Phoenix atlantica* was originally described from the Cape Verde islands by Chevalier (1935), but little attention has been paid to this taxon until recently. *Phoenix atlantica* was however accepted as a valid species by Govaerts and Dransfield (2005), and Henderson et al. (2006) showed that it was genetically isolated from putatively related species on the basis of microsatellite markers. The inclusion of *Phoenix atlantica* in the *P. dactylifera* clade still needs to be confirmed by a molecular phylogeny. In this article, the term '*Phoenix dactylifera* clade' refers to the *P. dactylifera*/*P. sylvestris*/*P. theophrasti* group, supported by both molecular and morphological analyses, whereas the 'date palm complex' refers to the wider group comprising the three aforementioned species plus *P. atlantica* and *P. canariensis*.

There have been three main hypotheses proposed to explain the origin of *P. dactylifera* (Beccari 1890, Corner 1966, Munier 1973, 1974, Popenoe 1973, Zohary and Spiegel-Roy 1975, Barrow 1998, Rossignol-Strick 2003).

1. *P. dactylifera* is a strictly cultivated plant which is derived by human selection from a known wild species. *Phoenix sylvestris* has often been regarded as the putative wild ancestor of the date palm, as well as *Phoenix atlantica* and *P. canariensis*.
2. *Phoenix dactylifera* is a strictly cultivated plant which results from a hybridization process between two or more wild species. Putative parent species mentioned in the litterature include *P. sylvestris*, *P. atlantica*, *P. canariensis* but also *P. reclinata*.
3. *Phoenix dactylifera* exists or existed in the wild as a distinct species in the Near East-Middle East area and was domesticated independently from other species.

The case of the eastern Mediterranean *Phoenix theophrasti* is still problematic. It is not clear whether it is a distinct species or a 'dedomesticated' or feral form of *P. dactylifera* (Barrow 1998). Other taxonomic questions in the genus include the distinction of *Phoenix caespitosa* from *P. reclinata* and the treatment of the *Phoenix loureiroi* complex.

The aim of the present study is to investigate species delimitations in the genus *Phoenix* using SSR markers, and in particular to determine the status of the date palm *Phoenix dactylifera*.

Materials And Methods

Genotyping Design—In a first analysis, we used a relatively balanced species sampling including 72 individuals representing 11 species (Appendix 1), with all 16 nuclear SSR loci from Billotte et al. (2004) and the chloroplast minisatellite CpfM from Henderson et al. (2006): *Phoenix acaulis* (2), *P. caespitosa* (1), *P. canariensis* (4), *P. dactylifera* (20), *P. loureiroi* (9), including *var. loureiroi* (7) and *var. pedunculata* (2), *P. pusilla* (4), *P. reclinata* (13), *P. roebelenii* (4), *P. rupicola* (4), *P. sylvestris* (6) and *P. theophrasti* (5). *Phoenix atlantica* was excluded from this first analysis because it has partially or completely shifted allelic ranges at some loci with respect to all other species (Henderson et al. 2006, Table 1 and Fig. 1). In a second analysis, we added two previously published datasets to the first matrix, one consisting of 49 accessions of *Phoenix dactylifera* from Tunisia (Zehdi et al. 2004b), the other including 24 samples of *Phoenix atlantica* from Cape Verde islands (Henderson et al. 2006), and an additional set of samples including 17 *Phoenix canariensis*, mostly cultivated in Sanremo, Italy, and 146 *P. dactylifera* samples from Tunisia (57), Italy (43) and various other origins including Oman, Djibouti, Niger, Senegal and Mauritania, giving a total of 308 samples. As some

of the above-mentioned subsets lacked loci CpfM, Pd44 and/or Pd 48, only the remaining 14 nuclear SSR loci were used in the enlarged analysis.

Two species were unobtainable for this study, namely *Phoenix paludosa* Roxb. and *P. andamanensis* S. Barrow.

Combination of datasets was made possible through a set of samples genotyped repeatedly in all experiments, which was used to standardize allele size scores (see Henderson et al. 2006).

DNA Extraction—DNA solutions from Kew DNA bank were kindly provided by Dr. M. W. Chase, in form of 30 µl total DNA aliquots, and used directly or after dilution at 5 ng/µl when needed. Other accessions were collected either in cultivation or in the wild (Appendix 1). In both cases, 10 g. of young, fresh leaf tissue were dehydrated in 200 g. anhydrous silica gel immediately after collection. The dry leaf material was ground into a fine powder using either an A10 analytical grinder (IKA, Staufen, Germany) with a star-shaped rotating cutter, at 20,000 rpm speed for one minute, or a bead-mill homogenizer TissueLyser (Qiagen, Courtaboeuf, France). The leaf powder was then subjected to DNA extraction using DNeasy Plant Maxi/Mini/96 Kits (Qiagen, Courtaboeuf, France), according to manufacturer's instructions and the resulting DNA solutions were stored at –20°C.

Amplification and Genotyping—For the nuclear $(GA)_n$ microsatellite loci, PCR amplification was performed using the primer pairs published by Billotte et al. (2004). The PCR conditions and infrared genotyping protocol performed using a LICOR automated sequencer were previously described by Billotte et al. (*op. cit.*) and the ABI automated sequencer protocol was described by Henderson et al. (2006). Allele size was determined with respect to a 50-350 bp molecular weight marker using the software SAGA GT 2.1 (LICOR Bioscience, Lincoln, NE, U.S.A) with LICOR-generated data, and with a non-commercial ladder and GeneMapper software v. 4.0 (Applied Biosystems, Foster City, CA, USA) for ABI-generated data.

The cpDNA minisatellite locus was identified within the *trnG(GCC)-trnfM(CAU)* intergenic spacer. Aligned sequences of the *trnS-trnfM* region (amplified with the universal primers of Demesure et al. 1995) from various palm species (Genbank accessions EU043466 to EU043497, Pintaud et al. unpubl.), indicated a repeat polymorphism of a 12 bp motif, which was part of an inverted repeat, similar in structure to the minisatellite reported in orchids by Cozzolino et al. (2003). A primer pair, previously reported in Henderson el al. (2006) was designed to amplify specifically the minisatellite locus for subsequent genotyping (CpfM-F: 5-CCG CCC ACG ATG AAG TAA TGT A-3' and CpfM-R 5'-GTC ACG GGT TCA AAT CCT GTC TC-3'). The PCR mix was prepared for 12,5 µl reactions with the following final concentrations of reagents: 1 X Failsafe premix E (including dNTPs, $MgCl_2$ and Betaine; Epicentre, Madison U.S.A); 0,2 µM each of forward and reverse primers; 0,625 U Failsafe enzyme mix; 5ng/µl template DNA. PCR conditions were the following: initial denaturation at 95°C

for 1 min; 35 cycles of 94°C for 30 s, 52°C for 45 s, and 72°C for 60 s; and a final elongation step at 72°C for 8 minutes. Genotyping protocol was the same as for nuclear microsatellites.

Genetic Data Analysis—The total number of alleles, number of private alleles (PA; defined as alleles found in a single species), observed heterozygosity (Ho), and nonbiased expected heterozygosity He (Nei 1987) were calculated for each species at all loci, using the program GENETIX 4.04 (Belkhir et al. 2000, www.univ-montp2.fr/~genetix/genetix/genetix.htm).

Clustering and Ordination of Genotypes—To depict the genetic relationships among individuals, phylograms were generated using the Neighbor-Joining (NJ) algorithm (Saitou and Nei 1987) with the program PHYLIP v.3.6 (Felsenstein 1995). The shared allele distance D_{AS} (Chakraborty and Jin 1993), an index based on an infinite allele model, was used to compute genetic distances because of its efficiency in differentiating between closely related populations or taxa (Goldstein and Pollock 1997; Estoup et al. 1995a). The genetic distance D_{AS} was calculated using MSA 3.10 (Dieringer and Schlötteer 2003) with 1000 bootstrap replications.

To combine nuclear and chloroplast information in ordination analyses, the haploid organellar data were coded as diploid homozygotes.

Results

Amplification success of the nuclear microsatellites across the genus exceeded 90%, except for loci Pd44 and Pd48 that caused amplification problems (Table 1). Locus Pd044 did not amplify at all in *P. canariensis*, *P. pusilla*, *P. reclinata*, *P. roebelenii* and *P. rupicola* but amplified in 100% of *P. atlantica* samples, displaying six alleles among 24 individuals. The behaviour of Pd044 in *P. dactylifera* is unpredictable. It amplified perfectly all Omani genotypes (Al-Ruqaishi et al. 2008 and present study) but failed with about 50% of Tunisian accessions and did not amplify at all in Mauritanian accessions. This pattern is probably due to the geographical distribution of a mutation in the annealing site of one of the primers.

Amplification success of the nuclear SSR loci outside the date palm complex dropped to 12-13 loci out of 16 in *Phoenix loureiroi*, *P. pusilla* and *P. reclinata*. A few individuals, irrespective of the species to which they belonged, had only 9-11 positive loci, most probably due to poor DNA quality. This, however, did not affect the extent to which these individuals clustered with others of the same species except in one case (rec4*, Fig. 3). Therefore not all loci used in this study are required to support species clusters. All loci showed an overall high level of genetic diversity. The mean allele number per locus, excluding the problematic Pd044 and Pd048 was 30, with a range from 23 to 41 (Table 1). However, several loci were monomorphic in some species, often with a private, possibly fixed allele. Locus Pd15 shows the same pattern in all *Phoenix canariensis* samples with

two bands, of 119 and 152 bp, suggesting a duplicated genome segment with a fixed allele for each copy.

Genetic diversity as measured by He was high in *P. dactylifera* (0.74), *P. reclinata* (0.73) and *P. loureiroi* (0.70). These species were also characterized by the absence of monomorphic loci.

The three species of the *Phoenix dactylifera* clade showed marked differences in allele frequency distribution (Fig. 1). *Phoenix dactylifera* and *P. sylvestris* showed much higher genetic diversity than *P. theophrasti* (Table 2).

The cpDNA minisatellite was amplified with a 100% success rate and showed five haplotypes. Although less polymorphic than the nuclear microsatellites, the cpDNA locus showed a pattern of variation strongly associated with species, including a private haplotype fixed in *Phoenix canariensis* (Table 3). Within the

Locus name	Repeat motif[1]	size[2]	# alleles	% amplification
mPdCIR010	$(GA)_{22}$	117-186	33	95%
mPdCIR015	$(GA)_{15}$	112-162	28	97%
		111-232	31	99% (all species)
mPdCIR016	$(GA)_{14}$	196-232	13	100% (*P. atlantica*)
		111-142	18	99% (other species)
		141-232	23	98% (all species)
mPdCIR025	$(GA)_{22}$	141-172	9	88% (*P. atlantica*)
		197-232	14	99% (other species)
mPdCIR032	$(GA)_{19}$	276-309	24	97%
mPdCIR035	$(GA)_{15}$	176-222	27	93%
mPdCIR044	$(GA)_{19}$	281-332	18	erratic amplification
mPdCIR048	$(GA)_{32}$	151-204	29	erratic amplification
mPdCIR050	$(GA)_{21}$	154-210	34	90%
mPdCIR057	$(GA)_{20}$	249-304	34	96%
mPdCIR063	$(GA)_{17}$	119-181	33	94%
mPdCIR070	$(GA)_{17}$	166-215	27	94%
mPdCIR078	$(GA)_{13}$	107-155	32	93%
mPdCIR085	$(GA)_{29}$	143-183	33	96%
mPdCIR090	$(GA)_{26}$	135-204	26	98%
mPdCIR093	$(GA)_{16}$	120-186	41	97%
CpfM	$(CTAACTACTATA)_{2-6}$	253-301	5	100%

Table 1. Summary of genotyping data for the 17 loci studied (12 species, 308 individuals). [1]Sequenced in *Phoenix dactylifera*; [2]amplicon size range in bp.

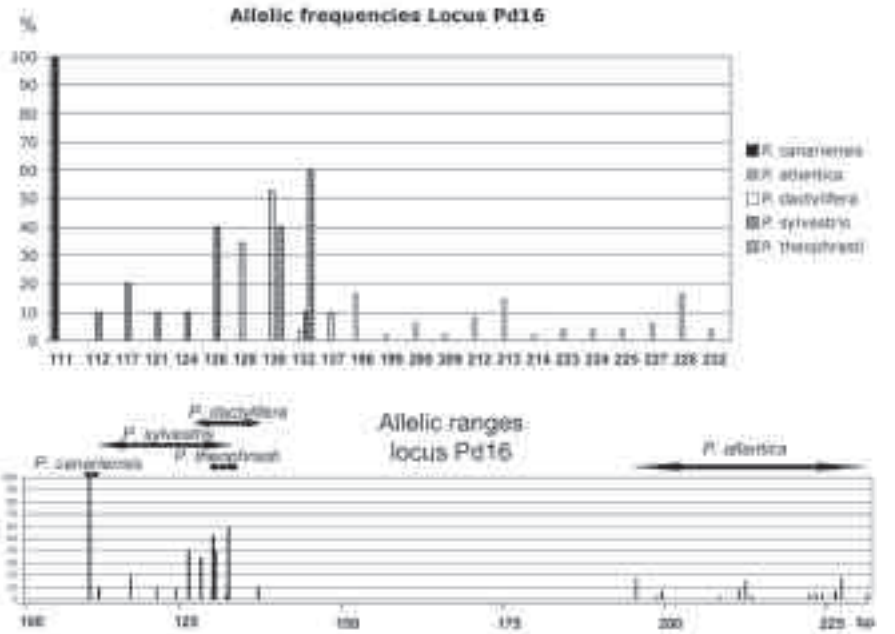

Fig. 1. Example of interspecific differentiation within the date palm complex, at locus Pd16. *Phoenix canariensis*: fixed private allele; *Phoenix sylvestris*, *P. dactylifera* and *P. theophrasti*: partially overlapping allelic ranges with private alleles or contrasting frequencies; *P. atlantica*: shifted range and high allelic diversity.

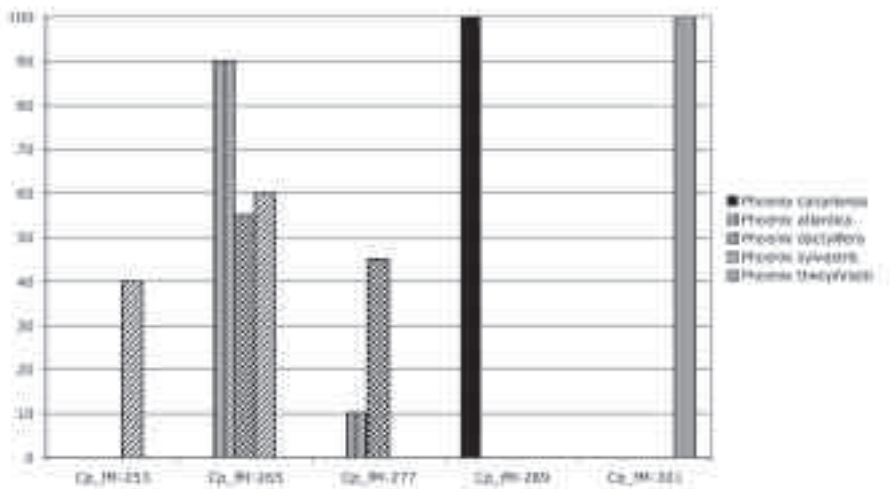

Fig. 2. Haplotype frequencies at the plastid minisatellite locus in the five species of the date palm complex.

He/Locus	*P. dactylifera*	*P. sylvestris*	*P. theophrasti*
Pd50	0.88	0.80	0.47
Pd63	0.76	0.75	0.71
Pd70	0.76	0.56	0.66
Pd78	0.87	0.68	0.50
Pd85	0.87	0.80	0.60
Pd90	0.83	0.00	0.00
Pd93	0.82	0.80	0.32
Pd15	0.77	0.52	0.66
Pd35	0.73	0.79	0.60
Pd44	0.20	0.61	0.72
Pd16	0.53	0.75	0.48
Pd48	0.76	0.80	0.00
Pd57	0.72	0.76	0.18
Pd10	0.88	0.76	0.46
Pd25	0.64	0.60	0.00
Pd32	0.84	0.66	0.00
# samples	**216**	**5**	**5**
He all loci	**0.74**	**0.66**	**0.40**
# alleles	**199**	**72**	**35**
# PA	**53**	**16**	**3**
%PA	**27**	**22**	**9**

Table 2. Genetic diversity measured by He for each locus and for all loci, total number of alleles, number and percentage of private alleles (PA) in each of the three species of the *P. dactylifera* clade.

date palm complex, *Phoenix canariensis* and *P. theophrasti* were separated on the basis of the minisatellite alone; *P. dactylifera* and *P. atlantica* had the same haplotype profile; and *P. sylvestris* had one haplotype shared with species outside the complex and one shared with *P. dactylifera* and *P. atlantica* (Table 3 and Fig. 2), the latter being the most common haplotype throughout the genus (Table 3). There were up to three haplotypes within a species (*P. roebelenii*).

The NJ trees based on the shared allele distance (D_{AS}), clearly separated all species of *Phoenix* (Fig. 3-4). Only one individual of *P. reclinata* (rec4) was misplaced, and this could be explained by missing data. Bootstrap values were,

Species	253 bp	265 bp	277 bp	289 bp	301 bp
P. pusilla	×				
P. loureiroi	×	×			
P. reclinata	×	×			
P. sylvestris	×	×			
P. roebelenii	×	×	×		
P. caespitosa		×			
P. acaulis		×			
P. dactylifera		×	×		
P. atlantica		×	×		
P. canariensis				×	
P. rupicola		×			×
P. theophrasti					×

Table 3. Distribution of haplotypes of the plastid minisatellite locus in the genus *Phoenix*.

however, often low on branches leading to species clusters, especially for the most variable ones (*P. dactylifera, P. reclinata, P. loureiroi*), but three species were strongly supported, with bootstrap values exceeding 90% (*P. canariensis, P. pusilla, P. rupicola*). The latter species have fixed private alleles at some loci and a generally low genetic diversity.

In the combined analysis, the *Phoenix dactylifera* cluster is perfectly maintained in spite of the wider sampling for this species which was increased by one order of magnitude (Fig. 4). Interspecific relationships did not receive significant bootstrap support and were highly unstable, depending on sampling (Figs. 3-4).

Discussion

Genetic Diversity—The microsatellite loci designed for *Phoenix dactylifera* used in this study showed good amplification and high polymorphism in most other species of *Phoenix*, thus confirming the good transferability indicated by Billotte et al. (2004). The only problematic loci were mPdCIR044, which has already been reported as producing erratic amplifications (Billotte et al. 2004, Ahmed and Al-Qaradawi 2009), and mPdCIR048 which could not be amplified in previous experiments (Zehdi et al. 2004b, Henderson et al. 2006). While all loci were initially selected for their polymorphism in the source species, *Phoenix dactylifera*, six of them revealed monomorphism in at least one of the other species. Such a loss of polymorphism in transferability experiments has often been

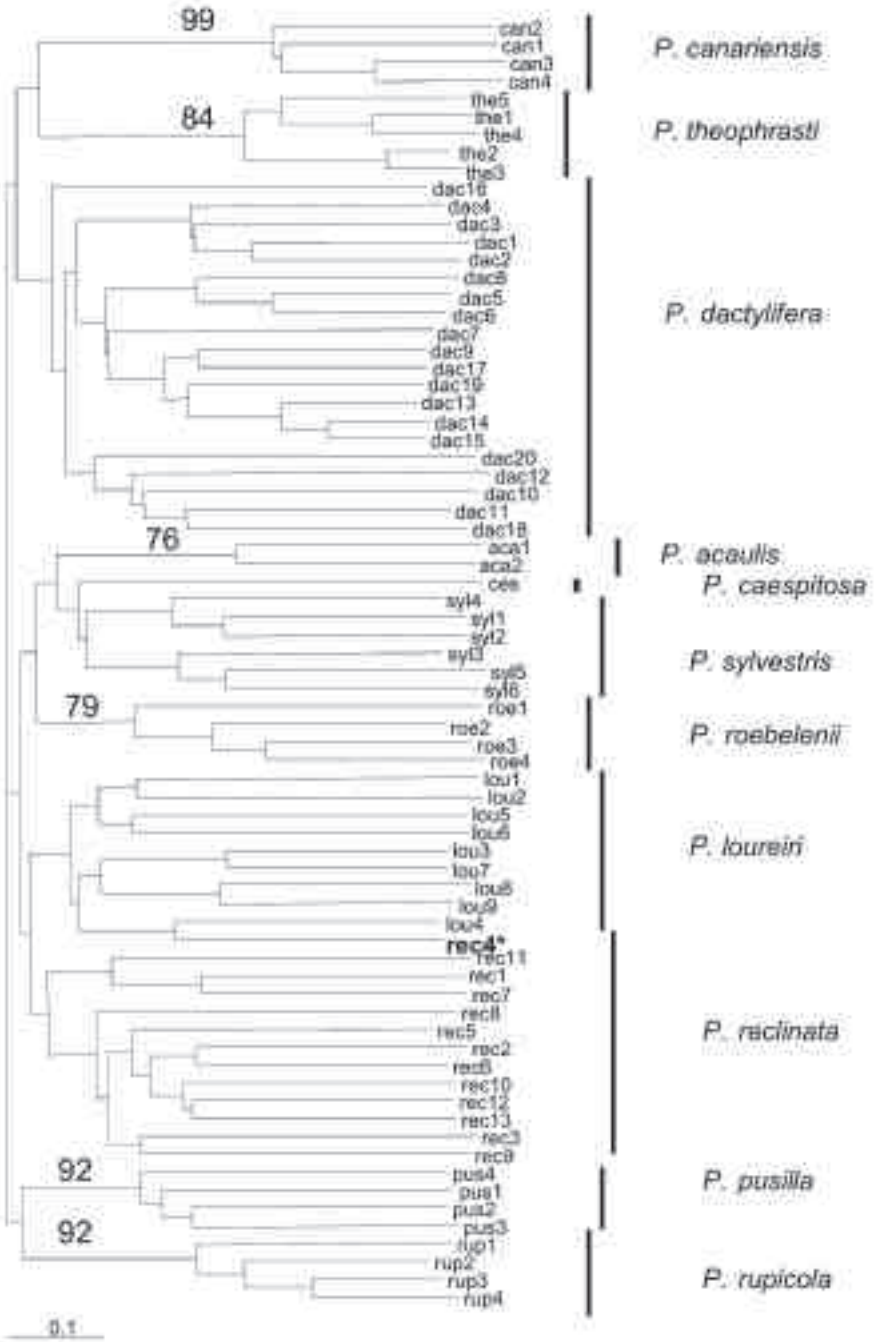

Fig. 3. Unrooted Neighbor Joining tree of 11 *Phoenix* species and 72 individuals. Bootstrap values (>70) at species level only, are shown above branches. * misplaced individual. See Appendix 1 for specimen abbreviation.

reported (Ellegren et al. 1997, Hutter et al. 1998, Butcher et al. 2000). The three widespread species (*P. dactylifera*, *P. reclinata* and *P. loureiroi*) showed high genetic diversity. These results are comparable to other tropical palm species such as *Euterpe edulis* (Gaiotto et al. 2003), which also showed high genetic diversity based on microsatellite markers. *Phoenix dactylifera* had a high number of private alleles (53), which strongly isolated it from other species in both analyses. For example, at locus Pd25, five out of eight alleles reported in *P. dactylifera* were private, three of them accounting for 84% of allelic frequencies at this locus. Various species with restricted distribution (*P. acaulis*, *P. canariensis*, *P. rupicola*, *P. pusilla*, *P. roebelenii*, *P. theophrasti*) had low genetic diversity and some fixed private alleles, a pattern consistent with an evolution of small populations in isolation. However, *Phoenix atlantica*, which is also an insular endemic from the Cape Verde archipelago, showed considerable genetic diversity, similar to that of *P. dactylifera* or even higher at some loci (Henderson et al. 2006), and generally associated with marked allelic range shifts with respect to other species (Fig. 1).

The high intra- and interspecific variability of the plastid minisatellite locus contrasts with the scarcity of nucleotide substitutions and indels in CpDNA within *Phoenix* (Asmussen et al. 2006). This indicates that not all structures are highly conserved within the CpDNA genome in *Phoenix*, with tandem repeats providing valuable information. This finding is in accordance with the pattern of variation of CpSSRs reported in *Bactris gasipaes* (Pintaud et al. 2008).

Species Delimitation–Micro-/minisatellite markers used in this study separated all species of *Phoenix* included (Fig. 3-4). All individuals of the same species cluster together, sometimes with high bootstrap support. Fixed private alleles are evidences of monophyly (synapomorphies) and genetic isolation of the corresponding species. Only the species having one or more monomorphic loci with a private allele formed clusters supported by significant bootstrap support in the NJ trees. These results largely confirm the morphological classification of Barrow (1998). However, little information is provided regarding relationships among species, due to the inherent limitations of the markers used (Estoup et al. 1995b, Garza et al. 1995, Viard et al. 1998), the kind of analysis that can be performed on such data, and the limited sampling of some of the species. The lack of a basis for interspecific comparison resulting from allelic size range shifts among species explains spurious relationships, which may be highly unstable depending on the sampling used. For the same reason is interspecific size homoplasy of little concern in delimitating species.

The *Phoenix dactylifera* clade, comprising *P. dactylifera*, *P. theophrasti* and *P. sylvestris* was identified in a previous phylogenetic analysis of sequence data (Barrow 1998). This grouping is not recovered by the analysis of microsatellite data, the three species being highly isolated in their allelic profiles, a fact that does not preclude a close phylogenetic affinity. Genetic isolation also characterizes *P. canariensis* and *P. atlantica*. *Phoenix canariensis* is the only species of the genus having a strictly private and fixed plastid haplotype. *Phoenix atlantica* has been

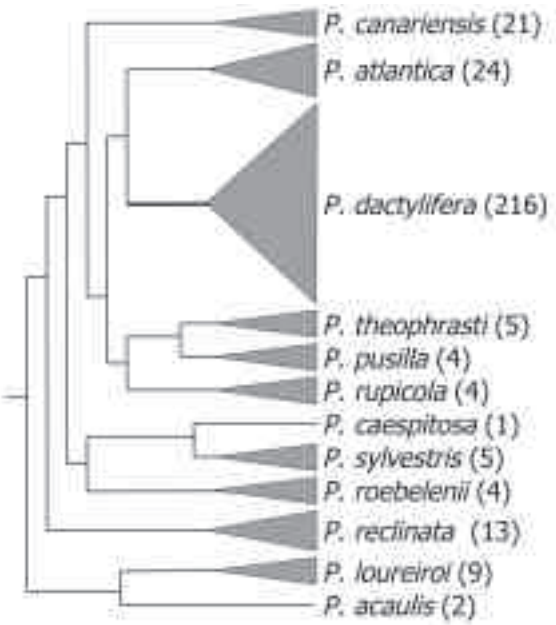

Fig. 4. Outline of the result of a Neighbor Joining analysis of the combined data sets representing a total of 308 individuals.

reported to display allelic range shifts with respect to other species, especially *P. dactylifera*, at various nuclear loci (Henderson et al. 2006), a phenomenon also seen in *P. canariensis*. However, the isolation of *Phoenix atlantica* from *P. dactylifera* is somewhat weakened with the extensive sampling of the latter species in the present analysis, revealing more shared polymorphisms. *Phoenix atlantica* is placed as sister to *P. dactylifera*, although without bootstrap support, in the second analysis. The fact that the two species share the same haplotype profile, different from all other species, at the plastid locus, may also be indicative of a sister relationship. The cultivated *Phoenix dactylifera* has a high number of private alleles, suggesting that it has been domesticated from wild populations of the same species. The status of wild populations of *P. dactylifera* is unclear, due to the ancient and widespread culture of the species, and the difficulty of distinguishing between truly wild populations and feral ones. However, the results of this study indicate that the search for wild populations of *Phoenix dactylifera* is essential to understanding the origin of the domestication of the date palm. The genetic diversity of *P. dactylifera* is high across its wide geographical distribution, and this is reflected in the morphological polymorphism of the species, which is probably the combined result of natural variation and human selection. Although the primary gene pool of the cultivated *Phoenix dactylifera* was most probably established without contributions from any other species,

careful analysis of shared alleles suggests that isolated hybridisation events with other species like *P. sylvestris*, *P. caespitosa* and *P. reclinata* are likely to have occurred during the expansion of date palm cultivation outside the Fertile Crescent, towards the Middle East and the southern limit of Sahara. Such a process could have resulted in particular local varieties having introgressed some allospecific genes.

Phoenix theophrasti, which is morphologically very similar to *P. dactylifera* was suggested by Barrow (1998) as possibly being a feral form of *P. dactylifera*. The present data show that these two species are highly distinct and divergent from each other. They do not share any cpDNA allele and have different alleles or distinct frequencies for shared alleles at the nuclear loci. *Phoenix theophrasti* is supported as a distinct species by high bootstrap support on the NJ tree. It appears to be an Aegean endemic, with a well-defined distribution in Crete, the Dodecanese archipelago and adjacent coastal Anatolia. Although the sampling is limited, it comprises individuals from Crete and Turkey and, contrary to herbaceous short-lived Aegean endemics with fragmented distribution (Edh et al. 2007), it has a very homogeneous genetic structure and low genetic diversity. Apart from life history considerations, this fact may also be due to relatively recent introduction of the species by man on some islands, from a single ancestral population.

The present data similarly resolve *Phoenix sylvestris* as a distinct species and indicate that *P. sylvestris*, like *P. theophrasti*, can be discarded as a possible ancestor of the cultivated *P. dactylifera*. The limited sampling of *P. sylvestris* suggests a high genetic diversity within this species, which still needs to be explored.

In conclusion, the analysis of hypervariable molecular markers has provided significant insights into the taxonomy of the genus *Phoenix*. Using a broader sampling, it is probable that markers of this type could accurately resolve the relationships between closely related taxa such as *Phoenix loureiroi* and *P. acaulis*, and could be very useful within the complex of cultivated, feral and wild populations of the date palm. The ease of distinguishing *P. dactylifera* from *P. theophrasti* with these markers will help considerably in studying the date palm complex in the Eastern Mediterranean region. Moreover, the numerous private alleles found in each species make these markers very suitable for introgression studies in areas of sympatry of two or more *Phoenix* species, especially since both nuclear and plastid loci are available.

This study indicates that microsatellites are useful markers for the evaluation of species limits, but a phylogenetic analysis based on sequence data showing an appropriate level of variation is still needed to precisely assess interspecific relationships within the genus *Phoenix*, which as yet remain poorly understood.

ACKNOWLEDGEMENTS. Part of the experiments were conducted at AMM, the DIA-PC genotyping and sequencing facility at INRA, in Montpellier, France, under supervision and help of S. Santoni and A. Weber. We also thank N. Mar-

seillac (CIRAD), C. Moreau, B. Malaurie (IRD) and S. Abdoulkader (CERD) for their contribution to laboratory work, data analysis and literature search. We are grateful to the following persons who provided samples: R. Castellana, M. W. Chase, A. Daher, C. Ducatillion,, M. Ferry, R. Huguenot, S. Ivorra, C. Littardi, C. Moricci, C. Newton, J.-F. Terral, L. Noblick, D. Sane and C. de la Torre. W. J. Hahn made useful comments on the manuscript. S. Zehdi benefited from a French Ministry of Foreign Affairs grant for scientific mobility between Tunisia and France.

Literature Cited

Ahmed, T. A. and A.Y. Al-Qaradawi. 2009. Molecular phylogeny of Qatari date palm genotypes using Simple Sequence Repeats markers. *Biotechnology* 8: 126-131.

Al-Ruqaishi, I. A., M. Davey, P. Alderson, and S. Mayes. 2008. Genetic relationships and genotype tracing in date palms (*Phoenix dactylifera* L.) in Oman, based on microsatellite markers. *Plant Genetic Resources: characterization and utilization* 6: 70-72.

Asmussen, C. B. and M. W. Chase. 2001. Coding and noncoding plastid DNA in palm systematics. *American Journal of Botany* 88: 1103-1117.

Asmussen, C. B., J. Dransfield, V. Deickmann, A. S. Barfod, J.-C. Pintaud, and W. J. Baker. 2006. A new subfamily classification of the palm family (Arecaceae): evidence from plastid DNA phylogeny. *Botanical Journal of the Linnean Society* 151: 15-38.

Barrow, S. 1998. A monograph of *Phoenix* L. (Palmae: Coryphoideae). *Kew Bulletin* 53: 513-575.

Barrow, S. 1999. Systematic studies in *Phoenix* L. (Palmae: Coryphoideae). Pp. 215-223 in *Evolution, variation and classification of palms*, eds. A. Henderson and F. Borchsenius. *Memoirs of the New York Botanical Garden* 83.

Beccari, O. 1890. Revista monografica delle species del genera *Phoenix* L. *Malesia* 3: 345-416.

Belkhir, K., J. Goudet, L. Chikhi, and F. Bonhomme. 2000. *Genetix (Ver. 4.01), logiciel sous WindowsTM pour la génétique des populations*, Laboratoire Génome et Population, Université Montpellier II, Montpellier.

Benaceur, M., C. Lanaud, M.-H. Chevallier and N. Bounaga. 1991. Genetic diversity of the date palm (*Phoenix dactylifera* L.) from Algeria revealed by enzyme markers. *Plant Breeding* 107: 56-57.

Billotte, N., N. Marseillac, P. Brottier, J.-L. Noyer, J.-P. Jacquemoud-Collet, C. Moreau, T. Couvreur, M.-H. Chevallier, J.-C. Pintaud, and A.-M. Risterucci. 2004. Nuclear microsatellite markers for the date palm (*Phoenix dactylifera* L.): characterization and utility across the genus *Phoenix* and in other palm genera. *Molecular Ecology Notes* 4: 256-258.

Boulos, L. 2005. *Flora of Egypt*. Vol. 4. Monocotyledons (Alismataceae – Orchidaceae). Cairo: Al-Hadara.

Butcher, P. A., S. Decroocq, Y. Gray and G. F. Moran. 2000. Development, inheritance and cross-species amplification of microsatellite markers from *Acacia mangium*. *Theoretical and Applied Genetics* 101: 1282-1290.

Chakraborty, R. and L. Jin. 1993. A unified approach to study hypervariable polymorphisms: statistical considerations of determining relatedness and population dis-

tances. Pp. 153-175 in *DNA fingerprinting: State of the Science,* eds. R. Chakraborty, J.T. Epplen and A.J. Jeffreys. Basel: Birkhäuser Verlag.

Chevalier, A. 1935. Plantes nouvelles de l'archipel des isles du Cap Vert. *Bulletin du Muséum National d'Histoire Naturelle*, *série II* 7: 137-139.

Corner, E. J. H. 1966. *The natural history of palms.* Berkeley: University of California Press.

Cornicquel, B. and L. Mercier. 1996. Identification of date palm (*Phoenix dactylifera* L.) cultivars by RFLP: partial characterization of a cDNA probe that contains a sequence encoding for a zinc finger motif. *International Journal of Plant Science* 158: 152-156.

Couvreur T. L. P., N. Billotte, A.-M. Risterucci, C. Lara, Y. Vigouroux, B. Ludeña, J.-L. Pham, and J.-C. Pintaud. 2006. Close genetic proximity between cultivated and wild *Bactris gasipaes* Kunth revealed by microsatellite markers in Western Ecuador. *Genetic Resources and Crop Evolution* 53: 1361-1373.

Couvreur T.L.P., W.J. Hahn, J.-J.de Granville, J.-L. Pham, B. Ludeña, and J.-C. Pintaud. 2007. Phylogenetic relationships of the cultivated Neotropical palm *Bactris gasiapes* (Arecaceae) with its wild relatives inferred from chloroplast and nuclear DNA polymorphisms. *Systematic Botany* 32: 519-530.

Cozzolino, S., D. Cafasso, G. Pellegrino, A. Musacchio, and A. Widmer. 2003. Molecular evolution of a plastid tandem repeat locus in an Orchid lineage. *Journal of Molecular Evolution* 57: 41-49.

Demesure, B., N. Sodzi, and R. J. Petit. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4: 129-131.

Diaz, S., C. Pire, J. Ferrer, and M. J. Bonete. 2003. Identification of *Phoenix dactylifera* L. varieties based on amplified fragment length polymorphism (AFLP) markers. *Cellular and Molecular Biology Letters* 8: 891-899.

Dieringer, D. and C. Schlötterer. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* 2**:** 1-3.

Dransfield, J., M. Rakotoarinivo, W.J. Baker, R.P. Bayton, J.B. Fisher, J. Horn, B. Leroy, and X. Metz. 2008a. A new Coryphoid palm genus from Madagascar. *Botanical Journal of the Linnean Society* 156: 79-91.

Dransfield J., N. W. Uhl, C. B. Asmussen, W. J. Baker, M. M. Harley, and C. E. Lewis. 2008b. *Genera Palmarum*, *the evolution and classification of palms*. Kew, U.K.: Royal Botanic Gardens.

Duminil, J., H. Caron, I. Scotti, S.-O. Cazal, and Petit R. 2006. Blind population genetics survey of tropical rainforest trees. *Molecular Ecology* 15: 3505-3513.

Edh, K., B. Widén, and A. Ceplitis. 2007. Nuclear and chloroplast microsatellites reveal extreme population differentiation and limited gene flow in the Aegean endemic *Brassica cretica* (Brassicaceae). *Molecular Ecology* 16: 4972-4983.

El-Assar, A. M., R. R. Krueger, P. S. Devanand, and C. T. Chao. 2003. Genetic analyses of date palms (*Phoenix dactylifera* L.) from Egypt using fluorescent-AFLP markers. *Hortscience* 38: 733-734.

Elhoumaizi, M. A., P. S. Devanand, J. Fang,and C.-C. T. Chao. 2006. Confirmation of 'Medjool' date as a landrace variety through genetic analysis of 'Medjool' accessions in Morocco; *Journal of the American Society of Horticulture Science* 131: 403-407.

Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics* 12: 551-558.

Ellegren, H., S. Moore, N. Robinson, K. Byrne, W. Ward and B.C. Sheldon. 1997. Microsatellite evolution: a reciprocal study of repeat lengths at homologous loci in cattle and sheep. *Molecular Biology and Evolution* 14: 854-860.

Elshibli, S. and H. Korpelainen. 2008. Microsatellite markers reveals high genetic diversity in date palm (*Phoenix dactylifera* L.) germplasm from Sudan. *Genetica* 134: 251-260.

Elshibli, S. and H. Korpelainen. 2009. Biodiversity of date palms (*Phoenix dactylifera* L.) in Sudan: chemical, morphological and DNA polymorphims of selected cultivars. *Plant Genetic Resources* (published online DOI: 10.1017/s1479262108197489)

Estoup, A., L. Garnery, M. Solignac, and J.-M. Cornuet. 1995a. Microsatellite variation in honey bee populations: Hierarchical genetic structure and test of the IAM and SMM. *Genetics* 140: 679-695.

Estoup, A., C. Tailliez, J. Cornuet, and M. Solignac. 1995b. Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). *Molecular Biology and Evolution* 12: 1074-1084.

Felsenstein, J. 1995. *Phylip (Phylogeny Inference Package)*. Seattle: University of Washington.

Garza, J., M. Slatkin, and N. Freimer. 1995. Microsatellite allele frequencies in humans and chimpanzees, with implications for constraints on allele size. *Molecular Biology and Evolution* 12: 594-603.

Gaiotto, F.A., D. grattapaglia, and R. Venkovsky. 2003. Genetic structure, mating system, and long-distance gene flow in heart of palm (*Euterpe edulis* Mart.). *Journal of Heredity* 94: 399-406.

Goldstein, D.B. and D.D. Pollock. 1997. Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. *Journal of Heredity* 88**:** 335-342.

Govaerts, R. and J. Dransfield. 2005. *World checklist of palms*. Kew, U.K.: Royal Botanic Gardens.

Hahn, W. J. 2002. A molecular phylogenetic study of the Palmae (Arecaceae) based on *atp*B, *rbc*L, and 18S nrDNA sequences. *Systematic Biology* 51: 92-112.

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

Hutter, C. M., M. D. Schug, and C. F. Aquadro. 1998. Microsatellite variation in *Drosophila melanogaster* and *Drosophila simulans*: a reciprocal test of the ascertainment bias hypothesis. *Molecular Biology and Evolution* 15: 1620-1636.

Kretzer, A. M., D. L. Luoma, R. Molina, and J. W. Spatafora. 2003. Taxonomy of the *Rhizopogon vinicolor* species complex based on analysis of ITS sequences and microsatellite loci. *Mycologia* 95: 480-487.

Munier, P. 1973. *Le palmier dattier*. Paris: Maisonneuve et Larose.

Munier, P. 1974. Le problème de l'origine du palmier-dattier et l'Atlantide. *Fruits* 29: 235-240.

Nei, M. 1987. *Molecular evolutionary genetics*. New-York: Columbia University Press.

Ouafi, S., R. Gaceb-Terrak, N. Bounaga and P. Lebreton. 1988. Les flavonoïdes, marqueurs infraspécifiques chez le palmier-dattier. *Comptes Rendus des Séances de l'Académie des Sciences, Paris*, T. 306, série III: 399-404.

Pintaud, J.-C., T. L. P. Couvreur, C. Lara, B. Ludeña, and J.-L. Pham. 2008. Reciprocal introgression between wild and cultivated compartments of *Bactris gasipaes* Kunth (Arecaceae) in Western Ecuador. Pp. 296-308 in *Crop Wild Relatives conservation and*

uses, ed. N. Maxted, B. Ford-Lloyd, S. Kell, J. Iriondo, E. Dulloo, and J. Turok. U.K.: CAB International Publishing.

Popenoe, P. 1973. *The date palm*. Edited by Henry Field. Miami: Field Research Projects.

Primmer, C. and H. Ellegren. 1998. Patterns of molecular evolution in avian microsatellites. *Molecular Biology and Evolution* 15: 997-1008.

Rhouma, S., S. Zehdi-Azouzi, A. O. M. Salem, A. Rhouma, M. Marrakchi and M. Trifi. 2007. Genetic diversity in ecotypes of Tunisian date-palm (*Phoenix dactylifera* L.) assessed by AFLP markers. *Journal of Horticultural Science and Biotechnology* 82: 929-933.

Rhouma, S., Dakhlaoui-Dkhil S., A. O. M. Salem, S. Zehdi-Azouzi, A. Rhouma, M. Marrakchi and M. Trifi. 2008. Genetic diversity and phylogenic relationships in date-palms (*Phoenix dactylifera* L.) as assessed by random amplified microsatellite polymorphism markers (RAMPOs). *Sciencia Horticulturae* 117: 53-57.

Rossignol-Strick, M. 2003. Climat et végétation sur le plateau iranien. *Dossiers d'Archéologie* 287: 5-17.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4**:** 406-425.

Sakka, H., S. Zehdi, A. O. M. Salem, A. Rhouma, M. Marrakchi and M. Trifi. 2004. Genetic polymorphism of plastid DNA in Tunisian date palm germplasm (*Phoenix dactylifera* L.) detected with PCR-RFLP. *Genetic Resources and Crop Evolution* 51: 479-487.

Salem, A. O. M., M. Trifi, H. Sakka, A. Rhouma and M. Marrakchi. 2001. Genetic inheritance analysis of four enzymes in date palm (*Phoenix dactylifera* L.). *Genetic Resources and Crop Evolution* 48: 361-368.

Sedra, M. H., P. Lashermes, P. Trouslot and M.-C. Combes. 1998. Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. *Euphytica* 103**:** 75-82.

Viard, F., P. Franck, M.P. Dubois, A. Estoup, and P. Jarne. 1998. Variation of microsatellite size homoplasy across electromorphs, loci, and populations in three invertebrate species. *Journal of Molecular Evolution* 47: 42-51.

Zahran, M. A. and A. J. Willis. 1992. *The vegetation of Egypt*. London: Chapman and Hall.

Zehdi S., J.-C. Pintaud, N. Billotte, A. Ould Mohamed Salem, H. Sakka, A. Rhouma, M. Marrakchi, and Trifi M. 2006. Etablissement d'une clé d'identification variétale chez le palmier dattier (*Phoenix dactylifera* L.) par les marqueurs microsatellites. *Plant Genetic Resources Newsletter* 145: 11-18.

Zehdi, S., H. Sakka, A. Rhouma, A. O. M. Salem, M. Marrakchi and M. Trifi. 2004a. Analysis of Tunisian date palm germplasm using simple sequence repeat primers. *African Journal of Biotechnology* 3: 215-219.

Zehdi, S., M. Trifi, N. Billotte, M. Marrakchi, and J.-C. Pintaud. 2004b. Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. *Hereditas* 141: 278-287.

Zehdi, S., M. Trifi, A. O. M. Salem, A. Rhouma and M. Marrakchi. 2002. Survey of inter simple sequence repeat polymorphisms in Tunisian date palms (*Phoenix dactylifera* L.). *Journal of Genetics and Breeding* 56: 77-83.

Zhu, Y., D. C. Queller, and J. E. Strassmann. 2000. A Phylogenetic Perspective onSequence Evolution in Microsatellite Loci. *Journal of Molecular Evolution* 50: 324-338.

Zohary, D. and M. Hopf. 2000. *Domestication of plants in the Old World. The origin and spread*

of cultivated plants in West Asia, Europe and the Nile Valley. 3rd Ed. New York: Oxford University Press.
Zohary, D. and P. Speigel-Roy. 1975. Beginnings of fruit growing in the Old World. *Science* 187: 319-327.

Appendix 1

Plant material used in this study. DNA bank reference: IRD = Laboratoire Genetrop, IRD, 34394 Montpellier, France; K = Jodrell Laboratory, Molecular Systemetics, Kew TW9 3AE, UK. Botanical gardens and germplasm collections accessions: MBC and RM = Montgomery Botanical Center, Old Cuttler Rd., Miami, U.S.A; Herbaria: K, G. Specimens codes in italics used in Fig. 2.

* * *

Phoenix acaulis Roxb.: *aca1*, cultivated U.S.A, seed from India, MBC 951193B, JCP 407 (IRD); *aca2*, cultivated U.S.A, seed from India, MBC 951193D, JCP 406 **(IRD). Phoenix caespitosa Chiov.:** *ces*, wild, Somalia, MWC 1802/Thulin 9016 (K). ***Phoenix canariensis* Chabaud**: *can1*, wild, La Gomera, Canary isl., JCP 169 (IRD); *can2*, wild, La Gomera, Canary isl., JCP 410 (IRD); *can3*, cultivated, Kew,U.K., MWC 1396/Kew 1984-4824 (K); *can4*, cultivated, Elche, Spain, MWC 1799/Barrow 75 (K). **Phoenix dactylifera L.:** *dac1*, cultivated, Dakar, Senegal, JCP 381 (IRD); *dac2*, cultivated, Atar, Mauritinia, cv 'Amsekshi', JCP 390 (IRD); *dac3*, cultivated, Atar, Mauritania, cv 'Ahmar', JCP 391 (IRD); *dac4*, cultivated, Atar, Mauritania, cv 'Idaghed', JCP 394 (IRD); *dac5*, cultivated, Atar, Mauritania, cv 'Tiguidert', JCP 395 (IRD); *dac6*, cultivated, Bordighera, Italy, cv 'Ebraica', JCP 422 (IRD); *dac7*, cultivated, Bordighera, Italy, cv 'Romana', JCP 423 (IRD); *dac8*, cultivated, Elche, Spain, cv 'Bou Feggous', HG 007, JCP 429 (IRD); *dac9*, cultivated, Elche, Spain, cv 'Zahidi', HG 014, JCP 430 (IRD); *dac10*, cultivated, Elche, Spain, cv 'Bou Sthami Black', HG015, JCP 431 (IRD); *dac11*, cultivated, Elche, Spain, cv 'Nabut Seif', HG 084, JCP 432 (IRD); *dac12*, cultivated, Elche, Spain, cv 'Medjoul', HG 051, JCP 447 (IRD); *dac13*, cultivated, Elche, Spain, cv 'Medjoul Ilicitan', HG 151, JCP 448 (IRD); *dac14*, cultivated, Elche, Spain, cv 'La Confitera', HG 223, JCP 449 (IRD); *dac15*, cultivated, Tunisia, cv 'Deglet Nour', SZ1 (IRD); *dac16*, cultivated, Kew, MWC 1395/PDJHL6 (K); *dac17*, cultivated, Elche, Spain, cv 'Zahidi', MWC 1800/ Barrow 77 (K); *dac 18*, cultivated, Kew, MWC 1891/Kew PDJHLI (K); *dac19*, cultivated, Kew, MWC 1398/Kew 1987-3379 (K); *dac20*, cultivated, Kew, MWC 1164 (K); **Phoenix loureiroi Kunth var. loureiroi:** *lou1*, cultivated (from Philippines), INRA Thuret, G2903, JCP 190 (IRD); *lou2*, wild, Hong Kong, MWC 1801/ Baker and Utteridge 009 (K); *lou3*, cultivated Kew (from Taiwan), MWC 1879/ Kew TFIT 1995-33; *lou5*, wild, Mae Sanam, Thailand, MWC 1187/Barrow 23 (K); *lou6*, wild, Trang, Thailand, MWC 1188/Barrow 32 (K); *lou8*, cultivated, U.S.A (from Vietnam), JCP 408 (IRD)/MBC 95255C; *lou9*, cultivated, U.S.A (from Vietnam), JCP 409 (IRD)/MBC 95255B; **Phoenix loureiroi Kunth var. pedunculata (Griff.)**

Govaerts: *lou4*, wild, Uttar Pradesh, India, MWC 1810/Barrow 44 (K); lou7, cultivated, U.K. (from India), JCP 221 (IRD)/JCP 579 (G); **Phoenix pusilla Gaertn.:** Sri Lanka, *pus1*, JCP 213 (IRD)/JCP 580 (G), *pus2*, MWC 1806/Barrow 66 (K), *pus3*, MWC 1807/Barrow 69 (K); *pus4*, MWC 1878/Barrow 70 (K); **Phoenix reclinata Jacq.:** *rec1*, cultivated, Italy, JCP 402 (IRD)/JCP 581 (G); *rec2*, cultivated, U.S.A. (from Zimbabwe), JCP 376 (IRD)/MBC 9712A; *rec3*, cultivated, U.S.A. (from Kenya), JCP 377 (IRD)/MWC 92165D; *rec4*, cultivated, U.S.A., JCP 378 (IRD)/RM 1037C; *rec5*, cultivated, France, JCP 188 (IRD)/JCP 582 (G); *rec6*, cultivated, France, JCP 194 (IRD)/JCP 583 (G); *rec7*, cultivated, France (from Tanzania), JCP 217 (IRD)/JCP 584 (G), *rec8*, wild, Senegal, JCP 388 (IRD); *rec9*, wild, Tanzania, MWC 1165/Dransfield 4828 (K); *rec10*, MWC 1193/Sambou et al. 1569 (K); *rec11*, cultivated, U.K. (from Botswana), MWC 1397/Kew 1991-228; *rec12*, wild Tanzania, MWC 1808/Goyder et al. 3928 (K); *rec13*, wild, Zimbabwe, MWC 1874/Wilkin 724 (K); **Phoenix roebelenii O'Brien:** *roe1*, cultivated, France, JCP 216 (IRD)/JCP 585 (G); *roe2*, cultivated, Thailand, MWC 1161/Barrow 26 (K); *roe3*, cultivated, U.K., MWC 1400/Kew 1987-530; *roe4*, cultivated, India, MWC 1805/Barrow 49 (K); **Phoenix rupicola T. Anderson:** *rup1*, cultivated, U.K. (from India), JCP 218 (IRD)/JCP 586 (G); *rup2*, wild, Samchi, Bhutan, MWC 1162/Grierson and Long 3414 (K); *rup3*, cultivated, U.K. (from India), MWC 1399/Kew 1989-75-PSSD; *rup4*, wild, West Bengal, India, MWC 1804/Barrow 73 (K); **Phoenix sylvestris (L.) Roxb.:** *syl1*, cultivated, Italy, JCP 258 (IRD)/JCP 591 (G); *syl2*, cultivated, Italy, JCP 404 (IRD)/JCP 592 (G); *syl3*, cultivated, U.K. (from India), JCP 214 (IRD)/JCP 588 (G); *syl4*, cultivated, France, JCP 405 (IRD)/JCP 593 (G); *syl5*, wild, Tamil Nadu, India, MWC 1803/Barrow 59 (K); *syl6*: wild, Tamil Nadu, India, MWC 1876/Barrow 58 (K); **Phoenix theophrasti Greuter.:** *the1*, cultivated, France (from Crete), JCP 215 (IRD)/JCP 589 (G); *the2*, cultivated, Spain (from Crete), JCP 261 (IRD)/JCP 594 (G); *the3*, cultivated, France (from Crete), JCP 259 (IRD)/JCP 595 (G); *the4*, wild, S.W. Anatolia, Turkey, MWC 1163/ Barrow 37 (K); *the5*, wild, S.W. Anatolia, Turkey, MWC 1191/Barrow 41 (K).