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# Conflicting patterns of nucleotide diversity between mtDNA and nDNA in the Moorish gecko, *Tarentola mauritanica*

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ABSTRACT

The gecko *Tarentola mauritanica* is a Mediterranean species that is widely distributed across southern Europe and North Africa. Initial phylogeographic studies based on mtDNA concluded that the European populations originated from recent colonizations from North Africa, possibly involving man-mediated introductions. A distinct lineage, found to be a sister taxon to the widespread European lineage, was subsequently reported from parts of the Iberian Peninsula. Like many gecko species, *T. mauritanica* contains several deep intraspecific genetic lineages within North Africa. However, in contrast to this diversity, a single mtDNA haplotype is widespread across a large part of its European range. In this work, we analysed a total of 834 base pairs from two mtDNA genes (12SrRNA and 16SrRNA) for 154 specimens, and a total of 1876 bp from three nuclear genes (ACM4, MC1R and Rag2) for 51 specimens to test the phylogenetic and phylogeographic patterns proposed for *T. mauritanica* based on mtDNA sequences. The mtDNA results reveal a Moroccan lineage basal to the common "European" haplotype, thus suggesting that the latter probably originated in Morocco. The remaining lineages obtained are the same as those observed in previous studies. In contrast, the nDNA data do not support the majority of the mtDNA phylogenetic relationships, possibly due to incomplete lineage sorting of these markers. Moreover, the mtDNA data suggest that the Iberian clade seems to have undergone a population expansion. Surprisingly, the European clade presents a higher nucleotide diversity for the nuclear genes when compared to the combined mtDNA dataset. These analyses suggest that the low mtDNA variability that characterises the European populations of *T. mauritanica* is the result of a selective sweep process and not solely due to a recent colonization event.



## 1. Introduction

The genus *Tarentola*, a member of the phyllodactylid group of geckos (Gamble et al., 2008b), is currently comprised of 21 species (Baha El Din, 1997; Carranza et al., 2002; Díaz and Hedges, 2008; Joger, 1984a,b,c; Schleich, 1984; Sprackland and Swinney, 1998), most of which display low levels of interspecific morphological variation. This genus is distributed across southern Europe, North Africa, and some of the Macaronesian islands (including Madeira, the Selvagens Islands, the Canary Islands and the Cape Verde archipelago), and is also found in the West Indies, particularly Cuba and the Bahamas (Schwartz and Henderson, 1991). The genus *Tarentola* has been the focus of various studies aimed at unravelling its phylogenetic relationships (Carranza et al., 2002, 2000; Harris et al., 2009; Jesus et al., 2002; Weiss and Hedges, 2007), phylogeographic patterns (Gübitz et al., 2000; Harris et al., 2004a,b; Perera and Harris, 2008; Vasconcelos et al., 2010), thermal ecology (Carretero, 2008), and foraging strategies (Hódar et al., 2006).

The Moorish gecko *Tarentola mauritanica* is one of the most common geckos in the Western Mediterranean Basin, and is distributed across southern Europe (Iberian Peninsula, France, Italy, the Balkans, and Greece), North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt), and most of the Mediterranean islands (Vogrin et al., 2008; see Fig. 1). *Tarentola mauritanica* is known to be sometimes associated to humanized environments, such as houses and stone walls, especially near artificial lights that attract insect prey (Arnold and Ovenden, 2002). This close relationship with humans, sometimes leads to accidental anthropogenic introductions of these geckos into new areas (Arnold and Ovenden, 2002), such as the Balearic Islands and Tenerife (Spain), the island of Madeira (Portugal), South America and United States (Vogrin et al., 2008).

This species has an extremely high genetic variability in North Africa, with at least eight different lineages (Harris et al.,

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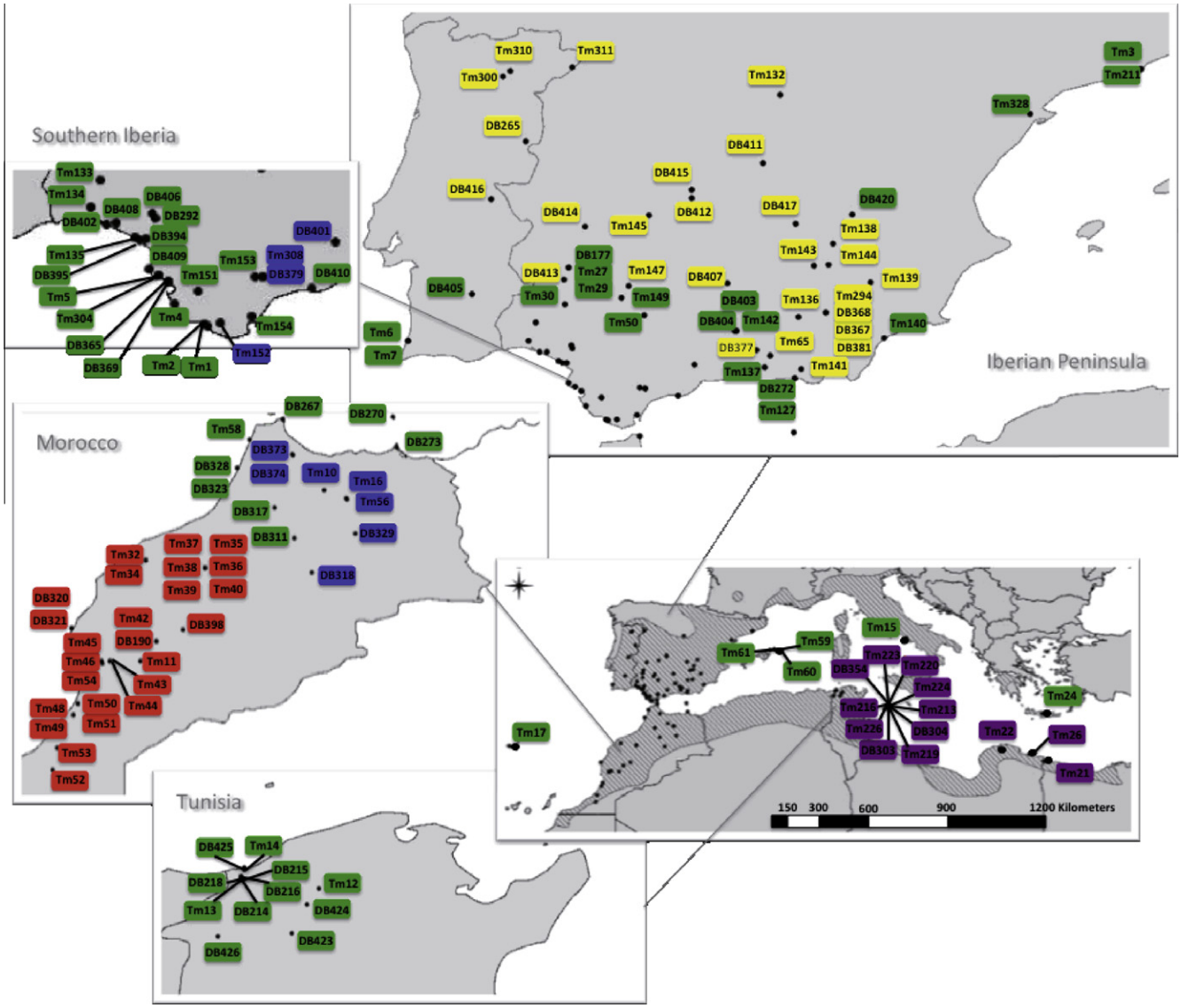


**Fig. 1.** Map showing all sampling localities of *Tarentola mauritanica* used in this study, and representation of the obtained clades from mtDNA according to the following colors: Green represents the specimens from Clade A; yellow indicates the individuals from Clade B; in red are the specimens from Clade C; blue represents the individuals from Clade D; and purple the individuals from Clade E. Specimens with codes starting with "Tm" represent individuals used in previous studies (Harris et al., 2004b,a, 2009; Perera and Harris, 2008). The dashed area in the bottom right map indicates the distribution range of *T. mauritanica* according to Vogrin et al. (2008). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

2004a,b). Specifically, two main different lineages have been identified in Morocco, one in the north, and another across central and southern regions. Taking into account the high genetic variability of *T. mauritanica*, and the typical morphological homogeneity of this group, it is thought that this could be a cryptic species complex (Harris et al., 2004a,b). Additionally, several studies repeatedly demonstrated the paraphyly of *T. mauritanica* with respect to *T. angustimentalis* from the Canary Islands (Carranza et al., 2000; Harris et al., 2004a,b, 2009; Perera and Harris, 2008). This pattern of morphological conservatism across divergent mtDNA lineages has also been found in *Tarentola* from the Cape Verde archipelago (Carranza et al., 2000; Jesus et al., 2002; Vasconcelos et al., 2010).

Surprisingly, the opposite pattern was initially observed in Europe, where a single common haplotype was found to be widespread across Portugal, Spain, continental Italy, Crete, Menorca, Morocco, and Tunisia (Harris et al., 2004a,b). This was initially thought to be the result of a very recent introduction from Tunisia followed by a rapid, possibly human-mediated expansion across southern Europe (Harris et al., 2004b). However, the discovery of a new distinct mtDNA lineage, widely distributed across Central Iberia (Harris et al., 2004a; Perera and Harris, 2008) sister to the common European and North African clade, indicated that the widespread European haplotype probably originated in Iberia and later spread rapidly to Tunisia and the rest of Europe (Harris et al., 2004a; Perera and Harris, 2008). Additionally, the recent finding of two distinct mtDNA lineages in Lampedusa and Conigli islands (Italy) suggests that this gecko arrived on these small islands from North Africa and not from southern Europe (Harris et al., 2009).

In the present study, as well as increasing the number of sampled specimens of *T. mauritanica*, segments of two mitochondrial markers (12SrRNA and 16SrRNA) and three protein-coding nuclear genes (ACM4, MC1R and Rag2) were analysed to test the phylogenetic and phylogeographic patterns proposed based on mtDNA sequences. The nuclear genes used in this study are typically employed in phylogenetic studies in reptiles (e.g. Vidal and Hedges, 2009), particularly geckos (e.g. Gamble et al., 2008b, 2008c), where they have been shown to be quite variable at the

intraspecific level (e.g. Perera and Harris, 2010; Rocha et al., 2009). We intend to investigate if the mtDNA lineages coincide with those derived from the nuclear markers, and particularly if these three nuclear genes also confirm the low level of genetic variability characteristic of the European lineage.

## 2. Material and methods

### 2.1. Samples and localities

Fifty-seven new samples of *T. mauritanica* were used in this work. The study was carried out using a total of 154 specimens from 120 localities comprised of: two *T. angustimentalis*, two *T. deserti*, one *T. boehmei*, one *T. boettgeri* and 91 specimens of *T. mauritanica* used in previously published studies (Harris et al., 2004a,b, 2009; Jesus, 2008; Perera and Harris, 2008). All sampling localities are represented in Fig. 1, and precise information on the specimens is given in Appendix A.

### 2.2. DNA extraction, amplification and sequencing

Tissue samples were collected from the specimen's tail, and total genomic DNA was extracted using standard saline methods (Sambrook et al., 1989). For the 57 new samples of *T. mauritanica* the Polymerase Chain Reaction (PCR) amplification and sequencing of two mtDNA gene fragments, the 12SrRNA and 16SrRNA, was performed using the primers 12Sa/12Sb and 16Sar/16Sbr from Kocher et al. (1989) and Palumbi (1996), respectively. PCR conditions were the same as those described in Harris et al. (1998). We also amplified and sequenced three nuclear protein-coding gene fragments: the acetylcholinergic receptor M4 (ACM4), the melanocortin 1 receptor (MC1R), and the recombination activating gene 2 (Rag2). Amplification and sequencing of these three nuclear genes was performed for 51 specimens of *T. mauritanica*, representing all known mtDNA groups. Regarding the ACM4 we used the primers tg-F and tg-R published by Gamble et al. (2008a) with PCR conditions following Gamble et al. (2008b). For the MC1R fragment the primers were MC1R_F and MC1R_R following conditions described in Pinho et al. (2009). For both ACM4 and MC1R the PCRs were carried out in 25 μl volumes, containing 5.0 μl of 10× reaction Buffer, 2.0 mM of $MgCl_2$, 0.5 mM each dNTP, 0.2 μM each primer, 1 U of Taq DNA polymerase (Invitrogen), and approximately 100 ng of template DNA. Finally, amplification and sequencing of the Rag2 gene fragment was performed using two sets of primers; 31FN.Venk/Lung.460R (amplification) and Lung.35F/Lung.320R (amplification and sequencing) published by Hoegg et al. (2004). PCR conditions were the same as described in Chiari et al. (2004). All amplified fragments were sequenced using an ABI310 automated sequencer (Applied Biosystems), following the manufacturer's protocols.

The obtained sequences were aligned using MAFFT v.5.861 (Katoh, 2008) with default parameters (gap opening penalty = 1.53; gap extension penalty = 0.123; progressive method = FFT-NS-2), and exported to Bioedit (Hall, 1999) to be checked and adjusted by hand.

### 2.3. Phylogenetic analyses

Previously published sequences of 12SrRNA and 16SrRNA (Harris et al., 2004a,b, 2009; Jesus, 2008; Perera and Harris, 2008) were added to our analyses, and *T. boettgeri* was designated as the outgroup. Calculation of the likelihood scores and choice of the best model of sequence evolution was carried out for each mtDNA gene using jModeltest v.0.1.1 (Posada, 2008), under the Akaike Information Criterion (following Posada and Buckley, 2004). We analysed the concatenated mtDNA dataset using Maximum Likelihood (ML) with the program GARLI 0.95 (Zwickl, 2006). Tree search was conducted using 5000 and 10,000 generations (*genthreshfortoposterm*) considering a stochastic algorithm, each resulting in a single best tree. The resulting likelihood values were compared, selecting from among these the tree with the highest likelihood score. Since no significant differences in likelihood scores were observed between the two searches, bootstrap support was calculated from 1000 bootstrap pseudo-replicates (Felsenstein, 1985), using 5000 *genthreshfortopoterm*. The 50% consensus tree was generated using the software PAUP* v.4.0d10 (Swofford, 2002). A Bayesian analysis was performed using Mr.Bayes v.3.1.2 (Huelsenbeck and Ronquist, 2001). This was carried out as a partitioned analysis of molecular data, since we are combining data from two different mtDNA regions. The Bayesian posterior probabilities were estimated using a Metropolis-coupled, Markov chain Monte Carlo (MC–MCMC) sampling approach. Both runs started with random starting trees and ran for $2 \times 10^6$ generations, saving one tree in each 100 generations. In both searches, the stationarity of the Markov chain was determined as the point when sampled log-likelihood values plotted against generation time, reached a stable mean equilibrium value. 25% of the samples (5000 samples) from the burnin were discarded. Using the software Tracer v1.4 (Rambaut and Drummond, 2007) was confirmed that all parameters had an ESS > 100 after burnin. The remaining trees were combined, and a 50% majority consensus tree was generated.

### 2.4. Population genetics analyses

In order to determine if each obtained mtDNA clade can be treated as a different population, we applied the *Snn* test (Hudson, 2000) on each nuclear gene fragment to test for genetic differentiation between the obtained mtDNA clades. Calculation of the *P*-value of the *Snn* was performed with a permutation test with 1000 replicates implemented in the software DnaSP v.5 (Librado and Rozas, 2009).

Haplotype diversity for each nuclear gene was represented using a haplotype network. Prior to this analysis, the existence of heterozygous positions was confirmed for all the nuclear gene fragments and an input file with that information was constructed using SeqPHASE (Flot, 2009). The phase of each haplotype and its reconstruction was carried out using PHASE v.2.1.1 (Stephens and Donnelly, 2003; Stephens et al., 2001) running the formerly built input file, and considering the default parameters of the software (thresholds are $p = q = 90\%$). This data file was used for the construction of a Median-Joining haplotype network, using the Fluxus Phylogenetic Network Analysis software v.4.5.1.0 (Bandelt et al., 1999; http://www.fluxus-engineering.com) with the parameter epsilon set to 0.

We calculated Tajima's *D* (Tajima, 1989) within each mtDNA and nDNA clade since it is expected that under a hitchhiking model the value of Tajima's *D* will be large and negative, indicating a skew toward rare variants (Braverman et al., 1995; Fay and Wu, 2000; Kim, 2006). To test the significance of Tajima's *D* values we performed 10,000 coalescent simulations with the software DnaSP v.5 (Librado and Rozas, 2009). Changes in population size were investigated for each nuclear and mitochondrial clade using the $R_2$ test, known to be very powerful in detecting population expansions in small sample sizes (Ramos-Onsins and Rozas, 2002). The $R_2$ test and its significance was performed using DnaSP v.5 (Librado and Rozas, 2009) with 10,000 coalescent simulations.

Nucleotide diversity ($\pi$) (with 95% confidence intervals) and uncorrected *p*-distances were calculated for each clade using the software DnaSP v.5 (Librado and Rozas, 2009) and MEGA v.4 (Tamura et al., 2007), respectively. The nucleotide diversity was estimated for both mitochondrial and nuclear data, and the uncorrected *p*-distances were only calculated between each mtDNA clade.

## 3. Results

A total of 834 bp (181 variable sites), corresponding to 344 bp for 12SrRNA and 490 bp for 16SrRNA, were used. Both ML and Bayesian analyses supported the same tree topology (Fig. 2), with the GTR + G model of molecular evolution fitting each gene partition best. Once again, and as previously reported (Carranza et al., 2000; Harris et al., 2004a,b, 2009; Perera and Harris, 2008),

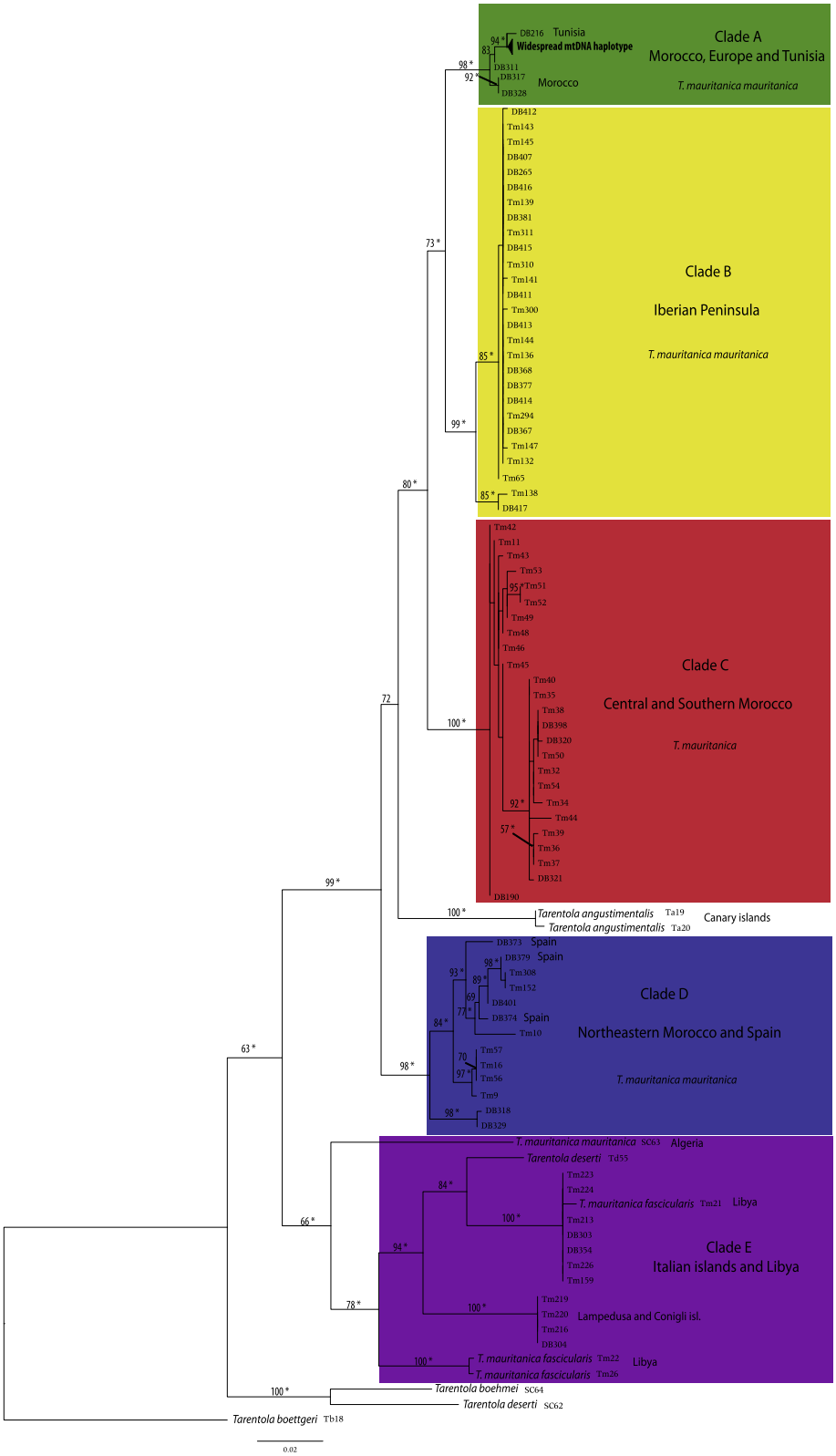


**Fig. 2.** ML phylogenetic tree for the 12SrRNA and 16SrRNA combined data, and with *Tarentola boettgeri* designated as outgroup. Values above the branches correspond to ML bootstraps, and (*) to the Bayesian posterior probabilities above 95%. The collapsed branch in Clade A corresponds to the common European haplotype. Specimens with this haplotype are indicated in Appendix A with the symbol (+).

*T. angustimentalis* from the Canary Islands was found to form a lineage inside *T. mauritanica*, thus making this species paraphyletic. *Tarentola deserti* is also polyphyletic, with one individual (SC62) being sister to *T. boehmei* and the other specimen of the same species (Td55) branching with Clade E of *T. mauritanica* from Libya, Lampedusa, and Conigli islands (Harris et al., 2004a,b, 2009; Perera and Harris, 2008).

The mtDNA tree can be divided into five distinct and well-supported evolutionary lineages (Fig. 2): (1) a European-Moroccan-Tunisian clade consisting of a collapsed lineage corresponding to the European widespread haplotype (64 specimens) along with a sister lineage from Tunisia and a basal lineage from north-western Morocco (Clade A); (2) an Iberian Peninsula clade (Clade B); (3) a clade from central and southern Morocco (Clade C); (4) another from north-eastern Morocco and Spain (Clade D); and finally, (5) a lineage from Libya and some Italian islands, namely Lampedusa and Conigli (Clade E). Clades C and E, with 9.0% of uncorrected genetic distance between them, are the most divergent, whereas Clades A and B, with 2.8% of genetic divergence, are the closest (Table 1). Clade A also has the lowest nucleotide diversity (0.00056), with Clade E showing the highest value (0.04031; Table 1).

A total of 51 specimens, which included representatives from all the defined mtDNA lineages, were sequenced for all three nuclear gene fragments. The obtained fragments had a length of 431 bp (14 variable sites) for ACM4, 668 bp (18 variable sites) for MC1R, and a total of 777 bp (19 variable sites) for Rag2. Heterozygous specimens were found for all three gene fragments (13 individuals for ACM4 and Rag2 and 7 specimens for MC1R). Somewhat unexpectedly, the Median-Joining networks for each nuclear gene (Fig. 3) do not support the majority of the mtDNA lineages or phylogenetic relationships, except for ACM4, where Clades A, C, D, and E are well separated from each other.

The results of the *Snn* test (Hudson, 2000) (Table 2) indicate that the clades defined by mtDNA are significantly differentiated for all nuclear genes. All clades were therefore treated as different populations, and demographic analyses were performed independently for each of them. Regarding the mtDNA results, the values obtained for Tajima's *D* neutrality test, and $R_2$ test were significant ($0.001 < P < 0.01$) only for Clades A and B, with Clade B presenting the highest negative value for Tajima's *D* (−1.97610; Table 1). Concerning the combined nuclear genes, no significant values were obtained for Tajima's *D* or $R_2$ test. Apart from Clade A, all other clades present a decrease in the nucleotide diversity for the combined nuclear genes compared to mtDNA. Moreover, only Clades A and E present non-overlapping 95% confidence intervals between their nDNA and mtDNA nucleotide diversity (Table 1).

## 4. Discussion

In light of the results obtained, it is surprising that given such a substructure and high genetic divergence between mitochondrial lineages, these are not corroborated by the nDNA genes, especially MC1R and Rag2. Such results suggest an incomplete lineage sorting of these nuclear markers, which would mean that, despite the highly divergent mtDNA lineages, the nuclear genome is not as distinct in the different clades. This lack of population structure in nuclear genealogies within highly differentiated mtDNA lineages is not, however, uncommon (e.g. Ballard et al., 2002), and has already been detected in amphibians (Velo-Antón et al., 2008) and other reptiles such as the wall lizards *Podarcis* (Pinho et al., 2007, 2008) and the whip snake *Hierophis viridiflavus* (Rato et al., 2009).

High levels of intraspecific genetic divergence for mtDNA have already been described for other geckos (Arnold et al., 2008; Austin et al., 2004; Jesus et al., 2005, 2006; Kasapidis et al., 2005; Perera and Harris, 2010; Rato and Harris, 2008), and when comparisons have been possible, variation within nuclear markers has not always been remarkable (Arnold et al., 2008; Austin et al., 2004), thus leading to the hypothesis that geckos may have a relatively faster rate of mtDNA evolution (Harris et al., 2004a; Jesus et al., 2005, 2006). On the other hand, the "normal" intraspecific levels used as yardsticks for mtDNA evolution may be biased (Harris and Froufe, 2005) or this could simply be a taxonomic artefact. Since geckos are characterized by a conservative morphology, it is likely that cryptic species have been overlooked, as suggested by some authors (Harris et al., 2004a; Perera and Harris, 2010). Another hypothesis is that such a high mtDNA evolutionary rate could be related to mitochondrial gene rearrangements, which are known to occur frequently in both amphibians and reptiles (Fonseca and Harris, 2008; Gissi et al., 2008).

On the basis of the hypothesis of high mtDNA evolutionary rate and high levels of diversity in North Africa, the finding of a single mitochondrial haplotype in *T. mauritanica* across a large geographic range was unexpected, suggesting a recent colonization and rapid expansion from Tunisia to southern Europe (Harris et al., 2004a). However, the discovery of different mtDNA lineages in Spain (Harris et al., 2004a; Perera and Harris, 2008) changed this scenario and suggested that the origin of this common "European"

**Table 1**
Uncorrected genetic distances (*p*) between combined mtDNA (12S + 16S) clades, and nucleotide diversity ($\pi$) (with 95% confidence interval), value of Tajima's *D* statistics (Tajima, 1989), and $R_2$ test (Ramos-Onsins and Rozas, 2002) estimated for both mtDNA and nDNA clades. The "*n*" corresponds to the number of haplotypes included in each clade. Statistic is abbreviated as ns (not significant).

| Clades | Uncorrected genetic distances (*p*) | | | | Nucleotide diversity ($\pi$) | Tajima's *D* | $R_2$ |
|---|---|---|---|---|---|---|---|
| | Clade A (%) | Clade B (%) | Clade C (%) | Clade D (%) | | | |
| *mtDNA* | | | | | | | |
| Clade A ($n = 68$) | – | – | – | – | 0.00056 (CI: 0.00–1.54) | −1.7123** | 0.0352* |
| Clade B ($n = 27$) | 2.8 | – | – | – | 0.00203 (CI: 0.15–4.21) | −1.9761** | 0.0479*** |
| Clade C ($n = 25$) | 3.6 | 3.6 | – | – | 0.01021 (CI: 2.20–18.55) | −1.4045 ns | 0.1178 ns |
| Clade D ($n = 13$) | 5.1 | 4.5 | 5.4 | – | 0.01750 (CI: 4.13–36.13) | −0.4319 ns | 0.1338 ns |
| Clade E ($n = 16$) | 8.2 | 8.5 | 9.0 | 8.6 | 0.04031 (CI: 11.11–74.77) | −0.0753 ns | 0.1568 ns |
| *nDNA* | | | | | | | |
| Clade A ($n = 54$) | | | | | 0.00136 (CI: 2.28–4.84) | −0.8590 ns | 0.0782 ns |
| Clade B ($n = 20$) | | | | | 0.00079 (CI: 0.76–2.11) | 0.1740 ns | 0.1456 ns |
| Clade C ($n = 10$) | | | | | 0.00134 (CI: 1.76–3.20) | 0.0628 ns | 0.1706 ns |
| Clade D ($n = 8$) | | | | | 0.00310 (CI: 4.11–5.93) | 0.8157 ns | 0.2158 ns |
| Clade E ($n = 10$) | | | | | 0.00252 (CI: 3.31–5.22) | 0.5234 ns | 0.1836 ns |

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

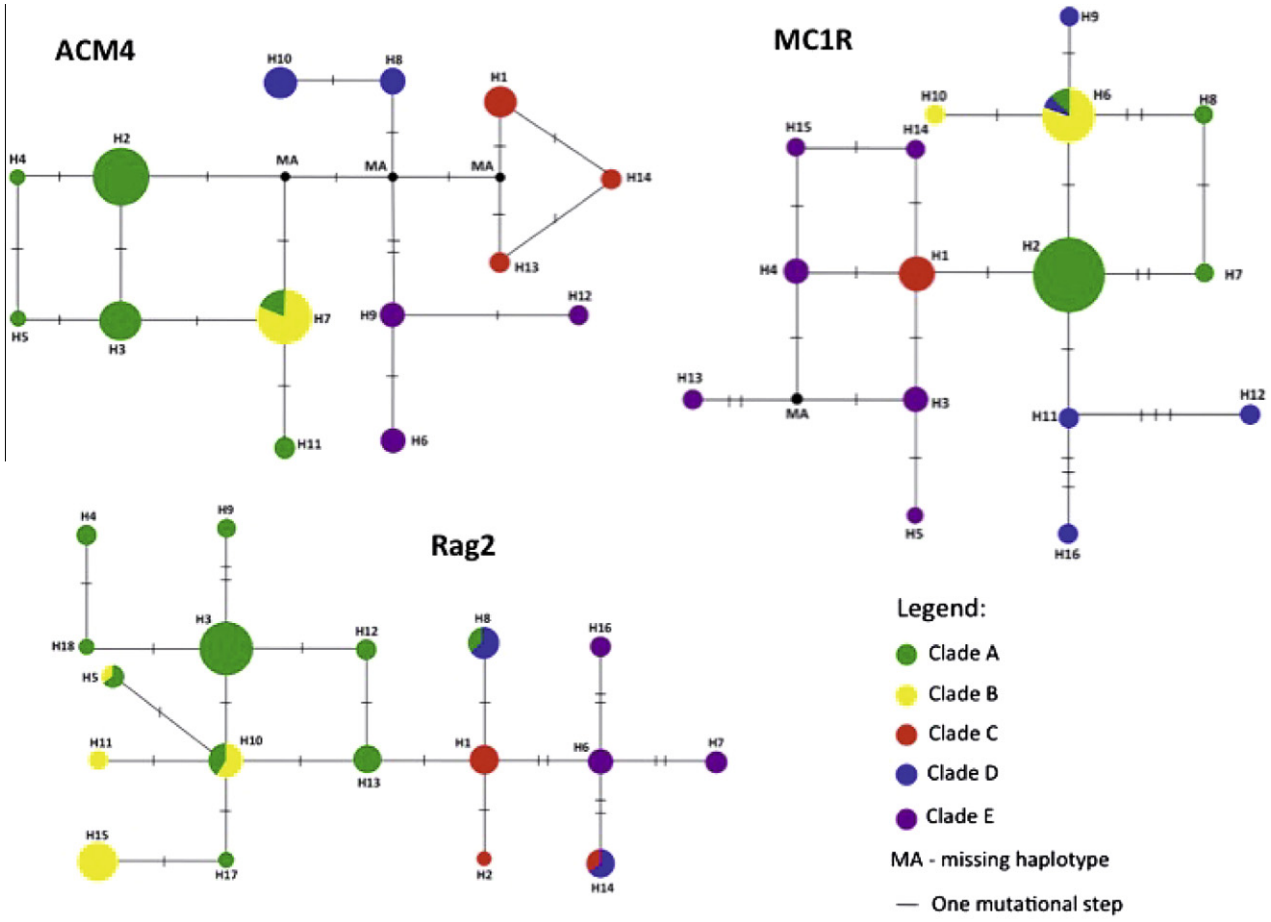


**Fig. 3.** Median-Joining networks of the protein-nuclear genes, acetylcholinergic receptor M4 (ACM4), the melanocortin 1 receptor (MC1R), and the recombination activating gene 2 (Rag2). The parameter epsilon was set to 0, and the considered clades correspond to the mtDNA lineages from Fig. 2.

haplotype was probably in Iberia, from where it spread to Tunisia and across Europe, presumably anthropogenically. Our data, however, reveal an older Moroccan lineage with a basal position (DB317 and DB328), which suggests that the common "European" haplotype possibly originated in Morocco.

A more striking aspect of our results is that when combining the nuclear genes these do not follow this lack of mtDNA variation. In contrast to the remaining clades, Clade A presents significantly higher nucleotide diversity for the nuclear markers with respect to the values for the combined mtDNA genes. This finding is unexpected and is not compatible with the hypothesis of being exclusively caused by a recent introduction or recent dispersal.

The finding of an mtDNA haplotype in such high frequencies in Europe might be explained by the occurrence of allele surfing during spatial expansion (Klopfstein et al., 2006). According to the surfing hypothesis, the haplotypes in the wave front can stochastically rise to high frequency during a population expansion and surf on the wave of advance, thus leading to a decreased genetic diversity at the limits of the species range (Klopfstein et al., 2006). However, this hypothesis does not seem to explain our results obtained for the European clade because of four reasons: (1) we do not observe an increase of the genetic differentiation between the newly formed European populations, since they all share a common haplotype. Even if we considered the hypothesis that maybe they belong to the same wave front (2) it seems unlikely that this has a geographic extension ranging from the Iberian Peninsula to Greece and Tunisia; (3) it is known that long distance colonizers, such as *T. mauritanica*, do not spread as a wave of advance but instead set up new populations distant from the parent population (Nichols and Hewitt, 1994); and (4) in the Iberian Peninsula there is more than one haplotype in the same geographic area as the common "European" haplotype (Fig. 1). Since one of the basic assumptions of allele surfing is that there is only one high frequency haplotype at the wave front, the occurrence of several others is highly contradictory.

An alternative possibility is that the nuclear marker patterns obtained could be due to a male-biased gene flow, with recurrent dispersion of males and rare dispersion of females leading to a contrasting pattern of nucleotide diversity between mtDNA and nDNA. *Tarentola* geckos are, however, usually long distance dispersers, rafting on buoyant vegetation and/or by human means, and have colonized the West Indies from West Africa and all the archipelagos of the Macaronesia, thereby suggesting that females are also very good passive dispersers (Carranza et al., 2000). However, taking into account that human mediated introductions of *T. mauritanica* do occur and are extensively reported (Vogrin et al., 2008), these seem to be purely accidental (Arnold and Ovenden, 2002). Even if human transport were intentional, it is very unlikely that it would be sex biased because habitat use by both sexes is similar.

A more plausible explanation would be that a selective sweep (Maynard-Smith and Haigh, 1974) of the mtDNA has led to the fixation of a single haplotype in much of Europe, with the exception of some populations from the Iberian Peninsula and Italian islands. It is widely accepted that mitochondrial DNA can often be under selection, thus challenging the postulation of neutrality assumed by several studies in phylogeography, speciation, and conservation genetics (Ballard and Whitlock, 2004; Bazin et al., 2006; Dowling et al., 2008). Selection can occur due to the adaption

**Table 2**
Nuclear genetic differentiation of ACM4, MC1R, and Rag2 between obtained mtDNA clades, using the *Snn* test (Hudson, 2000). The *P*-value of *Snn* is the probability obtained by the permutation test with 1000 replicates, and is abbreviated as ns (not significant).

| Clades | ACM4 | MC1R | Rag2 |
|---|---|---|---|
| A–B | 0.87*** | 0.91*** | 0.89*** |
| A–C | 1.00*** | 1.00*** | 1.00*** |
| A–D | 1.00*** | 0.95*** | 0.95*** |
| A–E | 1.00*** | 1.00*** | 1.00*** |
| B–C | 1.00*** | 1.00*** | 1.00*** |
| B–D | 1.00*** | 0.89*** | 1.00*** |
| B–E | 1.00*** | 1.00*** | 1.00*** |
| C–D | 1.00** | 1.00*** | 0.82** |
| C–E | 1.00*** | 0.95*** | 1.00*** |
| D–E | 1.00*** | 1.00*** | 1.00*** |

** $P < 0.01$.
*** $P < 0.001$.

of distinct mtDNA variants to different environmental conditions, co-evolution between mitochondrial and nuclear genes (Dowling et al., 2008), and selective sweeps of favoured mitochondrial mutations.

The hypothesis of an mtDNA selective sweep in Clade A is statistically supported by the significant results obtained with Tajima's *D* test, and $R_2$ test. According to Ramos-Onsins and Rozas (2002) significant results for the $R_2$ test could also be due to a genetic hitchhiking, since this process leaves the same footprint as a population expansion. The generation of specific patterns of sequence variation, such as a reduction in nucleotide variation (Maynard-Smith and Haigh, 1974) and a skew in the allele frequency distribution towards an increased frequency of rare and neutral mutations (Braverman et al., 1995; Fay and Wu, 2000; Kim, 2006), is expected after a recent selective sweep. This biased frequency spectrum was tested statistically using Tajima's *D* test (Tajima, 1989), and Clade A was found to follow both these two predictions, with a reduced mtDNA nucleotide diversity and a statistically significant highly negative value for Tajima's *D* (Table 1). Clade B does not show a decrease in the mtDNA diversity compared to the nuclear data, but according to Tajima's *D* statistics its mtDNA is not under a neutral selection model. These results and those obtained from the $R_2$ test strongly suggest that this lineage has undergone a population growth (Ramos-Onsins and Rozas, 2002), according to the mtDNA data. However, these results are not corroborated by the nuclear data, making further sampling in this geographic area necessary to assess this situation fully.

Other cases of possible selective sweeps have already been shown in various animal groups, such as birds (Bensch et al., 2006; Irwin et al., 2009) and insects (Shaw, 2002), and in all these studies the selective sweep was identified through the detection of a low genetic variability of the mtDNA compared to the nDNA. These findings reinforce the idea that mtDNA may not always accurately reflect nuclear genome variation patterns (Ballard and Whitlock, 2004), and that the sole use of this type of marker to infer a species' evolutionary history can be greatly misleading (e.g. Godinho et al., 2008; Renoult et al., 2009). In the particularly case of the Moorish gecko, the use of both nuclear and mitochondrial DNA markers allow us to conclude that the European populations are not the sole result of a recent colonization (human-mediated or not), but they are also being subjected to a selective sweep that is favouring a particular haplotype of mtDNA.

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## Appendix A

New and previously sampled specimens of *T. mauritanica* analysed in this study, and corresponding geographic locality, coordinates (in WGS84 coordinate system), and GenBank accession numbers. Regarding the individuals with starting code "Tm" the 12S and 16S sequences are already published, and corresponding reference is presented. Specimens marked with (+) have the common European mtDNA haplotype, and their branch appears in Fig. 2 as collapsed in Clade A.

| Code | Locality | Coordinates | Accession numbers (12S, 16S, ACM4, MC1R, Rag2) |
|---|---|---|---|
| DB177+ | Zafra, Spain | 38°24'N 6°25'W | HM014476, HM014533 |
| DB190 | 31 km from Azni, Morocco | 31°14'N 7°59'W | HM014477, HM014534, HM014590, HM014641, HM014692 |
| DB214+ | Guelta Safra, Tunisia | 36°54'N 8°43'E | HM014478, HM014535, HM014591, HM014642, HM014693 |
| DB215+ | Guelta Safra, Tunisia | 36°54'N 8°43'E | HM014479, HM014536, HM014592, HM014643, HM014694 |
| DB216 | Guelta Safra, Tunisia | 36°54'N 8°43'E | HM014480, HM014537, HM014593, HM014644, HM014695 |
| DB218+ | Guelta Safra, Tunisia | 36°54'N 8°43'E | HM014481, HM014538 |
| DB265 | Malcata, Portugal | 40°17'N 7°4'W | HM014482, HM014539 |
| DB267+ | Ceuta, Spain | 35°53'N 5°20'W | HM014483, HM014540 |
| DB270+ | Alborán islet, Spain | 35°56'N 3°2'W | HM014484, HM014541 |
| DB272+ | Adra, Almería, Spain | 36°44'N 3°1'W | HM014485, HM014542, HM014594, HM014645, HM014696 |
| DB273+ | Melilla, Spain | 35°18'N 2°57'W | HM014486, HM014543, HM014595, HM014646, HM014697 |
| DB292+ | Cantaritas, Sevilla, Spain | 37°12'N 6°21'W | HM014487, HM014544 |
| DB303 | Conigli Island, Italy | 35°30'N 12°33'E | HM014488, HM014545, HM014596, HM014647, HM014698 |
| DB304 | Conigli Island, Italy | 35°30'N 12°33'E | HM014489, HM014546, HM014597, HM014648, HM014699 |
| DB311 | Michliffen, Morocco | 33°24'N 5°61'W | HM014490, HM014547, HM014598, HM014649, HM014700 |
| DB317 | Moulay Idris, Morocco | 34°3'N 5°31'W | HM014491, HM014548 |

**Appendix A** (*continued*)

| Code | Locality | Coordinates | Accession numbers (12S, 16S, ACM4, MC1R, Rag2) |
|---|---|---|---|
| DB318 | Midelt, Morocco | 32°40'N 4°44'W | HM014492, HM014549 |
| DB320 | Essaouira, Morocco | 31°30'N 9°45'W | HM014493, HM014550 |
| DB321 | Essaouira, Morocco | 31°30'N 9°45'W | HM014494, HM014551 |
| DB323+ | Moulay Bousselham, Morocco | 34°52'N 6°17'W | HM014495, HM014552 |
| DB328 | Moulay Bousselham, Morocco | 34°52'N 6°17'W | HM014496, HM014553, HM014599, HM014650, HM014701 |
| DB329 | Tirmest, Morocco | 33°30'N 3°49'W | HM014497, HM014554, HM014600, HM014651, HM014702 |
| DB354 | Lampedusa, Italy | 35°29'N 12°38'E | HM014498, HM014555, HM014601, HM014652, HM014703 |
| DB365+ | Barrosa, Cádiz, Spain | 36°19'N 6°90'W | HM014499, HM014556 |
| DB367 | Galera, Granada, Spain | 37°44'N 2°33'W | HM014500, HM014557, HM014602, HM014653, HM014704 |
| DB368 | Galera, Granada, Spain | 37°44'N 2°33'W | HM014501, HM014558, HM014603, HM014654, HM014705 |
| DB369+ | Playa de la Barrosa, Cádiz, Spain | 36°19'N 6°90'W | HM014502, HM014559, HM014604, HM014655, HM014706 |
| DB373 | Talassetam, Morocco | 35°84'N 5°80'W | HM014503, HM014560, HM014605, HM014656, HM014707 |
| DB374 | Talassetam, Morocco | 35°94'N 5°84'W | HM014504, HM014561 |
| DB377 | Granada, Spain | 37°10'N 3°35'W | HM014505, HM014562 |
| DB379 | Benadalid, Málaga, Spain | 36°36'N 5°16'W | HM014506, HM014563 |
| DB381 | Galera, Granada, Spain | 37°44'N 2°33'W | HM014507, HM014564, HM014606, HM014657, HM014708 |
| DB394+ | Doñana National Park, Huelva, Spain | 36°59'N 6°26'W | HM014508, HM014565, HM014607, HM014658, HM014709 |
| DB395+ | Matalascañas, Huelva, Spain | 36°58'N 6°30'W | HM014509, HM014566, HM014608, HM014659, HM014710 |
| DB398 | 10km S of Touama, Morocco | 31°29'N 7°25'W | HM014510, HM014567 |
| DB401 | Torcal de Antequera, Málaga, Spain | 36°57'N 4°31'W | HM014511, HM014568, HM014609, HM014660, HM014711 |
| DB402+ | NW of Mazagón, Huelva, Spain | 37°85'N 6°51'W | HM014512, HM014569 |
| DB403+ | Alcalá la Real, Jaén, Spain | 37°27'N 3°53'W | HM014513, HM014570, HM014610, HM014661, HM014712 |
| DB404+ | Alcalá la Real, Jaén, Spain | 37°27'N 3°55'W | HM014514, HM014571, HM014611, HM014662, HM014713 |
| DB405+ | Beja, Portugal | 38°1'N 7°51'W | HM014515, HM014572, HM014612, HM014663, HM014714 |
| DB406+ | Doñana National Park, Huelva, Spain | 37°14'N 6°22'W | HM014516, HM014573 |
| DB407 | Andújar, Jaen, Spain | 38°10'N 4°2'W | HM014517, HM014574 |
| DB408+ | Moguer lagoons, Spain | 37°91'N 6°44'W | HM014518, HM014575, HM014613, HM014664, HM014715 |
| DB409+ | Ballena beach, Spain | 36°41'N 6°24'W | HM014519, HM014576 |
| DB410+ | Marbella, Málaga, Spain | 36°29'N 4°46'W | HM014520, HM014577, HM014614, HM014665, HM014716 |
| DB411 | Ocaña, Cuenca, Spain | 39°57'N 3°30'W | HM014521, HM014578, HM014615, HM014666, HM014717 |
| DB412 | Cabañeros National Park, Ciudad Real, Spain | 39°26'N 4°34'W | HM014522, HM014579, HM014616,HM014667, HM014718 |
| DB413 | Valencia del Ventoso, Badajoz, Spain | 38°13'N 6°28'W | HM014523, HM014580, HM014617, HM014668, HM014719 |
| DB414 | Cornalvo Natural Park, Badajoz, Spain | 39°1'N 6°10'W | HM014524, HM014581, HM014618, HM014669, HM014720 |
| DB415 | Cabañeros National Park, Ciudad Real, Spain | 39°34'N 4°34'W | HM014525, HM014582, HM014619, HM014670, HM014721 |
| DB416 | 7kms N from Castelo de Vide, Portugal | 39°25'N 7°34'W | HM014526, HM014583, HM014620, HM014671, HM014722 |
| DB417 | Ruidera lagoons, Albacete, Spain | 39°3'N 3°0'W | HM014527, HM014584, HM014621, HM014672, HM014723 |
| DB420+ | La Roda, Spain | 39°12'N 2°99'W | HM014528, HM014585 |
| DB423+ | Thibar, Tunisia | 36°30'N 9°4'E | HM014529, HM014586 |
| DB424+ | Beja National Park, Tunisia | 36°42'N 9°10'E | HM014530, HM014587, HM014622, HM014673, HM014724 |
| DB425+ | Tabarka, Tunisia | 36°57'N 8°45'E | HM014531, HM014588 |
| DB426+ | Chemtou, Tunisia | 36°29'N 8°34'E | HM014532, HM014589, HM014623, HM014674, HM014725 |
| Tm11 | Road to Tizi n'Test, Morocco | 30°49'N 8°19'W | Harris et al. (2004b), HM014624, HM014675, HM014726 |
| Tm127+ | Adra, Almería, Spain | 36°44'N 3°1'W | Perera and Harris (2008), HM014625, HM014676, HM014727 |
| Tm13+ | Kebir, Tunisia | 36°52'N 8°43'E | Harris et al. (2004b), HM014626, HM014677, HM014728 |
| Tm21 | Libya/Egypt border, Libya | 31°34'N 25°3'E | Harris et al. (2004b), HM014627, HM014678, HM014729 |
| Tm26 | Tobruk, Libya | 32°5'N 23°55'E | Harris et al. (2004b), HM014628, HM014679, HM014730 |
| Tm27+ | Zafra, Spain | 38°24'N 6°25'W | Harris et al. (2004a), HM014629, HM014680, HM014731 |
| Tm29+ | Zafra, Spain | 38°24'N 6°25'W | Harris et al. (2004a), HM014630, HM014681, HM014732 |
| Tm304+ | Chiclana de la Frontera, Cádiz, Spain | 36°37'N 6°19'W | Perera & Harris (2008), Perera & Harris (2008), HM014631, HM014682, HM014733 |
| Tm30+ | Huelva, Spain | 37°51'N 6°28'W | Harris et al. (2004a), HM014632, HM014683, HM014734 |
| Tm3+ | Barcelona, Spain | 41°22'N 2°8'E | Harris et al. (2004b), HM014633, HM014684, HM014735 |
| Tm43 | Azar, Morocco | 30°50'N 8°57'W | Harris et al. (2004a), HM014634, HM014685, HM014736 |
| Tm49 | Agadir-Tizni, Morocco | 29°42'N 9°43'W | Harris et al. (2004a), HM014635, HM014686, HM014737 |

(*continued on next page*)

**Appendix A** (*continued*)

| Code | Locality | Coordinates | Accession numbers (12S, 16S, ACM4, MC1R, Rag2) |
|---|---|---|---|
| Tm4+ | Playa de la Barrosa, Cádiz, Spain | 36°20'N 6°9'W | Harris et al. (2004b), HM014636, HM014687, HM014738 |
| Tm50 | Massa, Morocco | 29°56'N 9°37'W | Harris et al. (2004a), HM014637, HM014688, HM014739 |
| Tm56 | Taza, Morocco | 34°14'N 4°0'W | Harris et al. (2004a), HM014638, HM014689, HM014740 |
| Tm61+ | Lithica, Menorca, Spain | 40°0'N 3°52'E | Harris et al. (2004b), HM014639, HM014690, HM014741 |
| Tm6+ | Monte Clérigo, Portugal | 37°18'N 8°49'W | Harris et al. (2004b), HM014640, HM014691, HM014742 |

uellos for the tissue samples from Alborán islet, Melilla and Adra, Spain.

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