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When selection deceives phylogeographic interpretation: The case of the Mediterranean house gecko, *Hemidactylus turcicus* (Linnaeus, 1758)

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ABSTRACT

A previous study on *Hemidactylus turcicus* based on mtDNA makers indicated that this gecko has a Middle-East origin, and that the current phylogeographic pattern is the result of a very rapid spread from the east to the west of the species' range. The same study identified two distinct mitochondrial lineages with low differentiation and genetic diversity. Since *H. turcicus* is known to be closely associated to humanized environments, its present distribution range and phylogeography is frequently interpreted to be the result of recurrent human-mediated introductions. These conclusions used to be the same as those used to interpret the results obtained for the European populations of another gecko, *Tarentola mauritanica*. However, a recent study has revealed that the phylogeographic pattern of *T. mauritanica* is not solely the result of a recent colonization, but also of a mitochondrial selective sweep. Could the same be occurring in *H. turcicus*? To answer this question, two mitochondrial (12S rRNA and cytochrome *b*) and two nuclear genes (ACM4 and Rag2) were used in this study. From the mtDNA data we confirmed the existence of two distinct phylogeographic lineages; one occurring exclusively in the northern Mediterranean (Clade A), and another one more widespread that is the only lineage present in North Africa (Clade B). In light of these results, we could hypothesize that *H. turcicus* had its origin in Turkey, and from there Clade A moved to Europe and Clade B to North Africa spreading latter into Europe. However, Clade A presents significantly higher nucleotide diversity for the nuclear DNA compared to the mtDNA, and neutrality tests gave significant results for the mitochondrial data. These results suggest that the lack of mtDNA genetic diversity and structure in the European population of *H. turcicus* could also be due to a selective sweep, and not only because of a recent colonization. Together with the situation reported in *T. mauritanica*, the identification of a hitch-hiking process occurring in *H. turcicus*, represents two unprecedented cases of a selective sweep taking place in the same geographic area shaping the phylogeographic patterns of two unrelated genera of geckos.

1. Introduction

Hemidactylus geckos are one of the most species-rich genera of the Gekkonidae comprising over 85 species (Carranza and Arnold, 2006). Their distribution range includes tropical Asia and Africa, arid areas of northeast Africa and southwest Asia, and they also occur across the Mediterranean region. These geckos are very frequently associated to humanized habitats, living around or inside houses. Probably because of this, considerable evidence exists of possible human-mediated introductions of this genus, based on both direct observations and genetic markers (Carranza and Arnold, 2006; Jesus et al., 2005; Rocha et al., 2005; Vences et al., 2004).

Hemidactylus turcicus has a mainly circum-Mediterranean distribution including many islands and with populations extending to the south along the Nile River up to the border with Sudan (Mateus and Jacinto, 2008; Sindaco and Jeremcenko, 2008). They have also been introduced recently in the Canary islands (Geniez, 2002), Mexico, Cuba, Florida (Smith, 1946), and in other areas of the United States (Pianka and Vitt, 2003; White and Tumlison, 1999). Due to this rapid range expansion outside the Mediterranean region, Rödder and Lötters (2009) decided to assess the differences in climatic niches between the native and invaded ranges of *H. turcicus*, and to analyse which environmental variables are more conserved versus subject to niche shifts. Their results indicate that the degree of conservatism of niches in *H. turcicus* clearly depends on the predictors and variables considered *a priori*, and that these should always be tested with ecological niche models (ENMs). According to a recent phylogenetic study from Carranza and Arnold (2006), *H. turcicus* may have originated in the Middle East from

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where it moved westwards across the whole Mediterranean, reaching later the Atlantic Ocean. In this same study, the authors reported two distinct mtDNA lineages of *H. turcicus* with little genetic divergence between them, suggesting that the phylogeographic pattern obtained was the result of a very rapid and recent spread.

Tarentola mauritanica is another gecko whose European populations present a similar mtDNA genealogy to *H. turcicus*, with low genetic diversity (Harris et al., 2004a,b). However, recent studies (Rato et al., 2010) have demonstrated that this mtDNA pattern is not solely the result of a recent and rapid spread (possibly human-mediated), but also due to a genetic hitch-hiking process (Maynard-Smith and Haigh, 1974). This process occurs when neutral alleles increase their frequency due to gametic disequilibrium with mutations that are favoured by natural selection. This will lead to a selective sweep, which results in a decrease of polymorphism at the affected markers. Since mitochondrial genomes do not experience recombination, genetic hitch-hiking has the potential to cause strong selective sweeps (Hamilton, 2009).

In the present study, we intend to investigate if a similar pattern is happening in *H. turcicus*. Therefore, we have increased the geographic sampling of *H. turcicus* covering approximately its entire native range, and sequenced two nuclear DNA markers and two mitochondrial regions in order to evaluate if the low genetic diversity in mtDNA lineages is reflected by limited variation in the nuclear markers. Tests were then applied to identify if a selective sweep may also have occurred in this species.

2. Material and methods

2.1. Samples, DNA extraction and amplification

The study was carried out using a total of 100 specimens of *H. turcicus* from 78 distinct localities and one *Hemidactylus lemurinus* to root the tree (see details in Table 1 and Fig. 1). Sixty-seven of these specimens correspond to new samples, and the remaining ones are from Carranza and Arnold (2006). From this previous study, we did not include the specimens of *H.t. turcicus* and *H.t. lavadeserticus* from Jordan, since their taxonomy is not clear (S. Carranza, pers. observ.).

Total genomic DNA was extracted from tail tissue, using the DNeasy Extraction Kit from Qiagen, following the manufacturer's protocol. Fragments of two mitochondrial (12S rRNA and cytochrome *b*) and two nuclear exons (ACM4, and Rag2) were amplified by PCR for 67 and 61 individuals, respectively. For amplification and sequencing of the 12S and cytochrome *b* (cytb) partial sequences we used the primers 12Sa/12Sb, and cytochrome b1/cytochrome b2, respectively, both from Kocher et al. (1989). PCR conditions were the same as those described in Harris et al. (1998). The ACM4 nuclear gene fragment was amplified and sequenced using the primers tg-F and tg-R published by Gamble et al. (2008b), and PCR reactions were carried out as in Rato et al. (2010). 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). Amplified fragments were sequenced on an ABI3730XL automated capillary DNA sequencer. Sequences obtained for this study have been deposited in GenBank.

2.2. Sequence alignment and phylogenetic analyses

Sequences of 12SrRNA and cytb from *H. turcicus* previously published elsewhere (Carranza and Arnold, 2006) were added to our dataset, and *H. lemurinus* was designated as the outgroup, since it has been shown to be the sister taxa of *H. turcicus* (Arnold and Carranza, unpublished data). Alignment of the obtained sequences was performed using MAFFT v.6.811 (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.

The appropriate model of nucleotide substitution for each mtDNA gene, and concatenated data was determined using jModeltest v.0.1.1 (Posada, 2008), under the Akaike Information Criterion (Akaike, 1974). Maximum likelihood (ML) and Bayesian phylogenetic analyses were conducted on the combined mtDNA dataset, considering the model of sequence evolution estimated earlier. ML analysis was performed using GARLI v.1.0 (Zwickl, 2006). Tree search was conducted using 5000–10,000 generations (*genthreshfortopoterm*) considering a stochastic algorithm, each resulting in a single best tree. Since no significant differences in the topology were observed when the number of generations was increased, bootstrap support was calculated from 1000 bootstrap replicates using 6000 *genthreshfortopoterm*. A 50% majority-rule consensus tree was generated using the software PAUP* v.4.0d10 (Swofford, 2002). Partitioned Bayesian phylogenetic analyses were conducted using MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001). Both runs began with a random starting tree and ran for 2×10^6 generations, saving one tree in each 100 generations. Substitution-model parameters were always unlinked across partitions, and each subset was allowed to have its own rate (*prset* = variable). 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 sampled trees were discarded (burnin = 5000). Tracer v1.4 (Rambaut and Drummond, 2007) was used to confirm that all parameters had an ESS > 100 after burnin. The remaining trees were combined and a 50% majority consensus tree was generated.

2.3. Population genetic analyses

In order to investigate the haplotype diversity and structure within each marker, we built two haplotype networks; one for the combined mtDNA and another one for each nDNA gene. A median-joining haplotype network was constructed 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. Prior to this analysis, for each nuclear gene an input file with identification of all heterozygous positions was created using SeqPHASE (Flot, 2010). This generated file was then used to run PHASE v.2.1.1 (Stephens and Donnelly, 2003; Stephens et al., 2001) in order to reconstruct the phase of each haplotype, using the default parameters (thresholds are $p = q = 90\%$). Definition of the number of populations of *H. turcicus* was carried out with the mtDNA dataset using BAPS v.5 (Bayesian Analysis of Population Structure) (Corander et al., 2008). Genetic mixture analysis was performed at the individual level considering a spatial model. The *Snn* test (Hudson, 2000) was applied to each nuclear gene fragment to test for genetic differentiation between the obtained mtDNA clades. In order to calculate the *p*-value of the *Snn* we performed a permutation test with 1000 replicates implemented in the software DnaSP v.5 (Librado and Rozas, 2009). Within each of the defined populations we calculated Tajima's *D* (Tajima, 1989) for both mitochondrial and nuclear DNA, since it is expected that under a hitch-hiking 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). In order to compare the number of mutations in internal and external branches of a genealogy with the expectation of selective neutrality, we applied

Table 1
Codes, nDNA haplotypes, geographic locality, and GenBank Accession numbers of all specimens used in this study. All specimens are *Hemidactylus turcicus*, except for AO116, which is a *Hemidactylus lemurinus*. The numbers in superscript after the specimen codes correspond to the mtDNA haplotypes illustrated in Fig. 3. All GenBank Accession numbers starting with "DQ" correspond to sequences already published by Carranza and Arnold (2006).

Code	nDNA haplotypes		Locality	GenBank Accession nos.			
	ACM4	Rag2		Cytb	12S	ACM4	Rag2
AO116			Ayun Pools, Oman	HQ675988	HQ675860	–	–
E612.21[1]	1	1	Zaranick, north of Sinai, Egypt	DQ120127	DQ120298	HQ675927	HQ676055
E1008.13[2]	1,2	1	Andalucía, Spain	DQ120162	DQ120333	HQ675928	HQ676056
DB1570[3]	1	2,3	Houmt-Souk, Jerba island, Tunisia	HQ675989	HQ675861	HQ675929	HQ676057
DB1671[2]	2	3,4	Molyvos, Lesvos, Greece	HQ675990	HQ675862	HQ675930	HQ676058
DB1673[4]	1	5,6	Ancient Epidavros, Peloponnesus, Greece	HQ675991	HQ675863	HQ675931	HQ676059
DB1703[2]	1,2	5	One kilometer West of Cantoniera Mutrucone, Sardinia, Italy	HQ675992	HQ675864	HQ675932	HQ676060
DB3140[2]	1	5	Lampedusa island, Italy	HQ675993	HQ675865	HQ675933	HQ676061
DB3150[2]	1,2	7	Istanbul, Turkey	HQ675994	HQ675866	HQ675934	HQ676062
DB3154[2]	1	2,5	Bergama, Turkey	HQ675995	HQ675867	HQ675935	HQ676063
DB3155[2]	1	5	Kursunlu, Turkey	HQ675996	HQ675868	HQ675936	HQ676064
DB3156[2]	1	3,5	Istanbul, Turkey	HQ675997	HQ675869	HQ675937	HQ676065
DB3159[5]	1	5,8	Lampedusa island, Italy	HQ675998	HQ675870	HQ675938	HQ676066
DB3183[2]	1	1,9	Sanguedolce, Lampedusa island, Italy	HQ675999	HQ675871	HQ675939	HQ676067
DB3184[6]	1,2	5	Cala Galera, Lampedusa island, Italy	HQ676000	HQ675872	HQ675940	HQ676068
DB3189[7]	1,3	5	Lampedusa island, Italy	HQ676001	HQ675873	HQ675941	HQ676069
DB3197[2]	1	5	Lampedusa island, Italy	HQ676002	HQ675874	HQ675942	HQ676070
DB3205[2]	1	5	Lampedusa island, Italy	HQ676003	HQ675875	HQ675943	HQ676071
DB3212[2]	1	5	Corsica, France	HQ676004	HQ675876	HQ675944	HQ676072
DB3217[8]			Split, Croatia	HQ676005	HQ675877	–	–
DB3218[9]			Dubrovnik, Croatia	HQ676006	HQ675878	–	–
DB3219[9]			Dubrovnik, Croatia	HQ676007	HQ675879	–	–
DB3221[8]	1	2,3	Split, Croatia	HQ676008	HQ675880	HQ675945	HQ676073
DB3222[9]			Dubrovnik, Croatia	HQ676009	HQ675881	–	–
DB3225[8]			Split, Croatia	HQ676010	HQ675882	–	–
DB3226[9]			Hvar, Croatia	HQ676011	HQ675883	–	–
DB3228[2]			Zut, Croatia	HQ676012	HQ675884	–	–
DB3229[2]			Zut, Croatia	HQ676013	HQ675885	–	–
DB3230[9]	2	7	Hvar, Croatia	HQ676014	HQ675886	HQ675946	HQ676074
DB330[4]			Sanlúcar la Mayor, Sevilla, Spain	HQ676015	HQ675887	–	–
DB3824[3]			Laguna de La Mata National Park, Alicante, Spain	HQ676016	HQ675888	–	–
DB50[2]			La Muela, Andalucía, Spain	HQ676017	HQ675889	–	–
DB6[10]	1	1,5	Between Le Kef and Tourief, Tunisia	HQ676018	HQ675890	HQ675950	HQ676078
DB697[11]	1,2	2,3	Terra murata, Procida Island, Italy	HQ676019	HQ675891	HQ675947	HQ676075
DB698[11]	1,2	1,7	Terra murata, Procida Island, Italy	HQ676020	HQ675892	HQ675948	HQ676076
DB699[11]	1	1,7	Terra murata, Procida Island, Italy	HQ676021	HQ675893	HQ675949	HQ676077
DB700[12]	2	1,5	Terra murata, Procida Island, Italy	HQ676022	HQ675894	HQ675951	HQ676079
DB704[13]	4	1,10	Barano, Ischia Island, Italy	HQ676023	HQ675895	HQ675952	HQ676080
DB705[13]	1,2	1,5	Barano, Ischia Island, Italy	HQ676024	HQ675896	HQ675953	HQ676081
DB9[10]	1,2	1,5	Between Le Kef and Tourief, Tunisia	HQ676025	HQ675897	HQ675954	HQ676082
E612.28[4]			Las Palmas, Gran Canaria, Spain	DQ120128	DQ120299	–	–
IBE-1664[10]			Sardinia, Italy	HQ676026	HQ675898	–	–
IBE-S1966[2]	1	5	Monte Tassua, Sardinia, Italy	HQ676027	HQ675899	HQ675955	HQ676083
IBE-S2059[10]	1	5	Monte Tassua, Sardinia, Italy	HQ676028	HQ675900	HQ675956	HQ676084
IBE-S2179[2]	1,2	7,10	Sardinia, Italy	HQ676029	HQ675901	HQ675957	HQ676085
IBE-S2183[14]	1	5	Monte Tassua, Sardinia, Italy	HQ676030	HQ675902	HQ675958	HQ676086
IBE-S2224[2]			Punta sa Calanza Mine, Sardinia, Italy	HQ676031	HQ675903	–	–
IBE-S2225[2]			Punta sa Calanza Mine, Sardinia, Italy	HQ676032	HQ675904	–	–
IBE-S2228[2]	2	5,8	Punta sa Calanza Mine, Sardinia, Italy	HQ676033	HQ675905	HQ675959	HQ676087
E1008.8[2]			Heraklion, Crete, Greece	DQ120160	DQ120331	–	–
E1008.6[2]	2	5	Kos island, Greece	DQ120158	DQ120329	HQ675960	HQ676088
Lyra1[15]	1	10	Leros islet, near Patmos, Greece	HQ676034	HQ675906	HQ675961	HQ676089
E1008.9[2]	1,2	7	Marathi islet, near Patmos, Greece	DQ120161	DQ120332	HQ675962	HQ676090
E2505.16[10]	1	1	Qariat Arkmane, Morocco	DQ120144	DQ120315	HQ675963	HQ676091
E2505.15[10]	1	1	Qariat Arkmane, Morocco	DQ120143	DQ120314	HQ675964	HQ676092
E2505.14[10]	1	1	Qariat Arkmane, Morocco	DQ120141	DQ120312	HQ675965	HQ676093
Sa16[10]	2	5	Road to Sirri, Sardinia, Italy	HQ676035	HQ675907	HQ675966	HQ676094
Sa8[2]	2	5	San Pietro island, Sardinia, Italy	HQ676036	HQ675908	HQ675967	HQ676095
E1008.12[4]			El Garrobo, Sevilla, Spain	DQ120131	DQ120302	–	–
E2505.12[16]			South of Jeundouba, Tunisia	DQ120145	DQ120316	–	–
E2505.11[4]			Zaframagón, Cádiz, Spain	DQ120134	DQ120305	–	–
E2505.9[4]			Junqueira, Portugal	DQ120135	DQ120306	–	–
E2505.8[4]			Valdeinfierno, Córdoba, Spain	DQ120136	DQ120307	–	–
E2505.6[3]			Obejo, Córdoba, Spain	DQ120150	DQ120321	–	–
E2505.5[4]			Carmona, Sevilla, Spain	DQ120137	DQ120308	–	–
E2505.4[3]			El Alquian, Almería, Spain	DQ120149	DQ120320	–	–
E2505.2[9]	1,2	5	Erzin, Turkey	DQ120163	DQ120334	HQ675968	HQ676096
E2505.1[4]	1,2	1,5	Erzin, Turkey	DQ120138	DQ120309	HQ675969	HQ676097
SPM001629[17]			Torregorda, Cádiz, Spain	HQ676037	HQ675909	–	–
E1008.15[3]			Mahon, Menorca, Spain	DQ120156	DQ120327	–	–
E612.23[2]			Crete, Greece	DQ120159	DQ120330	–	–

(*continued on next page*)

Table 1 (*continued*)

Code	nDNA haplotypes		Locality	GenBank Accession nos.			
	ACM4	Rag2		Cytb	12S	ACM4	Rag2
E612.29[3]			Cuevas de Almanzora, Almería, Spain	DQ120148	DQ120319	–	–
E612.27[18]			Sant Andreu, Barcelona, Spain	DQ120142	DQ120313	–	–
E612.25[3]			Castillejos, Morocco	DQ120146	DQ120317	–	–
E612.26[3]	1	5,9	Castillejos, Morocco	DQ120147	DQ120318	HQ675970	HQ676098
SPM002387[19]			Zaranick, north of Sinai, Egypt	HQ676038	HQ675910	–	–
SPM002391[2]	2	3	Bodrum, Turkey	HQ676039	HQ675911	HQ675971	HQ676099
SPM002393[2]	1	1,3	Bodrum, Turkey	HQ676040	HQ675912	HQ675972	HQ676100
SPM002609[3]			Morocco	HQ676041	HQ675913	–	–
SPM002953[19]	1	1	Zaranick, north of Sinai, Egypt	HQ676042	HQ675914	HQ675973	HQ676101
SPM003052[3]	1	1	Granada, Spain	HQ676043	HQ675915	HQ675974	HQ676102
SPM003063[3]	1,2	1,5	Granada, Spain	HQ676044	HQ675916	HQ675975	HQ676103
SPM003117[20]	1	5	Vrysoules, Cyprus	HQ676045	HQ675917	HQ675976	HQ676104
SPM003134[21]	1,2	5,9	Vrysoules, Cyprus	HQ676046	HQ675918	HQ675977	HQ676105
SPM003733[4]	1	7	Port Cross island, France	HQ676047	HQ675919	HQ675978	HQ676106
SPM003741[2]	1	1,7	Levant island, France	HQ676048	HQ675920	HQ675979	HQ676107
SPM003742[2]			Levant island, France	HQ676049	HQ675921	–	–
SPM003744[2]	1	5	Monte Argentario, Italy	HQ676050	HQ675922	HQ675980	HQ676108
SPM003745[2]			Monte Argentario, Italy	HQ676051	HQ675923	–	–
SPM004429[4]	1	10	Platomona, Sardinia, Italy	HQ676052	HQ675924	HQ675981	HQ676109
SPM004999[2]	1	1,5	Lampedusa island, Italy	HQ676053	HQ675925	HQ675982	HQ676110
E2505.22[3]	1	5	Chergui island, Tunisia	DQ120155	DQ120326	HQ675983	HQ676111
E2505.21[3]			Chergui island, Tunisia	DQ120154	DQ120325	–	–
E2505.20[3]	1,2	5	Chergui island, Tunisia	DQ120153	DQ120324	HQ675984	HQ676112
E2505.19[3]	1,2	2,3	Chergui island, Tunisia	DQ120152	DQ120323	HQ675985	HQ676113
E2505.18[22]			Near Gafsa, Tunisia	DQ120157	DQ120328	–	–
E2505.17[3]	5	1	Five kilometer South of Le Kef, Tunisia	DQ120151	DQ120322	HQ675986	HQ676114
Volos1[4]			Kato Gatzea village, near city of Volos, Greece	HQ676054	HQ675926	–	–
E1008.3[23]	1,2	7,10	Kato Gatzea village, near city of Volos, Greece	DQ120139	DQ120310	HQ675987	HQ676115
E1008.4[4]			Kato Gatzea village, near city of Volos, Greece	DQ120130	DQ120301	–	–
E1008.2[4]			Kato Gatzea village, near city of Volos, Greece	DQ120129	DQ120300	–	–

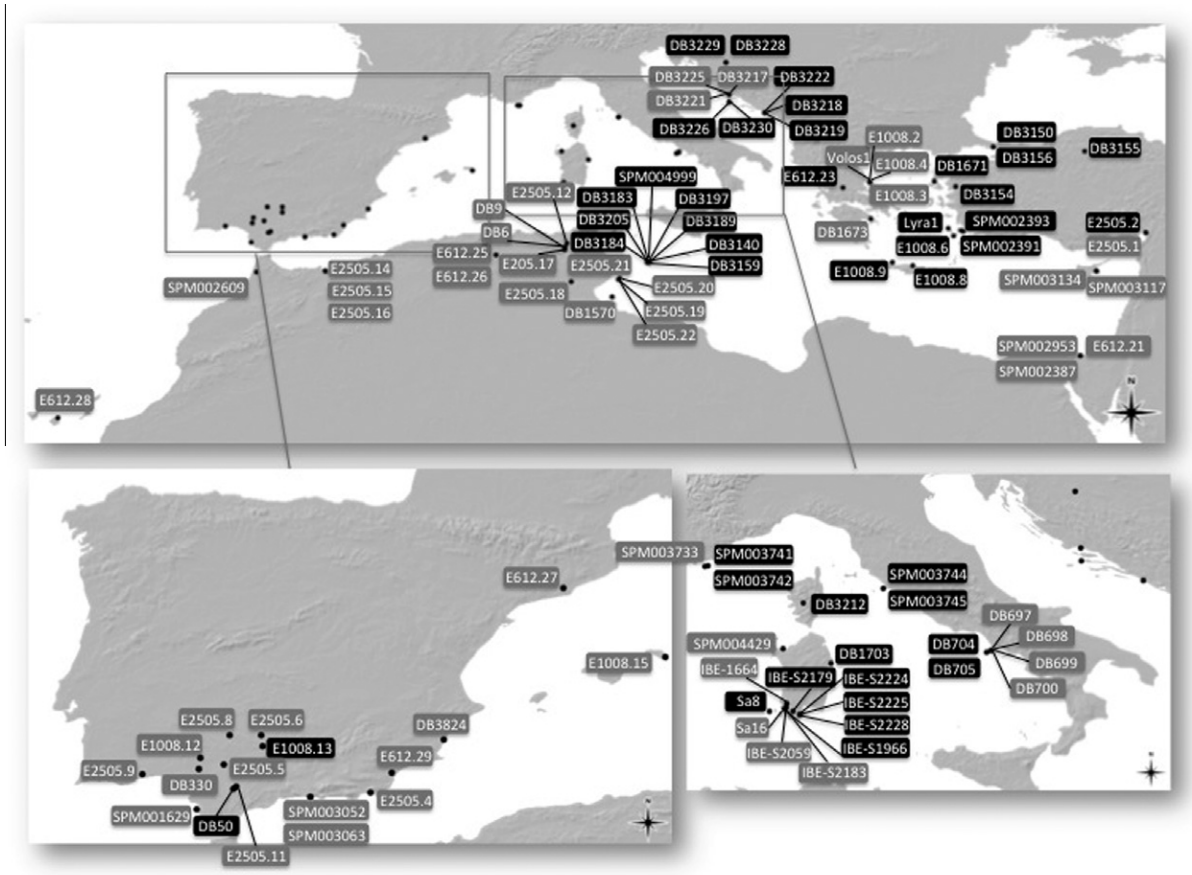

Fig. 1. Map showing all sampling localities of *Hemidactylus turcicus* used in this study, and a representation of the obtained clades in the mtDNA analyses. Black boxes correspond to individuals from Clade A and gray boxes to Clade B. Details of each individual's locality are shown in Table 1.

the Fu and Li's *D* test (Fu and Li, 1993) to each nuclear and mtDNA lineage, using the software DnaSP v.5 (Librado and Rozas, 2009). The significance of both Tajima's *D*, and Fu and Li's *D* values was calculated using 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 population 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 (π) (with 95% confidence intervals) and uncorrected *p*-distances were calculated for each population 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 cluster.

3. Results

Regarding the mtDNA, a total of 679 bp (377 bp of 12SrRNA and 302 bp of cytb) were obtained. The nuclear DNA dataset included 1174 bp corresponding to 423 bp for the ACM4 and 751 bp for the Rag2. Eighteen heterozygous individuals were observed in the ACM4 gene and 27 in the Rag2. Information about the number of variable and parsimony informative sites of both nuclear and mtDNA loci is explained in Table 2.

The models of sequence evolution selected by jModeltest were the GTR + I + G for the 12S rRNA, the SYM + G for the cytb and the TIM2 + G for the combined mitochondrial loci. The results of the phylogenetic analyses inferred using both ML and Bayesian analysis were identical and are presented in Fig. 2. The phylogenetic tree is comprised by two sister clades (A and B) separated by an uncorrected genetic divergence of 1.6%, as also obtained by Carranza and Arnold (2006). Despite the considerable sampling, and as noted by Carranza and Arnold (2006), there is minimal mtDNA genetic diversity within *H. turcicus*, with 23 haplotypes from 100 individuals. Specimens from Clade A occur only in Europe from Turkey to the Iberian Peninsula, including some of the Greek islands, Lampedusa, Corsica and Sardinia. Clade B occurs throughout the Mediterranean Basin, and is the only lineage present in North Africa. Both lineages present a low level of substructure, although clearly higher within Clade B and with some geographic coherency such as haplotype groups found in Egypt, Procida Island (Italy) and Split (Croacia).

The existence of these two main genetic lineages is also evident from the mtDNA haplotype network (Fig. 3). Ten mutational steps separate the two clades with a clear substructure and haplotype differentiation within Clade B, but not in Clade A.

Considering the results of the individual spatial mixture analysis from BAPS v.5 (Corander et al., 2008) the number of groups in the optimal partition is two (marginal likelihood = −475.1825), corresponding to the obtained mtDNA lineages. Therefore, both lineages were considered as distinct populations, and neutrality tests applied independently to each one of them. Clade A presents lower mtDNA nucleotide diversity compared to Clade B, and both clades have significant values for the Fu and Li's *D*, R_2 and Tajima's *D* tests (Table 3) with some exceptions when each gene fragment is analyzed independently; regarding Clade A all tests were not significant for cytb, and the R_2 test for 12S; in Clade B, no significant results were obtained for cytb concerning the R_2 or Tajima's *D* test. When we compare the results between nuclear and mtDNA, Clade A shows significantly higher nucleotide diversity for the nuclear markers compared to the mtDNA, with no overlap between both 95% confidence intervals (see Table 3). Moreover, both Clades A and B show no significant results for any of the neutrality tests carried out on the nDNA.

The haplotype networks for each of the nuclear loci (Fig. 4) do not show any phylogeographic pattern, nor do they support the existence of two distinct lineages of *H. turcicus*. Likewise, the *Snn* test does not support the two obtained mtDNA lineages for the nuclear data (*Snn* = 0.52, $p = 0.057$ for ACM4; *Snn* = 0.53, $p = 0.079$ for Rag2).

Table 2
Information about total sequence length, number of variable and parsimony informative sites for all loci used in this study.

Locus	Length (bp)	Number of variable sites	Number of parsimony informative sites
12S rRNA	377	31	5
cytb	302	56	16
ACM4	423	5	4
Rag2	751	7	7

4. Discussion

Although geckos are characterized by a conservative morphology, a number of studies have suggested that this group of reptiles usually presents high levels of intraspecific genetic divergence for mtDNA (Bauer et al., 2010; Carranza and Arnold, 2006; Harris et al., 2004a,b; Harris et al., 2009; Kasapidis et al., 2005; Perera and Harris, 2008, 2010), and *Hemidactylus* are not an exception (Arnold et al., 2008; Bansal and Karanth, 2010; Bauer et al., 2010; Jesus et al., 2005; Rocha et al., 2005, 2010; Vences et al., 2004). However, in the present study the divergence between the two lineages of *H. turcicus* is low, as well as the genetic substructure within each clade, especially in Clade A. Carranza and Arnold (2006) already suggested that this pattern in *H. turcicus* was likely to be the result of a recent and rapid spread across the Mediterranean from east to west. The nuclear genealogies obtained, and *Snn* test do not support the existence of two distinct lineages, suggesting an incomplete lineage sorting for these nuclear markers. This lack of genetic structure in the nuclear DNA within differentiated mitochondrial lineages is not uncommon. It has already been reported in various groups including amphibians (Velo-Antón et al., 2008), and several reptiles (Pinho et al., 2007, 2008; Rato et al., 2009) including geckos (Harris et al., 2004a; Jesus et al., 2005; Rato et al., 2010).

In light of the results obtained and taking into account that *H. turcicus* originated in the eastern Mediterranean Basin (Carranza and Arnold, 2006), it is plausible to assume the hypothesis that the two mtDNA lineages diverged in Turkey, where both still occur. From there, lineage A could have spread to Europe, and Clade B colonized North Africa, and latter Europe.

Hemidactylus geckos are frequently commensal with people, occurring very often in and around human houses, and in anthropogenically modified habitats. Therefore, human-mediated introductions are thought to be common (e.g. Carranza and Arnold, 2006; Jesus et al., 2005; Rocha et al., 2005; Vences et al., 2004). However, the phylogeographic conclusions of these studies were based primarily on mtDNA. After the finding of a selective sweep in *T. mauritanica* (Rato et al., 2010), these hypothesis, when based on minimal mtDNA genetic variation rather than direct observations, need to be re-examined. In *H. turcicus*, when the values of nucleotide diversity of each clade between mitochondrial and nuclear DNA are compared, it is notable that Clade A, which is characterized by a lack of mitochondrial diversity and substructure, presents significantly higher values of nucleotide diversity in the nDNA, with no overlap in the 95% confidence interval. These results could initially be interpreted as a result of a sex-biased gene flow,

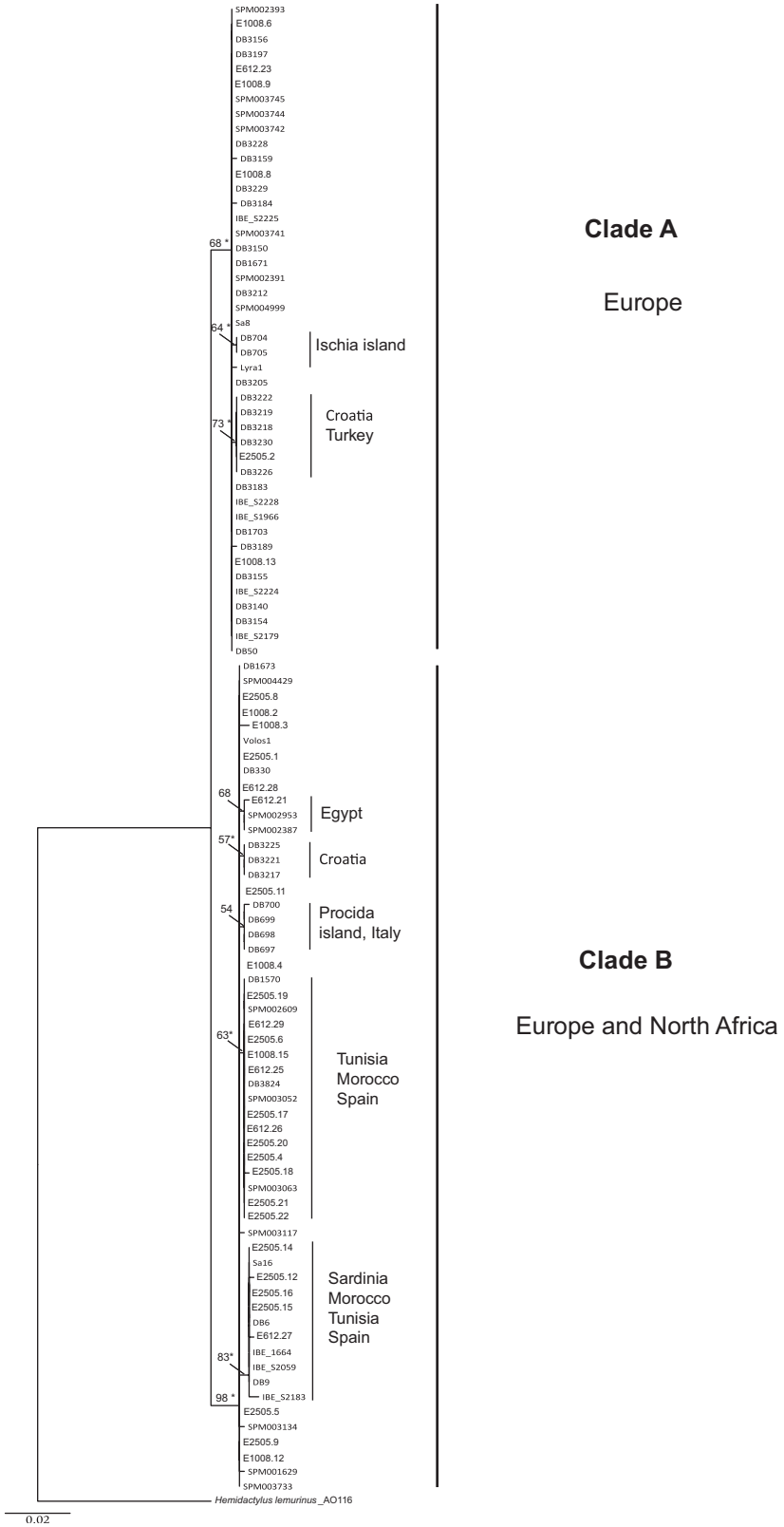

Fig. 2. ML phylogenetic tree for the 12SrRNA and cytb combined data with *Hemidactylus lemurinus* designated as outgroup. Numbers above the branches correspond to ML bootstrap values, and (*) to the Bayesian posterior probabilities above 95%.

with more dispersal of males than females. *Hemidactylus* geckos are characterized by occupying new niches by long-distance dispersal, frequently human-mediated (Carranza and Arnold, 2006; Locey and Stone, 2006), which is associated to a random colonization of both males and females. Therefore, it seems unlikely that a sex-biased gene flow could explain the pattern of nucleotide diversity observed in *H. turcicus*, since both males and females have the same opportunities to disperse.

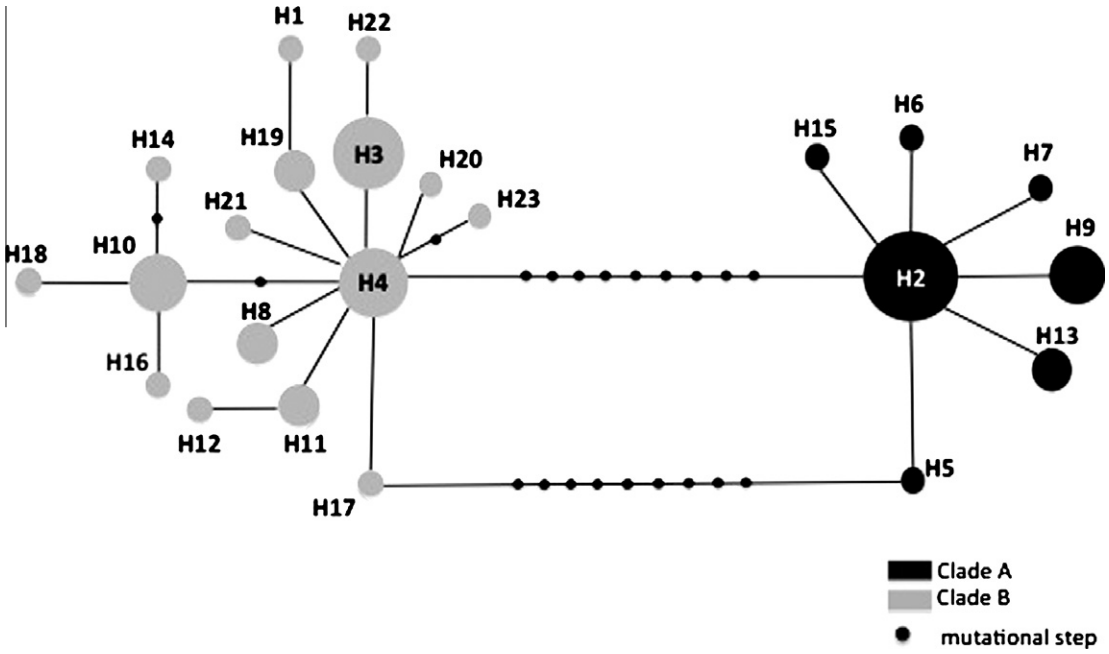

Fig. 3. Median-joining networks for the 12SrRNA and cytb combined data. The parameter epsilon was set to 0, and the considered clades correspond to the mtDNA lineages from Fig. 2.

Table 3
Nucleotide diversity (π) with 95% confidence interval, value of R_2 test (Ramos-Onsins and Rozas, 2002), Tajima's D statistics (Tajima, 1989), and Fu and Li's D (Fu and Li, 1993) estimated for both mtDNA and nDNA clades. The "n" corresponds to the number of haplotypes included in each clade.

	π [95% C.I.]	R_2	Tajima's D	Fu and Li's D
mtDNA				
Clade A (n = 44)	0.0008 [0.0004–0.0011]	0.0552*	−1.6445**	−2.30746*
12S		0.1041 ns	−1.47767*	−2.53467*
cytb		0.0664 ns	−1.26655 ns	−1.20857 ns
Clade B (n = 56)	0.0027 [0.0022–0.0033]	0.0504*	−1.54030*	−2.94688**
12S		0.0510*	−1.61619*	−1.93627*
cytb		0.0609 ns	−1.23886 ns	−2.59627*
nDNA				
Clade A (n = 62)	0.0016 [0.0013–0.0019]	0.0912 ns	−0.35675 ns	0.08754 ns
ACM4		0.0945 ns	−0.30500 ns	−0.49308 ns
Rag2		0.0933 ns	−0.30161 ns	0.41313 ns
Clade B (n = 60)	0.0019 [0.0016–0.0022]	0.1100 ns	0.07174 ns	1.44367 ns
ACM4		0.0854 ns	−0.40376 ns	0.87533 ns
Rag2		0.1205 ns	0.31746 ns	1.25849 ns

Statistics is abbreviated as ns (not significant)
* $0.01 < p < 0.05$
** $0.001 < p < 0.01$

A more likely hypothesis is that genetic hitch-hiking (Maynard-Smith and Haigh, 1974) is causing a selective sweep of the mitochondrial genome of Clade A. This hypothesis is corroborated by the significant results obtained for Fu and Li's D, Tajima's D and R_2 test, demonstrating that only mtDNA (and not nDNA) is under a selective pressure. Although significant results of these neutrality tests could also indicate a population expansion, since they leave the same footprint on DNA sequences, the differences of nucleotide diversity between mtDNA and nDNA, are a strong indicator that a hitch-hiking process has taken place, leading to an increase and fixation of a reduced number of mtDNA haplotypes across most of Europe. Moreover, the R_2 test, which is known to be extremely powerful in detecting population expansion (Ramos-Onsins and Rozas, 2002) failed for both 12S and cytb on Clade A, but not for 12S + cytb.

Clade B also presents significant results for all neutrality tests only for the mtDNA, but not a lower nucleotide diversity of the mitochondria compared to the nDNA. In fact, this lineage shows significantly higher nucleotide diversity for mtDNA compared to nDNA, with no confidence interval overlap (see Table 3). These results do not suggest that a genetic hitch-hiking process is occurring in the mtDNA, instead a population expansion is the more plausible explanation. This hypothesis is confirmed by the significant results obtained for the R_2 test for both 12S and total Clade B. The patchy distribution of Clade B in large parts of North Africa, especially in the northwest, and its particularly strong affiliation there with anthropogenically modified habitats (Bons and Geniez, 1996) seem to indicate that it was introduced into this region. Thus introductions may be further confusing the phylogeographic pattern within this species. If it is introduced in this region, models based on its "native distribution" (Rödder and Lötters, 2009) may also need to be reassessed.

The recognition of a selective sweep in the European populations of *H. turcicus* strongly parallels the case in *T. mauritanica*

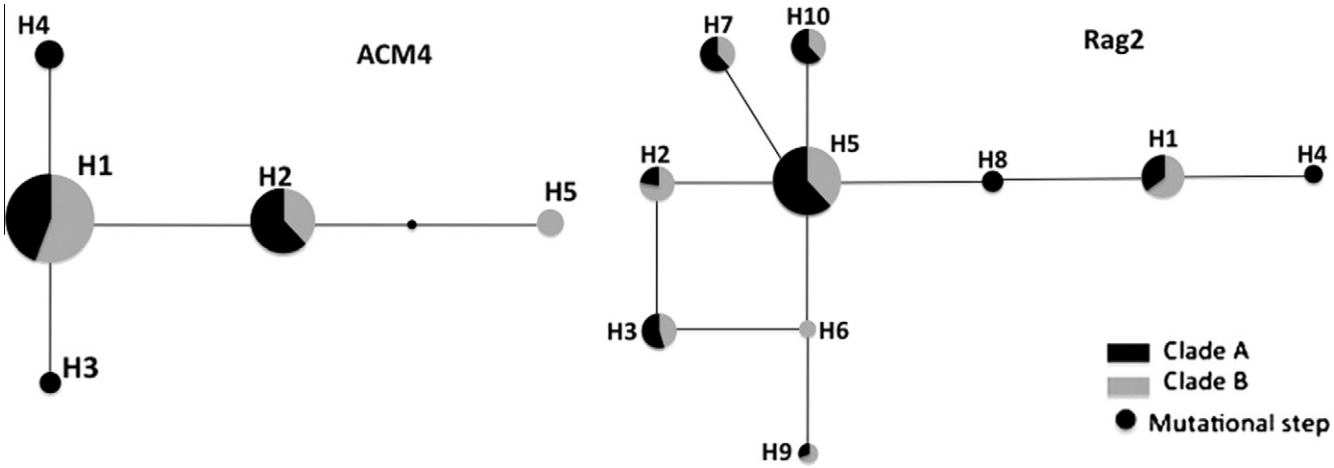

Fig. 4. Median-joining networks for the ACM4 and Rag2 nuclear genes. The parameter epsilon was set to 0, and the considered clades correspond to the mtDNA lineages from Fig. 2.

(Rato et al., 2010). These two situations represent an unprecedented discovery of a hitch-hiking process taking place in the same geographic area, shaping the mitochondrial genealogies of two different gecko families (Gekkonidae in the case of *Hemidacytlus* and Phyllodactylidae in the case of *Tarentola*; Gamble et al., 2008a). This example further highlights the problems that arise when single markers are used to infer species phylogeographic patterns and evolutionary histories (Godinho et al., 2008; Renoult et al., 2009), especially when selective forces are involved. Therefore, we need to be extremely cautious about the phylogeographic scenario proposed for *H. turcicus*, since this is only based on mtDNA, which has been shown to be under selective pressure. In order to make correct assumptions about the most likely phylogeographic pattern further nuclear markers need to be used.

In the future, it will be extremely important to discover which selective forces are driving these parallel selective sweeps, and to assess if the variables are the same or not for both *H. turcicus* and *T. mauritanica*. GIS modeling is an extremely powerful tool to unravel which are the environmental variables more likely to be causing this mtDNA selection in both gecko species. Likewise, as has been found with mammals (Luo et al., 2008) it is crucial to sequence the complete mitochondria for several intraspecific lineages, in order to assess the amino acid changes that might be being selected for.

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