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Origin and introduction history of the least weasel (*Mustela nivalis*) on Mediterranean and Atlantic islands inferred from genetic data

**Mónica Rodrigues · Arthur R. Bos · Patrick J. Schembri · Ricardo F. de Lima ·
Petros Lymberakis · Lluís Parpal · Michele Cento · Sandrine Ruette ·
Sakir O. Ozkurt · Margarida Santos-Reis · Juha Merilä ·
Carlos Fernandes**

Abstract Introduced mammalian predators have negatively affected native biota and ecological processes in ecosystems across the globe. The least weasel (*Mustela nivalis*) is a Holarctic mustelid carnivore that has been considered one of the world's worst invasive alien species. It has been introduced to several islands in the Mediterranean Sea and Atlantic Ocean, and here we present a genetic analysis of these insular populations, based on mitochondrial DNA and microsatellites and building upon a previous Western Palearctic phylogeographic study, with the aim of inferring their origin and introduction history. This is essential information for ecological and evolutionary studies of those populations and our understanding of their impacts on the islands' biodiversity. Among the Mediterranean insular populations, a substantial mitochondrial subdivision between weasels in Sicily-Corsica and Malta-Sardinia-Balearics is best explained by a dual colonization originating from the Eastern Mediterranean. Given the correspondence between the observed genetic partition and the differential geographic distribution of Greek and Phoenician settlements and influence during the last

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M. Rodrigues (✉) · R. F. de Lima · M. Santos-Reis ·
C. Fernandes
CE3C – Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal
e-mail: marodrigues@fc.ul.pt

M. Rodrigues · J. Merilä
Ecological Genetics Research Unit, Department of Biosciences, University of Helsinki, PO Box 65, 00014 Helsinki, Finland

A. R. Bos
The American University in Cairo,
PO Box 74, New Cairo 11835, Egypt

A. R. Bos
Naturalis Biodiversity Centre, PO Box 9517,
2300 RA Leiden, The Netherlands

P. J. Schembri
Department of Biology, University of Malta, Msida 2080, Malta

R. F. de Lima
Associação Monte Pico, Monte Café, São Tomé, São Tomé and Príncipe

P. Lymberakis
Natural History Museum of Crete, University of Crete, 71409 Iraklion, Crete, Greece

L. Parpal
Consorci per a la Recuperació de la Fauna de les Illes Balears (COFIB), Santa Eugènia, Spain

M. Cento
SROPU Bird Observation and Protection Station of Rome, 00165 Rome, Italy

millennium BC in those islands, we propose that weasels may have been introduced into the first group of islands by the Greeks and into the second group by the Phoenicians. There is strong support that the commercial and demographic expansions of Phoenicians and Greeks allowed the colonization of the western Mediterranean islands by the house mouse, and texts from Classical Antiquity show evidence that weasels were used for rodent control. Thus, weasels may have been intentionally introduced to the islands, with the aim of controlling commensal rodents. Besides indicating Eastern Mediterranean origins for weasels in Mediterranean islands, the mitochondrial data also revealed an unanticipated close genealogical connection between some of these populations and those from the Azores and São Tomé. The latter island populations showed no affinities with weasels from mainland Portugal, contrary to what might have been expected given that these Atlantic islands were settled by the Portuguese. The microsatellite data also supported the relationship between weasels in Atlantic and in Mediterranean islands. Considering geographic proximity and historical information, the most parsimonious explanation for the origin of the Atlantic weasels appears to be introduction from the Balearic Islands to the Azores and from there to São Tomé.

Introduction

Introduced mammals, through predation, competition, disease transmission and herbivory, have strongly impacted native fauna and flora and ecological processes on many of the world's islands (Courchamp et al. 2003). Several studies have shown that alien mammal species played (and play) a key role in the transformation of insular ecosystems (Traveset et al.

S. Ruette
Office National de la Chasse et de la Faune Sauvage, CNERA-PAD, 01330 Birieux, France

S. O. Ozkurt
Faculty of Education, Ahi Evran University, Kırşehir, Turkey

2009; Montgomery et al. 2015). The adverse effects of mammalian invasions on island biodiversity may be greater when naturally occurring terrestrial mammals are absent or few in number (Atkinson 2001), and endemics and long-isolated populations are the most vulnerable because of their greater naiveté (Berglund et al. 2009).

The introduction of alien predators is known to be the direct cause of severe reduction or extinction of numerous insular populations and species (Courchamp et al. 2003). The ubiquitous and devastating effects of rats *Rattus* sp. (Towns et al. 2006), domestic cat *Felis catus* (Medina et al. 2011), small Indian mongoose *Herpestes aeropunctatus* (Hays and Conant 2007), and dog *Canis familiaris* (Diamond 1989) are well documented. Other mammalian predators, such as the red fox *Vulpes vulpes* and some weasels *Mustela* spp., were introduced to fewer islands but also have caused significant damage to native species (e.g. Short 1998; Atkinson 2001) and are likewise included in the IUCN Invasive Species Specialist Group list of 100 of the world's worst invasive alien species (Lowe et al. 2004). The *Mustela* species in that list are the stoat *M. erminea*, the ferret *M. furo*, and the least weasel *M. nivalis*. Introduced least weasels have been shown to have a significant impact on insular faunas (Bover and Alcover 2008; Valenzuela and Alcover 2013), but their effects on potential prey species have not been investigated in most islands where *M. nivalis* is exotic (e.g. Dutton 1994).

The least weasel is widespread throughout the Holarctic ecozone. In the Western Palearctic, it is found in Great Britain, across the whole of continental Europe, in the Near East, and in Northwest Africa (Sheffield and King 1994; Tikhonov et al. 2008). The species also found its way to several islands in the Mediterranean Sea, including Crete, Malta, Sicily, Sardinia, Corsica, Minorca and Mallorca, and, in the Atlantic Ocean, the remote islands of São Miguel and Terceira in the Azores archipelago and São Tomé in the Gulf of Guinea (Mathias et al. 1998; De Marinis and Masseti 2003; Tikhonov et al. 2008). These insular populations are considered to be the result of human agency (Dutton 1994; Masseti 1995; Mathias et al. 1998), but their geographic origin and timing of introduction remain unknown. Historical records are absent or uninformative, and fossils, although present in some of the islands, generally lack direct dating (but see Valenzuela and Alcover 2013) and have not been

genetically analysed. As far as we know, with the exception of possibly some Aegean and Ionian islands (De Marinis and Masseti 2003), there is no evidence of modern introductions on any of the Mediterranean and Atlantic islands. The most comprehensive mitochondrial DNA (mtDNA) phylogeographic study available on the least weasel in the Western Palearctic only included samples from Crete, Sicily, Sardinia, Corsica and Minorca (Lebarbenchon et al. 2010). In that study, weasels in these islands clustered with samples from eastern Europe and the authors concluded that the insular populations resulted from human intervention, inferred to have occurred early in the Neolithic.

Genetic studies can be invaluable for reconstructing introduction histories, identifying potential geographic origins of introduced populations, evaluating hypotheses of single versus multiple introductions, inferring the demography of founder populations of invaders, and estimating introduction times (Kreiser et al. 2000; Ficetola et al. 2008; Muirhead et al. 2008; Johnson et al. 2011; Fitzpatrick et al. 2012; Cristescu 2015). Because physiological and environmental tolerances of species can potentially vary across their ranges, knowledge of the native-range sources of introductions may help to explain variation in establishment and spread success of exotics (Bastrop et al. 1998). The observation of extremely low genetic diversity in introduced populations suggests very small propagule size and, thus, high invasive potential (Ficetola et al. 2008; Puillandre et al. 2008; Eales and Thorpe 2010). Introduction time estimates may shed light on the chronology of faunal change and causes of extinction, trait evolution or temporal patterns of abundance of island populations affected by introduced species (Bover and Alcover 2008). They may also allow us to rule out a given alien species as a driver of a specific insular extinction (Ruffino and Vidal 2010).

In this study, we used phylogeographic and population genetic analyses of molecular marker variation to identify possible geographic origins and infer the introduction history of least weasels on all the major Mediterranean islands, with the exception of the Greek islands close to the mainland, and on the Azores and São Tomé Island in the Atlantic Ocean. We expanded the analysis of Mediterranean island weasels in Lebarbenchon et al. (2010) by including data from Mallorca and Malta, greatly increasing the sample size for Crete, and extending both the sample size and

geographic coverage around the Mediterranean basin. This is the first time that the weasel populations on the Azores and São Tomé have been analysed genetically.

We used sequences of the mitochondrial Cytochrome *b* (Cyt *b*) gene of least weasels sampled from the island populations and across the Western Palearctic, with a particular focus on the Mediterranean region. Because the timescale of nucleotide substitution in mtDNA is likely larger than that of human-mediated introductions (Fitzpatrick et al. 2012), we also used microsatellite loci, since their higher mutation rate may help to disentangle between potential source populations (Tsutsui et al. 2001; Muñoz-Fuentes et al. 2006; Vázquez-Domínguez et al. 2012). More generally, microsatellite data may provide historical information that can be compared with the genealogical patterns of mtDNA (Avise 2009).

Determining the location of the sources of the studied island populations of least weasels should improve our understanding of their patterns of morphological variation (Thomas 1901; Barrett-Hamilton 1904; Miller 1912; Frank 1985; Abramov and Baryshnikov 2000; Lebarbenchon et al. 2006) and enable comparisons between introduced populations and their native-range sources to test hypotheses about ecological and evolutionary changes in the invaded range (Colautti et al. 2004; Keller and Taylor 2008; Suarez and Tsutsui 2008). The results of this study may also provide clues about historical trade routes and cultural contacts that supplement our knowledge of human history on islands in the Mediterranean Sea and Atlantic Ocean.

Materials and methods

Sampling and DNA extraction

We collected tissue samples, mostly from road-kills and specimens in institutional collections, of least weasels from São Tomé (n = 5), the Azores (n = 4), Balearic Islands (n = 3), Sicily (n = 1), Malta (n = 8), Crete (n = 21), mainland southern Europe (Portugal, n = 32; Spain, n = 6; France, n = 3; Italy, n = 4; Greece, n = 2) and Near East (Turkey, n = 22; Egypt, n = 3). Samples were preserved in a salt-saturated solution of 20 % DMSO in water or in absolute ethanol and stored at −20 °C. For the

mtDNA analysis, we also included previously published Cyt *b* sequences of least weasels from the Western Palearctic (Lebarbenchon et al. 2010; McDevitt et al. 2012; Rodrigues et al. 2016). Most crucially for this study, the least weasels sampled by Lebarbenchon et al. (2010) included specimens from the Balearic Islands (n = 1), Sicily (n = 7), Sardinia (n = 2), Corsica (n = 6), mainland Spain (n = 2), mainland France (n = 6), mainland Italy (n = 10) and mainland Greece (n = 2). Locations and other details of the analysed samples and sequences are given in Fig. 1 and Table S1. We extracted genomic DNA using the DNeasy Blood & Tissue Kit (Qiagen) following the protocol for animal tissue.

Mitochondrial DNA

We amplified and sequenced the complete Cyt *b* gene (1140 bp) with two primer pairs generating overlapping fragments (L14979/H15791 and L15533/H16204; Fernandes et al. 2008). Polymerase chain reactions (PCRs) were carried out in a total volume of 15 μl containing 0.6 U of Surf Hot Taq DNA polymerase (Stabvida), 1X PCR buffer, 2 mM MgCl$_2$, 0.2 mM of each dNTP (Bioline), 0.5 μM of each primer and approximately 50 ng of DNA. The thermal cycling was as follows: initial denaturation at 95 °C for 15 min, followed by 10 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, 25 cycles of 94 °C

Fig. 1 Maps showing the geographic distribution of the Cyt *b* clades (see Fig. 2) found in the Western Palearctic (*top*) and São Tomé (*bottom*). *Black triangles* represent Clade I, *grey circles* represent Clade II, *white circles with black dot* represent Clade III, *white pentagons* represent Clade IV, and *white stars* represent Clade V

for 30 s, 50 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 10 min. PCR products were analysed by electrophoresis in 2 % agarose gels; positive results were purified using ExoSAP (Hanke and Wink 1994) and sequenced in both directions at Macrogen Inc. Sequences were assembled, aligned, and edited using SEQUENCER 4.7 (Gene Codes Corporation).

Microsatellites

We genotyped eight tetranucleotide microsatellite loci (Mn 1.5; Mn 1.6; Mn 1.27; Mn 4.1; Mn 4.14; Mn 5.11; Mn 6.9; Mn 6.14) selected from a panel previously developed for least weasels (Rodrigues et al. 2012), with PCR conditions as described in the same paper. GeneScan-500 ROX size standard (Applied Biosystems) was added to the PCR products and the fragment sizes were determined on an ABI 3100 Genetic Analyzer (Applied Biosystems) and analysed using GeneMapper 3.7 (Applied Biosystems).

Statistical analysis

The sequence alignment was analysed with FABOX 1.41 (Villesen 2007) to collapse identical sequences into haplotypes. Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) in MRBAYES 3.2.5 (Ronquist et al. 2012) and RAxML 7.2.8 (Stamatakis 2006), respectively. As outgroups, we used sequences of mountain weasel *M. altaica*, Siberian weasel *M. sibirica*, stoat and American mink *Neovison vison* from GenBank (accession numbers respectively: KC815122, HM106317, EF689078, EF689073). Substitution saturation was checked using the index of Xia et al. (2003) in DAMBE 5.3.74 (Xia 2013). The Cyt *b* alignment was partitioned by codon position, as this was the best-fit partitioning scheme according to the Bayesian information criterion (BIC) in PARTITIONFIN-DER 1.1.1 (Lanfear et al. 2012). Analyses in MRBAYES were conducted with two parallel runs, each with four Markov chains (one cold and three heated) and 20 million generations. The first five million generations were discarded as burn-in and, thereafter, chains were sampled every 500 generations. The entire general time-reversible (GTR) substitution model space was sampled within the analyses (Huelsenbeck et al. 2004). Convergence of the two runs was assumed

when the average standard deviation of the split frequencies was less than 0.01. Support for tree nodes was determined based on the values of Bayesian posterior probability (BPP) obtained from a majority-rule consensus tree (Holder et al. 2008). In RAxML we used a random starting tree, a GTR + Γ model of sequence evolution for each partition, and support for each node was evaluated by 1000 bootstrap replicates. Majority-rule consensus trees were calculated with SUMTREES 3.3.1 of the DendroPy library (Sukumaran and Holder 2010) and visualized and edited with TREEGRAPH 2.4.0 (Stöver and Müller 2010). Genealogical relationships among haplotypes were also estimated using a median-joining network (Bandelt et al. 1999) as implemented in POPART 1.7 (Leigh and Bryant 2015).

To estimate the genetic differentiation between island populations and geographically defined populations in the Mediterranean Basin, we computed the net average distance (D_a, Nei 1987) and pairwise Φ_{ST} (Excoffier et al. 1992) under the Tamura-Nei model (Tamura and Nei 1993) in ARLEQUIN 3.5.2.1 (Excoffier and Lischer 2010). The statistical significance of D_a and Φ_{ST} values was tested using 20,000 permutations. The D_a matrix and the neighbour-joining method (Saitou and Nei 1987) were used to construct a tree of populations in the software PAST version 3.07 (Hammer et al. 2001). For each population, we calculated haplotype diversity (h) and nucleotide diversity (π) in ARLEQUIN and haplotype richness (H_R) by rarefaction in CONTRIB 1.02 (Petit et al. 1998). We estimated the completeness of haplotype sampling in each population using the Stirling probability distribution and Bayes' theorem (Dixon 2006) in a program available at http://www.botanik.univie.ac.at/plantchorology/haplo.htm. Although this method is vulnerable to biases caused by uneven haplotype frequencies, it provides a way to evaluate the assumption that all haplotypes have been sampled.

The genetic affinities of populations were also estimated with the microsatellite data. The data was first checked using MICROCHECKER 2.2.3 (van Oosterhout et al. 2004) to identify possible genotyping errors due to large allele dropout, stuttering, null alleles and typographic errors. Within-population genetic diversity was characterized by the number of alleles (N_A), observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E) as calculated by GENALEX 6.501 (Peakall and Smouse 2012); allelic richness (A_R) was
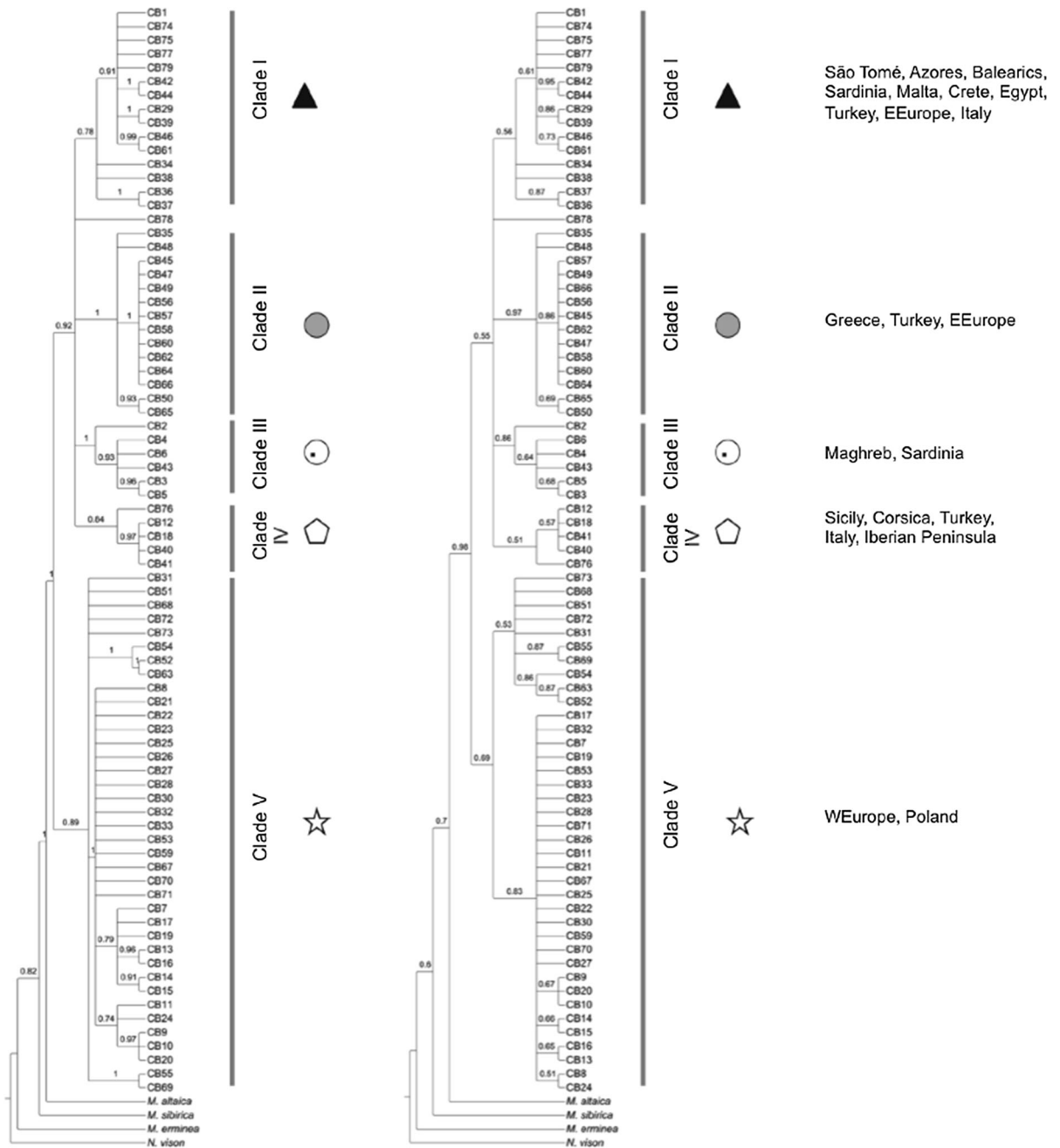
Fig. 2 Majority-rule consensus trees of the Bayesian (*left*) and maximum likelihood (*right*) analyses of Cyt *b* haplotypes. *Numbers above branches* are Bayesian posterior probabilities and bootstrap values, respectively. Haplotype codes are as in Table S1. *Symbols* next to clade names correspond to those used in Fig. 1. Geographic distribution of clades is given on the *right*

calculated in HP-RARE 1.0 (Kalinowski 2005) using a rarefaction procedure to account for unequal sample size between populations (Kalinowski 2004).

To visualize the genetic relationships among populations we used factorial correspondence analysis (FCA) in GENETIX 4.05 (Belkhir et al. 2004).

Ordination techniques allow for summarising relationships among samples without imposing a bifurcating evolutionary history. The FCA algorithm searches for orthogonal axes, defined by linear combinations of alleles, along which the total variance in allele frequency is maximized, and the factorial axes are ranked according to how much variance they explain. The dispersion of the populations along each axis can be related to linear combinations of single-locus F_{ST} values (Guinand 1996). Following the recommendation of Jombart et al. (2009), we represented the results of the FCA in a two-dimensional scatter plot instead of the more customary three-dimensional re-projection. Genetic associations between the sampled populations were further explored using nonmetric multidimensional scaling (NMDS), as implemented in PAST, on a matrix of pairwise Cavalli-Sforza and Edwards' (1967) chord distances (D_C) calculated in POPULATIONS 1.2.32 (Langella 1999). We also computed a NMDS of Nei's (1972) standard genetic distances (D_S), as this combination performed well in a previous study comparing the effectiveness of different ordination/distance combinations (Guiller et al. 1998). The fit of the data to the NMDS solution on two dimensions was measured by the stress value (Kruskal 1964). Finally, a perspective on the population affinities was obtained using individual-based Bayesian clustering implemented in the program STRUCTURE 2.3.4 (Pritchard et al. 2000). We applied the admixture model with independent allele frequencies and sample group information (LOCPRIOR model; Hubisz et al. 2009). The LOCPRIOR model does not tend to find structure when none is present and ignores sampling information when it is uncorrelated with individual ancestry (Hubisz et al. 2009). We ran 10 replicate runs for each value of K, the hypothesized number of genetic clusters, from 1 to 12 with one million Markov chain Monte Carlo (MCMC) iterations after a burn-in of 100,000. To infer the most likely K at the uppermost hierarchical level of population structure, we used STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt 2012) to summarise STRUCTURE output and calculate for each K the second-order rate of change of the likelihood function (ΔK, Evanno et al. 2005). For the most likely value of K, the cluster membership coefficients of each individual from the 10 replicate runs were aligned using the *FullSearch* algorithm in CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007), and

the resulting Q matrix was visualized using DISTRUCT 1.1 (Rosenberg 2004).

Results

Mitochondrial DNA

The Cyt *b* data set consisted of 222 sequences (218 least weasels plus four outgroups) of 1117 bp. The ingroup alignment contained 117 (10 %) polymorphic sites, of which 62 (6 %) were parsimony informative, and yielded 79 haplotypes. New haplotypes were deposited in GenBank (accession numbers KT737479–KT737491; Table S1). The index of substitution saturation (I_{ss}) for the whole data set was significantly lower than the critical value ($P = 0.000$), thus rejecting the null hypothesis of saturation (Xia et al. 2003).

The Bayesian and maximum likelihood inferences of the phylogenetic relationships of the Cyt *b* haplotypes are given in Fig. 2. The two topologies were congruent as evidenced by an overall similarity of 94 % calculated in the software Compare2Trees (Nye et al. 2006). The haplotype genealogy estimated using the median-joining algorithm (Fig. 3) was also consistent with the phylogenetic trees.

Among the island populations, weasels from São Tomé, São Miguel, Minorca, and Malta showed a single haplotype (CB1). This haplotype was also found in Crete, Turkey, and it was the only one observed in Egypt (Fig. 3). Haplotypes identified in Terceira (CB74) and Mallorca (CB44) were private but differed from haplotype CB1 by only one and three substitutions, respectively. In Sardinia, one haplotype (CB42) was four mutational steps away from haplotype CB1 within Clade I, while the other haplotype (CB43) clustered with the haplotypes from the Maghreb in Clade III. The population from Corsica was monomorphic for haplotype CB18, which was also detected in Sicily, mainland Italy, mainland Portugal and Turkey (Fig. 3). This haplotype grouped with the other two haplotypes recovered in Sicily (CB40 and CB41; both private) into a separate clade, Clade IV (Fig. 2). Other clades consistent between phylogenetic analyses included haplotypes from Eastern Europe (Clade II) and from across mainland Europe (Clade V) (Figs. 1 and 2).

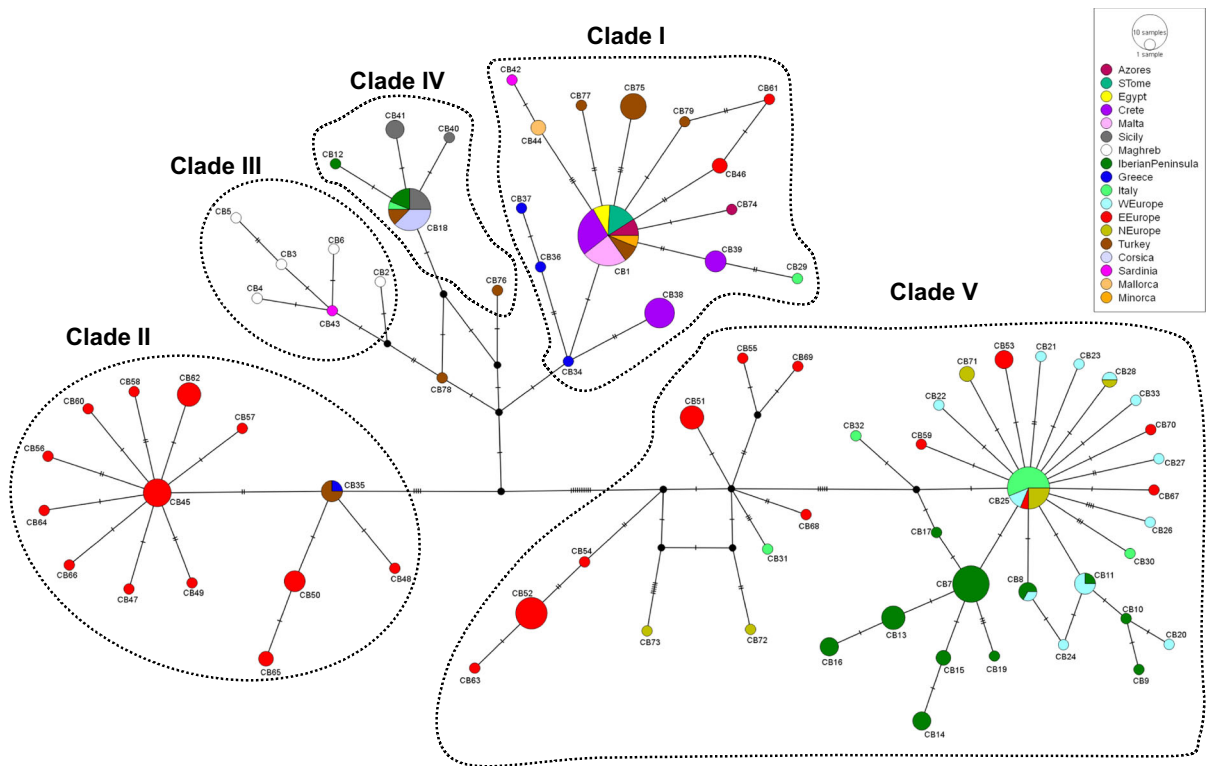Pairwise D_a distances among populations on the islands and the Mediterranean mainland varied from 0

Fig. 3 Median-joining network based on Cyt *b* haplotypes. *Circles* represent haplotypes and their size is proportional to the frequency observed. *Coloured pie* charts indicate the frequency of haplotypes in local or regional populations. *Small black circles* represent hypothetical haplotypes. *Dashes on lines* connecting haplotypes represent the number of nucleotide substitutions separating them. Haplotype codes are given in Table S1. The clades identified in the phylogenetic analyses are also indicated

to 2.21 %, and differentiation measured by Φ_{ST} ranged from 0 to 1 (Table S2). Correlation between the D_a and Φ_{ST} matrices was moderately strong (Mantel $r = 0.64$; $P = 0.0001$). Both estimates of genetic divergence indicated close relationships between weasels within three population groups: (i) São Tomé, São Miguel, Minorca, Malta, Crete, Egypt and Turkey; (ii) Sicily and Corsica; *iii*) Iberia, France and mainland Italy. This structure, already suggested by the overall phylogenetic analyses of haplotypes, was apparent in the neighbour-joining population tree based on D_a values (Fig. S1). In this tree, the Sardinian and Northwest African samples clustered together due to the presence of haplotype CB43 in Sardinia, but the other haplotype found in this island (CB42) was closely related to those observed in group (i) (Fig. 3).

Haplotype and nucleotide diversities were generally much lower in the insular populations than in the mainland ones, being zero in all the islands with the exception of Sardinia, Sicily and Crete (Table 1).

Among the island populations, the posterior probability that all existing haplotypes, or at least the most common ones (Dixon 2006), were sampled was greater than 90 % for São Tomé, Corsica, Malta and Crete, and about 60 % for São Miguel and Sicily. Diversity estimates for the Sardinian and Balearic populations should be taken with extra caution due to the extremely small sample sizes.

Microsatellites

Microsatellite variation, as measured by rarefied allelic richness averaged across loci (Table 1), was significantly lower in the island populations than in the mainland populations, even after excluding the Iberian sample because of its markedly larger size (Mann–Whitney U test, $n_i = 5$, $n_m = 5$, $U = 0.5$, $P = 0.016$). Similarly, the observed heterozygosity was lower in the island populations (Mann–Whitney U test, $n_i = 5$, $n_m = 5$, $U = 0$, $P = 0.008$), with mean

Table 1 Estimates of genetic diversity in the mitochondrial gene Cyt *b* and at eight microsatellite loci

Population	Cytochrome *b*					Microsatellites				
	n	n_H	H_R	h	π	n	N_A	A_R	H_E	H_O
S. Tomé	5	1*	0.00	0.00	0.000	5	1.88	1.66	0.34 ± 0.11	0.28 ± 0.08
S. Miguel	3	1	0.00	0.00	0.000	3	2.50	2.14	0.53 ± 0.12	0.38 ± 0.09
Mallorca	2	1	nd	0.00	0.000	2	1.75	1.75	0.42 ± 0.09	0.38 ± 0.13
Minorca	2	1	nd	0.00	0.000	–	–	–	–	–
Sardinia	2	2	nd	1.00 ± 0.50	0.010 ± 0.010	–	–	–	–	–
Malta	8	1*	0.00	0.00	0.000	8	2.38	1.66	0.33 ± 0.09	0.29 ± 0.09
Sicily	8	3	1.13	0.68 ± 0.12	0.001 ± 0.001	–	–	–	–	–
Corsica	6	1*	0.00	0.00	0.000	–	–	–	–	–
Crete	21	3*	1.11	0.67 ± 0.05	0.002 ± 0.001	21	5.13	2.30	0.59 ± 0.06	0.62 ± 0.06
Egypt	3	1	0.00	0.00	0.000	3	2.65	2.30	0.53 ± 0.16	0.63 ± 0.18
Turkey	18	8	1.60	0.86 ± 0.06	0.005 ± 0.003	19	6.88	2.83	0.73 ± 0.08	0.66 ± 0.07
Greece	4	4	2.00	1.00 ± 0.18	0.005 ± 0.003	2	2.75	2.75	0.73 ± 0.11	0.63 ± 0.16
Italy	14	6	1.04	0.60 ± 0.15	0.007 ± 0.004	4	3.88	2.77	0.74 ± 0.06	0.72 ± 0.07
France	9	7	1.76	0.92 ± 0.09	0.002 ± 0.001	3	4.25	3.25	0.87 ± 0.03	0.88 ± 0.06
Iberia	36	13	1.62	0.86 ± 0.04	0.006 ± 0.003	34	7.50	2.88	0.77 ± 0.01	0.71 ± 0.04
Maghreb	5	5	2.00	1.00 ± 0.13	0.003 ± 0.002	–	–	–	–	–

Asterisks indicate populations in which the estimated probability of haplotype sampling completeness was greater than 90 %. nd means not determined because the rarefaction size was set to three. Dashes indicate parameters that could not be estimated due to the lack of data. Sample sizes for mtDNA are larger in some populations due to the inclusion of Cyt *b* sequences published by Lebarbenchon et al. (2010)

n number of samples, n_H number of haplotypes, H_R haplotype richness, *h* haplotype diversity, π nucleotide diversity, N_A mean number of alleles, A_R average allelic richness, H_E mean expected heterozygosity, H_O mean observed heterozygosity

values over loci ranging from 0.28 (São Tomé) to 0.62 (Crete), while in the mainland they ranged from 0.63 (Egypt and Greece) to 0.88 (France).

The FCA of allelic composition showed a separation between weasels in the northern Mediterranean basin and those in the islands and Egypt, with all members of the two groups plotting on opposite sides of the first axis (Fig. 4a). In the NMDS plot, the two groups were also distinguished along the first dimension, with the exception of São Miguel, but this population was differentiated from the northern Mediterranean group by their scores on the second dimension (Fig. S2). In the analysis using D_S (not shown) the relative position of the samples in the reduced space was essentially the same as obtained using D_C, but with a higher stress value (0.18). The STRUCTURE clustering analysis of individuals based on their microsatellite genotypes identified two clusters (*K* = 2) as the uppermost hierarchical level of genetic partitioning between populations (Fig. S3). The mean posterior probability (Ln P(D)) for *K* = 2 was

−2704.74 ± 2.74, whereas for *K* = 1 was −2819.08 ± 0.04. The mean value of *r*, the parameter that estimates the informativeness of the sampling location data in the LOCPRIOR model, in the best run for *K* = 2 was 0.1654, indicating that the ancestry proportions vary substantially among locations (Hubisz et al. 2009). Similar to what was shown by the ordination analyses of population affinities, the individual-based STRUCTURE analysis, which is independent of population sample size, indicated a distinction between weasel populations in the northern Mediterranean basin and those in the islands and Egypt, with most individuals in each group having average membership coefficients >0.7 to their respective clusters (Fig. 4b). In the Egypt-islands group, only individuals from São Miguel had cluster membership probabilities around 0.5 for both clusters, and in the northern Mediterranean basin this was observed in samples from Italy and Turkey. The exceptions to the general pattern of the results may be due to lack of power in the dataset, sampling error, homoplasy, genetic drift, or, in
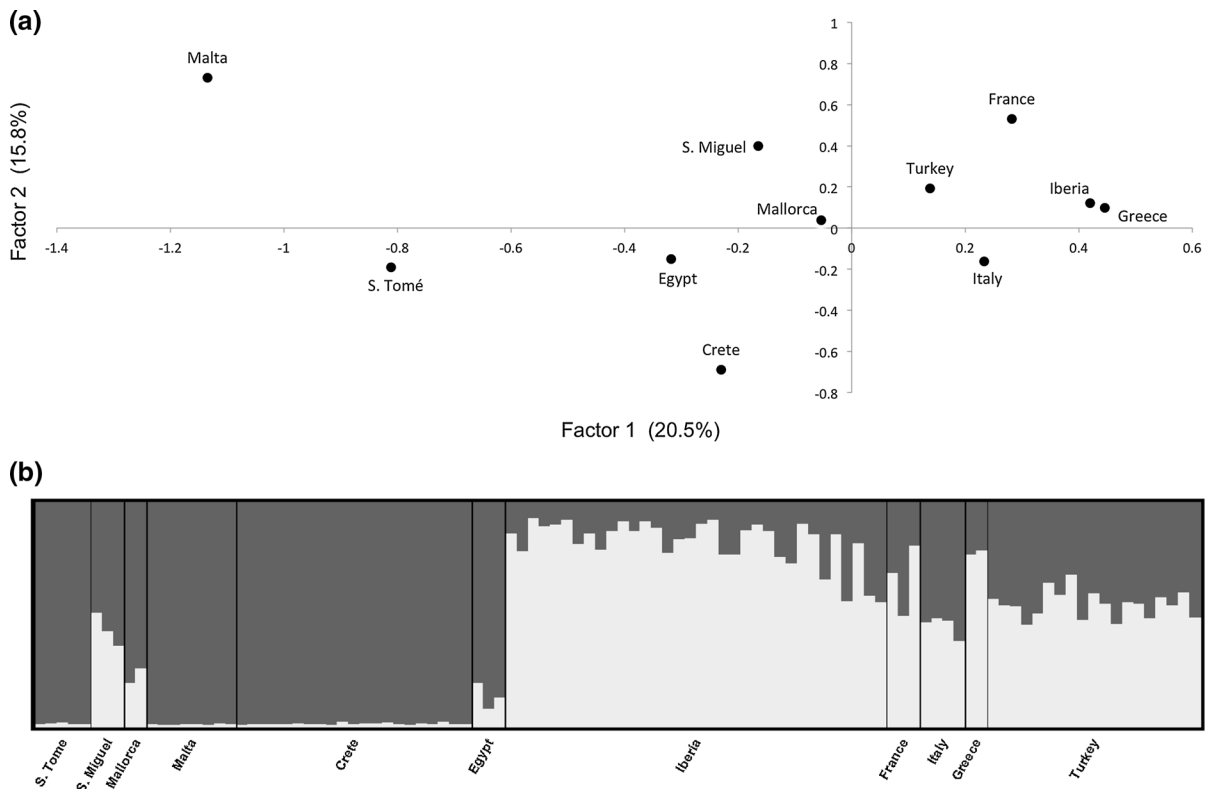
(a)

(b)

Fig. 4 **a** Two-dimensional plot of the factorial correspondence analysis (FCA) of microsatellite allele composition of eleven geographically defined weasel populations; the percentage of the total inertia accounted for by each of the first two factors is provided in parenthesis. **b** *Bar plot* of STRUCTURE results for $K = 2$ genetic groups. Each individual is depicted by a column that is partitioned into K segments, which length is proportional to the ancestry probability of the individual to each group. *Vertical black lines* separate different populations that are labelled below the figure

the case of Turkey, admixture as a result of gene flow from populations in Syria, Iran, and the Caucasus (Abramov and Baryshnikov 2000).

Thus, in agreement with the mtDNA results, the microsatellite data also indicated connections between weasels in the analysed Atlantic and Mediterranean islands and Egypt.

Discussion

In this study, we analysed mtDNA and microsatellite variation to identify possible geographic origins and introduction histories of least weasels on Mediterranean and Atlantic islands. Genetic diversity for both mtDNA and microsatellites was consistently low for several of the island populations (Table 1), suggesting that they may have experienced strong founder effects. This study, therefore, adds to the growing literature

indicating that founding bottlenecks may be common but not necessarily a limiting factor for the successful establishment of introduced species (Dlugosch and Parker 2008; Puillandre et al. 2008).

Our results provide evidence for a genetic link between weasels in the Atlantic islands, on some Mediterranean islands, and in Egypt, which agrees well with their morphological similarities noted by several authors. Specifically, Wright (1875) and Thomas (1895, 1901) pointed out the resemblance between specimens from Malta, Sardinia, Balearics and Egypt, while that between the latter and those from the Azores and São Tomé was also recognized early on (Barrett-Hamilton 1904; Miller 1912). Below we discuss how our results shed light and provide working hypotheses on the possible sources and introduction routes of the least weasel populations in the Mediterranean and Atlantic islands studied here.

Weasels on Mediterranean islands

Weasels occur on several Mediterranean islands where, in the light of palaeogeographic models and fossil evidence, their presence is commonly attributed to human introduction (Vigne 1992; Masseti 1995). These islands, with the exception of Sicily, Malta (via Sicily), Corfu and North Aegean islands, have not been connected to the mainland by land bridges for at least the last 125,000 years (Shackleton et al. 1984; van Andel 1989). We are not aware of any data on the swimming distance for weasels, but the maximum swimming range of the stoat, a closely related species with generally a larger body size, is 3–5 km (Veale et al. 2012), and hence that of the weasel is likely to be rather smaller, and very probably less than the post-glacial width of the Strait of Messina at its narrowest point (Antonioli et al. 2016). Furthermore, apart from a Sicilian fossil assigned to the terminal Pleistocene that might indicate natural dispersal via a land bridge during the Last Glacial Maximum (LGM; 26,500–20,000 years ago) (Masseti 1995), the oldest weasel remains from Mediterranean islands that have been dated with some confidence are from Late Bronze Age—Early Iron Age Cyprus (1600–1050 years BC; Lehmann and Nobis 1979). Masseti (1995) mentioned a femur of a small carnivore similar in size to a stoat that was found in the Xemxija tombs from the Copper Age in Malta (Pike 1971), and which he presumed to belong to a weasel. However, the site was subsequently used and the tombs were not sealed, and thus the bone may be a stratigraphic intrusion of later material, as proposed by Pike (1971) to explain the unexpected presence of rabbit *Oryctolagus cuniculus* bones in the same tombs. It is believed that weasels were introduced to control commensal rodents and their impact on crops, but the geographic origin of the Mediterranean insular populations and the identity of the peoples who introduced them remain unknown (Masseti 1995). In the first comprehensive mitochondrial phylogeographic study of the weasel in the Western Palearctic, Lebarbenchon et al. (2010) found that samples from Sicily, Corsica, Sardinia and Minorca clustered with samples from eastern Europe, and thus concluded that the presence of weasels in these western Mediterranean islands was due to human intervention. They found no traces of native lineages in Sicily, which could have descended from a LGM invasion, and estimated the age of the colonization of Sicily and

Corsica to be post-glacial, in particular suggesting that it may have occurred early in the Neolithic.

Dual origin of weasels on Mediterranean islands: Phoenicians and Greeks

In our study, the inclusion of samples of Egyptian and Turkish weasels allowed us to demonstrate that haplotypes in western and central Mediterranean islands are shared with, or are closely related to, haplotypes in the Near East. The mtDNA data showed a clear separation between weasels in Sicily-Corsica and Malta-Sardinia-Balearics but also suggest that both groups have their ancestry in the Eastern Mediterranean (Fig. 3). This subdivision indicates independence of the two introduction histories, since differentiation among introduced populations likely implies introduction from different sources (Fitzpatrick et al. 2012). The independence of the two introduction histories is also consistent with the geographic distribution of coat colour types, since weasels in the first group of islands have a pelage coloration of the 'nivalis' type, in which the demarcation line between the upper-brown and the lower-white colours is straight, while weasels in the second group of islands have a 'vulgaris' colour pattern in which the demarcation line is irregular (Frank 1985; Abramov and Baryshnikov 2000). The geographic distribution of these two basic types of weasel coloration seems to have some phylogeographic significance in the native range of the species (Frank 1985; Zima and Cenevová 2002), although there is also evidence for the independent appearance of the same pelage pattern in different populations, and in some areas both types occur sympatrically (Abramov and Baryshnikov 2000).

Given the correspondence between the observed mtDNA split among weasel populations in Malta-Sardinia-Balearics and Sicily-Corsica and the differential geographic distribution of Phoenician and Greek settlement and influence during the last millennium BC in these islands (Fig. 5), we propose that weasels may have been introduced into the first group of islands by the Phoenicians and into the second group by the Greeks. We consider it highly unlikely that the match between the mtDNA partition and the interspersed distribution of Phoenician and Greek major colonies and trading posts in those islands is coincidental. For instance, it would be remarkable that
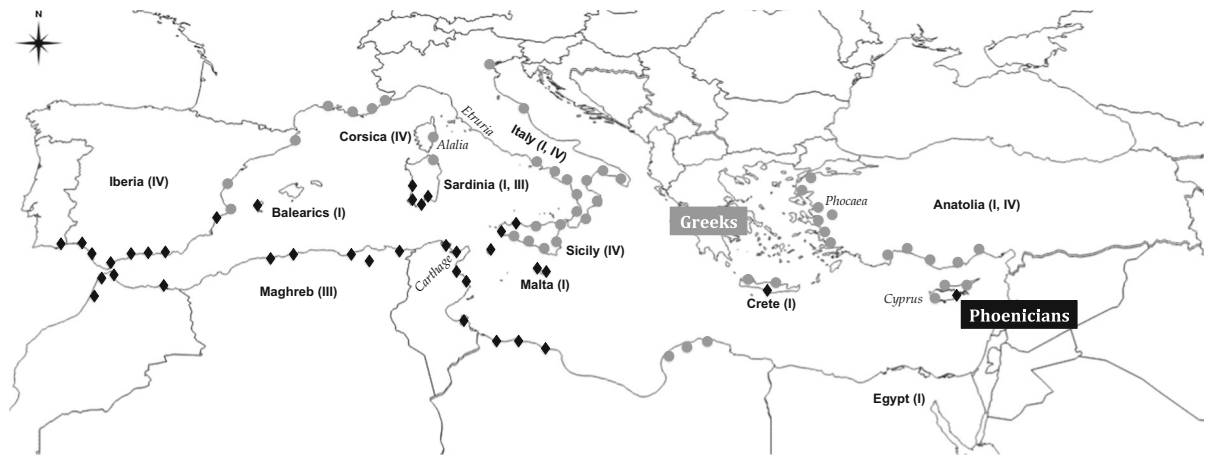
Fig. 5 Map showing the geographical distribution in the Mediterranean basin of the mtDNA clades found on the islands (clades I, III and IV), and the Greek (*grey circles*) and Phoenician (*black diamonds*) colonies established between the ninth and sixth centuries BC (map adapted from Cucchi and Vigne 2006). *Font in bold* are names of islands and mainland areas, followed in parenthesis by the clades found among those mentioned above. Also indicated (*in italics*) are locations referred to in the text

a common introduction history or introduction from the same source could have, by chance, produced a mosaic pattern of genetic affinities among the island populations that agrees with the pattern of Phoenician and Greek presence in the islands.

The commercial and demographic expansions of Phoenicians and Greeks during the last millennium BC allowed the colonization of the western Mediterranean islands by the house mouse *Mus musculus* (Cucchi and Vigne 2006), and possibly the black rat *Rattus rattus* (Ruffino and Vidal 2010). Texts from Classical Antiquity show evidence that weasels were used for rodent control (Frank 1985; Masseti 1995; Faure and Kitchener 2009). In the Near East, where rodents such as the house mouse and the black rat are commensal since at least the Neolithic and the Chalcolitic (Cucchi and Vigne 2006; Ruffino and Vidal 2010), weasels would have been attracted to cultivated fields and grain storages, as they are today in rural areas across their range (Sheffield and King 1994). Finally, the fact that the only known *M. nivalis* fossils from Cyprus are dated to the Late Bronze Age or Early Iron Age (Lehmann and Nobis 1979), a period in which Canaanite-Phoenician trade and presence in Cyprus intensified (Negbi 1992), can be interpreted as indicating transport of weasels by the Phoenicians. Taken together, these different lines of evidence support the idea that Phoenicians and Greeks may have intentionally introduced weasels into the Mediterranean islands, with the aim of controlling

commensal rodents following their stowaway introduction.

Thus, the proposed Phoenician-Greek hypothesis provides a parsimonious explanation for the inferred dual origin of weasels in Malta-Sardinia-Balearics and Sicily-Corsica that is consistent with the recorded human history in this area. Our results also provide hints on the possible location of the source region for each group of islands that are compatible with the hypothesis of introduction by Phoenicians and Greeks, respectively. In the Eastern Mediterranean, haplotype CB18 (the only haplotype found in Corsica and the most common in Sicily) was only found in Turkey, but this is not inconsistent with a Greek introduction of weasels into Sicily-Corsica since the Greek colonization of Sicily and Corsica was mainly carried out by Phocaean/Ionian and Dorian settlers arriving from western Anatolia (Rigsby 1987; Romano et al. 2003; King et al. 2011). On the other hand, the widespread haplotype CB1 (found in Malta and Minorca) was found both in Egypt and Turkey, suggesting that CB1 may be or may have been present in the Levant. In addition, despite the reasonable size of our Turkish sample, haplotypes CB42 (found in Sardinia) and CB44 (found in Mallorca), both closely related to CB1, were not observed in the native range, which raises the possibility that they may be present in the unsampled Levantine populations. Weasels are apparently rare in Lebanon and Syria (Masseti 2009; Abi-Said and Amr 2012) and we were unsuccessful in

obtaining access to specimens. Future analysis of samples from these populations and of the available fossils from Israel and Jordan, where the species was present at least until the first millennium BC (Bar-Oz and Dayan 2001), as well as of additional specimens from Egypt and mainland Greece, is warranted to confirm the above hypotheses and provide better resolution for identifying source populations within the Eastern Mediterranean.

A reasonably extensive sampling of the native range may allow the identification of a larger source region even if the mean number of individuals surveyed per population is not very large, since the total number of unique haplotypes recovered is positively related to the number of source populations sampled (Muirhead et al. 2008). The same study also found that errors associated with sampling a low number of individuals per source population are most acute when rare source haplotypes are dominant or fixed in the introduced populations. In our case, the most common haplotypes in the island populations, CB1 and CB18, were not rare in the Eastern Mediterranean (Fig. 3, Table S1). Furthermore, the results of Muirhead et al. (2008) indicated that the accuracy of assignment of introduced individuals to source populations is also strongly dependent on the degree of genetic differentiation between source populations. If the source populations exhibit high levels of genetic differentiation, the number of source individuals sampled will have less of an effect on the ability to correctly source individuals from the introduced population (Muirhead et al. 2008). In our study, there was a clear phylogeographic partitioning of haplotypes in the native range between mainland western Europe (almost all belonging to Clade V), eastern Europe (making up most of Clade II, and also a part of Clade V), Eastern Mediterranean (most of Clade I) and Maghreb (Clade III) (Fig. 3), and consequently a considerable degree of genetic differentiation among populations from these different regions (Table S2).

In the following, we discuss our results and evidence consistent with the Phoenician-Greek hypothesis of weasel introduction for the different Mediterranean islands.

Sicily and Corsica

Weasels in Sicily and Corsica clustered together in the same clade (Clade IV) (Fig. 2). The population from Corsica appeared fixed for haplotype CB18, which was also found in Sicily, Turkey, mainland Italy and mainland Portugal. The presence of CB18 in the latter two areas is likely the result of introduction, as explained below in the section 'Introductions into the Iberian and Italian Peninsulas'. The other two haplotypes recovered in Sicily (CB40 and CB41) were private and only one mutational step away from CB18 (Fig. 3). The mtDNA pattern in Sicily-Corsica fits best with a scenario of a single introduction from the Eastern Mediterranean to Sicily and from there to Corsica. If weasels in Sicily and Corsica had been introduced from mainland Italy or Iberia, it would be remarkable that the founder individuals carried exactly haplotype CB18, which occurs in both of the latter two areas and also in Turkey, and not any of the several other haplotypes belonging to the most common clade in mainland Italy and Iberia (Clade V, which is absent from Turkey) that were found in either of these two areas and not in the other (Fig. 3). We suggest that weasels may have been brought to Sicily and Corsica by the wave of Phocaean/Ionian and Dorian Greek settlers arriving from western Anatolia between the eighth and sixth centuries BC (Rigsby 1987; King et al. 2011). This proposal agrees with an assumed maximum age of 3000 years for the arrival of the weasel in Corsica (Vigne 1992). Although the Phoenicians also settled in western Sicily, the Greek hypothesis is supported by the facts that the Greek colonization of Sicily was massive and occupied most of the island (Romano et al. 2003), and that the Phocaeans had an important trading colony, Alalia (Alerie), in Corsica (Fig. 5). For instance, the introduction of the house mouse in Corsica, dated to the first half of the first millennium BC, has been associated with the Greek colonization of the island (Cucchi and Vigne 2006).

Malta, Sardinia and Balearics

The Maltese sample, like the Egyptian one, was monomorphic for haplotype CB1 and the STRUCTURE analysis also indicated genetic similarities between the two populations. These genetic patterns suggest a close relationship between Maltese and Egyptian weasels, which is consistent with a morphological resemblance already noted over a century ago by several authors (Thomas 1895; Barrett-Hamilton 1900; Miller 1912). Archaeological evidence shows

that the Phoenician colonization of Malta started at least in the eighth century BC (Vella 2005), developing into a fully-fledged colony by the end of the sixth century BC due to its central geographical position in the Mediterranean (Bonanno 2005). Given our results and the phenotypic similarity between weasels in Malta and in Egypt, a distinct possibility is that the Maltese weasel descends from Egyptian animals imported by Phoenician traders. There were intense and long-lasting commercial exchanges and cultural ties between Egypt and the Phoenicians since the Early Iron Age (Ballard et al. 2002; Evian 2011), and Phoenician Malta is characterized by a convergence of Egyptian and Levantine cultural influences (van Sister 2012). Genetic analysis of Levantine weasels is needed to test this hypothesis. In this context, it is worth noting that, unlike in Maltese and Egyptian weasels, the 'nivalis' coat pattern has been observed in Lebanese weasels (Harrison and Lewis 1964).

In Sardinia, one haplotype (CB42) fell in Clade I with haplotypes found in the Balearic Islands, Malta, Crete, Egypt, and Turkey, while the other haplotype (CB43) clustered with the haplotypes from the Maghreb in Clade III (Fig. 3). The haplotype CB43 in Sardinia is best explained as the result of introduction from the Maghreb, where it was not observed either because it was not sampled or because it has disappeared from this region, since the haplotype was not found elsewhere other than in Sardinia. At least by the ninth century BC, and probably as early as the tenth, the Phoenicians started founding several colonies in Sardinia as it became central in their pan-Mediterranean trading network (Negbi 1992). The Carthaginians gained control of these settlements in the sixth century BC (Carpenter 1958; van Dommelen 1997). This history of colonization is concordant with the introduction of haplotypes CB42 by the Phoenicians and CB43 by the Carthaginians (Fig. 5). Given the small size of the Sardinian sample, the absence of the common haplotype CB1 requires confirmation through additional sampling, as this could shed some light on the origin of the weasels introduced by the Phoenicians. Considering geographical proximity and the east–west direction of the Phoenician expansion in the Mediterranean, Malta would be a feasible source of the Sardinian population if further sampling finds haplotype CB1 in the latter island. Additional

sampling from the Eastern Mediterranean is also needed to try to detect haplotype CB42, as it was not observed in the native range.

In the Balearic Islands, weasels are present in Mallorca and Minorca (the Gymnesic Islands). While Lebarbenchon et al. (2010) analysed a single individual from Minorca, we included samples from the two islands and found that they harbor distinct but related haplotypes. Animals from Mallorca had haplotype CB44 and those from Minorca had haplotype CB1, with both haplotypes grouping into Clade I with haplotypes found in Sardinia, Malta, Crete, Egypt and Turkey (Fig. 3). As for other western Mediterranean islands, the introduction of the weasel to the Gymnesic Islands has traditionally been attributed to the Romans, but a specimen from Mallorca has recently been radiocarbon dated to the fourth-third century BC, prior to the Roman occupation of the Balearics (Valenzuela and Alcover 2013). These authors proposed that the weasel could have been brought by Talaiotics, the Iron Age settlers of the Balearics, serving as mercenaries with the Carthaginians during the Punic wars. These wars were fought in North Africa, the Iberian Peninsula, mainland Italy, Sicily and Sardinia. Among these possible origins for the Balearic weasels, only Sardinia is compatible with the available genetic data; haplotypes found in Mallorca (CB44) and Sardinia (CB42) differed by a single nucleotide substitution. Valenzuela and Alcover (2013) entertained the possibility that the Balearic weasels originated from North Africa because Lebarbenchon et al. (2010) only analysed samples from Morocco, but the result for our Tunisian sample further contradict a Maghreb origin for the Balearic weasels. Our results suggest a separate colonization of Mallorca and Minorca, with weasels in the first island carrying a haplotype closely related to one found in Sardinia and those in the second island sharing the same haplotype as populations in Malta and further east in the Mediterranean (Fig. 3). However, better sampling is required to verify that the widespread haplotype CB1 is absent from Mallorca and Sardinia, although it might have become extinct in these islands. At least by the eighth century BC, and for another five centuries until Sardinia and Malta became part of the Roman Republic, Phoenician travel and trade routes to southern Iberia from or via Sardinia included the Balearic Islands and contacts with the Talaiotics, even before Phoenicians established a permanent

settlement in Ibiza in the mid-seventh century BC (Bellard 1995). Those routes brought opportunities for passive transport of the house mouse, which apparently reached the Balearics during the first half of the first millennium BC (Cucchi and Vigne 2006), and for the subsequent introduction of weasels.

The absence of the weasel on Ibiza, given the Phoenician/Carthaginian presence from the mid-seventh to the mid-second century BC, is somewhat puzzling. Ibiza is the only large island (>100 km^2) in the western and central Mediterranean where the weasel is absent (De Marinis and Masseti 2003). The presence of the weasel on Mediterranean islands seems to be related to the number of potential rodent prey species available (De Marinis and Masseti 2003). During the period here proposed for the introduction of the weasel on Mediterranean islands (pre-Roman Iron Age), some of these islands harboured very few rodents other than possibly the house mouse (Cucchi and Vigne 2006; Traveset et al. 2009; Ruffino and Vidal 2010). This constraint, together with other factors such as rodent prey density, availability of suitable non-rodent prey, and demographic or environmental stochasticity (Sax and Brown 2000; Simberloff and Gibbons 2004) determined the success or failure of weasel introductions. Establishment failure or extinction of weasels introduced in antiquity has occurred for instance in Cyprus (Lehmann and Nobis 1979). Noteworthy is the fact that in the Gymnesic Islands the weasel found abundant food in the endemic Lilford's wall lizard *Podarcis lilfordi* and Mallorcan midwife toad *Alytes muletensis*, being apparently responsible for their collapse (Bover and Alcover 2008; Valenzuela and Alcover 2013). Conversely, in Ibiza, no amphibians other than the Balearic green toad *Bufo balearicus* were seemingly present at the time of the Phoenician/Carthaginian colonization (Pinya and Carretero 2011) and weasels do not appear to eat true toads (Sheffield and King 1994; Sidorovich and Pikulik 1997). Also, contrary to the tame Lilford's wall lizard, the Ibiza wall lizard *Podarcis pityusensis* retained its antipredatory behaviour, possibly because it was exposed to greater natural predation pressure by birds before human introduction of mammalian predators (Cooper Jr. and Pérez-Mellado 2012).

Crete

In Crete, contrary to what was inferred for the other Mediterranean islands, and in agreement with Lebarbenchon et al. (2010), the observed mtDNA variation is compatible with introduction from the nearest mainland: haplotype CB38 was closely related to a haplotype found in mainland Greece (CB34), haplotype CB1 was also present in Turkey, and haplotype CB39 was closely related to CB1 (Fig. 3). More samples from mainland Greece are needed to ascertain the absence of the common haplotype CB1, since its presence would fit the hypothesis of introduction exclusively from this area. Weasels in Crete and southern mainland Greece share a 'nivalis' type colour pattern (Bate 1905), which is also present in Turkey (but here the 'vulgaris' type also occurs) (Kasparek 1988; Abramov and Baryshnikov 2000). The microsatellite analyses suggested a greater genetic similarity between Cretan and Egyptian weasels than between the former and those in mainland Greece and Turkey (Figs. 4 and S2), even though the allele frequencies in the latter populations may have been affected by gene flow from neighbouring areas. In any case, this result raises the possibility that introductions from Egypt contributed to the gene pool of the Cretan population. This scenario is contradicted by the absence of shared control region (CR) haplotypes between Egypt and Crete, whereas there are CR haplotypes shared between Crete and mainland Greece or Turkey (Rodrigues et al. 2016). The Levant, where the 'nivalis' coloration type has been observed (Harrison and Lewis 1964), is a possible source of introduction that should be evaluated in future studies. Previous phylogeographic studies have found evidence suggesting that other mammals in Crete were introduced from the Levant (e.g. lesser white-toothed shrew *Crocidura suaveolens*, Dubey et al. 2007; Eurasian badger *Meles meles*, Marmi et al. 2006). The oldest weasel remains from Crete are post-Minoan, i.e. from the eleventh century BC onwards (Jarman 1996; Yannouli 2003). During the Early Iron Age, following the Minoan-Mycenaean period, Dorian Greeks colonized Crete and founded cities, but the Phoenicians also established permanent posts in the south of the island (Shaw 1989; Negbi 1992).

Introductions into the Iberian and Italian Peninsulas

As mentioned above when discussing the origin of Sicilian and Corsican weasels, we found haplotype CB18 in mainland Italy (Tuscany) and mainland Portugal (Alentejo), and haplotype CB12, which differs from CB18 by a single substitution, in mainland Spain (Barcelona). Other than CB12 and CB18 (and CB29, discussed further below), haplotypes found in Iberia and mainland Italy are very different from those found in Turkey, which suggests that southwestern European and Turkish populations have been isolated for a long time. Indeed, while Eastern Europe and Turkey appear to be areas of secondary contact between divergent lineages and/or with multiple refugia (McDevitt et al. 2012), the Iberian and Italian Peninsulas seem to have been isolated from eastern migration (Fig. 3). Accordingly, virtually all haplotypes observed in Iberia and mainland Italy belong to Clade V. The fact that, in contrast, haplotypes CB12 and CB18 either also occur in Turkey or are closely related to haplotypes found there, support the notion that Clade IV was introduced into the Iberian and Italian Peninsulas. The alternative view that the presence of haplotypes CB12 and CB18 in mainland southwestern Europe is the result of ancient natural dispersal from the east is also difficult to reconcile with the absence of multiple descendant haplotypes of CB12 and CB18 in the former region. On the other hand, to the best of our knowledge, we are not aware of any literature mentioning recent introductions of weasels to Iberia or mainland Italy. Given our results and historical knowledge, we believe that the presence of Clade IV haplotypes in the Iberian and Italian Peninsulas is the result of ancient introductions, probably from Corsica or Sicily (Figs. 3 and 5). Direct introductions from the Eastern Mediterranean seem unlikely since this would imply that haplotype CB18 had been sampled repeatedly, by chance alone, in several independent introductions. Likewise, a single simultaneous introduction event from the Eastern Mediterranean to Sicily, Corsica, mainland Italy and Iberia, seems improbable. The singleton CB12 may represent a mutation from haplotype CB18 that occurred after introduction to Iberia. It is true that the timescale of human-mediated introductions, even of those of short-lived organisms and which occurred millennia ago, is likely to be younger than the timescale of substitution in mtDNA; however, mutation is inherently stochastic, demographic expansion after introduction provides more opportunity for mutation, and short-term substitution rates may be measurably higher than phylogenetic-based estimates from the literature (Ho et al. 2007).

The fact that haplotypes found in Sardinia and the Balearics, where Phoenicians had colonies and trading posts involved in the western Mediterranean trade network, were not observed in Iberia and mainland Italy, is seemingly more consistent with weasel translocations into these regions carried out by Greek merchants or migrants coming from Corsica or Sicily. During the sixth century BC, Phocaean contact and trade with Etruria, the ancient region in central Italy that was the land of the Etruscans and which included modern Tuscany, were intense (Holland 1937; Malkin 2011) and, besides Alalia in Corsica, the Phocaeans founded colonies along the eastern Iberian coast (Fig. 5) and established trading relations with the Tartessians of southern Iberia (Bosch-Gimpera 1944; Cabrera 1998; Roller 2006; Miró and Santos 2014). It is noteworthy that, like weasels in Corsica and Sicily, southern Iberian weasels have a 'nivalis' type colour pattern, whereas populations elsewhere on the mainland of Western Europe typically show a 'vulgaris' coat pattern (Abramov and Baryshnikov 2000).

An individual sampled in central Italy (Abruzzo) carried a haplotype (CB29) that was highly distinct from all other Italian haplotypes but closely related to a Cretan haplotype (CB39) in Clade I (Fig. 3). Given this, and based on the same arguments presented above regarding the presence of Clade IV in mainland southwestern Europe, we suggest that haplotype CB29 may have been introduced into Italy from the Eastern Mediterranean. In particular, considering the genealogical affinity of haplotype CB29 and the history of human settlement in the Italian Peninsula, weasels may have been brought by Greek settlers or traders coming to *Magna Graecia*, the coastal Greek colonies in southern Italy, or the Dorian settlements in eastern Italy (Fig. 5). Ancient introductions of weasels to Italy as rodent controllers may be at the origin of the use of this practice by the Romans (Faure and Kitchener 2009).

Weasels on Atlantic islands

Azores

The Azores is a remote archipelago of nine volcanic islands situated in the North Atlantic Ocean that was rediscovered and settled by the Portuguese in the fifteenth century. The weasel is found in the islands of São Miguel and Terceira, while its present occurrence in the island of Faial (Godman 1870) has not been confirmed (Mathias et al. 1998). The origin and time of introduction of the weasel to the Azores are unknown, but the species is mentioned as being present in São Miguel and Terceira in the sixteenth century (Frutuoso 1873) and is assumed to have arrived with the first European colonists (Mathias et al. 1998).

Although there is evidence for knowledge of the Azorean islands in the fourteenth century (Davies 1964), suggestions of human presence before the Portuguese colonization of the archipelago, including theories of Phoenician occupation (Bikai and Bikai 1990), remain archaeologically unsubstantiated or controversial (but see Rodrigues 2015 and Rodrigues et al. 2015). Nevertheless, a recent genetic study of the Azorean house mice (Gabriel et al. 2015) found a remarkable mtDNA similarity between mice on some of the islands and mice from localities in Atlantic northern Europe, which was explained as the result of stowaway introduction during unrecorded visit(s) of Norwegian Vikings to the Azores (Kelley Jr. 1979). Thus, as also suggested by the results of Rodrigues et al. (2015), the Azores may have been visited and explored by mariners familiar with long-distance seafaring long before the Portuguese colonization.

We identified two haplotypes separated by a single substitution, one in São Miguel (CB1) and another in Terceira (CB74). Haplotype CB1 was also found in São Tomé, Minorca, Malta, Crete, Egypt and Turkey. Haplotype CB74 was private, but it may represent a post-introduction mutation from haplotype CB1. Importantly, the haplotypes recovered in the Azores were clearly distinct from all haplotypes detected in the Iberian Peninsula (Fig. 3). The relatively high estimates of probability of membership in the 'northern Mediterranean' cluster for the individuals from São Miguel in the STRUCTURE analysis (Fig. 4b) may be due to homoplasy (e.g. Hinten et al. 2003). The alternative hypothesis that the weasels in São Miguel are also descendants of individuals introduced from

mainland Europe is contradicted by the fact that all three individuals from São Miguel had haplotype CB1, but more specimens are needed to completely rule out this possibility. The observed mtDNA difference between Azorean and Iberian weasels was surprising, given the close demographic and cultural ties between people in the Azores and mainland Portugal. Likewise, the connection between weasels in the Azores and in some western Mediterranean islands, which we inferred to have been introduced by the Phoenicians, is intriguing. The mtDNA results, taken together with the geographical location of these islands and the available historical information, fit with the hypothesis that weasels were introduced to the Azores, sometime in the fifteenth or early sixteenth century, from Minorca. However, as mentioned above, analysis of more samples from Mallorca is needed to confirm the absence of haplotype CB1 there. In the settlement of the Azores, the islands of São Miguel and Terceira were among the first to be colonized and, being among the largest in the archipelago, became the most populated. House mice and black rats invaded the Azorean islands during the early stages of peopling, reaching plague levels in the sixteenth century (Frutuoso 1873; Mathias et al. 1998). Peopling of the Azores in the fifteenth century involved Portuguese and immigrants of different origins (Santos et al. 2003). From the late fifteenth and sixteenth centuries onwards, the Azores exported wheat and sugarcane and became an important port of call and trading post for ships sailing between Europe, Africa and the Americas. From the mid-fourteenth century onwards, Balearic sailors and merchants expanded their exploration and trading activities into the Atlantic, and the Balearic Islands became a major commercial hub (Abulafia 1991). Visits to the newly established colonies in São Miguel and Terceira by traders from the Balearic Islands, aware of the ancient tradition of using weasels for rodent control (Masseti 1995; Faure and Kitchener 2009), may have brought the founders of the Azorean weasel populations.

São Tomé

The island of São Tomé is the largest island of São Tomé and Príncipe, an island nation in the Gulf of Guinea off the western equatorial coast of Central Africa. The islands of São Tomé and Príncipe were apparently uninhabited before the arrival of the

Portuguese in the late fifteenth century, who promoted the rapid settlement of the islands due to their suitability for trade with the African mainland and for sugarcane plantations (Tomás et al. 2002). Associated with this colonization, about a dozen alien mammal species were introduced to these islands, including house mice and black rats (Dutton 1994). It is possible that weasels were introduced to control rodents early in the agricultural development of the islands, since by the first decades of the sixteenth century rodents reached plague numbers in the sugar cane plantations (Dutton 1994; Moore 2010). By the mid-sixteenth century the islands were Africa's foremost exporter of sugar. The value of weasels as rodent catchers is illustrated by Barboza du Bocage's (1895) comment on the objections raised by the farmers in São Tomé to the capture of specimens for study. All the five individuals we analysed had the same haplotype (CB1), which was also the only one present in the Azorean island of São Miguel and clearly separated from those in the Iberian Peninsula. Thus, our results indicate that the weasel in São Tomé was not introduced from mainland Portugal and the mtDNA data are consistent with the hypothesis of introduction from the Azores. This is in agreement with the morphological similarity between weasels in the Azores and São Tomé already noted by Barrett-Hamilton (1904), who also considered that the latter might have been derived by introduction from the former. Importation from the Balearic Islands is also a possibility, but São Tomé, unlike the Azores, was outside the main routes of Balearic Atlantic trade.

Conclusions

The least weasel is a Holarctic mustelid carnivore that has been introduced to several islands in the Mediterranean Sea and Atlantic Ocean. This study represents the first broad genetic analysis of the potential origin and introduction history of these insular populations. Among the Mediterranean island weasels, a major genetic partition between those in Sicily-Corsica and Malta-Sardinia-Balearics is best explained by a dual colonization originating from the Eastern Mediterranean. Given the correspondence between the observed genetic subdivision and the differential geographic distribution of Greek and Phoenician settlements and influence during the last millennium

BC in these islands, we propose that weasels may have been introduced into the first group of islands by the Greeks and into the second group by the Phoenicians. There is strong support that the commercial and demographic expansions of Phoenicians and Greeks allowed the colonization of the western Mediterranean islands by the house mouse (Cucchi and Vigne 2006), and texts from Classical Antiquity show evidence that weasels were used for rodent control (Frank 1985; Masseti 1995; Faure and Kitchener 2009). Thus, weasels may have been intentionally introduced to the islands with the aim of controlling commensal rodents following their inadvertent introduction. We also detected haplotypes in the Iberian and Italian Peninsulas that are likely non-native and that we suggest may be derived from weasels introduced by Greek immigrants or merchants during the colonial and commercial expansion of ancient Greece in the Mediterranean region. Despite the role of the Portuguese in the settlement of the Azores and São Tomé, weasels in these islands showed no genetic affinities with those in mainland Portugal. Instead, the most parsimonious explanation for the origin of the Atlantic weasels appears to be introduction from the Balearic Islands to the Azores, sometime in the fifteenth or early sixteenth century, to control rodents, and soon after from the Azores to São Tomé, to fulfil the same role in sugarcane plantations there. This study provides working hypotheses for future research. Analysis of Levantine populations and of additional material from mainland Greece, Sardinia, the Balearics and the Azores, as well as microsatellite data for the island populations not genotyped here, is warranted to evaluate proposed translocation scenarios, identify source populations in the Eastern Mediterranean, and reconstruct introduction routes.

We believe that the spread of the least weasel in the Mediterranean and Atlantic islands can be a useful model for invasion biology research. The least weasel is the most widespread wild carnivore on the islands of the Mediterranean basin (Masseti 1995), and it has been described as having a devastating effect on the native fauna of the Balearic Islands (Bover and Alcover 2008). The least weasel remains little studied on the other islands included in this investigation, especially concerning the historical and current impacts on the native biodiversity and on ecological processes, as well as on its interactions with other alien species (e.g. Dutton 1994). Besides the conservation

and management viewpoint, these isolated island weasel populations, exposed to different environmental conditions and species assemblages, offer opportunities to address research questions in ecology and evolutionary biology (Sax et al. 2007; Dlugosch and Parker 2008; Keller and Taylor 2008). Our work provides inferences on the origin and age of the introduced weasel populations, fundamental data for future ecological and evolutionary investigations in this study system.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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