

Cyprus as an ancient hub for house mice and humans

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Abstract

Aim: The distribution of the western house mouse (*Mus musculus domesticus*) around the world has been strongly influenced by the movement of humans. The close association between the house mouse and human phylogeography has been primarily studied in the peripheral distribution of the species. Here, we inferred the complex colonization history of Cyprus, situated close to the centre of the house mouse distribution and one of the first European islands to be colonized by the species. We investigated the resulting complexity of house mouse population genetics as well as considering the value of the house mouse as a bioproxy for studying modern human movement.

Location: The study was carried out on Cyprus.

Methods: The analysis was performed using 221 new mitochondrial D-loop sequences and assessed the fine-scale population genetic structure using 18 autosomal microsatellite loci from 191 modern house mice specimens.

Results: We found a high genetic variability in the island that is illustrated by the presence of individuals from 9 of the 11 previously identified house mouse haplogroups for the D-loop, reflecting the hub-like nature of the island to mice. Two main waves of mouse introductions were tentatively identified based on coalescent and mismatch analysis. The first is apparently related to the Bronze Age expansion and the second one to more recent human movements. Cyprus represents an island with high complexity due to different introductions related to human transport and activity.

Main conclusions: The dispersal of mice along with humans has left a complex footprint on the island with two main waves of introductions suggested. The phylogeography of the house mouse on Cyprus is in concordance with the complex human colonization history of the island and validates the use of the house mouse as a proxy to study human migration.

KEYWORDS

bioproxy, Cyprus, house mouse, human, mtDNA, phylogeography

Demetra Andreou and Jeremy S. Herman contributed equally to this work.

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1 | INTRODUCTION

Knowledge of human history has traditionally been inferred from documentary evidence, material artefacts and human remains. Remains from animals, particularly domesticated and commensal species, have also provided information about the human cultures with which they were associated. Recently, these approaches have been enriched by the use of genetic data from modern and ancient human DNA (Haak et al., 2010; Hervella et al., 2012; Lazaridis et al., 2017; Malmström et al., 2015). Many species have been linked with human migration and other anthropogenic activities and therefore may reflect a similar phylogeographic pattern (e.g., Heintzman et al., 2016; Herman et al., 2017; Jones, Eager, Gabriel, Jóhannesdóttir, & Searle, 2013; Thomson et al., 2014). These organisms are considered bioproxies or “living artefacts” of human migration history and can complement our knowledge of the archaeology and phylogeography of humans (Jones et al., 2013).

Several domestic species have already been used as bioproxies for human movement because of their close association with our species. For example, the spread of Neolithic culture to Europe has been corroborated by the domestication process of pigs (Larson et al., 2007) and goats (Naderi et al., 2007). Rats (Matisoo-Smith & Robins, 2004; Naderi et al., 2007; Wilmshurst, Anderson, Higham, & Worthy, 2008) and cats (Koch, Algar, Searle, Pfenninger, & Schwenk, 2015) have also been used as commensal and domestic bioproxies to track more recent movements. Furthermore, pathogens and parasites can also be used as proxies that show histories of colonization and demography (Jones et al., 2013).

The western house mouse (*Mus musculus domesticus*) has been a commensal species since the beginning of stored grain (Weissbrod et al., 2017). Humans and mice have migrated together for about 12,000 years (Bonhomme & Searle, 2012) travelling by land but also by boat (Cucchi & Vigne, 2006). The colonization history of the house mouse has been demonstrated to be informative in the study of the human population who transported them (Förster et al., 2009; Gabriel, Mathias, & Searle, 2015; Hardouin et al., 2010; Jones et al., 2012, 2013; Searle et al., 2009). One of the main examples of this close association between human and house mouse has been demonstrated for the Vikings. Viking mouse haplotypes were found on Madeira, suggesting a possible Viking visit to the island, unrecorded in historical records (Förster et al., 2009; Gündüz et al., 2001; Searle et al., 2009). Among subfossil house mouse remains on Madeira found in 2010, radiocarbon dates of a house mouse mandible predate Portuguese colonization and match the Viking hypothesis (Rando, Pieper, & Alcover, 2014). In this context, house mouse phylogeography has been a powerful complementary tool to aid archaeologists and historians to understand recent human movement.

Due to its location and richness in natural resources, Cyprus provides the key context for understanding the dynamics of human migration and trade, from mobile foragers to early farmers and later regional polities, sedentism and seafaring, together with the associated sociocultural changes in the Eastern Mediterranean (Knapp,

2013). Recent excavations (e.g., Simmons & Mandel, 2007) confirm early human activity in the Late Epipalaeolithic, identifying sites that suggest seafaring foragers and fishermen made seasonal return journeys from the Levantine shores to Cyprus between 11,000 and 9,000 cal BC to exploit local terrestrial and marine resources. The earliest Neolithic occupation and permanent settlement on Cyprus goes back to c. 9,000 cal BC, showing evidence for cultivated or even domesticated cereals and pulses virtually contemporaneous with earliest appearances in the Levant and Anatolia. Triggered by increasingly unstable environmental conditions on the mainland, this represents the “first successful overseas migration of farmers in the Mediterranean” (Knapp, 2013), which would have also entailed transport of plants and animals (Vigne et al., 2014). In the course of the prehistoric Bronze Age, regional interaction through seaborne trade contacts between Cyprus, Anatolia, and the Aegean increased, to become even more established, and extended to Egypt, during the protohistoric Bronze Age (Knapp, 2013).

The Mediterranean basin is an area of considerable importance in understanding the close relationship between humans and the western house mouse. The initial commensalism of the house mouse began in the Near East (Cucchi, Vigne, & Auffray, 2005). The range of house mice may have expanded slowly in the Near East by natural dispersal, but they had the potential to make rapid progress across the Mediterranean on boats (Cucchi, 2008). The earliest evidence of such human-mediated transport is the presence of house mouse remains at an archaeological site from the Early Preceramic Neolithic on the island of Cyprus (late 9,000 and 8,000 BC) (Cucchi, Vigne, Auffray, Croft, & Peltenburg, 2002).

Although the house mouse arrived in Knossos, Crete, during the Bronze Age (2,500–1,000 BC), it is only during the Iron Age (1,000 BC–300 AD) that the house mouse spread throughout the western Mediterranean basin and to Western Europe (Cucchi et al., 2005). Around 1,000 BC, the Phoenicians were the most prominent traders and they are therefore the most likely mediators of these mouse expansions (Bonhomme et al., 2011). House mice feed on stored grain but even though agriculture spread over much of Western Europe during the Neolithic, the species did not spread out of the extreme east of the Mediterranean at that time (Cucchi et al., 2005) probably because: (a) there was limited human maritime exchange between the eastern and western Mediterranean at this early stage, and (b) there was competition with the native wood mouse (*Apodemus sylvaticus*), which may also have been commensal in small Neolithic settlements (Cucchi et al., 2005), such as Skara Brae, Orkney (Romaniuk et al., 2016).

Being the first island in the Mediterranean colonized by mice makes Cyprus an interesting case study. Indeed, records from the archaeological site of Mylouthkia (situated close to Paphos on the west of the island) showed that the first introduction of the house mouse occurred during the Neolithic period, approximately 8,000 BC (Cucchi et al., 2002). The house mouse colonization history should therefore reflect the ancient migration of Neolithic people, Mycenaean Greeks, Phoenicians, Romans, Franks, and Ottomans. The substantial genetic diversity found in the mouse mitochondrial D-loop,

with seven differentiated haplogroups described in Cyprus, is a consequence of the island being at a maritime crossroads, with a consequently complex colonization process shaped by many introductions of house mice from several origins (Bonhomme et al., 2011; Macholán et al., 2007). Human trade and migration related to secondary colonizations can also be inferred through nuclear markers, such as microsatellites, through data on genetic relationship of populations and levels of genetic diversity (Hardouin et al., 2010; Jones, Jensen, et al., 2011).

The purpose of this study is threefold: (a) to establish the different sources of house mice that colonized Cyprus, using the mitochondrial D-loop, (b) to establish the timing of these migrations and (c) to investigate the population structure of the house mouse on the island using microsatellites. We then compare these results with the previously known pattern of human migration to and from the island and consider the value of the house mouse as a bioproxy for studying modern human movement.

2 | MATERIALS AND METHODS

2.1 | Sample collection

A total of 191 house mice (*Mus musculus domesticus*) were collected in Cyprus in 2013 and 2015 (Table S1.2 in Appendix S1). The mice were sampled by trapping at 27 sites distributed across the island (Figure 1), and an additional 33 samples were collected from Patras, Greece, due to the historical link between Cyprus and Greece. Farms and agricultural settings were targeted. The sampling follows the scheme from Ihle, Ravaoarimanana, Thomas, and Tautz (2006), and was made in order to minimize the sampling of highly related mice from the same family. All these samples were collected following local regulations for field collection of small mammals.

2.2 | Mitochondrial DNA sequencing and analysis

Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen), following manufacturers' instructions. Mitochondrial D-loop (= control region) sequences of 894 bp were generated using the primers and protocol previously described in Hardouin et al. (2010).

To infer the phylogenetic relationships of *M. m. domesticus* in the Mediterranean basin, northern Europe and the Near East, our dataset was combined with 1,319 previously published sequences downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/). The sequences were aligned using CODONCODEALIGNER 6.0.2 (CodonCode Corporation, Dedham, MA, USA), BioEDIT 7.2.5 (Hall, 1999) and SEAVIEW 4.5.4 (Gouy, Guindon, & Gascuel, 2010). A list with references of all the sequences used in the present study is available in the Appendix S1 in Supporting Information, Table S1.1.

There has been some divergence in the earlier literature regarding the assignment for the main haplogroups described for *M. m. domesticus* with two main nomenclatures independently developed (Bonhomme et al., 2011; Jones, Jóhannesdóttir, Gündüz, Richards, &

Searle, 2011). There is an almost exact correspondence between the main clades described (1 = C1; 2 = C; 3, 5, 9 = B; 4 = F; 7 = D; 8 = D1; 10 = A; 11 = E) (Bonhomme & Searle, 2012); the nomenclature of Bonhomme et al. (2011) was primarily used in this study, with occasional reference to the nomenclature by Jones, Jóhannesdóttir, et al. (2011).

Haplotype diversity and nucleotide diversity were calculated using DNASP 5.10.1 (Librado & Rozas, 2009). The substitution model, TN+G, was selected using JMODELTEST, 2.1.7 (Darriba, Taboada, Doallo, & Posada, 2012), based on the Akaike information criterion (AIC, cAIC) and the Bayesian information criterion (BIC). This model was used in subsequent phylogenetic and population genetic analyses. The phylogenetic tree was calculated using MRBAYES 3.2 (Ronquist et al., 2012) with a MCMC for two million generations, with the first 25% discarded as burn-in. *Mus musculus castaneus* (AF088879) and *Mus musculus musculus* (U47532) were used as outgroups. The aligned haplotypes were used to construct a NeighbourNet network with the hypothesis-poor algorithm of Huson and Bryant (2006) implemented in the 'Splitstree' package (4.10) with P distance as a default setting.

To gain insight into colonization of Cyprus by house mice, we conducted demographic analyses based on the mismatch distribution (MMD), using DNASP 5.10.1 (Librado & Rozas, 2009), and by coalescent analysis, using BEAST, 2.3.2 (Bouckaert et al., 2014). Further details are available in Appendix S2 in Supporting Information.

All the Cypriot and Greek house mouse mitochondrial D-loop sequences generated in the course of the study were deposited in GenBank (accession numbers MG937349–MG937536 and MG950367–MG950397).

2.3 | Microsatellite typing and analysis

All the Cypriot and Greek house mice were genotyped for 18 unlinked autosomal microsatellites as in Hardouin et al. (2010). The microsatellite data were analysed and scored with GENEMAPPER (Applied Bioscience). This dataset was combined with Hardouin et al. (2010) and Linnenbrink et al. (2013) in order to compare the Cypriot data with Western European and Iranian populations. In order to calibrate microsatellite allele size of the two datasets, subsamples of individuals from the previous studies were genotyped with the new samples, without any discrepancies observed. The heterozygosity and the mean allele number per locus were calculated using GENETIX 4.03 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004). Allelic richness was calculated using the rarefaction method available in HP-RARE (Kalinowski, 2005).

A discriminant analysis of principal components (DAPC) (Jombart, Devillard, & Balloux, 2010) was performed using the R-package 'adegenet' (Jombart, 2008); <http://www.r-project.org/>). This multivariate analysis derived the probability of individual membership in each different group. The software covered a range of possible clusters representing the total number of populations in the dataset. Principal components were retained as predictors for discriminant analysis in the individuals studied.

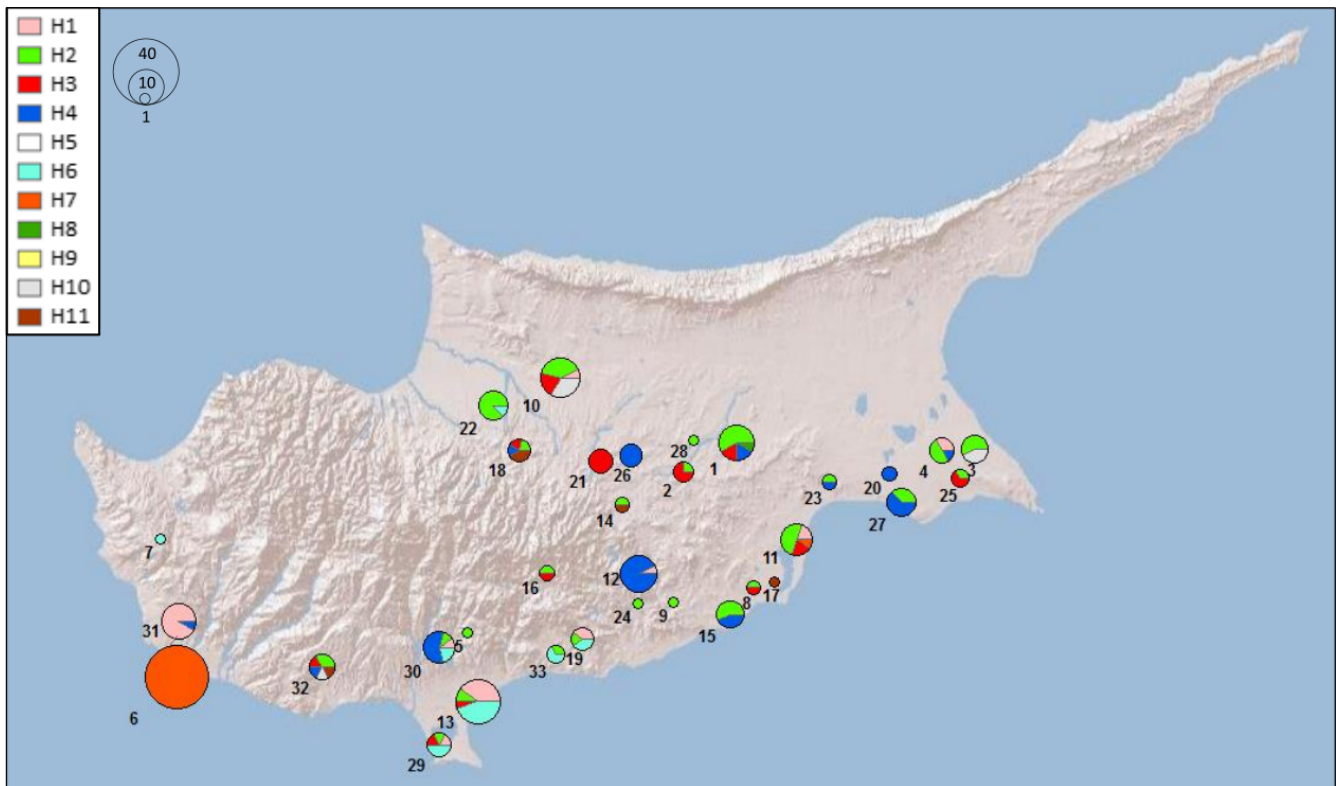


FIGURE 1 Map of *Mus musculus domesticus* sampling localities (numbered) on Cyprus and frequencies of each of the 11 different haplogroups (H1–H11) described by Bonhomme et al. (2011). These data incorporate new samples described here and those from Cucchi et al. (2006) and Bonhomme et al. (2011). 1. Athienou, 2. Dali, 3. Deryneia, 4. Frenaros, 5. Gerasa, 6. Geroskipou, 7. Kathikas, 8. Kiti, 9. Kofinou, 10. Kokkinotrimithia, 11. Larnaka, 12. Lefkara, 13. Limassol, 14. Lythrodontas, 15. Mazotos, 16. Melini, 17. Meneou, 18. Mitsero, 19. Monagroulli, 20. Ormideia, 21. Pera, 22. Peristerona, 23. Pyla, 24. Skarinou, 25. Sotira, 26. Tseri, 27. Xylophagou, 28. Agios Sozomenos, 29. Akrotiri, 30. Lemesos, 31. Paphos, 32. Post Geri, 33. Pyrgos. The size of the pie chart is related to sample size.

To address the differences within the island, *STRUCTURE* (Pritchard, Stephens, & Donnelly, 2000) was used. The software implemented a Bayesian clustering analysis. To find the possible number of clusters (K) into which our data can be divided, 10 runs for each cluster were performed and the likelihoods were recorded. To draw the *STRUCTURE* diagram, *CLUMPP* (1.1.2 (Jakobsson & Rosenberg, 2004)) and *DISTRUCT* (Rosenberg, 2004) software was used.

3 | RESULTS

3.1 | Phylogenetic analysis

A NeighbourNet network was drawn using 529 haplotypes derived from the 1,540 sequences, comprising our 221 new sequences (189 from Cyprus and 32 from Greece) and previously published data (Figure 2a). Figure 2a presents the 529 haplotype network showing 11 identifiable haplogroups. This analysis helped the haplogroup identification in the Bayesian phylogenetic tree (Figure 2b) which are defined based on the network analysis. The 11 haplogroups showed in this study correspond exactly with the ones described in Bonhomme et al. (2011).

The 189 Cypriot sequences collected for this study can be seen in Figure 1. A total of 32 haplotypes, belonging to 9 of the 11 described

haplogroups, were found in Cyprus (Figure 1). The haplotype diversity (h) and nucleotide diversity (π) were calculated for all the Cypriot samples together, giving values of 0.93 (h) and 0.00981 (π), respectively. The high values probably reflect the presence of unrelated mitochondrial DNA (mtDNA) sequences due to multiple house mouse introductions. This result is consistent with the high variability previously described on Cyprus (Bonhomme et al., 2011; Macholán et al., 2007).

A total of 65% of the Cypriot samples belong to three major haplogroups (H2, H4, and H7; i.e., clades C, F, and D of Jones, Jóhannesdóttir, et al. (2011)). H2 is high in frequency around the Mediterranean basin and in the Near East, whereas H4 is present in 17% of the Cypriot samples and was previously associated with the British Isles and Norway (Searle et al., 2009). H7 was only found on two locations in Cyprus, Geroskipou, and Larnaka (only one individual), (Figure 1; Table S1.2 in Appendix S1); however, it is distributed around the Mediterranean, with high frequency in North Africa, and also on the European continent (Figure 3).

3.2 | Demographic analysis and dating

In Cyprus, demographic expansions of haplogroups H1, H3, H4, and H6 began c. 500 years ago, according to the Bayesian skyline plots (BSPs; Figure S1.1 in Appendix S1) and unimodal MMDs (Figure S1.2

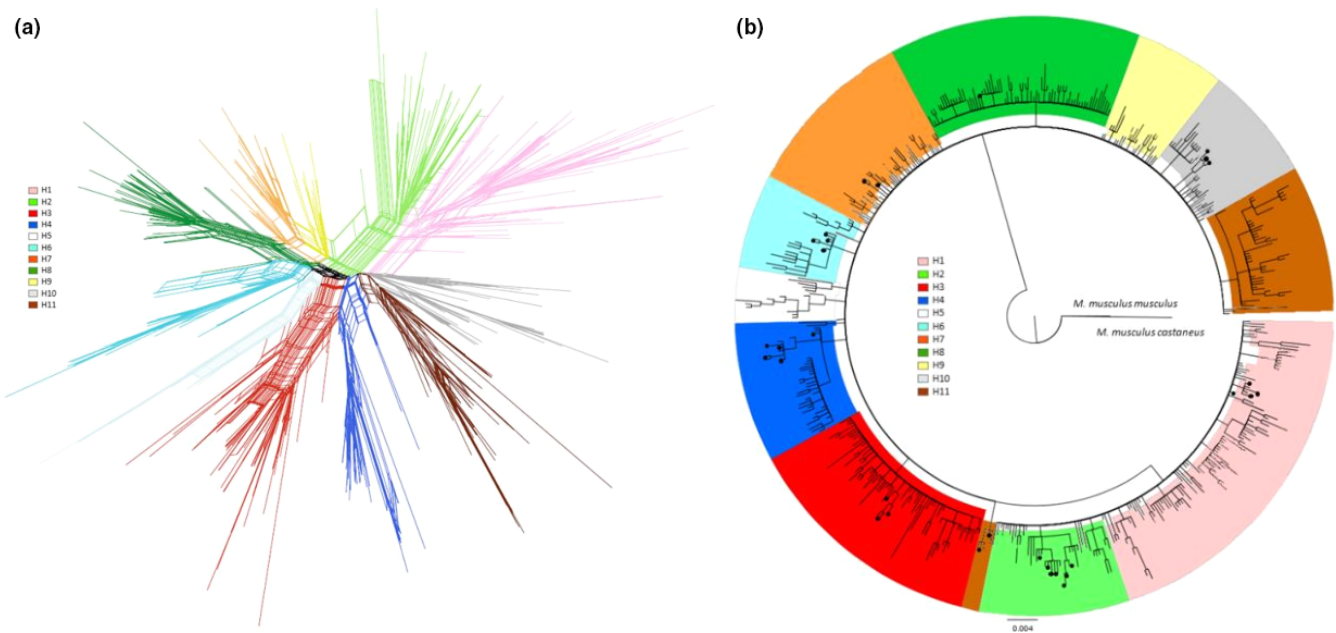


FIGURE 2 *Mus musculus domesticus* D-loop genealogy. (a) NeighbourNet network for the 529 haplotypes described from 1,540 individual mitochondrial D-loop sequences. (b) Bayesian tree generated with MrBayes for 529 house mouse D-loop haplotypes described here and previously published. The numbered haplogroups defined by Bonhomme et al. (2011) are displayed by different colours; some of these haplogroups are paraphyletic in our analysis. Haplotypes present on Cyprus are represented with black dots

in Appendix S1). The expansion times from the MMDs with two generations give the closest correspondence with the timings obtained with the coalescent model, which is measured in real time, rather than generations (Figure 4; Table S1.3 in Appendix S1). The respective tMRCA for these haplogroups are somewhat earlier, up to c. 900 years ago (Figure S1.1 and Table S1.3 in Appendix S1), but these latter dates refer to the coalescence of the haplogroup members from Cyprus within the overall population, that is the time at which their ancestors diverged from the remainder of the haplogroup, rather than the colonization or onset of demographic expansion on the island.

Demographic expansion of haplogroup H2 began about 1,400 years ago, according to both the skyline plot (Figure S1.1 in Appendix S1) and unimodal MMD (Figure S1.2 in Appendix S1) with two generations per year (Table S1.3 in Appendix S1), while the coalescence time was again earlier, c. 3,700 years. The coalescence of haplogroup H7 was c. 3,200 years ago (Figure S1.1 and Table S1.3 in Appendix S1), but this is due to the presence of a single divergent sequence from Larnaka, whereas the remaining 39 sequences are all identical and from Geroskipou (Figure 1; Table S1.2 in Appendix S1). This pattern of variation precludes the use of the MMD to estimate the timing of the demographic expansion and also confounds the skyline model (Figure S1.1 in Appendix S1); however, the MMD (Figure S1.2 in Appendix S1) and the Bayesian genealogy (not shown) are consistent with recent introductions of this haplogroup to Cyprus rather than the tMRCA of c. 3,000 years ago. The coalescence (tMRCA) of haplogroup H10 was about 2,400 years ago but the demographic expansion did not begin until c. 2,000 years ago, according to the MMD with two generations per year (Figure 4; Table S1.3 in Appendix S1). The fit to this model was poor (Figure S1.2 in

Appendix S1) and the coalescent model did not recover any signal of expansion (Figure S1.1 in Appendix S1), perhaps due to the rare appearance of this haplogroup in our sample (nine specimens). The coalescence and onset of demographic expansion for haplogroup H11 are recent, within the last 1,000 years, according to the Bayesian genealogy and mismatch models (Figure 4; Table S1.3 in Appendix S1) and although the latter appears to fit well (Figure S1.2 in Appendix S1), the skyline model did not recover any demographic change (Figure S1.1 in Appendix S1), presumably due to the small sample size of only five sequences. Only one sequence was attributed to haplogroup H8, precluding further analysis.

Tajima's *D* values were negative for all haplogroups except H10 (Table S1.3 in Appendix S1), although only one was statistically significant (H7), indicating an excess of rare nucleotide site variants compared to what would be expected under a neutral model of evolution. An excess of low frequency polymorphisms relative to expectation indicates a population size expansion (Tajima, 1989). All the haplogroups, except H10, showed evidence of recent expansion. The more sensitive Fu 's F_S also indicated expansion, except for H7, H10, and H11. This result is in agreement with BSP analysis; however, it might be due to subsequent replacement, given the rarity of H10 and H11 in the analysed sample, as mentioned before (H10 = 9 specimens; H11 = 5 specimens).

3.3 | Population structure on Cyprus

A total of 18 microsatellites were analysed for all newly collected samples (Table S1.4 in Appendix S1). Heterozygosities as well as mean numbers of alleles were calculated for Cyprus. These values

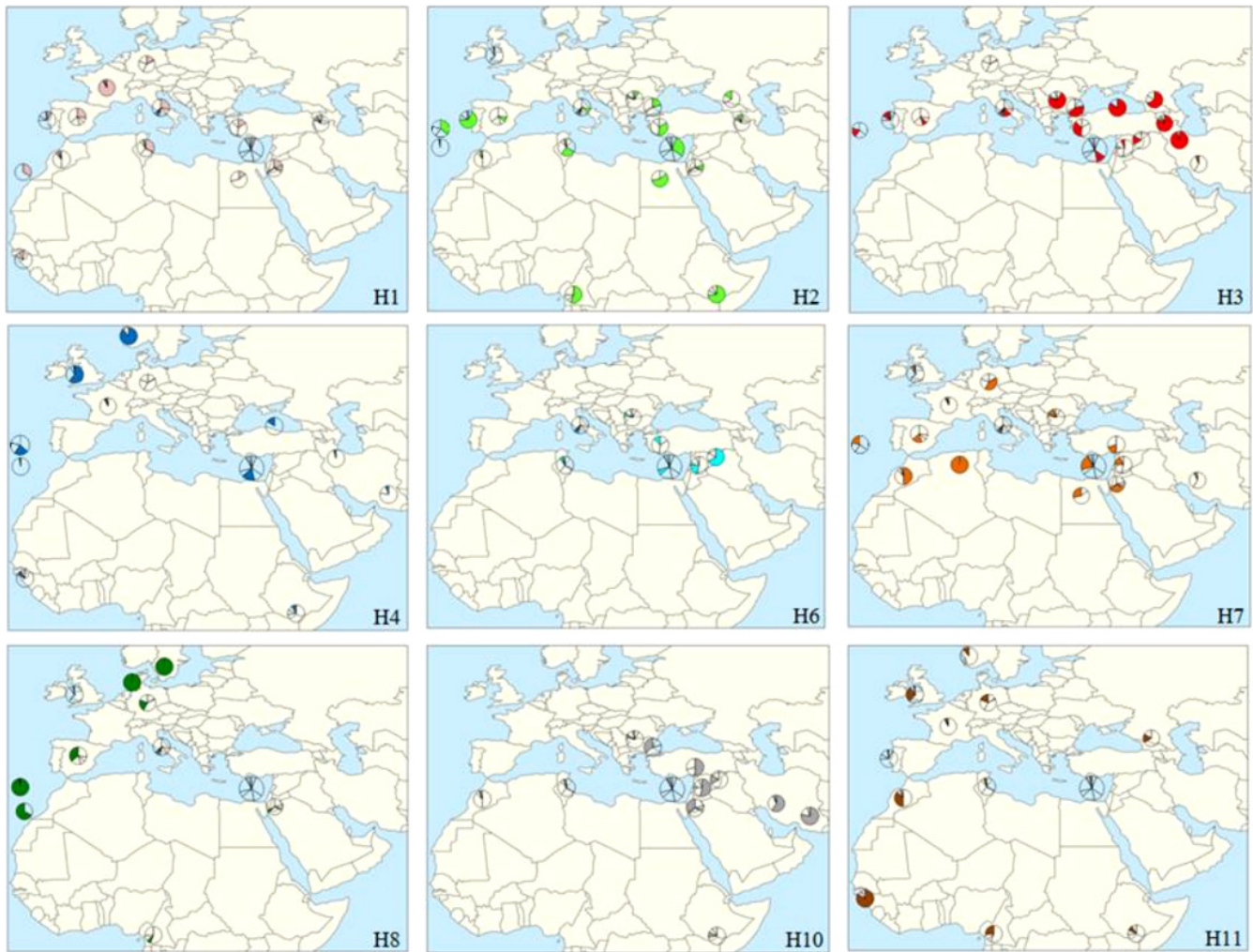


FIGURE 3 Geographical distributions in the Mediterranean and nearby areas for all the *Mus musculus domesticus* of all the D-loop haplogroups identified in Cyprus and constituent haplotypes. The pie charts display the proportion of individuals with the main haplogroups found on Cyprus

were compared with previous data from Hardouin et al. (2010) and Linnenbrink et al. (2013) to compare the Cypriot genetic diversity to that recorded for European and Iranian populations (Table 1). Those three datasets were calibrated using samples from Hardouin et al. (2010) that were resequenced. Mice from Cyprus displayed a very high observed heterozygosity (0.73) when compared to continental European populations (France, Germany, and Greece; Table 1). The genetic diversity found in Cyprus is comparable to that in Iran (expected heterozygosity 0.89, average number of alleles per locus 15.1—see Table 1). The two relatively recently founded populations of Cameroon (0.48) and Kerguelen (0.44) displayed low genetic diversity as expected (Table 1) (Hardouin et al., 2010; Ihle et al., 2006). The mean number of alleles per locus varied among localities across Cyprus from 1.38 to 8 with an overall mean of 4.06 (Table S1.4 in Appendix S1). The values for the expected heterozygosity within locations are similar to those from Western Europe, for example Cologne-Bonn, Germany (0.85), Massif Central, France (0.86), and Patras, Greece (0.83) (Table 1). The observed heterozygosity values

found on other islands are lower than on Cyprus (La Palma (0.75), Madagascar (0.67), Kerguelen (0.44), or Gough Island (0.70) (Bonhomme et al., 2011; Duplantier, Orth, Catalan, & Bonhomme, 2002; Gray et al., 2014; Hardouin et al., 2010).

Seven different clusters ($K = 7$) were identified in the DAPC (Figure S1.3a in Appendix S1). All the continental populations from France, Germany, Greece, and Iran clustered together, for axis 1 and 2 representations. The population from Cameroon formed a separate cluster, probably due to the relatively recent colonization event of this country by the house mouse. The Cyprus population also formed its own cluster, as expected for an island, but is more closely related to the European and the Iranian populations than the one from Cameroon (Figure S1.3a in Appendix S1).

The population structure on Cyprus was also investigated using DAPC (Figure S1.3b in Appendix S1). Four clusters ($K = 4$) were identified. The main cluster is composed by the populations collected in 22 locations. The specimens from Geroskipou formed a separate cluster, possibly because the mice were collected in a restricted

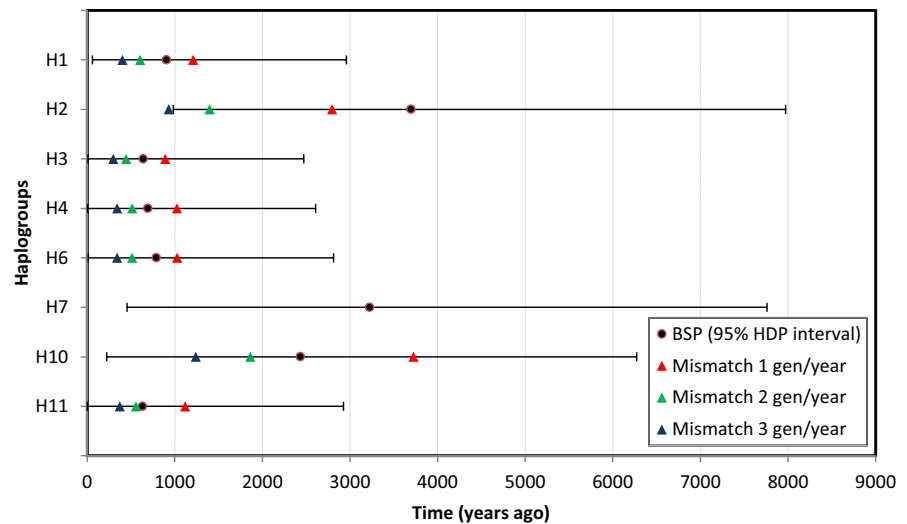


FIGURE 4 A summary of dates inferred from molecular data, for each *Mus musculus domesticus* haplogroup on Cyprus. Onset of demographic expansion estimated from mismatch distributions (MMD), using the mutation rate from Förster et al. (2009) and assuming one, two, or three generations per year. Median tMRCA, from Bayesian genealogy sampling in BEAST 2.3.2, is shown together with its 95% HPD limits

geographic area. Two other clusters were found, the first one is formed by specimens in Skarinou and Lefkara which are geographically close (8 km—Figure 1), which might explain the pattern. The second cluster is formed by Pyla and Gerasa although these populations are geographically distant (68 km—Figure 1). This pattern could be explained by a putative direct connection between the locations or as an artefact, due to the small sample size at both locations (Pyla = 2 specimens and Gerasa = 1 specimen).

A STRUCTURE analysis was performed on Cyprus to investigate population structure on the island. A value of $K = 4$ was also found, in accordance with the DAPC (Figure S1.3b in Appendix S1). The most differentiated subpopulation is Geroskipou. Limassol and Monagroulli formed a second cluster, Lefkara and Pera a third one, all the rest of the island clustered together (Figure 5). In order to investigate the population structure further, we decided to remove individuals sampled in the outlier population of Geroskipou. In this scenario, $K = 9$ was found. Locations like Limassol, Pera, Pyla, and Tseri form separate clusters. The rest of the populations were more admixed, probably reflecting high gene flow across the island.

4 | DISCUSSION

4.1 | Multiple mouse colonization events

The phylogenetic analyses revealed the presence of nine D-loop haplogroups on Cyprus out of the 11 haplogroups described for the western house mouse by Bonhomme et al. (2011). All five clades recognized in the alternative nomenclature scheme of Jones, Jóhannesdóttir, et al. (2011) were found on Cyprus. This mitochondrial diversity suggests a complex scenario with multiple colonization events. This result was expected, as Cyprus was the first island to be colonized by house mice in the Mediterranean basin (Cucchi et al., 2002). Due to its location in the eastern Mediterranean, Cyprus was a centre of commercial trade and this could have led to the high number of house mouse haplogroups found. House mouse

populations on islands are considered to be resilient to new introductions (Hardouin et al., 2010), suggesting that there were potentially many more introductions of house mice than are apparent from these nine possible successful colonization events. It is also likely that some of the haplogroup populations are derived from more than one introduction as well, as multiple colonization events are already implied by the presence of individuals from the different haplogroups. The signature of the founding females will generally be kept in the matrilineal line, being rare for invading females to successfully integrate into an existing population (Bonhomme & Searle, 2012) making mtDNA a good signature of founding females and providing an indicator of human exchanges (Jones et al., 2013).

The molecular dating suggests that there may have been two main waves of colonization (Figure 4; Table S1.3 in Appendix S1), given that the tMRCA and expansion dates from the MMDs for the haplogroups fall into two groups of broadly similar dates. However, the 95% HPD ranges in the coalescent analyses are wide and there is considerable overlap between them, so this suggestion must be treated with caution. Furthermore, it is important to bear in mind that the tMRCA represents the estimated coalescence times for the haplogroup members from Cyprus, but their divergence from the remainder of the respective haplogroup might have occurred before the colonization of Cyprus. This could be the case if members of the haplogroup successfully colonized Cyprus on more than one occasion, or this variability was present among the original colonists.

Nevertheless, the presence and timing of these two putative waves of colonization does seem plausible in the context of human history. The earlier wave, represented by members of haplogroups H2 and H10, dates to c. 2,400–3,700 years ago, according to the coalescent genealogy sampling (Figure 4; Table S1.3 in Appendix S1). These two haplogroups are present in the Near East (Figure 3), where *M. m. domesticus* originated, so this earlier colonization is plausible. The time of introduction coincides with the Bronze Age or Phoenician cultures, when the volume of trade may have increased in the eastern Mediterranean. According to the molecular dating,

TABLE 1 Population genetic parameters for the 18 microsatellite loci typed in *Mus musculus domesticus* on Cyprus and other localities (previous studies; see text). N = number of individuals, H_{exp} = expected heterozygosity, H_{obs} = observed heterozygosity

Countries/Island group	Location	N	H_{exp}	H_{obs}	Mean number of alleles	Allelic richness
Antipodes Island	Antipodes Island	18	0.44	0.51	3.06	2.79
Auckland Island	Auckland Island	13	0.42	0.39	3.17	2.93
Cameroon	Kumba	46	0.61	0.48	6.67	4.3
Cyprus	Cyprus	191	0.84	0.73	15.89	7.66
Falkland Islands	New Islands	12	0.44	0.41	3.20	3.09
France	Anjou	20	0.81	0.62	9.39	7.28
France	Divonne les Bains	12	0.80	0.60	8.44	7.57
France	Espelette	38	0.77	0.60	9.94	6.85
France	Louan-Villegruis	12	0.76	0.67	6.83	6.39
France	Severac le Château	65	0.86	0.73	13.11	8.16
France	Nancy	15	0.80	0.66	8.28	7.2
Germany	Cologne-Bonn	58	0.85	0.61	12.83	8.13
Germany	Schömborg/Langenbrand	18	0.79	0.55	8.06	6.82
Greece	Patras	33	0.83	0.67	10.61	7.52
Iran	Ahvaz	46	0.89	0.81	15.17	9.14
Kerguelen	Cochons/Cimetière	97	0.37	0.35	2.78	2.24
Kerguelen	Port-aux-Français	41	0.48	0.44	4.10	3.06
Macquarie Island	Macquarie island	40	0.42	0.38	3.33	2.61

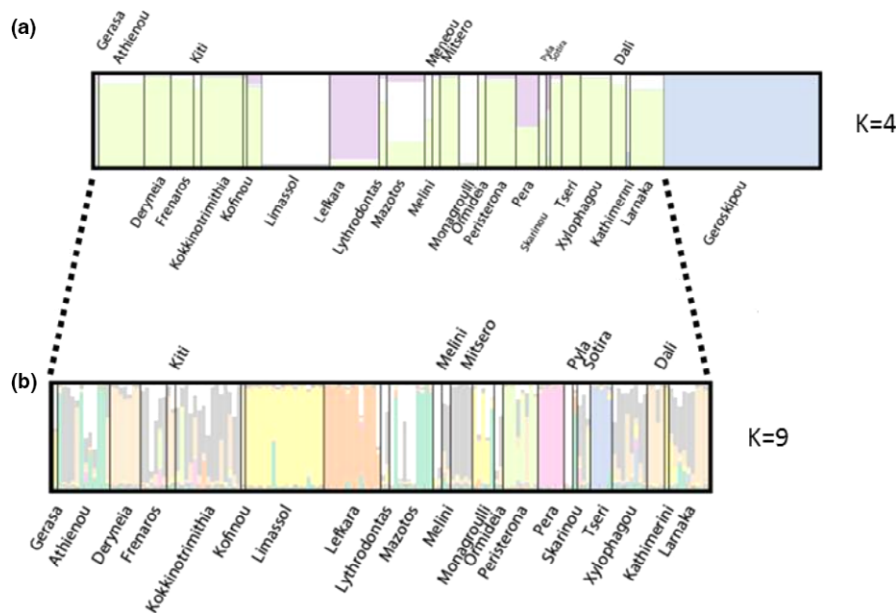


FIGURE 5 STRUCTURE analysis for the different locations across Cyprus. (a) The results for the STRUCTURE analysis with $K = 4$ are shown, represented by different colours. (b) STRUCTURE analysis without Geroskipou ($K = 9$), represented by different colours. Each vertical bar represents a single individual, as well as the likelihood to belong to a given population group

their introduction was much more recent than the Neolithic, whereas there is evidence from the zooarchaeological record that the house mouse was present in Cyprus already by c. 8,500 cal (Cucchi et al., 2002), suggesting that either the genetic signature of these earlier colonists has been replaced by that of more recent

introductions or that current sampling does not cover the full range of mitochondrial genetic variation on Cyprus.

The demographic expansion of haplogroups H2 and H10 was delayed until much more recently, c. 1–2,000 years ago (Figure S1.1 and Table S1.3 in Appendix S1). Once again, the 95% HPD margins



are broad, due to the nature of the coalescent modelling and the limited resolution of the data, therefore the signal and timing of this expansion must be treated with caution. Assuming that the dates are correct, the difference may be due to standing genetic variation within a single introduced population, more than one introduction from the source population, or delayed demographic expansion following introduction at the date of coalescence. The last of these could relate to changing ecological factors, such as increasing agriculture or urbanization, but is the least likely explanation, given the presence of clear splits within the trees inferred for each haplogroup.

The second wave of colonization involved members of five haplogroups (H1, H3, H4, H6, and H11) and coalescent genealogy sampling dates this to the last millennium, from c. 1,000 years ago (Figure 4; Table S1.3 in Appendix S1), although once again the uncertainty in this date should be acknowledged. If the date is accepted, this wave of introductions might be explained by the level of trade across the Mediterranean by that time. By then, the house mouse appears to have been arriving from two different directions, both the Near East and Western Europe, in the case of haplogroups H4 and H6 (Figure 3). Once again, the demographic expansion of these populations was more recent than their coalescence, in this case c. 500 years ago, and the most likely explanation is that there were multiple introductions from the source population.

Our results support the findings of Cucchi et al. (2006) and Bonhomme et al. (2011), concerning a complex introduction scenario with a notable presence of H2 and H4. The widespread H2 is geographically associated with the Near East and the Mediterranean basin. H4 (clade F) is found at highest frequency in Nordic countries and the British Isles (Figure 3) and is a lineage found in the Near East that apparently was spread around the northeast Atlantic by the Vikings (Searle et al., 2009). H8 (clade D) has also been associated with Scandinavia (Searle et al., 2009). Interestingly, H8 was detected in Madeira and the Canary Islands and could represent a possible Viking introduction (Förster et al., 2009). We are not suggesting a Viking introduction on Cyprus, although there are data suggesting trade between Cyprus and Scandinavia during the Bronze Age (Ling et al., 2014); the introduction of H8 is most likely due to more recent trade. Only 8.5% of our samples belonged to H1, although this haplogroup was the most common (37%) in Cyprus in Bonhomme et al. (2011). This difference may reflect our much increased sampling effort all across Cyprus (Figure 1). The high mitochondrial diversity suggests that the ecological conditions found on the island were favourable to establish large local populations of new migrants (Bonhomme et al., 2011). Propagule pressure must also have been high, due to the central location of Cyprus in the Mediterranean Sea.

4.2 | House mice as a proxy to study human movement and genetic diversity

Associations between house mice and human phylogeography have been well described and accepted, especially in the peripheral

distribution of the species, for example in northern and western Europe in association with Viking movements (Förster et al., 2009; Gündüz et al., 2001; Searle et al., 2009) or between Australia and the British Isles, demonstrating that the house mouse was brought to Australia during the British colonization (Gabriel, Stevens, Mathias, & Searle, 2011). Cyprus has a more complex history and it is more difficult to identify specific associations between human travellers and the house mouse haplogroups introduced. There are two possible reasons for this, firstly the high level of trade in the Mediterranean and secondly the geographical location of Cyprus, close to the origins of commensalism of *M. m. domesticus* with humans (Cucchi et al., 2005). However, even if the signal is not very clear, it does give a good insight into the relationships and trading activities of the island.

As house mice are moving using human-mediated transportation, their genetic diversity might correlate with human genetic diversity (Jones et al., 2013). The association between genetic diversity in mice and humans has been described in the Faroe Islands (Jones, Jensen, et al., 2011). In this particular case, low genetic diversity was found for both humans and mice. This relationship is also found on Cyprus where human genetic variability is relatively high, for example studies of Cypriot populations have revealed high mtDNA variability with six mtDNA haplogroups out of 10 present across the island and a high haplotype diversity (0.994) (Badro, Douaihy, Haber, Youhanna, & Salloum, 2013; Irwin et al., 2008). As expected from the geographical location of the island, Cypriot people are related to Near Eastern populations (Jordanians, Lebanese, Palestinians, and Syrians) (Badro et al., 2013). Future ancient DNA studies in this geographic region might help resolve the different waves of mouse introduction indicated by the present research.

4.3 | Mouse population structure in Cyprus

The 18 microsatellites genotyped for this study indicate a large mean number of alleles, high allelic richness, and high heterozygosity on Cyprus, when compared to other islands or even to continental populations (Hardouin et al., 2010; Linnenbrink et al., 2013). Indeed, similar high variability was, for example, present in Iran, which is also a phylogeographic melting pot for house mice (Hardouin et al., 2015). Furthermore, this similarity between Iran and Cyprus was confirmed in the DAPC analysis, which shows the population from Ahvaz in Iran closest to Cyprus (Figure S1.3a in Appendix S1). Interestingly, little population structure was found on the island (Figure 5; Figure S1.3b in Appendix S1), potentially because of a high level of goods transportation, and so mice, between farms all around the island.

5 | CONCLUSION

As expected for a commensal species, the western house mouse is characterized by a complex history shaped by founder events,

genetic drift, and admixture. Cyprus seems to be a good model that represents this complexity, due to different introductions that are related to human movements or transport. The substantial house mouse genetic variability found on the island reflects the level of human genetic diversity there. Two main waves of introductions could be tentatively identified and dated, the first one corresponding to the Bronze Age and the second one to more recent movements. Genetic variation in house mice from Cyprus does therefore appear to be concordant with the complex human history of the island. As a result, Cyprus is unusual, because genetic variation in populations on islands is often low, due to the genetic dominance of the first colonizations. Instead, Cyprus has high genetic diversity, reflecting the hub-like nature of the island with respect to traffic of both humans and mice.

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DATA ACCESSIBILITY

GenBank accession numbers are the following: MG937349–MG937536 (samples from Cyprus) and MG950367–MG950397 (samples from Greece).

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BIOSKETCH

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SUPPORTING INFORMATION

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