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Population genetics and demography of the endemic mouse species of Cyprus, *Mus cypriacus*

Emilie A. Hardouin¹ · Francesca Riccioli¹ · Demetra Andreou¹ · Miguel Baltazar-Soares¹ · Marin Cvitanović¹ · Nathan F. Williams¹ · Pascale Chevret² · Sabrina Renaud² · Oxala García-Rodríguez¹ · Eleftherios Hadjisterkotis³ · Despoina Miltiadou⁴ · Miloš Macholán⁵ · Linda Odenthal-Hesse⁶ · Sven Kuenzel⁶ · George P. Mitsainas⁷

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Abstract

Mus cypriacus is one of three small palaeoendemic mammals that have survived the Mediterranean islands' anthropization. This species, endemic to Cyprus, was described in 2006 and stands out as one of the last mammal species to have been discovered in Europe. Despite scarce data on its genetics, ecology, and life-history traits, *Mus cypriacus* is assessed as Least Concern LC in the IUCN Red List, partly due to its morphological similarity with the sympatric house mouse that prevented earlier identification. Our study uses mitochondrial and microsatellite markers to investigate this small rodent's population genetic structure and diversity. Our analysis did not identify any population genetic structure and suggested a high genetic diversity across Cyprus. When inferring habitat preference using sample locations, it appeared that *M. cypriacus* utilizes a diverse variety of habitats, covering more than 80% of the island. Although these results are encouraging for the conservation status of the species, they still need to be cautiously applied as potential threats may arise due to increasing habitat destruction and changes in land use. Consequently, our encouraging results should be applied judiciously. Additional ecological data are urgently needed to gain a more comprehensive understanding of this inconspicuous endemic species.

Keywords Mediterranean islands · Muridae · Rodentia · Endemic species · Microsatellites

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Introduction

Human activity is considered one of the primary causes of the current environmental changes, altering various ecological systems at a global level (Brooks et al. 2006; Pysek et al. 2010; Strassburg et al. 2012). Projections indicate that up to 50% of species will become extinct in the next 50 years (Koh et al. 2004; Thomas et al. 2004) as consequence attributed to human-mediated climate change and habitat destruction (Crutzen 2002; Zalasiewicz et al. 2011). In 2023, the International Union for Conservation of Nature (IUCN) Red List reported 42,100 species as threatened with extinction (<https://www.iucnredlist.org/>). The identified threats include habitat destruction, invasive species, pollution, overexploitation, and climate change (Baillie et al. 2004; Amori et al. 2008; Young et al. 2016; Tilman et al. 2017). Many species are endangered worldwide due to at least one of these factors (Capdevila et al. 2022), with endemic species being particularly susceptible to these threats (Purvis et al. 2000; Vié et al. 2008; Garcia and Di Marco 2020).

Cyprus is the third largest island in the Mediterranean basin. It is located in the Mediterranean biodiversity hotspot and hosts numerous endemic species due to its long-standing isolation since the Messinian Crisis (6–5.3 Myr—Hadjikyriakou and Hadjisterkotis 2002; Hadjisterkotis and Reese 2008; Hadjisterkotis 2012; Essl et al. 2013; Nicolaou et al. 2016). Among these, the endemic mouse *Mus cypriacus* (Rodentia: Muridae) was recently described on the island (Bonhomme et al. 2004; Cucchi et al. 2006). Notably, it represents one of the few terrestrial mammalian species discovered in Europe in the last 100 years (Nicolaou et al. 2016). It is of high conservation interest, as it is one of three small palaeoendemic mammals and, in fact, the only rodent species, which has survived the anthropization of Mediterranean islands. The other two are shrew species, i.e., *Crocidura sicula* and *C. zimmermanni* (Gippoliti and Amori 2006; Auffray and Britton-Davidian 2012; Frynta et al. 2015). Diverging from *Mus macedonicus* approximately 0.53 million years ago (Hadjisterkotis et al. 2000; Cucchi et al. 2006; Macholán et al. 2007), *Mus cypriacus* adds a compelling dimension to the island's evolutionary narrative.

Mus cypriacus is morphologically close to *M. macedonicus* with similar body size, but it displays a bigger skull with a longer lower tooth row (Cucchi et al. 2006; Macholán et al. 2008) and a longer tail (Kryštufek and Vohralík 2009). It is mainly found in cultivated areas at moderate altitudes between 300 and 900 m but occasionally reaching up to 1600 m. It sometimes occurs in syntopy with *M. m. domesticus* (Cucchi et al. 2006; Macholán et al. 2007; own observations), but it is almost absent from areas subjected to high anthropogenic pressure (Cucchi et al. 2006; Kryštufek and Vohralík 2009). Despite being assessed as Least Concern (LC), by IUCN (Amori 2017), this designation is based on scarce data, particularly regarding the ecology and life-history traits of *M. cypriacus*. Furthermore, critical knowledge gaps persist concerning its distribution on the island, and no research has been undertaken to understand potential threats to the species (Amori 2017). Addressing these gaps, the present study delves into the conservation status of *M. cypriacus* using molecular information to provide a more comprehensive understanding.

Materials and methods

Data collection

A total of 40 specimens of *M. cypriacus* collected during two sampling expeditions on Cyprus in 2005 and 2015 were used in the present study (Fig. 1). D-loop sequences and sampling locations of the specimens collected in 2005 were previously published in Macholán et al. (2007). Thirteen extra samples were collected in 6 localities from southern

Cyprus from the 03rd to the 17th of September 2015 using Sherman traps. Samples were mainly collected on stony-rocky substrate covered with garrigue/maquis vegetation during an intense sampling expedition for the study of *Acomys cahirinus* (sensu Renaud et al. 2020) in Cyprus. The new sampling locations can be found in Supplementary Material Table A. Samples were collected following local regulations for field collection of small mammals, and all procedures regarding animal handling complied with the approved guidelines by the American Society of Mammalogists (Sikes et al. 2011). Mitochondrial D-loop was sequenced for the specimens collected in 2015 using the protocol from Hardouin et al. (2010), and nucleotide sequences were deposited in GenBank (accession numbers OR227591 to OR227603). Those sequences were aligned with the sequences generated by Macholán et al. (2007), GenBank accession numbers EU106194–EU106216 and EU106278–EU106281. Twenty-one microsatellites were amplified on specimens captured in 2005 and 2015 using primers initially designed for *M. m. domesticus*, following the protocol of Hardouin et al. (2015). Microsatellite genotypes are provided in Supplementary Material Table B.

Molecular phylogeny and divergence time estimations

A median-joining haplotype network (Bandelt et al. 1999) was reconstructed using PopArt v.1.7 (Leigh and Bryant 2015) with the sequences generated in the present study and all *M. cypriacus* sequences available in GenBank (EU106194–EU106216, EU106278–EU106281—Macholán et al. 2007). The final alignment comprises 38 sequences with a length of 1066 bp. The number of haplotypes, haplotype diversity, nucleotide diversity, and mismatch distribution (MMD) were calculated using DNAsp (Librado and Rozas 2009). F_{ST} (Reynolds et al. 1983) and R_{ST} (Slatkin 1995) pairwise comparisons per sampling district, as well as Hardy–Weinberg equilibrium, were tested using Arlequin ver. 3.5.2.2. (Excoffier and Lischer 2010). Isolation by distance was investigated using a Mantel test performed using ade4 (Dray and Dufour 2007). To gain insight into the historical demography of *M. cypriacus*, a Bayesian Skyline Plot (BSP) was constructed using BEAST v2.6.7 (Bouckaert et al. 2014). The selected substitution model was GTR + G4 using jModelTest2 (Darriba et al. 2012). Since there is no current estimation of the number of substitution sites per generation, a strict molecular clock of 2.0×10^{-8} substitution sites per generation was used as it was found to be a conservative estimate for the close relative species *M. musculus* (Förster et al. 2009). The analysis was run for 75 million generations, with parameters and genealogies sampled every 5000 iterations and the first 10% discarded as burn-in. To assess convergence and the effective sample size

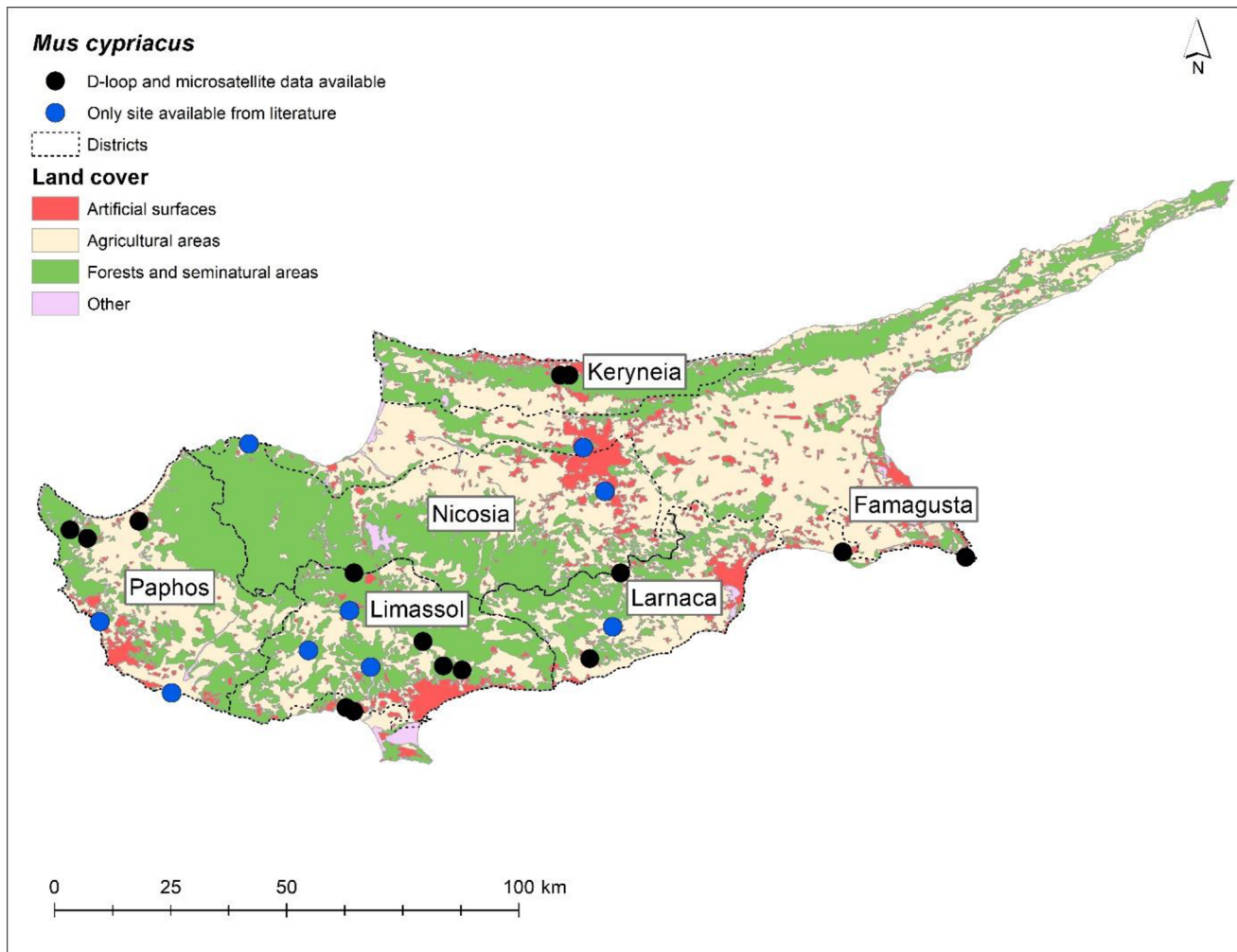


Fig. 1 Map of Cyprus with the different land covers and country districts. Black circles: sites with D-loop and microsatellite data available; blue circles: occurrence data from literature only (Bonhomme et al. 2004; Cucchi et al. 2006)

(ESS > 200) of each parameter, the log files were examined using Tracer v1.6 (Rambaut et al. 2014).

The divergence time between *M. cypriacus* and *M. macedonicus* was estimated through a phylogenetic tree analysis using BEAST 2.6 (Bouckaert et al. 2019). In the analysis, we incorporated D-loop and cytochrome b sequences of *M. cypriacus*, along with representatives from four subgenera of *Mus* (*Mus*, *Coelomys*, *Nannomys*, and *Pyromys*), as well as other murine genera (Supplementary Material Table C). *Arvicanthis niloticus* and *Otomys irroratus* were used as outgroups. For some species, we extracted the portion that includes cytochrome b and D-loop sequences from the complete mitochondrial genome. The sequences were aligned with MUSCLE (Edgar 2004), and gaps and ambiguous areas were excluded from the alignment using Gblocks (Castresana 2000) implemented in Seaview (Gouy et al. 2010). The complete alignment comprises 35 sequences and 2090 bp divided into two partitions to separate the coding cytochrome

b (1140 bp) and the non-coding portion of the alignment (950 bp). We used three calibration intervals defined in previous studies: 1) stem *Apodemini* ((Offset: 8.93, Log(Stdev): 1, Log(Mean): 4.5, (Aghová et al. 2018)), Most Recent Common Ancestor (MRCA) of *Mus* ((Offset: 7.29, Log(Stdev): 1, Log(Mean): 4.9, (Aghová et al. 2018)) and the divergence of *Apodemus mystacinus/Sylvaemus* (median prior age of 7 Myr (upper 95%, 5.96–12.37 Myr) (Fabre et al. 2013)). Bayesian model averaging was performed for each partition using the bModelTest package (Bouckaert and Drummond 2017) as implemented in BEAST v.2.6. We used a Birth Death Model speciation tree prior and assumed a log-normal relaxed molecular clock. Two independent runs were carried out with 20,000,000 Markov Chain Monte Carlo (MCMC) iterations, sampling trees, and log files every 1000 iterations. Result files were examined in Tracer v.1.7.1 (Rambaut et al. 2018) for each run to assess the chain and parameter convergence of independent runs and to verify that the overall

effective sample size (ESS) was > 200 . Tree files from separate runs were combined using LogCombiner v.2.6, and a consensus maximum clade credibility (MCC) tree was created using TreeAnnotator v.2.6 after removing 10% of initial trees and using median heights for nodes. The tree was visualized with FigTree v. 1.4 (Rambaut 2012).

Microsatellites

The presence of null alleles was investigated using MicroChecker (Van Oosterhout et al. 2004). Loci that significantly ($p < 0.05$) deviated from the Hardy–Weinberg proportions due to null alleles were discarded from the dataset. The observed and expected heterozygosity and the average number of alleles per locus were calculated using Genetix (Belkhir et al. 2004). Allelic richness was calculated using PopGenReport (Adamack and Gruber 2014) and F_{IS} using diveRsity (Keenan et al. 2013). STRUCTURE was used to infer population structure (Pritchard et al. 2000), with a burn-in period of 250,000 simulations, followed by a run length of 750,000 MCMC simulations and ten iterations for each K (number of clusters) with the admixture model. K from 1 to 5 was tested in our dataset. The results were analyzed using STRUCTURE HARVESTER (Earl and vonHoldt 2012). Results were summarized using CLUMPP (Jakobsson and Rosenberg 2007) and drawn using Distruct (Rosenberg 2004). A discriminant analysis of principal components (DAPC) (Jombart et al. 2010) was used to assess the population structure further using the ADEGENET package (Jombart 2008). The interaction number used was 1,000,000,000. Thirty principal components, explaining 93.2% of the total variance, were retained as predictors for the discriminant analysis. The presence of a population bottleneck was tested using BOTTLENECK (Piry et al. 1999), and the effective population size was estimated using NeEstimator V2.1 and the linkage disequilibrium method (Do et al. 2014).

Characterization of habitat occupation

To map the habitat preference of *M. cypriacus* on the island, we reviewed all known sampling locations of reported *M. cypriacus* specimens from Bonhomme et al. (2004), Cucchi et al. (2006), Macholán et al. (2007) and the present study. Land-cover analysis was performed for each reported location using the Corine Land Cover (CLC) data 2018 from the EU Observation programme Copernicus. The main land-cover categories used included artificial surfaces (corresponding to Corine class level code 1), agricultural areas (code 2), forest and seminatural areas (code 3), wetlands (code 4), and water bodies (code 5). The different land covers, except for wetlands and water bodies, represented in the category “other”, are displayed

in Fig. 1. The CLC data have a three-level hierarchical classification system, where the third level is the most detailed. For example, at Level 1, artificial surfaces are considered homogeneous. Still, at Level 3, they are split into eleven different categories (continuous and discontinuous urban fabric, industrial or commercial units, mineral extraction sites, green urban areas, etc.). Therefore, a more detailed analysis of the habitat preferences of *M. cypriacus* was conducted using both Level 2 and Level 3 classification systems (Supplementary Material Table D). All analyses were done in ArcMap 10.6 (ESRI).

Results

Phylogenetic analyses and divergence times

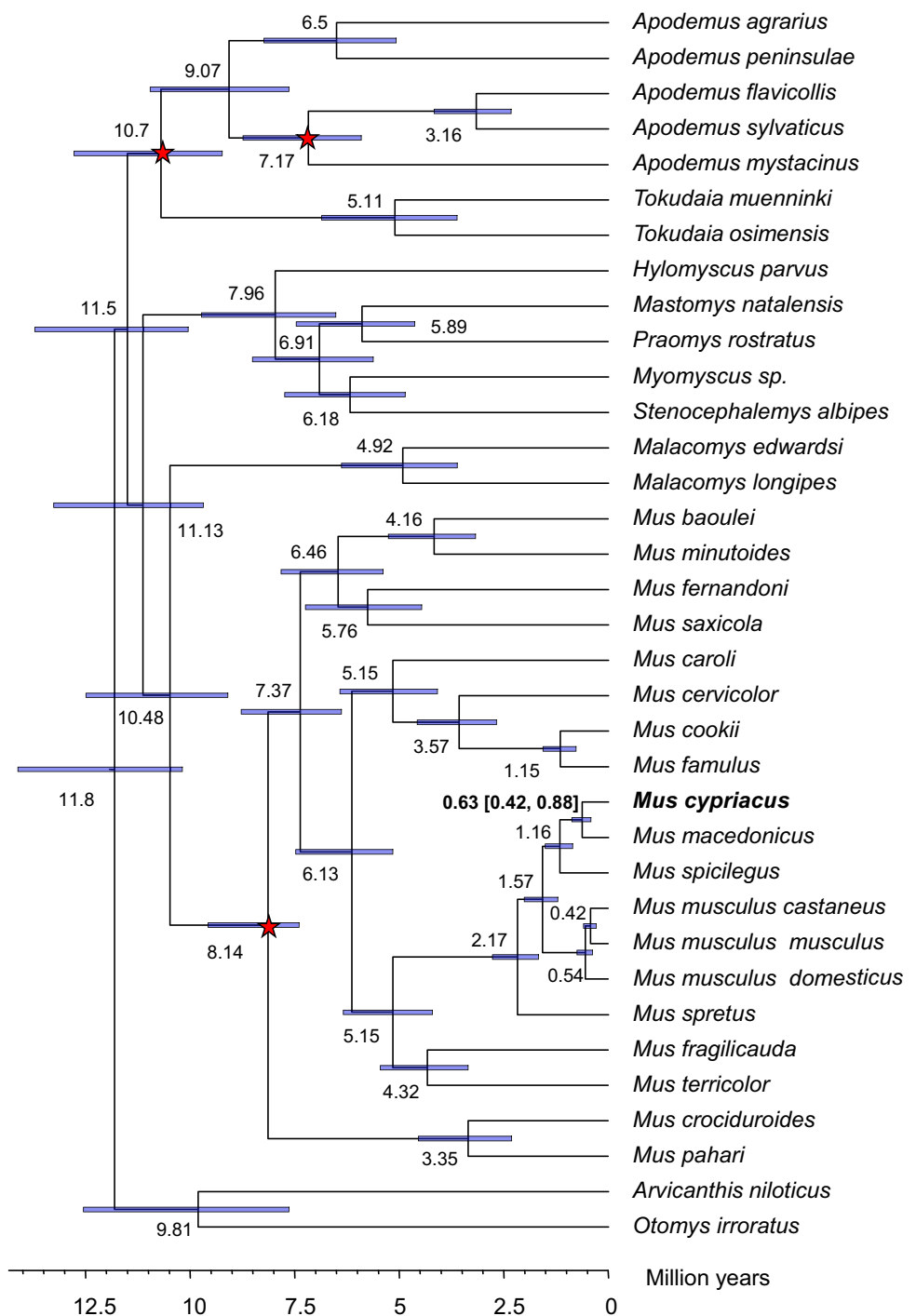
The divergence time between *M. cypriacus* and *M. macedonicus* was estimated using the chronogram obtained with BEAST, revealing that the mitochondrial DNA between the two species diverged 0.63 Mya [0.42, 0.88–95% highest posterior density interval, HPD] (Fig. 2).

Thirteen *M. cypriacus* mitochondrial D-loop sequences generated in the present study were aligned with the 27 sequences from Macholán et al. (2007) and analyzed together. Twenty-nine haplotypes and segregation sites were found in the dataset (Fig. 3A, Table 1). Average haplotype diversity was 0.979 (SD = 0.011), and nucleotide diversity (π) was 0.008 (SD = 0.0004). Haplotype diversity (Hd) was high in all studied districts (Hd between 1 and 0.89, Table 1). No geographic signal was found in our dataset (Fig. 3A, B). This was confirmed by pairwise F_{ST} values, which were low and non-significant between samples from all Cypriot districts except between Larnaca and Limassol (Table 2). The Mantel test did not detect a signature of isolation by distance (Mantel test: $r = -0.75$, $p = 1$).

A mismatch distribution (Fig. 4A) based on the 40 mitochondrial D-loop sequences available for this study was unimodal, indicative of population expansion (Rogers and Harpending 1992). τ was estimated to be 4.88 with $\theta_0 = 0.967$ and $\theta_1 = 1000$.

A Bayesian skyline plot was also produced to further investigate population demography at the island level. *M. cypriacus* was found to be stable for at least the last 25 generations (Fig. 4B). The number of generations per year is not known for this species. However, a value of two generations per year was found to be a conservative estimate for *Mus* species (Macholán et al. 2007) and was used in the present study, leading to the estimate of 25 generations representing 12.5 years. The effective population size was calculated to be 5895 with a very high posterior density interval (95% HPD = 113–31,882).

Fig. 2 Chronogram based on BEAST analysis. The three calibration points are indicated by a star (see method section for details); for each node, the mean and the 95% highest posterior density (blue bars) are indicated



Microsatellite-based population genetic analysis

A total of 21 microsatellites were genotyped on the samples collected in 2005 and 2015 (Fig. 1). One of those markers did not amplify, four were monomorphic, and two had null alleles. Those were discarded, and the remaining 14 loci were tested for Hardy–Weinberg equilibrium (HWE), revealing deviation in one microsatellite marker which was also excluded from the dataset. Hence, the final data set

contained 13 microsatellite loci. The mean allelic richness for the whole island was found to be 13.98. The possible genetic population structure was studied using STRUCTURE. Even though the best K was found to be $K=2$ using the Evanno method, the structure plot only reveals one population (Fig. 5). The Evanno method does not evaluate $K=1$, leading to an overestimation of the number of populations (Cunningham et al. 2020), which is most likely scenario here (Fig. 5). A DAPC analysis was also performed to further

Fig. 3 **A** D-loop haplotype network calculated using median-joining. The circle size represents the frequency of the respective haplotype, and the colours represent the populations of the individuals carrying a given haplotype. The hatch marks on the edge represent mutations between haplotypes and black circles represent hypothetical haplotypes. **B** Map of Cyprus with the geographical location of *M. cypricus* samples, with the same colour code as in (A)

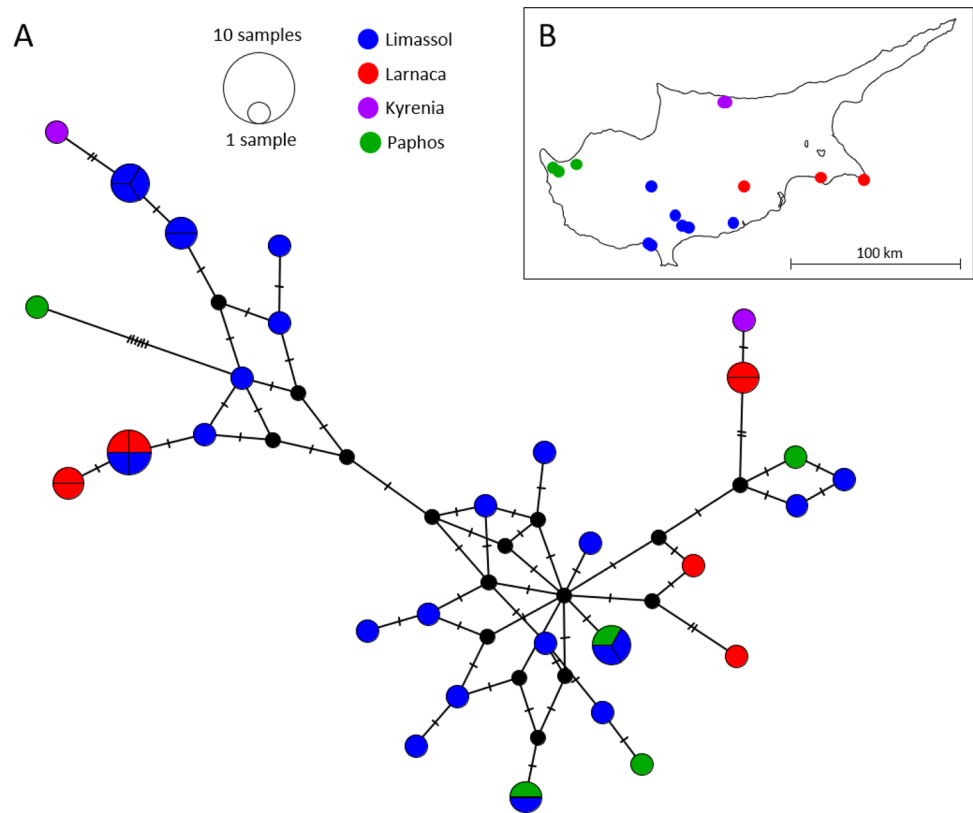


Table 1 Population genetic parameters for *Mus cypricus* by district

	Mitochondrial D-loop				Microsatellites					
	N	Number of haplotypes	Number of variable sites	Haplotype diversity (SD)	Nucleotide diversity (SD)	N	Hexp (SD)	Hobs (SD)	Average allele per locus	Mean allelic richness
Keryneia	2	2	9	1.00 (0.500)	0.012 (0.006)	4	0.69 (0.08)	0.90 (0.16)	4.23	3.33
Larnaca	8	5	11	0.89 (0.086)	0.007 (0.001)	6	0.78 (0.12)	0.87 (0.14)	6.77	5.37
Limassol	25	20	20	0.98 (0.017)	0.007 (0.0004)	24	0.85 (0.12)	0.88 (0.14)	13.15	10.96
Paphos	5	5	16	1.00 (0.126)	0.010 (0.002)	5	0.79 (0.05)	0.85 (0.15)	5.77	4.01
Total	40	29	29	0.98 (0.011)	0.008 (0.0004)	39	0.86 (0.11)	0.88 (0.11)	16.08	13.97

N number of individuals, SD standard deviation, H_{exp} expected heterozygosity, H_{obs} observed heterozygosity

Table 2 Pairwise F_{ST} values calculated using mitochondrial D-loop

	Keryneia	Limassol	Larnaca	Paphos
Keryneia	–			
Limassol	0.12	–		
Larnaca	0.03	0.09	–	
Paphos	0.02	0.03	0.12	–

Values corresponding to p values < 0.05 are highlighted in bold

investigate population structure; however, the lowest Bayesian information criterion (BIC) value (68.00) was found for $K = 1$, indicating no population structure. The first 10 PCs

of the PCA, explaining 50.5% of the total variance, were retained in the DAPC (Fig. 6). Populations were found to cluster together except Paphos and Keryneia which show slight divergence (Fig. 6). F_{ST} values between the population groups were low (Table 3).

As no population structure was found, population demography was investigated for *Mus cypricus* from the entire island. No recent bottleneck was identified using the one-tailed Wilcoxon test with the stepwise mutation model (SMM, $p = 0.95$), and also, no deficit or excess of heterozygotes was found using F_{IS} ($F_{IS} = -0.020, -0.052; 0.011$ 95% CI). The effective population size was calculated and found to be 109 (71.3–211.5, 95% CI).

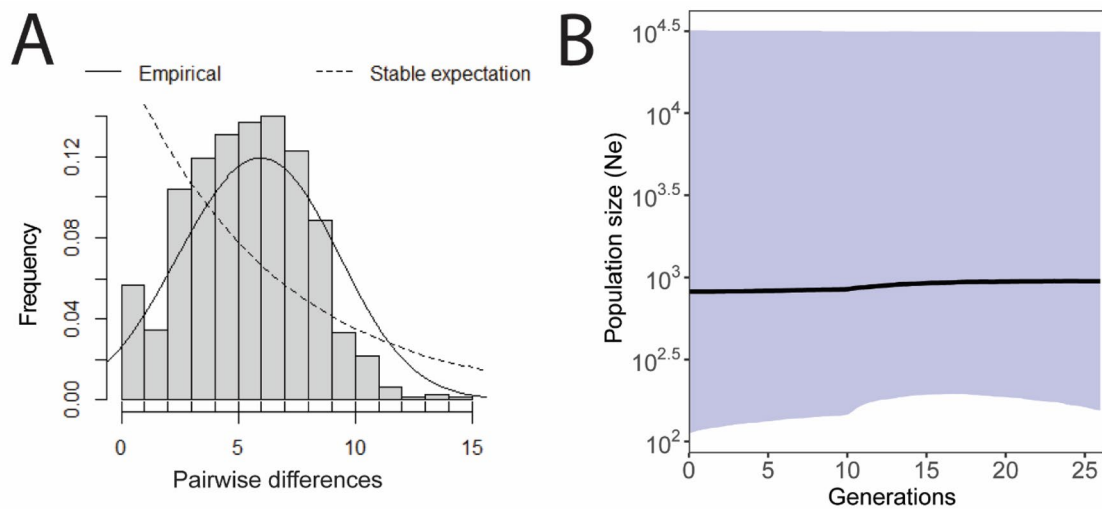


Fig. 4 **A** Mismatch distribution of the mitochondrial DNA D-loop. The observed mismatch distribution (bars) is compared with the expected mismatch distribution (dotted line) under a stable popu-

lation model. **B** Bayesian skyline plot of the mitochondrial DNA D-loop. The black line indicates the mean effective population size, and the blue shaded area represents the 95% HPD interval

Characterization of habitat preferences

Fifty-five sampling locations have been reported for *M. cypriacus* (Bonhomme et al. 2004; Cucchi et al. 2006; Macholán et al. 2007; present study). Corine Land Cover analysis identified that *M. cypriacus* was most commonly found in agricultural areas (41.82%, $N=23$), followed by forests and seminatural regions (36.36%, $N=20$), and artificial surfaces (21.82%, $N=12$; Supplementary Material Table B). A more detailed land-cover analysis (CLC Level 3) showed that *M. cypriacus* was primarily found in complex cultivation patterns (Corine Land Cover 2.4.2.–21.82%), followed by sclerophyllous vegetation (Corine Land Cover 3.2.3.–20%), and discontinuous urban environment (Corine Land Cover 1.1.2.–18.18%). In agreement with this tolerance to anthropized environments, 80% of *M. cypriacus* were captured within 2000 m from artificial surfaces. This could represent a sampling bias since traps are typically set in areas easily accessible via roads. Overall, the habitats from which *M. cypriacus* were observed cover 81.8% of the island (Supplementary Material Table D).

Discussion

Our results obtained using mitochondrial D-loop and microsatellites demonstrate that *M. cypriacus* is genetically highly diverse, with little or no population differentiation found on the island using these markers. We discuss reasons for this below. This result is in accordance with the previous literature indicating that *M. cypriacus* could be found in a wide

variety of habitats except in highly anthropogenic ones (Cucchi et al. 2006; Kryštufek and Vohralík 2009). The absence of *Mus cypriacus* from the latter might be due to competition with *M. m. domesticus* (García-Rodríguez et al. 2018) and the presence of high numbers of domestic and stray cats, which are significant predators of mice on the island (Heise-Pavlov and Hadjisterkotis 2009). Other ecological characteristics unique to these habitats could also contribute to *M. cypriacus* avoiding them.

Population demography

The mitochondrial divergence between *M. cypriacus* and *M. macedonicus* was estimated to be 0.63 million years [0.42, 0.88], consistent with a previous study that reported a divergence of 0.53 million years (Macholán et al. 2007). This timeframe aligns with the Mindel Glaciation (750,000–675,000 years ago), a period of low sea level during which the distance between Cyprus and the continent was reduced (Held 1989). This would have favoured the island's colonization by a common ancestor shared with *Mus macedonicus* (Bonhomme et al. 2004; Macholán et al. 2007; Auffray and Britton-Davidian 2012). Those mice would have then diverged from their continental counterpart, ultimately leading to the origin of *Mus cypriacus* (Macholán et al. 2007; Auffray and Britton-Davidian 2012). Population expansion growth has been dated at around 100,000 years (Macholán et al. 2007). While Bayesian skyline plots suggest a relatively stable mean effective population size in recent times, the wide range of the HPD interval implies that contemporary effective population sizes should be interpreted

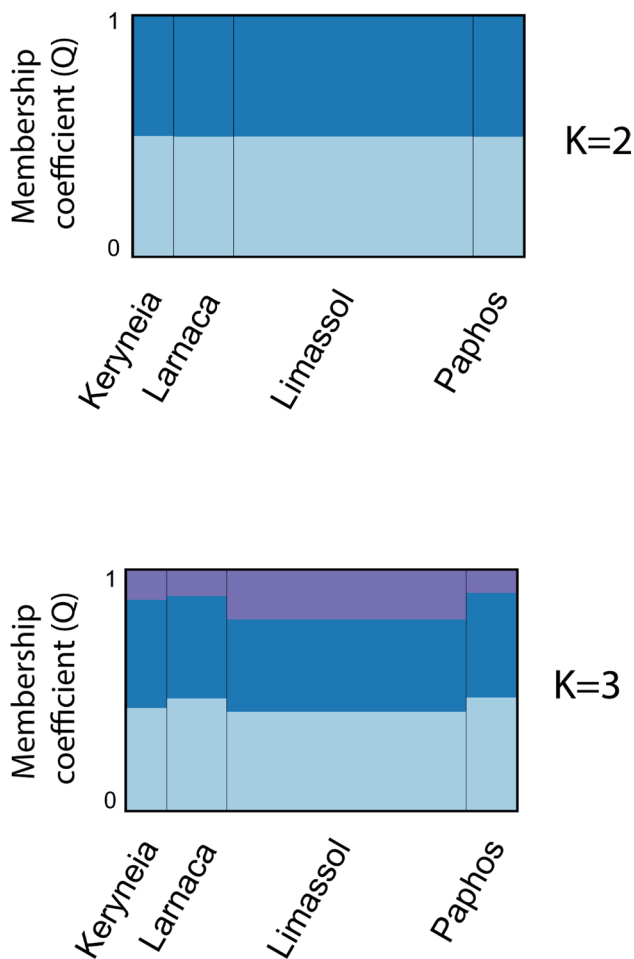


Fig. 5 Population structure analysis using 13 microsatellites. Results for $K=2$ and $K=3$ are presented. The colours correspond to different clusters identified by the structure analysis

with extreme caution. The discrepancy between the population expansion detected by the mismatch distribution and the stable population size suggested by the BSP may be due to differential sensitivity to sample size. Bayesian skyline plots and especially the detection of recent population changes are known to be affected by small sample sizes, while the mismatch distribution is less sensitive (Felsenstein 2006; Grant 2015). Specifically, Bayesian skyline plots based on less than 50 individuals often fail to capture population expansion, resulting in a flat shape (Grant 2015).

Genetic diversity

Mus cypriacus genetic diversity calculated using mitochondrial D-loop was high (haplotype diversity: 0.98). However, it may have been underestimated due to the low sample size and the uneven distribution across the island (e.g., sparse representation from the North and none from the North-East). Interestingly, the observed genetic diversity

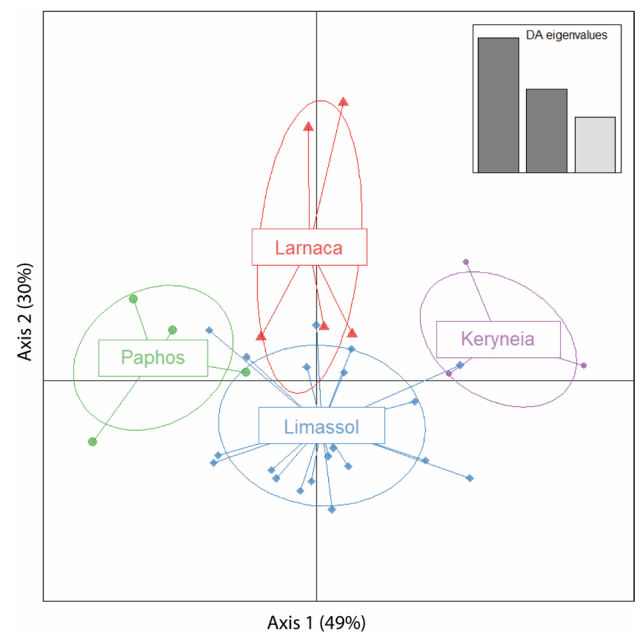


Fig. 6 Representation of the microsatellite variation on the first two axes of a DAPC. The percentage of the total variance explained by Axis 1 is 49%, and Axis 2 is 30%

Table 3 Pairwise R_{ST} values calculated using microsatellites

	Keryneia	Larnaca	Limassol	Paphos
Keryneia	–			
Larnaca	0.06	–		
Limassol	0.04	0.02	–	
Paphos	0.06	0.03	0.02	–

No p values < 0.05 were found

is comparable to its closely related species, *M. macedonicus* (haplotype diversity: 0.98) and *M. spicilegus* (haplotype diversity: 0.97), both of which, however, have a much broader distribution area on the continent (Macholán et al. 2007). These results are surprising at first glance, as island populations often exhibit lower genetic diversity compared to their mainland counterparts (e.g., Abdelkrim et al. 2005; White and Searle 2007; Hardouin et al. 2019, 2021; Conroy et al. 2021; Chevret et al. 2021; Sacks et al. 2022). However, counter-examples exist such as of *Oryzomys couesi cozumelae* or *Reithrodontomys spectabilis*, both endemic rodents on the island of Cozumel, the largest island of the Mexican Caribbean (with an area of c. 486 km²). These species exhibited unexpectedly high levels of genetic diversity (Vega et al. 2007; Espindola et al. 2014). Indeed, genetic diversity is known to be influenced by island size, with larger islands able to harbour a larger number of individuals (Frankham 1997). Accordingly, in Darwin's finches, a significant positive correlation exists between island size

and genomic diversity (Petren et al. 2005; Brüniche-Olsen et al. 2019). This pattern is also observed in the feral *M. m. domesticus* populations from the Kerguelen Archipelago (Hardouin et al. 2010) or the Orkney Islands (Chevret et al. 2021). With a substantial area of 9251 km², Cyprus is one of the largest Mediterranean islands and can probably sustain a large population size for a small rodent.

Absence of population fragmentation in *M. cypriacus*

No evidence of population fragmentation was found for *M. cypriacus* despite sampling localities covering distant areas across most of the island. Our habitat analysis further indicated that *M. cypriacus* is a generalist rodent utilizing a wide variety of habitats (Cucchi et al. 2006; Macholán et al. 2007; Hadjisterkotis pers. observations), which, when combined, cover more than 80% of the island area (Supplementary Table D). The generalist preferences of *M. cypriacus* and the availability of suitable habitats allow for efficient dispersal. This leads to an absence of genetic population structure and the maintenance of high genetic diversity. Given the rapid urban development experienced in Cyprus over the past 40 years, there is concern that it could disturb habitat connectivity, threatening the current situation. Presently, this threat still seems limited since areas with high anthropogenic pressure, from which *M. cypriacus* is absent, only cover 9.2% of the island's total area (Supplementary Material Table D).

Implications for conservation and management

Mus cypriacus has been classified as least concern by the IUCN despite the lack of information on the species. The present genetic data do not support an urgent reclassification. However, this species constitutes an example of the bias in perceiving different species and their importance for conservation (Brambilla et al. 2013). Small rodents are typically considered “uncharismatic” species, often leading to their significance being underestimated or overlooked (Amori et al. 2008). This bias arises from a negative perception, primarily driven by the damage caused by a few invasive species, such as the rat and the house mouse (Amori et al. 2008; Espindola et al. 2014). For example, the black rat (*Rattus rattus*) can threaten Cyprus's biodiversity by preying upon eggs of ground-nesting birds (Hadjisterkotis 2000, 2017), and the recently introduced brown rat (*Rattus norvegicus*) is known to kill poultry on the island (Hadjisterkotis et al. 2020).

Mus cypriacus may hold conservation significance simply by its survival into modern times as the sole extant endemic rodent among the past endemics of the Mediterranean islands. This is particularly noteworthy, considering

that even anciently introduced anthropochorous species are considered of conservation value as part of our “cultural heritage” (Masseti 2009). The success of *M. cypriacus* in persisting despite the range of habitat modifications (e.g., urbanization and the introduction of non-native species like cats and rats, to name only a few) that led to the extinction of all other endemic rodents on Mediterranean islands might be attributed to its evolution in a context that involved terrestrial predation by a local, endemic predator, the Cypriot genet *Genetta plesictoides* (Masseti 2009; Vigne et al. 2023). As a result, *M. cypriacus* was not naïve towards introduced terrestrial predators, showcasing a resilience that sets it apart.

The earliest evidence of domestic cats on the island dates back to approximately 9000 years ago from the Neolithic site of Shillourokambos (Vigne et al. 2004). African wildcats were, however, introduced to Cyprus at least 10,000 years ago by early Neolithic farmers, presumably to control house mouse pests (Cucchi et al. 2020). Consequently, the Cypriot mice have evolved in the presence of cats for the last 11,000 years. As a consequence, in response to cat odour, it displays avoidance behaviour similar to continental house mice (Frynta et al. 2015). This behavioural adaptation may have contributed to the species' ability to partially mitigate the impact of feral cats (*Felis catus*), which occur in high densities on Cyprus (Heise-Pavlov and Hadjisterkotis 2009).

A further possible evolutionary consequence of this permanent predation pressure may be that *M. cypriacus* does not display any trend towards body size gigantism (Masseti 2009). This characteristic contributes to the inconspicuous nature of *M. cypriacus*, allowing easy concealment and escape from predation. The small body size of this species (head + body length range: 75–91 mm according to Cucchi et al. 2006) also likely reduces potential competition with local populations of *Acomys cahirinus* (head + body length range: 98–119 mm according to Renaud et al. 2020). *A. cahirinus*, probably unintentionally introduced to Cyprus through human-mediated transport (Barome et al. 2001), now coexists with *M. cypriacus* in wild habitats. Both species face competition from the house mouse in anthropized environments.

Another aspect that emphasizes the need for efficient management measures to ensure the long-term conservation of *M. cypriacus* is its crucial role in maintaining ecological equilibrium on Cyprus. As an important prey item for predatory birds (Bonhomme et al. 2004; Hadjisterkotis pers. observation) and Cypriot snakes, including endemic taxa (*Hierophis cypriensis* and *Macrovipera lebetina lebetina*), *M. cypriacus* plays a significant role in the local food web. To comprehensively understand the factors contributing to its exceptional adaptability, further studies should focus on exploring the species' life-history traits and ecological habits. Additionally, adopting approaches that integrate

modern and archaeological data, such as using ancient DNA or employing geometric morphometrics, would provide valuable insights into the eco-evolutionary components that facilitated the survival and adaptability of the Cypriot mouse.

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Author contributions EAH, DA, EH, and GPM conceived the study; EAH, DA, EH, DM, and GPM conducted the fieldwork. EAH, FR, MBS, MC, NFW, PC, SR, OGR, LOH, SK, and MM performed the data collection and analysis. EAH, FR, and SR wrote the first draft of the manuscript. All authors were involved in the writing, data interpretation, and read and approved the final manuscript.

Data availability All the sequences generated in the present study were submitted to GenBank: the accession numbers are OR227591 to OR227603. Microsatellite genotype data are also provided as supplementary material (Table B) in a genepop format.

Declarations

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

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