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A new mitochondrial lineage in the edible dormouse, *Glis glis* (Rodentia: Gliridae), from Alonissos island (Sporades archipelago, Greece)

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Abstract. We performed a phylogeographic analysis of mitochondrial DNA in the edible dormouse, *Glis glis* (Linnaeus, 1766), including 15 known haplotypes obtained across large part the European species' range along with a fragment of the cytochrome *b* gene of one specimen from Alonissos island (Sporades archipelago, Greece). The haplotype of this specimen did not cluster with any other haplotypes, which were grouped into four lineages. The genetic divergence (mean K2P distance) between the Greek specimen and these four lineages, comprised between 3.3 % and 5.9 %, was comparable to the genetic divergence found between the aforesaid lineages (1.9-6.4 %). Accordingly, the specimen most likely falls into a new, never described before, Mediterranean mitochondrial lineage of *G. glis*.

Key words: Mediterranean island, phylogeography, mtDNA, cytochrome *b*

Introduction

The edible dormouse, *Glis glis* (Linnaeus, 1766), the largest species of dormouse is widespread in western, central, and southeastern Europe and Caucasus. The species has been also reported from many islands in the Mediterranean Sea, precisely in the Tyrrhenian, Adriatic, and Aegean Sea (Kryštufek 2010). Not long ago, two phylogeographic studies based on mtDNA sequences of specimens obtained from a large part of the species' range have outlined the genetic structure of the edible dormouse (Hürner et al. 2010, Lo Brutto et al. 2011). In brief, three main haplogroups have been identified: one haplogroup is widespread in Europe and Anatolia, while the others were found, respectively, in Sicily and Italy. Furthermore, a single divergent haplotype has been found in Macedonia, probably belonging to another lineage (Fig. 1). This pattern is presumed to result from the ancient fragmentation of the range occurred during the Quaternary glacial-interglacial climatic fluctuations in Europe (Hewitt 2000).

Recently, one more edible dormouse has been reported in an island of the Sporades, an archipelago in the Aegean Sea, namely Alonissos, which is the first record for this archipelago (Grano & Cattaneo, in press) (Fig. 1).

Here, we carried out a molecular analysis of this individual, sequencing a fragment of the cytochrome *b* (cyt *b*) gene and analyzing the sequence in the phylogeographic framework of the species.

Material and Methods

Total genomic DNA was extracted from a piece of muscle preserved in 80 % ethanol using the DNeasy tissue kit (Qiagen) according to the manufacturer's recommendations. A 1.140 bp mitochondrial DNA

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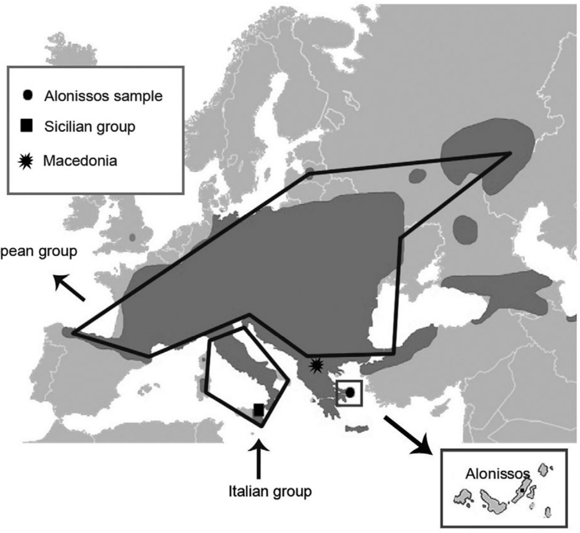

Fig. 1. *The geographic distribution of the mitochondrial lineages and haplotypes of the edible dormouse according to Hürner et al. (2010) and Lo Brutto et al. (2011). The location of the Alonissos specimen is boxed.*

fragment, encompassing the entire cyt *b* gene, was amplified by polymerase chain reaction (PCR) combining two primers, L14723 and H15915 (Irwin et al. 1991). DNA sequencing was performed by external service (Bio-Fab Research, Italy). The obtained 1.000 bp sequence was deposited in GenBank under the Accession Number JQ241278. For the phylogenetic analysis, the sequenced fragment was aligned with the dataset obtained by Hürner et al. (2010), including 15 haplotypes of specimens from a wide geographic area, which covered almost all the species' range in Europe. As outgroup, *Eliomys quercinus* was used (Gornung et al. 2010) (see Fig. 2 for accession numbers). Genetic divergence levels were evaluated with the Kimura two parameter model (K2P) (Kimura 1980) for comparative scopes using the MEGA 5 program (Tamura et al. 2011). Maximum likelihood (ML), maximum parsimony (MP), and neighbor-joining (NJ) methods were used to construct phylogenetic relationships among haplotypes. Bootstrap (1000 replicates) was used to assess the robustness of the nodes. ML trees were reconstructed using the PhyML 3.0 online program (Guindon et al. 2010). The appropriate model of substitution (GTR + Gamma) using the Akaike information criterion (AIC) was chosen, as implemented in Model Test 3.04 (Posada & Crandall 1998). The MP trees were obtained with Paup 4.0b10 using a heuristic search and tree-bisection-reconnection (TBR) and random addition of sequences (10 replicates). NJ trees were obtained with Paup 4.0b10 using the same model as in the ML analysis.

Results and Discussion

The final alignment of *G. glis* sequences includes 660 base pairs for each specimen. The trees obtained with the different methods (ML, MP and NJ) all show the same topology represented by the NJ tree

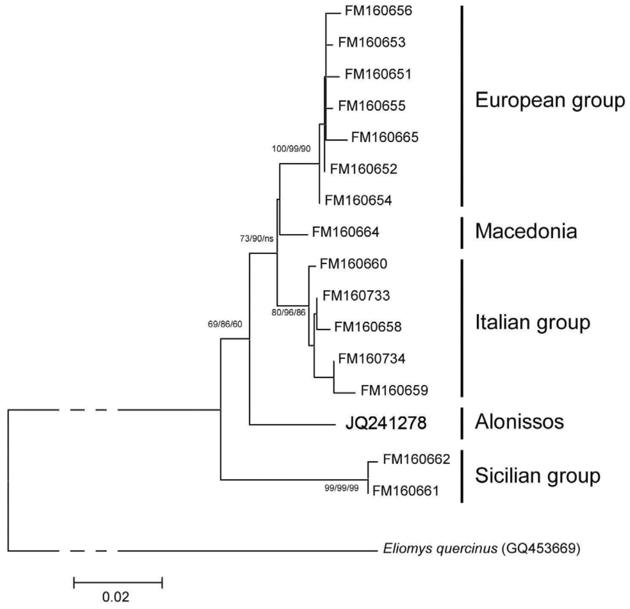

Fig. 2. *Neighbor-joining tree of haplotypes of the edible dormouse. The specimen from Alonissos is indicated in bold. Numbers at nodes specify bootstrap values at NJ /MP/ ML. Only basal nodes with values higher than 50 % are indicated.*

Table 1. *Pairwise Kimura 2 parameter distances (net below the diagonal; mean above the diagonal) between lineages of the edible dormouse. Standard errors are parenthesized. In bold are indicated the genetic distances between the new haplotype and the lineages found by Hürner et al. (2010).*

	Europe (N = 7)	Macedonia (N = 1)	Italy (N = 5)	Alonissos (N = 1)	Sicily (N = 2)
Europe	-	1.9 (0.5)	2.5 (0.5)	**3.8 (0.8)**	5.7 (0.9)
Macedonia	1.6 (0.5)	-	1.9 (0.5)	**3.3 (0.8)**	5.4 (0.9)
Italy	2.0 (0.5)	1.5 (0.5)	-	**4.0 (0.8)**	6.4 (1.0)
Alonissos	**3.5 (0.8)**	**3.3 (0.7)**	**3.7 (0.8)**	-	**5.9 (1.0)**
Sicily	5.4 (0.9)	5.3 (0.9)	6.0 (1.0)	**5.8 (1.0)**	-

in Fig. 2. We identified the same haplogroups as reported by Hürner et al. (2010), namely European, Italian, and Sicilian groups (Fig. 2), each of which is well supported (80-100 %). However, basal nodes generally show lower support (60-86 %). Interestingly, the Alonissos haplotype does not cluster with any of these groups and most probably belongs to a new, so far unreported, mitochondrial lineage. It shows a basal position, but with low support, with respect to the clade including the European group, the Italian group and Macedonia (Fig. 2). The distinctiveness of this haplotype is also evidenced by its genetic divergence with regard to the other clades (3.3 % and 5.9 %, mean K2P distance). These values are comparable to the average genetic divergence found among the other lineages (Table 1). However, the phylogenetic position of this lineage should be considered with caution, since it is represented by a single sequence.

The presence of such haplotype in the island can be explained in two different ways. First, the differences might be accumulated *in situ* due to the early isolation of the island population. In fact, the land connections between Alonissos and Northern Greece existed during the eustatic cycles of the Pleistocene. However, at 8000 yr BP and onward, the sea level was very close to its present position (Perissoratis & Conispoliatis 2003). The observed level of genetic divergence between the newly reported haplotype and the other mitochondrial lineages is high and comparable to the one observed among the main lineages, whose divergence was estimated between 0.48 mya, and 0.97 mya (middle-lower Pleistocene) (Hürner et al. 2010). Therefore, the presently characterized level of genetic divergence of the Greek specimen seems higher in respect to the time of isolation of the island. Following the second scenario, this specimen might has been introduced recently from the nearly mainland Greece. In fact, the edible dormouse is believed to be introduced in many Mediterranean islands by man in historical times (Masseti 2005). Further studies on genetic characterization of the edible dormouse populations are needed in the Balkanic area to better understand the real genetic diversity in this area and its role as a refugium for small mammals (e.g. Mitsainas et al. 2009).

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