Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 8 (1): 409-420 (2024) (http://www.wildlife-biodiversity.com/)

Short communication

Mitochondrial diversity in Algerian hedgehogs (*Atelerix algirus*) from Malta

Bazzoni Emanuela [1], Lecis Roberta [1*], Attard Vincent [2], Scandura Massimo[1]

[1]Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100 Sassari, Italy
[2]Nature Trust - FEE Malta, PO Box 9, Valletta VLT 1000, Malta
[*]Email: rlecis@uniss.it

Received: 05 October 2023 / Revised: 18 November 2023 / Accepted: 23 November 2023 Published online: 25 November 2023—Ministry of Sciences, Research, and Technology, Arak University, Iran.

How to cite: Emanuela, B., Roberta, L., Vincent, A., Massimo, S. (2024). Mitochondrial diversity in Algerian hedgehogs (*Atelerix algirus*) from Malta, Journal of Wildlife and Biodiversity, 8(1), 409-420. **DOI**: https://doi.org/10.5281/zenodo.10267065

Abstract

Island species are often understudied although being frequently represented by small and fragmented populations, potentially vulnerable to extinction. We investigated the phylogenetic position of hedgehogs living in Malta, using mitochondrial DNA control region analysis. A total of eleven Algerian hedgehog *Atelerix algirus* samples provided by a Wildlife Rescue Centre in Malta were processed for DNA extraction and sequencing. Phylogeographic analyses suggested the presence of different haplogroups within the species *A. algirus*: one endemic to Morocco (and the Canary Islands), another widely distributed in North Africa (reaching Spain and the Balearic Islands), and a third haplogroup represented by the two haplotypes detected in Malta, a diverging lineage typical of the island. We discuss management and conservation implications and put the basis for further research on Maltese hedgehogs.

Keywords: mtDNA, North African hedgehogs, genetic diversity, phylogeography

Introduction

The Algerian hedgehog (*Atelerix algirus*, Maltese: qanfud) is endemic to the Mediterranean region and occurs in Malta and Gozo, where it was historically introduced from North Africa probably by the Romans (Wild Fauna in the Maltese Islands – DRAFT, 2011). The species also occurs in the Northwest of Africa, on the Mediterranean coasts of Spain and in the Balearic and Canary Islands. Based on this zoogeographic pattern, the Algerian hedgehog was likely introduced into Europe by man (Best, 2018). The species usually inhabits coastal dry Mediterranean scrublands, and little is known about its genetic variability along its range. Recent studies investigated population genetic diversity across North Africa, Spain, Balearic and Canary Islands mainly by analyzing mitochondrial DNA (mtDNA) (Khaldi et al., 2016; Derouiche et al., 2016; Velo-Anton et al., 2019; El Farhati et al., 2021), but no genetic studies have been conducted on individuals from Malta.

The origin and taxonomic position of hedgehogs living on the Maltese Islands was uncertain for a long time. The species was first recorded by Gulia (1858), and erroneously classified as *Erinaceus europaeus*. After a taxonomic revision of Maltese specimens (Lanfranco, 1969), today all hedgehog populations occurring in the Maltese archipelago are assigned to the species *Atelerix algirus* (Sciberras et al., 2012). Other studies (Storch, 1970; Malec & Storch 1972) suggested that *A. algirus* was introduced into Malta by a man from the neighbouring African mainland, recognizing two morphotypes on the island: a light-coloured variety with whitish spines and a dark-coloured one with spines presenting a median dark band. The Algerian hedgehog is listed under the Habitat Directive (Annex IVa), the Bern Convention (Appendix II) and protected by Maltese

legislation. In this work, we investigated mitochondrial diversity in Maltese hedgehogs, utilizing sequencing and analyzing the control region, potentially useful to highlight phylogenetic relationships within this species due to its fast evolutionary rate.

Materials and methods

Eleven hedgehog tissue samples originating from roadkills and animals hosted in a Wildlife Rescue Centre in Malta were collected. Recovered animals were formerly found and rescued from various localities on the island (Fig. 1). Among the sampled hedgehogs, seven belonged to the light-coloured morphotype, while four individuals were dark-coloured.

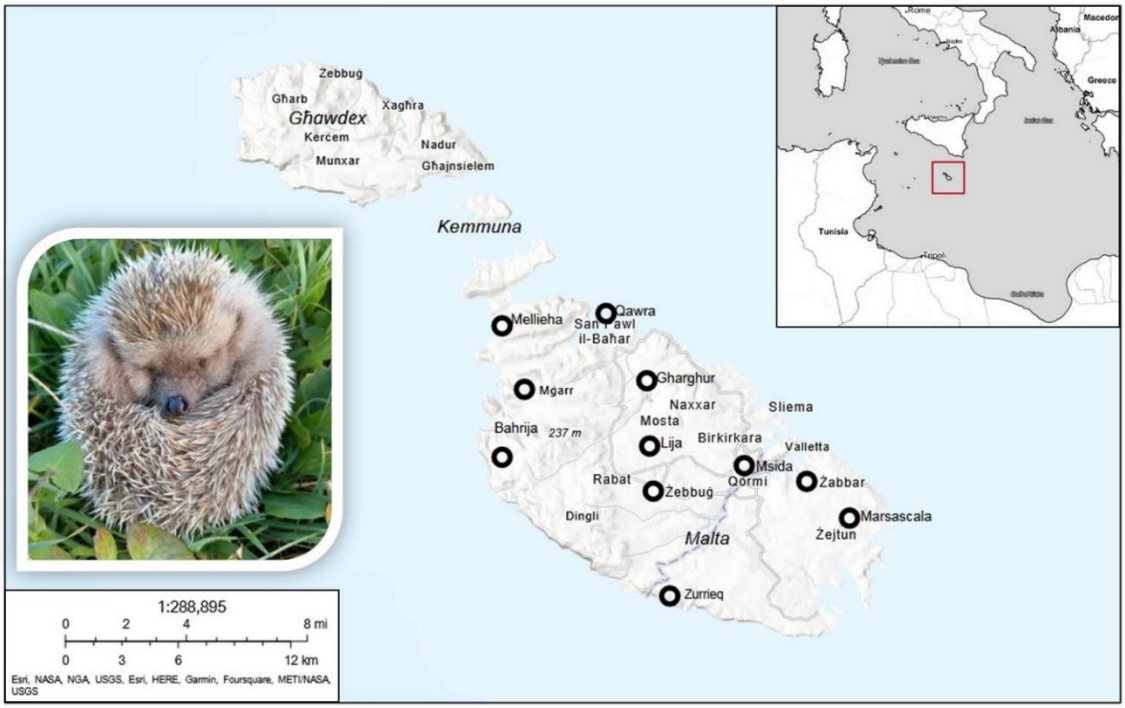

Figure 1. Sampling sites of Maltese hedgehogs analyzed in this study (Photo: Angelique Lofaro Wildlife Rescuers Nature Trust FEE Malta)

We performed genomic DNA extraction using a commercial kit (Sigma GenElute Mammalian Genomic DNA Miniprep Kit). Approximately 465 base pairs of the mitochondrial control region (CR) were amplified by a specific PCR, using the following primer pair for *A.algirus*: CR_F: 5'-CATCAACACCCAAAGTTG-3' and CR_R: 5'-TGAAGAAAGAACCAGATG-3', previously applied to characterize hedgehogs from Algeria (Derouiche et al., 2016). Amplified products were purified using the EXO/SAP enzymatic method and sequencing was performed by Macrogen Inc., Netherlands.

DNAsp v.6 (Rozas et al., 2017) was used to investigate genetic diversity indexes. Differentiation from other African hedgehog populations was investigated, comparing the obtained sequences with 90 homologous sequences available in GenBank (belonging to *A. algirus* and *A. albiventris* originating from Northern and Central Africa, Spain, Balearic and Canary Islands; Derouiche et al., 2016; Velo-Anton et al., 2019; El Farhati et al., 2021) and two outgroup sequences (*Erinaceus europaeus* and *E. roumanicus*), in order to clarify the phylogeographic position of Maltese hedgehogs. Sequences of 395 bp were aligned in MEGA X software (Molecular Evolutionary Genetics Analysis, Kumar et al., 2018) and considered for further analyses. A total of 55 haplotypes were identified using Fabox DNA Collapser (Villesen, 2007).

Phylogenetic analyses were conducted using MEGA X. A model selection was performed to identify the best evolutionary model describing our data. The phylogenetic tree was built using the Maximum Likelihood (ML) method and the selected model HKY + G + I (Hasegawa et al., 1985). Initial tree(s) for the heuristic search were obtained by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology

with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4535)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 66.86% sites). To statistically support the tree topology, 1000 bootstrap replicates were applied (Felsenstein, 1985).

Results and discussion

MtDNA CR sequences obtained from the eleven Maltese samples corresponded to two different haplotypes, differing for one mutation in position 33. Apparently, there was no correlation between the two detected haplotypes and the morphological traits (light or dark morphotypes). Sequences were deposited in GenBank under accession numbers OQ448875 and OQ448876. The overall estimated genetic diversity was rather low (π-nucleotide diversity- 0,0005, H-haplotype diversity- 0,222). As shown in the obtained phylogenetic tree (Fig. 2), a certain degree of genetic differentiation was found among *A. algirus* populations, as the available CR sequences belong to separate and well-diverging haplogroups. Comparing the haplotypes found in Malta with a wide array of available haplotypes from previously analyzed hedgehog populations in North Africa, Spain, Balearic and the Canary Islands, we found a high degree of phylogenetic distance of the Maltese population from any other reference population (Fig. 2).

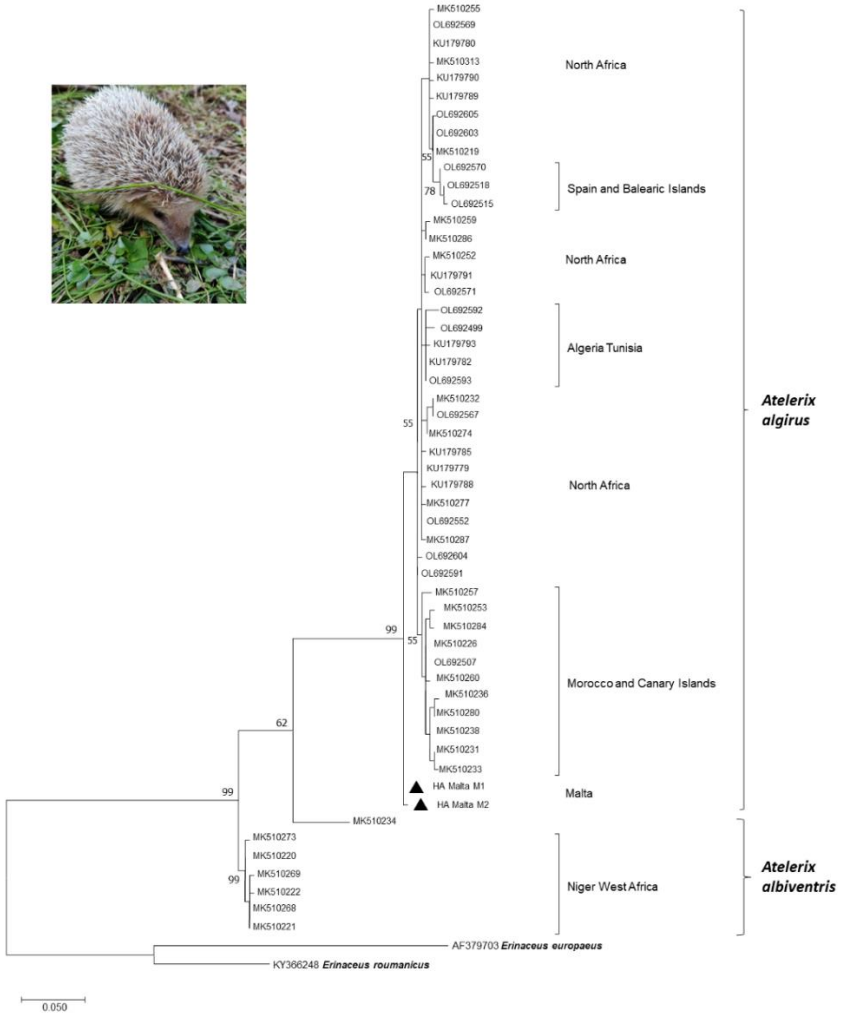

Figure 2. Maximum Likelihood tree (HKY+G+I model) based on 395-bp control region hedgehog sequences obtained in this study and retrieved from GenBank. New haplotypes identified in Malta are indicated by black triangles. Numbers at nodes express the bootstrap support to branches (Photo: Angelique Lofaro Wildlife Rescuers Nature Trust FEE Malta)

As reported in a previous evolutionary study on mtDNA sequences from African hedgehogs (El-Farhati et al., 2021), our results highlight the existence of diverging hedgehog clades in North Africa (*A. algirus*) and in central Africa (*A. albiventris*), and the presence of a distinct and apparently ancestral lineage in Malta. According to El-Farhati et al. (2021), two distantly related lineages occur in North Africa, both

corresponding to *A. algirus*: one group occurring over a larger range spanning from Morocco to Libya (and including Algeria and Tunisia), and one detected only in Morocco and the Canary Islands. Our phylogenetic tree confirmed that the Maltese group is related to the *A. algirus* clade, although sharply distinct from the other haplogroups. These results are congruent with a previous study (Khaldi et al., 2016), showing low genetic diversity in *A. algirus* across its geographic range. The low diversity found in Maltese sampled hedgehogs and their differentiation from North African samples can lead to multiple interpretations: either the local population is ancient and had colonized the island in the far past, then diverging from the source mainland populations, or it was introduced more recently but from a geographic area in North Africa that was not included in past samplings (therefore not represented by reference *A. algirus* sequences) or from a population that went extinct.

Previous molecular studies on mammal species inhabiting North Africa and Mediterranean Europe found shared genetic lineages, despite the potential barrier effect of the Mediterranean Sea. In most cases, one or more historical (thus phylogenetically recent) human-mediated dispersal events across the Mediterranean Sea were suggested as explanation (e.g. *Lepus capensis*, Scandura et al., 2007; *Cervus elaphus*. Doan et al., 2017). In other taxa, however, remarkable splits were detected (e.g., *Herpestes ichneumon*, Gaubert et al., 2011), which are often more cumbersome to interpret. Southern European genetic lineages were often nested within North African clades, and many taxa showed exceptionally high genetic variability and differentiation in this region (Husemann et al., 2014). The regions around the sea straits of Gibraltar and Sicily acted as important biogeographical links between North Africa and Europe at different times

(Husemann et al., 2014). Based on stratigraphic studies on small-sized mammals 'remains in the Ghar Dalam cave in Malta, Hunt & Schembri (1999) recognised an impressive overlap of layers, the most superficial included a lower (prehistoric) stage or Layer III dated as Holocene, and an upper (historic) stage or Layers II and I dating from Phoenician (c.2700 BP) to modern times. The upper layer yielded remains of hedgehogs, together with rats, mice, bats and shrews. It also yielded remains of domestic animals including cattle, sheep, goats, pigs, cats, pigeons, and chickens (Hunt & Schembri 1999).

The appearance of Algerian hedgehogs in Malta could hence be dated between Phoenician and modern times, with a small population likely affected by a founder effect. Maltese hedgehogs probably had the time for genetic differentiation since the beginning of their geographic isolation on the island. Under a founder effect, the evolution may have been rapid, and the traits that distinguish Maltese hedgehogs may have appeared within a few thousand years. Further sampling in Malta and in Gozo is needed to better analyze the degree of genetic differentiation in the local hedgehogs and between them and the other *A. algirus* populations, also by using other mtDNA and/or nuclear markers. A careful morphological characterization of individuals occurring in the two islands would also be worthwhile. Our preliminary results might support the hypothesis that Maltese hedgehogs represent a distinct ESU (evolutionarily significant unit) and should be therefore managed and protected accordingly. Studying their genetic variation might shed light on the potential for survival and long-term adaptation and can be fundamental for this species' management in such a limited range.

Acknowledgements

We thank the Environment & Resources Authority (ERA, Malta) for issuing the Environmental permit (Permit number: EP 1290/21) to allow hedgehog sample collection and genetic analyses.

References

Best, T.L. (2018). Family Erinaceidae (Hedgehogs and Gymnures). In: Wilson DE, Mittermeier RA (Eds) Handbook of the Mammals of the World. Lynx Edicions, Barcelona, 288-331.

Derouiche, L., Bouhadad, R., & Fernandes, C. (2016). Mitochondrial DNA and morphological analysis of hedgehogs (Eulipotyphla: Erinaceidae) in Algeria. Biochemical Systematics and Ecology, 64, 57-64.

Doan, K., Zachos, F.E., Wilkens, B., Vigne, J.D., Piotrowska, N., Stankovic, A., Jedrzejewska, B., Stefaniak, K., & Niedzialkowska, M. (2017). Phylogeography of the Tyrrhenian red deer (*Cervus elaphus corsicanus*) resolved using ancient DNA of radiocarbon-dated subfossils. Scientific Reports, 7, 2331.

El-Farhati, H., Khaldi, M., Ribas, A., Wassim Hizem, M., Nouira, S., & Nicolas, V. (2021). Evolutionary history of the two North African hedgehogs (Mammalia: Erinaceidae) *Atelerix algirus* and *Paraechinus aethiopicus* based on phylogeography and species distribution modelling. Vertebrate Zoology, 71, 799–811.

Felsenstein, J. (1985). Confidence Limits in Phylogenies: An Approach Using the Bootstrap. Evolution, 39(4), 713-791.

Gaubert, P., Machordom, A., Morales, A., Vicente Lopez-Bao, J., Veron, G., Amin, M., Barros, T., ... & Palomares, F. (2011). Comparative phylogeography of two African carnivorans presumably introduced into Europe: disentangling natural versus human-mediated dispersal across the Strait of Gibraltar. Journal of Biogeography, 38, 341–358.

Gulia, G. (1858). Repertorio di storia naturale. AngloMaltese: Malta.

Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, 22(2), 160-74.

Hunt, C.O., & Schembri, P.J. (1999). Quaternary environments and biogeography of the Maltese Islands. In A. Mifsud and C. Savona Ventura (Eds.), Facets of Maltese prehistory (pp. 41-75).

Husemann, M., Schmitt, T., Zachos, F.E., Ulrich, W., & Habel, J.S. (2014). Palaearctic biogeography revisited: evidence for the existence of a North African refugium for Western Palaearctic biota. Journal of Biogeography, 41, 81-94.

Khaldi, M., Ribas, A., Barech, G., Hugot, J.P., Benyettou, M., Albane, L., Arrizabalaga, A., & Nicolas, V. (2016). Molecular evidence supports recent anthropogenic introduction of the Algerian hedgehog *Atelerix algirus* in Spain, Balearic and Canary Islands from North Africa. Mammalia, 80(3), 313-320.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35(6), 1547-1549.

Lanfranco, G.G. (1969). Maltese mammals (Central Mediterranean). Progress Press: Malta, 28pp. + Pts. I - 8.

Malec, F., & Storch, G. (1972). Der Wanderigel, Erinaceus algirus Duvernoy et Lereboullet 1842, von Malta und seine Beziehungen zum nordafrikanischen Herkunftsgebiet. Saugetierkund. Mitt., 20, 146-15 1. München

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. Molecular Biology and Evolution, 34, 3299-3302. DOI: 10.1093/molbev/msx248

Scandura, M., Iacolina, L., Ben Slimen, H., Suchentrunk, F., & Apollonio, M. (2007). Mitochondrial CR-1 variation in Sardinian hares and its relationship with other Old-World hares (genus *Lepus*). Biochemical Genetics, 45, 305-323.

Sciberras, A., Sciberras, J., Sammut, M., & Aloise, G. (2012). A contribution to the knowledge of the terrestrial Mammalian fauna of Comino and its satellite islets (Maltese Archipelago). Biodiversity Journal 3(3), 191-200.

Storch, G. (1970). Holozane Kleinsaugerfunde aus der Ghar Dalam-Hohle Malta (Mammalia, Insectivora, Chiroptera, Rodentia). Senckenbergiana bioL, 51 (3-4): 135-145. Frankfurt

Velo-Anton, G., Boratyński, Z., Ferreira, C.M., Lima, V.O., Alves, P.C., & Brito, J.C. (2019). Intraspecific genetic diversity and distribution of North African hedgehogs (Mammalia: Erinaceidae). Biological Journal Linnean Society, 127, 156-163.

Villesen, P. (2007). FaBox: an online toolbox for Fasta sequences. Molecular Ecology Notes, 7(6), 965-968.

Wild Fauna in the Maltese Islands: Capture, Killing and Exploitation – DRAFT (2011) Ecosystems Management Unit Environment Protection Directorate Malta Environment and Planning Authority Policy on Nature Protection, pp 13-14 (DraftDossier-WildFaunaMalteseIslands-2011.pdf (era.org.mt)