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Islands as Time Capsules for Genetic Diversity Conservation: The Case of the Giglio Island Mouflon

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Abstract: The use of multidisciplinary approaches of investigation including biological, biogeographical, historical, morphological, and genetic analysis, can be useful in identifying and preserving biodiversity. The present study focuses on the characterisation and conservation of a mouflon population (*Ovis gmelini musimon*) from the Mediterranean island of Giglio. Here we provide the first molecular data on the Giglio population and compare it with mouflons from Sardinia, Elba, and Corsica using both nuclear and mitochondrial markers. Our results suggest that the Giglio mouflon harbours genetic variability likely of Sardinian origin but not represented in the current Sardinian mouflon diversity. Although not presenting the typical characteristics of an invasive alien species, the Giglio mouflon is being subjected to eradication through culling or trapping and surgical sterilization. The molecular evidence we report highlights that such actions are causing the irremediable loss of ancestral genetic variants of the genus *Ovis*. Finally, we highlight how a multidisciplinary approach is necessary to aid the conservation and management of the anthropochorous populations of Mediterranean mammals.

Keywords: mouflon; conservation; insularity; eradication; biodiversity

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1. Introduction

The Tyrrhenian mouflon, *Ovis gmelini musimon* (Pallas, 1811), is a medium-sized ungulate occurring in Sardinia and Corsica, where it was introduced by humans in the Neolithic [1–4]. In these Mediterranean islands, the species is protected by law. In the present paper, we follow the taxonomic definition of the species *Ovis gmelini* Blyth, 1841 [5,6]. Due to intense poaching and habitat loss, the Sardinian population suffered a dangerous census decline during the 20th century, which lasted until the 1970s [7], at which time it was estimated that just a few hundred individuals survived [8,9].

At the initiative of some Italian authorities in zoology (including A. Ghigi, A. Toschi, and R. Videsott), a small group of mouflons was transferred to the island of Giglio (2,380 ha) in the Tuscan Archipelago in the mid-1950s to avert their anticipated extinction in Sardinia and Corsica [10–12]. This small population was hosted in a private, fenced property approximately 90 ha in size, on the Franco promontory (Figure 1A). Its initial nucleus was formed in 1955 with seven animals: four females and three males, of which one young male and two females were collected from Germany "… from Corso-Sardinian

stock, first imported to Hungary around 1800 and fortified, while maintaining breed purity in Germany" [10]. The other individuals were all collected from reserves in Sardinia. The animals thrived in the Mediterranean climate of Giglio Island and by 1960–1961, the headcount reached 24 individuals. Due to breaches in the original fence, mouflons began to be sighted outside the reserve in the early 1980s, first in the northern part of the Franco promontory and then in the southwestern portion of the island (Figure 1B). Although no official estimate of the current population size exists, anecdotal records suggest a population size ranging from 24 to 96 individuals [13]. Currently, the Giglio mouflon is being subjected to an introduced species eradication project mediated by the local government [13]. However, no prior biological and ecological studies, and no genetic evaluation have been undertaken to assess the value of this population in terms of biodiversity. Here, we provide the preliminary findings obtained by the genetic characterisation of the Giglio mouflon via investigation of mitochondrial DNA (mtDNA) and microsatellite markers.

Figure 1. Geographic location of the Giglio Island. (**A**) The dark grey area represents the range of the original enclosure in the Franco promontory. (**B**) Two adult males of mouflon roaming in Franco promontory. Photo courtesy of Amy Bond, 2021.

2. Materials and Methods

Overall, 24 mouflon specimens were sampled, including 15 from Giglio Island, one from Elba, seven from Sardinia, and seven from Corsica. Importantly, the Sardinian individuals were selected from a previous study [14] to maximise their representativeness of the Sardinian mouflon diversity and contrast the small sample size. Additionally, two individuals of Sarda, a modern domestic Sardinian breed, were included. Whole peripheral blood was collected by competent veterinarians from the specimens of Sardinia and Corsica. Non-invasive hair sampling was performed on Elba and Giglio individuals translocated to natural reserves located in mainland Tuscany, Italy. Genomic DNA was extracted from blood and hair using the GenElute Blood genomic DNA and the GenElute Mammalian genomic DNA Minipreps kits (Merck), respectively, according to the manufacturer's protocol. Sample quality and DNA concentration were determined using an ND-8000 spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific, MA, USA).

The primer pair CR1-CR2 (Table S2, Supplementary Materials) was used to amplify the entire mtDNA D-loop region, following the protocol described by Satta and colleagues

(2021). The PCR products were purified using a Montage Gel Extraction Kit (Merck Millipore), sequenced by terminator chemistry (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems) and subjected to capillary electrophoresis on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Multiple sequence alignment was performed using Bioedit v7.2.5 [15].

The dataset was extended including 88 *O. gmelini* sequences retrieved from Satta and colleagues [14] to better cover the geographical distribution of the species. We included sequences representative of mouflons from the islands of Sardinia (54) and Corsica (2), and from mainland Europe (3). Three additional homologous sequences from urial (*O. vignei*) were included as outgroups. Sequence data were analysed using DnaSP v6.10.03 to assess the genetic diversity [16]. The genetic relationship among haplotypes was investigated by means of median-joining network using PopART v1.750 [17]. Pairwise genetic distances were estimated with the software MEGA v11 [18].

To evaluate the population diversity and structure, samples were genotyped at 14 Simple Sequence Repeats (SSR) loci retrieved from literature [14]. The amplification products were obtained according to Scali and colleagues [19] and subjected to fine sizing by capillary electrophoresis using an ABI 3130 DNA analyser (Applied Biosystems). The single sample from Elba Island was not subjected to population-aimed analyses. To investigate the ordinal relationship between populations and individuals, we performed a Principal Components Analysis (PCA) as implemented in the ade4 v1.7-19 R package [20]. For each SSR locus, we assessed whether any allele was private to the Giglio population rather than shared across populations.

3. Results

The sequencing reaction for the D-loop region failed for six samples from Giglio, which were consequently excluded from further analyses. The nucleotide sequencing of the PCR products provided nine Giglio, one Elba, and eight Corsica sequences of heterogeneous length ranging from 630 to 1,180 nucleotides (GB#ON960213-22). The whole dataset counted 109 sequences. The analyses were carried out on the hypervariable domain I of the mitochondrial D-loop region to estimate the genetic variability on the available population sample. Two novel haplotypes were detected among the nine Giglio samples, Hpt-6 found in eight samples and Hpt-7 in the remaining one, separated by three-step mutations, with haplotype diversity Hd = 0.222 and nucleotide diversity π = 0.0021. Hpt-6 and Hpt-7 were found in Giglio with frequency 89% and 11%, respectively.

Among the eight Corsican samples analysed in the present study, six shared the same haplotypes identified in a Corsican mouflon sample (h11) by Sanna and colleagues [21], whereas two showed a new haplotype (Hpt-8). Within the ten Corsican samples, we found three mtDNA haplotypes separated by one-step mutations (Hd = 0.511; π = 0.0018). The Elba sample harboured a private haplotype (Hpt-9). To the best of our knowledge, none of these haplotypes had previously been described in domestic sheep breeds.

The median-joining network detected three main mtDNA haplotype clusters characterised by a strong geographical component (Figure 2). The 18 mouflon sequences clustered in five haplotypes (Hpt-6 to 9 and h11), according to the results inferred from the analysis of the genetic variability and haplotype diversity previously reported. Hpt-6 and Hpt-7 were found to be closely related to haplotypes Hpt-1 and Hpt-4, both previously described in the Sardinian mouflon [14], whereas Hpt-9 appeared closer to continental Europe mouflons.

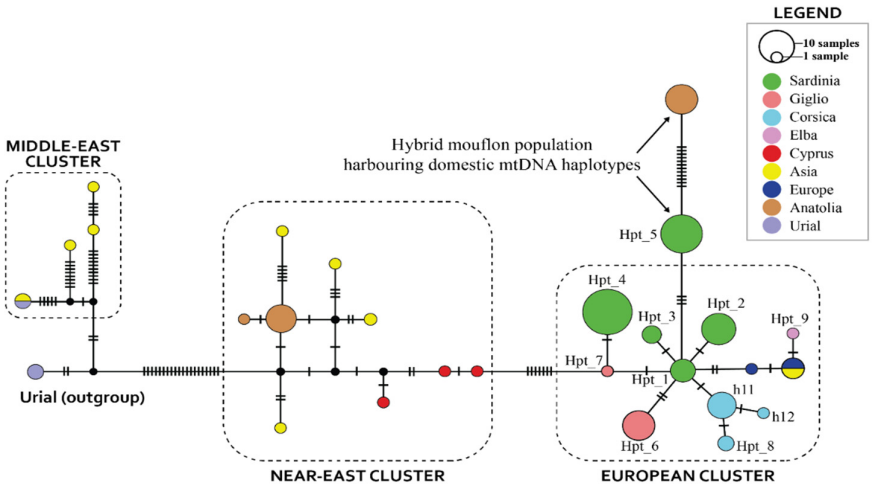

Figure 2. Median-joining network showing the relationship among mouflon mtDNA haplotypes.

To quantify the differences among the haplotypes within the European Cluster (n = 62), pairwise genetic distances between groups were calculated. Hpt-6 was found more related to Hpt-1 (0.0063 ± 0.005) than Hpt-7 (0.0096 ± 0.005), the latter being equidistant from Hpt-1 and Hpt-4 (0.0032 ± 0.003). For both Hpt-6 and Hpt-7, the maximum distance was recorded from Hpt-9 (0.0193 ± 0.003).

The PCA performed on SSR genotypes discriminated the dataset into three distinct clusters, overlapping with the geographic origin of the samples: Sardinia, Corsica, and Giglio Island (Figure 3), with the domestic individuals included within the Sardinian cluster. Importantly, we found ten Giglio individuals harbouring two private SSR alleles at two loci (MCM150-112 and MNS5-185; Table S1, Supplementary Materials).

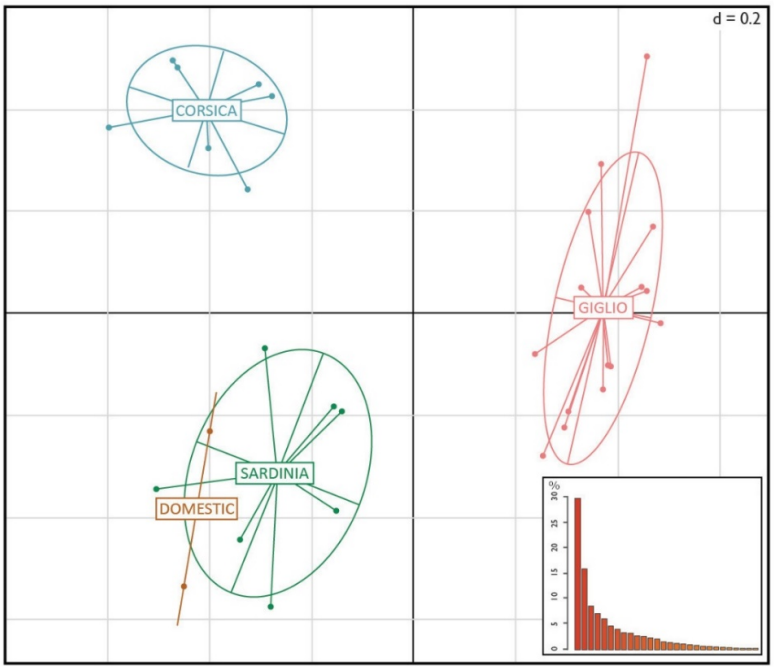

Figure 3. PCA of four mouflon and one domestic population, X and Y axes represent the first and second PCs, respectively. The inset shows the importance of each PC in terms of percentage of total variance explained.

4. Discussion

The biodiversity existing on islands can be extremely rich as geographic isolation might simultaneously promote evolutionary diversity as well as host formerly widespread lineages of species [22–24]. Our results confirm the role of the Mediterranean islands as a genetic reservoir, as already highlighted for: the Cretan shrew and sparrow [25,26], the Sicilian shrew [27], the Rhodian fallow deer [28,29], the Cypriot mouse and mouflon [21,30], the European griffon vulture [31], the Sardinian miniature horses and red deer [32,33], the Pianosa hare [34], and the viper and goat of Montecristo [35,36].

The two mtDNA haplotypes, Hpt-6 and Hpt-7, grouped within the European mouflon cluster and have not been previously described in domestic sheep breeds. Hpt-6 appeared closely related to Hpt-1, which is the oldest mtDNA haplotype identified within the European mouflon cluster, and possibly the one from which the current mouflon diversity originated [37]. Hpt-7 is positioned between Hpt-1 and Hpt-4 in the median-joining network (Figure 2). Both Hpt-1 and Hpt-4 are of Sardinian origin, suggesting that Hpt-7 represents a residual trace of the original Sardinian mouflon variability [14]. Similarly, SSR analyses showed private alleles in the Giglio mouflons, indicating that this population harbours exclusive genetic variability. The low incidence of Hpt-7 in the Giglio population highlights its inherent frailty, hence requiring adequate and urgent protection measures to prevent its irreversible disappearance. The haplotype harboured by the Elba sample descends from the lineage ancestor of the European haplotypes; this is consistent with the historical sources which trace back the origin of the Elban colony to the introduction in 1975 of six females and three males from continental Europe [12].

The translocation of Sardinian mouflons to Giglio occurred in the mid-1950s, when the Sardinian population was experiencing a severe bottleneck due to habitat erosion and extensive poaching, with only ~300 remaining individuals recorded around 1978 [8,38]. According to the available scans of the Sardinian mouflon diversity [14,21], the Sardinian lineages translocated to Giglio did not survive in the source population. The occurrence of genetic isolates stemming from a widespread species is not uncommon, as seen in the present-day fallow deer population inhabiting the island of Rhodes in the eastern Aegean Sea, which retains some genetic traits absent both in the current, neighbouring Turkish population, and in the remaining world population of the species [28,29]. Over the following decades, the enactment of several laws aimed at protecting local wildlife, coupled with the institution of natural reserves, allowed the Sardinian mouflon to increase in number. However, the population bottleneck experienced in the first half of the 20th century eroded a proportion of the original diversity, a part of which has been fortuitously preserved on the island of Giglio.

Eradication can be a useful tool to prevent the spread of invasive alien species and mitigate their impact on native biodiversity, ecosystem functions, animal and plant health, and human economies [39–41]. However, along with evaluating the impacts of removing a species from an ecosystem, the irreversible nature of eradication should also impose an in-depth evaluation of the genetic value of the focal population, and whether it represents a genetic isolate of a formerly widespread population [39]. Our results suggest that the Giglio mouflon population is of a high conservation priority as it represents an invaluable and irreplaceable genetic resource.

In 2014, the European Union provided guidelines to define 'invasive alien species or race' (IAS; Regulation EU 1143/2014). IAS, often introduced as a result of globalisation, are defined as those species or breeds inserted outside their natural geographical range, intended as a macro-area, accidentally or intentionally, perhaps for commercial exchanges. IAS are known to negatively affect island biota due to their tendency to become demographically out of control in a relatively short period of time and alter the structure and functioning of entire ecosystems [42,43]. The main consequences are significant economic impacts and serious/major problems for human health [42,44]. However, no negative impact on the environment or on other species has been assessed through sound and documented studies to establish containment measures of the Giglio

mouflon. The only notified disturbance due to the mouflon seems to be some negligible damage to crops (amounting to a few hundred euros over the last 19 years).

This study demonstrates that the mouflon population on Giglio Island harbours genetic diversity undetected in the Sardinian stock. Like a time capsule, the island of Giglio might have preserved a proportion of the ancestral DNA of the Sardinian mouflon, as the extant population represents a snapshot of the autochthonous Sardinian pool at the time of translocation. Our results suggest an urgent need to interrupt the eradication activities and simultaneously conceive conservation plans to preserve what is left of the Giglio population, whilst allowing larger-scale genomic investigations to confirm and add detail to this lost genetic resource. The original enclosure in the Franco promontory could be restored to allow the monitoring of the population, while sound and documented investigations on the impact of the mouflon on Giglio Island can be performed [45]. Importantly, the *in situ* conservation strategy would allow the Giglio authorities to leverage the presence of the local mouflon population to promote biodiversity awareness as an added feature of the highly tourism-based local economy, ultimately converting the presence of mouflon from an alleged nuisance to an enriching feature of Giglio Island. The data obtained on the enclosed population would enable educated choices to either allow the Giglio mouflon back to unmanaged conditions, or whether to develop alternative *in situ* and *ex situ* conservation plans. In the long term, following the necessary genomic assessment and the implementation of a dedicated restocking program, it would be possible to reintegrate the once lost lineages represented in the Giglio mouflon back into the Sardinian mouflon population. Moreover, the availability of these unexplored mouflon genetic variants is promising to add detail to the general understanding on the evolution under human cultural control of the genus *Ovis* [46–48]. This additional information will likely increase the detection power on studies aimed at assessing the levels of selection, genomic adaptation to environmental challenges [49–52], and the fine-grained ancestry and occurrence of crossbreeding [3,53,54].

5. Conclusions

The Mediterranean has been intensively influenced by human activity over a prolonged period, with no ecosystems left untouched. Over the past 12,000 years, this geographical area has been characterised by the multiple stratification of successive anthropogenic cultural events that have deeply altered the composition of its original biocenoses. Such complex events led to multiple occurrences of introduced populations likely harbouring unique genetic diversity, such as the one discussed here. The case of the Giglio mouflon suggests that whenever irreversible conservation actions are under consideration, the precautionary principle should be embraced, and thorough multidisciplinary investigations should be implemented to assess the benefit–risk ratio [45].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14080609/s1, Table S1: SSR scores, Table S2: CR1-CR2 primers sequence

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Data Availability Statement: The mtDNA sequences generated in this work are publicly available in GenBank at GB# ON960213-22; the SSR scores are provided as Supplementary Table S1.

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References

1. Poplin, F. Origine Du Mouflone de Corse Dans Une Nouvelle Perspective Paléontologique: Par Marronnage. *Ann. Genet. Sel. Anim.* **1979**, *11*, 133–143.
2. Masseti, M. The Prehistoric Diffusion of the Asiatic Mouflon, Ovis Gmelini Blyth, 1841, and the Bezoar Goat, Capra Aegagrus Erxleben, 1777, in the Mediterranean Area beyond Their Natural Distributions. In *Proceedings of the Second International Symposium on Mediterranean Mouflon "The Mediterranean mouflon: Management and conservation"*; Hadjisterkotis, E., Ed.; Game Fund of Cyprus/IUCN Species Survival Commission, Caprinae Specialist Group: Nicosia, Cyprus, 1997.
3. Barbato, M.; Hailer, F.; Orozco-terWengel, P.; Kijas, J.W.; Mereu, P.; Cabras, P.; Mazza, R.; Pirastru, M.; Bruford, M.W. Genomic Signatures of Adaptive Introgression from European Mouflon into Domestic Sheep. *Sci. Rep.* **2017**, *7*, 7623. https://doi.org/10.1038/s41598-017-07382-7.
4. Ciani, E.; Mastrangelo, S.; da Silva, A.; Marroni, F.; Ferenčaković, M.; Ajmone-Marsan, P.; Baird, H.; Barbato, M.; Colli, L.; Delvento, C.; et al. On the Origin of European Sheep as Revealed by the Diversity of the Balkan Breeds and by Optimizing Population-Genetic Analysis Tools. *Genet. Sel. Evol.* **2020**, *52*, 25. https://doi.org/10.1186/s12711-020-00545-7.
5. Cugnasse, J.-M. Révision Taxonomique Des Mouflons Des Îles Méditerranée. *Mammalia* **1994**, *58*, 507–512.
6. Michel, S.; Ghoddousi, A. *Ovis Vignei. The IUCN Red List of Threatened Species 2020*; E. T54940655A54940728; International Union for Conservation of Nature and Natural Resources: Gland, Switzerland, 2020.
7. Perco, F. *Ungulati*; Carlo Lorenzini editore: Udine, Italy, 1987.
8. Cassola, F. *Il Muflone Di Sardegna: Importanza, Stato Attuale e Problemi Di Conservazione*; World Wildlife Fund: Roma, Italy, 1976.
9. Perco, F. *Il Muflone*; Edagricole: Bologna, Italy, 1977.
10. Masserini, V. *Diana*. Editoriale Olimpia, Firenze, Italy, 1958, pp. 11–14.
11. Baldacci, U. *La riserva di Caccia*; Ente Produttori Selvaggina, Roma, Italy, 1975, pp. 1–4.
12. Masseti, M. *Fauna Toscana. Galliformi Non Migratori, Lagormorfi e Artiodattili*; Agenzia Regionale per lo Sviluppo e l'Innovazione nel settore Agricolo-forestale (ARSIA): Firenze, Italy, 2003; ISBN 8882950441.
13. LifeGoGiglio. Available online: https://www.lifegogiglio.eu/en/highlights/actions/ (accessed on 8 July 2022).
14. Satta, V.; Mereu, P.; Barbato, M.; Pirastru, M.; Bassu, G.; Manca, L.; Naitana, S.; Leoni, G.G. Genetic Characterization and Implications for Conservation of the Last Autochthonous Mouflon Population in Europe. *Sci. Rep.* **2021**, *11*, 14729. https://doi.org/10.1038/s41598-021-94134-3.
15. Hall, T. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
16. Rozas, J.; Ferrer-Mata, A.; Sánchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E.; Sánchez-Gracia, A. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Mol. Biol. Evol.* **2017**, *34*, 3299–3302. https://doi.org/10.1093/molbev/msx248.
17. Bandelt, H.J.; Forster, P.; Rohl, A. Median-Joining Networks for Inferring Intraspecific Phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036.
18. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. https://doi.org/10.1093/molbev/msab120.

19. Scali, M.; Vignani, R.; Quintetti, M.; Bigliazzi, J.; Paolucci, E.; Cresti, M. Genetic Traceability of Cinta Senese Pig (*Sus scrofa domesticus* L.): A Study of the Meat and Processed Products by Microsatellite Markers. *Food Nutr. Sci.* **2015**, *06*, 712–726. https://doi.org/10.4236/fns.2015.68074.
20. Dray, S.; Dufour, A.-B. The Ade4 Package: Implementing the Duality Diagram for Ecologists. *J. Stat. Softw.* **2007**, *22*, 1–20. https://doi.org/10.18637/jss.v022.i04.
21. Sanna, D.; Barbato, M.; Hadjisterkotis, E.; Cossu, P.; Decandia, L.; Trova, S.; Pirastru, M.; Leoni, G.G.; Naitana, S.; Francalacci, P.; et al. The First Mitogenome of the Cyprus Mouflon (Ovis Gmelini Ophion): New Insights into the Phylogeny of the Genus Ovis. *PLoS ONE* **2015**, *10*, e0144257. https://doi.org/10.1371/journal.pone.0144257.
22. Drake, D.R.; Mulder, C.P.H.; Towns, D.R.; Daugherty, C.H. The Biology of Insularity: An Introduction. *J. Biogeogr.* **2002**, *29*, 563–569. https://doi.org/10.1046/j.1365-2699.2002.00706.x.
23. Hébert, K.; Millien, V.; Lessard, J.-P.; Masters, J. Source Pool Diversity and Proximity Shape the Compositional Uniqueness of Insular Mammal Assemblages Worldwide. *J. Biogeogr.* **2021**, *48*, 2337–2349. https://doi.org/10.1111/jbi.14156.
24. Fernández-Palacios, J.M.; Kreft, H.; Irl, S.D.H.; Norder, S.; Ah-Peng, C.; Borges, P.A.V.; Burns, K.C.; de Nascimento, L.; Meyer, J.Y.; Montes, E.; et al. Scientists' Warning—The Outstanding Biodiversity of Islands Is in Peril. *Glob. Ecol. Conserv.* **2021**, *31*, e01847. https://doi.org/10.1016/j.gecco.2021.e01847.
25. Reumer, J.W.F. Shrews (Soricidae) on Islands, with Special Reference to Crocidura Zimmermanni from Crete. In *Pleistocene and Holocene Fauna of Crete and its First Settlers*; Prehistory Press: Madison, WI, USA, 1996; pp. 173–179.
26. Masseti, M. Mammals of the Mediterranean Islands: Homogenisation and the Loss of Biodiversity. *Mammalia* **2009**, *73*, 169-202.
27. Sarà, M.; Vitturi, R. Crocidura Populations (Mammalia, Soricidae) from the Sicilian-Maltese Insular Area. *Hystrix Ital. J. Mammal.* **1996**, *8 (1-2), 121-132*. https://doi.org/10.4404/hystrix-8.1-2-4101.
28. Masseti, M.; Vernesi, C. Historic Zoology of the European Fallow Deer, Dama Dama Dama: Evidence from Biogeography, Archaeology and Genetics. In *Deer and People. Oxbow books, Oxford*, 2014; pp. 13–22.
29. Masseti, M.; Pecchioli, E.; Vernesi, C. Phylogeography of the Last Surviving Populations of Rhodian and Anatolian Fallow Deer (*Dama dama dama* L., 1758). *Biol. J. Linn. Soc.* **2008**, *93*, 835–844. https://doi.org/10.1111/j.1095-8312.2007.00951.x.
30. Cucchi, T.; Orth, A.; Auffray, J.-C.; Renaud, S.; Fabre, L.; Catalan, J.; Hadjisterkotis, E.; Bonhomme, F.; Vigne, J.-D. A New Endemic Species of the Subgenus Mus (Rodentia, Mammalia) on the Island of Cyprus. *Zootaxa* **2006**, 1241, 1–36.
31. Mereu, P.; Satta, V.; Frongia, G.N.; Berlinguer, F.; Muzzeddu, M.; Campus, A.; Decandia, L.; Pirastru, M.; Manca, L.; Naitana, S.; et al. The Complete MtDNA Sequence of the Griffon Vulture (Gyps Fulvus): Phylogenetic Analysis and Haplotype Frequency Variations after Restocking in the Sardinian Population. *Biol. Conserv.* **2017**, *214*, 195–205. https://doi.org/10.1016/j.biocon.2017.08.017.
32. Morelli, L.; Useli, A.; Sanna, D.; Barbato, M.; Contu, D.; Pala, M.; Cancedda, M.; Francalacci, P. Mitochondrial DNA Lineages of Italian Giara and Sarcidano Horses. *Genet. Mol. Res.* **2014**, *13*, 8241–8257. https://doi.org/10.4238/2014.October.20.1 ABSTRACT.
33. Doan, K.; Zachos, F.E.; Wilkens, B.; Vigne, J.-D.; Piotrowska, N.; Stanković, A.; Jędrzejewska, B.; Stefaniak, K.; Niedziałkowska, M. Phylogeography of the Tyrrhenian Red Deer (*Cervus elaphus corsicanus*) Resolved Using Ancient DNA of Radiocarbon-Dated Subfossils. *Sci. Rep.* **2017**, *7*, 2331. https://doi.org/10.1038/s41598-017-02359-y.
34. Hobohm, C. Nature-Culture Dichotomy and Environmental Consciousness: Do We Fear the Right Things? In *Perspectives for Biodiversity and Ecosystems*; Springer: Cham, Switzerland, 2021; pp. 17–41.
35. Masseti, M.; Zuffi, M.A.L. On the Origin of the Population of Asp Viper, Vipera Aspis Hugyi Schinz, 1833, of the Island of Montecristo, in the Northern Tyrrhenian Sea (Tuscan Archipelago, Italy). *Herpetol. Bull.* **2011**, 117.
36. Somenzi, E.; Senczuk, G.; Ciampolini, R.; Cortellari, M.; Vajana, E.; Tosser-Klopp, G.; Pilla, F.; Ajmone-Marsan, P.; Crepaldi, P.; Colli, L. The SNP-Based Profiling of Montecristo Feral Goat Populations Reveals a History of Isolation, Bottlenecks, and the Effects of Management. *Genes* **2022**, *13*, 213. https://doi.org/10.3390/genes13020213.
37. Mereu, P.; Pirastru, M.; Barbato, M.; Satta, V.; Hadjisterkotis, E.; Manca, L.; Naitana, S.; Leoni, G.G. Identification of an Ancestral Haplotype in the Mitochondrial Phylogeny of the Ovine Haplogroup B. *PeerJ* **2019**, *7*, e7895. https://doi.org/10.7717/peerj.7895.
38. CASSOLA, F. Management and Conservation of the Sardinian Moufflon (Ovis Musimon). In *The Biology and Management of Mountain Ungulates*; Croom-Helm: London, UK, 1985; p. 197.
39. Myers, J.H.; Simberloff, D.; Kuris, A.M.; Carey, J.R. Eradication Revisited: Dealing with Exotic Species. *Trends Ecol. Evol.* **2000**, *15*, 316–320. https://doi.org/10.1016/S0169-5347(00)01914-5.
40. Hess, S.C.; Kawakami, B.; Okita, D.; Medeiros, K. *A Preliminary Assessment of Mouflon Abundance at the Kahuku Unit of Hawai'i Volcanoes National Park*; U.S. Geological Survey: Reston, VA, USA, 2006.
41. Bertolino, S.; Sciandra, C.; Bosso, L.; Russo, D.; Lurz, P.W.W.; di Febbraro, M. Spatially Explicit Models as Tools for Implementing Effective Management Strategies for Invasive Alien Mammals. *Mammal Rev.* **2020**, *50*, 187–199. https://doi.org/10.1111/mam.12185.
42. Sundseth, K. *Invasive Alien Species: A European Union Response*; Publications Office of the European Union: Luxembourg, 2014; ISBN 9279354868.
43. Hadjisterkotis, E.; Konstantinou, G.; Sanna, D.; Pirastru, M.; Mereu, P. First Mtdna Sequences and Body Measurements for Rattus Norvegicus from the Mediterranean Island of Cyprus. *Life* **2020**, *10*, 136. https://doi.org/10.3390/life10080136.
44. Bani, L.; Orioli, V.; Trasforini, S.; Puzzi, C.M.; Sibilia, A.; Dondina, O.; Tirozzi, P. The Spread of Exotic Fish Species in Italian Rivers and Their Effect on Native Fish Fauna since 1990. *Biodiversity* **2021**, *22*, 4–12. https://doi.org/10.1080/14888386.2020.1837014.

45. Shackleton, D. *Objectives, Format and Limitations of the Caprinae Survey and Action Plan*; IUCN International Union for Conservation of Nature: Gland, Switzerland, 1997; ISBN 2-8317-0353-0.
46. Kijas, J.W.; Lenstra, J.A.; Hayes, B.J.; Boitard, S.; Porto Neto, L.R.; San Cristobal, M.; Servin, B.; McCulloch, R.; Whan, V.; Gietzen, K.; et al. Genome-Wide Analysis of the World's Sheep Breeds Reveals High Levels of Historic Mixture and Strong Recent Selection. *PLoS Biol.* **2012**, *10*, e1001258. https://doi.org/10.1371/journal.pbio.1001258.
47. Dotsev, A. v.; Kunz, E.; Shakhin, A.V.; Petrov, S.N.; Kostyunina, O.V.; Okhlopkov, I.M.; Deniskova, T.E.; Barbato, M.; Bagirov, V.A.; Medvedev, D.G.; et al. The First Complete Mitochondrial Genomes of Snow Sheep (*Ovis nivicola*) and Thinhorn Sheep (*Ovis dalli*) and Their Phylogenetic Implications for the Genus Ovis. *Mitochondrial DNA Part B* **2019**, *4*, 1332–1333. https://doi.org/10.1080/23802359.2018.1535849.
48. Cao, Y.-H.; Xu, S.-S.; Shen, M.; Chen, Z.-H.; Gao, L.; Lv, F.-H.; Xie, X.-L.; Wang, X.-H.; Yang, H.; Liu, C.-B.; et al. Historical Introgression from Wild Relatives Enhanced Climatic Adaptation and Resistance to Pneumonia in Sheep. *Mol. Biol. Evol.* **2020**, *38*, 838–855. https://doi.org/10.1093/molbev/msaa236.
49. Lv, F.-H.; Cao, Y.-H.; Liu, G.-J.; Luo, L.-Y.; Lu, R.; Liu, M.-J.; Li, W.-R.; Zhou, P.; Wang, X.-H.; Shen, M.; et al. Whole-Genome Resequencing of Worldwide Wild and Domestic Sheep Elucidates Genetic Diversity, Introgression and Agronomically Important Loci. *Mol. Biol. Evol.* **2021**, *39*, msab353. https://doi.org/10.1093/molbev/msab353.
50. Alberto, F.J.; Boyer, F.; Orozco-terWengel, P.; Streeter, I.; Servin, B.; de Villemereuil, P.; Benjelloun, B.; Librado, P.; Biscarini, F.; Colli, L.; et al. Convergent Genomic Signatures of Domestication in Sheep and Goats. *Nat. Commun.* **2018**, *9*, 813. https://doi.org/10.1038/s41467-018-03206-y.
51. Cortellari, M.; Barbato, M.; Talenti, A.; Bionda, A.; Carta, A.; Ciampolini, R.; Ciani, E.; Crisà, A.; Frattini, S.; Lasagna, E.; et al. The Climatic and Genetic Heritage of Italian Goat Breeds with Genomic SNP Data. *Sci. Rep.* **2021**, *11*, 10986. https://doi.org/10.1038/s41598-021-89900-2.
52. Passamonti, M.M.; Somenzi, E.; Barbato, M.; Chillemi, G.; Colli, L.; Joost, S.; Milanesi, M.; Negrini, R.; Santini, M.; Vajana, E.; et al. The Quest for Genes Involved in Adaptation to Climate Change in Ruminant Livestock. *Animals* **2021**, *11*, 2833. https://doi.org/10.3390/ani11102833.
53. Somenzi, E.; Ajmone-Marsan, P.; Barbato, M. Identification of Ancestry Informative Marker (AIM) Panels to Assess Hybridisation between Feral and Domestic Sheep. *Animals* **2020**, *10*, 582. https://doi.org/10.3390/ani10040582.
54. Lorenzini, R.; Cabras, P.; Fanelli, R.; Carboni, G.L. Wildlife Molecular Forensics: Identification of the Sardinian Mouflon Using STR Profiling and the Bayesian Assignment Test. *Forensic Sci. Int. Genet.* **2011**, *5*, 345–349. https://doi.org/10.1016/j.fsigen.2011.01.012.