

Reevaluating the phylogenetic position of *Prolagus sardus* (Wagner, 1829): Insights from ancient DNA and paleogenomics

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Abstract. This news and views article critically examines the recent phylogenetic reassessment of the extinct Sardinian pika, *Prolagus sardus* (Wagner, 1829), based on ancient DNA analysis. The study highlights the significance of integrating molecular and paleontological evidence in resolving long-standing taxonomic debates. By analyzing mitochondrial DNA extracted from a Neolithic specimen, researchers determined that *P. sardus* represents a distinct evolutionary lineage, separate from modern pikas of the *Ochotona* genus. Molecular clock analysis suggests that *Prolagus* diverged from the *Ochotona* lineage approximately 30 million years ago, supporting the hypothesis that it belongs to a separate family, Prolagidae. These findings refine our understanding of lagomorph evolution, biogeography, and extinction dynamics in Mediterranean island species. The study underscores the importance of paleogenomic approaches in reconstructing evolutionary histories and calls for further research to expand genetic data on *P. sardus*.

Key Words: biogeography, conservation genetics, evolutionary lineage, extinction dynamics, insular species, mitochondrial DNA, molecular phylogenetics, taxonomic classification, *Prolagus sardus*.

This news and views article critically examines the recent phylogenetic reassessment of the extinct Sardinian pika, *Prolagus sardus* (Wagner, 1829) based on ancient DNA analysis. It highlights the significance of the study's findings in resolving long-standing taxonomic debates, discusses their implications for evolutionary biology and conservation genetics, and explores broader questions about island species' extinction dynamics and evolutionary trajectories.

A recent study, conducted by a team of 13 researchers, including Valerio Joe Utzeri, Elisabetta Cilli, Francesco Fontani, Daniel Zoboli, Massimiliano Orsini, and others, represents a collaboration among several prestigious institutions such as the University of Bologna, the Max Planck Institute for Evolutionary Anthropology, the University of Cagliari, and the A.N. Severtsov Institute of Ecology and Evolution. Their research focused on the extinct Sardinian pika (Figure 1), an enigmatic lagomorph species that once inhabited the Mediterranean islands of Sardinia and Corsica (Fernández-Bejarano et al 2024) before becoming extinct in the Holocene (Vigne 1992; Angelone et al 2014; Valenzuela et al 2022). The primary objective of the study was to reassess the phylogenetic position of *P. sardus* using ancient DNA (aDNA) analysis, a modern genetic approach that allows for the reconstruction of evolutionary relationships among extinct species (Orsini et al 2023). Given the ongoing debate regarding whether *Prolagus* belongs to the Ochotonidae family (which includes modern pikas) or should be classified separately under the family Prolagidae, Orsini et al (2023) aimed to provide new molecular evidence to clarify this uncertainty.

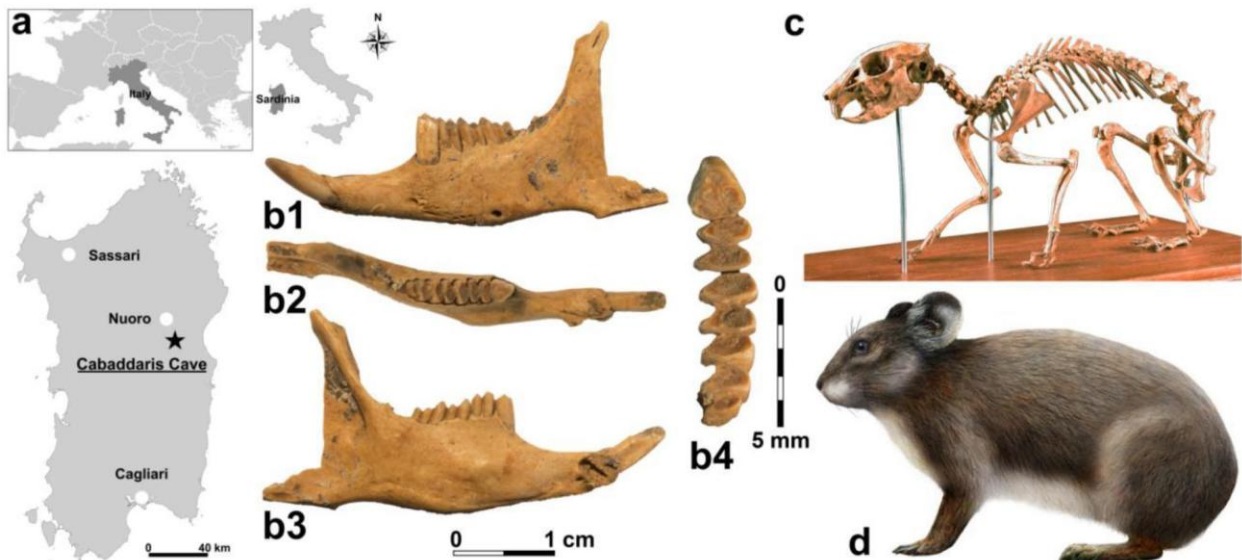


Figure 1. (a) Geographic location of the Cabaddaris Cave (Sardinia, Italy); (b) left hemimandible of *P. sardus* used for DNA extraction and radiocarbon dating, from different perspectives (b1: buccal, b2: occlusal, b3: lingual and b4: occlusal view of the cheek teeth); (c) composite skeleton of *P. sardus* (Museo Sardo di Geologia e Paleontologia "D. Lovisato", University of Cagliari); (d) palaeo-artistic reconstruction of *P. sardus* (drawing by D. Zoboli, source: Orsini et al 2023).

To achieve their goal, the team successfully extracted and sequenced a portion of the mitochondrial genome from a *P. sardus* specimen recovered from the Cabaddaris cave (Figure 1), an archaeological site in Sardinia (Orsini et al 2023). The bone sample was radiocarbon dated to the Neolithic period, approximately 7500 years before present. The researchers applied state-of-the-art paleogenomic methodologies, including next-generation sequencing and bioinformatics analyses, to recover and analyze the genetic material (Orsini et al 2023). Despite the challenges posed by the degradation of ancient DNA, particularly in warm Mediterranean climates where preservation conditions are less favorable than in permafrost environments, the team managed to retrieve a significant portion of the mitochondrial genome. This dataset allowed them to conduct detailed phylogenetic analyses and estimate divergence times between *Prolagus* and its closest living relatives (Orsini et al 2023).

The results of the study indicate that *P. sardus* represents a distinct evolutionary lineage, separate from the modern pikas of the genus *Ochotona* (Orsini et al 2023). Molecular clock analysis suggests that *Prolagus* diverged from the *Ochotona* lineage approximately 30 million years ago (Orsini et al 2023). This finding challenges previous classifications that grouped *Prolagus* within the family Ochotonidae and supports the alternative hypothesis that it belongs to a separate family, Prolagidae. The study's phylogenetic trees consistently placed *P. sardus* as a sister taxon to Ochotonidae, reinforcing the idea that it followed an independent evolutionary trajectory. These molecular results align with previous paleontological evidence, which highlighted unique morphological traits in *Prolagus*, such as the absence of the third lower molar (M3), distinguishing it from other lagomorphs (Orsini et al 2023).

The implications of these findings are significant for multiple fields of research, including evolutionary biology, paleontology, and conservation genetics. First, they provide a more refined understanding of lagomorph evolution by clarifying the placement of an extinct lineage that has long been debated. By integrating genetic data with fossil evidence, the study demonstrates the power of paleogenomics in resolving taxonomic uncertainties and reconstructing evolutionary histories that would otherwise remain speculative. Additionally, the research offers new insights into the biogeography and extinction dynamics of Mediterranean island species. *P. sardus* was once abundant in Sardinia and Corsica, where it coexisted with endemic predators and early human

populations. Its eventual extinction, likely influenced by the introduction of new predators and ecological competitors, provides a case study in how insular species are particularly vulnerable to environmental changes and anthropogenic pressures.

Moreover, the study underscores the importance of continued exploration of ancient DNA from fossil remains to fill gaps in our understanding of past biodiversity. While the researchers successfully recovered partial mitochondrial genome sequences, they emphasize the need for further studies to obtain a complete genomic profile of *P. sardus* (Orsini et al 2023). Future research could involve analyzing additional specimens from different time periods and locations to assess genetic diversity within the species and track potential evolutionary changes over time. This approach could also help determine whether the genetic isolation of *Prolagus* contributed to its extinction or if other factors, such as habitat loss and climate fluctuations, played a more significant role.

In conclusion, this study represents a milestone in the phylogenetic reassessment of *P. sardus*, providing compelling molecular evidence that supports its classification as a separate lineage from Ochotonidae. The integration of paleogenomics and traditional paleontology offers a more comprehensive perspective on the evolutionary history of this extinct species, reinforcing the need for multidisciplinary approaches in the study of biodiversity and extinction. By shedding light on the genetic identity of *Prolagus*, the research not only resolves a long-standing debate but also contributes to broader discussions on the evolutionary trajectories of island-endemic mammals and their responses to ecological pressures.

Conflict of Interest. The authors declare that there is no conflict of interest.

References

- Angelone C., Prieto J., Gross M., 2014 Complement to the study of the pikas (Lagomorpha, Ochotonidae) from the Middle Miocene of Gratkorn, Austria. *Palaeobiodiv. Palaeoenviro* 94:125-134.
- Fernández-Bejarano E., Blanco A., Angelone C., Zhang Z., Moncunill-Solé B., 2024 Bone histology of the Late Pleistocene *Prolagus sardus* (Lagomorpha: Mammalia) provides further insights into life-history strategy of insular giant small mammals. *Zoological Journal of the Linnean Society* 201(1):169-183.
- Orsini M., Ribani A., Latorre A., Lisovsky A., Pillola G. L., Bovo S., et al, 2023 Ancient DNA re-opens the question of the phylogenetic position of the Sardinian pika *Prolagus sardus* (Wagner, 1829), an extinct lagomorph. *Scientific Reports* 13:13635.
- Valenzuela A., Torres-Roig E., Zoboli D., Pillola G. L., Alcover J. A., 2022 Asynchronous ecological upheavals on the Western Mediterranean islands: New insights on the extinction of their autochthonous small mammals. *Holocene* 32:137-146.
- Vigne J.-D., 1992 Zooarchaeology and the biogeographical history of the mammals of Corsica and Sardinia since the last ice age. *Mammal Review* 22(2):87-96.

Received: 09 November 2024. Accepted: 28 December 2024. Published online: 30 December 2024.

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How to cite this article:

Petrescu-Mag I. V., Oroian C., 2024 Reevaluating the phylogenetic position of *Prolagus sardus* (Wagner, 1829): Insights from ancient DNA and paleogenomics. *Rabbit Gen* 14(1):38-40.