



First mitochondrial genome of the marbled polecat *Vormela peregusna* (Carnivora, Mustelidae)

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ABSTRACT

The marbled polecat, *Vormela peregusna*, is one of the least studied species in the Mustelidae family, especially with regard to phylogeography and genetic diversity. In this study, we determined the mitochondrial genome sequence of *V. peregusna* and investigated its position within the Mustelidae phylogeny. The generated mitogenome is 15,982 bp in length; it consists of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and a control region.

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The marbled polecat, *Vormela peregusna* Gldenstaedt (1770), is a small mammal species in the Mustelidae family. It is listed as Vulnerable (VU) according to the IUCN Redlist (IUCN 2020). The species is distributed from southeast Europe, throughout the Middle East, to several parts of Asia (Gorsuch and Larivière 2005; IUCN 2020). Despite its wide distribution in the Middle East (Ellerman and Morrison-Scott 1951; Harrison 1968; Wilson and Reeder 2005; Ibiş and Tez 2014), previous genetic studies of marbled polecats are scarce and do not include Lebanese specimens (Koepfli et al. 2008).

In this study, we sequenced the mitochondrial genome of *V. peregusna* using historical DNA extracted from a Lebanese museum specimen to provide more genetic information about this species and to investigate its phylogenetic relationship with other species of the Mustelidae family. The sequence was submitted to GenBank (accession number MW013133). This is the first study to produce the mitochondrial genome of the species *Vormela peregusna*, and the first sequenced mitogenome of a species belonging to the Ictonychinae subfamily of Mustelidae.

A footpad tissue sample was obtained from a preserved museum specimen (voucher number MOQ20) from the Museum of Birds, Mammals and Butterflies of Qobayat-Lebanon (34°34'00"N, 36°16'45"E) using sterile instruments. DNA was extracted using a modified silica-column extraction protocol (McDonough et al. 2018) at the ancient DNA

laboratory of the Smithsonian Center for Conservation Genomics (CCG) in Washington, DC where the DNA is stored. We quantified the DNA extract using a Qubit[®] fluorometer (Life Technologies) and estimated the DNA fragment size using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) with High Sensitivity DNA kits. We then used the Illumina blunt-end single-tube library preparation method for degraded DNA (Carøe et al. 2018) to prepare a DNA library for sequencing. We determined the number of index PCR cycles by performing qPCR on the library, and then performed a dual indexing PCR with TruSeq-style indices (Meyer and Kircher 2010) using Kapa HiFi Uracil+ (Kapa Biosystems). The library was sequenced with 2 × 150 bp paired-end reads using an Illumina MiSeq[®] platform at the CCG.

PCR duplicates and poor-quality reads were removed from the raw sequence data with prinseq-lite-0.20.4; adapter contamination was removed using TrimGalore version 0.4.1. Mitogenome assembly, consensus generation, and annotation were performed with Geneious v9.1.2 software (Biomatters Ltd.). Quality-filtered reads were mapped to the complete mitogenome of the closest phylogenetic relative available, the small-toothed ferret-badger *Melogale moschata* (NC_020644). The generated consensus sequence was aligned to the small-toothed ferret-badger reference sequence using the MAFFT v7.450 plug-in (Katoh and Standley 2013), and

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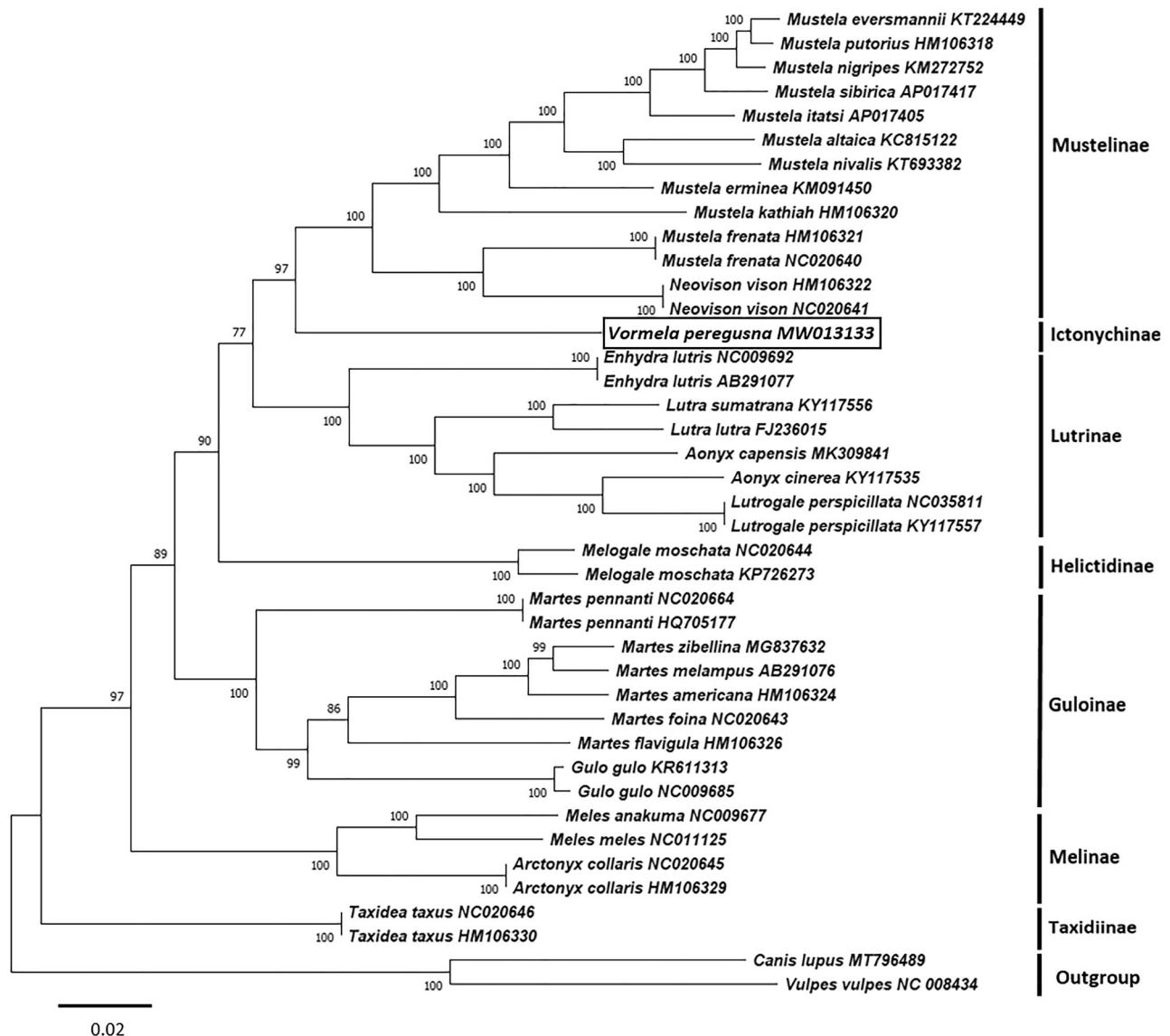


Figure 1. Maximum-likelihood phylogenetic tree of *Vormela peregusna* and species of Mustelidae and Canidae families based on complete mitogenome sequences.

annotations were transferred from the reference. The sequence was translated to check for stop codons.

The generated mitochondrial genome length of *V. peregusna* is 15,982 bp which covers 99.7% of the reference sequence. The average sequencing depth is $12.5 \times (0-34 \times)$. The sequence contains 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, 13 protein-coding genes (PCGs), and 1 control region. The base composition is 33.2% A, 26% C, 12.9% G, 27.4% T and 0.5% N; the GC content is 38.9%, which is approximately accordant with other Mustelidae species (Jeon et al. 2017; Gao et al. 2020). The small gaps observed, especially in the *16S rRNA*, *ATP6* and *ND5* genes, are likely due to the degraded quality of the historical DNA extract.

To determine the phylogenetic position of *V. peregusna* with respect to other species in the family, and to investigate the position of the Ictonychinae subfamily in the Mustelidae phylogenetic tree, we constructed a maximum-likelihood (ML) tree using the software MEGA X (Kumar et al. 2018) with 1000 bootstrap replicates including our generated

sequence and complete mitogenome sequences of several Mustelidae species obtained from GenBank (Figure 1). The mitogenome sequence of two Canidae species (*Canis lupus* and *Vulpes vulpes*) were used as an outgroup. The phylogenetic tree showed that the Ictonychinae subfamily was sister to the clade composed of Mustelinae species. These results are concordant with the topology in Koepfli et al. (2008) obtained from nuclear genes and the *cytb* mitochondrial gene. Moreover, previous phylogenetic analysis based on mitochondrial and nuclear genes (Law et al. 2018) showed the position of Ictonychinae subfamily in a sister clade to the Mustelinae and Lutrinae subfamilies clade. This is partly in accordance with our results which revealed the close relationship between Mustelinae and Ictonychinae subfamilies species, but also the relatively far distance-relation between these two subfamilies and the Lutrinae subfamily.

Museum specimens are sometimes the only accessible material to study extinct and endangered species, or rare and elusive species such as the marbled polecat (Castañeda-Rico et al. 2020). Adding to the body of literature on a rare

species from an understudied region of the world, our results are essential for deciphering the evolutionary history of this species and for future phylogeographical and population genetic studies.









Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, reference number MW013133.

References

- Carøe C, Gopalakrishnan S, Vinner L, Mak SS, Sinding MHS, Samaniego JA, Wales N, Sicheritz-Pontén T, Gilbert MTP. 2018. Single-tube library preparation for degraded DNA. *Methods Ecol Evol.* 9(2):410–419.
- Castañeda-Rico S, León-Paniagua L, Edwards CW, Maldonado JE. 2020. Ancient DNA from museum specimens and next generation sequencing help resolve the controversial evolutionary history of the critically endangered Puebla Deer Mouse. *Front Ecol Evol.* 8:94.
- Ellerman JR, Morrison-Scott TCS. 1951. Checklist of Palaearctic and Indian mammals, 1758–1946 (Vol. 3). London: Trustees of the British Museum (Natural History)
- Gao W, Lu Z, Liang Y, Ren Z-M. 2020. Complete mitochondrial genome of *Mustela sibirica* (Carnivora: Mustelidae), a protected and endangered species in China. *Mitochondrial DNA B Resour.* 5(1):1081–1083.
- Gorsuch WA, Larivière S. 2005. *Vormela peregusna*. *Mamm Species.* 779: 1–5.
- Harrison D. 1968. *Mammals of Arabia*. Vol. 2. London: Ernst-Benn Ltd.
- Ibiş O, Tez C. 2014. Phylogenetic status and genetic diversity of the Turkish marbled polecat, *Vormela peregusna*, (Mustelidae: Carnivora: Mammalia), inferred from the mitochondrial cytochrome b gene. *Vert Zool.* 62(4):285–294.
- IUCN 2020. The IUCN Red List of Threatened Species. Version 2020-1. <http://www.iucnredlist.org>
- Jeon MG, Kim HR, Min JH, Kim HJ, Park YC. 2017. Complete mitochondrial genome of the Asian badger *Meles leucurus* (Mustelidae) from Korea. *Mitochondrial DNA B Resour.* 2(2):556–557.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 30(4):772–780.
- Koepfli KP, Deere KA, Slater GJ, Begg C, Begg K, Grassman L, Lucherini M, Veron G, Wayne RK. 2008. Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. *BMC Biol.* 6(1):10.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol.* 35(6):1547–1549.
- Law CJ, Slater GJ, Mehta RS. 2018. Lineage diversity and size disparity in Musteloidea: testing patterns of adaptive radiation using molecular and fossil-based methods. *Syst Biol.* 67(1):127–144.
- McDonough MM, Parker LD, Rotzel McInerney N, Campana MG, Maldonado JE. 2018. Performance of commonly requested destructive museum samples for mammalian genomic studies. *J Mammal.* 99(4): 789–802.
- Meyer M, Kircher M. 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb Protoc.* 2010(6):pdb.prot5448.
- Wilson DE, Reeder DM. (eds.). 2005. *Mammal Species of the World: A Taxonomic and Geographic Reference*. 3rd edition. Johns Hopkins University Press, Baltimore, Maryland, 2142 pp.