The marbled polecat, *Vormela peregusna* Güldenstaedt (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; İbîş and Tez 2014), previous genetic studies of marbled polecats are scarce and do not include Lebanese specimens (Koepfl 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 MQ020) 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 (Caroe 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
annotations were transferred from the reference. The sequence was translated to check for stop codons.

The generated mitochondrial genome length of V. peregrusna is 15,982 bp which covers 99.7% of the reference sequence. The average sequencing depth is 12.5 × (0–34×). 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. peregrusna 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 (Castaneda-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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**ORCID**

Liliane Boukhdoud [http://orcid.org/0000-0001-5391-2992]
Lillian D Parker [http://orcid.org/0000-0003-3370-9473]
Nancy Rotzel McInerney [http://orcid.org/0000-0002-6519-7671]
Carole Saliba [http://orcid.org/0000-0002-2927-5046]
Rhea Kahale [http://orcid.org/0000-0002-3784-4739]
Hugh Cross [http://orcid.org/0000-0002-6745-9479]
Elizabeth Matisoo-Smith [http://orcid.org/0000-0002-3370-9473]
Nancy Rotzel McInerney [http://orcid.org/0000-0002-6519-7671]
Carole Saliba [http://orcid.org/0000-0002-2927-5046]
Rhea Kahale [http://orcid.org/0000-0002-3784-4739]
Hugh Cross [http://orcid.org/0000-0002-6745-9479]
Elizabeth Matisoo-Smith [http://orcid.org/0000-0002-3370-9473]

**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.

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