

Genetic analysis reveals occurrence of Pallas's cat *Otocolobus manul* in the Karakoram Wildlife Sanctuary, Ladakh

CHAMBA WANGMO¹, SHAHID HAMEED^{1, 2, 3}
MD. NIAMAT ALI¹ and TAWQIR BASHIR^{4*} 

Abstract In the Indian Trans-Himalaya, Pallas's cat *Otocolobus manul* is reported exclusively from Ladakh, Himachal Pradesh, Uttarakhand and Sikkim. In Ladakh, it has been reported primarily from the south-east, with no prior evidence of its occurrence in the north. In this study, we documented the first record of Pallas's cat in the Karakoram Wildlife Sanctuary in the Nubra Valley, northern Ladakh. We identified the species based on the non-invasive genetic analysis of a morphologically ambiguous scat sample collected during a study on dietary patterns of sympatric carnivores. DNA-based species identification confirmed the presence of Pallas's cat. We also identified prey remains, of pika *Ochotona* spp. and Himalayan marmot *Marmota himalayana*, in the scat. Our record extends the known range of Pallas's cat into northern Ladakh and adds a new mammal species to the documented fauna of the Karakoram Wildlife Sanctuary. We recommend a comprehensive assessment of distribution, population, diet, habitat and potential threats to this elusive species to ensure its long-term survival in this landscape.

Keywords DNA, Ladakh, Nubra valley, *Otocolobus manul*, Pallas's cat, range extension, scat, small carnivore

Small felids play a critical role in the ecosystems they inhabit by regulating populations of small prey, facilitating nutrient cycling and maintaining habitat health (Litvaitis & Villafuerte, 1996; Crooks & Soulé, 1999; Bandyopadhyay et al., 2024). However, despite their ecological importance and the threats they face, these species often remain underrepresented in research and conservation, especially compared to charismatic large carnivores (Nowell & Jackson, 1996; Mugerwa et al., 2020; Bandyopadhyay et al., 2024). One such species is Pallas's cat *Otocolobus manul*, a small, elusive felid with a wide but fragmented distribution

across Central Asia from Iran to Mongolia and southern Siberia (Greenspan & Giordano, 2021; Lorestani et al., 2022). Studies of the species' potential habitat have enhanced knowledge of its distribution in Afghanistan, Tibet and parts of India, Nepal, China, Bhutan and Pakistan (Hameed et al., 2014; Shrestha et al., 2014; Dendup et al., 2019; Ross et al., 2020). The species typically inhabits rocky areas, montane grasslands, shrublands and cold montane deserts with extreme continental climate, low rainfall, low humidity and large temperature variations (Nowell & Jackson, 1996; Munkhtsog et al., 2004; Ross et al., 2020). Pallas's cat plays an important role in cold dry montane ecosystems by regulating small mammal populations, particularly pikas *Ochotona* spp. (Greenspan & Giordano, 2021), the availability of which has a strong influence on the distribution of this felid (Seimon et al., 2022). Pallas's cat is categorized as Least Concern on the IUCN Red List, but data on its occurrence comprises mostly individual records and thus there have been no estimates of population size or trend (Ross et al., 2020).

The c. 15,000 km² Nubra Valley lies in the northernmost region of the district of Leh within the Union Territory of Ladakh, in the Trans-Himalayan biogeographical zone of India. The Ladakh mountain range lies to its south and the Karakoram Range to the north, with some permanently glaciated areas. The valley was designated as a protected area in 1987, as the Karakoram (Nubra-Shayok) Wildlife Sanctuary (Fig. 1), spanning an elevation range of 2,690–8,572 m. This region also harbours the snow leopard *Panthera uncia*, Central Asian lynx *Lynx lynx isabellinus*, Tibetan wolf *Canis lupus chanco*, Siberian ibex *Capra sibirica*, blue sheep *Pseudois nayaur* and Ladakh urial *Ovis vignei vignei*. However, as a result of the remoteness of the area and the extreme climate, there has been no comprehensive documentation of the fauna of the region.

During our study on the dietary patterns of sympatric carnivores in the Karakoram Wildlife Sanctuary from February 2021 to December 2022, we encountered an ambiguous scat sample in the Hunder Dok area, at an altitude of 4,424 m, which we could not assign to any of the known carnivore species in the area based on its morphological characteristics. We collected the scat in a sterile falcon tube, sprayed it with absolute ethanol, and stored it at the Wildlife Biology Laboratory at the University of Kashmir, at –20 °C, for later genetic analysis.

*Corresponding author, tawqir84@gmail.com

¹Centre of Research for Development, University of Kashmir, Srinagar, Jammu and Kashmir, India

²Department of Environmental Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India

³Nature Conservation Foundation, Mysore, Karnataka, India

⁴Division of Wildlife Sciences, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Jammu and Kashmir, India

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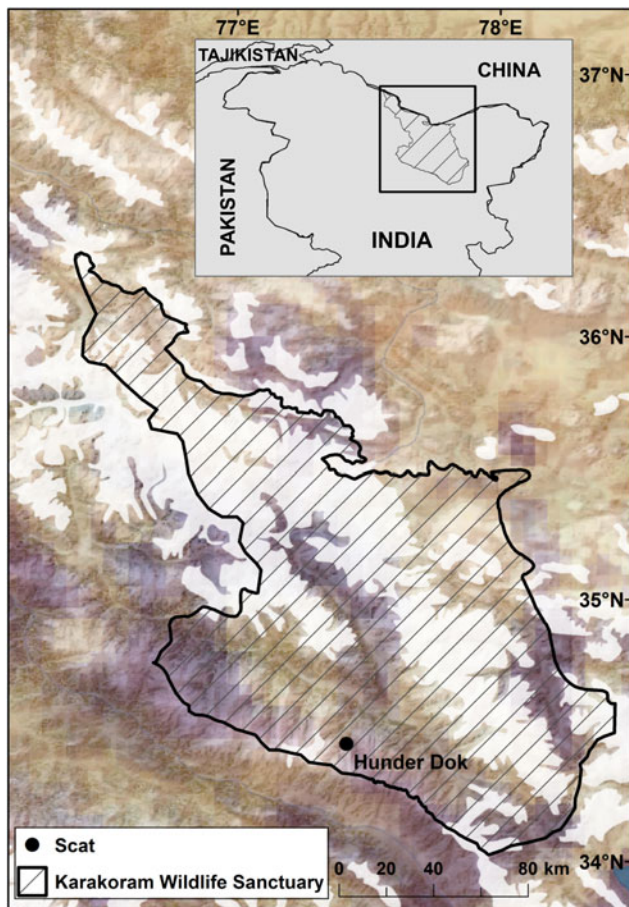


FIG. 1 Nubra Valley, northern Ladakh, India, showing the location (Hunder Dok) in which we collected a scat of Pallas's cat *Otocolobus manul*.

We extracted genomic DNA from the scat using the QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Germany) following the manufacturer's protocol. The extracted DNA was PCR amplified using the carnivore-specific primer targeting the 148-bp mitochondrial Cytochrome-b gene with the forward sequence 5' -TA TTCTTTATCTGCCTATACATRCACG-3' and reverse sequence 5' AAAGTGCAGCCCCTCAGAATGATATTT GTCCTCA-3' (Farrell et al., 2000). We conducted a PCR reaction in a total volume of 15 µl, containing 7.5 µl of 2X Premix EmeraldAmp GT PCR Master Mix (Takara, Japan), 5.9 µl of ddH₂O, 0.3 µl of each forward and reverse primer, and 1.0 µl of template DNA. The PCR assays entailed an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, elongation at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes. We used electrophoresis with 1.2% agarose gel to confirm the success of the PCR amplification, and sequenced the successful PCR amplicon using a BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI 3730XL sequencer (Applied Biosystems) in both the forward and reverse

directions, with the same primer pair used for PCR amplification. We manually edited sequences to remove low-quality bases and primer sequences at the ends, corrected any base-calling errors, and also generated contig and consensus sequences using *CodonCode Aligner* (CodonCode Corporation, USA). To identify the species, we did an NCBI nucleotide blast of the consensus DNA sequence. The cytochrome b sequence that we generated matched the reference sequences of Pallas's cat in GenBank (top three hits: MH978908.1, KR132585.1, KF990330.1, identity > 98.70%). We submitted the DNA sequence to NCBI to generate an accession number (PQ066009.1).

We then used standard micro-histology of mammalian hair remains in the scat (Vogel et al., 2019) to identify any prey, using the modified point-frame method (Ciucci et al., 2004). Oven-dried scat samples were evenly spread in a gridded tray, and 40–50 hairs located nearest to the grid intersections were randomly selected for microscopic examination. Under a microscope, the cuticular and medullary patterns of these hairs were compared with reference slides of potential prey species in the region (Bahuguna et al., 2010). Using this method, we found undigested hair remains of pikas and the Himalayan marmot *Marmota himalayana* in the scat.

To our knowledge, this is the first confirmed occurrence of Pallas's cat in the Karakoram Wildlife Sanctuary in the Trans-Himalayan region of northern Ladakh. In India, the species was previously reported only from the Trans-Himalayan areas of Ladakh (Mahar et al., 2017), Himachal Pradesh (Sharma et al., 2024), Uttarakhand (Pal et al., 2019) and Sikkim (Chanchani, 2008; Menon, 2023). Within Ladakh, previous records have confirmed its presence in the Hanle, Staklung and Lal Pahari areas of the Changthang Wildlife Sanctuary and in the Rupshu region (Mallon, 1991; Pfister, 2004; Mahar et al., 2017; Maeshwari et al., 2023). Nubra Valley shares its eastern boundary with the Changthang plateau, where there have been frequent sightings of the species (Mahar et al., 2017; Maeshwari et al., 2023), suggesting that Pallas's cat in this area may be ecologically connected to the population on the adjoining Tibetan Plateau. Our finding expands the known distribution of Pallas's cat into northern Ladakh and suggests biogeographical connectivity with eastern Ladakh. The new record also provides evidence to support connectivity of this region with other parts of the Indian Trans-Himalaya where the species has already been documented, and with neighbouring countries, including China (Mallon, 2002; Fox & Dorji, 2007) and Nepal (Shrestha et al., 2014; Werhahn et al., 2018, 2024), and Gilgit-Baltistan in Pakistan (Hameed et al., 2014).

Similar biogeographical extensions have also been observed in other high-altitude carnivores, such as the Tibetan sand fox *Vulpes ferrilata*, which was first reported from the Indian Trans-Himalaya in Changthang, Ladakh



PLATE 1 Habitat of Pallas's cat *Otocolobus manul* in the Karakoram Wildlife Sanctuary, Nubra Valley, Ladakh, India. Photo: Chamba Wangmo.

(Namgail et al., 2005), expanding its known range beyond the Tibetan Plateau. The finding of pika and marmot hairs in the scat of Pallas's cat reaffirms the species' dependence on small-sized prey and its potential role in regulating the population of small mammals in this high altitude ecosystem (Ross et al., 2010, 2020). Given the limitations of a single sample, however, we recommend a detailed study to provide a baseline of the species' distribution, status and diet. Our finding nevertheless suggests this region may provide suitable habitat for Pallas's cat (Plate 1), and provides scope for monitoring to inform species conservation and habitat management in the region.

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Conflicts of interest None.

Ethical standards This study is based on field data collected through non-invasive sampling. Our research received the necessary permit from the Department of Wildlife Protection, Ladakh (Permit No. CWLW/Research/2021/1506-08), for conducting the field surveys. No ethical approval was required for this research, and it otherwise abided by the *Oryx* guidelines on ethical standards.

Data availability The data supporting our findings are available on request from the corresponding author.

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