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Original contributions and short notes about wild cats are welcome

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Eurasian lynx and Pallas's cat in Dolpa district of Nepal: genetics, distribution and diet

The Eurasian lynx *Lynx lynx* and the Pallas's cat *Otocolobus manul* are elusive felids which are little studied in the Himalayas of Nepal where they seem to occur at low densities. We present mtDNA genomic and dietary data of six Eurasian lynx scats and one Pallas's cat scat collected in Dolpa district of Nepal. The Eurasian lynx scats were found in alpine shrub lands at elevations from 4,738 - 4,818 m. Dietary analysis based on microscopic frequency of occurrence revealed that the six Eurasian lynx scats contained 56 % woolly hare *Lepus oiostolus*, 17.7 % pika *Ochotona* sp. and rodent *Alticola* sp., 9.7 % Himalayan marmot *Marmota himalayana*, 7 % vegetation and 3.3 % domestic goat *Capra aegagrus hircus*. The Pallas's cat scat found in a rocky boulder field contained 76 % pika hair, 18 % woolly hare hair and 4 % vegetation. This study provides the first scientific record of Eurasian lynx in Shey-Phoksundo National Park SPNP. The finding on Pallas's cat represents an expansion of the IUCN distribution range in Nepal, presents the second locality record of the species in the country, and with the sample collected at 5,539 m is the highest elevation record for the species to date. Further researches into population status, ecology, genetics and conservation of these elusive felids in the Himalayas are recommended.

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The field work for this study was conducted in the arid high Himalayan landscape of upper Dolpa (29°-29°34'48" N / 82°53'24"-83°26'24" E) at elevations ranging from 3,600 m - 5,600 m. The study area comprised alpine grassland and alpine steppe habitats (Miehe et al. 2016). In search of predator signs an area of approximately 1,088 km² was covered by walking 810 km between May to July 2016. The genetic analyses of the collected predator scats

were conducted at the Center for Molecular Dynamics-Nepal with the genetics protocol developed by The Royal Zoological Society of Scotland WildGenes Laboratory (Werhahn et al. 2017). For species identification, samples were amplified and sequenced at the cytochrome *b* region of the mitochondrial DNA (generic MCB primer pairs MCB_398_F:TACCATGAGGACAAATATCATTCTG and MCB_869_R:CCTCCTAGTTTGTAGGGATTGATCG; Verma & Singh 2003). The lynx sam-

ples were also amplified and sequenced at the D-loop region of the mitochondrial DNA (mtU: CTTTGGTCTTGTAACCAAAAAA; and R3: TAAGAACCAGATGCCAGGTA; Rueness et al. 2003). The sequences were then examined with Geneious version 9.0.5 and compared with available sequences on the NCBI GenBank database using the Basic Local Alignment Search Tool BLAST to identify the species.

The microscopic diet analysis was conducted with a microscope (Bresser Science TRM-301,40x-1000x) applying a modified point-frame method: 50 hairs closest to the intersections in a gridded tray per scat were identified based on cuticular cell arrangements, medullary patterns and relative lengths with a reference collection and literature (Teerink 1991, Ciucci et al. 2004, Bahuguna et al. 2010, Klare et al. 2011). We then used the method frequency of occurrence per food item to provide qualitative dietary results (Klare et al. 2011).

Eurasian lynx: Results and Discussion

Six genetically verified scats of Eurasian lynx were found in alpine shrub and grasslands in two areas in upper Dolpa: three scats were found southwest of Bhijer village (29°23'42" - 29°26'9.6" N / 82°56'45.6" - 82°59'2.4" E; 4738 - 4818 m), and three scats east of Koma village (29°24'25.2" - 29°26'56.4" N / 83°7'22.8" - 83°8'24" E; 4330 - 4645 m; Fig. 1 & 2). The generated Eurasian lynx cytochrome *b* mtDNA sequences were 311 bp long, and all six scat sequences belonged to the same Eurasian lynx cytochrome *b* haplotype already deposited on NCBI GenBank, i.e. identity overlap of 100% with accessions KR132581 and



Fig. 1. Eurasian lynx (light blue) and Pallas's cat (dark blue) scat collection locations in Dolpa district, Nepal (modified from Himalayan Map House (2010)).



Fig. 2. Eurasian lynx habitat in the Himalayas of Dolpa, Nepal. Alpine shrubland at ≥ 4400 m above sea level (Photo G. Werhahn).

KM982549 (Paijmans et al. 2016), KF990332 (unpublished, originating from Mongolia) and KP202283 (Li et al. 2016). The 466 bp long D-loop mtDNA sequence of the five successfully amplified samples presents a new haplotype with 99.6 % identity overlap with accessions EU818861, EU818858 and EU818856 (which all belong to the South clade in Rueness et al. 2014); and 99.4% identity overlap with accession KR919624 (Ning et al. 2016). This Eurasian lynx D-loop haplotype newly found in Dolpa of western Nepal is deposited in GenBank under the NCBI accession MF997606.

The frequency of occurrence analysis of these six Eurasian lynx scats revealed a dietary content of 56% woolly hare hair, 17.7% pika and rodent *Alticola* sp. (hair, bones, jaw, and claw), 9.7% Himalayan marmot hair, 7% vegetation, 3.3% debris, 3.3% domestic goat hair and 3% unidentifiable bone parts.

Globally the Eurasian lynx is classified as Least Concern by the IUCN Red List and the subspecies reported for the Himalayas is *Lynx lynx isabellinus* (Kitchener et al. 2017). The IUCN national Red List series of Nepal classifies the Eurasian lynx as Vulnerable in the country due to indications for decreasing population trends (Jnawali et al. 2011). Only infrequent Eurasian lynx observations and reports are available, and very little is known about population size, distribution and the genetics of this elusive species in Nepal (Jnawali et al. 2011, Breitenmoser et al. 2015). Major threats to the Eurasian lynx in the Himalayas are persecution and retaliatory killings in response to livestock depredation and poaching for its fur, and these threats are likely to be also in effect across the border in China (Din & Nawaz 2010, Jnawali et al. 2011). Local people around Koma and Bhijer village have reported occasional livestock depredation incidences by lynx during social surveys conducted for this study. We recommend to include the Eurasian lynx in the legal framework of the wildlife damage relief guidelines of Nepal (GoN 2015) and in other conservation payment schemes which are locally already in place for snow leopards *Panthera uncia*, to encourage coexistence with predators in the Himalayan high-altitude habitats (Dickman et al. 2011, GoN 2015). We also recommend further researches into the population status, ecology, conservation status, and phylogeny of the Eurasian lynx in the Himalayas (Jnawali et al. 2011, Rueness et al. 2014).

Pallas's cat: Results and Discussion

A genetically verified Pallas's cat scat was collected at 5,593 m (29°18'43.2" N / 83°25'33.6" E) in barren rocky habitat (Figs. 1, 3, 4) towards northeast of Tinkyu village of upper Dolpa. The generated 352 bp long mtDNA sequence matched with mitochondrial DNA sequences of *Otocolobus manul* deposited in GenBank (identity overlap of 99.7% with KR132585 published in Paijmans et al. (2016), 99.0% with KP202295 originating from Mongolia and published in Li et al. (2016), and 99.0% with KF990330 (unpublished, originating from Mongolia). Matches to other felid species were at much lower identity overlap (i.e. the next closest thereafter was < 92% identity overlap with Bay cat *Catopuma badia*). This Pallas's cat sample represents a new haplotype, not previously submitted to NCBI GenBank, and is available under the NCBI accession MF997607. The revised taxonomy of felids mentions the Pallas's cat subspecies *Otocolobus manul nigripictus* to be found in the Himalayas (Kitchener et al. 2017) which our haplotype is likely to represent.

The optimal Pallas's cat habitat is reported to be a mix of grassland and shrub steppe with rocky cover, ravines and hill-slopes (Ross et al. 2016). Hence in addition to the genetic verification, habitat and prey availability were also supportive of the scat originating from a Pallas's cat: we found the Pallas's cat scat in a very rocky hillslope within montane grassland steppe, with pikas regularly sighted among the rocks (Fig. 4). This Pallas's cat scat contained 76% pika hair, 18% woolly hare hair, 4% vegetation, and 2% debris. The presence of the Pallas's cats in the Himalayas of Nepal was documented for the first time in 2014 through camera trap images

from the Annapurna Conservation Area in Manang at two locations, namely Thorkya (4,200 m) and Angumila Lapche (4,650 m; Shrestha et al. 2014). The historical presence of the species in the area is suggested by a pelt photographed in the year 1987 in a household in Nyesyang valley in Manang district (Lama et al. 2016). Our record of the species from Dolpa district holds significance for Pallas's cat conservation in Nepal in that it provides the second locality record situated 90 km northeast of the first documented location in Manang. This implies the existence of a population, presumably at low density, across a larger range than previously known in the country and it also extends the known IUCN global distribution for the species westwards in Nepal (Ross et al. 2016; Fig. 3).

The Pallas's cat is globally classified by the IUCN Red List as Near Threatened. The global population trend is decreasing with main threats being habitat degradation, hunting for fur and depletion of prey base (Ross et al. 2016). The Pallas's cat is known to inhabit montane grassland and shrub land steppe of Central Asia with records up to 5,050 m in the northwestern Tibetan Plateau of China (Fox & Dorji 2007, Ross et al. 2016). This Pallas's cat record at 5,539 m provides the new highest elevation record of the species to our knowledge. However climate, habitat and prey base appear to be the main determinants for its presence (Ross et al. 2016).

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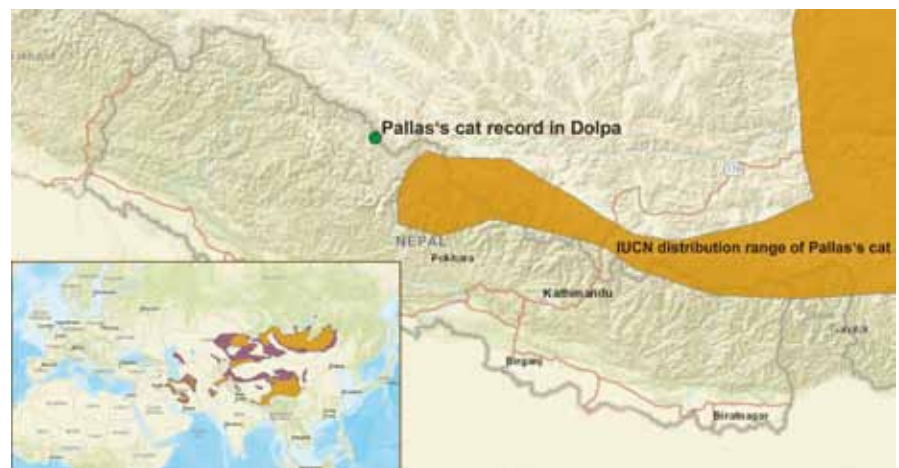


Fig. 3. Pallas's cat scat location in Dolpa district of Nepal (green dot) and the current IUCN distribution (orange: range; violet: possible range; map modified from Ross et al. (2016)).



Fig. 4. Pallas's cat habitat in rocky landscape at 5,593 m above sea level in Dolpa, Nepal (Photo G. Werhahn).

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