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Incidental discovery of non-focal carnivore species during genetic study of Bengal tiger (*Panthera tigris tigris*) and snow leopard (*Panthera uncia*) in Nepal

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ABSTRACT

The Bengal tiger (*Panthera tigris tigris*) and the snow leopard (*Panthera uncia)* are highly endangered apex predators. These charismatic animals are categorized as flagship and umbrella species, and hence are the focus of many conservation programs. Protecting tiger and snow leopard also safeguards the entire habitat in which they reside, including other non-focal sympatric carnivores. Our non-invasive sampling based genetic studies on these flagship species have detected other non-focal carnivore species that co-share the same habitat. In our non-invasive genetic study of the tiger in the Chitwan National Park (2011-2013), of all collected scat samples (n=420), only 62% (n=262) were of tiger. The remaining non- tiger samples (n=158) included common leopard (*Panthera Pardus fusca*, n=74), leopard cat (*Prionailurus bengalensis,* n=10), fox (*Vulpes ssp.,* n=5) and jungle cat (*Felis catus,* n=1). Of 48 putative snow leopard scat samples collected from the Mustang region (2010-2011), only 65% (n= 31) were of snow leopard. We identified red fox (*Vulpes vulpes*, n=7), leopard cat (*Prionailurus bengalensis,* n=2), wolf (*Canis lupus,* n=1), common leopard (*Panthera Pardus fusca*, n=1) and lynx (*Lynx lynx,* n=1) from the remaining samples using target DNA amplification and sequencing. This study, which was integrated in our overall genetic studies of tiger and snow leopard, has significantly increased our understanding of the carnivore community in the Terai and Himalaya region of Nepal. We were also able to document lynx at the highest elevation (3935 m) and detect the presence of an illusive himalayan wolf and leopard cat for the first time in the snow leopard habitat of Nepal.

Key words: Bengal tiger, carnivore species, Chitwan National Park , leopard

INTRODUCTION

Amid concerns over the loss of biodiversity, experts have been increasingly seeking clues to understand ecosystems and biodiversity (Cardinale *et al*., 2012). The prevailing conservation and management practices, globally, include coarse-filter and fine-filter approaches (Noss, 1987), the former aims to preserve entire communities of plants and animals by protecting large extents of habitat (ecosystem approach) whilst the latter focuses to protect species (species approach).

Nepal established several protected areas based on species and ecosystem approaches for wildlife and habitat management following prevailing conservation trends in the 1970s (Heinen and Shrestha, 2006). The ecosystem conservation and management approach has largely been boosted by research on mega species such as the greater one horned rhinoceros (*Rhinoceros unicornis*), Bengal tiger (*Panthera tigris tigris,* hereafter referred as tiger) and snow leopard (*Panthera uncia*). Maintaining viable populations of these mega species, that include apex predators, is important for ecosystem integrity and resilience (Soulé and Terborgh, 1999). This has been demonstrated well by successful programs with landscape level approach to conservation like the Terai Arc Landscape project in Nepal, which has recently contributed to the gradual recovery in the tiger population (GoN, 2013) including other mega fauna such as rhinoceros and elephants (GoN, 2009, Thapa *et al*., 2013).

Current burgeoning conservation needs and limited availability of resources often forces conservation managers and planners to rely on information on the occurrence of apex species to serve as ecosystem health indicators (Simberloff, 1998). Tiger in the Terai arc landscape and snow leopard in the high Himalaya including the trans-himalayan region of Nepal are such apex predators (Forrest *et al*., 2012).

Tiger and snow leopard are both endangered species (IUCN, 2013). They are also charismatic and umbrella species (Clucas *et al*., 2008). Therefore, they

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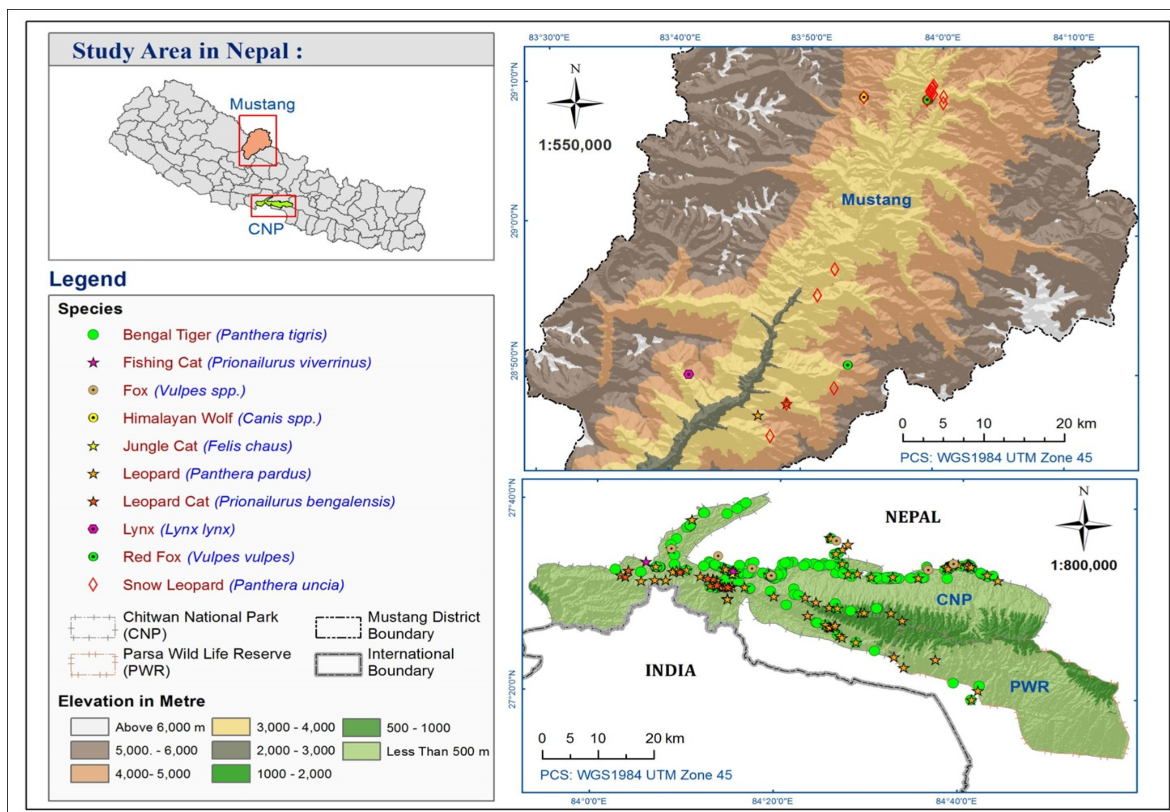

Figure 1. Map of the study sites.1) Chitwan National Park located in the plains of Terai. Tiger tends to occupy the lowland area of Nepal 2) Mustang is located within the Annapurna Conservation Area. Snow leopards are known to occur throughout trans-himalayan range of Nepal 3) Locations showing the distribution of scat samples and identified species from the study sites in Chitwan National Park, and Mustang Region.

are herded as both means for and targets of conservation. By protecting tiger and snow leopard, it is hoped that the entire community and ecosystem they reside in can be protected. Being charismatic, they also have traits and qualities that appeal to target audiences to raise funds and awareness for reducing the biodiversity loss (Verissimo *et al*., 2013).

Core tiger populations are fragmented and found mainly in lowland areas across the four protected areas of Parsa, Chitwan, Bardia and Shuklaphanta and occupy 36% of 26,000 sq. km of the forested landscape of Terai Arc Landscape, Nepal (Barber-Meyer *et al*., 2013, Shrestha, 1997, Smith *et al*., 1998). This habitat is also shared with leopard (*Panthera pardus*), dhole (*Cuon alpinus*), sloth bear (*Melursus ursinus*) and striped hyena (*Hyaena hyaena*) within the Terai Arc Landscape (Joshi *et al*., 1995, Odden *et al*., 2010, Seidensticker 1976, Thapa *et al*., 2013). The trans-himalayan region of Nepal is prime habitat for snow leopard covering an area of 20,000 sq. km(Forrest *et al*., 2012, Jackson and Ahlborn 1989) and including other carnivore species, such as wolf (*Canis lupus*) and lynx (*Lynx lynx*) (Mishra *et al*., 2003, Namgail *et al*., 2007).

In the past, there have been numerous studies conducted on the status of the flagship species (GoN, 2013, Karki *et al*., 2013, Subedi *et al*., 2013). However, there has been limited information available on the degree to which the non-focal species have benefited while working with these flagship species. Use of non-invasive sampling for genetic studies utilizing molecular scatology techniques (Kelly *et al*., 2012, Reed *et al*., 1997) primarily on flagship species could be beneficial in identifying the occurrence of the non-focal species along with the species of interest , thereby helping to map and document the spatial distribution of species.

Scat (fecal) surveys are often used to detect and monitor elusive and low-density carnivore species (Janečka *et al*., 2008, Karmacharya *et al.,* 2011, Waits and Paetkau, 2005). However, there is a high degree of misidentification of scats during field collection, with many scats not belonging to the target species of interest (Janečka *et al.,* 2008, Karmacharya *et al.,* 2011, Spiering *et al.,* 2009). Genetic analysis of DNA extracted from fecal samples can address this problem. One common approach is to identify a species of interest using species-specific PCR primers for identification (Fernandes *et al*., 2008, Kurose *et al*., 2005, Nagata *et al*., 2005, *Oliveira et al.,* 2010) for the selected pools of samples. However, this approach does not provide identification of any of the non-focused species. For these samples, DNA sequence data can help identify the species: this is the basis of DNA barcoding for species identification (Baselga *et al*., 2013).

During our study on tiger in the Chitwan National Park (CNP) in the lowland areas of Terai Arc under Nepal Tiger Genome Project (NTGP) in 2011-2013 and on snow leopard in the high elevation of the Mustang region of Annapurna Conservation area in Nepal (Fig. 1), there was significant misidentification of collected scats (Karmacharya *et al*., 2011). Using DNA barcoding technique, we were able to genetically profile non-focal carnivore species sharing tiger and snow leopard habitats,

thereby enhancing our understanding of the carnivore community across the lowland of Terai and the high elevations in Himalayan landscapes of Nepal. For broader tiger conservation effort, it is essential that we have landscape level of ecological data to understand conservation from ecological perspective. This study aims to add additional understanding of major fauna in the tiger and snow leopard habitat.

MATERIALS AND METHODS

Putative tiger samples (n=420) were collected from CNP in 2011-2013. Samples were opportunistically collected across selected routes (fire-lines, trails and riverbanks) within core areas of CNP (Fig. 1).

Putative snow leopard scat samples (n= 48) were opportunistically collected in 2010-2011 from Annapurna Conservation Area (Mustang) of Nepal. The study sites were selected based on suitable habitat and recently reported high activities for snow leopard (Fig. 1).

Collected samples were stored in DET buffer (20% DMSO, 0.25 M Sodium-EDTA, NaCl to saturation, pH 7.5) and transported in ambient temperature to the Center for Molecular Dynamics Nepal laboratory in Kathmandu. DNA was extracted from all scat samples using commercially available QIAamp DNA stool mini kit (QIAGEN, Inc.) following the manufacturer's instructions. Extracted DNA was stored at -20^{0}C and handled carefully to avoid any cross contamination between samples.

Species Identification: tiger and snow leopard

Species identification on samples was done by Polymerase Chain Reaction (PCR) by targeted amplification of tiger-specific region (162 bp) within Cytochrome b of mitochondrial DNA using PCR primers (TIF/TIR, Table 1, Fig. 2) (Bhagavatula and Singh 2006) in tiger; and targeting a 150 bp region of Cytochrome b (CYTB-SCT-PUN-F / CYTB-SCT-PUN-R, Table 1, Fig. 3)

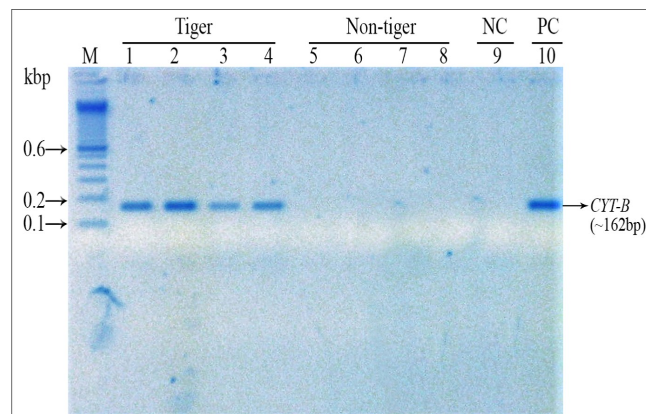

Figure 2. Tiger species identification using PCR. The tiger-specific PCR primers amplified a 162bp portion of Cytochrome b region of mitochondrial DNA. NTC= no DNA (negative control); L= 100bp DNA ladder; Post Ctrl= known tiger DNA. Samples W-TG-SU050 and W-TG-SU051 are non- tiger samples. The remaining samples are of tiger.

(Janečka et al. 2008) in snow leopard. PCR reactions were carried out in a final volume of 7 µl containing 3.5 µl Qiagen mastermix (2X), 0.7 µl Q-solution, 0.07 µl of each forward and reverse primer at a final concentration of 0.2 µM, 0.66 µl nuclease free water and 2 µl template DNA for tiger samples and 1.16 µl nuclease free water and 1.5 µl DNA for snow leopard samples, respectively. DNA from known tiger and snow leopard tissue samples was used as a positive control. PCR reactions were carried out in MJ Research PTC-225 Thermal Cycler with the conditions shown in Table 2. The amplified PCR products (162 bp for tiger and 150bp for snow leopard) were visualized on 1.8 % agarose gel by electrophoresis.

Tiger negative samples were further subjected to common leopard species identification PCR to quickly identify common leopard scats. For this, common leopard-specific primers (NADH4-F and NADH4-R) targeting NADH-4 region of mitochondrial DNA were used (Mondol *et al*., 2009) (Table 1, Fig. 4). PCR conditions were the same as above (Table 2).

Table 1. PCR primers used in this study

Identification	Primers	Primer Sequence (5'-3')	References
Tiger	TIF TIR	5'-ATAAAAAATCAGGAATGGTG-3' 5'-TGGCGGGGATGTAGTTATCA-3'	(Bhagavatula and Singh, 2006)
Common leopard	NADH4-F NADH4-R	5'-TRATAGCTGCYTGATGAC-3' 5'-GTTTGTGCCTATAAGGAC-3'	(Mondol *et al*.,2009)
Snow leopard	CYTB-SCT-PUN-F CYTB-SCT-PUN-R	5'-TGGCTGAATTATCCGATACC-3' 5'-AGCCATGACTGCGAGCAATA-3'	(Janečka *et al*., 2008)
Carnivore	CYTB-SCT- F CYTB-SCT- R	5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3' 5'-TATTCTTTATCTGCCTATACATRCACG-3'	(Farrell *et al*., 2000)

General Carnivore Identification

Carnivore specific PCR was performed on samples that were not identified as tiger, common leopard or snow leopard. For carnivore specific PCR, a unique primer set (CYTB-SCT- F and CYTB-SCT- R) was used which targets a 146 bp region of Cytochrome b (Table 1, Fig. 5) (Farrell et al. 2000).

Table 2. Species specific PCR conditions used in this study

Species specific PCR	Temperature	Time	Cycles
Tiger	95°C	15 minutes	1
	94°C	30 seconds	35
	59°C	90 seconds	
	72°C	90 seconds	
	72°C	10 minutes	1
Common leopard	95°C	15 minutes	1
	94°C	15 seconds	50
	56°C	30 seconds	
	72°C	30 seconds	
	72°C	10 minutes	1
Snow leopard	95°C	15 minutes	1
	95°C	30 seconds	45
	60°C	15 seconds	
	72°C	60 seconds	
	72°C	10 minutes	1
Carnivore	95°C	15 minutes	1
	94°C	30 seconds	45
	55°C	30 seconds	
	72°C	60 seconds	
	72°C	10 minutes	1

PCR was performed in a final 25 µl reaction mixture containing 12.5 µl Qiagen mastermix, 2.5 µl Q-solution, 0.63 µl each forward and reverse primers at a final concentration of 0.5 µlM, 6.74 µl nuclease free water and 2 µ DNA. PCR reactions were carried out in MJ Research PTC-225 Thermal Cycler with the conditions as shown in Table 2. The PCR products, along with incorporated known carnivore sample as positive control, were visualized on 2% agarose gel.

The carnivore positive PCR amplicons were sequenced on an ABI 310 machine using the forward primer (CYTB -SCT- F); DNA sequences were BLAST searched in the NCBI database to identify carnivore species. Species identification on samples using BLAST results were based on Cytochrome b DNA sequence data of 100 bp or more, maximum identity of 95% or higher and query coverage of more than 95%.

RESULTS

Tiger genetic study

Based on tiger specific PCR identification, we identified 62% (n=262) of the total collected samples (n=420) as tiger. Of the 84 samples, we were able to identify leopard cat (*Prionailurus bengalensis*, n=10), fox (*Vulpes ssp*,n=5) and jungle cat (*Felis catus*, n=1) (Table 3). We could not identify the remaining (n=68) samples due to poor DNA quality. This suggests there was 26% field misidentification of target species (without including non-identified samples).

Snow leopard genetic study

Based on snow leopard specific PCR based identification, we were able to identify 65% (31) of the total samples (n=48) as snow leopard. Out of the 17 non-snow leopard samples, we identified red fox (*Vulpes vulpes,* (n=7), leopard cat (n= 2), wolf (n=1), leopard (n=1) and lynx (n=1) (Table 4). Some samples (n=5) yielded poor DNA for PCR amplification. Overall there was 28% field misidentification of target samples (without including non-identified samples).

DISCUSSION

In our tiger study, 26% field misidentification of target species was higher than reported by Mondol *et al*., 2009 (8%) (Mondol *et al*., 2009) and Borthakur *et al*. 2011 (4%) (Borthakur *et al*., 2011). The cause for high misidentification could be due to the difficulty in distinguishing juvenile tiger and adult leopard scats in the field, low level of expertise in scat identification by field personnel, the opportunistic sample collection strategy and high degree of non-identification (16%) of the total samples due to poor DNA quality.

For snow leopard, our accuracy of 65% for the target species was higher than 39% reported in Kanchenjunga Conservation Area and Shey Phoksundo National Park of Nepal (Karmacharya *et al*., 2011) and 33% reported in Gobi desert in Mongolia (Janecka *et al*., 2011).

Table 3. NCBI BLAST results of target amplification of Cytochrome b region of mtDNA in non-tiger samples.

Sample	PCR DNA length (bp)	Percentage Match	Query %	Species Name	Common Name
1	89	100	96	*Vulpes vulpes*	red fox
2	74	100	99	*Panthera pardus fusca*	leopard
3	101	99	100	*Prionailurus bengalensis*	leopard cat
4	92	100	98	*Felis chaus*	jungle cat

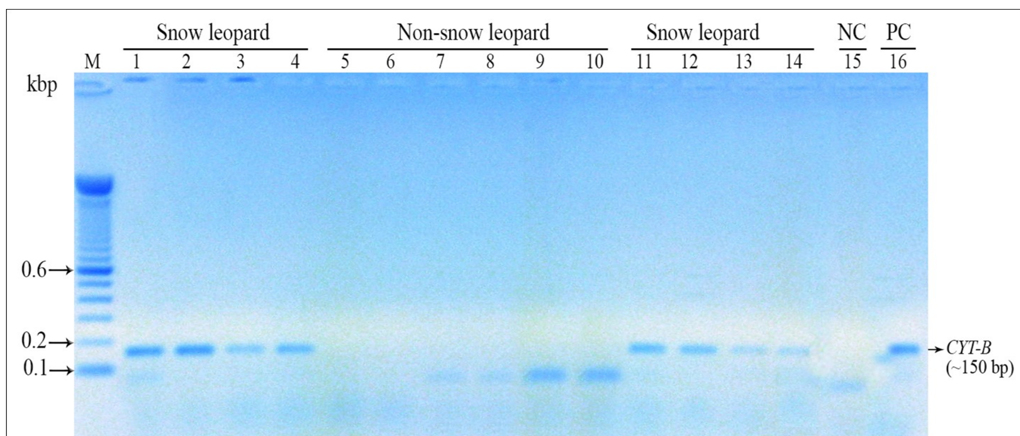

Figure 3. Snow leopard species identification using PCR. The snow leopard specific PCR primers amplified a 150-bp region of Cytochrome b of mitochondrial DNA. NTC, NEC6, NEC7 = no DNA (negative control); L = 100bp DNA ladder; POS (SLPM35) = known snow leopard sample. W-BS-SL1317, 1318, 1319 are of non- snow leopard; and others are snow leopard samples.

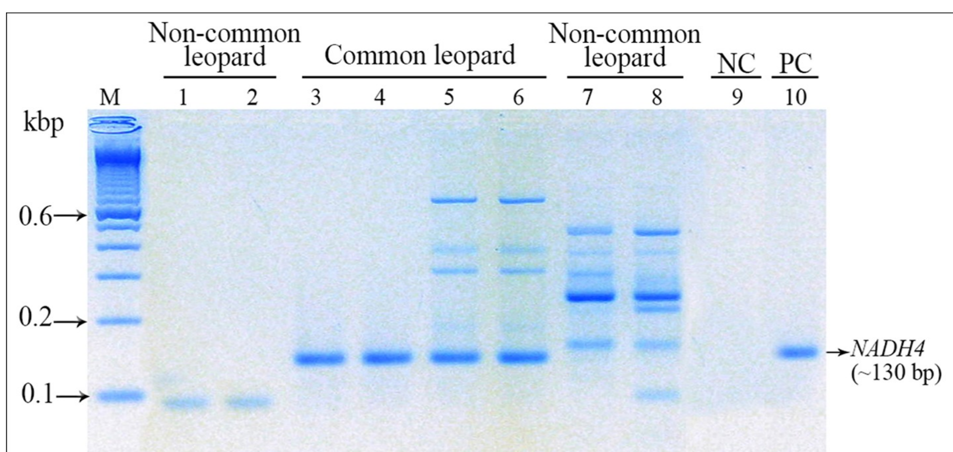

Figure 4. Common leopard species identification using PCR. The common leopard specific PCR primers amplified a 130-bp region of NADH4 region of mitochondrial DNA. NTC = no DNA (negative control); L = 100bp DNA ladder; Post Ctrl= known common leopard sample. Samples W-SU-TG021, & -022 are common leopard and rest are non-common leopard.

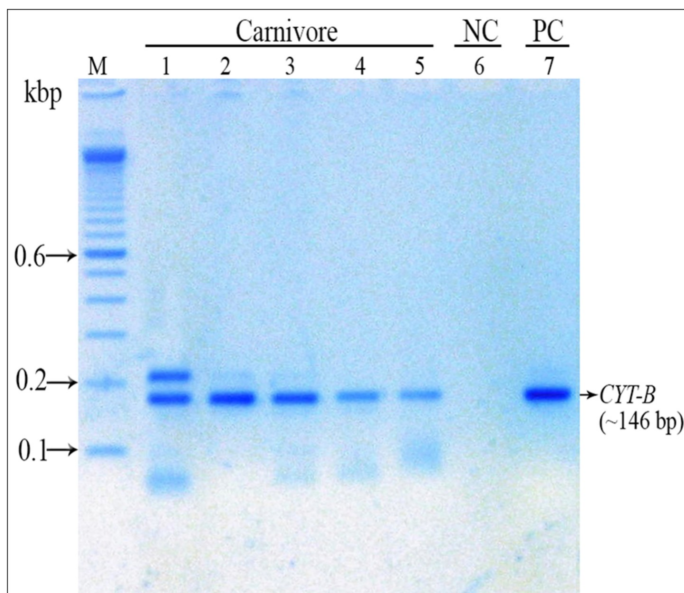

Figure 5. Carnivore identification using PCR and DNA sequencing. Carnivore-specific primers amplified around 146 bp region of Cytochrome b of mitochondrial DNA. NTC= no DNA (negative control); L= 100bp DNA ladder; Pos Ctrl= known carnivore sample. Samples W-SS-SL895, -896, 897, 898 & 900 are of carnivore species.

Table 4. NCBI BLAST results of target amplification of Cytochrome b region of mtDNA in non-snow leopard samples.

Sample	PCR DNA length (bp)	Percentage Match	Query %	Species Name	Common Name
1	90	98	100	*Vulpes vulpes*	red fox
2	85	97	100	*Prionailurus bengalensis*	leopard cat
3	105	97	97	*Canis lupus*	wolf
4	88	98	100	*Panthera pardus*	common leopard
5	92	98	98	*Lynx lynx*	lynx

The use of species identification data from scat DNA for species distributions (Cossíos *et al*., 2007, *Palomares et al*., 2002) is common: however, distribution studies based solely on scat morphology can be misleading (Davison *et al*., 2002) because sympatric carnivore species often have indistinguishable scat morphologies (Farrell *et al*., 2000). In most studies, only scats thought to be from target species based on morphology are collected. DNA analysis frequently reveals that many collected samples are from non-focal species (Farrell *et al*., 2000, Janečka *et al*., 2008, Perez *et al*., 2006, Sugimoto *et al*., 2012), as was found in our study.

While field misidentification can be a disadvantage when looking for specific species, we can make the best out of all other samples we have collected non-invasively by identifying them using a segment of mitochondrial DNA and sequencing it, followed by BLAST search in the NCBI database (Table 3 and Table 4) (Madden, 2002). We were able to identify one meso-predator (leopard) and five small carnivore species (lynx, wolf, leopard cat, fox and jungle cat) that occur in tiger and snow leopard habitat across the environmental gradient (Fig. 2). Hence, this is the first study to identify non-focal carnivore species during non-invasive sampling for genetic studies of tiger and snow leopard in Nepal.

More research is needed to understand the type of interaction between the carnivore species existing in the Terai and trans-himalaya landscapes. This kind of work provides us with opportunities to understand more about species that have not been previously reported in an area. We have identified the presence of an unknown species of fox in the Terai landscape. DNA sequence of identified species closely matched with the red fox (98% match; 100% query on 90bp PCR amplicon DNA sequence data) but red foxes are not known to occur in the Terai landscape. We believe that we have detected Bengal fox (*Vulpes bengalensis*), which is known to inhabit the Terai area of Nepal and India [10], but there were no DNA sequences for Bengal fox in the NCBI database, so further study is needed to verify our assumption.

This kind of study is important to build baseline genetic information on species including those that are currently not being studied. By analyzing misidentified samples, we have gathered information on co-occurring carnivore species, including the first genetic evidence of the presence of elusive species such as wolf, lynx, jungle cat and leopard cat in our study sites. Lynx and leopard cat are two species that are under the NPCA 1973 protected animal list and least studied carnivores in Nepal. Our study documents the highest record for lynx in its entire range at an elevation of 3935 m in Mustang region. Leopard cats are found in a broad range of habitat types from tropical lowland rain forests to coniferous forests in the Himalayas (Mohamed *et al*., 2013), their occurrence in our survey sites agrees with this distribution range from sub-tropical deciduous forest (elevation 150- 800 m) in plains of Terai across CNP to alpine forest (elevation 3876 m) in the high Himalaya across the Mustang region near the Sagarmatha National Park. Our study documents the presence of the leopard cat at the highest elevation (4474 m) across the snow leopard habitat at the Kanchangunga Conservation Area (Thapa, 2013).

In Mustang, red fox and wolf species were found sharing the same habitat as snow leopard. Similarly, leopard cat, leopard and lynx also occur in snow leopard territory. In Mustang, red fox, snow leopard and leopard cat were found to share the habitat. In Chitwan, tigers and leopards were found in abundance sharing the same area. The remaining carnivore species, such as the unknown species of fox, leopard cat and jungle cat, were also found in tiger habitat. These findings confirm that there is some degree of overlap (with some areas showing overlap of multiple species) among these carnivore species and provide us with a rough distribution map of these species, hence increasing our knowledge of the carnivore community in our study sites.

CONCLUSION

In the on-going debate on whether focusing solely on an umbrella and/or flagship species is beneficial (Andelman and Fagan 2000), this study shows that even while conducting research on an umbrella and/or flagship species such as tiger or snow leopard, there is ample opportunity to collect data on other, often overlooked non-focal carnivore species which often co-occur. This emphasizes the importance of holistic sampling and monitoring approaches that capture broader ecological information. This non-invasive genetic sampling approach, integrated in our studies of tiger and snow leopard, has significantly increased our understanding the carnivore community in the Terai and Himalaya regions of Nepal.

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REFERENCES

Andelman, S. J. and Fagan, W. F. 2000. Umbrellas and flagships: efficient conservation surrogates or expensive mistakes? - Proceedings of the National Academy of Sciences of the United States of America 97: 5954-5959.

Barber-Meyer, S., et al. 2013. Influence of prey depletion and human disturbance on tiger occupancy in Nepal. - Journal of Zoology 289: 10-18.

Baselga, A., et al. 2013. Whole-community DNA barcoding reveals a spatio-temporal continuum of biodiversity at species and genetic levels. - Nature communications 4: 1892.

Bhagavatula, J. and Singh, L. 2006. Genotyping faecal samples of Bengal tiger Panthera tigris tigris for population estimation: a pilot study. - BMC genetics 7: 48.

Borthakur, U., et al. 2011. Noninvasive genetic monitoring of tiger (Panthera tigris tigris) population of Orang National Park in the Brahmaputra floodplain, Assam, India. - European Journal of Wildlife Research 57: 603-613.

Cardinale, B. J., et al. 2012. Biodiversity loss and its impact on humanity. - Nature 486: 59-67.

Clucas, B., et al. 2008. Flagship species on covers of US conservation and nature magazines. - Biodiversity and Conservation 17: 1517-1528.

Cossíos, E. D., et al. 2007. Update on the distribution of the Andean cat Oreailurus jacobita and the pampas cat Lynchailurus colocolo in Peru. -.

Davison, A., et al. 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. - Journal of Zoology 257: 141-143.

Farrell, L. E., et al. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. - Mol Ecol 9: 1583-1590.

Fernandes, C. A., et al. 2008. Species-specific mitochondrial DNA markers for identification of non-invasive samples from sympatric carnivores in the Iberian Peninsula. - Conservation genetics 9: 681-690.

Forrest, J. L., et al. 2012. Conservation and climate change: Assessing the vulnerability of snow leopard habitat to treeline shift in the Himalaya. - Biological Conservation 150: 129-135.

GoN. 2009. Elephant Conservation Action Plan for Nepal.

GoN. 2013. Summary Report : Status of Tiger and Prey-Base Population in Nepal.

Heinen, J. T. and Shrestha, S. K. 2006. Evolving policies for conservation: an historical profile of the protected area system of Nepal. - Journal of Environmental Planning and Management 49: 41-58.

IUCN. 2013. The IUCN Red List of Threatened Species. Version 2013.1 http://www.iucnredlist.org. Downloaded on 24 September 2013.

Jackson, R. and Ahlborn, G. 1989. Snow leopards (Panthera uncia) in Nepal: home range and movements. - National Geographic Research 5: 161-175.

Janečka, J., et al. 2008. Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. - Animal Conservation 11: 401-411.

Janecka, J. E., et al. 2011. Comparison of noninvasive genetic and camera-trapping techniques for surveying snow leopards. - Journal of Mammalogy 92: 771-783.

Joshi, A. R., et al. 1995. Home ranges of sloth bears in Nepal: implications for conservation. - The Journal of wildlife management: 204-214.

Karki, J. B., et al. 2013. Estimating the abundance of Nepal's largest population of tigers Panthera tigris. - Oryx FirstView: 1-7.

Karmacharya, D. B., et al. 2011. Noninvasive genetic population survey of snow leopards (Panthera uncia) in Kangchenjunga conservation area, Shey Phoksundo National Park and surrounding buffer zones of Nepal. - BMC research notes 4: 516.

Kelly, M. J., et al. 2012. Noninvasive sampling for carnivores. - Carnivore ecology and conservation: a hand-book of techniques. Oxford University Press, Oxford: 47-67.

Kurose, N., et al. 2005. Fecal DNA analysis for identifying species and sex of sympatric carnivores: a noninvasive method for conservation on the Tsushima Islands, Japan. - The Journal of heredity 96: 688-697.

Madden, T. 2002. The BLAST sequence analysis tool. - The NCBI Handbook [Internet]. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda, MD.

Mishra, C., et al. 2003. The role of incentive programs in conserving the snow leopard. - Conservation Biology 17: 1512-1520.

Mohamed, A., et al. 2013. Density and habitat use of the leopard cat (Prionailurus bengalensis) in three commercial forest reserves in Sabah, Malaysian Borneo. - Journal of Mammalogy 94: 82-89.

Mondol, S., et al. 2009. A panel of microsatellites to individually identify leopards and its application to leopard monitoring in human dominated landscapes. - BMC genetics 10: 79.

Mondol, S., et al. 2009. Evaluation of non-invasive genetic sampling methods for estimating tiger population size. - Biological Conservation 142: 2350-2360.

Nagata, J., et al. 2005. Fecal genetic analysis using PCR-RFLP of cytochrome b to identify sympatric carnivores, the tiger Panthera tigris and the leopard Panthera pardus, in far eastern Russia. - Conservation Genetics 6: 863-866.

Namgail, T., et al. 2007. Carnivore-caused livestock mortality in Trans-Himalaya. - Environmental management 39: 490-496.

Noss, R. F. 1987. From plant communities to landscapes in conservation inventories: a look at The Nature Conservancy (USA). - Biological Conservation 41: 11-37.

Odden, M., et al. 2010. Do tigers displace leopards? If so, why? - Ecological research 25: 875-881.

Oliveira, R., et al. 2010. Species identification using a small nuclear gene fragment: application to sympatric wild carnivores from South-western Europe. - Conservation Genetics 11: 1023-1032.

Palomares, F., et al. 2002. Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. - Mol Ecol 11: 2171-2182.

Perez, I., et al. 2006. Critically Endangered Arabian leopards Panthera pardus nimr in Israel: estimating population parameters using molecular scatology. - Oryx 40: 295-301.

Reed, J., et al. 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. - Molecular ecology 6: 225-234.

Seidensticker, J. 1976. On the ecological separation between tigers and leopards. - Biotropica: 225-234.

Shrestha, N. 1997. Protected wildlife species of Nepal: an introductory handbook. - IUCN.

Simberloff, D. 1998. Flagships, umbrellas, and keystones: Is single-species management passé in the landscape era? - Biological conservation 83: 247-257.

Smith, J. L. D., et al. 1998. Landscape analysis of tiger distribution and habitat quality in Nepal. - Conservation Biology 12: 1338-1346.

Soulé, M. E. and Terborgh, J. W. 1999. Continental conservation: scientific foundations of regional reserve networks. - Island Press.

Spiering, P. A., et al. 2009. Sampling error in noninvasive genetic analyses of an endangered social carnivore. - Conservation Genetics 10: 2005-2007.

Subedi, N., et al. 2013. Population status, structure and distribution of the greater one-horned rhinoceros Rhinoceros unicornis in Nepal. - Oryx 47: 352-360.

Sugimoto, T., et al. 2012. Population size estimation of Amur tigers in Russian Far East using noninvasive genetic samples. - Journal of Mammalogy 93: 93-101.

Thapa, K., et al. 2013. Distribution update. -.

Thapa, K., et al. 2013. Past, present and future conservation of the greater one-horned rhinoceros Rhinoceros unicornis in Nepal. - Oryx 47: 345-351.

Thapa, K., Pradhan, NMB, Barker, J, Dhakal, M, Bhandari, AR, Gurung, GS, Rai, DP, Thapa, GJ, Shrestha, S, Singh, GR. 2013. High elevation record of a leopard cat in the Kanchenjunga Conservation Area, Nepal. - Cat News 58.

Verissimo, D., et al. 2013. Using a Systematic Approach to Select Flagship Species for Bird Conservation. - Conservation biology : the journal of the Society for Conservation Biology.

Waits, L. P. and Paetkau, D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. - Journal of Wildlife Management 69: 1419-1433.