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Genetics, ecology and conservation of Himalayan black bears (*Ursus thibetanus laniger)* in Annapurna Conservation Area of Nepal

（ネパール・アンナプルナ保護区における

ツキノワグマ（***Ursus thibetanus laniger***）の

遺伝子、生態および保全について）

Rabin Kadariya

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ABBREVIATIONS

ACA	Annapurna Conservation Area
ACAP	Annapurna Conservation Area Project
ADO	Allele dropout
AICc	Corrected Akaike information criterion
BI	Bayesian inference
BPP	Bayesian posterior probabilities
CAMCs	Conservation Area Management Committees
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CR	Control region
DBH	Diameter at breast height
DNA	Deoxyribonucleic acid
DNPWC	Department of National Parks and Wildlife Conservation
FA	False alleles
F_{IS}	Wright's inbreeding coefficient
Fnull	Frequency of null alleles
GENETUP	German-Nepal Tuberculosis Project
GPS	Global Positioning System
GTR	General Time Reversible
H	Haplotype diversity
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HWE	Hardy-Weinberg equilibrium

IUCN	International Union for Conservation of Nature
Ka BP	Kilo annum before present
LD	Linkage disequilibrium
MCMC	Markov Chain Monte Carlo
ML	Maximum likelihood
MoFSC	Ministry of Forest and Soil Conservation
MP	Multiplex primer
MtDNA	Mitochondrial DNA
MUs	Management units
Mya	Million years ago
N_A	Number of alleles
NCBI	National Center for Biotechnology Information
N_E	Effective no of allele
NE	Northeast
NK	North Korea
NTNC	National Trust for Nature Conservation
NW	Northwest
PCR	Polymerase chain reaction
PFO	Percent frequency of occurrence
PIC	Polymorphic information content
P_{ID}	Probability of identity
P_{ID}Sibs	Probability of identity of siblings
PV	Percent Volume
rRNAs	Ribosomal ribonucleic acids

S	South
SD	Standard deviation
SE	Standard error
SK	South Korea
Ta	Annealing temperature (ºC)
tRNAs	Transfer ribonucleic acids
uH_E	Unbiased expected heterozygosity
UK	Unknown
USA	United States of America
VNTRs	Variable number of tandem repeats

PREFACE

Asiatic black bears (*Ursus thibetanus*) have wide range distribution over west, south and southeast Asia, parts of China, far-east Russia, Korea and Japan (Garshelis and Steinmetz 2016). The Asiatic black bear is one of the three bear species found in Nepal (Jnawali et al. 2011). Two species of bears are found in the mountain region of Nepal namely, the Asiatic/Himalayan black bears and the Tibetan brown bears (*Ursus arctos isabellinus*). It is distributed in middle and high mountain region of Nepal from east to west and rarely recorded in the Churia region (Stubblefield and Shrestha 2007, Jnawali et al. 2011, Yadav et al. 2017). The taxonomic identification of Asiatic black bears in Nepal is unknown whether it is same as previous reported Tibetan black bears (*Ursus thibetanus thibetanus*) from china or new Himalayan subspecies (*Ursus thibetanus laniger*).

Asiatic black bears are opportunistic omnivores. They mainly feed on fruits, and in some extent to vegetation, insect, and animal matter (Schaller et al. 1989, Hwang et al. 2002, Hashimoto 2003, Sathyakumar and Viswanath 2003, Koike 2010). Asiatic black bears occupy a variety of forested habitats, both broad-leaved and coniferous, and occasionally recorded in open alpine meadows (Sathyakumar et al. 2013). Knowledge of their food habits is important to understand the bear distribution and habitat use (MacHutchon and Wellwood 2003). Diversity of habitats and food is important in providing alternative foods when one food source fails. Information on the habitat uses and food ecology of the black bear in Nepal is still unavailable whereas studies are available from much of its range countries.

Depredation of crops, killing of livestock and in extreme cases of fatal attacks on humans are the main conflict issues related to this species (Charoo et al. 2011), which resulted to retaliate through traps, snares, poisoning and even through muzzle loaded guns. Illegal killing of bears, trade and habitat degradation are global threats of bear conservation, and the remaining populations are patchily distributed in fragmented habitat (Charoo et al. 2011, Sathyakumar et al. 2013). Japan appears to be the only range country that has documented an increasing number of Asiatic black bears, reflected by an increasing area of occupied range (Oi and Yamazaki 2006). Considering this, it may be assumed that the population and density estimates proposed for different countries need a proper review (Garshelis and Steinmetz 2016). It is evident that global population of Asiatic black bears is showing signs of decline over the years and this has led IUCN to include this species under the globally vulnerable (Garshelis and Steinmetz 2016) and endangered in Nepal (Jnawali et al. 2011). Subspecies of Asiatic black bears, including the Himalayan black bears, have not been separately assessed yet in the IUCN Red List. The Asiatic black bear has received little attention in Nepal from the conservation community compared to other charismatic and endangered species such as Royal Bengal tiger (*Panthera tigris tigris*), Asian elephant (*Elephas maximus*), greater one-horned rhino (*Rhinoceros unicornis*), snow leopard (*Panthera uncia*) etc. (Jnawali et al. 2011).

Habitat degradation and poaching were major threats of bear survival in Nepal before 1980s, whereas human-bear conflict, especially crop raiding and retribution killing (Stubblefield and Shrestha 2007) are major threats of bear conservation during last decades when wildlife conservation started by establishing protected areas in Nepal. In recent years, Annapurna Conservation Area (ACA) has been considered as major habitat for black bears. Despite, considerable efforts for the conservation, human-bear conflict remains as unresolved issues in local communities (Bista and Aryal 2013). Therefore, community-based authorities i.e. Conservation Area Management Committees (CAMCs) and Annapurna Conservation Area

Project (ACAP) are facing a growing demand to establish effective plans and measures for the management and conservation of bear populations. However, the status of black bear population and its ecological and genetic information from Nepal is still in ambiguity. The frequency of bear visit in cropland and its extent of diet are helpful for the development of effective conservation plan.

Genetic characteristics can be used to understand the response of species to the landscape and anthropogenic features even in the absence of ecological and biological details on bears. Knowledge of the genetic structure and gene flow in wild populations is an important component of adaptability and crucial for making decisions to improve their sustainability. A decrease in genetic variation due to habitat fragmentation, human-bear conflict and other significant threats could lead to extinction of the population. Understanding the genetic structuring patterns of species, their ecology and evolution provides information for the development of conservation and management strategies toward maintaining stable populations. The Asiatic black bear is one of the key mammals of the Himalayan forests and shares its home with a great diversity of other species, both plants and animals. The conservation of bears would also help in conserving other associated species as bear is the umbrella species of mountain ecosystem of Nepal.

Non-invasive genetic studies are increasingly being used to monitor the conservation status of Asiatic black bears (Saito et al. 2008, Latham et al. 2012, Uno et al. 2012, Malcolma et al. 2014, Mukesh et al. 2015), as the direct observation, capture and tagging of bears are difficult and costly in mountain landscapes. Furthermore, this approach is favored because non-invasive sampling reduces the risk of injury to endangered wildlife and reduces burden on field works. Mitochondrial DNA (mtDNA) is widely used for the study of the phylogeny of ursidae. Phylogeographic analysis suggested that the significant genetic differentiation has occurred among fragmented populations (Ishibashi and Saitoh 2004), which indicated that it is essential to take effective actions for conservation of remaining bear populations. Additionally, mtDNA sequence data provides important information about taxonomy, maternal gene flow patterns, past isolation events, natural re-colonization events, and evolutionary history (Onorato et al. 2004). Microsatellite markers are useful in getting the data of genetic diversity within the species because of their abundance, high polymorphism content, co-dominance and bi-parentally inherited characteristics (Li et al. 2002, McRae et al. 2005). Genetic analyses give important insights into the population structure and connectivity among such wide-ranging animals, historic and current levels of gene flow among populations, as well as information about genetic diversity, kinship, and movement patterns within populations (Paetkau et al. 1998, Woods et al. 1999).

The studies in this thesis provided the genetic and ecological information of Himalayan black bears in Annapurna Conservation Area, Nepal. In the first chapter, I have described the ancient lineages of Himalayan black bears (*U. t. laniger*), minimum population, genetic diversity and population structure by using both mtDNA and microsatellite markers. The non-invasive samples were collected from six management units of ACA. Three novel haplotypes and whole mitogenome sequence were reported from wild populations of Himalayan black bears. In the second chapter, I have investigated the seasonal diet by fecal analysis and habitat characteristics and existing conservation threats by organizing transect survey in bear habitat. This is the first genetic and ecological study on Himalayan black bears in Nepal.

CHAPTER I

High genetic diversity and distinct ancient lineage of Asiatic black bears revealed by non-invasive surveys in the Annapurna Conservation Area, Nepal

Introduction

The Asiatic black bear (*Ursus thibetanus)* has a broad geographic distribution across south and southeast Asia, China, Russia, Korea, and Japan (Garshelis and Steinmetz 2016). There are seven recognized subspecies: Japanese black bears (*U. t. japonicus)* in Japan (Ohnishi et al. 2009, Wu et al. 2015), Ussuri black bears (*U. t. ussuricus)* in far-east Russia, northeast China, and Korea (Heptner and Naumov 1998, Hwang et al. 2008, Choi et al. 2010), Formosan black bears (*U. t. formosanus*) in Taiwan (Wilson and Reeder 2005, Tsai et al. 2009), Indochinese/Sichuan black bears (*U. t. mupinensis*) in southwest China (Hou et al. 2007), Baluchistan black bears (*U. t. gedrosianus)* in south Pakistan and Iran, Tibetan black bears (*U. t. thibetanus)* in the eastern Himalayas and southeast Asia, and Himalayan black bears (*U. t. laniger*) in the western Himalayas (Pocock 1941, Lan et al. 2017). A small population of *U. t. laniger* is patchily distributed across Pakistan, northwest India, and likely northeast India and Nepal as well (Pocock 1941). *U. t. laniger* may overlap with the distribution of *U. t. thibetanus* across Myanmar and northeast India to possibly Nepal in the Himalayan range. *U. t. laniger* is distinguished from *U. t. thibetanus* by its longer and thicker fur with abundant under-wool, and chest marks that are usually smaller and whiter. Recently, evidence for the presence of Himalayan black bears was found using a paw sample collected from a monastery at the Nepal-Tibet border and a fecal sample from a zoo in Pakistan, although the geographic origins of both samples were unknown (Lan et al. 2017). Although the range of *U. t. thibetanus* and *U. t. laniger* may overlap in Nepal (Pocock 1941), no molecular data from wild populations have been available to date to elucidate the phylogeny of black bears inhabiting the mountainous region of Nepal and throughout their distribution range.

In Nepal, black bears are distributed most broadly across mid- to high-elevation mountains (≤ 4,200 m), including 13 protected areas (Fig. 1-1A) (Stubblefield and Shrestha 2007, Jnawali et al. 2011, Yadav et al. 2012). Recent records showed a range overlap with sloth bears in hilly areas (237 m) in Bardia National Park (Jnawali et al. 2011, Yadav et al. 2017), but no overlap with brown bears (*U. arctos pruinosus*) in Nepal (Garshelis and Steinmetz 2016). The Asiatic black bear is threatened in much of its habitat by poaching, habitat degradation and fragmentation, and is designated as "vulnerable" in the International Union for Conservation of Nature (IUCN) Red List (Garshelis and Steinmetz 2016), and as an endangered mammal in Nepal (Jnawali et al. 2011). It is also listed in CITES Appendix I, indicating that this bear species requires comprehensive protection to address the grave conservation threats it faces. The status of the black bear population and its genetic characteristics are still poorly understood in Nepal.

Genetic information can be used to better understand the population response of bears to human-induced landscape changes even in the absence of detailed ecological and biological data. Large mammals such as bears often occur at low densities, requiring expansive and continuous landscapes to maintain viable populations (Proctor et al. 2012). Small, isolated populations are susceptible to extinction through the loss of genetic variation and inbreeding depression

(Allendorf and Lundquist 2003, Frankham 2009). Inbreeding depression has been documented in both captive and wild animal populations due to reproduction between genetically related individuals (Jimenez et al. 1994, Saccheri et al. 1998, Crnokrak and Roff 1999). An isolated population may benefit from genetic variants introduced from other populations of the same species (Allendorf et al. 2013). This situation is also applicable for newly recovered bear populations in the mountain landscapes of Nepal (Acharya et al. 2016). Natural barriers (steep terrain and river) and scattered human settlements have led to habitat fragmentation, which might hinder the movement of bears, impeding gene flow among different populations. The amount of gene flow between bear populations in the forests of the northern high mountains and the adjacent southern middle mountains of Nepal is virtually unknown. Analyzing the genetics of the populations in this region is important for understanding the demographic consequences of habitat isolation and helps to inform the implementation of appropriate conservation action.

Recent records indicate that the wild populations in Nepal are presumably undergoing rapid changes in population size (Acharya et al. 2016, Bhattarai et al. 2017). Endangered or vulnerable species not only require a large enough population size, but also sufficient genetic variation for long-term persistence of the species (Lande and Barrowclough 1987, Frankham 2009). Considering the conservation and management of endangered species, it is important to evaluate the level of genetic diversity within populations which are supposed to be affected by significant changes in population size. Information on genetic structure and gene flow is crucial for ensuring the sustainability of wild populations. Community-based conservation has been initiated in the Annapurna Conservation Area (ACA) since the late 1980s by Annapurna Conservation Area Project (ACAP) and Conservation Area Management Committees (CAMCs). The conservation awareness, planting of fuelwood species, provision of alternative energy resources, legal prosecution on illegal extraction of forest resources and wildlife poaching, ban on harvesting from standing trees, alternative income generation activities and eco-tourism promotion have been effective in conserving wildlife populations, protecting forest resources, and enhancing livelihoods in local communities (Bajracharya et al. 2005), but no studies have been conducted on the effect of these conservation measures on the genetic diversity of the local bear population.

This study incorporates both microsatellite markers which are widely used in conservation genetics to quantify genetic diversity, relatedness, population structure and population monitoring, and mitochondrial DNA (mtDNA) sequences which are mainly used to infer phylogenetic relations, evolutionary history and resolving taxonomic issues. This study investigates the present levels of genetic diversity, population structure, and evolutionary relationships of Himalayan black bears in the ACA using both microsatellite markers and mtDNA. The hypotheses of the study were (i) the remaining populations of bears inhabiting the northern and southern forests would exhibit genetic substructuring and low levels of geneflow due to the dispersal barriers of high mountains, rivers, habitat fragmentation, and human settlements in the area, and (ii) a distinct lineage of Himalayan black bear (*U. t. laniger*) inhabits the mountain range in Nepal.

Materials and methods

Study area

This study was carried out in the southern middle and high mountain forests of the ACA (Fig. 1-1B). The ACA, which is bordered in the north by Tibet (China), is a major part of the

Chitwan Annapurna landscape, extending from the north to the south in Nepal. It is the first conservation area (7,629 km^2) established in Nepal, representing 22% of the protected areas in the country. Within the ACA, the forest, which is the preferred habitat of Asiatic black bears, comprise 1,160 km^2 (15%) from the southern mid-altitude to the north high-altitude mountain region (NTNC 2017). The forests in the ACA are highly diverse, ranging from subtropical broad-leaved forests to subalpine forests, and mostly consists of *Schima-Castanopsis*, *Pinus roxburghii*, *Quercus* spp., *Acer* spp., *Pinus wallichiana*, *Abies spectabilis*, *Betula utilis*, *Aurndinaria* spp., and *Rhododendron* spp. ACA forests support several endangered species: Himalayan black bears (*U. t. laniger*), red panda (*Ailurus fulgens*), musk deer (*Moschus chrysogaster*), and clouded leopards (*Neofelis nebulosa*) in the study site and snow leopards (*Panthera unica*), Tibetan brown bears (*U. actor pruinosus*), and Himalayan wolves (*Canis lupus chanco*) in the upper Himalayan region. Traditionally, the people of in this region have been highly dependent on natural resources, particularly the mountain forests (Bajracharya et al. 2006), which have been greatly altered by human migration and tourism activities. The majority of local human inhabitants still rely on the local forest ecosystems for subsistence, for collecting wood, fodder, medicinal plants, and wild foods, for grazing, and as cultural and spiritual sites.

Sampling

Intensive sampling was conducted in six management units of the ACA during the rainy (July-September) and autumn (October-December) seasons of 2015 and 2016. Four and two of the management units were located in the middle and high mountain regions of the ACA, respectively. Fecal and hair samples were searched within 21 grids (5×5 km^2) over the six units and in the adjoining cropland. Additional fecal samples were opportunistically collected from the agricultural land locating adjacent the bear habitats. The grids were selected based on accessibility, geographic representation, and forest type to better represent the bear population for genetic assessment. Foot walk was organized along animal trails, villagers' routes, and mountain ridges to cover the different habitats of bears. If bear signs were observed, the search for fresh feces was intensified as these yield higher-quality DNA (Prugh et al. 2005, Smith et al. 2006). The exterior surface of each fresh samples of putative bear feces was rubbed lightly using a cotton swab, which was then stored in a 15-ml vial containing 10 ml of 100% ethanol (Wako, Osaka, Japan) (Wasser et al. 1997, Murphy et al. 2007). The "freshness" of the fecal sample was estimated based on visual examination and on the external characteristics based on acquired experience of the collector and substantiated by the presence of surroundings bear sign and information from observation of local villagers. Honey-baited hair traps that consisted of a single barbed wire strand with an automated camera (Model #: 119537C, Trophy Cam HD, Bushnell Outdoor Products, Kansas, USA) were placed in 11 locations (Fig. 1-1B). A wire, approximately 20 m long, was wrapped around at least 4 trees, 40–50 cm above the ground at each location (Woods et al. 1999, Uno et al. 2012). Traps were visited every 10 days during a one-month trapping season. Bear signs were not detected at all of the traps, and thus discontinued the placement of hair traps at additional sites. Hair samples, which were visually identified as bear hairs, were collected from the barbed wire fencing of agricultural land and from the broken branches of trees where bears nested. Hair samples were stored in paper envelopes containing silica desiccant beads (Sigma, silica gel, Type II, 3.5 mm bead size). Detailed information about the habitat characteristics, conservation threats and GPS locations (GPS 72H, Garmin, Garmin Corporation, Taipei, Taiwan) were noted for each fecal and hair sample. Disposable latex gloves were replaced after each sample was collected and forceps were immediately rinsed with bleach and 75% ethanol after collecting each hair sample to avoid any possibility of sample cross-contamination.

Genetic analysis

DNA extraction

Genomic DNA was extracted using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen Inc.) for fecal samples and the Wako DNA Extractor FM Kit (Wako, Osaka, Japan) for hair samples according to the manufacturers' instructions. Negative controls were included, and DNA samples were processed under sterile conditions to avoid cross-contamination.

Microsatellite genotyping

For the microsatellite analysis in this study, 27 loci were initially tested using 16 fecal samples to determine genotypes; 11 loci from *U. americanus* (Paetkau et al. 1995, Paetkau et al. 1998, Meredith et al. 2009), 10 loci from *U. arctos* (Taberlet et al. 1997), and 6 loci from *U. t. japonicus* (Kitahara et al. 2000). The primers and combination of these primers for multiplex PCR are listed in Table 1-1. Eight loci were finally selected (see result) for further analyses based on the ease of use in multiplex PCR, amplification efficiency, readability and success, having a relatively small (< 0.25) probability of identity (P_{ID}), and having a high value (> 0.5) of polymorphic information content (PIC) (Uno et al. 2012). *Amelogenin* gene was used with the primers SE47/SE48 for molecular sexing of individual Asiatic black bears (Ennis and Gallagher 1994, Yamamoto et al. 2002, Mukesh et al. 2013). Samples were first multiplexed with the primer sets of three highly variable loci (MU23, G10B and MU50), and then discarded if they did not produce scorable results at any locus. Samples that amplified at all three loci were further amplified using primers for an additional five loci and sex primers. Using TaKaRa Multiplex PCR Assay Kit (TaKaRa Bio Inc., Shiga, Japan), PCR reactions were carried out in 3 multiplex reactions, each in a final volume of 15 µl, containing 7.5 µl of 2x multiplex PCR buffer (Mg^{2+}, dNTP plus), 75 nl of multiplex PCR enzyme mix, 0.50 µl of primer mix of each 7.5 µM, 1 µl of DNA extract and remaining RNase-free water. Amplification was performed in Applied Biosystems Veriti Thermal Cycler (Applied Biosystems, USA) with an initial denaturation at 94 ºC for 1 minute, followed by 40 cycles of 94 ºC for 30 seconds, 50-55 ºC (Table 1-2) for 1 minute, 72 ºC for 1 minute, and a final extension at 72 ºC for 10 minutes. PCR products were visualized on ABI Prism 310 Genetic Analyzer using the GeneScan software. Following PCR, products were diluted 1:50-1:80, and 1 µl was mixed with an internal lane size standard according to the manufacturer's instructions (GeneScan-500 LIZ Size Standard, Applied Biosystems, USA). Microsatellite allele sizes were estimated by comparison to size standard using GeneMapper version 4.1 (Applied Biosystems, USA).

Genotyping errors

Each sample that was successfully amplified in the first multiplex set of three loci was genotyped two additional times to control for genotyping errors. Poor-quality DNA from fecal samples could lead to the misidentification of individuals and biased estimates (Taberlet et al. 1996, Taberlet et al. 1999), and so samples, which were not successful in the first round of amplification, were discarded. If a sample produced a consensus genotype without ambiguous amplifications in both rounds, additional reactions were not carried out. Otherwise, three to four amplifications were conducted for each sample to confirm any allele that was inconsistently scored. A consensus genotype was constructed if at least two replicates were matched in eight loci for each sample; samples missing any locus were excluded from the data set. In some cases, an additional singleplex PCR was performed for the final confirmation of the allele. Maximum likelihood allele dropout (ADO) and false alleles (FA) were calculated from PEDANT version 1.0 with 10,000 search steps for enumerating error (Johnson and Haydon 2007). Genotyping errors such as stutter bands, null alleles, and large allele dropouts were verified using MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004).

Mitochondrial DNA sequencing

The left variable region of the mitochondrial control region (CR)/D-loop (approximately 675 bp) was amplified of all identified individuals using the bear-specific primer pairs 11H2 and 11L2, and BED 1-2 and BED 3-3 (Table 1-3). The PCR amplification was carried out in 25 µl reaction volume which contained 2.5 µl of 10X *Ex Taq* Buffer, 2 µl of dNTP Mixture, 125 nl of *Ex Taq* polymerase (TaKaRa Bio Inc., Shiga, Japan), 0.5 µl each of 10 µM forward and reverse primers, 1 µl of DNA template and remaining PCR-grade water. After denaturation at 94 ºC for 1 minute, 35 cycles were performed as 94 ºC for 30 seconds, 55 ºC for 30 seconds, 72 ºC for 90 seconds, and a final extension at 72 ºC for 10 minutes. Five microliter of PCR aliquots were run on a 1.5% agarose gel and visualized by ethidium bromide staining under ultraviolet illumination to confirm the amplification of targeted region by primers. Nested PCR was conducted by using internal primer pair (BED 1-2 and BED 3-3) following the same procedures only for those fragments which were not amplified by the first primer pair. PCR products were purified using FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co. Ltd), measured DNA concentration using a spectrometer (NanoDrop 2000, Thermo scientific) and sequenced in both directions using same amplify primers with an ABI 3730 XL DNA Analyzer following the manufacture's protocol. Amplification and sequencing were repeated in case of ambiguous results.

To resolve the polytomy of phylogenetic relationships of closely-related subspecies of Asiatic black bears based on CR and cytochrome b, whole mitogenome sequencing was performed for two samples that were determined to have different haplotypes for the CR. Bear-specific primers were designed for the amplification of fragments covering the entire mitochondrial genome as in Hirata et al. (2013). In the case of poor PCR performance with these primers, additional primers were also designed to complete the sequencing of each fragment in both directions (Table 1-3). The amplification and sequencing were carried out following above mentioned procedures for CR haplotypes.

Data analysis

Genetic diversity and probability of identity

Samples that matched at all eight loci were pooled to create individual genotypes using GIMLET version 1.3.3 (Valière 2002). Genetic diversity (mean number of alleles per locus (N_A), effective no of alleles (N_E), observed heterozygosity (H_O), expected heterozygosity (H_E) and Unbiased expected heterozygosity (uH_E)) and Wright's inbreeding coefficient (F_{IS}) (Weir and Cockerham 1984) were calculated with GenALEx version 6.5 (Peakall and Smouse 2012). The Hardy-Weinberg equilibrium (HWE) of eight microsatellite loci following the exact test and linkage disequilibrium (LD) between all pairs of loci were tested using the web-based program GENEPOP version 4.2 (Rousset 2008). Bonferroni corrections were applied for multiple comparisons. P_{ID}, the probability of identity of siblings (P_{ID}Sibs), PIC, and the null allele frequency (Fnull) of each locus, were calculated using CERVUS version 3.0.7 (Kalinowski et al. 2007). The GPS coordinates of each genotype and their recaptures were mapped using ArcMap version 10.0 (ESRI, Inc.).

Estimation of relatedness and analysis of population genetic structure

Pairwise relatedness values (r) were calculated for all individual bears using the estimator developed by Queller and Goodnight (Queller and Goodnight 1989) in GenALEx. To determine the patterns of population genetic substructure for the high and middle mountain populations of the ACA, a Bayesian clustering analysis was used in STRUCTURE version 2.3.4 (Pritchard et al. 2000). The same program was also used to detect any genetic structure caused by barriers to bear movement in the conservation area. The range of possible clusters (K) ranged from 1 to 5, and five independent runs were performed with and without prior information of sampling locations. The admixture model with correlated frequencies was run with burn-in periods of 50,000 and 500,000 Markov Chain Monte Carlo (MCMC) iterations. Each individual bear was assigned to a cluster if its membership coefficient (q) was above 0.7, or classified as admixed if q was less than 0.7. To determine the most probable value of K, mean LnProb values as in Pritchard et al. (2000) was used as implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Mitochondrial DNA sequencing and phylogenetic inference

Sequences were visually inspected for errors, multiple peaks, and heteroplasmy using FinchTV version 1.4.0 (Geospiza Inc.), and aligned with Clustal W (Thompson 1994) in MEGA version 7.0.26 (Kumar et al. 2015). The exact length of the CR could not be determined due to the presence of a variable number of tandem repeats (VNTRs). The genomic positions of 2 rRNAs, 22 tRNAs, 13 protein-coding genes, and the CR were determined by the whole mitogenome sequence of Asiatic black bears from the Yunnan province of China (GenBank accession no. NC_009971, Yu et al. 2007). All sequences were deposited in the NCBI GenBank database (Accession number MH262297–MH262299 for the CR haplotypes and MH281753 for the complete mitochondrial genome). Haplotype and nucleotide diversity of the CR were analyzed using DnaSP version 6.10.04 (Rozas et al. 2017).

The complete mitogenome and the haplotypes sequences of six subspecies of Asiatic black bears were obtained from the GenBank (Annex 1). The Japanese black bear (Accession no AB863014, Wu et al. 2015) was used as an outgroup for the phylogenetic analysis of Asiatic black bears. Insertions and deletions of nucleotides, VNTR sequences of 10-bp units in the CR, and ambiguous sites were excluded from the analyses. Three data sets comprising (1) the left domain (675 bp) of the CR, (2) cytochrome b (1,140 bp), (3) the whole mitogenome except VNTRs of the CR (16,363 bp), and (4) a combination of 12 protein-coding and 2 rRNA genes (12,663 bp) were analyzed. A phylogenetic network tree was constructed using the median joining network (Bandelt et al. 1999) in PopART (http://popart.otago.ac.nz) to investigate the possible relationships between CR haplotypes of Asiatic black bears and haplotypes generated from Nepal. Phylogenetic trees were prepared using maximum likelihood (ML) and Bayesian inference (BI) with the programs RAxML version 8.2.10 (Stamatakis 2014), CIPRES Science Gateway version 3.3, and MrBayes version 3.2.6 (Ronquist et al. 2012), respectively. The GTR substitution model (the only model provided in RAxML) was used to infer the maximum likelihood phylogeny using 1,000 bootstrap replicates in RAxML. The best-fit model of nucleotide substitution for BI was selected by jModelTest version 2.1.10 (Darrib et al. 2012), using corrected Akaike information criterion (AICc) values. The Bayesian analysis included two independent runs of four chains for 1,000,000 MCMC generations sampled every 1,000 generations. Nodal support on the BI tree was measured by Bayesian posterior probabilities (BPP). Tracer version 1.7 (Rambaut et al. 2018) was used to verify that effective sample size of the underlying posterior distribution was large enough (> 200) for a meaningful estimation of parameters. Both trees were visualized in FigTree version 1.4.3. Additional genealogical relationships among observed mtDNA haplotypes were inferred using the neighbor-joining method (Saitou and Nei 1987) in the program MEGA, with the genetic distance between haplotypes calculated using Kimura's two parametric method (Kimura 1980). No gamma correction was made for the difference in substitution rates of different pairs of nucleotides, as the observed sequence divergence was too low to have a significant effect on estimates (Nei and Kumar 2000). A maximum parsimony analysis was also conducted in MEGA for comparison.

Results

Non-invasive sampling and genotyping of microsatellite loci

A total of 126 fecal and 21 hair samples were collected in five management units in the ACA from elevations ranging from 1,725 to 3,580 m. Of 126 fecal samples, 44 and 82 were collected during the rainy and autumn seasons, respectively. Twenty-four samples that did not produce usable DNA extracts, and 26 additional samples that amplified inconsistently for several loci or did not yield complete genotypes even after the third round of PCR were removed from further analyses (Table 1-4). Amplification was unsuccessful at two loci (MSUT5 and MSUT6) for 16 fecal samples while following the original annealing temperature with slight adjustment to the protocol. Four loci (G1A, MU51, MU09, and MU59) amplified for some samples only whereas the remaining 21 loci were successfully amplified for all samples. All 25 loci were polymorphic, ranging from 2 (G10X) to 7 (MU50) alleles each. Three loci (G10P, G1A and G10B) deviated from HWE, but the differences were not statistically significant after Bonferroni corrections. Two loci (MSUT4 and G10X) had higher P_{ID} (> 0.250) and lower PIC (< 0.5) values than the other loci. Following amplification, eight of the 19 polymorphic loci were selected with high amplification success, also taking into account their ease of use in multiplex PCR and readability of their allelic peaks (Table 1-2). These eight microsatellites were successfully genotyped using at least two PCR experiments from 73% (n = 92) of the fecal samples and 24% (n = 5) of the hair samples. An overall genotyping success was 66% (n = 97) for both hair and fecal samples (Table 1-4) with 0–4% allelic dropout errors, with negligible presence of false alleles. The Micro-checker program did not detect any errors due to stutter bands, large allele dropouts or deficits of heterozygotes. The proportion of successfully amplified fecal samples collected in autumn (84%) was noticeably higher than that of samples collected during the rainy season (52%).

Genetic variability and individual distribution

The number of alleles (N_A) for the eight microsatellite loci ranged from 5 to 10, and averaged 7.6, which was higher than the effective numbers of alleles (4.4). MU23 and MU50 were highly polymorphic with 10 alleles whereas G10B and UamB5 were the least polymorphic with 5 alleles (Table 1-5). In the Jomsom unit, an individual with seven recaptures had a base-pair difference mutation in MU23 (Annex 2). Combined across all loci, the average values of observed (H_O) and expected (H_E) heterozygosity were 0.798 and 0.760, respectively. No significant deviation from HWE ($P > 0.306$) or LD was detected between pairs of the eight microsatellite loci ($P > 0.07$). Three pairs had slight deviations that were not statistically significant after Bonferroni corrections ($P > 0.02$). The cumulative P_{ID} and P_{ID}Sibs were 3.17×10^{-09} and 5.5×10^{-04}, respectively, which indicated that the power of the markers selected was more than sufficient to discriminate among individuals. Similarly, PIC averaged 0.727, and ranged from 0.617 to 0.816. The value of F_{IS} was −0.041, indicating no signs of inbreeding among populations. The negative value of F_{IS} also indicates an excess of heterozygous individuals in the region, a finding supported by the lower proportion (< 0.1) of null alleles.

From the 97 samples that were successfully genotyped, 60 individuals were identified, of which 20 were sampled multiple times (Annex 2). Two individuals were detected up to seven times, while 40 individuals (67%) were recorded only once during the sampling period. Sex determination identified 32 (54%) males and 28 (46%) females. Most of the bears (n = 48) were recorded in three management units in the southern forest during multiple surveys. Similarly, ten and two individuals were recorded in two high mountain management units located in Mustang and Manang districts, respectively. In total, 60 individuals were found in approximately 525 km^2 (11.5 individuals/100 km^2) of the ACA that included both forests and surrounding farmlands.

Genetic relatedness and population structure

The average pairwise relatedness value for 1,770 pairs among 60 individuals was less than 0.05 ($r = -0.017$, SE = 0.005), which suggests that individuals were highly unrelated to one another. Models with and without LOCPRIOR showed that no individual was assigned with high posterior probability ($q \geq 0.7$) to any of the two clusters at $K = 2$ (Fig. 1-2). Individuals from the ACA never formed a separate cluster even with increasing values of K. Results from STRUCTURE and the log probability of all individuals revealed that the value of K is most likely 1 (Fig. 1-3). High variance between iterations and large standard deviations were observed with other estimated values of K (Table 1-6). These results indicated that black bears from the ACA effectively constituted a single randomly-mating population without subdivision and that all individuals were highly admixed.

Sequence characteristics, haplotypes in the mtDNA CR, and geographic distribution of haplotypes

For all 60 individuals, the left domain of the CR (675 bp) was successfully sequenced. Three haplotypes were reported, which were distinguished by two transition substitutions of adenine (A) and guanine (G) and a repeated-number variation at a T-repeat site (Table 1-7). Haplotypes NEP-A1 ($n = 24$) and NEP-A2 ($n = 21$) were widely distributed in the study area from the northwest to south while NEP-A3 ($n = 15$) was detected in the northeast to the south of the ACA. All three haplotypes were found in the middle of the southern area of the ACA (Fig. 1-1C). The overall haplotype diversity (H) and nucleotide diversity (π) from the CR were 0.381 (± 0.057) and 0.113% (± 0.017%), respectively, which were calculated by excluding the T-repeat motif. Haplotype and nucleotide diversity are relatively low ($H < 0.5$ and $\pi < 0.1\%$) considering only the population of the ACA. This study produced first mitogenome (16,771 bp) sequenced from natural populations of *U. t. laniger* using hair and fecal samples, although a mitogenome of *U. t. laniger* was previously sequenced from an old skin found in a monastery near the Nepal-Tibet border (Lan et al. 2017). VNTRs located in the CR were too long to be sequenced directly to represent the actual length of the mitogenome. The K2P distances among Asiatic black bears calculated using MEGA ranged from 0.8 to 5.6% for cytochrome b (average 2.5%), 1.6 to 7.4% for the CR (average 3.4%), 0.8 to 3.8% (average 1.9%) for the complete data set except VNTRs of the CR, and 0.7 to 3.7% for the 12 protein-coding and 2 rRNA genes (average 1.9%). Compared to sequences from Nepal, sequences from Japanese black bears showed higher divergence, while sequences from black bears on the main Asian continent showed less divergence with the exception of those from *U. t. mupinensis* (Table 1-8).

Phylogenetic relationships

The GTR+G model was selected as the best model using the AICc approach in jModelTest 2 for cytochrome b, genes in the complete dataset and protein-coding genes, while the HKY+I model was selected for the CR. Maximum likelihood and Bayesian phylogenetic analyses revealed that *U. t. laniger* from the ACA of Nepal formed a monophyletic clade with *U. t. thibetanus* and with respect to other reported Asiatic black bear subspecies. A similar tree topology was obtained using mitogenomes (excluding the VNTRs of the CR) and the dataset of 12 protein-coding and 2 rRNA genes (Fig. 1-4). Identical well-resolved and strongly-supported tree topologies (BP 92–100% and BPP 1) consistent with previous studies were obtained in both maximum likelihood and Bayesian analyses using mitogenomes of the Asiatic black bear subspecies (Wu et al. 2015, Lan et al. 2017). However, tree topologies obtained using the cytochrome b gene and the left domain of the CR did not resolve the phylogeny of *U. thibetanus* subspecies (Fig. 1-5), likely because of homoplasy caused by the high substitution rate in this region, and the lack of phylogenetically informative sites (Wu et al. 2015). Therefore, a median joining network was used, which has been demonstrated to better visualize intraspecific sequence variation in the CR. In the network, the haplotypes from the ACA of Nepal were grouped separately from *U. t. thibetanus* and other subspecies (Fig. 1-6). Although unknown source of Asiatic black bear mitogenome (FM177759) contributed to minor differences in phylogenetic resolution, overall, haplotypes of *U. t. laniger* formed a clade basal to all other Asiatic black bears subspecies on the Asian continent in analyses using different methods and sequence sets. Neighbor-joining and maximum parsimony analyses of cytochrome b sequences produced similar topologies to those generated with mitogenomes. However, the basal placement of haplotypes of *U. t. laniger* had weak bootstrap support (Fig. 1-7), while the topology based on the CR had weak or no bootstrap support.

Discussion

Genetic diversity of Himalayan black bears in Nepal

Valuable information on genetic diversity was obtained in this first study on the population genetics of Himalayan black bears from Nepal. The selected polymorphic microsatellite loci, which were highly informative, can be used for population estimation, and evaluating genetic substructure, gene flow, and other genetic parameters of Asiatic black bear populations using non-invasive samples. This study revealed that there is no any evidence to suggest that the genetic diversity and geneflow of Himalayan black bears in Annapurna region of Nepal are inadequate, and any evidence of inbreeding in the population was not detected. The low rate of genotyping success from hair samples was possibly due to the poor quality of DNA extracts from samples that were old. Although collecting fecal samples from agricultural land and the nearby bamboo forest during the rainy season was relatively manageable, it was difficult to obtain high-quality DNA due to the high levels of moisture in the samples (Brinkman et al. 2010). Future fecal sampling is recommended to be conducted during the dry season (Murphy et al. 2007). In addition, systematic fecal sample collection from the mountain range would help to identify extant populations and the levels of genetic differentiation between the different protected areas. Non-invasive genetic samples can also provide useful information about the relationships between population function, gene flow, and habitat connectivity (Dutta et al. 2015).

No significant differences between observed and expected heterozygosity were found, low levels of relatedness among bear individuals, and a lack of population substructure. The average expected heterozygosity was similar to that found in Indian (0.72, Mukesh et al. 2015) and Russian (0.65–0.76, Kim et al. 2011, Latham et al. 2012) bear populations, and higher than the heterozygosity found in Japanese (0.66, Saito et al. 2008; 0.68, Uno et al. 2015; 0.53–0.70, Ohnishi et al. 2007) and North Korean (0.67, Kim et al. 2011) populations. The higher levels of genetic diversity in the ACA could also be attributed to the continuity of suitable habitats between the Chitwan-Annapurna landscape in the south, and the eastern and western mountain forests where community forestry programs (e.g. plantation, control on grazing and on illegal extraction of forest resources, regulations for forest product collection) have been implemented for the last four decades (MoFSC, 2016). No other studies are available for comparisons among broad geographic regions in Nepal. The ACA is located in the central part of a continuous mountain landscape and the genetic diversity in the ACA could represent the high levels of connectivity at a wide scale among black bear populations in Nepal. The estimated genetic diversity of black bears in the ACA is similar to those of populations of North American bears in continuous habitats, such as the population of brown bear (*U. arctos*) in Kluane (Paetkau et al. 1998). As these North American populations are regarded as stable (Servheen et al. 1998), the genetic diversity of black bears in the ACA may be sufficient to maintain a stable population.

Population genetic structure

No evidence of population substructure was found among the ACA populations, indicating a high degree of admixture throughout the conservation area. The results are similar to those reported for the population in Dachigam National Park in northwest India (Mukesh et al. 2015). The results may also be due to a large proportion of the genomes of bears in the ACA being admixed ancestrally. This study found substantial evidence for gene flow between the high mountain and the southern middle mountain forest regions, suggesting that bears in this conservation area are panmictic, freely moving long distances between regions and breeding effectively in both areas. Male-biased dispersal and female-biased philopatry are known in most mammalian species (Greenwood 1980). As reported in the American black bear (White et al. 2000) and Himalayan black bear (Mukesh et al. 2015), male black bears have a larger home range and disperse further than females. The lack of genetic substructure within the dataset indicates that bears have no effective barriers to movement within the ACA, although signatures of at least two subpopulations were expected between the northwest and southern region of the ACA, due to the potential migration barriers of rivers, high elevation mountains, and human settlements in this area. The result showed that either these landscape features have not restricted the movement of bears between the northwest and southern regions or, alternatively, it could be that there has been a more recent restriction of movement between these regions but not enough time has elapsed to leave a genetic signature of population structure. This also provides further support for population connectivity within the neighboring protected areas via contiguous forest and highlights their importance in the overall management of the species. It is highly recommended that further genetic studies in other protected areas be carried out using the molecular methods developed here to evaluate admixing ancestry and population substructuring over a broader geographic range. Additional factors such as forest cover, food availability, human impact, road networks, and other human infrastructure can be assessed in relation to the movement of bears (Dutta et al. 2015).

Phylogenetic relationships of Asiatic black bears

This is the first study using genetic and geographic data from the mitogenome sequences from six subspecies and two Asiatic black bears of unknown origin. The only subspecies that was not represented in this phylogeny was *U. t. gedrosinus*. In previous studies, the lack of phylogenetic resolution for Asiatic black bears was primarily due to the low level of variation in the much shorter sequences of cytochrome b or the CR. Previous studies also placed the Japanese subspecies (*U. t. japonicus*) as the basal group to all other *U. thibetanus* subspecies (Yasukochi et al. 2009, Wu et al. 2015). In this phylogenetic analysis, the individual FM177759 (Krause et al. 2008) branched off more basally than the clade of *U. t. laniger*. However, the geographical origin of this individual is unknown. Based on the largest mtDNA dataset available from Asiatic black bears, the mitogenome-based phylogeny provides strong evidence that *U. t. laniger* is the most basal of all black bear subspecies on the Asian continent. The Japanese black bear was estimated to have diverged from continental black bears approximately 1.48 Mya (Wu et al. 2015) and the ancestor of *U. t. laniger* diverged from other lineages of *U. thibetanus* at 475 Ka BP (Lan et al. 2017). Individuals of the northeast Asian *U. t. ussuricus* also clustered into a single clade (Choi et al. 2010, Kim et al. 2011).

The mitogenome analysis strongly supports previous finding of a monophyletic lineage of *U. t. laniger* in the ACA that is considered ancient with respect to all other mainland Asiatic black bears subspecies (Wu et al. 2015, Lan et al. 2017) and does not support the results of Choi et al. (2010) that places *U. t. mupinensis* as basal to all other *U. thibetanus* in their Clade A. Although the sequences used in Choi et al. (2010) were from the same mitogenome regions used in this analysis, the *U. t. mupinensis* in this phylogenetic tree was placed monophyletic to the other subspecies of *U. thibetanus* but the *U. t. laniger* sequences were the basal group. The phylogenetic analyses inferred from mitogenomes of two unknown and six known subspecies suggesting that whole mitogenomes (without the VNTRs of the CR) were more informative and provide a high resolution for Asiatic black bear phylogeny. The CR and cytochrome b genes are not sufficiently informative to resolve phylogenetic relationships in Asiatic black bears, particularly among such closely related subspecies. Although the left domain of CR is considered the most appropriate region to use in constructing phylogenetic trees (Hwang et al. 2008), this analysis did not obtain sufficient resolution. Additional caution is needed when analyzing data from samples of unknown origin as interbreeding between *U. t. japnonicus* and *U. t. ussuricus* from China (Yasukochi et al. 2009), and *U. thibetanus* and *U. malayanus* in northern Cambodia (Galbreath et al. 2008) have been reported. This may affect the interpretation of results from phylogenetic analyses of samples from these regions. Samples of southeast Asiatic black bears from Vietnam and Taiwan were taken from a rescue center and a zoo, respectively. Phylogenetic analyses that included these samples produced complex topologies, suggesting a complicated structuring of populations in these regions (Kim et al. 2011).

The phylogenetic analyses demonstrated that lineages of the Himalayan black bears in Nepal are ancient and genetically distinct from lineages of other subspecies of Asiatic black bears from the main continent (China, Taiwan and southeast Asia). The result corroborates the findings of previous studies of *U. t. laniger* from the Himalayan region based on the specimen from the Mustang district monastery (Lan et al. 2017). Mitogenomes from the samples NEP-A1 and NEP-A2 were similar and closely related to mitogenome haplotype derived from the sample collected at the monastery, which is located far away from the black bears' natural habitat and outside the known distribution of Asiatic black bears. The T-repeat region and VNTRs of the CR were excluded from the original sequence (MG066704) reported in GenBank, so unable to compare the T-repeat region of the haplotypes of NEP-A1 and NEP-A2 with monastery sample. The bear skin at the monastery could have originated from the southern part of the district, which is highly populated by Himalayan black bears based on the microsatellite analysis. These three novel haplotypes came from samples collected from the wild mountain habitats where black bears were never recorded as being reintroduced. Individuals of *U. t. laniger* from India and Pakistan also formed a well-supported clade with other continental bear subspecies using the amplicon sequence of cytochrome b (Lan et al. 2017).

Based on this study, *U. t. laniger* is likely distributed throughout the western Himalayan mountain range from Nepal to Pakistan, including the northwest India. Further sampling along the eastern Himalayas in Nepal, India, Bhutan, and Myanmar may help to more clearly define the distribution range of *U. t. laniger* and *U. t. thibetanus* in the east. There was no evidence of a habitat overlap between the Tibetan brown bear (*U. a. pruinosus*), the sloth bear (*U. ursinus*), and *U. t. laniger* in ACA. The brown bear has been sporadically recorded in the northern part of the ACA and sloth bears inhabit southern Chitwan National Park of the Chitwan-Annapurna landscape (Jnawali et al. 2011).

Population status, human-bear conflict, and conservation threats

Available data indicates that there are approximately 500 black bear individuals in Nepal (Jnawali et al. 2011). From this study, a minimum of 60 individuals is estimated to inhabit a single conservation area based on non-invasively collected samples. The estimated minimum density of black bears in the ACA (11.5 bears/100 km^2) was less than previous estimates from the national parks of the western Himalayas (17 bears/100 km^2, Mukesh et al. 2015) and Thailand (8–29 bears/100 km^2, Ngoprasert et al. 2012, Ngoprasert et al. 2013), which could not be extrapolated to estimate the bear population outside protected areas. Black bear density from studies in Russia was estimated to be 8–11 bears/100 km^2 (Aramilev 2006). Future systematic surveys conducted throughout the potential distribution range of black bears will likely reveal that the total population is much higher in ACA. Sampling in this study was mainly opportunistic and geographically biased, and individual bears were not found repeatedly due to the large geographic distances. In addition, all individuals, which may include cubs, were included in the calculations. In future studies, a standardized capture-recapture approach may help to improve the dataset and estimate a more precise population size. In general, the results support the ACA as being a highly suitable habitat for bears, and also support the potential for the bear population to increase in the future.

The locations of bears and their frequent recaptures in agricultural lands indicate that they involve in damaging crops and altercations with humans in the conservation area. Bears visited agricultural land during the rainy season but were not observed during the autumn. This might be due to the sufficient food being available in nearby forests during autumn. The highest number of bears were recorded on agricultural land during the rainy season, contributing to negative human-bear interactions. One male bear was recorded in 2015 in the *Quercus* forest in the Sikles unit and was recorded again in 2016 on a maize farm during the rainy season. Later, the bear was furtively killed by villagers using a gun. Human-bear interactions have poor outcomes when bears frequently visit agricultural lands, with observations of poisoning, snares, gunshots, and even retaliatory killings of bears in these areas. Despite mountain forests being suitable habitats for Himalayan black bears, human-bear conflicts were recorded throughout most of the bears habitat in this range. Increasing rates of human-bear conflict further exacerbate the negative responses of local villagers towards bears. The study showed that 25% (n = 16) of the individual bears sampled were recorded from agricultural land, demonstrating the potential for human-bear conflict in the rainy season. Proper management to reduce crop losses to bears will help secure the future survival of bear populations and maintain their genetic diversity in the long-term.

Globally, habitat fragmentation and degradation are major causes of population decline and species extinction (Crooks et al. 2011), which also affect genetic variation and population viability. A fragmented landscape means that individuals have to expend more effort to move through their habitat, which potentially decreases overall movement and genetic exchange (Dutta et al. 2015). During the last few decades, middle mountain forests have been well managed in Nepal, resulting in increased forest cover due mainly to the community forestry program throughout the regions (Niraula et al. 2013, MoFSC 2016) and the community-based conservation program in the ACA (Bajracharya et al. 2005). The improved forest conditions include increased habitat connectivity, which may have played a crucial role in the maintenance of genetic diversity in bear populations here, by facilitating the free movement of the Himalayan black bears. However, the construction of roads and hydropower facilities, increasing human-bear conflict, and retaliatory killing of bears are emerging threats for bear populations in the mountainous habitats of Nepal.

Conclusions

The intraspecific phylogeny demonstrates that the wild population of Himalayan black bears (*U. t. laniger*) from Nepal is genetically distinct and belongs to an ancient lineage of continental Asiatic black bears. The minimum number of bears in the ACA population was estimated from non-invasive samples, which can be extended to estimate the total population for future management of Himalayan black bears. The genetic analyses revealed that no management interventions are currently needed to maintain genetic diversity in the population, but human-bear conflict has to be addressed for long-term survival of the bears. Genetic diversity in the bear population in central Nepal is relatively high as bear habitats are interconnected, enabling high levels of gene flow and admixture. Further, comparisons with Asiatic black bears from Japan revealed that genetic variation in isolated populations was lower than in continuous populations (Ohnishi et al. 2007). This study indicates that community-based conservation in the ACA may have contributed to the viability of the bear population here, with low levels of population substructuring. Still, results were based on samples collected within a single conservation area. Additional non-invasive studies on evolutionary lineage, population status, genetic diversity, and genetic structure should be conducted over the entire mountain landscape of Nepal with sufficient sample replicates to represent this widespread species.

Table 1-1. Microsatellite loci and primers tested for genotyping fecal and hair samples collected non-invasively for the Himalayan black bears in Annapurna Conservation Area, Nepal.

Locus	Multiplex	Labelling dye		Primer sequence (5'–3') Forward (F)/Reverse(R)	Ta	References
G1A	MP2	FAM	F:	GACCCTGCATACTCTCCTCTGATG	55	Paetkau et al. 1995
			R:	GCACTGTCCTTGCGTAGAAGTGAC		
G10B	MP1	VIC	F:	GCCTTTTAATGTTCTGTTGAATTTG	55	Paetkau et al. 1995
			R:	GACAAATCACAGAAACCTCCATCC		
G10C	MP3	NED	F:	AAAGCAGAAGGCCTTGATTTCCTG	55	Paetkau et al. 1995
			R:	GGGGACATAAACACCGAGACAGC		
G1D	MP5	VIC	F:	GATCTGTGGGTTTATAGGTTACA	55	Paetkau et al. 1995
			R:	CTACTCTTCCTACTCTTTAAGAG		
G10J	MP8	PET	F:	GATCAGATATTTTCAGCTTT	55	Paetkau et al. 1998
			R:	AACCCCTCACACTCCACTTC		
G10L	MP7	NED	F:	GTACTGATTTAATTCACATTTCCC	55	Paetkau et al. 1995
			R:	GAAGATACAGAAACCTACCCATGC		
G10M†	MP4	FAM	F:	TTCCCCTCATCGTAGGTTGTA	55	Paetkau et al. 1995
			R:	TTTCCAAATAATTTAAATGCATCC		
G10P	MP3	VIC	F:	AGGAGGAAGAAAGATGGAAAAC	55	Paetkau et al. 1995
			R:	TCATGTGGGGAAATACTCTGAA		
G10X	MP3	FAM*	F:	CCACCTTCTTCCAATTCTC	55	Paetkau et al. 1998
			R:	TCAGTTATCTGTGAAATCAAAA		
MU05	MP2	VIC*	F:	GTGATTTTTCTTGTAGCCTAGG	55	Taberlet et al. 1997
			R:	GAAACTTGTTATGGGAACCA		
MU09	MP4	VIC*	F:	TTGAAGTTCAGGGTAAATGC	55	Taberlet et al. 1997
			R:	ATATAGCAGCATATTTTTGGCT		
MU10	MP5	NED	F:	TTCAGATTTCATCAGTTTGAC	55	Taberlet et al. 1997
			R:	CAGCATAGTTACACAAATCTCC		
MU23	MP1	NED	F:	GCCTGTGTGCTATTTTATCC	55	Taberlet et al. 1997
			R:	AATGGGTTTCTTGTTTAATTAC		
MU26	MP7	VIC	F:	GCCTCAAATGACAAGATTTC	55	Taberlet et al. 1997
			R:	TCAATTAAAATAGGAAGCAGC		

Locus	Multiplex	Labelling dye		Primer sequence (5'–3') Forward (F)/Reverse(R)	Ta	References
MU50	MP1	FAM	F:	TCTCTGTCATTTCCCCATC	55	Taberlet et al. 1997
			R:	AAAGGCAATGCAGATATTGT		
MU51	MP2	NED	F:	GCCAGAATCCTAAGAGACCT	55	Taberlet et al. 1997
			R:	AAGAGAAGGGACAGGAGGTA		
MU59	MP4	NED	F:	GCTCCTTTGGGACATTGTAA	55	Taberlet et al. 1997
			R:	GACTGTCACCAGCAGGAG		
MU61	MP5	FAM	F:	ACCCAGAGAAGTCCGATTAC	55	Taberlet et al. 1997
			R:	CTGCTACCTTTCATCAGCAT		
MU64	MP8	NED	F:	ACTCAACACAACCATTAAATCA	55	Taberlet et al. 1997
			R:	AGGACCCAAATGACACTACA		
UamB5	MP6	NED	F:	CCGGTGGATCTATCTCAGAGT	55	Meredith et al. 2009
			R:	GGGATCTTGTCTATCCTGCTC		
UamD2	MP6	VIC	F:	ACACCTGTCTTCCCTTCCTAAC	55	Meredith et al. 2009
			R:	TTCCATCTGAGAGGCTGAAC		
MSUT2	MP9N	PET	F:	AGTGAATCCTAAACAGGTTA	55	Kitahara et al. 2000
			R:	TAATATGAATATGGTGTGCT		
MSUT4	MP10	VIC	F:	GTGTCCAACTGTAGATGA	50	Kitahara et al. 2000
			R:	TGAGTAATATTCTTTTCTCT		
MSUT5	MP9	VIC	F:	GGGACTGAGCCTCTCATC	50/55	Kitahara et al. 2000
			R:	TCCAATATTTTGTCTGAGTG		
MSUT6	MP9	FAM	F:	CATATGGTGACTAAGATAAC	50/55	Kitahara et al. 2000
			R:	AAGAGATGATTTCTGTCTC		
MSUT7	MP10	NED	F:	TGGAAAATATTCTCATTC	50	Kitahara et al. 2000
			R:	TTGTAGGTTACTGGTTAC		
MSUT8	MP9N	FAM	F:	GATCCTGGGACTTCTCAG	55	Kitahara et al. 2000
			R:	TCCAGAGAAAGAGGACTG		
Amelogenin	MP6	PET	F:	CAGCCAAACCTCCCTCTGC	55	Yamamoto et al. 2002
			R:	CCCGCTTGGTCTTGTCTGTTGC		

Ta, annealing temperature (^{o}C)

†Primer sequence was modified based on GenBank #U22089.

*Reverse primers were labelled with a fluorescence dye.

Table 1-2. Characteristics of microsatellite markers tested in the present study.

Multiplex set	Locus	Labelling dye	Ta	Allele size	N_A	Amplification efficiency*	Amplification readability*	Amplification success (%)	H_O	H_E	PIC	P_{ID}	P_{ID}Sibs	P
MP1	**MU50**	FAM	55	212–224	7	Good	good	100%	0.875	0.817	0.735	0.086	0.389	0.867
	G10B	VIC	55	154–166	5	Good	good	100%	0.500	0.800	0.711	0.102	0.400	0.037
	MU23	NED	55	110–124	6	Good	good	100%	0.875	0.858	0.776	0.067	0.364	0.424
MP2	G1A	FAM	55	192–196	3	Good	good	44%	0.000	0.714	0.555	0.211	0.490	0.031
	MU05	VIC	55	136–146	6	Good	good	100%	0.875	0.817	0.730	0.090	0.390	0.842
	MU51	NED	55	108–122	4	Fair	good	63%	0.625	0.617	0.510	0.246	0.523	0.610
MP3	G10X	FAM	55	186–188	2	Fair	good	100%	0.375	0.325	0.258	0.530	0.730	1.000
	G10P	VIC	55	152–160	4	Good	good	100%	0.250	0.758	0.658	0.136	0.429	0.003
	G10C	NED	55	102–118	6	Good	good	100%	1.000	0.850	0.766	0.072	0.370	1.000
MP4	G10M	FAM	55	204–210	4	Good	good	100%	0.625	0.592	0.510	0.243	0.533	0.778
	MU09	VIC	55	130–148	5	Good	good	81%	1.000	0.817	0.727	0.094	0.391	1.000
	MU59	NED	55	106–130	6	Fair	good	88%	1.000	0.842	0.759	0.075	0.374	1.000
MP5	**MU61**	FAM	55	201–219	6	Good	good	100%	0.875	0.850	0.766	0.072	0.370	0.742
	G1D	VIC	55	180–186	3	Good	good	100%	0.500	0.633	0.511	0.248	0.515	0.270
	MU10	NED	55	122–128	4	Good	fair	100%	0.500	0.725	0.618	0.164	0.451	0.097
MP6	**UamD2**	VIC	55	206–226	5	Good	good	100%	0.750	0.792	0.701	0.108	0.406	0.456
	UamB5	NED	55	144–160	4	Good	good	100%	1.000	0.692	0.592	0.180	0.471	1.000
MP7	MU26	VIC	55	190–210	4	Good	good	100%	0.750	0.700	0.605	0.169	0.464	0.796
	G10L	NED	55	124–142	6	Good	good	100%	0.625	0.775	0.691	0.110	0.414	0.181
MP8	MU64	FAM	55	191–203	5	Good	good	100%	0.750	0.700	0.595	0.180	0.467	0.731
	G10J	VIC	55	98–112	6	Good	good	100%	0.625	0.733	0.654	0.131	0.439	0.350
MP9N	MSUT8	FAM	55	114–124	4	Good	good	100%	1.000	0.758	0.658	0.136	0.429	1.000
	MSUT2	PET	55	77–99	6	Good	good	100%	0.750	0.833	0.748	0.081	0.380	0.131
MP10N	MSUT7	NED	50	118–138	4	Good	good	100%	0.625	0.692	0.582	0.191	0.473	0.488
	MSUT4	VIC	50	92–102	5	Good	good	100%	0.500	0.533	0.474	0.276	0.569	0.608
Mean/combined					**4.8**					**0.729**	**0.636**	**3.53×10^{-22}**	**1.45×10^{-09}**	

Ta, annealing temperature °C; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content; P_{ID}, probability of identity; P_{ID}Sibs, probability of identity of siblings; P, P values for exact test of Hardy-Weinberg equilibrium (level of significance, $\alpha = 0.05$).

*Amplification efficiency and readability were estimated following Uno et al. 2012.

The highlighted loci were selected for amplification of all samples.

Analysis were carried out based on 16 fecal samples which later identified 8 unique genotypes.

Table 1-3. Primers designed for mitochondrial genome and control region (CR) sequencing.

mtDNA fragment	Prime name[a]		Primer sequence (5'-3') Forward (F)/Reverse(R)	Position[b]	References
Amplifying primers					
1	mtDNA_1H	F:	CAAATGGGACATCTCGATGGACTA	16754	Delisle and Strobeck 2002
	mtDNA_1L	R:	CAGCTATCACCAGGCTCGTTAG	2347	Delisle and Strobeck 2002
2	mtDNA_2AH_2	F:	GAGAAAGTACCGCAAGGGAAC	2105	This study
	mtDNA_2BL_2	R:	CACCCTAACAAAGCCCTGTC	3582	This study
3	mtDNA_3AH	F:	AGCAATCCAGGTCGGTTTCTATC	3416	Hirata et al. 2013
	mtDNA_3BL	R:	GTGAGCGATTGAAGAGTATGCTAG	5373	Hirata et al. 2013
4	mtDNA_4H_2	F:	AAGTCACACAAGGCGTTCCT	5165	This study
	mtDNA_4L_2	R:	GACAGTCCACCCAGTTCCTG	6626	This study
5	mtDNA_5H_2	F:	GCTCTCAGCCTTTTGATTCG	6336	This study
	mtDNA_5L_3	R:	TTGATTCGTGCCGAACTAGG	6348	This study
6	mtDNA_6AH_2	F:	TTCCGACTATCCAGATGCCTAC	7562	This study
	mtDNA_6BL	R:	TAGGCCTGAATGAGGGCTAC	9479	Hirata et al. 2013
7	mtDNA_7H_2	F:	TTGGCTCACTTTCTACCTCAAG	9217	This study
	mtDNA_7BL	R:	GCTGATAGGGAGTCGGTAAAG	11271	Hirata et al. 2013
8	mtDNA_8H_2	F:	CTGCGAAGCAGCACTAGGAC	10997	This study
	mtDNA_8L_2	R:	TGCTGACTGCGAAAGCATAG	12801	This study
9	mtDNA_9H_2	F:	AGCATCAAGCCATCCTTCAC	12356	This study
	mtDNA_9L_2	R:	TGGCGGTCTCGATAATTAGG	13857	This study

mtDNA fragment	Prime name[a]	Primer sequence (5'-3) Forward (F)/Reverse(R)		Position[b]	References
10_1	mtDNA_10H_2	F:	AAGATTATTGCCTTCTCCACCTC	13557	This study
	mtDNA_10L_2	R:	TGCTGGGAGATCAATGAGTG	15133	This study
10_2	mtDNA_10BH_2	F:	AAAACCCCACAAAACTCATCAC	14931	This study
	mtDNA_10CL_2	R:	TGCTTCTTCCTTGAGTCTTGG	16287	This study
11	mtDNA_11H_2[c]	F:	ATGAATCGGAGGACAACCAG	16072	This study
	mtDNA_11L_2[c]	R:	AGCTACATAAACGCGGTTGG	902	This study
CR	mtDNA_BED1-2[c]	F:	TACCCTCCCCAAGACTCAAG	16271	This study
CR	mtDNA_BED3-3[c]	R:	GGAAAAATCAATAGGAGGGAGAC	823	This study
Sequencing primers					
1	mtDNA_1_seq1	R:	GATTAGCAAGGGGTGGTGAG	1573	This study
3	mtDNA_3_seq1	F:	CCGTAGCCCAGACAATTTCA	4054	This study
6	mtDNA_6_seq1	F:	AATTGCACTCCCATCGCTAC	8151	This study
7	mtDNA_7_seq1	F:	CCTGAACCCGCTAGAAGTACC	9893	This study
8	mtDNA_8_seq1	R:	TTGATGGTAGGAGGGAGTGC	12204	This study
10	mtDNA_10_seq1	F:	CGTCTCCAACCAGAAAGGAC	14387	This study

[a]The letter codes H and L refer to the heavy and light strands of the mitochondrial DNA, respectively and the name was given following the primer name of Hirata et al. (2013).

[b] Position corresponds to nucleotide numbers of Asiatic black bears (NC009971) from Yunnan province of China (Yu et al. 2007).

[c]These region represented the amplification of CR haplotypes.

Amplifying and sequencing primers were used for direct sequencing of the purified PCR product.

Table 1-4. Details of non-invasive sample size, genotyping success and the number of individuals located in forest vs. farmland in each management unit.

Management Unit	Total sample†	Genotype status					Individual location	
		DNA unsuccess	Ambiguous genotype	Success (no)	Success (%)	Unique genotype	Forest	Farmland
Jomsom	56 (41)	18 (8)	16 (15)	22 (18)	39 (44)	10 (10)*	1	9
Ghandruk	22 (17)	5 (1)	5 (5)	12 (11)	55 (65)	10 (9)	7	3
Lwang	29 (29)	1 (1)	1 (1)	27 (27)	93 (93)	20 (20)	19	1
Sikles	35 (34)		2 (1)	33 (33)	94 (97)	18 (18)	18	1#
Manang	5 (5)		2 (2)	3 (3)	60 (60)	2 (2)		2
Total	**147 (126)**	**24 (10)**	**26 (24)**	**97 (92)**	**66 (73)**	**60 (59)**	**45**	**16**

†Sixty one samples (16 hairs and 45 feces) were collected from the agricultural land.

*One identical genotype represented by both feces and hair sample.

#One individual found both in farmland and forest.

Numbers in parentheses were represented by feces only.

Table 1-5. Genetic variability parameters for the 8 microsatellite loci used to evaluate the population of Annapurna Conservation Area, Nepal (N = 60 individuals).

Locus	Multiplex	N_A	N_E	H_O	H_E	uH_E	PIC	P_{ID}	P_{ID}Sibs	F_{IS}	P	Fnull	ADO
MU23	MP1	10	5.00	0.833	0.800	0.807	0.772	0.069	0.367	-0.033	0.759	-0.02	0.0
G10B	MP1	5	3.59	0.650	0.721	0.727	0.684	0.115	0.418	0.107	0.112	0.06	0.0
MU50	MP1	10	5.23	0.883	0.809	0.816	0.784	0.062	0.361	-0.084	0.941	-0.05	0.6
G10C	MP2	9	6.11	0.917	0.836	0.843	0.816	0.047	0.344	-0.088	0.986	-0.04	0.0
MU05	MP2	8	4.31	0.917	0.768	0.775	0.736	0.086	0.387	-0.185	1.000	-0.10	0.0
MU61	MP2	8	5.17	0.800	0.807	0.813	0.780	0.064	0.363	0.017	0.353	0.01	3.8
UamB5	MP3	5	2.95	0.717	0.662	0.667	0.617	0.160	0.459	-0.075	0.785	-0.04	0.0
UamD2	MP3	6	3.13	0.667	0.680	0.686	0.627	0.155	0.449	0.029	0.306	0.01	1.6
Mean/Cum		**7.63**	**4.44**	**0.798**	**0.760**	**0.767**	**0.727**	**$3.17 X 10^{-9}$**	**$5.5 X 10^{-4}$**	**-0.041**			
SE		0.73	0.40	0.038	0.023	0.023							

N_A, observed no of allele; N_E, effective no of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; uH_E, unbiased expected heterozygosity; PIC, polymorphic information content; P_{ID}, probability of identity (locus); P_{ID}Sibs, probability of siblings identity (locus); F_{IS}, Wright's inbreeding coefficient; P, P values for exact tests of Hardy-Weinberg equilibrium (level of significance, $\alpha = 0.05$); Fnull, predicted frequency of null alleles; ADO, allele drop out (%); MP, multiplex primer; SE, standard error.

Table 1-6. Summary and raw STRUCTURE HARVESTER output for K = 1 to K = 5 local population of ACA.

a. Summary of STRUCTURE HARVESTER output

K	Repetitions	mean estimated LnP(Data)	SD of estimated LnP(Data)
1	5	-1608.42	0.342053
2	5	-1629.94	13.248132
3	5	-1647.58	45.652076
4	5	-1648.82	18.235323
5	5	-1666.06	53.710222

b. Raw STRUCTURE HARVESTER output

K	Run No	Estimated Ln prob. of data	Mean value of Ln likelihood	Variance of Ln likelihood
1	2	-1608	-1596	24.1
1	5	-1608.1	-1595.7	24.7
1	3	-1608.6	-1595.9	25.3
1	4	-1608.7	-1595.8	25.7
1	1	-1608.7	-1595.9	25.6
2	7	-1630.1	-1593.3	73.7
2	10	-1644.3	-1591.5	105.8
2	9	-1611.8	-1594.5	34.6
2	6	-1640.7	-1591.6	98.1
2	8	-1622.8	-1593.7	58.1
3	14	-1616.5	-1593.9	45.3
3	13	-1610.2	-1594.5	31.4
3	12	-1616.2	-1594	44.4
3	15	-1699	-1589.4	219.2
3	11	-1696	-1588.6	214.7
4	16	-1635.1	-1591.9	86.4
4	19	-1642.1	-1592.4	99.5
4	18	-1639.8	-1592.4	94.7
4	17	-1680.6	-1590.5	180.2
4	20	-1646.5	-1592.4	108.3
5	24	-1609	-1594.5	28.9
5	25	-1716.6	-1587.2	258.8
5	21	-1729.2	-1589	280.4
5	23	-1646.1	-1590.9	110.4
5	22	-1629.4	-1593.2	72.4

Each K was performed 5 iterations and a mean value was estimated. The lowest value of Ln likelihood and the low variance of Ln likelihood suggest that the true value of K = 1, implying that Himalayan back bears of ACA are one breeding population and that there is gene flow between northern high mountains and southern mid hills.

Table 1-7. Variable positions and observed frequencies of the left domain of the CR for the 3 haplotypes Himalayan black bears from ACA in Nepal.

Haplotypes	Used length	Position no#			Population			Individuals	
		105	166	558	NW	S	NE	No	Percentage
NEP-A1	675	T	G	A	9	15	0	24	40
NEP-A2	674	–	.	.	1	20	0	21	35
NEP-A3	674	–	A	G	0	13	2	15	25

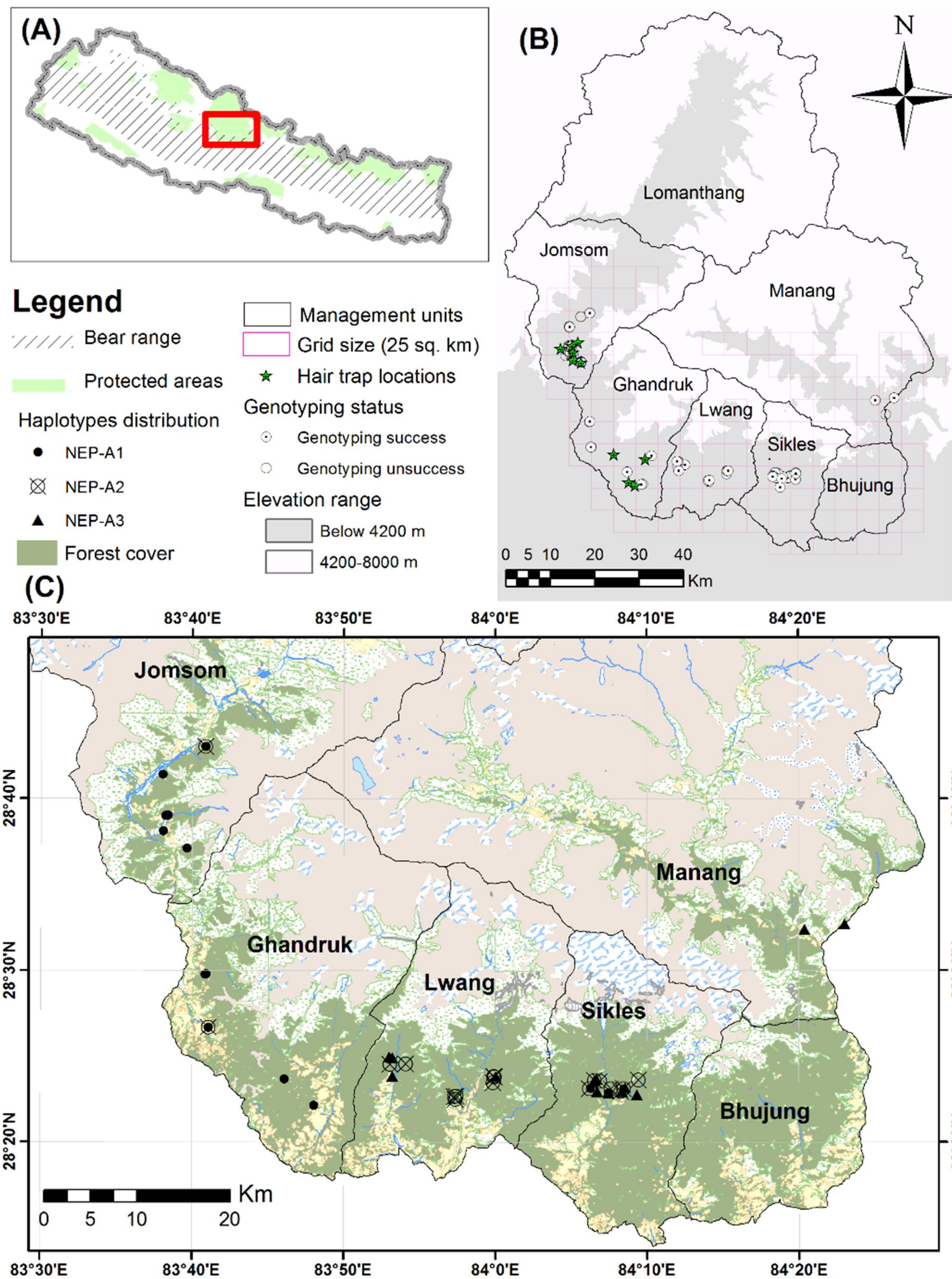
NW, northwest (Jomsom); S, south (Ghandruk, Lwang, and Sikles); NE, northeast (Manang).

#The positions were numbered for a haplotype NEP-A1 from the 5' end of sequence. Dot indicate identity with the nucleotides of NEP-A1. Dash indicates variation in the number of Ts at a T-repeat site (98–105 for NEP-A1).

Table 1-8. Evolutionary distances among *U. thibetanus* for control region (CR), cytochrome b and mitogenome of mtDNA sequences.

	U. t. laniger			***U. t. thibetanus***			***U. t. formosanus***			***U. t. mupinensis***			***U. t. ussuricus***			***U. thibetanus* (YUN)**			***U. thibetanus* (UK)**		
	CR	**Cytb**	**Mtge**	**CR**	**Cytb**	**Mtge**	**CR**	**Cytb**	**Mtge**	**CR**	**Cytb**	**Mtge**	**CR**	**Cytb**	**Mtge**	**CR**	**Cytb**	**Mtge**	**CR**	**Cytb**	**Mtge**
U. t. thibetanus	0.021	0.028	0.019																		
U. t. formosanus	0.024	0.025	0.019	0.022	0.012	0.011															
U. t. mupinensis	0.060	0.030	0.025	0.066	0.014	0.017	0.067	0.013	0.019												
U. t. ussuricus	0.017	0.026	0.019	0.016	0.011	0.009	0.019	0.010	0.012	0.060	0.009	0.013									
U thibetanus (YUN)	0.024	0.025	0.019	0.019	0.010	0.009	0.022	0.009	0.012	0.052	0.012	0.014	0.019	0.008	0.008						
U thibetansus (UK)	0.025	0.034	0.021	0.034	0.024	0.020	0.037	0.020	0.021	0.071	0.026	0.027	0.037	0.022	0.021	0.037	0.020	0.020			
U. t. japonicus	0.040	0.054	0.032	0.044	0.049	0.032	0.050	0.044	0.033	0.074	0.050	0.038	0.044	0.047	0.033	0.047	0.044	0.032	0.049	0.056	0.034

CR, Control region; Cytb, Cytochrome b; Mtge, Mitogenome; U. t., Ursus thibetanus; YUN, Yunnan province of China; UK, Unknown source.



(A)
(B)
N
Lomanthang
Jomsom
Manang
Ghandruk
Lwang
Sikles
Bhujung
0 5 10 20 30 40
Km
Legend
Bear range
Protected areas
Haplotypes distribution
NEP-A1
NEP-A2
NEP-A3
Forest cover
Management units
Grid size (25 sq. km)
Hair trap locations
Genotyping status
Genotyping success
Genotyping unsuccess
Elevation range
Below 4200 m
4200-8000 m
(C)
83°30'E
83°40'E
83°50'E
84°0'E
84°10'E
84°20'E
28°40'N
28°30'N
28°20'N
Jomsom
Manang
Ghandruk
Lwang
Sikles
Bhujung
Km
0 5 10 20

Figure 1-1. (A) Distribution range of Himalayan black bears in Nepal (B) Distribution of noninvasive samples with genotyping status and location of hair traps (C) Geographic distribution of three haplotypes in forest habitats of 5 management units. A total of 147 samples were collected from Annapurna Conservation Area (ACA). The grey color represents maximum elevation range (< 4,200 m) of Himalayan black bears except Lomanthang unit. Stars indicate the location of hair traps (n = 11) and white circles with black dots indicate samples (n = 97) which were analyzed for this study. The black circles (n = 24), circles with cross (n = 21) and triangles (n = 15) represent the distribution of control region (CR) haplotypes NEP-A1, NEP-A2 and NEP-A3, respectively. (Data source: Shapefiles and topographical maps, survey department, Government of Nepal, http://nationalgeoportal.gov.np; SRTM DEM, the Earth Explorer, https://earthexplorer.usgs.gov)

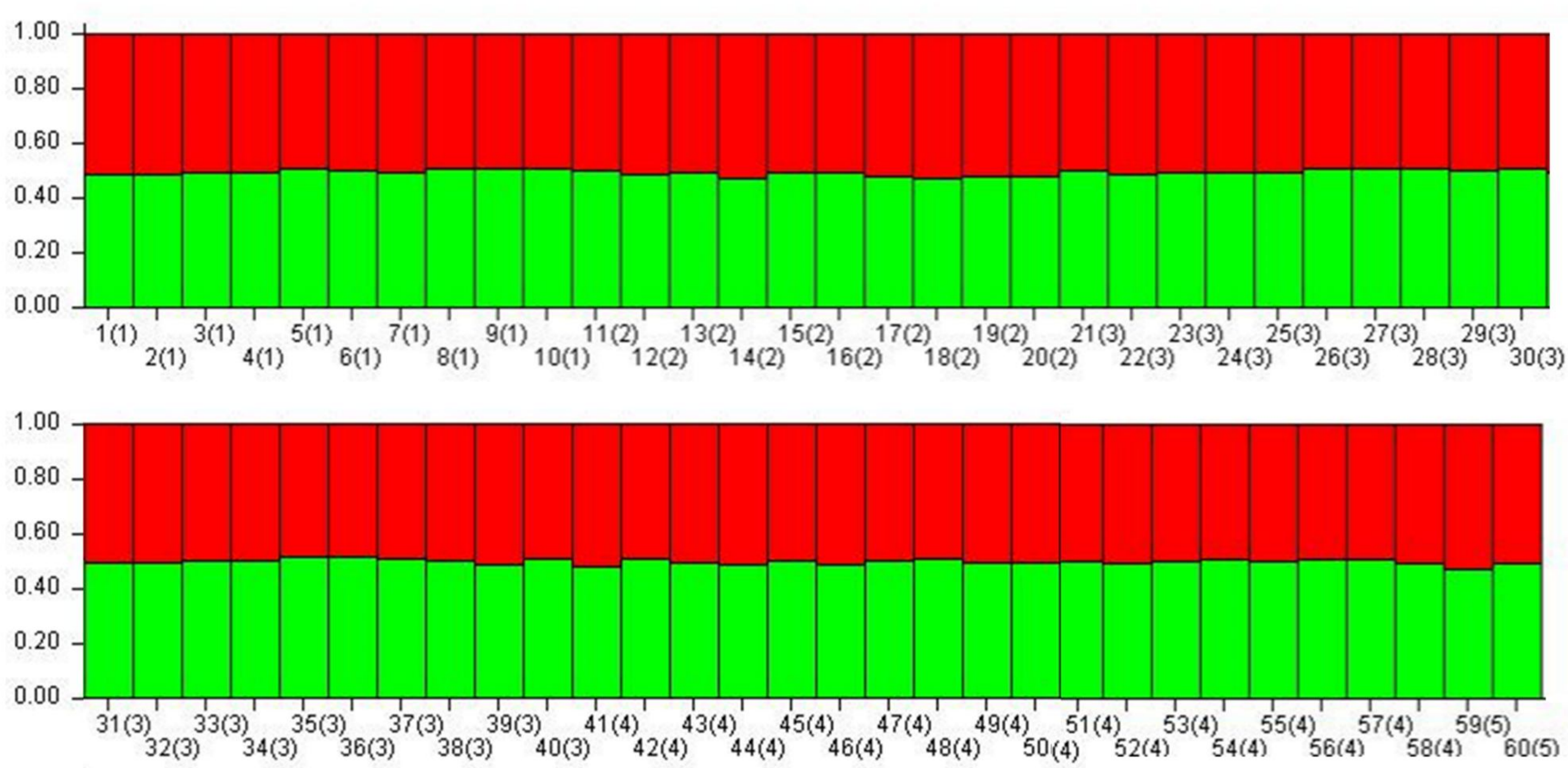


Figure 1-2. Population structure of Himalayan black bears from ACA, Nepal without prior sampling information. Vertical bar represented individual bears and color represented membership coefficient (q) of each individual which was less than 0.7 when K = 2; (1) = Jomsom, (2) = Ghandruk, (3) = Lwang, (4) = Sikes, (5) = Manang management units, and no 1–60 represents the individual identified from microsatellite analysis; Similar patterns were observed with prior information of location model.

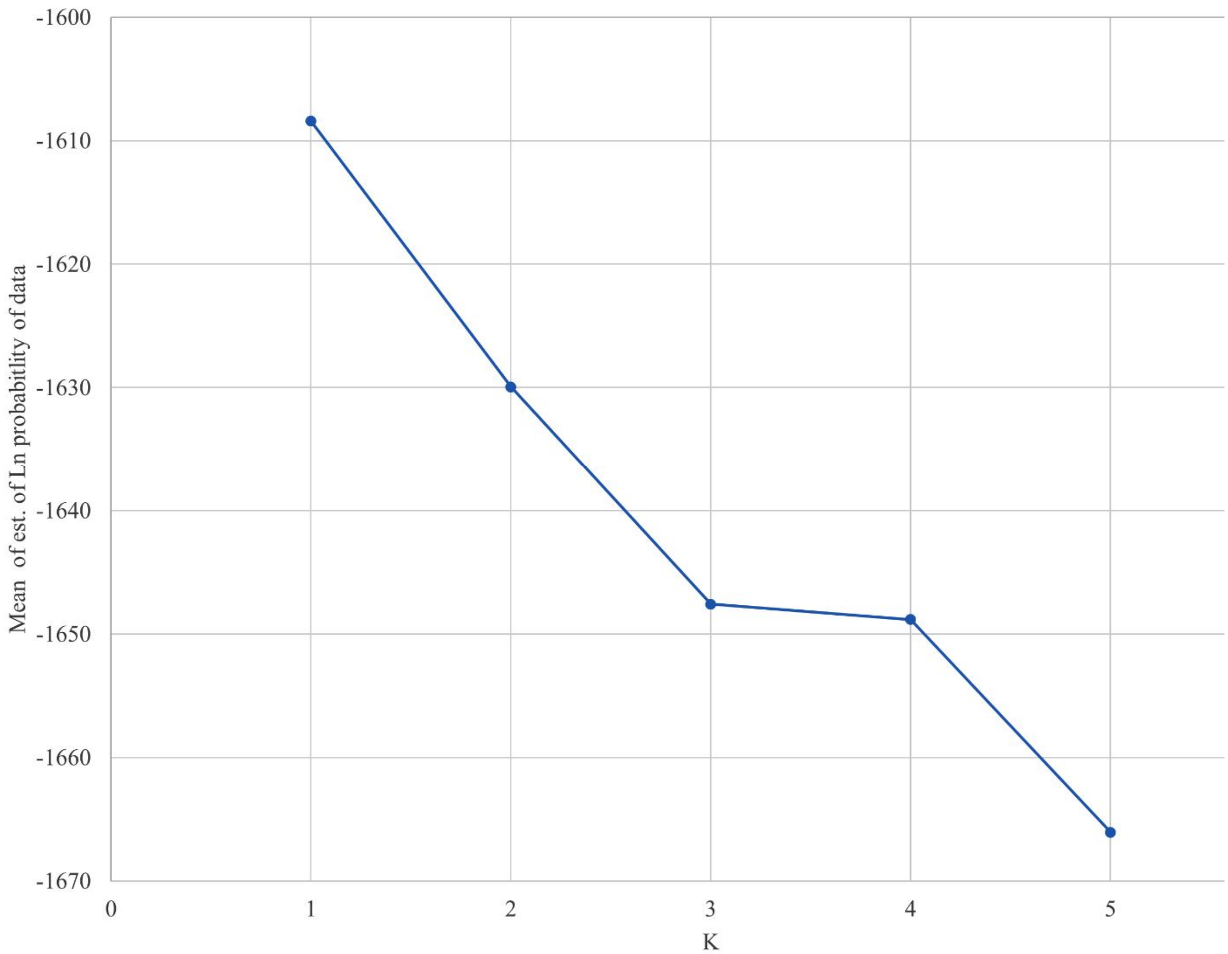


Figure 1-3. Structure results of 60 individuals from 5 different management units of ACA.

The mean of estimated Ln probability of data is higher when population sub cluster K = 1. Y axis values are fixed from -1600 to -1670 for clear presentation of graph.

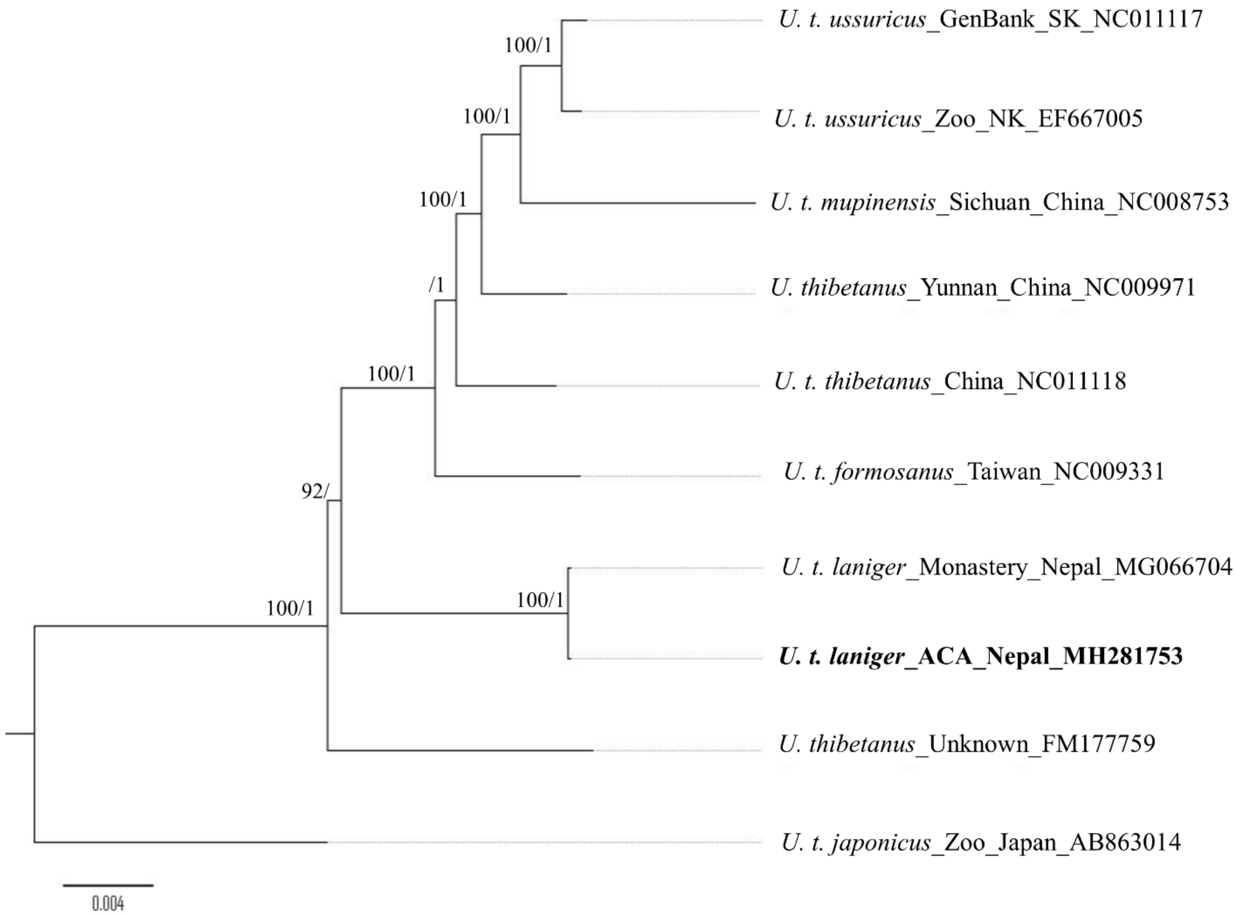


Figure 1-4. Phylogenetic relationships among Asiatic black bears using mitogenomes excluding the VNTR's of the CR (16,363 bp). The number at the nodes of the branches indicate bootstrap supporting values in percentage (BP) and Bayesian posterior probabilities (BPP) based on maximum likelihood and Bayesian analyses, respectively. Identical tree topology was also obtained from 12 mitochondrial protein-coding genes and two rRNA genes (12,663 bp). The mtDNA sequence of *U. t. japonicus* (AB863014) was used as an outgroup. Only bootstrap values over 75% and BPP over 0.95 are shown. Sequences are identified by the subspecies name, origin and country name, followed by the GenBank accession number (NK, North Korea; SK, South Korea). The position of mitogenome haplotypes from *U. t. laniger* generated in this study is shown in bold face.

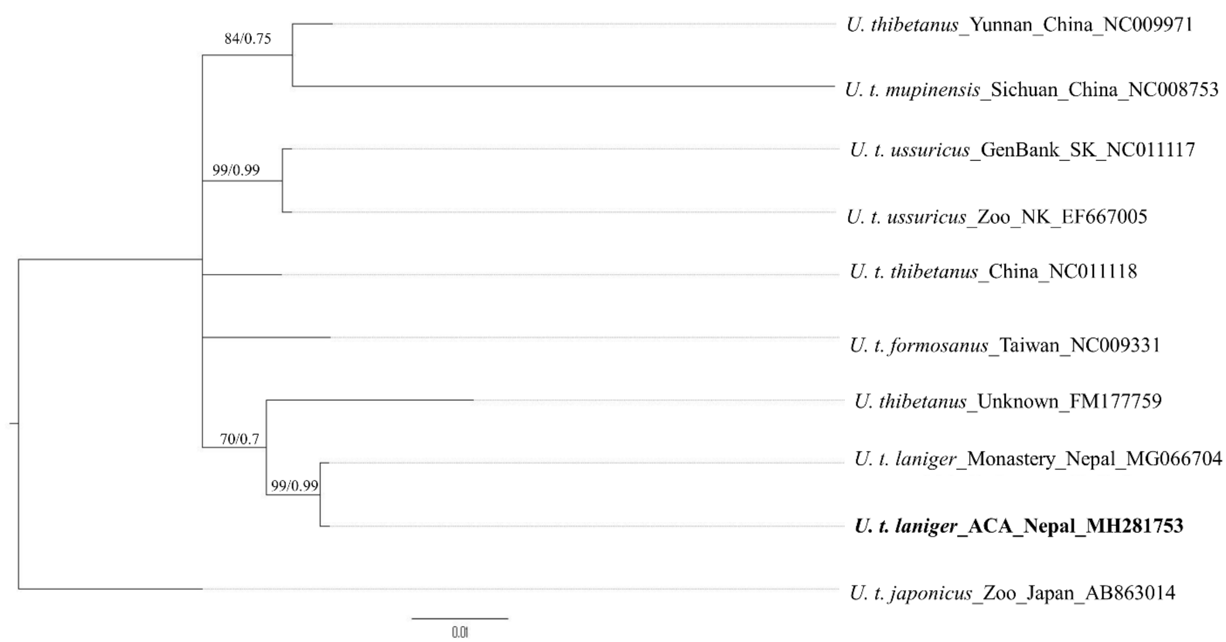


Figure 1-5. Phylogenetic relationship of Asiatic black bears based on the nucleotide sequence of CR (675 bp). The numbers at the nodes of the branches indicate bootstrap supporting values in percentage (BP) and Bayesian posterior probabilities (BPP) based on maximum likelihood and Bayesian analyses, respectively. The mtDNA sequence of *U. t. japonicus* (AB863014) was used as an outgroup. Only bootstrap values over 50% and BPP over 0.5 are shown. Sequences are identified by the subspecies name, origin and country name, followed by the GenBank accession number (NK, North Korea; SK, South Korea). The position of mitogenome haplotypes from *U. t. laniger* generated in this study is shown in bold face.

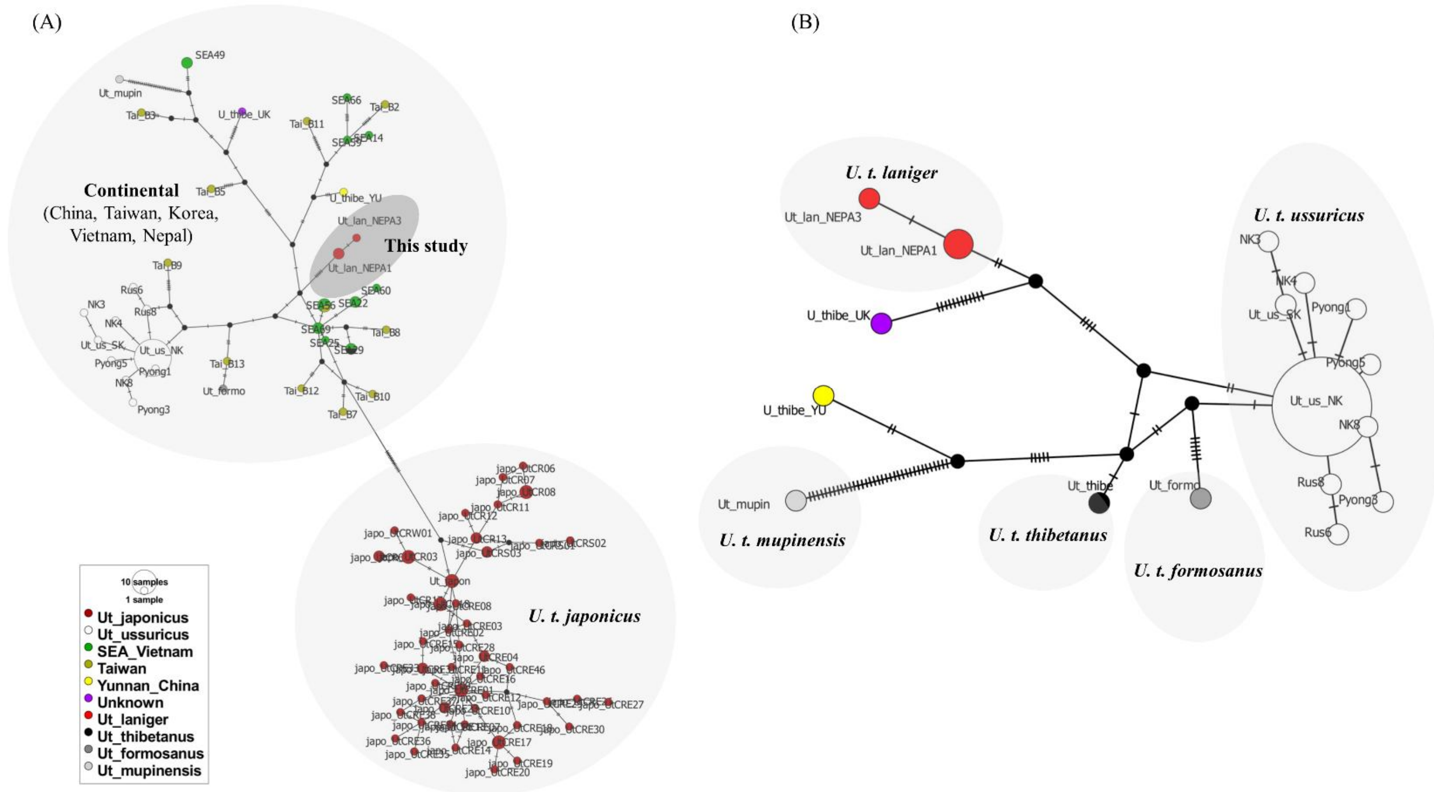


Figure 1-6. Median joining network based on CR haplotypes by using POPART. A total of 523 positions were included in the final analysis after complete deletion. Network tree was generated using all published haplotypes for 6 subspecies of Asiatic black bears, unknown haplotypes from southeast Asia (Vietnam), Taiwan, Yunnan province of China and unknown origin; (A) for 130 haplotypes of Asiatic black bears and (B) based on continental subspecies of 40 haplotypes after excluding the haplotypes from Japan, Taiwan zoo and Vietnam's rescue center. The detail information of each haplotypes is presented in Annex 1.

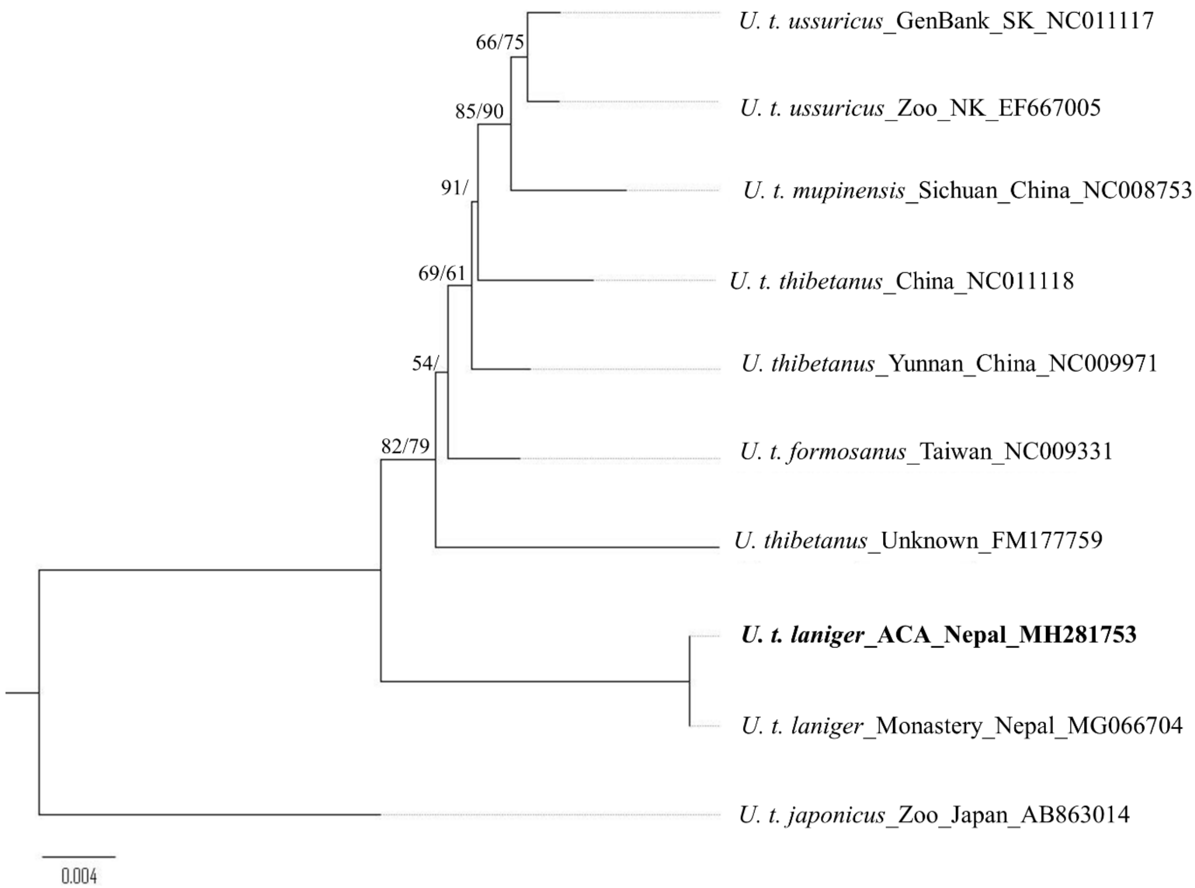


Figure 1-7. Phylogenetic tree based on the complete cytochrome b gene (1,140 bp). The numbers denoted at the node are the bootstrap values based on Neighbor joining/Maximum parsimony methods, respectively. Only the values greater than 50% are shown. Sequences are identified by the subspecies name, origin and country name, followed by the GenBank accession number (NK, North Korea; SK, South Korea). The mitogenome generated in this study is marked in bold face.

CHAPTER II

Seasonal diet and general habitat characteristics of Himalayan black bears in Annapurna Conservation Area of Nepal

Introduction

The Himalayan black bear (*Ursus thibetanus laniger*) is one of three bear species found in Nepal, however; it has never received conservation importance (Jnawali et al. 2011) in the mountain regions of the country. Asiatic black bears which live predominantly in forest areas, especially in hills and mountainous areas, are found over a wide area of south and southeastern Asia, Korea, China, Russia, and Japan (Garshelis and Steinmetz 2016). Separate assessment of Himalayan black bears has not been conducted yet. The Asiatic black bear is listed as vulnerable by IUCN Red List (Garshelis and Steinmetz 2016) and Appendix I of CITES (CITES 2017). Their range overlaps the brown bear (*Ursus arctos isabellinus*) in the western Himalayas however, it does not appear to overlap with the brown bear (*U. a. pruinosus*) in Nepal (Garshelis and Steinmetz 2016).

Information on the habitat uses and food ecology of the black bear in Nepal is still unavailable whereas, studies are available from much of its range countries. Asiatic black bears are opportunistic omnivores. They mainly feed on fruits, and to some extent to vegetation, insect, and animal matter (Schaller et al. 1989, Hwang et al. 2002, Hashimoto 2003, Sathyakumar and

Viswanath 2003, Koike 2010). Food habits of Asiatic black bears differ substantially in geographic range, seasonal availability, palatability, and nutrient content (Hwang et al. 2010, Sharma et al. 2010, Nakajima et al. 2012). Asiatic black bears occupy a variety of forested habitats, both broad-leaved and coniferous and occasionally recorded in open alpine meadows (Sathyakumar et al. 2013). Individual bears seasonally move to different habitats and elevations (Izumiyama and Shiraishi 2004). Knowledge of their food habits is important to understand the bear distribution and habitat use (MacHutchon and Wellwood 2003). Black bears have a major role in the maintenance of healthy ecosystems as they are seed dispersers and predators (Sathyakumar and Viswanath 2003). Their conservation helps in conserving habitats for many other important species of mountainous regions.

The aim of this study was to acquire information about bear seasonal food habit to better understand their ecology. The knowledge may be useful in improving habitat quality in terms of food resources, population management, and reducing human-bear conflict adjacent to bear habitats. It was difficult to collect direct observation data in the mountain terrain. The setting of radio collars is costly and a time burden, so fecal analysis, bear sign survey, and interviews with local herders were conducted to document the seasonal food habits of Himalayan black bears. The information on food items and the occurrence of crops in the bear's food and its extent are helpful for the development of effective conservation plans. Successful conservation of Asiatic black bears critically depends on proper management and protection of bears and their habitat.

Materials and Methods

Study area

This study was conducted in the southern region of Annapurna Conservation Area (ACA), Nepal (Fig. 2-1). ACA's rich biological diversity owes much to its location. The intersection of the two major bioregions between east and west Himalayas supports 22 forest types with 1,352 plant species. The forest region covers an area of 1,160 km^2 which is 15% of the land use of the total area of ACA. The forest is highly diversified from moist subtropical broad-leaved forests to dry conifer forests (1,000–2,000 m), temperate forests (2,000–3,000 m) to subalpine forests (3,000–4,100 m). Dominant plant types within the ACA mostly consist of *Schima-Castanopsis*, *Pinus roxburghii*, *Alnus nepalensis*, *Persea* spp., *Rhododendron* spp., *Aurndinaria* spp., *Lyonia ovalifolia*, *Berberis* spp., *Lindera* spp., *Viburnum* spp., *Daphniphyllum himalayensis*, *Quercus* spp., *Acer* spp., *Mahonia nepaulensis*, *Coriaria nepalensis*, *Prunus* spp., *Myrsine semiserrata*, *Sorbus cuspidata*, *Daphne bholua*, *Pinus wallichiana*, *Taxus baccata*, *Abies spectabilis*, *Betula utilis*, etc. The Annapurna region features an outstanding variety of wildlife that includes 23 species of amphibians, 41 species of reptiles, 488 species of birds, and 105 species of mammals (NTNC 2017). The seasonal climate is dominated by the southerly monsoon occurring between June and September. The southern Annapurna region with 3,000 mm of annual rainfall, has the highest precipitation in the country, whereas the Trans-Himalayan region has average annual rainfall of 193 mm (NTNC 2017). The elevation ranges from under 1,000 m to over 8,000 m.

Sign survey

Bear sign surveys were carried out by selecting five management units (MUs) in ACA. In each unit, a minimum of four to a maximum of eleven sign surveys (transects) were laid in forest areas, based on accessibility, habitat type and geographic representation in each unit. The average length of the transect was 3.4 km, ranged from 0.9 km to 7 km, and covered altogether 116.4 km ground efforts in the ACA forest. Sign surveys were organized along animal or human trails, up-step ridge structures, and up and down hillsides, to cover the wide range of topographical variation and different habitats of Himalayan black bears. Thirty four surveys were organized between October and December, of which, 24 surveys in 2015 and 10 surveys in 2016. Surveys could not organize during the monsoon season (July through September). Feces surveys were quite difficult during the monsoon season due to increased vegetation cover, lower scat visibility, frequent heavy rainfall, and floods (Huygens et al. 2003). Available feces were opportunistically collected from farmland and surrounding areas to analyze monsoon diets. Different types of bear signs were recorded that consisted of feces, climbed trees (claw marks, platforms, broken branches, hairs), insect foraging (ground diggings, broken logs, raided bees nests), ground markings (foot prints, resting place, feeding sign), and carrion debris. The claw marks and hair on platforms or broken branches were considered a single sign. All recorded signs were aged as very fresh (≤ 7 days), fresh (8–30 days), old (1–3 months) and very old (> 3 months). The age of sings was visually estimated based on freshness, color, the presence of leaves, and fungus in the marks. The fecal age was additionally estimated following the level of decomposition, moisture content, the actual condition of food items, comparison with other feces, the status of foraging signs, and the local herder experiences. Trees or shrubs found with signs were identified to the lowest possible taxon, and DBH (diameter at breast height), height, and platform height were measured. All bear signs were noted; these were habitat types, dominant plant species, availability of wild fruits, GPS location, elevation, forest age (< 30 years, 30–100 years, and > 100 years), canopy cover, habitat condition (visibility) and conservation threats such as grazing, human presence, firewood collection, fodder lopping, log extraction and fire. In transect, additional plant information such as suspected bear food items, phenological stage, and status of the ground mast were recorded. Local herders were asked for the detailed descriptions of bear food plants, their fruiting phenology and other food items of bears. Fruit production was subjectively rated as frequent (high), sporadic (moderate), or rare (low), based on the amount of fruit observed on trees/bushes and grounds. Bear feeding signs could not identify on ground grasses because many wildlife and livestock consumed these grasses. The bear signs, which were recorded near to each other, were regrouped for analysis of habitat characteristics and conservation threats.

Fecal analysis

In the study area, the Himalayan black bear is the largest wild mammal, so feces were easily identified on the basis of size, odor, color, and content of foods. Additional fresh bear signs around the feces also assisted in the confirmation of bear feces. Feces found in the field were carefully identified, collected with caution to avoid ground debris and placed in plastic bags. While collecting feces, plastic bags were labeled with GPS location, estimated age, collector name, date, and ID. Feces were sun-dried and stored properly in a dry place if they were not processed immediately. Feces were washed in running water through 2-mm and 1-mm mesh sieves to separate individual food items. Undigested food remains were identified to species level by referring to plant/seed samples collected from the study area. Some items were not identified in the lower taxonomy of plant fragments and animal matter due to the lack of sufficient references, and, in some cases, such diet items constituted in trace amounts. The non-food items (bear hairs, stones, soils, and woody debris) were not included in the analysis. Plant materials were classified into three categories: wild fruits, vegetation and agricultural crops; and two other categories insects and animals. The relative volume of each food item was visually estimated and assigned an exact volume because most of the feces contained only 2–3 food items. Items found in trace amounts were given an arbitrary volume of 1–2%. The percent frequency of occurrence (PFO) and percent volume (PV) of each food item was determined. The percent frequency of occurrence (PFO) for each food item is the proportion of all feces collected that contain particular food items: PFO = (Frequency of item/Total number of feces) × 100, where Frequency of item = Number of feces containing the same item. The formula for Percent Volume is (PV) = Total percent volume of item/ Total number of feces. In this study, food items of feces were examined for six months (July-December), and these variation on monsoon (July-September) and autumn (October-December) season. The monsoon season data were biased, collecting agricultural land and surroundings, but seemed helpful to understand general trends of diet in monsoon season. A potential bias of the sampling design was possible oversampling in areas where feces were readily observed and under sampling where feces were less obvious. Biased hard mast bear feces collection is common with Asiatic black bear feces surveys in general (Hwang 2002, Hashimoto et al. 2003). So, only one fecal sample was collected from one location to avoid over-representation. The specific habitat parameters and seasonal diet variations were determined using the chi-square ($\chi 2$) test. The land use map and management units of the study area were used in ArcMap version 10.0 (ESRI, Inc.) to prepare the distribution map of black bears.

Results

Distribution and habitat characteristics of Himalayan black bears

On average three signs were observed per kilometer whereas in total 345 (ranged, 1–30) bear signs were found in 85% (n = 29) transects (Table 2-1). Of which, 314 fresh signs (≤ 30 days) were found in 82% (n = 28) transects in five MUs in ACA, but could not find any signs on transect laid in pure coniferous forests, and fresh signs in an additional five transects of mixed broad-leaved forests where fruits were not available during the survey period. Besides, 71 bear feces were opportunistically collected from different agricultural land (n = 14 sites) (Fig. 2-1) during monsoon and early autumn season. In total, 416 bear signs, of which 63% feces, 20% climbed trees, 11% insect foraging, and 6% ground mark were recorded (Table 2-2). It could not find any bear rubbing trees in ACA. Black bears were well distributed in six management units of ACA (Fig 1). Plant species such as *Quercus* spp. (55%), *Arundinaria* spp. (11%) and *Rhododendron* spp. (8%) were found to be dominant in bear habitat. Other common plant species were *Lindera* spp., *Symplocos ramosissima, Berberis* spp., *Juglans regia, Sorbus cuspidate, Rhus wallichi, Daphniphyllum himalayensis, Pinus wallichiana, Cornus capitate, Ilex dipyrena, Betula alnoides*, and *Cotoneaster frigidus.* Additionally, black bears generally inhabited dense bamboo understory forest (32%). The presence of rhesus macaque (*Macaca mulatta),* langur *(Semnopithecus entellus),* Himalyan serow *(Capricornis sumatraensis),* Himalayan goral (*Naemorhedus goral),* barking deer *(Muntiacus muntjac),* common leopard (*Panthera pardus),* marten (*Martes flavigula),* and jungle cat (*Felis chaus)* were recorded in the bear habitat.

Occurrence of bear sign was highest in mixed board-leaved forest (77%), followed by mixed coniferous forest (14%) and agricultural land (9%) (χ^2 = 86; df = 2; p < .001). Black bears mainly used old age forest (94% in > 100 years old forest), higher canopy cover (66% in > 60% crown cover), and no association with the visibility of forest ($\chi^2 = 0.77$; df = 2; p = 0.68) (Table 2-3). Although a survey was conducted in the elevation range 1,250–3,650 m, bear signs were recorded in the elevation range 1,680–3,580 m. The maximum signs (61%) were found in the range of 2,000–2,500 m ($\chi^2 = 80$; df = 3; $p < .001$) (Table 2-3). Below 2,000 m, most signs were found in mixed broad-leaved forests, whereas above 3,000 m they were found in mixed coniferous forest with respect to the availability of these forests in this elevation range. The frequent livestock grazing, fodder looping, and firewood collection were recorded near the village whereas the intensity was observed to be decreased far from village except livestock grazing, which was common in all area of ACA. Anthropogenic characteristics such as grazing (59%), human walk (29%), fodder lopping (19%), firewood collection (16%), and logging (5%) overlapped in resources used by bears, although the intensity was not severe in many locations.

Determining bear diets

The 261 feces (monsoon = 68 (26%), autumn = 193 (74%)), which were collected from six MUs in ACA, were analyzed. Of these, 190 feces were systematically collected from bear forest habitats, and 71 feces were opportunistically collected from crop fields and their surroundings. The largest percentage of feces (63%, n = 165) was identified as very fresh (≤ 7 days) to a minimum (2.7%, n = 7) in the old category (1–3 months). In terms of land use, 166 (64%), 24 (9%) and 71 (27%) feces were found in mixed broad-leaved forests, mixed coniferous forest, and agricultural land, respectively. Individual feces contained one to four food items, where 66% had only one item, 26% had two items, 7% had three items, and only 1% had four items (mean = 1.43, SD = 0.67). Identified food items from feces included five categories: wild fruits (twenty species), crops (four species), vegetations (three species and fragments), insects (two species) and animal matters (hairs and bones) (Table 2-4). No evidence of conifer tree species was discovered in bears diet. The PFO of wild fruits was 68.6%, followed by vegetation, agricultural crops, animal, and insect with 27.2%, 24.9%, 4.6%, and 3.8%, respectively in six months (July-December). A similar composition was found in percent volume (PV) of food items ($p = 0.19$) where wild fruits, crops, and vegetation were 62%, 22.4%, and 14.3%, respectively (Fig. 2-2). The Himalayan bamboo shoot was found in 11.1% feces (PV = 5.3%) whereas *Arisaema* spp. was found in 8% of collected feces (PV = 5.5%) during the entire period of six months. Bamboos and *Arisaema* spp. were major items during a food deficient period. The whole part of *Arisaema* spp. (fruits, leaves, and rhizomes) was recorded in feces when fresh shoots of bamboo were found. The fragments of plants found in 10.3% feces (PV = 3.5%), which was a small amount in most of the feces, could not identify. The evidence of mammal hair/bone occurrences were found in 12 samples but were unable to identify species. A significant difference in the diet of black bears was observed among the seasons ($P < 0.001$).

In monsoon (July-September), agricultural crops (maize, rice, and apple), vegetation (*Arundinaria* spp., *Arisaema* spp., and fragments), and wild fruits (*Ficus auriculate*, *Juglans regia*, and *Prunus persica*) were recorded food items. The diet was dominated by crops (85.3%), followed by vegetation (36.8%), and wild fruits (5.9%). No feces of insects and 1.5% of animal hairs were recorded. The PV of crops, vegetation, and wild fruits were 76.4%, 21.9%, and 1.8%, respectively. Majority of feces (PFO = 63.2%, PV = 58.4%) predominantly contained maize, followed by the shoots of Himalayan bamboos (PFO = 19.1%, PV = 10%) and *Arisaema* spp. (PFO = 16.2%, PV = 9%).

In autumn (October-December), four species of oak were consumed by bears: *Lithocarpus elegans, Q. lamellose, Q. glauca*, and *Q. incana*. Wild fruit (eighteen items), crops (three items), vegetation (two items and fragments), insects (two items), and animal matter of unknown species were recorded. The PFO of wild fruits (hard/soft mast), vegetations, animals, insects and crops were 90.7%, 23.8%, 5.7%, 5.2%, and 3.6% (Table 2-4) and similar trend in calculation of PV 83.2%, 11.6%, 1.2%, 0.6%, and 3.5%, respectively ($p = 0.11$). Oaks (PFO = 43.7%, PV = 53%) were dominant food items, whereas some wild fruits (S*orbus microphylla*, *Cotoneaster frigidus*, *Juglans regia*, *Lindera pulcherrima*, *Lindera* spp., *Cornus capitate,* and unidentified seeds), crops (buckwheat, apple), and insects (beetle, wasp) were less frequently detected (PV < 1%) in the bear diet.

Feeding sign and availability of bear food

It was found that > 134 occurrences of bear feeding signs on transects, including 22 species of wild fruits, three species of vegetation, four items of insects, three species of crops, and two animal carrions (Annex 3). Most of the climbed trees were observed in mixed board-leaved forests (75%, n = 63), followed by mixed coniferous forests (25%, n = 21). Bear signs were not recorded in conifer tree species. Feeding signs were primarily found on oak trees (50%), where several nest structures (platform) were observed in many single trees. The DBH and height of climbed trees ranged from 5 to 170 cm (mean = 51.7, SD = 33.8) and 4 to 70 m (mean = 31.5, SD = 18.2), respectively. The platform height was recorded from 3 to 60 m (mean = 26.7, SD = 16.6). Under the climbed tree, there was an ample amount of fallen nuts and fecal piles (up to 15 numbers) observed. Bear signs on *Berberis aristate* were not observed, although it was a common berry tree in transects. The parts of *Maesa chisia* consumed by bears could not identify. In addition, beetles were found in 24 broken logs, hornet/wasps in 19 ground holes, a single case of bumble bees, a raided bee nest, and two animal (ox and mule) carcasses. It supports the bear preference of insects in mixed broad-leafed forests during the autumn season. Many cases of crop damage especially maize, apple, and rice were observed in the monsoon season. Additional 35 lists of bear food items were recorded from the local herders, which were not recorded in feces analysis and feeding sign surveys, of which, 28 species were wild fruits, five species were vegetation, and wo species were crops (Annex 3).

Based on the bear feces analysis, feeding signs, and interviews with local herders, 76 food items were recorded including 57 species of wild fruits, eight species of vegetation, four species of insects, six species of agricultural crops, and a few occurrences of animal matter. Availability varied in different management units (Annex 3). The availability of plant species was higher in autumn (n = 36), followed by spring (n = 17) and monsoon (n = 13) seasons (χ^2 = 13.7; df = 2; $p < .001$), but no plant food was recorded for the winter season. Except for *Arisaema* spp. and *Arundinaria* spp., the availability of bear food plants in mixed coniferous forests during monsoon season was very rare.

Discussion

Habitat use by Himalayan black bears

This study revealed that the Himalayan black bear shared habitats with grazing livestock and the occasional human presence, as such disturbances were found in almost all bear habitats to some extent. Bears were abundantly observed in old age forests, in forests with high canopy cover, and in areas with a relatively high abundance of fruit trees and their availability. Previous studies reported similar results in terms of availability of fruit trees and fruiting (Steinmetz et al. 2011, Sathyakumar et al. 2013). Bear presence was frequently found in forests having bamboo as a dominant understory species where the probability of human presence is higher for collecting bamboo shoots, sticks, and fodder. It may lead to increased incidences of bear/human encounters. During a food shortage period, black bears widely disperse in search of food towards croplands which also lead to frequent human encounters (Johnsingh 2003, Sathyakumar and Choudhury 2007, Sathyakumar et al. 2013). Himalayan black bears preferred mixed broad-leaved, particularly *Quercus* dominant forests in ACA having a variety of foods and high abundance, which showed a similar forest habitat in Manaslu Conservation Area (Chhetri 2013). Both coniferous and broad-leaved forests are common in the study area, but more coverage were organized in the latter category during the field survey. Any signs were not found in the pure coniferous forest, nor any conifer tree species were listed as foods of Himalayan black bears. Rare cases of *Pinus* seed were recorded in Japan when other masts were not available (Huygens et al. 2003). Coniferous forests, although not clearly defined as either mixed or pure, is reported as a more suitable habitat than broad-leaved forests in the Makau Barun National Park (Bista et al. 2018), where coniferous forests coverage is available three times more than broad-leaved forests. Bears were found in an elevation range of 1,680–3,582 m, where it was previously reported in the elevation range of 1,600–3,200 m (Bista and Aryal 2013) in the southeastern region of ACA. It was reported in forested habitats of Indian Himalayas up to 4,300 m (Sathyakumar et al. 2013) bear might be distributed in upper elevation ranges; however, this study was confined 1,256–3,647 m.

Food habit in monsoon

Fecal analysis has been widely used for the dietary composition of Asiatic black bears, but some variation on analytical methods occurred in its range countries. The Himalayan black bears were found as omnivores as previously reported other Asiatic black bears: feeds were mainly on fruits, crops, and vegetation of plant species. A small amount of animal matter and insects were also recorded, which vary from season and location (Hwang et al. 2002, Huygens et al. 2003, Steinmetz et al. 2011, Sathyakumar et al. 2013). Fecal analysis revealed that a severe amount of maize was observed in bear diet during crop harvesting time in ACA. Results in monsoon were based on opportunistically collected feces from cropland and surroundings, probably biased to adequately document bear diet. The composition might change if feces were collected systematically, but it is hard to organize surveys in monsoon due to heavy rain, big floods, and dense forest. Similarly, heavy rain immediately washes most parts of feces, so it cannot detect. Feces are difficult to see in dense forests, and there are high chances of decomposition of feces mostly composed of green vegetation (Hwang et al. 2002, Huygens et al. 2003). Fresh succulent plants and grasses were common in monsoon, and the limited access on wild fruits shifted bears to maize, probably a supply of high energy. In ACA, the available twelve varieties of *Arisaema* spp. (NTNC 2017) and eight varieties of bamboo (this study) seem to be major alternative food sources when other fruits are not available. Black bears tend to rely on more green vegetation, agricultural crops, or riskier foods when food becomes scarce (Hwang et al. 2002, Sathyakumar et al. 2013). Bears showed hyperphagia in both the monsoon and autumn for key foods available in particular locations, as most of the feces contained only one food item. The crop damage was prominent in two MUs where the surrounding forests are dominated by pure coniferous or mixed coniferous forests.

Food habit in autumn

In ACA, fruits were the most preferred food item in the autumn, which were higher than mountain protected areas of northwestern India (Sathyakumar et al. 2013). Black bears particularly consumed *Quercus* acorns according to their relative availability during autumn which is almost a similar trend in range countries (Hwang et al. 2002, Koike 2010, Sathyakumar et al. 2013). The fruiting time of acorns (*Quercus* spp.) and millet crops were same in ACA, but any millet traces were not found in the feces. There was no report on millet depredation, either. The relative importance of wild fruits in bear feces during autumn is seasonal fruiting of many fruit trees and abundance. This study also supports that Himalayan black bears feed according to opportunity and availability of food (Sathyakumar and Viswanath 2003). Acorns are relatively high in fat and carbohydrates which black bears prefer before hibernation in the temperate forest of its range (Schaller 1969, Hashimoto et al. 2003, Sathyakumar et al. 2013). Previous studies showed that Asiatic black bears shifted from leafy material in the early summer diet to fleshy fruits and then to fat rich fruits before hibernation (Manjrekar 1989, Schaller et al. 1989, Hwang et al. 2002, Sathyakumar and Viswanath 2003). They tend to prefer fat based food (oak and insects) in autumn but use other food depending on the condition.

Animal matter and insects have been found particularly during autumn in smaller quantities and were reported in all bear range countries. Significant portions of broken logs and grounds holes were mostly associated with beetle and wasp/hornet, respectively, in this study. The contributions of animal and insect materials are mostly underestimated in fecal analyses as these materials are more easily digested (Hewitt and Robbins 1996). Some feces in ACA contained animal matter, which, was supported by sign survey and local herder observation. The common wildlife of ACA: rhesus macaque (*M. mulatta)*, langur *(S. entellus)*, Himalayan serow *(C. sumatraensis)*, Himalayan goral (*N. goral)*, barking deer *(M. muntjac)* were not confirmed in bear diet, whereas Hanglu deer (*Cervus elaphus hanglu*), langur (*S. ajax*), takin (*Budorcas taxicolor*), bamboo rat (*Rhizomys sinense*), Chinese muntjac (*M. reevesi*), serow (*N. swinhoei*), and Japanese serow (*C. crispus*) were reported in other bear range countries (Schaller et al. 1989, Hwang et al. 2002, Hugens et al. 2003, Trent 2010, Sathyakumar et al. 2013). Many soft lying logs were home for beetles and easily broken after softening during monsoon. Black bears prefer to eat insects when they found them because these insects are nutritious (Beck and Beck 1955).

No information is available on bear food, even from herders' information, and its movement during the winter season, but perpetually falling acorns were reported in the southern part of ACA in late autumn, which may be a probable food item for winter. Black bears were reported to feed on fallen acorn (Schaller 1969, Hwang et al. 2002, Satyakurmar and Viswanath 2003). Although the hibernation of Himalayan black bears from Nepal is unknown, they undergo hibernation in the western Himalayas to avoid harsh winter (Prater 1971, Sathykurma et al. 2013), whereas in the eastern Himalayas black bears remain active year-round (Roberts 1977). Further studies are needed to know whether Himalayan black bears either go for hibernation in high elevations or shifting toward downhill to escape cold winter.

Conservation of Himalaya black bears

Bamboo subspecies are varied in geographic locations and elevations, have been traditionally exploited by local communities to support their livelihoods for multiple uses (Panthi et al. 2017). Except for bamboo, other bear food items did not seem to be extracted by local people, whereas, sporadic looping of oak trees for fodder was observed. The community-based conservation seems effective for the protection of Himalayan black bear habitat. Although a ban on the collection of timbers and fuelwoods from standing trees was observed as common practices in the conservation area, the livestock grazing, i.e. release of unproductive buffaloes, ox and mule year-round in the forests, halting of sheep/goats in November and May while shifting the herds in a traditional rotational grazing system, and altitudinal shifting of buffaloes/cow herds occurred in most of the bear habitats. Contrary to the expectation, bear presence seemed to be highly co-existed with livestock grazing, perhaps due to temporal differences in foraging of livestock and bears. The conservation awareness, planting of fuelwood species, and the provision of alternative energy resources has contributed to the reduction in fuel wood collection in ACA (Bajracharya et al. 2005). CAMCs have regulated the commercial harvesting of bamboo sticks and shoots, to some extent having extracted a block system using a rotation, but cannot avoid future excessive extraction due to the growing demand for these products. Bamboo extraction, if not regulated properly, can be a major long-lasting threat for bear survival. Other anthropogenic disturbances are relatively low in ACA due to conservation initiatives by CAMC and ACAP, high migrations to nearby cities, shifts from agricultural practices to other jobs, and low level of infrastructures as most of the forests are located at the end of human settlements. In recent years, many farmlands have been abandoned, after migration from the rural areas. These factors have widened the habitat of the Himalayan back bears, leading to an increase in the numbers of bears visiting farmlands. The Wildlife Damage Compensation Relief Guideline (2013) has allocated monetary provision for human causalities; however, existing policies are not considering bears to minimize crop damage. Even ACAP could not make any provision for a direct compensation program to offset the economic burden of bear damage. The implementation of community-based crop damage compensation system, bear based eco-tourism promotion and adoption of alternative crops are recommended to resolve the crop damage by bears. These interventions may extend the favorable environment for the future maintenance of bear populations. Long-term studies are also recommended to adequately report the food habits and habitat use of Himalayan black bears, and complete understanding of seasonal variation.

Conclusions

This study suggested that Himalayan black bears are omnivores and preferred the food items based on its availability. The acorns and agricultural crops are major food items in autumn and monsoon season, respectively. The vegetation, especially bamboos and *Arisaema* spp. are major accompanying foods during both seasons. Although crucial information was identified on monsoon diet, the contribution of items might be different if systematic transect survey could organize in the dense forest. As contrary to expectation, bear seemed to be co-existed with anthropogenic disturbances, especially with grazing livestock in the mountain forest. The mixed broad-leaved forests are preferred habitat in the ACA. Intensive field survey in all seasons are recommended for detail evaluation of seasonal changes in bear diet, habitat use and conservation threats.

Table 2-1. General descriptions of sign surveys (transects) and bear signs.

MUs	Total transect (presence)	Total length (km)	Elevation range (m)	Total signs (fresh signs)	Sign /km	Sign location (n)	Forest types	Fruit availability	Feces (Ag sites)
Jomsom	5 (4)	8.9	2168–3120	32 (26)	4	17	MC, C	Sporadic	38 (5)
Ghandruk	11 (8)	45.8	1787–3647	61 (54)	1	25	MBL, MC	Rare	12 (2)
Lwang	6 (6)	20.0	1256–2467	96 (95)	5	43	MBL	Frequent	4 (1)
Sikles	8 (8)	28.8	1422–2782	151 (135)	5	53	MBL	Frequent	6 (1)
Bhujung	4 (3)	12.8	1705–2843	5 (4)		5	MBL	Very rare	
Manang			1688–2633						11 (5)
Total	34 (29)	116.4	1256–3647	345 (314)	3	143			71 (14)

MC, Mixed coniferous forest; C, coniferous forest; MBL, Mixed broad-leaved forest; Ag, Agricultural

Table 2-2. Different signs of Himalayan black bears recorded in transects (n = 34) and opportunistic collected from agricultural land (n = 14) in Annapurna Conservation Area, 2015-2016.

Sign category	Likely food	No of signs		Season		Habitat types		
		Total (%)	Fresh (%)	Monsoon	Autumn	Broad-leaved	Mixed coniferous	Ag
Climbed tree	Fruit	84 (20%)	65 (78%)	3	79	63	21	
Ground mark	Grass	24* (6%)	24 (100%)		24	22	2	
Insects foraging	Beetle, wasp, bee	45 (11%)	41 (91%)		45	45		
Carrion debris	ox, mule	2	1 (50%)		2	2		
Feces#	All	261 (63%)	254 (97%)	68	193	166	24	71
Total		416	385 (93%)	71	343	298	47	71

* Feeding sign on grasses and *Arisaema* spp.; #71 feces were opportunistically collected from agricultural land; Fresh indicates a month old age sign.

Table 2-3. Habitat characteristics of Himalayan black bears in ACA.

Habitat parameters	Total no of sign	Percent of bear sign
a. Habitat types (n = 157)		
Mixed broad-leaved forest	121	77
Mixed coniferous forest	22	14
Agricultural land	14	9
b. Canopy cover (n = 143)		
Low crown cover (< 30%)	5	3
Normal crown cover (30–60%)	44	31
High crown cover (> 60%)	94	66
c. Forest ages (n = 143)		
0–30 years		
31–100 years	9	6
Old growth forest (> 100 years)	134	94
d. Visibility of forest (n = 143, p = 0.68		
Sparse (> 60% visibility)	49	34
Normal (30–60% visibility)	52	36
Dense (< 30% visibility)	42	29
e. Elevation range (n = 157)		
< 2000 m elevation	14	9
2000–2500 m elevation	95	61
2501–3000 m elevation	43	27
> 3000 m elevation	5	3
f. Conservation threats (n = 143)		

Habitat parameters	Total no of sign	Percent of bear sign
Grazing	84	59
Human presences	29	20
Fodder collection	19	13
Firewood collection	16	11
Log extraction	5	3
Fire occurrence	2	1
g. Dominant plant species (n = 143)		
Quercus spp.	79	55
Rhododendron spp.	16	11
Arundinaria spp.	12	8
Lindera spp.	8	6
Symplocos ramosissima	7	5
Berberis spp.	5	3
Juglans regia	3	2
Sorbus cuspidate	3	2
Rhus wallichi	2	1
Daphniphyllum himalayensis	2	1
Pinus wallichiana	2	1
Cornus capitate	1	1
Ilex dipyrena	1	1
Betula alnoides	1	1
Cotoneaster frigidus	1	1

Table 2-4. Percent frequency of occurrence (PFO) and percent volume (PV) of food items of half year (July-December), monsoon (July-September) and autumn (October-December) in Annapurna Conservation Area, Nepal.

Food items (No)	July-December (n = 261)*			July-September (n = 68)**			October-December (n = 193)***		
	F	PFO	PV	F	PFO	PV	F	PFO	PV
Wild fruit (20)	**179**	**68.6**	**62.0**	**4**	**5.9**	**1.8**	**175**	**90.7**	**83.2**
Small leaf rowan (*Sorbus microphylla*)	1	0.4	0.4				1	0.5	0.5
Tree cotoneaster (*Cotoneaster frigidus*)	2	0.8	0.6				2	1.0	0.8
Indian barberry (*Berberis aristata*)	7	2.7	2.1				7	3.6	2.8
Oak (*Lithocarpus elegans*)	2	0.8	0.8				2	1.0	1.0
Oak-bull (*Quercus lamellosa*)	64	24.5	22.0				64	33.2	29.7
Oak-ring cupped (*Quercus glauca*)	7	2.7	2.5				7	3.6	3.3
Oak-woolly-leaved (*Quercus incana/lanata*)	41	15.7	13.9				41	21.2	18.9
Fig tree (*Ficus auriculata*)	1	0.4	0.4	1	1.5	1.5			
†Denga (*Sorbus cuspidate)*	20	7.7	5.4				20	10.4	7.3
Walnut (*Juglans regia*)	2	0.8	0.0	1	1.5	0.1	1	0.5	0.0
Peach-wild (*Prunus persica*)	2	0.8	0.0	2	2.9	0.1			
Himalayan bird cherry (*Prunus cornuta)*	16	6.1	3.6				16	8.3	4.8
†Sisi (*Lindera pulcherrima*)	3	1.1	0.6				3	1.6	0.8
†Sibas (*Lindera* spp.)	7	2.7	2.7				7	3.6	3.6
†Dabdabe (*Symplocos ramosissima*)	6	2.3	1.1				6	3.1	1.5
†Lokar (*Lindera* spp.)	3	1.1	0.5				3	1.6	0.6
Himalayan Holly (*Ilex dipyrena*)	12	4.6	4.0				12	6.2	5.3
†Goban (*Saurauia napaulensis*)	3	1.1	0.8				3	1.6	1.1
Dogwood (*Cornus capitata*)	1	0.4	0.0				1	0.5	0.0
#Unidentified (NA); one species	8	3.1	0.7				8	4.1	1.0
Agricultural crop (4)	**65**	**24.9**	**22.4**	**58**	**85.3**	**76.4**	**7**	**3.6**	**3.5**
Buck wehat (*Fagopyrum tataricum*)	3	1.1	0.7				3	1.6	0.9
Maize (*Zea mays*)	46	17.6	16.4	43	63.2	58.4	3	1.6	1.6
Rice (*Oryza sativa*)	4	1.5	0.9	4	5.9	3.4			
Apple (*Malus pumila*)	13	5.0	4.5	11	16.2	14.6	2	1.0	1.0
Animal (UK)	**12**	**4.6**	**0.9**	**1**	**1.5**	**0.0**	**11**	**5.7**	**1.2**
^Hair and bones	12	4.6	0.9	1	1.5	0.0	11	5.7	1.2
Insect (2)	**10**	**3.8**	**0.4**				**10**	**5.2**	**0.6**
Bettle (Coleoptera)	9	3.4	0.2				9	4.7	0.2
Wasp (Vespidae)	1	0.4	0.2				1	0.5	0.3
Vegetation (2, UK)	**71**	**27.2**	**14.3**	**25**	**36.8**	**21.9**	**46**	**23.8**	**11.6**
Himalayan bamboo (*Arundinaria* spp.)	29	11.1	5.3	13	19.1	10.0	16	8.3	3.6
Cobra lily (*Arisaema* spp.)	21	8.0	5.5	11	16.2	9.0	10	5.2	4.2
^Fragment of plants (Graminoid)	27	10.3	3.5	3	4.4	2.9	24	12.4	3.8

F = Frequency; PFO = Percent frequency of occurrence; PV = Percent of volume; †native name; No significant different between PFO and PV (*P = 0.19; **P = 0.38; ***p = 0.11); UK, Unknown

#All seeds of 8 samples belonged to same species but could not match with references.

^Could not identify the species level due to lack of reference materials for micro-histology.

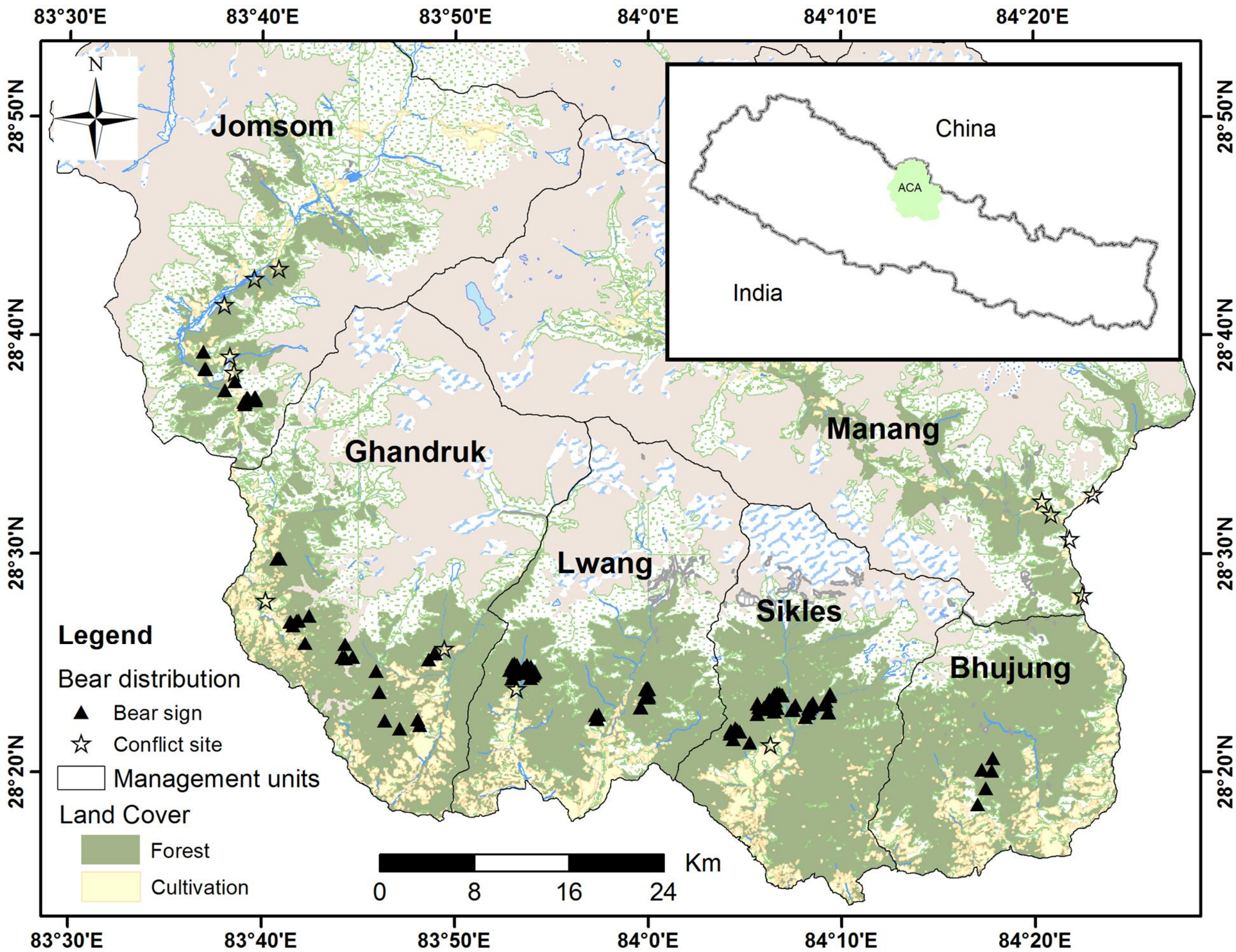


Figure 2-1. Distribution of Himalayan black bears in Annapurna Conservation Area.

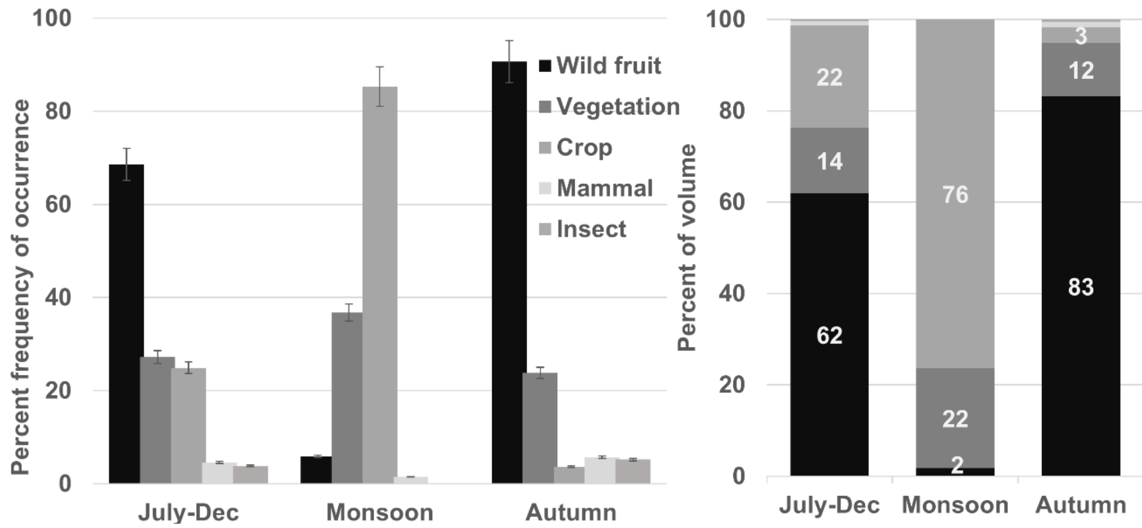


Figure 2-2. Seasonal changes (monsoon: July-September; autumn: October-December) in the diet of Himalayan black bears based on percent frequency of occurrence and percent volume of food items found in feces (n = 261), Annapurna Conservation Area, Nepal.

SUMMARY

Asiatic black bears (*Ursus thibetanus*) have a widespread distribution in mountain landscapes, and are considered vulnerable globally, but are low-priority species for conservation in Nepal. Habitat fragmentation, illegal hunting, and human-bear conflict are the major threats to Asiatic black bears across their global range. Having an adequate level of genetic variation in a population helps with adapting to rapidly changing environments, and thus is important for the long-term health of bear populations. Sound understanding of ecological requirements is needed to develop effective policies for the conservation of Himalayan black bears.

In Chapter I, genetic diversity, genetic structure, and the phylogenetic relationship of Asiatic black bears were elucidated from the ACA of Nepal to other subspecies by organizing non-invasive surveys. To assess levels of genetic diversity and population genetic structure, eight microsatellite loci were genotyped using 147 samples and 60 individuals were identified in an area of approximately 525 km^2. The Asiatic black bear population in the ACA has maintained high levels of genetic diversity (H_E = 0.76) as compared to other bear populations from range countries. A signature of population substructure was not detected among sampling localities and this suggests that animals are moving freely across the landscape within the ACA. A moderate population size was detected that may increase with the availability of suitable habitat in the ACA, so bear-related conflict should be addressed to ensure the long-term viability of this expanding bear populations. Primers specific to bears were designed to amplify a 675 bp fragment of the mitochondrial control region from the collected samples. Three haplotypes were observed from the entire conservation area. The complete mitochondrial genome (16,771 bp), the first obtained from wild populations of the Himalayan black bear (*U. t. laniger)*, was also sequenced to resolve the phylogenetic relationships of closely related subspecies of Asiatic black bears. The resulting phylogeny indicated that Himalayan black bear populations in Nepal are evolutionary distinct from other known subspecies of Asiatic black bears.

In chapter II, habitat characteristics, seasonality, and occurrence of cultivated crops in the diet of black bears were described in the ACA, Nepal. An analysis of 261 feces showed wild fruits (21 species), grasses (two items), agricultural crops (four items), insects (two items), and animal matter. The occurrence of agricultural crops and wild fruits in bear diets were reversed in monsoon and autumn season. Agricultural crops constituted 85.3% (Percent Volume: PV = 76.3%) in monsoon and dropped to 3.6% (PV = 3.4%) in autumn whereas wild fruits constituted 5.9% (PV = 1.8%) in monsoon and increased to 90.7 % (PV = 83.2%) in autumn. A small amount of feces contained insects (3.8% occurrence, 0.4% volume) and animal matter (5.7% occurrence, 1.2% volume), particularly in autumn. Although bears were omnivores, acorns were the most preferred food item in autumn and maize during monsoon season, whereas bamboo shoots and *Arisaema* spp. were supplemental foods during both seasons and fulfilled fruit deficiency. Himalayan black bears vary their diet seasonally, reflecting changes in food availability. They depredate crops when fruits are not accessible in the forest. Timely crop damage mitigation programs should be addressed to seek community support in bear conservation. Black bears were well distributed in forested areas of ACA with an elevation range of 1,680 to 3,582 m, in highly preferred mixed broad-leaved forests. They seemed to co-exist with anthropogenic disturbance, especially livestock grazing.

This is the first genetic and ecological assessment of Asiatic black bears in Nepal and the information herein should be incorporated in Nepal’s national assessment, and when revising the IUCN status and conservation action plan for Asiatic black bears. This study also confirmed the existence of an ancient lineage of Himalayan black bears in western Himalayas that deserves consideration in future conservation plans.

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REFERENCES

1. Acharya KP, Paudel PK, Neupane PR, Köhl M. 2016. Human-wildlife conflicts in Nepal: patterns of human fatalities and injuries caused by large mammals. PLOS ONE 11(9):e0161717. doi: 10.1371/journal.pone.0161717
2. Allendorf FW, Luikart G, Aitken SN. 2013. Conservation and the genetics of populations, 2nd edition. Wiley-Blackwell Publishing.
3. Allendorf FW, Lundquist LL. 2003. Introduction: population biology, evolution, and control of invasive species. Conservation Biology 17(1):24–30. doi: 10.1046/j.1523-1739.2003.02365.x
4. Aramilev VV. 2006. The conservation status of Asiatic black bears in the Russian Far East. In: Japan Bear Network, editor. Understanding Asian bears to secure their future. Japan Bear Network, Ibaraki, Japan, pp. 86–89.
5. Bajracharya SB, Furley PA, Newton AC. 2005. Effectiveness of community involvement in delivering conservation benefits to the Annapurna Conservation Area, Nepal. Environmental Conservation 32(03): 239–247. doi: 10.1017/S0376892905002298
6. Bajracharya SD, Furley PA, Newton AC. 2006. Impacts of community-based conservation on local communities in the Annapurna Conservation Area, Nepal. Biodiversity and Conservation 15:2765–2786. doi: 10.1007/s10531-005-1343-x
7. Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1):37–48. doi: 10.1093/oxfordjournals.molbev.a026036
8. Beck JR, Beck DO. 1955. A method for nutritional evaluation of wildlife foods. Journal of Wildlife Management 19:198–205.

9. Bhattarai BR, Wright W, Poudel BS, Aryal A, Yadav B, Wagle R. 2017. Shifting paradigms for Nepal's protected areas: history, challenges and relationships. Journal of Mountain Science 4(5):964–979. doi: 10.1007/s11629-016-3980-9

10. Bista M, Panthi S, Weiskopf SR. 2018. Habitat overlap between Asiatic black bear *Ursus thibetanus* and red panda *Ailurus fulgens* in Himalaya. PLOS ONE 13(9):e0203697.

11. Bista R, Aryal A. 2013. Status of the Asiatic black bear *Ursus thibetanus* in the southeastern region of the Annapurna Conservation Area, Nepal. Zoology and Ecology 23(1):83–87.

12. Brinkman TJ, Schwartz MK, Person DK, Pilgrim KL, Hundertmark KJ. 2010. Effects of time and rainfall on PCR success using DNA extracted from deer fecal pellets. Conservation Genetics 11(4):1547–1552. doi: 10.1007/s10592-009-9928-7

13. Charoo SA, Sharma LK, Sathyakumar S. 2011. Asiatic black bear-human interactions around Dachigam National Park, Kashmir, India. Ursus 11(22):106–113. doi:10.2192/URSUS-D-10-00021.1

14. Chhetri M. 2013. Distribution and abundance of Himalayan black bear and brown bear conflict in Manaslu Conservation Area, Nepal. National Trust for Nature Conservation-Manaslu Conservation Area Project, Nepal.

15. Choi EH, Kim SK, Ryu SH, Jang KH, Hwang UW. 2010. Mitochondrial genome phylogeny among Asiatic black bear *Ursus thibetanus* subspecies and comprehensive analysis of their control regions. Mitochondrial DNA 21(3–4):105–114. doi: 10.3109/19401736.2010.491864

16. CITES. 2017. Appendices I, II and III. Convention on international trade in endangered species of wild fauna and flora.

17. Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. Heredity 83:260–270. doi: 10.1038/sj.hdy.6885530

18. Crooks KR, Burdett CL, Theobald DM, Rondinini C, Boitani L. 2011. Global patterns of fragmentation and connectivity of mammalian carnivore habitat. Philos Trans R Soc B Biol Sci 366:2642–2651. doi:10.1098/rstb.2011.0120

19. Darriba D, Taboada GL, Doallo R, Posada D. 2012. JModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8):772. doi: 10.1038/nmeth.2109.

20. Delisle I, Strobeck C. 2002. Conserved primers for rapid sequencing of the complete mitochondrial genome from carnivores, applied to three species of bears. Molecular Biology and Evolution 9(3):357–361. doi: 10.1093/oxfordjournals.molbev.a004090

21. Dutta T, Sharma S, Maldonado JE, Panwar HS, Seidensticker J. 2015. Genetic variation, structure, and gene glow in a sloth bear (*Melursus ursinus*) meta-population in the Satpura-Maikal landscape of central India. PLOS ONE 10(8):e0132005. doi: 10.1371/journal.pone.0132005

22. Earl DA, vonHoldt BM. 2012. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4(2):359–361. doi: 10.1007/s12686-011-9548-7

23. Ennis S, Gallagher TF. 1994. A PCR-based sex-determination assay in cattle based on the bovine Amelogenin locus. Anim Genet 25(6):425–427. doi: 10.1111/j.1365-2052.1994.tb00533.x

24. Frankham R, Ballou JD, Briscoe DA. 2009. Introduction to conservation genetics. Cambridge University Press, Cambridge.

25. Galbreath GJ, Hunt M, Clements T, Waits LP. 2008. An apparent hybrid wild bear from Cambodia. Ursus 9(1):85–86. doi: 10.2192/07SC007R2.1

26. Garshelis D, Steinmetz R. 2016. *Ursus thibetanus* (errata version published in 2017). The

IUCN Red List of Threatened Species 2016:e.T22824A114252336. Available from: http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22824A45034242.en. Cited 24 October 2018.

27. Greenwood PJ. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behavior 28(4):1140–1162. doi: 10.1016/S0003-3472(80)80103-5

28. Hashimoto Y, Kaji M, Sawada H, Takatsuki S. 2003. Five-year study on the autumn food habits of the Asiatic black bear in relation to nut production. Ecological Research 18:485–492.

29. Heptner VG, Naumov NP. 1998. Mammals of the Soviet Union, Vol. II Part 1a, Sirenia and Carnivora (Sea cows; Wolves and Bears). Smithsonian Institution libraries and National Science Foundation, Washington DC, USA.

30. Hewitt DG, Robbins CT. 1996. Estimating grizzly bear food habits from fecal analysis. Wildlife Society Bulletin 24:547–550.

31. Hilderbrand GV, Jenkins SG, Hanley TA, Robbins CT. 1999. Effect of seasonal differences in dietary meat intake on changes in body mass and composition in wild and captive brown bears. Canadian Journal of Zoology 77:1623–1630.

32. Hirata D, Mano T, Abramov AV, Baryshnikov GF, Kosintsev PA, Vorobiev AA, Evgeny G, Raichev EV, Tsunoda H, Kaneko Y, Murata K, Fukui D, Masuda R. 2013. Molecular phylogeography of the brown bear (*Ursus arctos*) in Northeastern Asia based on analyses of complete mitochondrial DNA sequences. Mol Biol Evol 30(7):1644–1652. doi: 10.1093/molbev/mst077.

33. Hou WR, Chen Y, Wu X, Hu JC, Peng ZS, Yang J, Tang ZX, Zhou CQ, Li YM, Yang SK, Du YJ, Kong LI, Ren ZI, Zhang HY, Shuai S. 2007. A complete mitochondrial genome sequence

of Asian black bear Sichuan subspecies (*Ursus thibetanus mupinensis*). Int J Biol Sci. 3:85–90.

34. Huygens OC, Miyashita T, Dahle B, Carr M, Izumiyama S, Sugawara T, Hayashai H. 2003. Diet and feeding habits of Asiatic black bears in the Northern Japanese Alps. Ursus 14:236–245.
35. Hwang DS, Ki JS, Jeong DH, Kim BH, Lee BK, Han SH, Lee JS. 2008. A comprehensive analysis of three Asiatic black bear mitochondrial genomes (subspecies *ussuricus, formosanus* and *mupinensis*), with emphasis on the complete mtDNA sequence of *Ursus thibetanus ussuricus* (Ursidae). Mitochondrial DNA 19(4):418–429. doi:10.1080/19401730802389525
36. Hwang MH, Garshelis DL, Wang Y. 2002. Diets of Asiatic black bears in Taiwan, with methodological and geographical comparisons. Ursus 13:111–125.
37. Hwang MH, Garshelis DL, Wu YH, Wang Y. 2010. Home ranges of Asiatic black bears in the central mountains of Taiwan: Gauging whether a reserve is big enough. Ursus. 21 (1):81–96.
38. Ishibashi Y, Saitoh T. 2004. Phylogenetic relationships among fragmented Asian black bear (*Ursus thibetanus*) populations in western Japan. Conservation Genetics 5:311–323.
39. Izumiyama S, Shiraishi T. 2004. Seasonal changes in elevation and habitat use of the Asiatic black bear (*Ursus thibetanus*) in the Northern Japan Alps. Mammal Study 29:1–8.
40. Jimenez JA, Hughes KA, Alaks G, Graham L, Lacy RC. 1994. An experimental study of inbreeding depression in a natural habitat. Science 266(5183):271–273. doi: 10.1126/science.7939661
41. Jnawali SR, Baral HS, Lee S, Acharya KP, Upadhyay GP, Pandey M, Shrestha R, Joshi D, Laminchhane BR, Griffiths J, Khatiwada AP, Subedi N, Amin R, editors. 2011. The status of

Nepal mammals: The national red list series. Department of National Parks and Wildlife Conservation, Kathmandu, Nepal.

42. Johnsingh AJT. 2003. Bear conservation in India. Journal of Bombay Natural History Society. 100:190–201.

43. Johnson PCD, Haydon DT. 2007. Maximum likelihood estimation of allelic dropout and false allele error rates from microsatellite genotypes in the absence of reference data. Genetics 175 (2):827–842. doi:10.1534/genetics.106.064618

44. Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16(5):1099–1106. doi:10.1111/j.1365-294X.2007.03089.x

45. Kim YK, Hong YJ, Min MS, Kim KS, Kim YJ, Voloshina I, Myslenkov A, Smith GJ, Cuong ND, Tho HH, Han SH, Yang DH, Kim CB, Lee H. 2011. Genetic status of Asiatic black bear (*Ursus thibetanus*) reintroduced into South Korea based on mitochondrial DNA and microsatellite loci analysis. J Hered 102(2):165–174. doi:10.1093/jhered/esq121

46. Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide. J Mol Evol 16(2):111–120.

47. Kitahara EY, Isagi Y, Ishibashi Y, Saitoh T. 2000. Polymorphic microsatellite DNA markers in the Asiatic black bear Ursus thibetanus. Mol Ecol 9(10):1661–1689. doi:10.1046/j.1365-294x.2000.01030.x

48. Koike S. 2010. Long-term trends in food habits of Asiatic black bear in the Misaka Mountains on the Pacific coast of central Japan. Mammalian biology 74:17–28.

49. Krause J, Unger T, Nocon A, Malaspinas AS, Kolokotronis SO, Stiller M, Soibelzon L, Spriggs H, Dear PH, Briggs AW, Bray SCE, O'Brien SJ, Rabeder G, Matheus P, Cooper A,

Slatkin M, Paabo S, Hofreiter M. 2008. Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. BMC Evolutionary Biology 8:220. doi: 10.1186/1471-2148-8-220

50. Kumar S, Stecher G, Tamura K. 2015. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33 (7):1870–1874. doi:10.1093/molbev/msw054
51. Lan T, Gill S, Bellemain E, Bischof R, Nawaz MA, Lindqvist C. 2017. Evolutionary history of enigmatic bears in the Tibetan Plateau-Himalaya region and the identity of the yeti. Proc R Soc B 284:20171804. doi:10.1098/rspb.2017.1804
52. Lande R, Barrowclough GF. 1987. Effective population size, genetic variation, and their use in population management. In: Soule ME, editor. Viable populations for conservation. Cambridge University Press, Cambridge, pp. 87–123.
53. Latham E, Stetz JB, Seryodkin I, Miquelle D, Gibeau ML. 2012. Non-invasive genetic sampling of brown bears and Asiatic black bears in the Russian Far East: A pilot study. Ursus 23(2):145–158. doi: 10.2192/URSUS-D-11-00022R2.1
54. Li YC, Korol AB, Fahima T, Beiles A, Nevo E. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: A review. Molecular Ecology 11:2453–2465.
55. Machutchon AG, Wellwood DW. 2003. Grizzly bear food habits in the northern Yukon, Canada. Ursus 14(2):225–235
56. Malcolma KD, McSheab WJ, Garshelis DL, Luod SJ, Van Deelen TR, Liu F, Li S, Miaod L, Wangf D, Brown JL. 2014. Increased stress in Asiatic black bears relates to food limitation, crop raiding, and foraging beyond nature reserve boundaries in China. Global Ecology and Conservation 2:267–276. doi: 10.1016/j.gecco.2014.09.010

57. Manjrekar N. 1989. Feeding ecology of the Himalayan Black bear (*Selenarctos thibetanus*) in Dachigam National Park. M.Sc. thesis. Saurashtra University, Rajkot, Gujarat, India.

58. McRae SB, Emlen ST, Rubenstein DR, Bogdanowicz SM. 2005. Polymorphic microsatellite loci in a plural breeder, the grey-capped social weaver (*Pseudonigrita arnaudi),* isolated with an improved enrichment protocol using fragment size selection. Molecular Ecology Notes 5:16–20.

59. Meredith EP, Rodzen JA, Banks JD, Jones KC. 2009. Characterization of 29 tetranucleotide microsatellite loci in black bear (*Ursus americanus*) for use in forensic and population applications. Conserv Genet 10(3):693–696. doi:10.1007/s10592-008-9617-y

60. Ministry of Forests and Soil Conservation. 2016. Conservation landscapes of Nepal. Ministry of Forest and Soil Conservation, Kathmandu, Nepal

61. Mukesh, Sharma LK, Charoo SA, Sathyakumar S. 2013. An improved and reliable molecular sexing technique for Asiatic black bears, *Ursus thibetanus*. Conservation Genet Resour 5(4):1079–1082. doi:10.1007/s12686-013-9988-3

62. Mukesh, Sharma LK, Charoo SA, Sathyakumar S. 2015. Conflict bear translocation: investigating population genetics and fate of bear translocation in Dachigam National Park, Jammu and Kashmir, India. PLOS ONE 10(8):e0132005. doi: 10.1371/journal.pone.0132005

63. Murphy MA, Kendall KC, Robinson A, Waits LP. 2007. The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. Conserv Genet 8(5):1219–1224. doi: 10.1007/s10592-006-9264-0

64. Nakajima A., Koile S, Masaki T, Shimada T, Kozaki C, Nemoto Y. 2012. Spatial and elevation variation in fruiting phenology of a deciduous oak (*Quercus crispula*) and its effect on foraging behavior of the Asiatic black bear (*Ursus thibetanus*). Ecological Research

27:529–538.

65. Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford.
66. Ngoprasert D, Reed DH, Steinmetz R, Gale GA. 2012. Density estimation of Asian bears using photographic capture-recapture sampling based on chest marks. Ursus 23:117–133.
67. Ngoprasert D, Steinmetz R, Gale GA. 2013. Estimating the abundance of Asiatic black bears and sun bears in Thailand. International Bear News 22(3):30–32
68. Niraula RR, Gilani H, Pokharel BK, Qamer FM. 2013. Measuring impacts of community forestry program through repeat photography and satellite remote sensing in the Dolakha district of Nepal. Journal of Environmental Management 126:20–29. doi: 10.1016/j.jenvman.2013.04.006
69. NTNC. 2017. Annapurna conservation area management plan. 2017–2021. Lalitpur: National Trust for Nature Conservation, Kathmandu, Nepal.
70. Ohnishi N, Uno R, Ishibashi Y, Tamate HB, Oi T. 2019. The influence of climatic oscillations during the quaternary era on the genetic structure of Asian black bears in Japan. Heredity (Edinb) 102(6):579–589. doi: 10.1038/hdy.2009.28
71. Ohnishi N. Saitoh T, Ishibashi Y, Oi T. 2007. Low genetic diversities in isolated populations of the Asian black bear (*Ursus thibetanus*) in Japan, in comparison with large stable populations. Conserv Genet 8 (6):1331–1337. doi: 10.1007/s10592-006-9281-z
72. Oi T, Yamazaki K. 2006. The status of Asiatic black bears in Japan. In: Japan Bear Network, editor. Understanding Asian bears to secure their future. Japan Bear Network, Ibaraki, Japan, pp. 122–133.
73. Onorato DP, Hellgren EC, Van Den Bussche RA, Skiles JR. 2004. Paternity and relatedness

of American black bears recolonizing a desert montane island. Canadian Journal of Zoology 82:1201–1210.

74. Paetkau D, Calvert W, Stirling I, Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4(3):347–354. doi: 10.1111/j.1365-294X.1995.tb00227.x
75. Paetkau D, Shields GF, Strobeck C. 1998. Gene flow between insular, coastal and interior populations of brown bears in Alaska. Molecular Ecology 7(10):1283–1292. doi: 10.1046/j.1365-294x.1998.00440.x
76. Paetkau D, Waits LP, Clarkson PL, Craighead L, Vyse E, Ward R, Strobeck C. 1998. Variation in genetic diversity across the range of North American brown bears. Conserv Biol 12(2):418–429. doi: 10.1111/j.1523-1739.1998.96457.x
77. Panthi S, Khanal G, Acharya KP, Aryal A, Srivathsa A. 2017. Large anthropogenic impacts on a charismatic small carnivore: Insights from distribution surveys of red panda *Ailurus fulgens* in Nepal. PLOS ONE 12(7):e0180978.
78. Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics 28(19):2537–2539. doi:10.1093/bioinformatics/bts460
79. Pocock RI. 1941. The fauna of British India, including Ceylon and Burma. Mammalia. Volume 2. Taylor and Francis, London, UK.
80. Prater SN. 1971. The book of Indian animals. Third edition. Bombay Natural History Society, Bombay.
81. Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multi-locus genotype data. Genetics 155(2):945–959.

82. Proctor MF, Paetkau D, Mclellan BN, Stenhouse GB, Kendall KC, Mace RD, Kasworm WF, Servheen C, Lausen CL, Michael L. Gibeau ML, Wakkinen WL, Haroldson MA, Mowat GM, Apps CD, Ciarniello LM, Barclay RMR, Boyce MS, Schwartz CC, Strobeck C. 2012. Population fragmentation and inter-ecosystem movements of grizzly bears in western Canada and the northern United States. Wildlife Monographs 180 (1):1–46. doi:10.1002/wmon.6

83. Prugh LR, Ritland CE, Arthur SM, Krebs CJ. 2005. Monitoring coyote population dynamics by genotyping faeces. Molecular Ecology 14(5):1585–1596. doi: 10.1111/j.1365-294X.2005.02533.x

84. Queller DC and Goodnight KF. 1989. Estimating relatedness using genetic markers. Evolution 43(2):258–275. doi: 10.1111/j.1558-5646.1989.tb04226.x

85. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Tracer v1.7. Available from: http://tree.bio.ed.ac.uk/software/tracer/. Cited 9 March 2018.

86. Roberts TJ. 1977. The mammals of Pakistan. Oxford University Press, Oxford, UK.

87. Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3):539–542. doi:10.1093/sysbio/sys029

88. Rousset F. 2008. GENEPOP’007: a complete reimplementation of the GENEPOP software for windows and Linux. Mol Ecol Resour 8(1):103–106. doi: 10.1111/j.1471-8286.2007.01931.x

89. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA Sequence polymorphism analysis of large data sets. Mol Biol Evol 34(12):3299–3302. doi: 10.1093/molbev/msx248

90. Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494. doi: 10.1038/33136

91. Saito M, Yamauchi K, Aoi T. 2008. Individual identification of Asiatic black bears using extracted DNA from damaged crops. Ursus 19(2):162–167. doi: 10.2192/08GR006.1

92. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425.

93. Sathyakumar S, Choudhury A. 2007. Distribution and status of Asiatic black bear in India. Journal of Bombay Natural History Society. 104(3):316–323.

94. Sathyakumar S, Sharma LK, Charoo SA. 2013. Ecology of Asiatic black bear in Dachigam National Park, Kashmir, India. Final project report. Wildlife Institute of India, Dehradun, India.

95. Sathyakumar S, Viswanath S. 2003. Observations on food habits of Asiatic black bear in Kedarnath Wildlife Sanctuary, India: Preliminary evidence on their role in seed germination and dispersal. Ursus 14:103–108.

96. Schaller GB, Qitao T, Johnson KG, Xiaoming W, Heming S, Jinchu H. 1989. The feeding ecology of giant pandas and Asiatic black bears in the Tangjiahe Reserve, China. In: Gittleman JL, editor. Carnivore behavior, ecology, and evolution. Cornell University Press, New York, pp. 164–182.

97. Schaller GB. 1969. Food habits of the Himalayan black bear (*Selenarctos thibetanus*) in Dachigam Sanctuary, Kashmir. Journal of Bombay Natural History Society 66:156–159.

98. Servheen C, Herrero S, Peyton B, editors. 1998. Bears: Status survey and conservation action plan. IUCN/SSC Bear and Polar Bear Specialist Groups, IUCN, Gland, Switzerland and Cambridge, UK.

99. Sharma LK, Charoo SA, Sathyakumar S. 2010. Investigation on the ecology and behavior of Asiatic black bear using satellite telemetry: A case study from Dachigam National Park, Kashmir, India. Telemetry in wildlife science 13 (1):85–94.

100. Smith DA, Ralls K, Hurt A, Adams B, Parker M, Maldonado JE. 2006. Assessing reliability of microsatellite genotypes from kit fox faecal samples using genetic and GIS analyses. Molecular Ecology 15(2):387–406. doi: 10.1111/j.1365-294X.2005.02841.x

101. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313. doi: 10.1093/bioinformatics/btu033

102. Steinmetz R, Garshelis DL, Chutipong W, Seuaturien N. 2011. The shared preference niche of sympatric Asiatic black bears and sun bears in a tropical forest mosaic. PLOS ONE 6(1):e14509.

103. Stubblefield CH, Shrestha M. 2007. Status of Asiatic black bears in protected areas of Nepal and the effects of political turmoil. Ursus 18(1):101–108. doi:10.2192/1537-6176(2007)18[101:SOABBI]2.0.CO;2

104. Taberlet P, Camarra JJ, Griffin S, Uhres E, Hanotte O, Waits LP, Paganon CD, Burke T, Bouvet J. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology 6(9):869–876. doi: 10.1111/j.1365-294X.1997.tb00141.x

105. Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24(16):3189–3194. doi: 10.1093/nar/24.16.3189

106. Taberlet P, Waits LP, Luikart G. 1999. Non-invasive genetic sampling: Look before you leap. Trends in Ecology & Evolution 14(8):323–327. doi: 10.1016/S0169-5347(99)01637-7

107. Takahashi K, Shiota T, Tamatani H, Koyama M, Washitani I. 2008. Seasonal variation in

fleshy fruit use and seed dispersal by the Japanese black bear (*Ursus thibetanus japonicus*). Ecological Research 23:471–478.

108. Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673–4680. doi: 10.1093/nar/22.22.4673

109. Trent JA. 2010. Ecology, habitat use, and conservation of Asiatic black bears in the Min mountains of Sichuan province, China. M.Sc. thesis. Virginia Polytechnic Institute and State University. Blacksburg, Virginia Tech, USA.

110. Tsai CL, Chou YC, Shih CC, Cheng HC, Yang CC, Kao HW. 2009. The complete mitochondrial genome of the Formosan black bear (*Ursus thibetanus formosanus*). Zootaxa 1971:50–58.

111. Uno R, Doko T, Ohnishi N, Tamate HB. 2015. Population genetic structure of the Asian black bears (*Ursus thibetanus*) within and across management units in northern Japan. Mammal Study 40(4):231–244. doi: 10.3106/041.040.0404

112. Uno R, Kondo M, Yuasa T, Yamauchi K, Tsuruga H, Tamate HB, Yoneda M. 2012. Assessment of genotyping accuracy in a non-invasive DNA-based population survey of Asiatic black bears (*Ursus thibetanus*): lessons from a large-scale pilot study in Iwate prefecture, northern Japan. Popul Ecol 54(4):509–519. doi: 10.1007/s10144-012-0328-3

113. Valière N. 2002. GIMLET: a computer program for analysing genetic identification data. Mol Ecol Notes 2(3):377–379. doi: 10.1046/j.1471-8286.2002.00228.x-i2

114. Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol

Notes 4(3):535–538. doi: 10.1111/j.1471-8286.2004.00684.x

115. Wasser SK, Houston CS, Koehiler GM, Cadd GG. 1997. Techniques for applications of faecal DNA methods to field studies of *Ursids*. Molecular Ecology 6(11):1091–1097. doi: 10.1046/j.1365-294X.1997.00281.x

116. Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6):1358–1370. doi: 10.1111/j.1558-5646.1984.tb05657.x

117. White THJ, Bowman JL, Leopold BD, Jacobson HA, Smith WP, Vilella FJ. 2000. Influence of Mississippi alluvial valley rivers on black bear movements and dispersal: implications for Louisiana black bear recovery. Biol Conserv 95(3):323–331. doi: 10.1016/S0006-3207(00)00024-0

118. Wilson DE, Reeder DM. 2005. Mammal species of the world, a taxonomic and geographic reference, 3rd edition. The Johns Hopkins University Press, Baltimore, USA.

119. Woods JG, Paetkau D, Lewis D, Mclellan BN, Proctor P, Strobeck C. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27(3):616-627.

120. Wu J, Kohno N, Mano S, Fukumoto Y, Tanabe H, Hasegawa M, Yonezawa T. 2015. Phylogeographic and demographic analysis of the Asian black bear (*Ursus thibetanus*) based on mitochondrial DNA. PLOS ONE 10(9):e0136398. doi: 10.1371/journal.pone.0136398

121. Yadav BP, Aryal A, Poudel DP, Pandey P, Thapalia BP. 2012. Asiatic black bear killed in Rara National Park, Nepal. International Bear News. 21(3):20–22.

122. Yadav SK, Lamichhane BR, Subedi N, Dhakal M, Thapa RK, Poudyal L. 2017. Himalayan black bear discovered in Babai valley of Bardia National Park, Nepal, co-occurring with sloth bears. International Bear News 26(3):23–25.

123. Yamamoto K, Tsubota T, Komatsu T, Katayama A, Murase T, Kita I, Kudo T. 2002. Sex

identification of Japanese black bear, *Ursus thibetanus japonicus*, by PCR based on Amelogenin gene. J Vet Med Sci 64(6):505–508. doi: 10.1292/jvms.64.505

124. Yasukochi Y, Nishida S, Han SH, Kurosaki T, Yoneda M, and Koike H. 2009. Genetic structure of the Asiatic black bear in Japan using mitochondrial DNA analysis. Journal of Heredity 100(3):297–308. doi: 10.1093/jhered/esn097

125. Yu L, Li YW, Ryder OA, Zhang YP. 2007. Analysis of complete mitochondrial genome sequences increases phylogenetic resolution of bears (*Ursidae*), a mammalian family that experienced rapid speciation. BMC Evol Biol 7:198. doi: 10.1186/1471-2148-7-198

ANNEXES

Annex 1. Overview of the sequence/haplotypes used for the phylogenetic analysis.

Species/subspecies	NCBI accession no	Haplotype name	Source site	Country/place	Mitochondrial region	Total bp
U. t. laniger	MG066704	Ut_lan_MON	Monastery	Nepal/Mustang	mitogenome	16395
U. t. laniger	MG131881	Ut_lan_PAK	Zoo	Pakistan/Lahore	CR	180
U. t. japonicus	AB863014	Ut_japon	Zoo	Japan/Hirosima	mitogenome	16748
U. t. japonicus	AB101520	japo_UtCR01				706
	AB101521	japo_UtCR02				705
	AB101522	japo_UtCR03				704
	AB101523	japo_UtCR04				703
	AB101524	japo_UtCR05				702
	AB101525	japo_UtCR06				705
	AB101526	japo_UtCR07				704
	AB101527	japo_UtCR08				705
	AB101528	japo_UtCR09				704
U. t. japonicus	AB101529	japo_UtCR10	Natural habitat	Japan/Honsu southern	tRNA-CR	703
	AB101530	japo_UtCR11				704
	AB101531	japo_UtCR12				703
	AB101532	japo_UtCR13				704
	AB101533	japo_UtCR14				703
	AB101534	japo_UtCR15				704
	AB101535	japo_UtCR16				704
	AB101536	japo_UtCR17				703
	AB101537	japo_UtCR18				703
	AB101538	japo_UtCR19				702
	AB101539	japo_UtCR20				703
	AB859728	japo_UtCRE46	Natural habitat	Japan/Honsu northern	tRNA-CR	705
	AB441772	japo_UtCRE01				706
	AB441773	japo_UtCRE02				706
	AB441774	japo_UtCRE03				706
	AB441775	japo_UtCRE04				706
	AB441776	japo_UtCRE05				706
	AB441777	japo_UtCRE06				706
	AB441778	japo_UtCRE07				706
	AB441779	japo_UtCRE08				706
	AB441780	japo_UtCRE09				706
	AB441781	japo_UtCRE10				706
	AB441782	japo_UtCRE11				706
	AB441783	japo_UtCRE12				706
U. t. japonicus	AB441784	japo_UtCRE13	Natural habitat	Japan/All	tRNA-CR	706
	AB441785	japo_UtCRE14				706
	AB441786	japo_UtCRE15				706
	AB441787	japo_UtCRE16				706
	AB441788	japo_UtCRE17				706
	AB441789	japo_UtCRE18				706
	AB441790	japo_UtCRE19				706
	AB441791	japo_UtCRE20				706
	AB441792	japo_UtCRE21				706
	AB441793	japo_UtCRE22				706
	AB441794	japo_UtCRE23				706
	AB441795	japo_UtCRE24				706
	AB441796	japo_UtCRE25				706

Species/subspecies	NCBI accession no	Haplotype name	Source site	Country/place	Mitochondrial region	Total bp
	AB441797	japo_UtCRE26				706
	AB441798	japo_UtCRE27				706
	AB441799	japo_UtCRE28				706
	AB441800	japo_UtCRE29				706
	AB441801	japo_UtCRE30				706
	AB441802	japo_UtCRE31				706
	AB441803	japo_UtCRE32				706
	AB441804	japo_UtCRE33				706
	AB441805	japo_UtCRE34				706
	AB441806	japo_UtCRE35				706
	AB441807	japo_UtCRE36				706
	AB441808	japo_UtCRE37				706
	AB441809	japo_UtCRE38				706
	AB441810	japo_UtCRS01				706
	AB441811	japo_UtCRS02				706
	AB441812	japo_UtCRS03				706
	AB441813	japo_UtCRS04				706
	AB441814	japo_UtCRW01				706
U. t. japonicus	AB360915	J1c	Natural habitat	Japan/All	Left Domain-CR	240
	AB360916	J2c				240
	AB360917	J3b				239
	AB360918	J3c				240
	AB360919	J4c				240
	AB360920	J5bN				238
	AB360921	J5a				238
	AB360922	J5b				239
	AB360923	J6b				239
	AB360924	J7b				239
	AB360925	J7c				240
	AB360926	J8d				241
	AB360927	J9b				239
	AB360928	J10b				239
	AB360929	J10c				240
	AB360930	J10d				241
	AB360931	J10e				242
	AB360932	J11b				239
	AB360933	J11c				240
	AB360934	J11d				241
	AB360935	J12c				240
	AB360936	J13c				240
	AB360937	J14c				240
	AB360938	J14d				241
	AB360939	J15b				239
	AB360940	J16a				238
	AB360941	J16c				240
	AB360942	J17c				240
	AB360943	J18c				240
	AB360944	J19c				240
	AB360945	J19d				241
	AB360946	J20c				240
	AB360947	J21b				239
	AB360948	J21c				240
	AB360949	J22b				239
	AB360950	J23a				238
	AB360951	J23b				239
U. t. ussuricus	AB360952	R1c		Russia		240
	AB360953	R2b				239

Species/subspecies	NCBI accession no	Haplotype name	Source site	Country/place	Mitochondrial region	Total bp
	AB360954	R2c				240
	AB360955	R3c_K1c		Russia/Korea		240
U. thibetanus	AB360956	C1c		China		240
U. t. ussuricus	EF667005	Ut_us_NK	Zoo	NK/Pyongyang	mitogenome	16701
U. t. ussuricus	EU573177	Pyong1	Zoo	NK/Pyongyang	CR	1286
	EU573178	Pyong2				1306
	EU573179	Pyong3				1282
	EU573180	Pyong4				1316
	EU573181	Pyong5				1304
	EU573182	Pyong6				1276
U. t. ussuricus	NC_011117	Ut_us_SK	Genbank	South Korea	mitogenome	16824
U. t. ussuricus	EU264506	Rus1	Translocated	Russia Primorsky Krai	CR	1344
	EU264507	Rus2				1355
	EU264508	Rus3				1355
	EU264509	Rus4				1355
	/EU264510	Rus5				1355
	EU264521	Rus6				1324
	EU264523	Rus7				1315
	EU264524	Rus8				1325
	EU264525	Rus9				1325
	EU264526	Rus10				1355
	EU264527	Rus11				1325
	HM135178	Rus12				1325
	HM135179	NK1		North Korea		998
	EU264503	NK2				1325
	EU264504	NK3				1325
	EU264505	NK4				1325
	EU264512	NK5				1325
	EU264513	NK6				1335
	EU264514	NK7				1325
	EU264515	NK8				1325
	EU264516	NK9				1345
	EU264517	NK10				1355
	EU264518	NK11				1335
	EU264519	NK12				1335
U. thibetanus	HM135180	SEA22	Rescue center	Vietnam	CR	615
	HM135181	SEA28				615
	HM135182	SEA60				615
	HM135183	SEA69				615
	HM135184	SEA56				615
	HM135185	SEA6				615
	HM135186	SEA49				615
	HM135187	SEA48				615
	HM135188	SEA29				615
	HM135189	SEA25				615
	HM135190	SEA70				615
	HM135191	SEA59				615
	HM135192	SEA66				615
	HM135193	SEA14				615
U. t. formosanus	NC_009331	Ut_formo	UK	Taiwan	mitogenome	17044
U. thibetanus	AF319154	Tai_B2	Zoo/National Park	Taiwan/Taipeo/Yushan	CR	531
	AF319155	Tai_B3				532
	AF319156	Tai_B4				532
	AF319157	Tai_B5				532
	AF319158	Tai_B7				532
	AF319159	Tai_B8				531
	AF319160	Tai_B9				531

Species/subspecies	NCBI accession no	Haplotype name	Source site	Country/place	Mitochondrial region	Total bp
	AF319161	Tai_B10				532
	AF319162	Tai_B11				532
	AF319163	Tai_B12				532
	AF319164	Tai_B13				531
U. t. mupinensis	NC_008753	Ut_mupin	Company	China/Sichuan	mitogenome	16868
U. t. thibetanus	NC_011118	Ut_thibe	UK	UK	mitogenome	17034
U. thibetanus	NC_009971	U_thibe_YU	Frozen	China/Yunnan	mitogenome	16795
U. thibetanus	FM177759	U_thibe_UK	Lab	USA/Maryland	mitogenome	16893
U. thibetanus	MF041989	Ut_lan_IND	UK	India	Cytochrome b	435
U. t. laniger	MG131869	Ut_lan_PAK	Zoo	Pakistan/Lahore	Cytochrome	191
U. t. mupinensis	AY522429	Ut_mupin_AY	UK	UK	Cytochrome b	1140
U. t. ussuricus	AY522430	Ut_us_AY	UK	UK	Cytochrome b	1140
U. t. ussuricus	AB360957	Ut_us_K1	Not specify	Korea	Cytochrome b	1140
U. t. ussuricus	EU573171	Ut_us_NK33	Zoo	NK/Pyongyang	Cytochrome b	1140
	EU573172	Ut_us_N43				1140
	EU573173	Ut_us_NK9				1140
	EU573174	Ut_us_NK21				1140
	EU573175	Ut_us_NK24				1140
	EU573176	Ut_us_NK16				1140
U. t. japonicus	AB360959	Ut_japo_cbJ1	Natural habitat	Japan/All	Cytochrome b	1140
	AB360960	Ut_japo_cbJ2				1140
	AB360961	Ut_japo_cbJ3				1140
	AB360962	Ut_japo_cbJ4				1140
	AB360963	Ut_japo_cbJ5				1140
	AB360964	Ut_japo_cbJ6				1140
U. thibetanus	U23558	U_thibe_U	Personal	USA	Cytochrome b	1140
U. thibetnaus	AB360958	U_thibe_mcbC1	Not specify	China	Cytochrome b	1140
U. americanus	NC003426	U_ameri	UK	USA	mitogenome	16841

Annex 2. Database of 60 Himalayan black bears for 8 microsatellites in 5 management units of Annapurna Conservation Area, Nepal.

S.N.	MU	MU23a	MU23b	G10Ba	G10Bb	MU50a	MU50b	G10Ca	G10Cb	MU05a	MU05b	MU61a	Mu61b	UamB5a	UamB5b	UamD2a	UamD2b	Sex
1	Ghandruk	110	112	156	156	218	220	108	112	136	140	213	215	144	156	206	210	F
2	Ghandruk	118	124	154	156	222	224	102	116	136	140	209	213	152	156	210	210	M
3	Ghandruk	110	110	156	156	212	224	108	114	142	146	213	215	152	156	206	210	F
4	Ghandruk	118	128	158	160	218	224	102	112	140	142	209	213	156	160	206	210	F
5	Ghandruk	118	124	154	160	212	218	102	118	140	142	213	213	156	156	206	210	M
6	Ghandruk	112	124	156	166	218	222	112	114	136	140	213	215	156	156	206	206	F
7	Ghandruk	110	124	156	156	212	220	108	116	142	146	201	219	156	160	206	210	F
8	Ghandruk	114	124	156	166	220	224	106	116	136	142	213	219	156	164	210	210	F
9	Ghandruk	114	124	158	166	218	220	108	116	136	146	201	219	156	164	206	210	F
10	Ghandruk	114	124	158	166	218	220	108	116	142	144	209	219	160	164	206	210	M
11	Jomsom	110	124	160	160	212	228	102	112	140	144	209	213	156	156	206	206	M
12	Jomsom	121	124	156	160	222	222	102	102	140	142	209	217	156	160	206	218	F
13	Jomsom	114	124	160	160	216	218	106	118	140	144	213	215	156	156	206	214	M
14	Jomsom	114	124	156	160	220	220	102	118	136	140	209	221	152	156	206	222	M
15	Jomsom	122	124	156	156	218	222	102	106	140	144	213	217	152	160	206	218	M
16	Jomsom	114	124	156	160	212	222	106	112	140	144	213	217	152	156	206	206	F
17	Jomsom	114	124	160	160	220	222	106	112	140	144	209	213	156	160	210	210	M
18	Jomsom	110	124	156	160	218	222	102	106	140	144	209	217	152	156	206	218	F
19	Jomsom	114	124	156	160	212	220	106	118	136	140	213	213	144	156	210	210	M
20	Jomsom	122	124	156	156	218	218	102	114	140	144	209	217	156	156	206	218	F
21	Lwang	110	124	156	166	220	220	112	114	136	140	201	213	156	156	210	210	F
22	Lwang	114	124	156	158	210	220	106	114	138	140	201	209	156	156	210	214	F
23	Lwang	114	124	154	158	220	226	106	114	136	140	209	217	144	156	206	210	F
24	Lwang	110	124	160	166	210	220	102	114	140	144	217	219	152	156	210	218	M
25	Lwang	110	124	154	156	212	222	102	108	140	148	215	219	144	152	210	214	M
26	Lwang	122	124	158	160	212	218	112	118	140	140	209	215	156	156	206	210	M
27	Lwang	112	124	154	156	216	222	106	114	140	144	215	217	156	156	210	214	M
28	Lwang	110	124	156	158	220	222	108	118	136	144	209	215	156	160	210	218	M
29	Lwang	114	124	156	158	220	224	112	118	140	144	209	209	144	152	206	210	M

S.N.	MU	MU23a	MU23b	G10Ba	G10Bb	MU50a	MU50b	G10Ca	G10Cb	MU05a	MU05b	MU61a	Mu61b	UamB5a	UamB5b	UamD2a	UamD2b	Sex
30	Lwang	110	124	156	156	212	220	102	102	138	146	215	217	156	156	210	210	M
31	Lwang	122	124	158	166	220	220	102	118	140	148	209	219	156	156	206	210	M
32	Lwang	110	124	156	158	222	226	112	114	136	146	217	219	156	160	210	210	F
33	Lwang	110	124	156	156	220	222	102	112	138	146	215	219	152	156	210	210	F
34	Lwang	110	124	160	160	212	224	102	112	140	144	213	215	152	156	206	210	F
35	Lwang	114	124	156	156	212	220	118	118	136	140	209	209	156	156	206	210	F
36	Lwang	122	124	154	156	212	218	108	118	136	140	209	215	156	156	210	222	M
37	Lwang	114	124	156	156	212	212	114	118	136	138	215	219	144	156	218	222	F
38	Lwang	114	124	156	160	212	218	108	118	136	138	209	219	152	156	210	222	M
39	Lwang	110	124	158	166	220	222	108	114	136	140	201	209	152	156	206	226	M
40	Lwang	112	124	156	158	216	222	106	118	140	140	215	217	152	156	210	222	M
41	Manang	114	124	158	158	212	220	118	122	140	144	207	213	152	152	206	206	F
42	Manang	114	124	156	156	214	220	102	106	136	136	219	219	156	160	206	222	M
43	Sikles	110	124	158	160	210	222	102	108	138	142	209	209	144	144	206	206	M
44	Sikles	110	124	156	156	212	220	102	106	144	146	215	219	152	156	210	210	M
45	Sikles	110	124	158	166	220	220	108	112	136	140	201	213	152	156	206	206	M
46	Sikles	110	124	156	156	218	220	114	118	144	150	209	217	156	160	218	226	M
47	Sikles	112	124	156	158	218	220	114	118	136	140	215	219	144	156	206	218	M
48	Sikles	110	124	156	156	216	224	102	112	146	148	209	219	152	156	210	214	F
49	Sikles	110	124	156	160	220	222	108	118	136	140	213	217	144	160	206	214	F
50	Sikles	112	124	156	156	212	216	106	118	140	140	215	215	152	160	222	222	M
51	Sikles	110	124	156	160	212	224	112	118	136	140	209	209	156	156	206	210	M
52	Sikles	112	124	156	160	212	220	104	106	136	140	209	209	152	156	206	210	M
53	Sikles	112	124	156	166	220	222	102	102	138	140	209	217	152	160	206	210	F
54	Sikles	114	124	158	160	212	220	102	102	136	146	201	201	144	152	206	210	M
55	Sikles	110	124	154	166	218	222	102	108	136	136	209	209	152	160	210	210	F
56	Sikles	110	124	154	156	212	220	102	118	136	140	215	215	144	160	210	214	F
57	Sikles	110	124	156	166	218	220	102	114	136	140	213	213	152	152	210	210	F
58	Sikles	114	124	154	154	212	220	102	118	136	144	209	215	156	160	210	210	F
59	Sikles	122	124	154	154	212	222	102	106	136	140	209	213	156	160	210	218	M
60	Sikles	112	124	154	158	212	214	114	118	136	144	209	219	156	160	206	206	F

Annex 3. Food items with foraging evidence of Himalayan back bears based on feces analysis (n = 2 61), record of bear sign during 34 transect survey (n > 134), and checklist of food from herders.

Category	Native name	English Name	Scientific name	Feces[a]	Sign[b]	Interview[c]	Period[d]	Avaliability[e]	Remarks
Fruit	Aishelu	Himalayan raspberry	*Rubus ellipticus*			√	2	3	
	Aishelu Gade	Raspberry	*Rubus* spp.			√	2	4	
	Aishelu-Lekh	Raspberry	*Rubus* spp.			√	2	1	
	Amphi		*Pyrularia edulis*			√	4	4	
	Angeri	Lyonia	*Lyonia Ovalifolia*			√	3	4	
	Arkhalo	Oak	*Lithocarpus elegans*	2	2	√	4	2	Hard mast
	Banjh	Himalayan oak	*Quercus leucotrichophora*			√	3	1	Hard mast
	Bankakri	Creeping cucumber	*Solena heterophylla*			√	3	4	
	Bhakkimplo	Chinese sumac	*Rhus javanica*			√	4	3	
	Bhalayo/Rum		*Rhus Wallichi*			√	4	3	
	Bhalyao Rani	Wax tree	*Rhus succedanea*			√	4	4	
	Bilaune		*Maesa chisia*		1		4	3	
	Charchare					√	3	1	
	Chutro Kattike	Indian barberry	*Berberis aristate*	7		√	4	6	
	Jamanemandro		*Mahonia napaulensis*		1	√	2	5	
	Denga		*Sorbus cuspidate*	20	4	√	4	4	
	Dhatelo		*Prinsepia utilis*			√	2	2	
	Dimmar	Himalayan dogwood	*Cornus capitate*	1	3	√	4	1	
	Dosi				1		4	1	
	Dudhilo	Fig	*Ficus nerifolia*			√	4	4	
	Rakchan		*Daphniphyllum himalayensis*			√	4	5	
	Kalikath Seto		*Myrsine semiserrata*		3	√	4	3	
	Dabdabe		*Symplocos ramosissima*	6	1	√	4	5	
	Kaulo Kathe	Machilus	*Persea duthii*			√	3	2	
	Kopile				1	√	3	2	
	Lokar			1	2	√	4	4	
	Sibas		*Lindera spp.*	7	2	√	4	1	
	Sisi		*Lindera pulcherrima*	3	2	√	4	4	
	Ghagaru	Fire thorn	*Pyracantha crenulata*			√	3	2	
	Ghuyali	Oleaster	*Elaegnus parvifolia*			√	2	6	
	Goban		*Saurauia napaulensis*	3		√	4	3	
	Gophla	Sausage vine	*Holboellia latifolia*			√	4	1	
	Rayas	Cotoneaster	*Cotoneaster frigidus*	2	1	√	4	4	
	Jhyo					√	4	1	
	Puri	Black cherry	*Prunus nepalensis*	16	4	√	4	5	
	Kharsu	Khashru oak	*Quercus semecarpifolia*		1	√	3	2	Hard mast
	Kukurdaino	Green briers	*Smilax ferox*			√	4	2	
	Lise	Himalayan holly	*Ilex dipyrena*	12	1	√	4	5	
	Lokta	Paper plant	*Danphe bholua*			√	2	3	
	Machhaino	Masuri Berry	*Coriaria nepalensis*		2	√	3	2	
	Mel	Himalayan pear	*Pyrus pashia*			√	4	1	

Category	Native name	English Name	Scientific name	Feces[a]	Sign[b]	Interview[c]	Period[d]	Avaliability[e]	Remarks
	Mallo	Starry viburnum	*Viburnum mullaha*			√	4	5	
	Narga	Reddish viburnum	*Viburnum erubescens*			√	4	5	
	Nimaro	Fig tree	*Ficus auriculata*	1		√	3	3	
	Okhar	Walnut	*Juglans regia*	2	1	√	4	4	Hard mast
	Paiya	Himalayan birch	*Betula alnoides*			√	2	2	
	Paiyeu	Himalayan cherry	*Prunus cerasoides*		1	√	2	4	
	Phalat Sano	Oak-woolly-leaved	*Quercus incana*	41	26	√	4	5	Hard mast
	Phalat Thulo	Oak-bull	*Quercus Lamellosa*	64	10	√	4	6	Hard mast
	Phalat Majhaula	Oak-ring cupped	*Quercus glauca*	7		√	4	1	Hard mast
	Phirchi	Berry				√	3	2	
	Rainsh	Small leaf rowan	*Sorbus microphylla*	1	8	√	4	1	
	Range					√	2	1	
	Saingi	Berry				√	4	1	
	Simalto					√	2	1	
	Aru Jungali	Peach-wild	*Prunus persica*	2		√	4	1	
		Unknown		8					
Vegetation	Phulchuk		*Leucosceptrum canum*			√	4	3	Flower?
	Saire					√	2	1	Flower?
	Allo	Himalayan nettle	*Girardinia diversifolia*			√	2	6	Soft leaf
	Bako (12 types)	Cobra lilly	*Arisaema* spp.	21	2	√	2,3,4	6	Leaf, fruit, rhizome
	Nigalo (8 types)	Himalyan bamboo	*Arundinaria* spp.	29	6	√	2,3	6	Shoot
	Sisno	Common nettle	*Utrica dioica*			√	2	6	Soft leaf
	Thosne	Sikkim Knotweed	*Aconogonum mollie*			√	2	2	Succulent
	Ghas	Grasses (UK)	Graminoid	27	1	√		6	
Crop	Fapar	Buck wehat	*Fagopyrum esculentum*	3		√	4	6	
	Makai	Maize	*Zea mays*	46	√	√	3,4	6	
	Dhan	Rice	*Oryza sativa*	4	√	√	3	4	
	Syau	Apple	*Malus pumila*	13	√	√	3,4	2	
	Kodo	Millet	*Eleusine coracana*			√	4	4	
	Pharsi	Pumpkin	*Cucurbita maxima*			√	3	4	Stored
Animal		Small livestock				√		6	2 locals reported
		Carrion			2	√			
		Hair and bones		12					
Insect	Khapate kira	Beetle	Coleoptera	9	24	√		6	1 feces contained caterpillar
	Barulo/Aringal	Wasp/Hornet	*Vespa* spp.	1	19	√		6	
	Marui	Honey bee	*Abis* spp.		1	√		6	
	Bhamara	Bumble bee	*Bombus* spp.		1	√		6	

a = no of feces; b = no of fruit producing trees, vegetation, insect and carrion found during sign survey; c = check lists of bear food items after discussion with local herders; d = Period (season) of bear forage, 1: Jan-March (winter), 2: April-June (spring), 3: July-September (monsoon), 4: October-December (autumn); e = recorded in no of management units (maximum = 6)