Sloth bears (*Melursus ursinus*) in Nepal: Ecology, genetic diversity, and human-sloth bear conflict

（ネパールにおけるナマケグマ（*Melursus ursinus*）：

生態、遺伝的多様性および人との軋轢）

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ABBREVIATIONS

ADO	Allele Dropout
AIC	Akaike Information Criterion
BBC	Banke-Bardiya Complex
BZ	Buffer Zone
BZUC	Buffer Zone User Committee
BNP	Bardiya National Park
CNP	Chitwan National Park
CPC	Chitwan-Parsa Complex
CR	Control Region
Dist	Disturbance
DNA	Deoxyribonucleic Acid
DNPWC	Department of National Parks and Wildlife Conservation
EVI	Enhanced Vegetation Index
FA	False Allele
F_{IS}	Wright's Inbreeding Coefficient
Frut	Fruit
G	Grassland
GPS	Global Positioning System
H_E	Expected Heterozygosity
H_O	Observed Heterozygosity
HWC	Human Wildlife Conflict
HWE	Hardy-Weinberg Equilibrium
IUCN	International Union for Conservation of Nature
K	Number of Parameters
LBF	Lamahi-Bhaluwang Forest
LD	Linkage disequilibrium
MCMC	Markov Chain Monte Carlo
MF	Mixed Forest
ML	Maximum Likelihood
MtDNA	Mitochondrial DNA
N_A	Number of Allele
NCBI	National Center for Biotechnology Information
N_E	Effective Number of Allele

NTNC	National Trust for Nature Conservation
P	Probability of Detection
PCR	Polymerase Chain Reaction
PFO	Percent Frequency of Occurrence
PIC	Polymorphic Information Content
PID	Probability of Identity
PIDSibs	Probability of Identity of Siblings
RF	Riverine Forest
SD	Standard Deviation
SE	Standard Error
SF	Sal Forest
Ta	Annealing Temperature
Tcov	Tree Cover
Term	Termite
TJF	Trijuga Forest
TRI	Terrain Ruggedness Index
$_{U}H_{E}$	Unbiased Expected Heterozygosity
UK	Unknown
UNESCO	United Nations Educational, Scientific and Cultural Organization
VNTR	Variable Number of Tandem Repeats
W_i	AIC model weight
ΔAIC	AIC values difference between each model and the model with the lowest AIC
Ψ	Probability of Occupancy

NOTES

Publication related to the dissertation

1. **Paudel RP**, Kadariya R, Lamichhane BR, Subedi N, Sashika M, Shimozuru M, and Tsubota T (2022) Habitat occupancy of sloth bear *Melursus ursinus* in Chitwan National Park, Nepal. Ecology and Evolution 12(3): e8699

The contents of chapter I have been published as publication 1.

PREFACE

The sloth bear (*Melursus ursinus*, Shaw, **1791)** is a medium-sized bear species in the Ursidae family of Carnivora order. It stands at 2 - 3 ft at the shoulder and 4 - 6 cm ft from nose to tail. Adult males are heavier (80-145 kg) than females (55-95 kg) (Garshelis et al., **1999**). It was first described as a species of American sloth (*Bradypus spp.)* because of its peculiar claws and teeth formation (Shaw & Nodder, **1791**). Crooked figure, long shaggy black coat, hairy ears, long-curved claws, naked lengthened snout, protrusible lips and tongue, closeable nostrils, and loss of upper incisor teeth make it a peculiar ursid (Owen, **1833**). Two sub-species of sloth bears are recognized, *Melursus ursinus ursinus* sub-species is currently found in Nepal and India and is physically larger and more prolonged than the *Melursus ursinus inornatus* sub-species endemic to Sri Lanka (Pocock, **1941**). They are primarily allopatric with other bear species within their distribution range, although co-occurrence with Asiatic black bears (*Ursus thibetanus*) and sun bears (*Helarctos malayanus*) *(*Bargali et al., **2012**; Choudhary, **2011**, **2013**; Garshelis et al., **2022**; Yadav et al., **2017**) have been reported. The molecular analysis suggests a close evolutionary relationship between these Asian bears (Kitchener et al., **2020;** Kumar et al., **2017**).

Sloth bears have a variable home range (9–14 km^2 in CNP and 12–85 km^2 in India) according to habitat conditions (Joshi et al., **1995;** Yoganand, **2005**). They occur in a range of habitats below 2000 m, including dry or moist forests, savannah, scrublands, and grasslands (Dhariya et al., **2020**; Garshelis et al., **1999**). Mating occurs from May to July, during which a female mate with multiple males in a hierarchical order of dominance. Females enter the den dug out on ground for giving birth and remain there without foraging for about 2 months. A litter size of two cubs is usually produced between November and January. Females emerge out of den from December to January and carry the cubs on its back until 6-9 months. Sloth bear cubs are nursed for 12-14 months. Cubs become independent after staying with the mother for 1.5 to 2.5 years before the mother starts mating again (Joshi et al., **1999**).

The composition of food items varies significantly according to place and season but is chiefly composed of insects and plants. They mostly dig for subterranean or mound-building termites and ants. Foraging on fallen fruits on the ground or climbing trees to obtain fruits and honey is also common. They rarely hunt mammals but feeding on livestock and carrion of wild mammals is reported. Feeding on agricultural crops and human food waste is recorded mostly from human-dominated landscapes.

Direct threats to adult sloth bears from a natural predator like the tiger (*Panthera tigris*) is rare. Sloth bear cubs could be killed by a tiger and other medium and large-sized carnivores like a

leopard (*Panthera pardus*) and dhole (*Cuon alpinus).* Sloth bears are illegally killed mostly for their bile, and cubs are removed from the wild to be trained as bears for street entertainment. The indirect but largest threat is from human-induced land use and land cover change. Connectivity between their habitat is getting lost due to habitat fragmentation and the remaining sloth bear populations are becoming small and isolated.

Globally, their populations have declined by almost 50% over the last three decades, and the species is categorized as "vulnerable" in IUCN Red List of Threatened Species (Dhariya et al., **2020**). Despite being recognized as an endangered species within Nepal, they are not listed in Appendix I list of protected wildlife in Nepal by the national parks and wildlife conservation act, 1973 and no conservation measures are currently in place. Detailed observation of sloth bear behavior from Nepal was reported by Laurie and Seidensticker (**1977**) and the initial intensive study on sloth bear ecology was conducted by Joshi (**1996**), who provided the first estimates of sloth bears home range, relative abundance, sociobiology and feeding ecology from Nepal. Sharma et al. (**2013**) and Dutta et al. (**2015**) provided the initial information on the genetic diversity of sloth bears from central India. Gene flow between the populations through functional corridors helped maintain a moderate genetic diversity in the sloth bear meta-population in central India. The status of sloth bear genetic diversity and population structure in Nepal is unknown, and the determinants of their habitat occupancy and interaction with humans are inadequately explored.

In this context, studies in this thesis explore the ecology, genetics and conservation of sloth bears from Nepal. In the first chapter, I explored the distribution and determinants of habitat use by sloth bears using an occupancy framework. In Chapter II, I used non-invasive DNA samples from feces and opportunistically obtained hairs to explore the genetic diversity and structure of the sloth bear population in Nepal. In chapter III, I investigated the diet of sloth bears using the fecal analysis. In chapter IV, I explored the human-sloth bear interaction by analyzing the incidents of human death and injuries from bear attacks. This is the first of its kind study exploring the genetics, ecology and conservation of sloth bears in Nepal.

CHAPTER I

DISTRIBUTION AND DETERMINANTS OF HABITAT USE BY SLOTH BEARS

INTRODUCTION

The sloth bear *Melursus ursinus* (Shaw, 1791; **Figure 1.1**) is an endemic mammal of the Indian subcontinent that occurs in a wide range of habitats, including dry or moist forest, savannah, scrublands, and grasslands (Garshelis et al., **1999**). Their populations have declined by almost 50% over the last three decades and the species is categorized as "vulnerable" in IUCN Red List of Threatened Species (Dharaiya et al., **2020**). Sloth bears have been extirpated from Bangladesh (Islam et al., **2013**) and possibly Bhutan (Dharaiya et al., **2020**; Garshelis et al., **1999**). They were once present along a continuous strip of forest and grasslands in southern Nepal until the 1950s, when the expansion of human settlement and agriculture confined them primarily to a few protected areas (Amin et al., **2018**; Jnawali et al., **2011**). They exist at a higher density in the central habitat at the Chitwan-Parsa complex (CPC). West of CPC, sloth bears exist at a much lower density in forest areas around Lamahi-Bhaluwang and the Banke-Bardiya complex (BBC) (Grashelis et al., **1999a**; Subdei et al., **2021**; Yadav et al., **2017**). Further west of BNP, they have been sighted in Shuklaphanta national park (Yadav et al., **2016**). In the east of CPC, a small sloth bear population persists in Trijuga forest near Koshi-Tappu Wildlife Reserve (Pokharel et al., **2022**; Shah et al., **2018**). Rugged foothills of the Himalayas, also known as 'Siwalk'or 'Churia' is considered to provide critical habitat for sloth bears (Subedi et al., **2021**).

Species distribution and habitat use are primarily determined by the availability and spatial variation of food resources and the extent of natural and anthropogenic threats (Ceballos & Ehrlich, **2002**; Schipper et al., **2008**). Unlike other carnivores, sloth bears are specially adapted for a myrmecophagous diet (Joshi et al., **1997**, **1999**; Sacco & Valkenburgh, **2004**). The availability of termites and fruits is important for sloth bear diet and influences their habitat use (Bargali et al., **2004**; Baskaran et al., **2015**; Joshi et al., **1997**; Khanal & Thapa, **2014**; Laurie & Seidensticker, **1977**; Mewada, **2015**; Mewada et al., **2019**; Palei et al., **2014**, **2020**; Philip et al., **2021**; Rather et al., **2020**; Sukhadiya et al., **2013**). In fruit-rich areas, sloth bears play an important role in dispersing seeds and regeneration of fruit plants, thereby aiding in the maintenance of forest structure and composition (Sreekumar & Balakrishnan, **2002**). Reports of sloth bear from human-dominated landscapes (Akhtar et al., **2004**, **2007**; Bargali et

al., **2012**; Puri et al., **2015**) and the prevalence of human–sloth bear conflict in India (Bargali et al., **2005**; Debata et al., **2017**; Dhamorikar et al., **2017**; Garcia et al., **2016**; Ratnayeke et al., **2014**; Sharp et al., **2020**) and Nepal (Acharya et al., **2016**; Lamichhane et al., **2018**; Pokharel & Aryal, **2020**; Silwal et al., **2017**) suggest a high nexus between humans and sloth bears. They largely prefer habitats away from human disturbance (Babu et al., **2015**; Ghimire & Thapa, **2014**; Joshi et al., **1999**; Ratnayeke et al., **2007**; **2007a**). Removal of the individuals through poaching or live capture for use as "dancing bears" is not common, but maybe detrimental enough for a population that is already small, isolated, and threatened.

Chitwan National Park (CNP) is a key for wildlife habitat in Nepal. The highest density of sloth bears in Nepal is reported to occur in CNP (Garshelis et al., **1999a**). Translocation of this species from areas of high occupancy to suitable habitats outside CNP is recommended for its long-term conservation (Jnawali et al., **2011**). However, the lack of recent information on sloth bear distribution and habitat use patterns has hindered its conservation and management. Estimating their density and abundance is challenging due to their elusive nature and the difficulty in identifying individuals. The application of conventional methods such as camera traps, telemetry, and genetic analysis can provide valuable information, but are logistically challenging and resource intensive. In contrast, occupancy methods account for imperfect detection to provide reliable ecological information when species research and monitoring are resource constrained or logistically challenging. This study was the first of its kind to use occupancy models to study the distribution and habitat use of sloth bears in Nepal. This study established the current presence of sloth bears across the park and provided information on their distribution, habitat use, and associated covariates. The results will have far-reaching implications for the research, management, and conservation of sloth bears in Nepal.

MATERIAL AND METHODS

Study area

This study was carried out in CNP, Nepal. CNP, a UNESCO world heritage site, was the first area in Nepal to receive protected status and covers 953 km^2 (**Figure 1.2**). The park is located in the south-central part of Nepal along the floodplains of the Rapti, Reu, and Narayani rivers. The major vegetation cover consists of deciduous sal (*Shorea robusta*) forest (70%) followed by grassland (10%), riverine forest (7%), mixed forest (7%), and wetlands (4%). The successional gradient of the park is formed of 10 grassland and 3 forest associations (Lehmkuhl, **1999**). Temperatures reach a maximum of 38°C during the summer and drop to a

minimum of 6°C in winter. The average annual rainfall in the area is 2400 mm, most of which occurs during the summer monsoon. The matrix of different habitat conditions and climates makes this area a biodiversity hotspot. CNP harbors the largest populations of rhinos (*Rhinoceros unicornis*), tigers (*Panthera tigris*), sloth bears, and many other threatened flora and fauna in Nepal. The park is also a part of the Terai-Duar savanna and grasslands ecoregion, which is listed among the 200 most important areas globally (Dinerstein et al., **2017**). Its resources are also of great importance to the livelihood of local people who depend strongly on forest resources for farming and livestock (Stræde & Treue, **2006**). Local people are allowed to enter the core area of the park for approximately 2 weeks annually to collect grass, but the pressure for illegal access to park resources persists throughout the year (Sharma & Shaw, **1993**; Stræde & Helles, **2000**). The 750 km^2 area surrounding the park is delineated as a buffer zone. The buffer zone provides an extended habitat for wildlife and forest products for local communities, and also serves as an important area for eco-tourism activities. Although poaching has not been excessive in recent years, human–wildlife conflicts are frequent in and around the park (Acharya et al., **2016**; Lamichhane et al., **2018**; Silwal et al., **2017**). Furthermore, the impacts of global climate change on the local flora and fauna are predicted to intensify (Thapa et al., **2015**).

Study design and field methods

Square grids of 4 × 4 km was laid over a map of the study area using QGIS 3.16. With a random starting position, I surveyed the grids in a checkboard pattern, sampling every other gird at a systematic spacing of 4 km. This checkerboard sampling design minimized autocorrelation between sampling grids, facilitated the concentration of survey efforts, ensured an even coverage of the large and hostile study area, and was suitable for studying medium-to-large mammals with relative ease. The same sampling method has been used to study elephants (*Elephas maximus*) (Thapa et al., **2019**), tigers (Thapa & Kelly, **2017**), and four-horned antelope (*Tetracerus quadricornis*) (Krishna et al., **2008**). This method yielded a total of 45 grids which covered 720 km^2 (43% coverage of the park and buffer area). The grid size was comparable to the home range of sloth bears, which is estimated to be 9 and 14 km^2 for male and female sloth bears, respectively (Joshi et al., **1995**). Sign surveys were conducted within the 45 grids, with a sampling effort of 4 km in each grid. Field team searched for sloth bear signs along a 4-km-long random walking trail that was divided into 20 continuous segments of 200 m. Using the handheld GPS, grids on the ground were identified and navigated after randomly selected a starting point in the first segment. Within these segments, sloth bear

detection/non-detection data and associated ecological, landscape, and anthropogenic variables were collected. Detection of signs and covariates detected in a segment was recorded as "1", otherwise "0". If sampling could not proceed due to logistic reasons, or the area was outside park jurisdiction or under intense human use, the segment was treated as a missing observation. To standardize the detection process, avoid biases that may arise from the duplication, misidentification, and decay of signs, and adhere to the closure assumption in occupancy studies, only the first encounter of fresh sloth bear signs, that is, direct sightings, footprints, and scat along sample trails were included (Karanth et al., **2011**; Morin et al., **2016**; Putman, **1984**; Rota et al., **2009**). Field surveys were carried out between March and June 2020. Sloth bears and Asiatic black bears are sympatric in the landscape further west of the current study area, particularly in the outer Himalaya and the intervening valleys in Uttarakhand (India) and possibly in BNP (Nepal) (Bargali, **2012**; Kadariya et al., **2018**; Seidensticker et al., **2011**; Yadav et al., **2017**). However, Asiatic black bears have not been recorded in the present study area (Jnawali et al., **2011**; Subedi et al., **2021**; Lamichhane et al., **2016**). The field team involved trained wildlife technicians who were able to unambiguously identify signs of bear presence.

Covariate selection

A mix of six plausible remotely sensed and ground-based variables that reflected the characteristics of the landscape, habitat conditions, and persistent anthropogenic pressures, as well as the availability of major food resources, based on a review of the available literature, was selected. For a small study area with a few sample sites, the model loses its power of explanation and the number of unwanted errors increases as the number of variables is increased in the model. It is generally advised to use 1 variable per 10 sites in an occupancy model. Thus, following the principles of parsimony, three site covariates and three sample covariates were included (**Table 1.1**). Termites, fruits, and disturbance were included as sample covariates and measured them in the field. It was predicted that the presence of termites and fruit trees would have a positive influence on bear detection and occupancy. In each segment, the presence/absence of termite mounds and fruit plants that were frequently consumed by sloth bears during the dry season in the study area was recorded (Khanal & Thapa, **2014**). These variables were quantified at the grid level as the proportion of replicate segments in which they were present. Sloth bears have been reported to avoid human and livestock disturbances (Babu et al., **2015**; Puri et al., **2015**), but they have also been reported from human-dominated landscapes with degraded habitats (Bargali et al., **2012**). Human

disturbance, livestock disturbance, and fire in along search trails was combined as a measure of disturbance. A single disturbance score was prepared by taking the average value across segments. Sloth bears are thought to prefer relatively dry, rugged, and forested habitats (Puri et al., **2015**; Srivathsa et al., **2018**). Enhanced vegetation index (EVI) was extracted from Landsat 8 satellite data as a measure of vegetation productivity. Topographic ruggedness index was computed using the SRTM digital elevation model (Riley et al., **1999**). In Nepal, it has been reported that sloth bears move to grasslands during the dry season and prefer to remain in forests during the wet season (Joshi et al., **1995**). Tree cover data prepared by Hansen et al. (**2013**) was extracted using QGIS 3.16 as a proxy of habitat condition, with a higher cover indicating a forested habitat and a lower cover indicating a grassland habitat. All site covariates were first checked for collinearity. The results showed that none of the covariates were significantly correlated (Pearson's $|r| = <0.5$). All site covariates were scaled and normalized before running occupancy models (Krishna et al., **2008**; Panthi et al., **2017**). Based on the literature on sloth bear ecology, it was hypothesized that sloth bear occupancy would increase with the increasing presence of termites and fruits and in dry, forested, and heterogeneous habitats.

Occupancy estimation and modeling the effects of covariates

Spatial replication can serve as a good surrogate for temporal replication in occupancy studies of sloth bears if an appropriate modeling framework is used to account for the particular sampling process (Srivathsa et al., **2018**). Standard occupancy models (MacKenzie et al., **2002**) that assume independence between replicates to separate non-detection from absence were not suitable for single-season dataset collected along adjacent trail segments. However, Hines et al. (**2010**) modeling approach accounts for such spatial dependence between replicates. This approach does not assume that in an occupied grid all spatial replicates are occupied but rather estimates two additional parameters, θ^0 and θ^1, representing the replicate-level presence of the species, which is conditional on signs being absent or present in the previous replicate, respectively. Standard single-season occupancy model and correlated detection model was compared to identify an appropriate model for the data. These models were compared based on the Akaike information criterion (AIC) and the model with the lowest AIC score was selected (Burnham & Anderson, **1998**). This comparison indicated the spatial dependencies in sign detection in the replicate segments, with a lower AIC value (better model performance) for the spatial correlation model than the standard occupancy model (**Table 1.2**). Therefore, spatial correlation model (Hines et al., **2010**) was used for further analysis. Single-species

single-season occupancy analysis using a maximum likelihood-based approach wa run in the PRESENCE 2.12.31 software (Hines, **2006**). While modeling covariate effects, possibility that covariates influencing sloth bear presence would also affect sloth bear detectability due to occupancy–abundance relationships could not be ignored so, covariates influencing occupancy were also included to test for their effect on detectability. Two-step process was followed to estimate the probability of detection (p) and probability of bear occurrence (ψ). First, detection was modeled by keeping a global covariate structure for the occupancy model as ψ (Global). This global model included all six covariates (i.e., termites, fruits, disturbance, tree cover, terrain heterogeneity, and vegetation productivity) that could influence the probability of bear occurrence. Different combinations of the detectability covariates were modeled for ψ (Global) and the best model was selected based on the minimum AIC. In the second step, the probability of occupancy (ψ) was modeled by keeping the top detection model from the previous step as a constant structure in the detection model (Doherty et al., **2012**; Panthi et al., **2017**; Srivathsa et al., **2018**). Covariates were modeled stepwise beginning with the univariate model structure. If the addition of covariates improved the model fit, then it was retained to be combined with the other covariates in multivariate models. The candidate model set included either the single or additive effects of two or more covariates to investigate the influence of covariates on occurrence. Model fit was assessed using the parametric bootstrap procedure (MacKenzie & Bailey, **2004**). The covariate models were compared and ranked using an information theoretic approach, relying on the AIC for testing relative model fits. Due to the inherent advantage of model averaging (Burnham & Anderson, **1998**), the final occupancy estimates and associated standard error were averaged across the model set. To infer the relative influence of covariates on occurrence, the estimated β-coefficients of the model containing the particular covariate was used.

RESULTS

First, the standard occupancy model (MacKenzie et al., **2002**) and spatial correlation model were compared (Hines et al., **2010**). The model developed by Hines et al. (**2010**), which accounted for spatial dependencies in sign detection along the replicates, received more support from the data compared to MacKenzie et al. (**2002**) modeling approach (ΔAIC of ψ (.),p(.) =16.7, relative to ψ(.) th0(.),th1(.),p(.),th0pi(.)). Models with different combinations of the detectability (p) covariates were fitted, keeping the global covariate structure for occupancy ψ (Global) (**Table 1.2**). All candidate models had some level of support based on the AIC values

and corresponding model weights, and no single model received unequivocal support from the data. Detectability was estimated from the best performing model with the lowest AIC value (p = .25 ± $0.05_{SE,}$ W_i = 0.37). This detectability model suggested that sloth bear detection increased with an increase in the presence of termite mounds (β_{Term} = 0.75 ± 0.34_{SE}), drier habitats (β_{EVI} = −0.46 ± 0.19_{SE}), and non-heterogeneous terrain (β_{TRI} = −0.36 ± 0.25_{SE}). This detectability model was used in subsequent analyses to model occupancy probability. Occupancy models were fitted in a stepwise additive process (**Table 1.3**). All covariate structures were ran for modeling occupancy using the next best detection model (Term + EVI, ΔAIC = 0.13, W_i = 0.35) as it also received similar support from the data. Among the set of candidate models, the model including termites (β_{Term} = 1.08 ± $0.60_{SE,}$ W_i = 0.76) was the best occupancy model. Because of the inherent advantages of model averaging (Burnham & Anderson, **1998**), probability of sloth bear occupancy (ψ = 0.69 ± 0.24_{SE}) was averaged across all models.The model-specific β-coefficient value from the occupancy models for termites (β_{Term} = 1.08 ± 0.60_{SE}), fruit (β_{Frut} = 0.10 ± 0.14_{SE}), and terrain heterogeneity (β_{TRI} = 0.50 ± 0.29_{SE}) indicated their positive influence on sloth bear occupancy, whereas the negative β-coefficients for disturbance (β_{Dist} = −0.26 ± 0.16_{SE}), tree cover (β_{Tcov} = −0.14 ± 0.14_{SE}), and vegetation productivity (β_{EVI} = −0.31 ± 0.23_{SE}) indicated their negative associations with sloth bear habitat occupancy (**Table 1.4**).

DISCUSSION

Occupancy and detection

This study provided the first occupancy estimate for sloth bears from CNP, Nepal. Their signs were detected in 21 of the 45 grids sampled, giving a naive occupancy of 46%. By explicitly incorporating the imperfect detection of animals into the occupancy estimate, the proportion of area occupied by sloth bears in CNP substantially increased to 69% with a model-averaged detection probability of 0.25. Hines et al. (**2010**) approach estimates the probability of detecting the species in a spatial replicate, given its presence in the site as well as its presence in the replicate, while the MacKenzie et al. (**2002**) approach calculates the probability of detecting the species in a site given its presence in the site. Because of this additional conditioning on presence in the spatial replicate, estimates from Hines et al. (**2010**) tend to be higher than from the MacKenzie et al. (**2002**) approach. The large increase in habitat occupancy over the naive estimate highlights the importance of considering the imperfect detection using an appropriate occupancy approach when studying sloth bears.

Estimates of habitat occupancy by sloth bears and effects of covariates vary across studies within its distribution range. Discrepancies in the landscape composition, scale of the study, nature of data, and methods used may preclude direct comparisons of occupancy estimates and the effect of covariates across studies in different landscapes. In India, habitat occupancy was estimated at 57% in Bhadra Wildlife Sanctuary (Srivathsa et al., **2018**), 61% in the Malenad region (Puri et al., **2015**), 79% in different regions of northeastern Karnataka (Das et al., **2014**), and 83% in the Mudumalai Tiger Reserve (Ramesh et al., **2012**). Most of the reported studies of sloth bear occupancy in India are from the Western Ghats, which has large blocks of contiguous forest cover and a diversity of habitat conditions, with semi-evergreen, tropical moist, dry deciduous, thorny forest, and scrub landscapes interspersed with agricultural areas and rocky outcrops, while current study area was relatively homogenous with small grasslands patches interspersed in a deciduous forest habitat. Sloth bears have a small home range (9–14 km^2) in CNP (Joshi et al., **1995**) compared to Central India (12–85 km^2) (Yoganand, **2005**), indicating a possible availability of resource-rich habitat for sloth bears in CNP. In the unprotected Trijuga forest area of Udaypur and Saptari districts, approximately 200 km east of CNP, the probability of habitat use was estimated much lower at 43% (Pokharel et al., **2022**). Variation in patterns of habitat use by sloth bears is a characteristic of most bear species; bears exhibit high diversity, complexity, and adaptability in their use of habitat mostly depending on the diversity and quantity of foods, and habitat conditions providing shelter and safety from human and non-human predators like tigers (Garshelis, **2022**). Species tend to exhibit occupancy–abundance relationships (Gaston et al., **2000**; Zuckerberg et al., **2009**), particularly in small and homogenous areas (Hui et al., **2009**). This indicates that sloth bears are fairly abundant and have a wide distribution throughout the park. Relatively high-occupancy areas (psi > 0.70) were located in the central-north area of the park (**Figure 1.3**). Both Laurie and Seidensticker (**1977**), as well as Garshelis et al. (**1999**), recognized that there was an uneven distribution of sloth bears with a high density in the alluvial floodplains and a relatively lower density in the rest of the park, which is dominated by upland Sal forest.

Influence of covariates

The importance of different covariates was assessed based on the magnitude of the estimated β-coefficients. The summed AIC weight from the models could not be used to determine the relative importance of covariates because the model set was not balanced with respect to the representation of covariates across the models. Because occupancy covariates were scaled and normalized, their β-coefficient represented the change in logit (ψ) for 1 standard deviation

change in the covariate. The model-specific β-coefficient value from the occupancy models indicated that termites, fruit, and terrain heterogeneity had positive influences on sloth bear occupancy, whereas disturbance, tree cover, and vegetation productivity had negative associations with sloth bear habitat occupancy.

The food resources of sloth bears, particularly termites, had a relatively strong influence on sloth bear occupancy. This was expected because sloth bears are opportunistic omnivores that are specialized for a myrmecophagous diet (Joshi et al., **1997**, **1999**). Studies of their feeding ecology have shown that termites are the most frequent dietary item throughout the year, while fruit consumption is dependent on seasonal availability (Bargali et al., **2004**; Palei et al., **2014**, **2020**; Ramesh et al., **2012**; Rather et al., **2020**; Yoganand, **2005**). In Chitwan, fruits are available for a short period from April to August, while termites tend to increasingly dominate the sloth bear's diet. Their presence was detected in 52% of scats in the 1970s (Laurie & Seidensticker, **1977**), 81% during the 1990s (Joshi et al., **1997**), and 92% in the 2010s (Khanal & Thapa, **2014**). The presence of sloth bears was negatively associated with tree cover, indicating a preference for open grassland habitats. Forest and grassland associations provide a habitat mosaic and are a key determinant of mammalian abundance in CNP (Bhattarai & Kindlmann, **2012**; Lehmkuhl, **1999**). Another study in CNP suggested that an abundant food supply during the dry season would prompt the movement of sloth bears from dense sal forests to open grassland areas (Joshi et al., **1995**). Despite the higher density of termite mounds in sal forest compared to mixed or open habitats (Axelsson & Andersson., **2012**; Chakraborty & Singh, **2020**), based on their diggings there was more evidence of sloth bears in grassland habitats during the dry season (Garshelis et al., **1999**). During the dry season, the soil in upland sal forest habitats becomes stiff (Malla & Karki, **2016**). Termites excavate deeper into the ground to seek moisture (Ahmed & Pradhan, **2018**; Sen-Sarma, **1974**). Obtaining termites from stiff mounds becomes difficult in forests compared to grassland habitats where the soil is relatively loose, making it less likely that sloth bears will dig into mounds and underground colonies of termites and ants (Garshelis et al., **1999**; Joshi et al., **1995**, **1997**). It seems likely that the distribution of sloth bears in CNP is seasonal, and depends on the seasonal variation of food sources. Therefore, results in this study may have differed if multi-season sampling were used. There may also be negative associations with tree cover because the sampling design may have resulted in higher coverage of peripheral areas that consist of grasslands, riverine forests, and buffer zones, while most of the dense forest lies in the core of the park.

Habitat occupancy was negatively associated with disturbance, indicating that sloth bears avoid disturbed and degraded habitats. Human activities are the predominant factors that determine areas of occupancy within the sloth bear range (Seidensticker et al., **2011**). Multiple factors, such as individual behavior and evolutionary history, as well as the frequency, duration, and scale of disturbance events, influence species occupancy (Graham et al., **2021**; Iwasaki & Noda, **2018**; Sousa, **1984**). In relatively intact landscapes, such as the Western Ghats in India, sloth bears have been shown to avoid disturbance (Babu et al., **2015**; Das et al., **2014**; Puri et al., **2015**), while in human-dominated landscapes they have been reported to tolerate some degree of disturbance (Bargali et al., **2012**), often consuming cultivated crops (Palei et al., **2020**) and human food waste (Prajapati et al., **2021**), and causing conflicts with humans (Debata et al., **2017**; Dhamorikar et al., **2017**). Human–sloth bear conflict is common throughout the year in CNP, suggesting that sloth bears perceive humans as a threat (Acharya et al., **2016**; Lamichhane et al., **2018**; Silwal et al., **2017**). Previous reports of sloth bears from degraded forests were likely because the study was conducted in an area of degraded forests and should not be taken as the norm in terms of sloth bear ecology (Rather et al., **2021**) but rather as the manifestation of a high nexus between sloth bears and humans in the landscape. Sloth bears might use disturbed habitats in moderation for food, water, and shelter. In a few instances, sloth bears and their signs were sighted in fissures and crevices along the forest, and along river paths used by humans. A rugged terrain provides sloth bears with resting and denning sites (Akhtar et al., **2007**; Bargali et al., **2012**; Baskaran et al., **2015**), as well as cover to hide their cubs from potential predators, such as tigers. Terrain heterogeneity was positively related to the habitat occupancy of sloth bears. Enhanced vegetation productivity was negatively associated with sloth bear occupancy, suggesting a preference for dry habitats. A similar preference for heterogeneous and dry habitats was reported for sloth bears in India (Puri et al., **2015**).

The 95% confidence interval of β-coefficients for the occupancy covariates overlapped zero indicating weak statistical support for the magnitude of influence of variables. Current study results were limited by the small sample size and single-season sampling. The scale of study, use of grid size comparable to the home range of sloth bears in the study area, and adoption of a checkerboard sampling design for wider coverage, and efficient sampling amid logistic challenges resulted in a relatively small sample size. While few studies from small areas report estimates based on small sample size (Lamichhane et al., **2020**; Thapa et al., **2017**), others use smaller sampling units (Babu et al., **2015**; Das et al., **2014**;) or use occupancy estimates as the

intensity of habitat use (Thapa & Kelly, **2017**; Thapa et al., **2019**). Sampling units should be larger than the estimated home range of species to measure the true estimate of occupancy (Karanth et al., **2011**; MacKenzie & Royle, **2005**). It is suggested that for a rare species, it is more efficient to survey more sampling units less intensively, while for a common species fewer sampling units should be surveyed more intensively (MacKenzie & Royle, **2005**). Limited sample and poor detectability make it difficult to disentangle the occupancy and detection process, and fully retrieve species–environment relationships (Guillera-Arroita et al., **2014**; MacKenzie & Bailey, **2004**). Furthermore, the use of a step-wise modeling approach may increase the risk of the possible overfitting of data that might not hold up to generalizations. Cautious application of occupancy methods by sampling in more sites with larger replication may produce more precise and robust inferences. The additional quantified measurement of active termite mounds, underground colonies of termites and ants, fruit-bearing trees, and disturbance intensity may be required to provide a deeper understanding of the ecological interactions and behavioral responses of the sloth bear. The results would likely change if standard multi-season sampling were adopted. Nevertheless, findings from this study fill an important information gap on sloth bears in Nepal, while many contemporary wildlife research and conservation programs are focused on large and charismatic species.

CONCLUSIONS

This study provides the first occupancy estimates for sloth bears in CNP. Sloth bears were widely spread but elusive. The use of habitat was strongly and positively influenced by the presence of termites in the habitat. mounds. Sloth bears prefer dry and rugged habitats and avoid disturbed and degraded habitats. Multi-season occupancy analysis using quantified measurements of habitat parameters is recommended to understand seasonal variations in occupancy.

Figure 1.1. Picture of the sloth bear female with cubs. The sloth bear are the only ursid exhibiting this behaviour of carrying their cubs in its back.

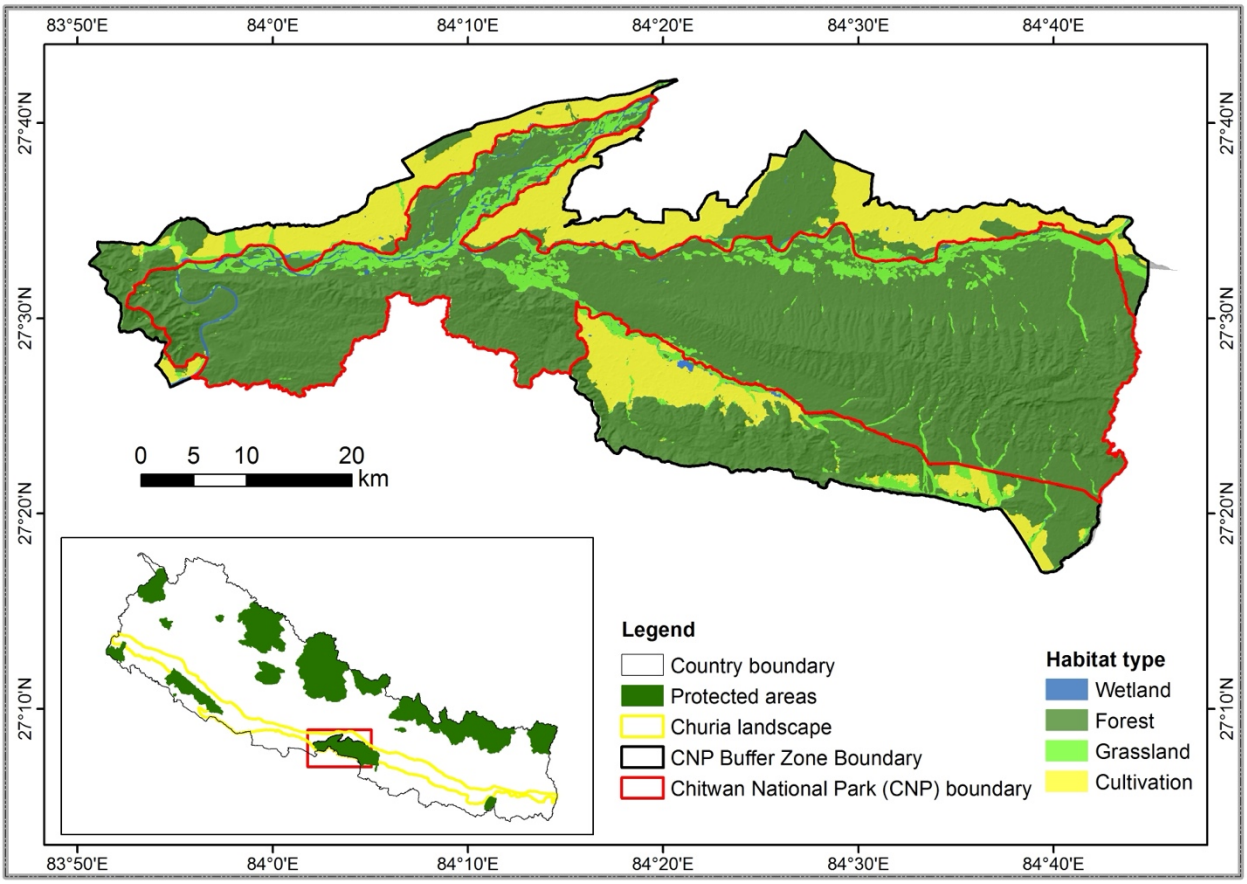


Figure 1.2. Study area map for sloth bear occupancy study. Major habitat types in Chitwan national park and its buffer zone is shown. Green areas represent the forest and grassland habitat, yellow areas indicate human settlement and cultivation .

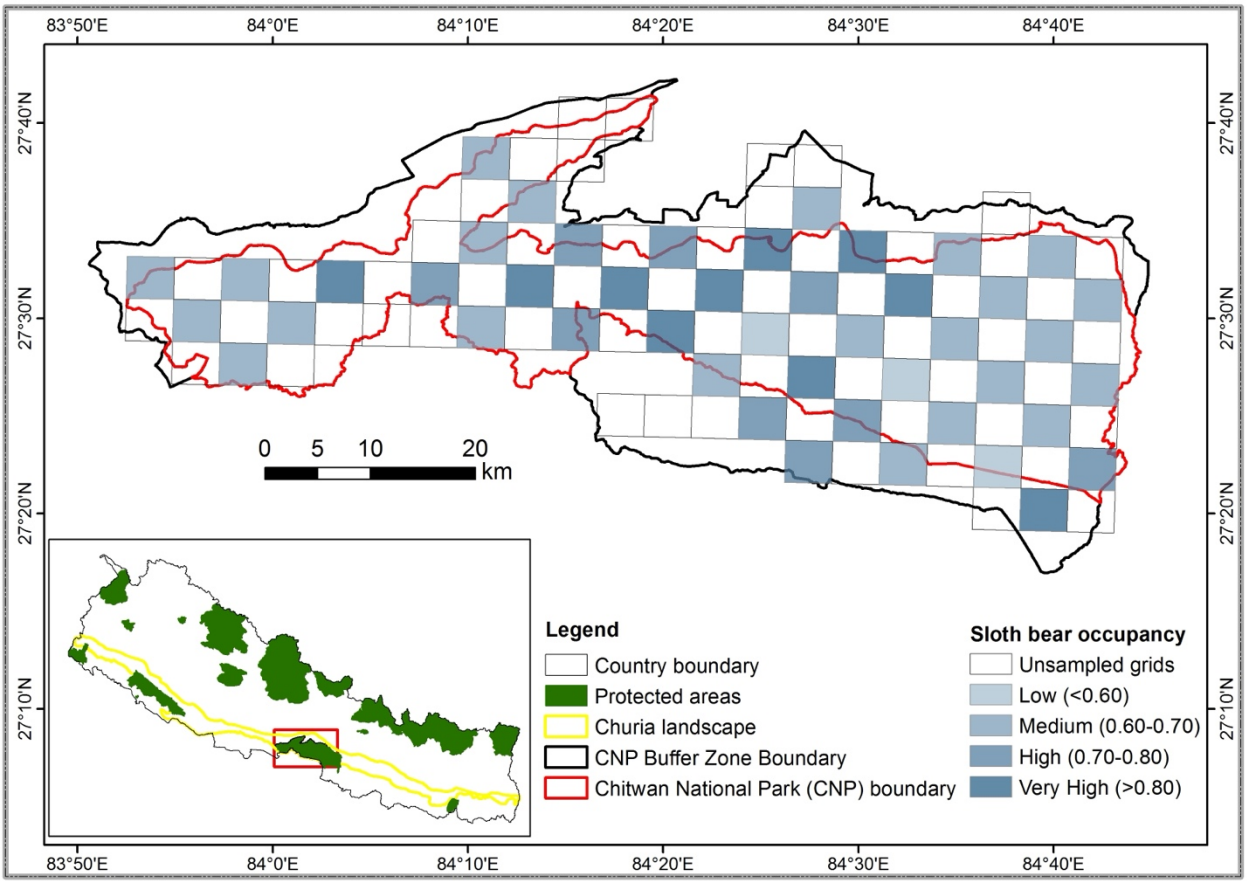


Figure 1.3. Habitat occupancy probability map of sloth bear in CNP. Sloth bears had moderate to high occupancy with most high occupancy areas in the north and central part of CNP.

Table 1.1 Description of variables used in occupancy analysis of sloth bears. The expected positive or negative influence on detection (p) and occupancy (ψ) probability is presented based on reference literatures.

Covariate	Description	ψ	p	Reference
Enhanced vegetation index (EVI)	The EVI is similar to the normalized difference vegetation index but with a correction for some atmospheric conditions and canopy background noise, and is more sensitive in areas with dense vegetation cover. The EVI was derived from Landsat 8 thematic mapper imagery. A high EVI indicates moist and more productive areas, while a low EVI indicates drier areas.	−	−	Sloth bears prefer relatively dry habitats and areas with a high vegetation productivity negatively influence sloth bear occupancy (Puri et al. 2015; Seidensticker et al. 2011).
Tree cover (Tcov)	Tcov was derived from data prepared by Hansen et al. (2013) and downloaded from the Global Forest Change website. A high Tcov indicates forested habitat, while a low Tcov indicates relatively open lowland habitats, such as grasslands.	+	+	Sloth bears have been reported in a wide range of habitats, mostly forests, with some seasonal variation depending on the availability of food resources (Dharaiya et al. 2020; Joshi et al. 1995).
Terrain ruggedness index (TRI)	The TRI was computed using the Shuttle Radar Topography Mission digital elevation model (Riley et al. 1999) in QGIS 3.16. High coefficient of variation values in TRI indicated a large heterogeneity in terrain.	+	+	The rugged terrain provides sloth bears with resting and denning refuge and positively influences sloth bear occupancy (Yoganand et al. 2005; Akhtar et al. 2007; Puri et al.2015).
Disturbance (Dist)	Presence/absence scores of humans, livestock, and fire were recorded in the field and pooled to obtain an average Dist score as a surrogate for human impact. A high Dist score indicated more human impact, while a low score indicated less human impact on the habitat.	−	−	Sloth bears largely prefer habitats away from human disturbance (Joshi et al. 1999; Das et al. 2014; Babu et al. 2015, Puri et al. 2015; Baskaran et al. 2015).
Fruit (Frut)	The presence/absence of fruit plants most frequently consumed during the dry season in Chitwan (Khanal & Thapa, 2014) were pooled to obtain an average fruit score for each grid and recorded as the proportion of trail segments with the presence of fruit trees.	+	+	Termites and fruits are the major components of sloth bear diet that influence its distribution and habitat use (Dhariya et al. 2020; Das et al. 2014; Khanal & Thapa, 2014; Joshi et al. 1997; Laurie and Seidensticker, 1977).
Termite (Term)	The presence/absence of termites was recorded in the field and a single score for each grid was obtained by quantifying it as the proportion of trail segments with the presence of termite mounds.	+	+	

Table 1.2. Summary of the model selection process for factors influencing detection probability of sloth bears

Model	AIC	ΔAIC	Wi	ML	Model
ψ (Global),th0(),th1(),p(Term+EVI+TRI),th0pi()	468.46	0.00	0.37	1.00	14.00
ψ (Global),th0(),th1(),p(Term+EVI),th0pi()	468.59	0.13	0.35	0.94	13.00
ψ (Global),th0(),th1(),p(Term),th0pi()	470.71	2.25	0.12	0.32	12.00
ψ (Global),th0(),th1(),p(EVI),th0pi()	472.83	4.37	0.04	0.11	12.00
ψ (Global),th0(),th1(),p(.),th0pi()	472.99	4.53	0.04	0.10	11.00
ψ (Global),th0(),th1(),p(TRI),th0pi()	473.93	5.47	0.02	0.06	12.00
ψ (Global),th0(),th1(),p(Dist),th0pi()	474.35	5.89	0.02	0.05	12.00
ψ (Global),th0(),th1(),p(Frut),th0pi()	474.39	5.93	0.02	0.05	12.00
ψ (Global),th0(),th1(),p(Tcov),th0pi()	474.94	6.48	0.01	0.04	12.00

Table 1.3. Summary of the model selection process for factors influencing occupancy probability of sloth bears

Model	AIC	ΔAIC	W_i	ML	K
ψ (Term),th0(),th1(), p(Term+EV+TRI),th0pi()	465.85	0	0.76	1	9
ψ (Dist),th0(),th1(), p(Term+EVI+TRI),th0pi()	470.95	5.10	0.06	0.08	9
Ψ(.),th0(),th1(), p(Term+EVI),th0pi()	471.63	5.78	0.04	0.06	7
ψ (EVI),th0(),th1(), p(Term+EV+TRI),th0pi()	471.93	6.08	0.04	0.05	9
ψ (.),th0(),th1(), p(Term+EV+TRI),th0pi()	472.00	6.15	0.04	0.05	8
ψ (TRI),th0(),th1(), p(Term+EV+TRI),th0pi()	472.03	6.18	0.03	0.05	9
ψ (Tcov),th0(),th1(), p(Term+EV+TRI),th0pi()	473.04	7.19	0.02	0.03	9
ψ (Frut),th0(),th1(), p(Term+EV+TRI),th0pi()	473.44	7.59	0.02	0.02	9

Table 1.4. Comparison of the relative strength of covariate influence on sloth bear occupancy and detection.

Covariates	Occupancy			Detection		
	β(SE)	LCI	UCI	β (SE)	LCI	UCI
Termite	1.08 (0.60)	-0.09	2.25	0.75 (0.34)	0.09	1.41
Fruit	0.10 (0.14)	-0.17	0.38	0.27 (0.35)	-0.42	0.96
Disturbance	-0.26 (0.16)	-0.56	0.05	0.69 (0.87)	-1.01	2.39
Tree cover	-0.14 (0.14)	-0.42	0.14	0.04 (0.16)	-0.27	0.35
Terrain ruggedness	0.50 (0.29)	-0.08	1.07	-0.30 (0.31)	-0.91	0.31
Vegetation productivity	-0.31 (0.23)	-0.76	0.13	-0.35 (0.20)	-0.74	0.04

CHAPTER II

GENETIC DIVERSITY AND POPULATION STRUCTURE OF SLOTH BEARS FROM NEPAL

INTRODUCTION

The sloth bear *Melursus ursinus* (Shaw, **1791**), locally known as Kathe Bhalu in the Nepali language, is among many large carnivores globally threatened with extinction. In general, large mammals like sloth bears are highly vulnerable to negative genetic consequences from habitat changes due to their specialized feeding behavior and requirements for substantial and intact habitats. Habitat fragmentation can reduce dispersal, resulting in the reduction of gene flow, an increase in random genetic drift, and inbreeding which produces a negative effect on genetic variation (Frankham et al., **2002**; Frankham, **2010)**. The situation becomes particularly concerning for sloth bears as their population and geographic range have declined sharply, remaining habitats are patchy, and they face greater impacts of human footprints (Dhariya et al., **2020**; Wolf & Ripple, **2018**).

Sloth bears occur at the highest density in CNP and at a much lower density in BNP in the west and TJF in the east (Jnawali et al., **2011**). In recent years, improved law enforcement and community-based conservation have contributed to the expansion of forest cover and the recovery of the population of some large mammals like tigers (DNPWC & DFSC, **2022**). An increase in population can contribute to maintaining a high genetic diversity if there is adequate gene flow. An unstable population that has undergone a sharp decline and has an inadequate genetic exchange, may not be able to maintain similar levels of genetic diversity and population structure (Jansson et al., **2012**). Large and well-connected habitats are essential to facilitate genetic exchange between populations and to maintain greater genetic diversity in sloth bears (Dutta et al., **2015**). Previous studies on sloth bears in Nepal have reported a relatively smaller home range size, greater dependence on the myrmecophagous diet, and valuable insights into its sociobiology (Garshelis et al., **1999**; Joshi et al., **1995**, **1997**, **1999).** However, evidence for the maintenance of genetic diversity and population structure is nonexistent, as bears, in general, and sloth bears, in particular, have received very little research and conservation priority in Nepal.

Greater mobility over large distances and utilization of wider resources facilitates the maintenance of high genetic diversity and little population clustering in large carnivores (Pečnerová et al., **2021**). The natural and anthropogenic barriers in their dispersal can bring

changes in genetic diversity and population structure (Dixon et al., **2007**; Lino et al., **2019**; Ohnishi et al., **2007**; Silva et al., **2018**; Vaeokhaw et al., **2020**). Habitat specialist species that depend on specific resources are expected to have low genetic diversity and high genetic differentiation compared to habitat generalists that can consume various food resources and survive on a wide range of habitat conditions (Pasinelli, **2022**). Further, male-biased dispersal and female-biased philopatry are known to exert a considerable influence on the genetic structure of the bear population by limiting mating between closely related individuals (Shirane et al., **2019**). Sex and life-stage-specific differences in habitat use by sloth bears is also reported (Garshelis et al., **1999**; Joshi et al., **1995**, **1999**) that can influence their genetic diversity and structure. Understanding the genetic diversity and structure of existing sloth bear populations is an important and effective way to allocate limited resources to the conservation and management of wildlife. Failure to account for existing patterns of genetic variation in conservation and management increases the risk of gene homogenization, thereby reducing the resilience and adaptability of the species.

In this context, the genetic status of sloth bears in Nepal was explored using non-invasive DNA samples. Genetic data acquired without disturbing individuals (through feces and hair samples) has been widely used to obtain valuable ecological and genetic information on wildlife species (Dutta et al., **2014**; Kadariya et al., **2018**; Thapa et al., **2018**). DNA obtained from non-invasive samples are usually degraded compared to blood or tissue samples. However, careful collection, storage, and transportation of fresh non-invasive samples can provide comparable results and is a suitable method when the species under study is endangered, challenging to capture or the study has limited resources. In this first-of-its-kind study on sloth bears from Nepal, I aimed to understand the current genetic diversity and population structure of sloth bears in Nepal. Microsatellite markers and mitochondrial DNA analysis were used to reveal the genetic variation, structure, and evolutionary relationships of sloth bears from Nepal.

MATERIAL AND METHODS

Study Area

The study was carried out along the Churia-Terai region of the outer Himalayan landscape in Nepal (**Figure 2.1**). It consists of a geologically fragile mountain range along the foothills of the Himalayas known as ‘Siwalik’ or ‘Churia’, and alluvial flood plains formed by tributaries of the Ganges River and the associated valleys. Field sampling for genetic data was TJF, CNP and BNP. CNP covers 953 km^2 and is in the south-central part of Nepal along the floodplains

of the Rapti, Reu, and Narayani rivers. Bardia National Park covers 986 km^2 and is situated in the southwest part of Nepal along the flood plains of the Karnali and Babai rivers. TJF covers 430 Km^2 and is located in the southeastern part of Nepal along the bank of the Triyuga and Koshi rivers.

Bio-climatic conditions: The region experiences a sub-tropical monsoonal climate. The annual rainfall ranges between 1138 mm and 2680 mm, with over 80% of the rain occurring during the monsoon months (June-September). The altitudinal range lies between 60 and 1500 m above sea level. Currently, around 50% of the landscape is under agriculture and settlement, and another 50% comprises forest, shrublands, grasslands, and river and riverbeds (Ram et al., **2021**). A diverse matrix of habitats can be found locally characterized by the different associations of grassland and forest tree species (Lehmkuhl, **1999**). The major forest cover consists of deciduous Sal (*Shorea robusta*) forest that dominates most of the upland areas. Alluvium flood plains are characterized by tall and short grassland species like *Narenga spp., Saccharum spp.* and *Imperata spp.* and riverine forest species like *Trewia spp., Bombax spp.* and *Acacia spp.*

Wildlife diversity: Tiger *Panthera tigris* is the dominant carnivore of the landscape, although other carnivores like the common leopard, striped hyena *Hyaena hyaena* dhole coexist in lower density. Charismatic ungulates of the landscape include Greater one-horned Rhinoceros and Asian Elephants. Pangolins *Manis pentadactyla* and *M. crassicaudata* are other myrmecophagous species present in the landscape. The landscape is also a home to various small mammals, herpetofauna, bats, butterflies, and birds.

Local communities, livelihood, and conservation threats: The landscape was densely forested with sparse human habitation until the 1950s. It has a high human population density with an average of 392 persons/km^2 now. Most people depend on subsistence agriculture and are involved in farm and off-farm-based livelihood activities. The forest and park resources are also of great importance to the livelihood of local people who depend intensely on forest resources for farming and livestock (Stræde & Treue, **2006**). The collection of forest resources is regulated by the government and local communities under different management regimes, but the pressure for illegal access to resources persists throughout the year (Sharma & Shaw, **1993**; Straede & Helles, **2000**). Some fruit species like *Ficus sps., Syzygium sps. Zizyphus sps., Aegle marmelos, Cassia fistula, Phoenix sps., Magnifera indica, Bridelia retusa, and Bombax ceiba* are used both by local people for their livelihood and sloth bears as

an essential component of its diet (Shah et al., **2018**). Expansion of agriculture and settlement, linear infrastructure development, and mining activities are the significant drivers of deforestation and habitat fragmentation that threaten sloth bears in the landscape. Although poaching has not been excessive in recent years, livestock and human disturbance in wildlife habitats and human-wildlife conflicts are frequent (Acharya et al., **2016**; Lamichhane et al., **2018**; Silwal et al., **2017**).

Sampling design and data collection

An intensive search for sloth bear feces in the study area was conducted along forest trails, rivers, and animal tracks between 2019-2021. Hair samples were opportunistically collected when available mostly from termite mounds, ground diggings, rubbing, and scrapes in trees, and den sites. Sampling effort was concentrated within (4×4 km^2) grids laid over the study area map based on the estimated home range of sloth bears in CNP. This design was adopted to maximize search effort and coverage to better represent the sloth bear population for genetic assessment. The sloth bear feces mainly were distinguished based on feces contents, such as the presence of termites, ants, and fruit remains. Presence of pugmarks, the freshness of diggings, scrapes, and termite mound feedings in the nearby surroundings, and local knowledge of wildlife distribution based on previous reports of sightings aided in distinguishing fresh sloth bear samples. Experienced wildlife technicians involved in the survey determined the freshness of sloth bear signs based on the visual patterns of the exterior surface and experience. When a fresh putative bear fecal sample was encountered, the outer surface was rubbed multiple times to ensure the mucus layer was attached to the swab. The swab was stored in a 2.5ml vial containing InhibitEX buffer solution (Qiagen Inc., Tokyo). Hair samples were collected using sterilized forceps and were stored in paper envelopes. Disposable latex gloves were replaced after each sample was collected and forceps were immediately rinsed with 75% ethanol to avoid contamination. The GPS location (Garmin) and environmental characteristics of the sample location were recorded. Samples were stored at -20^{o}C until analysis.

Genetic methods

DNA extraction: Genomic DNA was extracted from the fecal swab and hair samples using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen Inc.) for fecal samples and the Isohair Easy kit (Nippon Gene, Inc. Tokyo, Japan) for hair samples following the manufacturer's instructions. Negative controls were included and DNA samples were processed under sterile conditions to avoid cross-contamination.

Microsatellite loci selection: Seven polymorphic nuclear microsatellite loci optimized for the sloth bear study (Bellemain & Taberlet, **2004**; Cronin et al., **2009**; Ostander et al., **1993**; Paetkau et al., **1995**, **1998**; Poissant & Davis, **2011**; Sharma et al., **2013;** Taberlet et al., **1997**) were initially selected to determine the genotypes. Samples were first multiplexed with the primer set of two loci (MU26 and G10L) and then discarded if they did not produce scorable results at any locus, even after multiple rounds of PCR. Samples amplified at any loci were further amplified using primers for an additional five loci (G1A, G10B, G10J, CXX203, and UMAR2) and sex primers. In the initial set of 7 microsatellites, MU26 locus was monomorphic, and the number of different alleles was lower than that of Sharma et al. (**2013)**. So, additional eight microsatellite loci (G10H, CXX20, G10C, G1D, MU05, MU09, MU59, G10M) previously used for studies in bears were included to check if increasing microsatellite loci improved the results. Sex-specific genes were used for the molecular sexing of individual of sloth bears (Bidon et al., **2013**). Monomorphic loci (MU26, G10C, G1D) were excluded, and twelve polymorphic loci were finally considered for further genetic analysis.

Polymerase Chain Reaction (PCR): PCR was performed using multiplex PCR Assay Kit Ver.2 (TaKaRa Bio Inc., Shiga, Japan) at a 15-µl volume containing 14 µl master mix (PCR water, Buffer, Multiplex primer, PCR enzyme) and 1 µl DNA template. Amplification was performed in an Applied Biosystems Veriti Thermal Cycler with an initial denaturation at 94°C for 1 min, followed by 40 cycles of 94°C for 30 sec, 55–58°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were diluted and processed for separation using capillary electrophoresis on an ABI sequencer (Applied Biosystems, Inc. USA). Allele data were obtained using GeneScan-500 LIZ Size Standard and scored on GeneMapper software (version 4.1). The multi-tube approach was adopted to improve detection accuracy as fecal samples are usually characterized by the poor quality of DNA. Each PCR reaction was repeated at least two times. In some cases, an additional singleplex PCR was performed to confirm the allele that provided ambiguous reading in multiplex PCR. If the sample reading did not have ambiguous amplifications, additional reactions were not conducted.

Mitochondrial DNA analysis: The left variable region of the mitochondrial control region (CR)/D-loop (approximately 675 bp) of all identified individuals was amplified. mtDNA is widely used for phylogenetic analysis because of its maternal inheritance, non-recombination, and rapid evolution. Within the non-coding regions of mtDNA genome, the control region is the most polymorphic region and is preferred for population-level studies. Bear-specific primer pairs was used to amplify the CR region. Each PCR reaction was conducted in a total volume

of 25 µl that contained 2.5 µl 10x Taq buffer, 2µl dNTP mix, 1 µl each of the forward primer and reverse primer,0.5 µl Ex-Taq polymerase-HS enzyme (TaKaRa Bio Inc., Shiga, Japan), 17.4 µl of PCR water and 1 µl of DNA template. Initial PCR was carried out with an initial denaturation at 94°C for 1 min, 40 cycles each with denaturation at 94°C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72°C for 1 min followed by a final extension at 72°C for 5 min. PCR products were tested in 1.5% agarose gel. Nested PCR was conducted if amplification was not successful in the first PCR. Nested PCR was carried out with an initial denaturation at 94°C for 1 min, 35 cycles each with denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min followed by a final extension at 72°C for 5 min. PCR products were tested in 1.5% agarose gel, and the successful amplicons were purified and sequenced in both directions using a BigDye Terminator Cycle Kit v.3.1 (ThermoFisher Inc.). The reaction was carried out with an initial denaturation at 94°C for 1 min, 25 cycles each with denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec, and extension at 60°C for 3 min followed by a final extension at 60°C for 3 min. Sequence reading was conducted on an ABI sequencer (Applied Biosystems, USA).

Data analysis

Microsatellite analysis: Samples amplified for all seven loci were pooled to create consensus genotypes. Identical consensus genotypes were grouped to identify the number of different individuals using GIMLET software version 1.3.3 (Valiere N, **2002**). The GPS coordinates of each genotype and their recaptures were mapped using QGIS version 3.16. 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}) were calculated with GenALEx version 6.5 (Peakall & Smouse, **2006**). The Hardy-Weinberg equilibrium (HWE) of loci following exact test and linage 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. The probability of identity (P_{ID}), the probability of identity of siblings (P_{ID} Sibs), mean polymorphic information content (P_{IC}), and the null allele frequency (F_{null}) of each locus, were calculated using CERVUS version 3.0.7 (Kalinowski et al., **2007**). To determine the patterns of population genetic structure of the sloth bears population, a Bayesian clustering analysis in STRUCTURE version 2.3.4 (Pritchard et al., **2000**) was used. The admixture model was run with burn-in periods of 50,000 and 500,000 Markov Chain Monte Carlo (MCMC) iterations. The range of possible clusters (K) ranged from 1 to 6, and five independent runs

were performed with and without prior information of sampling locations. Each 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, the mean LnProb values as in Pritchard et al., (2000), implemented in STRUCTURE HARVESTER (Earl & VonHoldt, **2012**) was used.

Mitochondrial analysis: 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**). Reference sequence for the control region was obtained from the mitogenome sequence of sloth bears deposited in the NCBI GenBank database (Accession no EF196662.1, NC009970.1). The Himalayan black bear (Accession no NC009331) was used as an outgroup for the phylogenetic analysis of sloth bears. Base substitutions and the C and T-repeat variation were used in calculating haplotypes. Sequence alignment, haplotype identification and phylogenetic tree construction was done using MEGA-X software (Kumar et al., **2018**). The evolutionary history was inferred by using the maximum likelihood method and the Kimura 2-parameter model. The bootstrap consensus tree was inferred from 1000 replicates. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. Initial trees for the heuristic search were obtained automatically by applying neighbor-join, and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with a superior log likelihood value. All positions containing gaps and missing data were eliminated.

RESULTS

Non-invasive sampling and genotyping

A total of 116 fecal and 11 hair samples were collected from approximately 1000 km^2 of the area surveyed at three locations (**Table 2.1**). Sixty samples produced reliable readings, 19 were ambiguous for several loci, and the remaining samples did not yield complete genotypes. Overall genotyping success was 47% with negligible errors due to allelic dropout error and false alleles. A total of 37 unique individuals were identified from these 60 genotypes. Seven females and 18 males were identified. The sex of 12 individuals could not be determined. 23 samples were repeated and belonged to 13 individuals. Most of the individuals were recorded from the central habitat (CNP, n = 32), and very few individuals were recorded from the east (TJF, n = 3) and west (BNP, n = 2). Of 127 samples, most samples were obtained in spring (n = 90) and winter (n = 35) and a few during monsoon/summer (n = 2). Almost an equal

percentage of samples were obtained from the forest (53.5 %) and grassland habitats (46.5%) despite the difference in area of their land cover.

Genetic diversity

The average allelic richness across 12 polymorphic loci was 3.58 (SE = 0.42), and the number of effective alleles was 2.15 (SE = 0.24). Three loci (MU26, G10C, G1D) were monomorphic and excluded from the analysis. Other loci were polymorphic with two (MU59, MU09, G10B), three (G10L, UMAR2, G10M), four (MU05, G10H, CXX203, CXX20), five (G1A) or more alleles (G10J) per locus (**Table 2.2**). The observed heterozygosity (0.44, SE = 0.05) was lower than the expected heterozygosity (0.48, SE = 0.05). No significant deviation from Hardy Weinberg Equilibrium (HWE) ($p > 0.05$) was detected between the microsatellite loci. No significant linkage disequilibrium was observed between microsatellite loci except for CXX203 and CXX20, which persisted even after Bonferroni correction. The mean polymorphic information content (PIC) was 0.42, ranging from 0.12 to 0.71. The cumulative P_{ID} and P_{ID}Sibs were 1.02×10^{-6} and 1.72×10^{-3}. The fixation index F_{ST} was 0.07 (SE=0.02), indicating little differentiation among populations. The Weir and Cockerham (1984) measure of the inbreeding coefficient (F_{IS}) was 0.08 and positive for most loci, indicating signs of possible inbreeding and excess homozygous individuals in the area.

Population structure

Visualization of results from the STRUCTURE using Structure Harvester showed the highest mean LnProb value for K = 1 (**Figure 2.2, Table 2.4**), indicating a single genetic structure of the sloth bear population. The membership coefficient (q) did not show absolute values (0 or 1). No individuals were assigned with high posterior probability ($q \geq 0.70$) to any of the clusters at K = 2 for both models with and without prior location information of individuals (**Figure 2.3**). These results indicated that individuals were admixed, and no visible genetic differentiation of the sloth bear population could be observed.

Haplotype distribution and phylogenetic relationship

A consensus sequence using the forward and reverse primers was obtained for the control region of mitochondrial DNA. The base substitutions at two variable positions and the repeat number variation at the thymine (T) and cytosine (C) repeat sites defined four unique haplotypes (**Table 2.5**). The base substitution detected in this analysis was a single position

transition of adenine (A)- Guanine (G) and C-T. No insertion or deletion was observed except for the T and C repeat number variation. Multiple substitutions were not observed at any variable positions. The substitutions were observed only in the samples from the eastern study area (the Trijuga Forest). This eastern haplotype (MUNEP-E1, n= 3) is distributed approximately 200 km east of the central population in CNP. Variation in the T repeat site was observed in the samples from the BNP. This western haplotype (MUNEP-B1, n = 2) is distributed approximately 300 km west of the central population in CNP. All other individuals belonged to the (MUNEP-C1, n=15 and MUNEP-C2, n= 17) haplotype. Maximum likelihood phylogenetic analysis revealed that the haplotypes from Nepal formed a distinct clade compared to the reference sequence of sloth bears mitochondrial genome available in the GeneBank (**Figure 2.4**).

DISCUSSION

Genotyping success

Valuable information on sloth bear genetics was obtained for the first time from the present study on the conservation genetics of sloth bears from Nepal. Success rate for obtaining complete genotypes from the samples was about 50%. This might be because feces and hair samples are vulnerable to rapid degradation in hot and humid conditions and are characterized by low DNA quality. Such non-invasively collected genetic samples are prone to high rates of incomplete genotyping, allelic dropouts, and false alleles (Taberlet et al., **1996**; Kunde et al., **2020**). Genotyping success rate could have been maximized if better DNA samples were obtained using blood or tissues. However, it required capturing and handling wild sloth bears, which was possible but logistically challenging in this study. Non-invasive sampling techniques were better suited for this research that provided a more cost-effective option to obtain large samples within a short time frame in remote and challenging habitats. Repeated genotyping using a multi-tube approach reduced genotyping errors and increased the useability of the non-invasive samples for genetic analysis (Bourgeois et al., **2019**; Shimozuru et al., **2019**).

Genetic diversity

Evidence from the seven microsatellite markers indicates that the genetic diversity of sloth bears from Nepal is relatively lower compared to existing information on their genetics across their distribution range (**Table 2.3**). On average, fewer alleles (N_A = 3.5) were observed in this

study from Nepal compared to that observed for the sloth bear population from Central India (N_A = 8.8). The expected heterozygosity (H_E = 0.36) was also lower for the sloth bear population in Nepal compared to that in central India using the same loci (H_E = 0.72) (Dutta et al., **2015**) as well as different additional loci (H_E = 0.51) (Thatte et al., **2020**). A moderate level of genetic diversity (H_E = 0.61) was detected in tigers from the same landscape in Nepal (Thapa et al., **2018**), while a high level of genetic diversity was observed in Himalayan black bear (H_E = 0.76) from the Annapurna conservation area in Nepal (Kadariya et al., **2018**). Sun bears in Cambodia also exhibited low genetic diversity (H_E = 0.58) (Kunde et al., **2020**).

The pattern of genetic variation depends on the dispersal ability, density of the study species, and the human footprint on its habitat (Thatte et al., **2020**). Sloth bears have a small home range with extensive overlap within and between sexes (Joshi et al., **1995**; Ratnayeke et al., **2007a**; Yoganand, **2005**). High mutual tolerance and limited dispersal may contribute to increased inbreeding and a loss in genetic diversity over the years. Small but positive F_{IS} values in this study suggest that sloth bears may be inbreeding. Inbreeding is accelerated when habitats become isolated because of natural or human-induced sharp barriers like the construction of roads. Sloth bears suffer the highest conservation risk of roads among all apex predators (Quintana et al., **2022**). Rapid habitat fragmentation over the decades has isolated wildlife habitats in Nepal (Ram et al., **2021**). Within these isolated habitats, their occupancy is further determined by the fine-scale habitat features, particularly the distribution of termites and fruits that are not uniformly accessible to sloth bears. Fruits are seasonal and insects like ants and termites are more accessible to sloth bears in alluvial grassland habitats (Garshelis et al., **1999**; Joshi et al., **1997**). A large number of feces samples collected in this study from grassland habitats, despite the lower proportion of this habitat type, indicate the importance of this habitat despite their rarity. The low genetic diversity of sloth bears in this study area may result from limited dispersal imposed by habitat fragmentation, myrmecophagous behavior, and patchy distribution of food resources. Although a direct comparison of diversity parameters between different species or the same species from different areas may not be adequate to draw concrete conclusions, genetic diversity results amidst limited genetic studies on sloth bears indicate a low genetic diversity and the need for further molecular investigations.

Population differentiation

Microsatellite information obtained in this study for sloth bears from Nepal did not exhibit significant evidence of population differentiation. The variance of Ln likelihood and standard deviation for other values of K compared to K = 1 and the low difference in magnitude of delta

K and membership coefficients < 0.7 for K = 2 suggested that K = 1 was most meaningful for the dataset in this study (**Table 2.4**). The ability of the algorithm to detect the actual number of clusters of individuals correctly is often limited by the number and type of marker used, parameters used for simulations and the number of individuals genotyped. Thus, it is advised that population differentiation results using such programs should be cautiously interpreted with all the possible biological explanations (Cullingham et al., **2019**). The fixation index for the population using microsatellite data (F_{ST}) was 0.16 (SE = 0.03), which also indicated minimum support for population structuring. The results are different from that reported for the population of sloth bears in central India (Dutta et al., **2015**). Sloth bears in this central Indian landscape were interconnected by corridors that facilitated genetic exchange and prevented further genetic sub-structuring of the population.

Signature of population differentiation was expected to be detected in this study because of the previous report of small home range size for sloth bears, large geographic distance, presence of human settlements, rivers, and escarpments between the studied habitats. However, the existence of a single population cluster indicates that these landscape features have yet to exert strong resistance to genetically isolate the sloth bear population. When location information was included during the structural analysis, the fractional membership coefficient (q) of individuals, particularly TJF and BNP, increased slightly but was < 0.7 to be assigned to a different cluster. Presence of some alleles unique to the TJF and BNP individuals indicate that increase in sample size from these areas may change current results and reveal the population differentiation. Not enough time may have passed since the population with a large proportion of ancestrally admixed genomes became isolated to detect the signature of population differentiation. Much of the human modification of landscape, particularly the development and expansion of infrastructures, agriculture, and settlement, have occurred within the past few decades (<100 years). On an evolutionary timescale, more than this period may be needed to leave a detectable genetic signature on the population as it requires many generations of long-lived mammal-like sloth bears to pass their genes. Large carnivores, including tigers, leopards, and sloth bears have been recently reported from the outer Himalayas, also known as 'Siwalik' or 'Chure' that connects current sampling locations. This might suggest that sloth bears may have been able to maintain their gene exchange using this corridor habitats.

Additionally, male-biased dispersal and female-biased philopatry have been reported to influence the genetic characteristics in other bear populations (Shirane et al., **2019**). Female sloth bears mate with multiple males multiple times in a hierarchal order and remain in their habitat with young cubs, but adult males show seasonal migration between habitats and have

an overlapping home range with multiple females and other males (Joshi et al., **1995**, **1999**; Garshelis et al., **1999**). The sex and life-stage-specific behavior of sloth bears may also influence their genetic diversity and population differentiation in sloth bears. Sex identification on sloth bears has not been well established. There were more males than female sloth bears in this study, but the sex of many samples could not be identified with certainty. Future studies with large sample sizes to adequately represent existing genetic variability from different sub-populations may be able to further clarify genetic differentiation in sloth bear populations from Nepal.

Haplotypes and phylogenetic relationship

Phylogenetic analysis based on the control region (CR) of mitochondrial DNA showed that sloth bear individuals in this study formed a distinct clade compared to reference samples. Four haplotypes were identified based on base substitutions and a variable number of T and C repeats. Samples from the eastern region, which is located approximately 200 km away from the central sloth bear population in CNP, clustered together, forming a unique (MUNEP-E1) haplotype. Similarly, the BNP population, which is located approximately 300 km away from the central sloth bear population in CNP, belonged to a different haplotype (MUNEP-B1) based on the variation in the T and C repeat regions. The central population also showed variation in the T and C-repat regions forming two different haplotypes (MUNEP-C1 and MUNEP-C2). Phylogenetic analysis using the maximum likelihood method for sequences of the mtDNA control region demonstrated that genetic distance among these lineages was very low. It suggests that sloth bear populations BNP, CNP, and TJF have a shared ancestry and slight genetic variation since divergence. The lack of genetic differentiation also supports that these populations may have diverged very recently to detect signatures of genetic differences at the population level.

CONCLUSIONS

The findings from this study indicate that sloth bears in Nepal are evolutionary unique and are characterized by having a low genetic diversity across their distribution range. A minimum of 37 sloth bear individuals were genetically identified using non-invasive samples which can be extended to estimate the total population for future management of sloth bears in the park. The results suggest that incidental conservation programs targeted at other species may not be adequate for sloth bear conservation. Sloth bear specific conservation interventions may be required to enhance the genetic diversity in the sloth bear population from Nepal. However,

current results are based on low number of individuals genotyped from feces samples collected mostly from CNP. Long-term monitoring of the sloth bear population and analysis of genetic information using sufficient hair samples from diverse sub-populations is suggested for a deeper understanding of its genetic status.

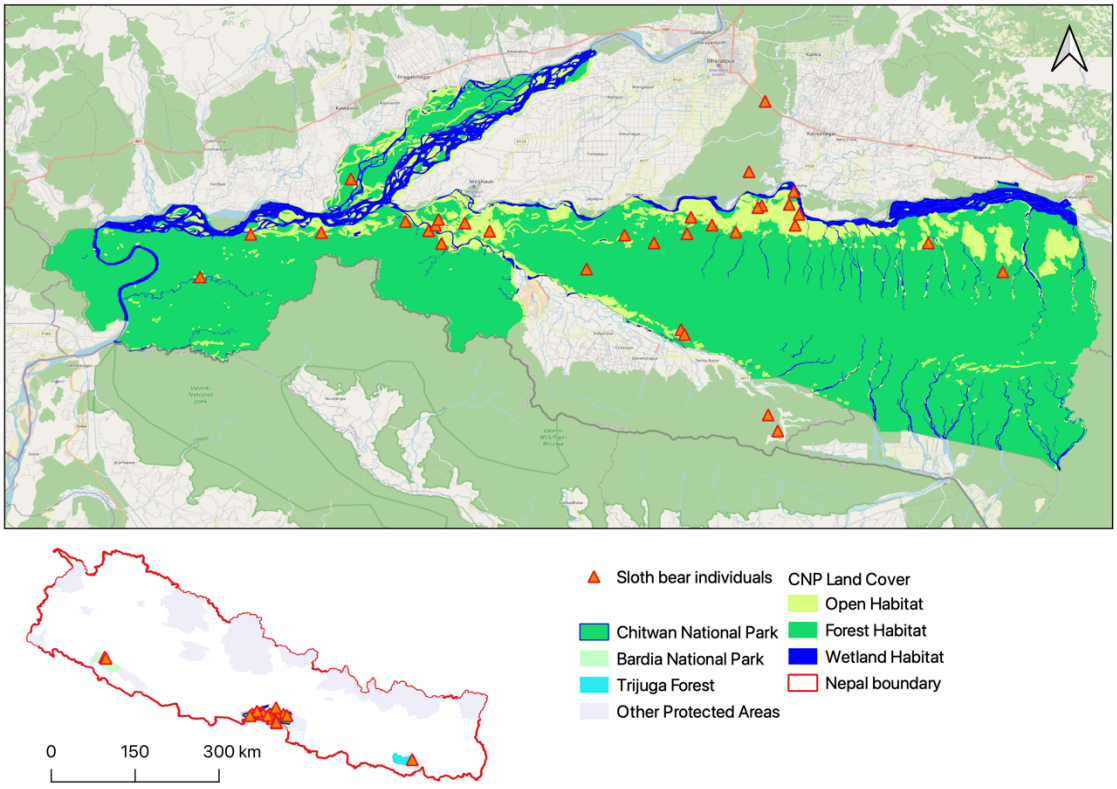


Figure 2.1 Study area map showing distribution of genetic samples

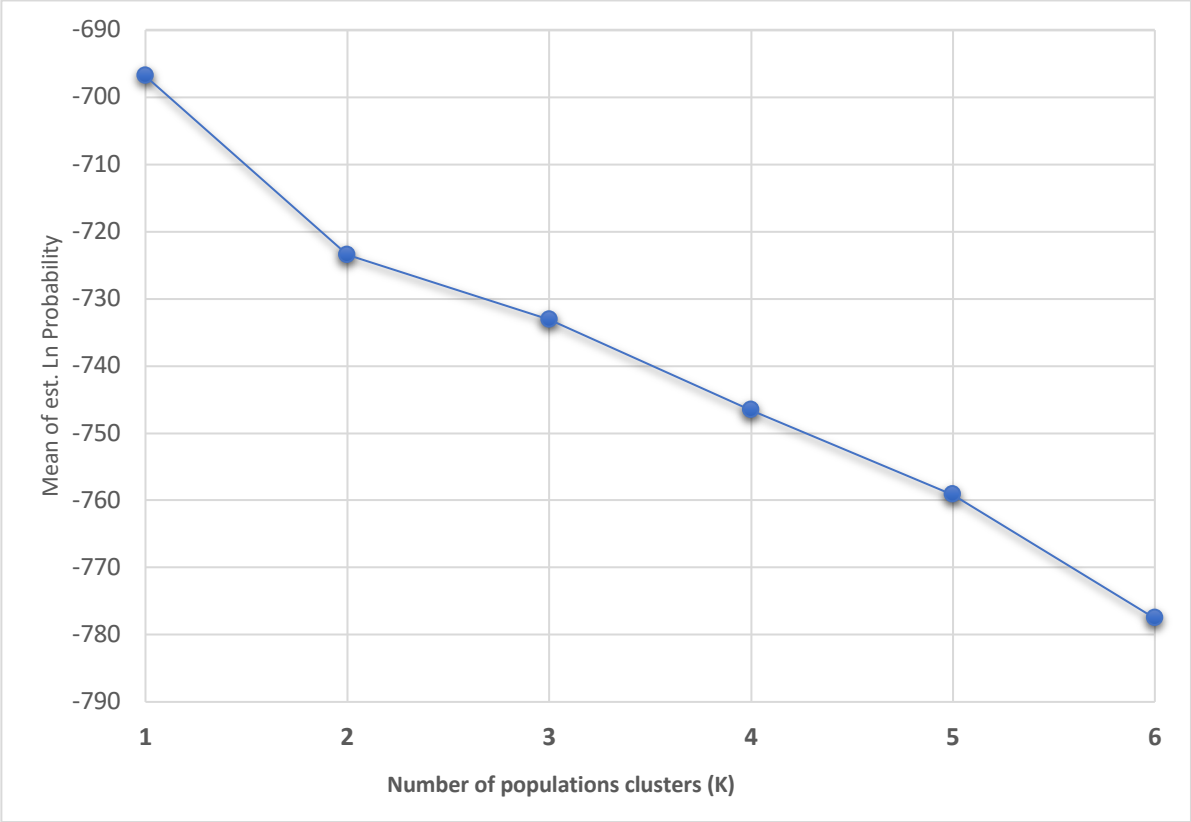
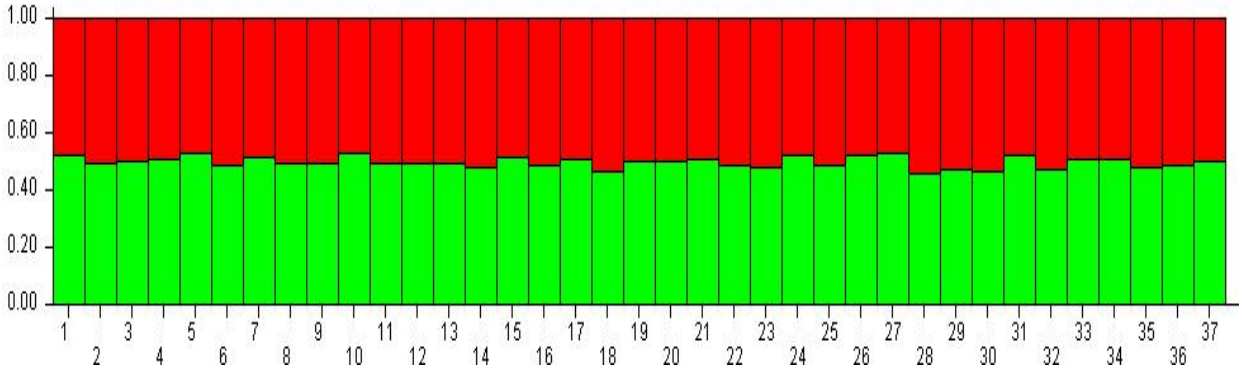
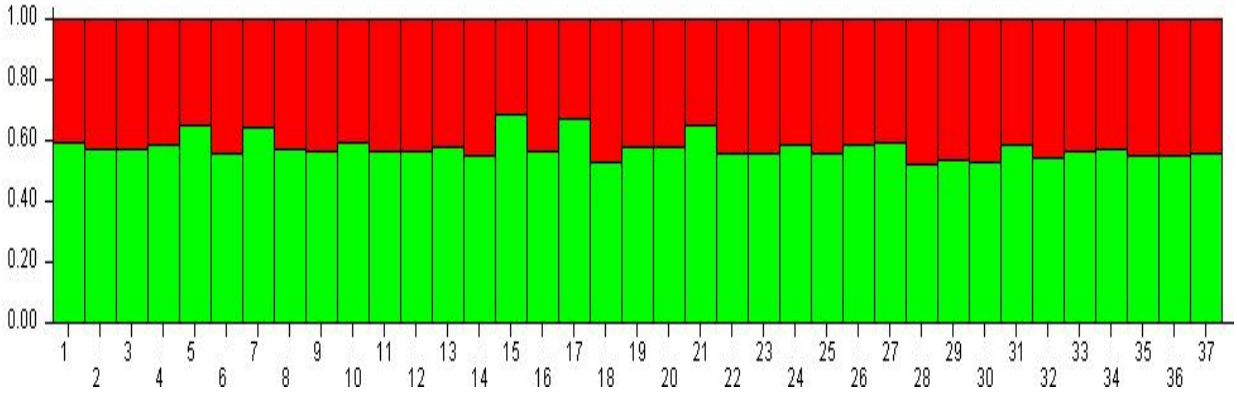


Figure 2.2 Number of population clusters. Structure results of 37 individuals from three locations. The mean of estimated Ln probability of data is higher when population sub cluster K = 1. Y-axis values are fixed from -790 to -690 for clear presentation of graph



A: *Population structure of sloth bear in Nepal without prior location information. Vertical bar represent individual bears and color represent membership coefficient (q) of each individual which was taken less than 0.70 when K = 2. Number 1-37 represents the individual identified from microsatellite analysis.*



B: *Population structure of sloth bear in Nepal with prior location information. Vertical bar represent individual bears and color represent membership coefficient (q) of each individual which was taken less than 0.70 when K = 2. Number 1-37 represents the individual identified from microsatellite analysis. Number 5, 7 and 21 individuals were from TJF, number 15, 17 were from BNP and all other individuals from CNP.*

Figure 2.3. Population structure plots for sloth bears in Nepal

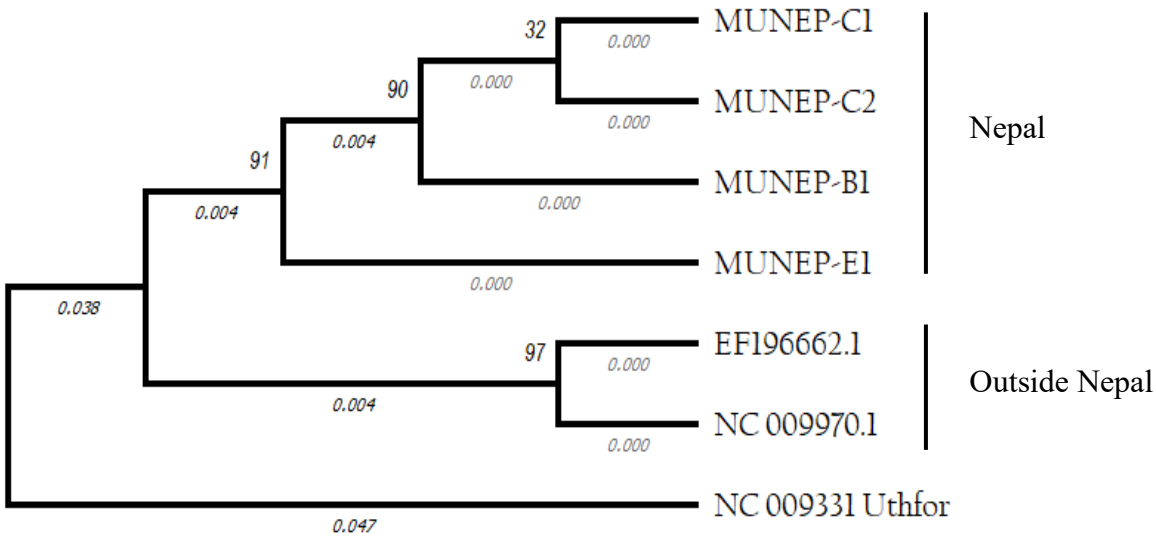


Figure 2.4 Phylogenetic relationship between sloth bears in Nepal

Table 2.1 Details of samples, genotyping success, and the sample origin. The total number of the unique individuals identified from forest and grassland habitats are included in parentheses

Location	Total	Incomplete	Ambiguous	Complete	% Success	Unique	Forest	Grassland
CNP	107	41	18	48	45	32	48 [17]	59 [15]
BNP	12	5	0	7	58	2	12 [2]	0
TJF	8	2	1	5	63	3	8 [3]	0
Total	127	48	19	60	47	43	68 [22]	59 [15]

Table 2. 2 Genetic diversity parameters for the 12 polymorphic microsatellite loci used to evaluate the population of the sloth bears from Nepal

Locus	Multiplex	N_A	N_E	H_O	H_E	$_UH_E$	PIC	PID	PIDSibs	F_{IS}	P	Fnull	ADO
G10L	MP1	3	1.69	0.35	0.41	0.41	0.36	0.40	0.65	0.15	0.29	0.10	0.00
G1A	MP2	5	2.10	0.51	0.52	0.53	0.47	0.28	0.56	0.03	0.28	0.04	0.00
G10B	MP2	2	1.14	0.14	0.13	0.13	0.12	0.77	0.88	-0.06	1.00	-0.03	0.00
G10J	MP2	7	4.03	0.68	0.75	0.76	0.71	0.10	0.40	0.12	0.36	0.05	0.00
CXX203	MP3	4	2.13	0.46	0.53	0.54	0.47	0.28	0.56	0.15	0.15	0.10	0.00
UMAR2	MP3	3	1.24	0.16	0.20	0.20	0.18	0.66	0.82	0.18	0.36	0.09	0.00
G10H	MP4	4	3.56	0.62	0.72	0.73	0.67	0.13	0.42	0.15	0.07	0.07	0.00
Cxx20	MP4	4	2.05	0.46	0.51	0.52	0.45	0.30	0.57	0.11	0.43	0.07	0.00
MU05	MP4	4	2.18	0.60	0.54	0.55	0.44	0.31	0.56	-0.08	0.75	-0.05	0.00
MU09	MP5	2	1.95	0.46	0.49	0.49	0.37	0.38	0.60	0.07	0.74	0.03	0.00
MU59	MP5	2	2.00	0.46	0.50	0.51	0.38	0.38	0.59	0.09	0.74	0.04	2.99
G10M	MP5	3	1.77	0.42	0.43	0.44	0.39	0.37	0.63	0.05	0.39	0.02	0.00
Mean/Cum		3.58	2.15	0.44	0.48	0.48	0.42	1.02×10^{-6}	1.72×10^{-3}				
SE		0.42	0.24	0.05	0.05	0.05							

N_A observed number of allele; N_E effective number of allele; H_O, observed heterozygosity; H_E expected heterozygosity; $_UH_E$ unbiased expected heterozygosity; **PIC**, polymorphic information content; **PID,** probability of identity (locus); **PIDSibs**, probability of siblings identity (locus); F_{IS}, Wright's inbreeding coefficient; **P**, p values for exact tests of Hardy-Weinberg equilibrium ; **Fnull**, predicted frequency of null alleles; **ADO,** allele dropout %; **SE**, standard error

Table 2.3 Comparison of genetic diversity parameters in current study with similar studies.

A. Comparison of genetic diversity parameters across common microsatellite loci

	This Study(Nepal)			Dutta et al. 2015 (India)		
Locus	**N_A**	**H_O**	**H_E**	**N_A**	**H_O**	**H_E**
MU26	1	0.00	0.00	7	0.38	0.69
G10L	3	0.35	0.41	7	0.89	0.68
G1A	5	0.51	0.52	11	0.38	0.72
G10B	2	0.14	0.13	4	0.27	0.47
G10J	7	0.68	0.75	12	0.58	0.88
CXX203	4	0.46	0.53	12	0.65	0.86
UMAR2	3	0.16	0.20	9	0.58	0.71
Mean	**3.57**	**0.33**	**0.36**	**8.86**	**0.53**	**0.72**

B. Comparison of genetic diversity parameters for sloth bears with other studies in Nepal and India

Study	**Location, Species**	**N_A**	**H_O**	**H_E**
This Study	Sloth bears in this study (using 7 loci)	**3.57**	**0.33**	**0.36**
	Sloth bears in this study (using 12 loci)	**3.58**	**0.44**	**0.48**
Dutta et al., 2015 (Sloth bears in Central India)	Kanha (n=9), 940 km^2	5.71	0.62	0.75
	Pench (n=8), 293 km^2	5.29	0.52	0.61
	Satpura (n=16), 646 km^2	6.29	0.55	0.64
	Melghat (n= 22), 1677 km^2	6.29	0.49	0.65
	Average Central India (using 7 loci)	**8.86**	**0.53**	**0.72**
Thatte et al., 2020 (Central India)	Sloth bears (n=104, 11 loci)		**0.39**	**0.51**
	Tigers (n= 117, 12 loci)		**0.52**	**0.72**
Thapa et al., 2018 (Tigers in Nepal)	CNP (n=37), 953 km^2	4	0.58	0.57
	BNP (n=25), 986 km^2	4	0.57	0.55
	ShuNP (n=16), 305 km^2	3	0.46	0.52
	Average in Nepal (using 8 loci)	**3.51**	**0.54**	**0.61**
Kadariya et al., 2018 (Black bears in Nepal)	Annapurna Conservation Area, Nepal	**7.63**	**0.79**	**0.76**

N_A, observed number of allele; **H_O**, observed heterozygosity; **H_E**, expected heterozygosity

Table 2.4 Summary and raw STRUCTURE Harvester outputs

a. Summary of STRUCTURE Harvester output

K	Repetitions	Mean estimated LnP (Data)	SD of est. LnP(Data)
1	5	-696.86	0.151658
2	5	-723.5	8.359725
3	5	-733.12	16.802738
4	5	-746.58	28.960093
5	5	-759.18	17.991303
6	5	-777.56	37.713366

b. Raw STRUCTURE Harvester output

K	Run No	Est.Ln probability of data	Mean value of Ln Likelihood	Varience of Ln likelihood
1	3	-697	-690.1	13.8
1	4	-696.7	-690	13.4
1	5	-696.7	-690	13.5
1	1	-697	-690	14.1
1	2	-696.9	-690.1	13.8
2	6	-715.9	-687.3	57.2
2	10	-731.9	-682.6	98.6
2	7	-728.2	-683.7	89
2	8	-728.3	-684.7	87.1
2	9	-713.2	-686.9	52.6
3	15	-750.7	-681.9 1	137.7
3	12	-735.5	-685.2 1	100.6
3	11	-729.7	-684.6	90.3
3	14	-706.6	-689.2	34.7
3	13	-743.1	-683.6 1	119
4	19	-725.5	-686.3	78.4
4	18	-779.5	-680.6 1	197.7
4	16	-709.4	-688	42.8
4	17	-751.4	-683.8 1	135.4
4	20	-767.1	-679.7 1	174.8
5	25	-782.6	-679.6 2	206.1
5	23	-752.7	-685.6 1	134.2
5	22	-766.9	-682.1 1	169.5
5	24	-759.9	-682.0 1	155.7
5	21	-733.8	-684.8	98
6	29	-734.1	-686.5	95.3
6	30	-830.2	-678.8 3	302.7
6	28	-749.1	-682.8 1	132.8
6	26	-791.3	-679.2 2	224.2
6	27	-783.1	-682.6 2	200.9

K, number of population cluster; **Lnp**, Log likelihood; **Ln**, natural logarithm

Table 2. 5 Variable positions and observed frequencies of the left domain of the CR for four haplotypes of Sloth bears from Nepal. Dot indicates identity with the nucleotides of MUNEP-B1. Dash indicates variation in the number of Ts and Cs.

Haplotype	**Sequence length**	**Position no**				**Individuals**	**Location**
		07	59	60	190		
MUNEP-B1	466	G	T	C	C	2	BNP
MUNEP-E1	466	A	—	•	T	3	TJF
MUNEP-C1	466	•	—	—	•	15	CNP
MUNEP-C2	466	•	—	•	•	17	CNP

CHAPTER III

DIET OF SLOTH BEARS (*Melursus ursinus*) FROM NEPAL

INTRODUCTION

The knowledge of dietary composition can provide crucial insights into species' behavior and environmental interactions (Putman, **1984**). Generalist species feed on a diversity of foods that enables them to survive in a variety of habitats. At the same time, the specialist species have a narrow dietary niche and thus more specific habitat requirements. The availability and quality of food influence species' behavior, body condition and reproduction success and thus exerts bottom-up regulation of population growth. Thus, successful management and conservation of species requires wildlife managers to have an adequate understanding of specific dietary habits.

Bears are highly adaptive and vary their behavior according to the availability of food resources and the extent of risk from humans and non-human predators (Garshelis, **2022**). Knowledge of the ursids in general, and particularly sloth bears, is limited in Nepal. Sloth bears are mostly allopatric and occur in tropical habitats compared to the other two species of bears, viz., Brown bears and Asiatic black bears, that occur in the temperate and alpine habitats, respectively in Nepal. They are distributed along the forest and grassland habitat in the Chure-Terai landscape mostly within national parks and few forest patches outside protected areas. They have a high density in CNP because of the resource-rich habitat matrix of forest and grasslands and a lower density outside CNP. Outside Nepal, they are present in at least 174 protected areas in India and forested habitats in Srilanka (Philip et al., **2021**). Their population is declining across their distribution range because of the habitat loss and fragmentation, decreasing food resources, and adverse interactions with humans (Dhariya et al., **2020**). The species is listed as globally vulnerable by the IUCN and included in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

The abundance of nutrient-rich food across different seasons is critical for bears entering hibernation. Sloth bears are not true hibernators, but females are reported to fast for several weeks during parturition and neonatal development (Joshi et al., **1999**). A constant food supply is essential to meet their energy requirements throughout the year, including the energetically demanding periods of reproduction and growth. Sloth bears are omnivores but do not hunt

mammals, instead, they are adapted for myrmecophagy. Like other bears but unlike most other myrmecophagous mammals, sloth bears exhibit significant dietary plasticity with varying food habitats across habitats and seasons according to food availability (Joshi et al., **1997**; Philip et al., **2021**; Rabari & Dhariya, **2022**). They engineer their ecosystems through their role as important seed dispersers and pest controllers in the Terai-Duar savanna and grassland ecoregion at the base of Himalayas. In human-dominated landscapes, they are reported to feed on agriculture crops and human food waste (Prajapati et al., **2021**). Human-bear conflicts have been reported in areas where such maladaptation exists. The presence of crops and human food waste is not reported in Nepal (Joshi et al.,**1997**; Khanal & Thapa, **2014**), although some crops were consumed in the 1970s when the park still had human habituation (Laurie & Seidensticker, **1977**).

Significant changes have occurred over the past decades in the sloth bear distribution range in Nepal along the Chure-Terai landscape (Ram et al., **2021**). However, current information on the food habits of sloth bears is still poorly understood. This study aimed to clarify the feeding ecology of sloth bears from Nepal with a focus on dietary diversity. The knowledge about their ecology and feeding behavior is essential to plan effective bear conservation strategies. It will provide valuable insights to forest and wildlife managers for efficient habitat management in terms of provisioning food resources, regulating population growth and movement, and promoting human-bear co-existence. While molecular and animal-borne video cameras are increasingly being used as a modern technique for studies on feeding ecology and diet (De Barba et al., **2013**; Jimbo et al., **2022**; Nawaz et al., **2019**; Tezuka et al., **2022**), this study focused on using non-invasive and less resource-intensive methods. Scat analysis is a widely used, cost-effective method for elucidating the food and feeding behavior of wildlife. As the study area experiences distinct seasons viz. winter (December-January-February), spring (March-April-May), summer/monsoon (June-July-August), and autumn (September-October-November), it was hypothesized that the diet of sloth bears would vary seasonally.

MATERIAL AND METHODS

Study Area

The study was carried out along the Churia-Terai (CT) region of the outer Himalayan landscape in Nepal **(Figure 3.1).** It consists of a geologically fragile mountain range along the foothills of the Himalayas known as ‘Siwalik’ or ‘Churia’ and alluvial flood plains formed by tributaries of the Ganges River and the associated valleys. Field sampling was concentrated in the CNP,

BNP and TJF. CNP covers 953 km^2 and is in the south-central part of Nepal along the floodplains of the Rapti, Reu, and Narayani rivers. BNP covers 986 km^2 and is situated in the southwest part of Nepal along the flood plains of the Karnali and Babai rivers. TJF covers 430 km^2 and is located in the southeastern part of Nepal along the bank of the Triyuga and Koshi rivers. These study sites are geographically at least 200 km apart from each other. The altitudinal range lies between 60 and 1500 m above sea level, and the study area experiences a sub-tropical monsoonal climate. The annual rainfall ranges between 1138 mm and 2680 mm, with over 80% of the rain occurring during the monsoon months. The landscape was densely forested with sparse human habitation until the 1950s. Eradication of malaria disease, land reform, and development projects with the advent of democracy led to rapid deforestation for the expansion of agriculture and human settlement in the area. Currently, around 50% of the landscape is under agriculture and settlement, and another 50% comprises forests, shrublands, grasslands, and riverbeds (Ram et al., **2021**).

The major forest cover consists of deciduous Sal (*Shorea robusta*) forest. Alluvium flood plains are characterized by tall and short grassland species like *Narenga spp., Saccharum spp. and Imperata spp.* and riverine forest species like *Trewia spp., Bombax spp., and Acacia spp.* Tiger *Panthera tigris* is the dominant carnivore of the landscape that co-occurs with other mega herbivores like Greater one-horned Rhinoceros *Rhinoceros unicornis* and Asian elephants *Elephas maximus*. Pangolins *Manis pentadactyla* and *M. crassicaudata* are other myrmecophagous species present in the landscape. The landscape has high human population density, and the biological resources are of great importance to the livelihood of local people who depend intensely on forest resources for farming and livestock (Stræde & Treue, **2006**). Some fruit species like *Ficus spp., Syzygium spp. Zizyphus spp., Aegle marmelos, Cassia fistula, Phoenix sps., Magnifera indica, Bridelia retusa, and Bombax ceiba* are used both by local people for their livelihood and sloth bears as an essential component of their diet (Shah et al., **2018**). The collection of forest resources is regulated by the government and local communities under different management regimes. Expansion of agriculture and settlement, linear infrastructure development, and mining activities are the significant drivers of deforestation and habitat fragmentation in the landscape. The sloth bear suffers the highest risk of all apex predators from the roads (Quintana et al., **2022**). Although poaching has not been excessive in recent years, livestock and human disturbance in wildlife habitats and human-wildlife conflicts are frequent (Acharya et al., **2016**; Lamichhane et al., **2018**; Silwal et al., **2017**).

Feces collection

Sloth bear feces collection was conducted from May 2019 to March 2021. Sampling effort was concentrated along the 4 km walking transects laid within (4 × 4 km^2) grids covering major habitat types in the CNP. Feces were also collected opportunistically when available outside the grids. Most of the scats were collected along the linear features like roads and riverbanks, forest trails, and wildlife tracks. Presence of pugmarks, the freshness of diggings, scrapes, and termite mound feedings in the nearby surroundings, and local knowledge of wildlife distribution based on previous reports of sightings aided in distinguishing fresh sloth bear samples. Feces were carefully identified in the field and collected cautiously to avoid ground debris. They were georeferenced (Garmin GPS) and were placed in zip-loc bags with appropriate labels. Feces were sun-dried and stored properly in a dry place before processing them for subsequent analysis.

Feces analysis

Feces were soaked with tap water inside a plastic container for 24 hours. Thoroughly soaked samples were washed repeatedly under tap water in an aluminum sieve of mesh size 0.7 mm and 2 mm to separate the food items. This process washed away most of the fine soil and digested materials. All the undigested food remains in the sieves were evenly sprinkled on a white tray containing a transparent grided sheet at the bottom. Food materials were classified into five categories: plants, red ants, black ants, termites, and others. Food items were identified by referring to the reference seed samples and using a microscope when needed. The percent frequency of occurrence for each item was calculated as the proportion of all feces collected that contain a particular food item, i.e., PFO = (Frequency of food item/ total number of feces) × 100 (Khanal & Thapa, **2014**; Philip et al., **2021**). The seasonal variation in dietary composition was determined using the Chi-square test. Not all food items are digested to the same extent, food items that are more difficult to digest might be overestimated, and easily digestible food items may be underestimated. Plant fragments other than seeds, insect parts other than heads, and non-food items that required further micro-histological or molecular techniques for identification were discarded.

RESULTS

A total of 194 sloth bear feces was collected by surveying an area of approximately 1000 km^2. Most of the samples were collected from CNP (78.9% n = 153), and a few samples originated

from BNP (12.9%, n = 25) and TJF (8.2 %, n = 16). Most of the samples were collected in the spring season (46.9%, n = 91), followed by winter (29.4 %, n = 57) and monsoon (23.7%, n = 46). No samples were collected in the autumn season. Samples from the dry season (76.3 %, n = 148) were higher than those from the wet season (23.7%, n = 46). An almost equal number of feces were collected from the forests (49.5%) and grassland (50.5%) habitats. All the BNP and TJF originated in forest habitats, while 64% of the samples from CNP were from the grassland habitat.

Fecal analysis revealed the dominance of insect composition (95.4%) in the sloth bear diet throughout the year in all seasons (**Figure 3.2**). Termites were the most frequently consumed (86.1%) food item, followed by red ants (62.9%) and black ants (44.8%). Fruits occurred in 25.8% of the fecal samples (**Table 3.1**). Among the plant food, the fruits of *Ficus spp.*, *Ziziphus spp*., and *Syzygium spp.* were most prevalent in the sloth bear scat. On average, 2.29 ± 0.94 (mean ± SD) number of food items were present in the scats. The number of food items was higher in spring (2.41 ± 0.97), followed by winter (2.35 ± 0.85) and summer (1.98 ± 0.92). The average number of food items was 2.24 ± 0.86 in BNP, 2.25 ± 0.94 in CNP, and 2.69 ± 0.98 outside PAs.

The food composition varied between seasons, locations, and habitats. The Chi-square test indicated a significant difference in fruit proportion between seasons ($p < 0.01$) but not for insects (termites and ants). Insects had a high percentage frequency of occurrence across seasons viz. spring (99.9%), summer (86.9%), and winter (96.5%). Fruits occurred in higher proportion in BNP during the monsoon and winter seasons compared to other locations (**Table 3.2**). It occurred in markedly higher proportions in forest habitats during the summer/monsoon season in CNP (**Table 3.3**). Food items of human origin or cultivated crops was not detected in the sloth bear feces.

DISCUSSION

This study highlight the insectivorous dietary composition of sloth bears in Nepal, dominated by myrmecophagy. These results align with previous findings that have highlighted the myrmecophagous diet of sloth bears (Joshi et al., **1997**; Khanal &Thapa, **2014**). Sloth bears consume 10 insect species belonging to at least five different orders and seven different families i.e., Apidae, Tenebrionidae, Termitidae, Cicadidae, Formicidae, Gryllotalpidae and Scarabaeidae (**Figure 3.3**). Insect feeding by bears is fairly common and has been widely reported. Malayan sun bears in the North-East part of India consumed at least 14 different

insect species, mostly in the family Coleoptera, Hymenoptera, and Isoptera (Sethy and Chauhan, **2018**). Apennine brown bears in central Italy are recorded to consume a great diversity of ant species comprising 15 genera and at least 42 species (Tosoni et al., **2018).** Japanese black bears were recorded to eat 9 different species of ants with an estimated 50,000–60,000 mg (dry weight) per day of ants in the grasslands of the Nikko National Park, Japan (Yamazaki et al., **2012**). In Dhorpatan hunting reserve in Nepal Himalayan black bears consumed ants throughout the year but termites were consumed at a less frequency during autumn season (Panthi et al., **2019**). Myrmecophagy has not been reported in brown bears from Nepal and limited dietary studies have shown it to predominantly feed on small mammals like marmots, followed by ungulates, livestock, plants, and birds (Aryal et al., **2012**).

Hair of wild mammals, livestock, agriculture crops or other foods of human origin was not detected. The occurrence of wild mammals in the sloth bear diet is rare as sloth bears do not prey on wild ungulates. Previous reports of mammalian hairs was attributed to scavenging on carcass of wild animals (Ramesh et al., **2009;** Rabari & Dharaiya, **2022**). A sloth bear was reported to kill at least 90 goats to feed on their visceral organs in CNP (Khadka, **2021**) but the cause for this behavior is unclear. Sloth bears have been reported to feed on plants cultivated in agriculture fields, mostly from the human-dominated landscapes in India where natural habitats are degraded or face immense pressure from human disturbance (Chhangani, **2002**; Mewada, **2015**; Palei et al., **2020**). An instance of this species foraging for human-generated rubbish within an urban area was also reported in India (Prajapati et al., **2021**). Laurie and Seidenssticker (**1977**) reported sloth bears feeding on agricultural crops in CNP but they were not reported by succeeding studies by Joshi et al. (**1997**) and Khanal & Thapa (**2014**) from the same area. It is possible that this study may have missed such instances as sampling efforts were mostly concentrated in the parks that are relatively resource rich and free from intense human pressures. These instances of foraging on crops and human foods highlight increasing human interference in bear habitat rather than bear's preference of human-modified habitat and foods.

Compared to an animal diet a plant-based diet was only very prevalent among sloth bears in this study during summer/monsoon season. High myrmecophagy and relatively low frugivory may suggest sloth bears' adaptation of feeding behavior according to changing food availability. Termites, particularly those living in grassland savannah habitats, are known to be better adapted to cope with changing environmental conditions (Woon et al., **2022**) and thus represent a more reliable food source. Fruits production, on the other hand, is seasonal and is

affected by changing patterns of land use and climate (Burkle et al., **2013**; Shrestha et al., **2012**), making it less dependable source of food. When available, sloth bears have widely consumed various fruits across their distribution range. At least 120 plant species from 20 plant order belonging to 38 different families and 86 genus were found to be consumed by sloth bears across its natural distribution range (**Figure 3.4**). Most plant species consumed belonged to the Fabaceae, Moraceae and Poaceae plant families, that are among the family with wide distribution and large species diversity. Most of the current grassland habitats in CNP were under human settlement and agriculture until it was restored by moving human settlements outside of the park in 1970s. This change in habitat may have favored abundance of insects in grassland habitats. It has been demonstrated that reforestation of abandoned farmlands can provide better foraging habitat for bears by increasing the availability of foods like cicads nymphs for brown bears in Japan (Tomita & Hiura, **2021**). Higher proportion of fruits and fewer insects during monsoon/summer may be because of higher availability of seasonal fruits and lower accessibility to underground colonies of termites and because of flooding in grassland habitats (Joshi et al., **1997**). Additionally, the differences may be because of the different nutritional values of food items. It was found that caloric value obtained by sloth bears from termites (5.12-7. 32Kcal.g) and ants (5.60 ± 0.06) was higher than fruits (3.8-4.98Kcal/g) (Yoganand, **2005**). Sloth bears can obtain highly nutritious and immobile queen pupae in spring compared to patchily distributed fruits. It may be energetically demanding for sloth bears to climb and eat fruits from trees scattered across the habitat, especially during the spring and winter when fruits are not abundant. Thus, insect feeding may be beneficial in terms of foraging efficiency as well as meeting the energetic requirements.

The dependence of sloth bears on the insectivorous diet may further increase as the availability of fruits becomes uncertain due to ongoing changes in land use, land cover, and climate. Diet composition in bears can vary according to the sex and life stage according to differences in their energetic requirements and foraging experience (Jimbo et al., **2022**; Naganuma et al., **2020**). Long-term monitoring of dietary composition and foraging observations can provide valuable clues into such dynamics of sloth bears feeding ecology. Use of animal-borne video systems, stable isotope analysis and metabarcoding may be required for a more accurate and individual-level differences in sloth bear diet. Estimating the availability and contribution of food items to the diet in terms of the ingested mass and energy content may be essential to deepen the knowledge of the sloth bear diet. Still, the rank of species according to their relative contribution may be similar. The frequency-based method may overestimate items that are

difficult to digest and underestimate the easily digestible food items (Baskaran & Desai, **2010;** Shirane et al., **2021**). However, frequency-based methods can still provide much-needed ecological information to conservation managers when time and resources are constrained. This is a typical scenario when studying non-charismatic species in remote and less developed areas.

CONCLUSIONS

This study has strengthened the unique feeding ecology of sloth bears. Unlike other ursids, sloth bears are highly insectivorous with a myrmecophagous diet and occasionally on seasonally available fruits. Unique dietary niche and diversification in food items may have helped sloth bears adapt to the spatial and temporal uncertainty in food availability and avoid potential competition with sympatric large carnivores like tigers while meeting their energetic requirements. The current flagship species (e.g., tiger and rhinos) based conservation approach may not be adequate for the long-term conservation of sloth bears in Nepal. Conservation efforts aimed at sloth bears should ensure resource-rich habitats with an adequate abundance of termites and fruits. Information on distribution patterns, abundance, and nutrition may be required for a more profound understanding of the importance of different food items in sloth bears' diets. More intensive sampling and use of animal-borne video collars and microscopic and molecular techniques to identify easily digested food items may provide a deeper understanding of the feeding ecology of sloth bears.

Table 3.1. Sloth bear food items and their seasonal occurrence percentage

FOOD ITEMS	Spring (n = 91)	Summer (n = 46)	Winter (n = 57)	Overall (n=194)
INSECTS	98.90	86.96	96.49	95.36
Ants and Termites	97.80	84.78	96.49	94.33
Termite	90.11	67.39	94.74	86.08
Ants	83.52	71.74	85.96	81.44
Red Ant	60.44	58.70	70.18	62.89
Black Ant	57.14	19.57	45.61	44.85
Honey Bee	6.59	2.17	0.00	3.61
Other Insects	8.79	2.17	1.75	5.15
FRUITS	17.58	52.17	17.54	25.77
Ficus spp.	2.20	21.74	10.53	9.28
Schizium spp.	0.00	13.04	0.00	3.09
Cassia spp.	2.20	6.52	0.00	2.58
Ziziphus spp.	2.20	0.00	7.02	3.09
Agele marmelos	3.30	0.00	0.00	1.55
Bridelia spp.	4.40	2.17	0.00	2.58
Other Fruits	1.10	0.00	5.26	2.06
Unidentified fruits	6.59	0.00	3.51	6.19
Plant fragments	3.30	0.00	1.75	2.06

Table 3.2. Percentage frequency of occurrence of sloth bear fo d items in different seasons across locations

Location	Season	Fruits	Insects	Termites	Ants	Red Ants	Black Ants
CNP	Springr	17.58	98.90	90.11	83.52	60.44	57.14
CNP	Summer	39.39	90.91	69.70	72.73	57.58	27.27
CNP	Winter	10.34	100.00	96.55	82.76	55.17	51.72
BNP	Springr	–	–	–	–	–	–
BNP	Summer	84.62	76.92	53.85	69.23	61.54	0.00
BNP	Winter	33.33	83.33	83.33	83.33	83.33	41.67
TJF	Springr	–	–	–	–	–	–
TJF	Summer	–	–	–	–	–	–
TJF	Winter	3.00	100.00	62.50	56.25	50.00	37.50

Table 3.3. Percentage frequency of occurrence of sloth bear food items in different seasons across different habitat types in CNP

	Fruits	Insects	Termites	Ants	Red Ants	Black Ants
Grassland habitat						
Spring	16.67	98.15	88.89	81.48	68.52	57.41
Summer	26.09	86.96	65.22	78.26	52.17	39.13
Winter	14.29	100.00	95.24	80.95	47.62	42.86
Forest habitat						
Spring	18.92	100.00	91.89	86.49	48.65	56.76
Summer	70.00	100.00	80.00	60.00	60.00	0.00
Winter	0.00	100.00	100.00	87.50	75.00	50.00

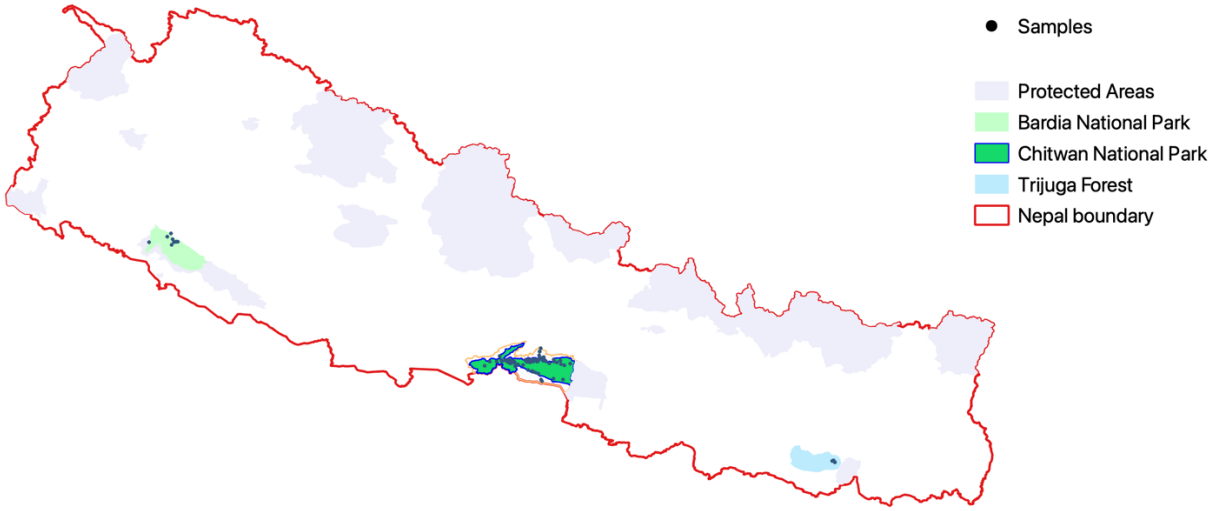


Figure 3.1. Study area map showing locations of feces collection for diet study in Nepal

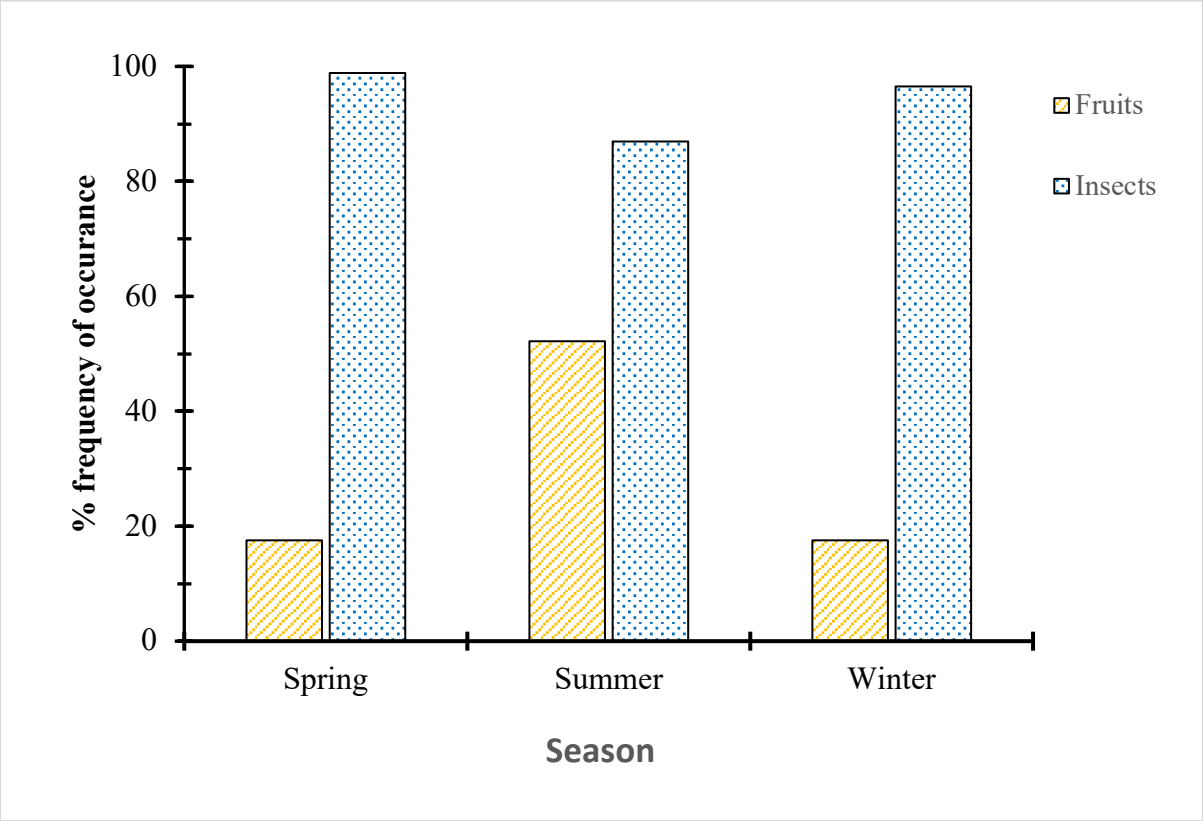


Figure 3.2. Seasonal pattern of percent frequency of occurrence of insects and fruit food items in sloth bear diet

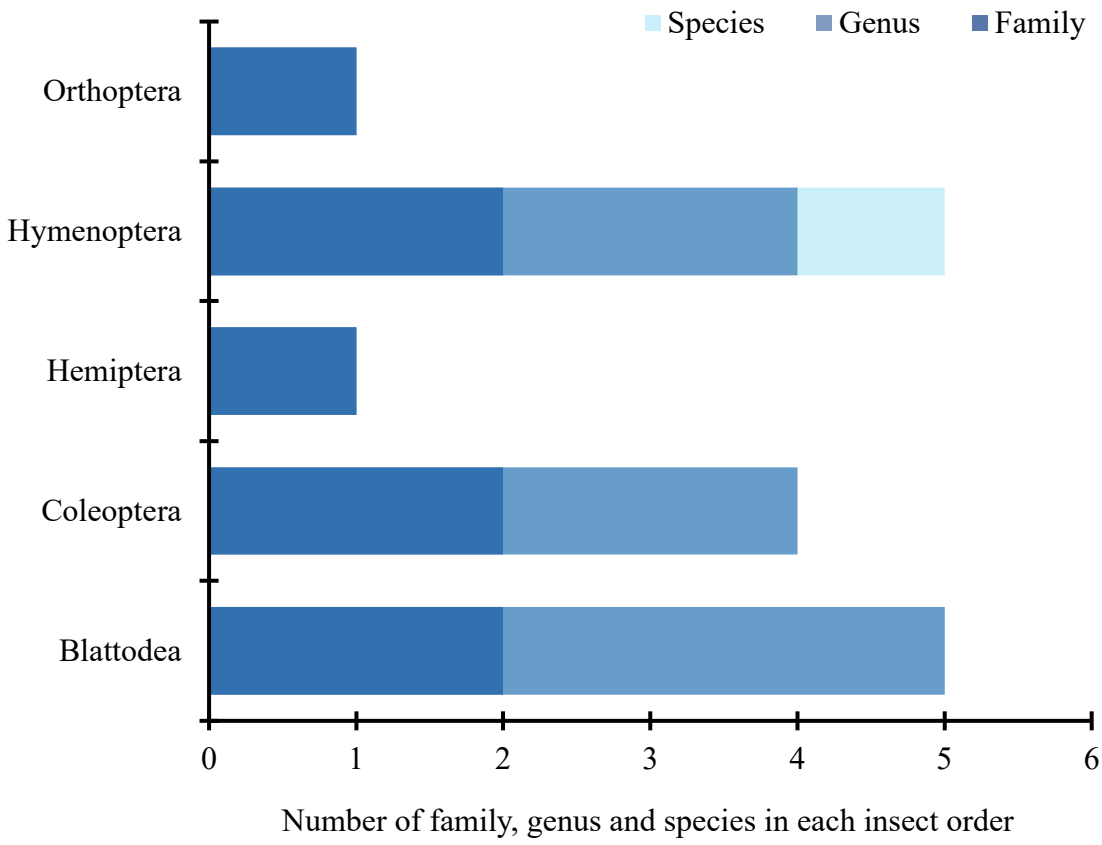


Figure 3.3. Taxonomic diversity of insects in sloth bear diet

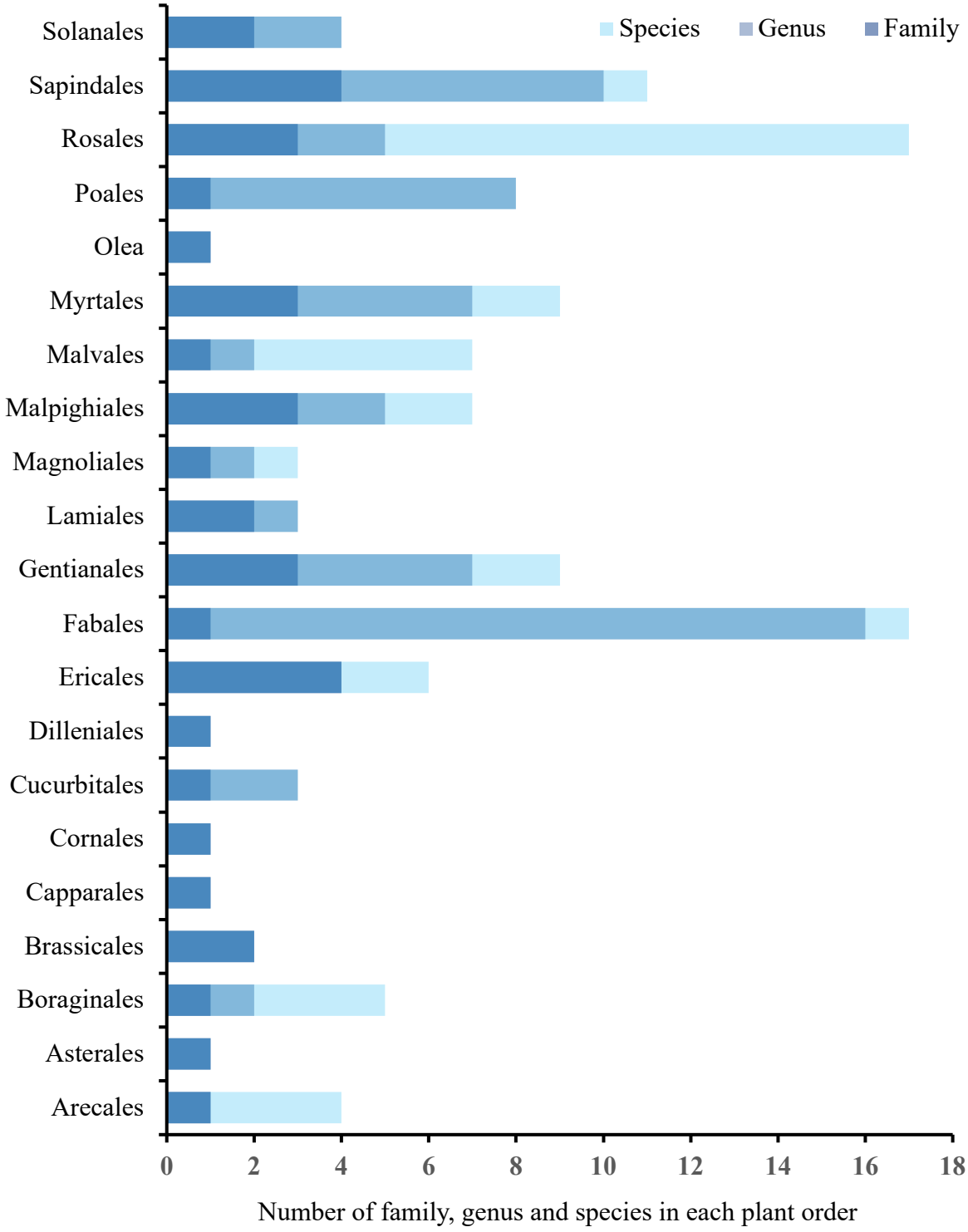


Figure 3.4. Taxonomic diversity of plants in sloth bear diet

CHAPTER IV

HUMAN-SLOTH BEAR CONFLICTS IN CHITWAN NATIONAL PARK, NEPAL

INTRODUCTION

Effective conservation of many threatened species depends on the successful co-existence of people with wildlife (Carter et al., **2012**, Frank et al., **2019**; Woodroffe et al., **2005**). Increasing human-wildlife conflicts (HWC) and other conservation threats are pushing Asian bears to extinction (Gomez et al., **2021**). HWC arises when the requirements of people and wildlife overlap, creating costs for both (Inskip & Zimmermann, **2009**). Human casualties are the dominant cost of human-wildlife conflict, particularly for rural people living near wildlife-rich areas (Gulati et al., **2021**).

Population growth and associated forest loss and fragmentation in Nepal have increased the proximity of wildlife to humans (Ram et al., **2021**). Human settlements are increasingly becoming conflict hotspots with increased conflict incidents involving mega-herbivores and large carnivores (Acharya et al., **2016**). This phenomenon is prominent in CNP, which has a dense human population outside its boundary and a significant abundance and diversity of large carnivores like tigers, leopards, and megaherbivores like elephants and rhinos. A growing number of studies report human-wildlife conflicts, particularly involving tigers and leopards (Dhungana et al., **2018**; Lamichhane et al., **2018**; Pokherel & Wegge, **2019**), elephants (Neupane et al., **2017;** Pradhan et al., **2011**) and rhinos (Pant et al., **2020**; Subedi et al., **2017**). Despite being recognized as one of the most unpredictable and feared wildlife species (DNPWC, **2017**), studies exploring the trend and characteristics of human-sloth bear conflict are almost non-existent (Garshelis et al., **1999**; Joshi et al., **1997**; Laurie and Seidensticker, **1977**; Lamichhane et al., **2016**). In contrast, studies in India have recognized the dynamic nature of human-sloth conflict and it's implications for conservation (Dhamorikar et al., **2017;** Garcia et al., **2016**; Sharp et al., **2020**). Disregarding HWC in a human-dominated landscape pose a challenge to the conservation of threatened species. An increase in the population of species increases the probability of adverse encounters that can lead to death and injury of humans and damage to crops and properties. Such conflict events can further induce retaliatory killings and may require the removal of conflict animals despite their rarity and importance. In

this context, this study explores the spatial-temporal patterns of human casualties from sloth bears.

MATERIAL AND METHODS

Study Area

The study was conducted in CNP (**Figure 4.1**). It is Nepal's first protected area and designated UNESCO world heritage site. CNP is a hotspot for biodiversity and harbors the world's second-largest population of greater one-horned rhinoceros, Nepal's largest population of tigers and many wild Asian elephants. Land cover of CNP is mainly dominated by *Shorea robusta* forest (73%), followed by grasslands (12%), riverine forest (7%), and wetland and associated areas (8%). The core area of the park is 952 km^2 and borders Parsa National Park in the east and Valmiki Tiger Reserve (India) in the south. An area of 730 km^2 surrounding the park is designated as buffer zone. Buffer zone serves as a transition zone between the core park habitat and human-dominated landscape that facilitates the dual role of provisioning ecosystem services to the local communities as well as additional habitat for wildlife. Local communities engage in participatory conservation and development activities in their respective BZ areas through the 22-buffer zone user committee (BZUC). Most of the local people are traditionally associated with forests for sustaining their agrarian livelihood.

Data collection and analysis

Research permission for the study was obtained from the DNPWC and CNP. Human-wildlife conflicts in CNP have been recorded since its establishment, however, details including the date of incidents are available only for recent years (Lamichhane et al., **2018**). Protected area authorities objectively verify incidents of human casualties and other damages by wildlife before providing financial support to the victims or their dependents according to the guideline (Acharya et al., **2016**). Database of adverse human-wildlife interaction incidents involving death or injury of people caused by sloth bears over 12 years (2008-2019) in CNP was collected for the study. For each incidence, the type of conflict (death or injury) and time of the incident (year, month, and day) was extracted. Additional information on the characteristics of the victims and the incidents were obtained through a survey of 100 households involved in adverse human-sloth bear interactions between 2008-2019. Household heads or adult family members were interviewed using a pre-structured questionnaire in March-April, 2020 after obtaining their verbal consent to participate. The survey collected information on the

demographic background, livelihood practices, the activity of bears and victims during the incidents, and their perception of human-sloth bear existence. Seasons were defined as winter (December-January-February), spring (March-April-May), summer (June-July-August) and autumn (September-October-November). Simple linear regression and χ^2 goodness-of-fit test was used to examine temporal and spatial patterns of human casualties. Statistical analysis and chart preparations was done in MS Excel (Microsoft Office). QGIS3.16.8 was used to extract co-variates information and prepare map showing the study area and the distribution of human casualties.

RESULTS

During the study period, 74 incidents of adverse human-sloth bear encounters were recorded (**Figure 4.2**). Only one of these encounters was related to human death, while all other events were associated with human injuries.

Temporal patterns of conflicts Human sloth bear conflict was prevalent throughout the year. The human injuries caused by the sloth bear attacks showed a decreasing trend but at a statistically non-significant rate (-0.42, R^2=0.13, $p > 0.05$) during the study period. On average, 6.17 (SD = 2.96) human casualties from sloth bear encounters occurred every year. The prevalence of adverse interactions resulting in casualties varied across seasons. The greatest human casualties from sloth bear attacks occurred in winter (35%) and spring (31%). Human sloth bear conflict was comparatively lower in summer (15%) and autumn (19%) (**Figure 4.3**).

Spatial patterns of conflicts The recorded human-sloth bear encounters were concentrated in a few buffer areas surrounding the park. Among all these human-sloth bear conflict, 80% of incidents occurred in the southwest sector of park in Madi municipality. Within this area, more than half of all the human casualties from the sloth bear attacks belonged to the *Aayodhyapuri* and *Rewa* buffer zone forest user communities (**Figure 4.4**).

Characteristics of conflicts Majority of the victims of human-sloth bear conflicts were dependent on the forest-livestock and agriculture-based farming activities (**Table 4.1**). Most of the human casualties occurred when victims were walking alone in and around their village (40%), followed by activities in the forest (39%), farm activities (7%) and regular activities at home (14%) (**Table 4.2**, **Figure 4.5**). Single bear was involved in 59% of the conflict events while in 31% of events a mother with a cub was involved. Respondents identified 40% of bears involved in the conflict as females, 12% as males, and 48% were not identified. More than half of the conflict events (59%) were reported to have occurred in the evening (17:00-19:00),

followed by morning (5:00-10:00, 16%), afternoon (14:00-16:00, 14%), noon (11:00-13:00,6%) and night (20:00-5:00,5%).

Relief and compensation The amount of relief and compensation provided to the human-sloth bear victims increased over the years. A total of US$ 22,041 was provided as compensation and relief to the victims or their families during the study period (**Figure 4.6**). On average, the protected area authority provided US$ 1,837 annually as relief and compensation to the victims of human sloth bear conflict.

DISCUSSION

The findings from this study indicates the prevalence of human-sloth bear conflict in CNP and its buffer zone areas. Significant patterns of decrease in the conflict trends during the study period was not detected. Results showed no significant difference in the human-sloth bear conflict between seasons. These findings contrast the trend reported from many areas of sloth bear habitat in India (Debata et al., **2017**; Garcia et al., **2016**; Rajpurohit & Krausman, **2000**; Singh et al., **2018**). Many reported increases in human-sloth bear conflicts are primarily from the central Indian landscape, which harbors the largest sloth bear population in its range (Rajpurohit & Krausman, **2000**). Sloth bears have larger home range and greater movement between habitats, as indicated by relatively higher genetic diversity and gene flow between meta-populations (Dutta et al., **2015**; Thatte et al., **2020**,). Comparatively, sloth bears in CNP have a smaller home range indicating a resource-rich habitat (Joshi et al., **1995**). Majority of the human-bear conflicts in India were reported from a relatively degraded landscape where sloth bears raided the crops and/or areas where local people visited the forest for livestock grazing, collection of wild fruits, mushrooms or for open defecation (Bargali et al., **2005**; Debata et al., **2017**; Dhamorikar et al., **2017**; Garcia et al., **2016**; Singh et al., **2018**; Sharp et al., **2020**). The sloth bears in CNP has a pronounced myrmecophagous diet than a plant-based diet and no indication of crop-raiding in recent decades (Joshi et al., **1997**; Khanal & Thapa, **2014**), suggesting that limitations of food resources may not be the major cause for these conflicts.

The population trend of sloth bears in the area needs to be better understood but about 250 adult sloth bears were estimated to be present in the park in the 1990s (Garshelis et al., **1999**). Personal observations and incidental capture in camera traps suggested researchers to believe an increase in sloth bear populations in area, but only 39 individuals were sighted during rhino survey in 2015 (Lamichhane et al., **2016**). More individuals were observed in the eastern and

central sectors of the park, providing an index of sloth bear population and its distribution (Lamichhane et al., **2016**). However, over 80% of the human-sloth bear conflicts occurred in the southwestern area under Madi management sector of the park. Incidental observations of sloth bears done during targeted surveys of other species may not adequately capture the population trends of sloth bears, thus, limiting the association between the population of sloth bears and conflict trends in the study area. A study from the area showed that neither the trends of the human population nor the wildlife population have a significant influence on the frequency of death and injuries from attacks (Lamichhane et al., **2018**). Conflict-causing individual animals can differ from the rest of the population, with physically impaired or transient animals without territory being more likely to be involved in conflicts (Lamichhane et al., **2017**).

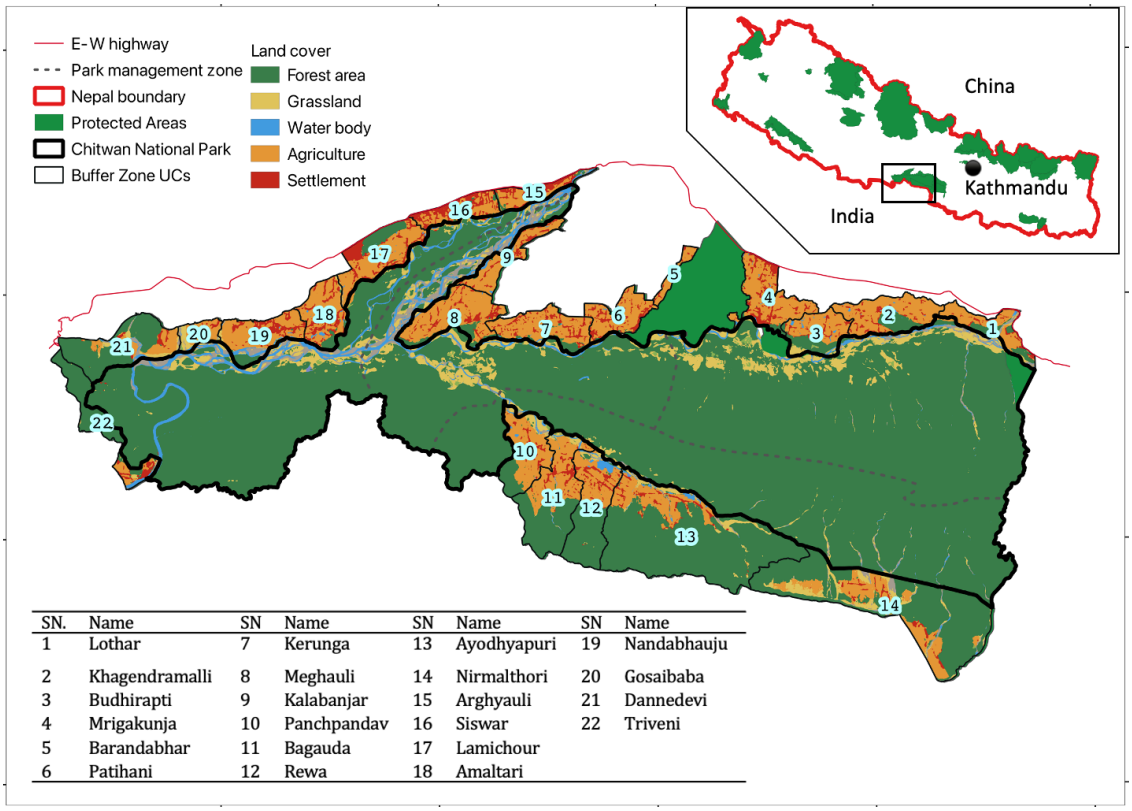
The human-sloth-bear conflict was higher in the winter season in the study area, which is similar to findings reported from Karnataka (Sharp et al., **2020**) but different from that reported for monsoon at Dnyanganga wildlife sanctuary (Singh et al., **2018**) and north Bilaspur Forest division (Bargeli et al., **2005**) in Central India. High conflicts in winter may be due to reduced visibility and increased activity of bears. Female sloth bears emerge out of their den with cubs to start foraging during the winter season (Joshi et al., **1999**). Interviews with the victims showed that multiple bears were present during 41% of the conflict events, with mothers with cubs reported being present in 31% of events. In winter, the temperature is relatively lower (12-26^{o}C), leading to decreased visibility and increased concentration and overlap of human and sloth bear activity during the daytime. Visibility is significantly reduced because of foggy weather and an increase in understory vegetation cover. Regeneration and establishment of bushes of invasive species like the *Lantana camera* along the walking routes reduce visibility and provide hiding and resting areas for sloth bears which can increase human-sloth bear encounters (Debata et al., **2017**; Sharp et al., **2020**; Singh et al., **2018**).

Besides demographic and ecological factors associated with wildlife, social factors can be more critical in driving human-wildlife conflicts (Dickman et al., **2010**). Characteristics of respondents and encounter events in from the questionnaire survey indicate that high dependence on forest and farm-based activities for livelihood may be a risk factor for human-bear conflicts. Most bear attacks were sudden and defensive attacks by female bears likely to protect the cubs nearby. Similar reports of a high number of attacks from sloth bears occurred in areas with forest-dependent tribal communities when the victims were busy with outdoor farm and forest activities (Bargali et al., **2005**; Debata et al., **2017**; Garcia et al., **2016**; Singh

et al., **2018**). The human-sloth-bear conflict was highest when human activity in the forest was greatest and the season and time of highest human activity also varied significantly by region (Sharp et al., **2022**). The buffer zone areas of the national park provide extended habitat for wildlife and forest products for local communities and thus are becoming hotspots of conflicts. Discrepancies in the trend of conflict and the amount of compensation may be because of increase in the serious nature of conflict events and the rise in the maximum amount that can be claimed from the government. Guidelines for relief and compensation to be claimed from the government started recently, and the procedures are often arduous and time-consuming (Acharya et al., **2016**; Baral et al., **2021**), thus actual cost and loss involved may be much greater. Extensive investigations of such habitat features, along with human and bear activity at the edges of their habitat, may be required for a deeper understanding of the causes of the conflicts.

CONCLUSIONS

This study shows that human-sloth bear conflict was prevalent, but the annual and seasonal trends of conflicts was not statistically significant. Madi valley was the hotspot of most of the human-sloth bear conflicts in the park. Factors pertaining behavior of sloth bears, activities of humans, and habitat features may drive these conflicts, but such drivers could not be identified in this study. If conflicts between sloth bears and people persist economic cost would increase, and the local support for the conservation would likely decrease, threatening achievements made in the area. Promoting human-bear co-existence in the future requires conservation efforts to focus adequately on identifying the social and ecological dimensions involved.



SN.	Name	SN	Name	SN	Name	SN	Name
1	Lothar	7	Kerunga	13	Ayodhyapuri	19	Nandabhauju
2	Khagendramalli	8	Meghauli	14	Nirmalthori	20	Gosaibaba
3	Budhirapti	9	Kalabanjar	15	Arghyauli	21	Dannedevi
4	Mrigakunja	10	Panchpandav	16	Siswar	22	Triveni
5	Barandabhar	11	Bagauda	17	Lamichour		
6	Patihani	12	Rewa	18	Amaltari		

Figure 4.1 Study area map of CNP showing the land cover and buffer zone areas

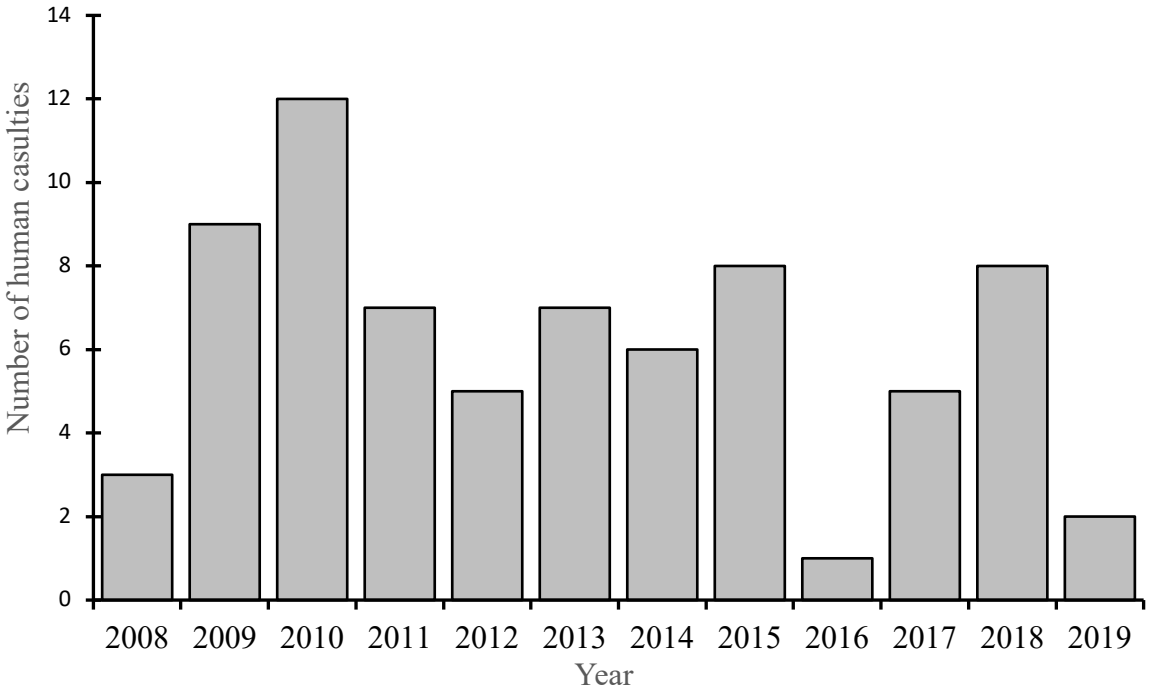


Figure 4.2 Annual trend of human-sloth bear conflicts (2008-2019)

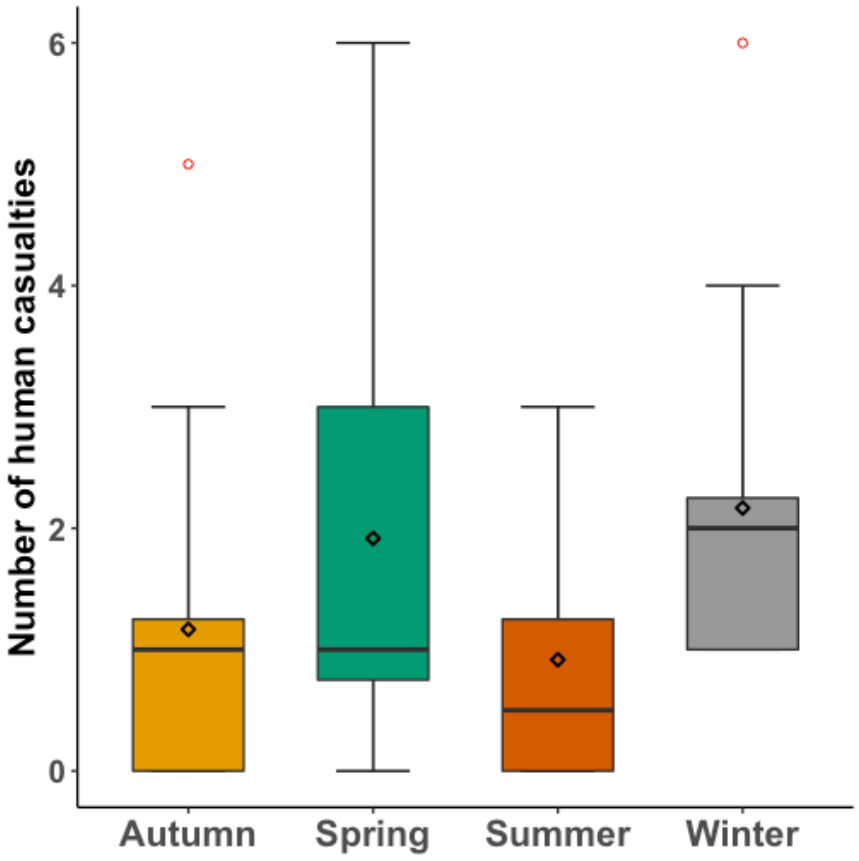


Figure 4.3. Seasonal distribution of the pattern of human casualties by the sloth bear. The cross mark inside the box indicates the mean value of human casualties

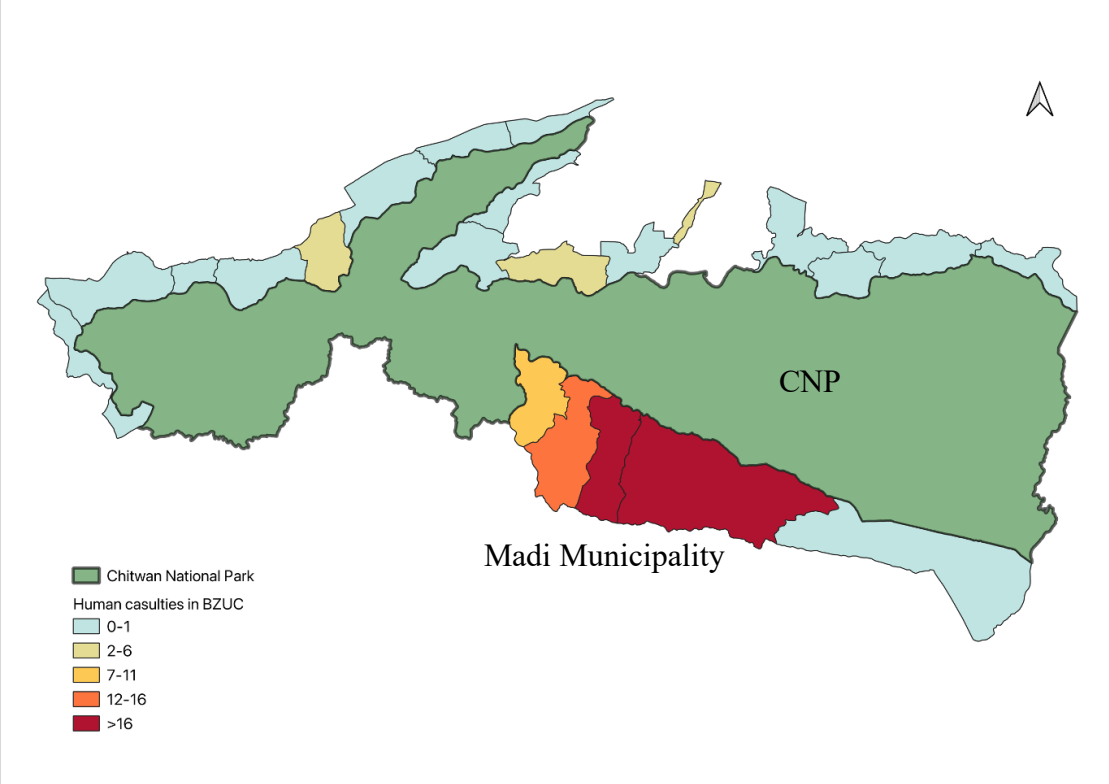


Figure 4.4 Spatial distribution of human casualties from sloth bear in CNP

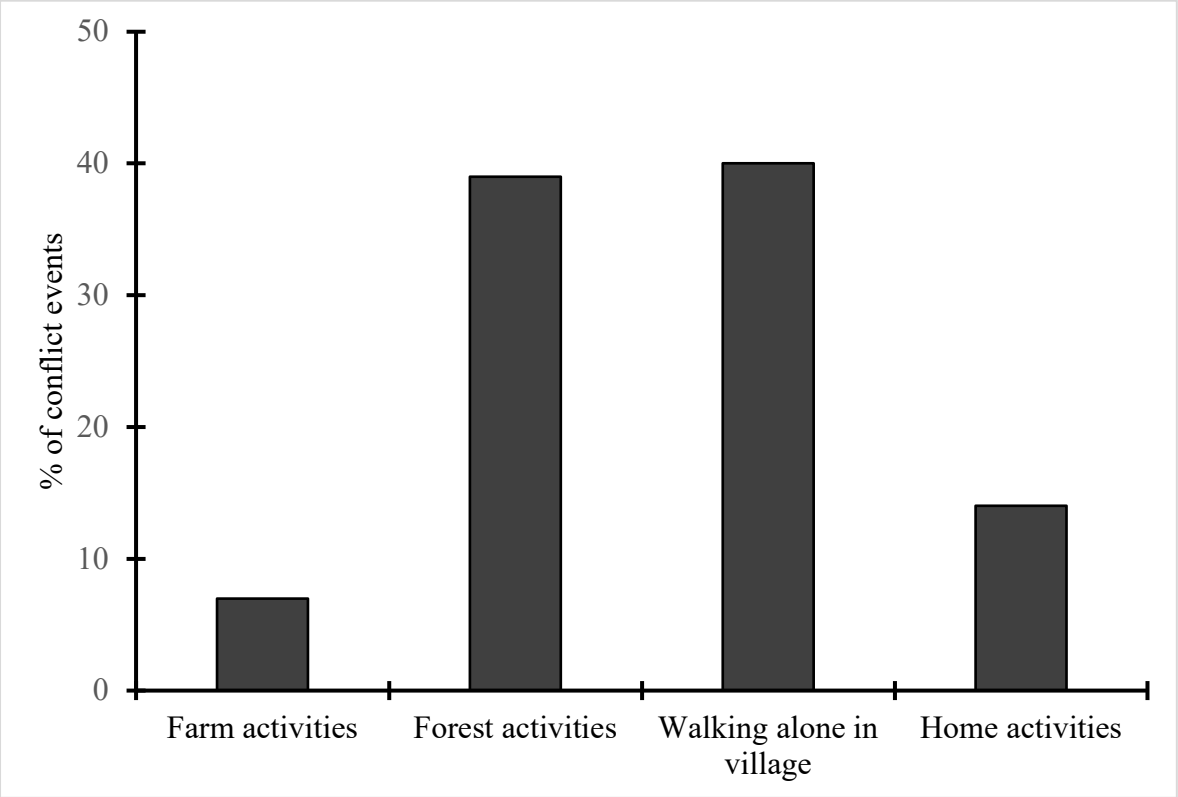


Figure 4.5 Activity of victims during adverse interactions with sloth bear

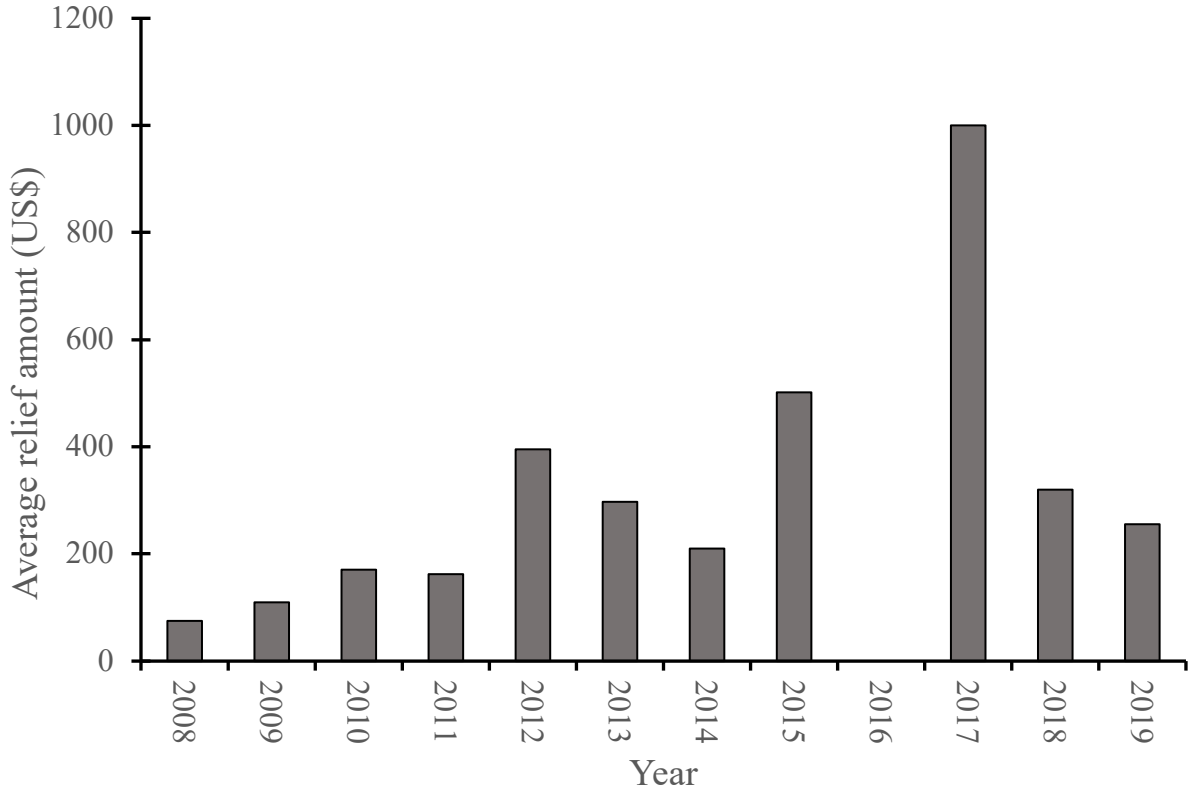


Figure 4.6. Pattern of the average annual amount of relief and compensation provided to the sloth bear victims

Table 4.1 Characteristics of the respondents involved in the survey

Characteristics of the respondents	Percentage (%)
Gender	
Male	70
Female	30
Age Group	
≤ 30	10
30-40	10
40-50	15
50-60	34
60-70	21
≥ 70	10
Education level	
No formal education	40
≤ 5 years of formal education	22
upto 10 years of formal education	34
≥ 12 or more years of formal education	4
Years of residency in the locality	
≤ 30 years	11
30-40 years	17
40-50 years	24
50-60 years	27
≥ 60 years	21
Major source for livelihood	
On Farm (Forest-Livestock-Agriculture)	79
Off-Farm (Pvt./Gov. Jobs & Business)	10
Average contribution for livelihood	
On-farm activities	
≤ 40%	7
40-60 %	13
60-80%	33
≥ 80%	47
Off-farmactivities	
≤ 20%	83
20-40	9
40-60	7
≥ 60%	1
Frequency of forest vist	
Daily	36
Weekely	26
Monthly	29
Rarely	9

Table 4.2. Characteristics of human sloth bear conflict events

Characteristics of human-bear conflicts	Percentage (%)
Human activity at the time of conflict	
Farm activities	7
Forest activities	39
Walking alone in village	40
Home activities	14
Bear number during conflict event	
Single bear	59
Multiple bear	41
(Mother with cubs)	31
Sex of bear involved in attack	
Female	40
Male	12
Uncertain	48
Bear activity during encounter	
Already attacking	24
Feeding	9
Resting	6
Walking	57
Uncertain	4
End of Encounter	
Bear ran away	57
People chased away bear	41
Animal chased away bear	2
Claimed for Relief & Compensation	
Yes	81
No	19
Human injury type	
Serious	75
Normal	25

CONCLUSION AND CONSERVATION IMPLICATIONS

The sloth bear population in Nepal is characterized by the presence of relatively low genetic diversity and unique haplotypes. The population consists of a minimum of 37 sloth bear individuals, with most individuals in CNP and a few in BNP and TJF. They have a wide distribution with a high probability of habitat occupancy in the north and central areas of the CNP. Within this habitat, their probability of occupying the habitat is most significantly influenced by the presence of termites. Sloth bears are likely to increase their habitat use when it is rugged, dry, not too dense, have fruit plants, and are free from high human disturbance. Unlike other ursids, sloth bears are highly insectivorous with a myrmecophagous diet and seasonally frugivorous. Conflict with humans is prevalent, with no significant patterns over seasons and years. Different ecological and social factors are responsible for these conflicts. Most conflicts likely occur due to sudden encounters between humans and bears when victims walk alone or are involved in forest-related activities. Most attacks by bears are likely a defensive response by female sloth bear to protect the cubs.

Wildlife and their habitats in Nepal are already under pressure from human activities, which is further exacerbated by the increasing impacts of climate change (MFSC, **2014**; Pant et al., **2020**). The long-term viability of sloth bears in Nepal depends on safeguarding the existing population and ensuring connectivity through adequate forest cover, food resources (termites, ants, and fruiting plants), and safety (minimal risk from human pressure, road, traffic, and other infrastructures) along the corridors. The functionality of the 'Siwalik' corridor will be crucial in facilitating the gene flow and maintaining genetic diversity in sloth bears from Nepal. Studies on tigers (Subedi et al., **2021**; Thapa et al., **2017**, **2018**) suggest these corridors can facilitate dispersal, genetic exchange, and maintaining the meta-population dynamics of large mammals. Tigers and sloth bears co-occur in Nepal, where the former's population has doubled since 2009 (DNPWC & DFSC, **2022**). Direct threats to sloth bear populations through predation by tigers are low (Joshi et al., **1999**), but indirect consequences of habitat alteration due to tiger-focused management can be expected.

Unique habitat requirements and low genetic diversity suggest that incidental conservation measures aimed at other species may not be adequate for sloth bear conservation in Nepal. Management actions should be geared toward creating connected habitat that enables sloth bears to access their foods throughout the year, disperse and successfully reproduce. Its unique characteristics and ecological importance make the sloth bear a potential umbrella species (Puri

et al., **2015**; Ratnayeke & Manen, **2012**). Current results and the recent reports of sloth bears outside the protected area along the Churia landscape (Pokharel et al., **2022**; Subedi et al., **2021**) hint to such a possibility in Nepal. Rigorous assessments of its population and ecological interactions are essential to strengthen current knowledge on sloth bears in Nepal. Additional genetic sampling using hair traps will further strengthen current results on sloth bear genetics. Direct or indirect foraging observations using animal-borne video collars and microscopic and molecular techniques to identify easily digested food items may produce additional information on the feeding ecology of sloth bears. Intensive sampling in the human-dominated landscape is recommended for a detailed evaluation of its foraging behavior and co-existence with humans. If conflicts between sloth bears and people persist, the local community support for the conservation would likely decrease, threatening achievements in the area. Promoting human-bear co-existence in the future requires conservation efforts to focus adequately on identifying the social and ecological dimensions of conflicts and judicious management of the population with adequate consideration to the genetic health. Current findings provide a valuable baseline for future actions and strategies aimed at sloth bear conservation and management in Nepal.

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SUMMARY

The sloth bear (*Melursus ursinus*) is listed as a globally 'Vulnerable' species but has received very low conservation attention in Nepal despite their rarity and ecological importance. Their populations have declined across their distribution range mainly because of habitat deterioration and adverse human-bear interactions, including poaching and retaliatory killings. Large and well-connected habitats provide opportunities for gene flow and maintenance of high genetic diversity. Adequate genetic variation in a population is essential to increase their resilience against disease and pest, and flexibility to adapt in a changing environment. Habitat related features can regulate population growth through changes in availability of food, cover and safety. Thus, a thorough understanding of ecological requirements and genetic status is fundamental for developing effective strategies for sloth bear conservation.

In Chapter I, I describe the distribution and determinants of habitat use by sloth bears. I used the occupancy method to account for imperfect detections during sign surveys and provide robust estimates of habitat occupancy. The model-averaged habitat occupancy estimate was 69% and the detection probability was 0.25. The probability of habitat occupancy by sloth bears increased with the presence of termites and fruits and in rugged, dry, open, undisturbed habitats. Results indicate that the sloth bear had a wide distribution in CNP with high occupancy in the central and northern parts of the park.

In Chapter II, I explore the genetic status of sloth bears from Nepal. To elucidate the levels of genetic diversity and population genetic structure, I genotyped 127 samples using twelve microsatellite loci, identifying 37 individuals in an area of approximately 1000 km^2. The sloth bear population in Nepal has a relatively low genetic diversity ($H_E = 0.48$) compared to other bear populations across its range. I did not detect adequate evidence of genetic sub-structuring of the population across the landscape. Primers specific to bears were designed to amplify the fragment of mitochondrial control region from collected samples. Four haplotypes were observed with two haplotypes in CNP and one each in BNP and TJF. The resulting phylogeny indicated that sloth bears from Nepal are evolutionarily distinct from the other known sloth bear populations.

In Chapter III, I elucidated the dietary composition of sloth bears from Nepal. An analysis of 194 fecal samples showed a high myrmecophagous diet dominated by termites and ants. Insect occurred in 95.36% of the feces and the fruits occurred in 25.77% of the feces samples. Insects had a high percentage frequency of occurrence across seasons. Fruits occurred at a higher

proportion in monsoon season in CNP and its proportion was high in both monsoon and winter seasons in BNP. I did not detect food items of human origin or cultivated crops in the sloth bear feces.

In Chapter IV, I explore the human-sloth bear interactions in Chitwan National Park. I report 74 incidents of adverse human-sloth bear encounters in 12 years period. Although 6.17 (SD = 2.96) human casualties from sloth bear encounters occurred every year, I did not detect significant trend of variation in conflicts with time or season. 80% of all these human-sloth bear conflict incidents were reported from the southwest sector of park in Madi municipality. Interviews with victims of human-sloth bear conflicts reveled their high dependence on forest and farm activities, low level of education. Most of the human casualties occurred when victims were walking alone in and around their village (40%), followed by activities in the forest (39%). Single bear was involved in 59% of the conflict events while in 31% of events a mother with a cub was involved.

This is the first genetic study of sloth bears from Nepal. It is also a first-of-its-kind study combining occupancy methods, diet, and conflict to evaluate the ecological status of the sloth bears in Nepal. The information herein have important conservation implications. Reduction of genetic diversity can have severe consequences on individual fitness whereby their potential to adapt and evolve with changing habitat conditions may be seriously compromised. Safeguarding existing habitats and connecting habitat patches with corridors can be a key landscape-level conservation intervention. At a fine scale, identifying suitable habitats and conserving them to ensure that they do not pose a high risk from human and non-human predators, provide adequate shelter, and most importantly, supply diverse food resources in abundance (particularly termites, ants and fruit plants) can be a major intervention. Conservation of sloth bears in its northern distribution range can contribute to the enhancement of biodiversity and ecosystem services throughout the Gangetic plains, as they have been argued to be a better umbrella species and a proxy for carnivore monitoring. This study have breached the long information barrier on genetic and ecological aspects of sloth bears from Nepal that should be adequately considered in future strategies and action plans aimed at bear management and conservation.

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ANNEXES

Annex 1: Presence-absence data of sloth bears in 200m segments for occupancy analysis

Site	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex 1: Presence-absence data of sloth bears in 200m segments for occupancy analysis

Site	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5
24	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	1	1	1	1	1
28	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	1	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0
32	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0
34	1	1	0	1	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
39	1	0	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0
40	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1
41	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex 2. Untransformed site level data for field based and remotely sensed co-variates

Site	Term	Frut	Dist	Tcov	EVI	TRI
1	0.25	0.15	0.72	61.45	0.47	18.05
2	0.15	0.10	0.02	68.95	0.46	7.46
3	0.00	0.40	0.25	38.05	0.44	13.83
4	0.00	0.05	0.95	27.45	0.43	10.79
5	0.10	0.05	0.10	41.45	0.48	20.44
6	0.10	0.15	0.73	61.40	0.43	19.73
7	0.15	0.20	0.33	53.60	0.45	8.32
8	0.05	0.35	0.13	41.70	0.47	3.48
9	0.00	0.55	0.00	66.55	0.45	20.60
10	0.05	0.20	0.00	69.75	0.49	8.03
11	0.00	0.10	0.31	46.40	0.43	20.52
12	0.30	0.05	0.33	72.15	0.48	21.32
13	0.00	0.20	0.33	57.95	0.45	8.26
14	0.00	0.45	0.13	53.35	0.43	22.76
15	0.05	0.05	0.00	58.85	0.46	10.39
16	0.05	0.45	0.07	63.10	0.45	4.00
17	0.00	0.20	0.00	68.80	0.46	15.80
18	0.00	0.80	0.00	68.40	0.49	25.45
19	0.10	0.25	0.44	51.05	0.45	12.71
20	0.60	0.10	0.03	65.20	0.46	12.36
21	0.00	0.00	0.17	76.35	0.48	5.66
22	0.00	0.25	0.00	62.75	0.46	5.95
23	0.00	0.00	0.00	58.85	0.45	4.98
24	0.00	0.20	0.00	71.35	0.46	14.52
25	0.05	0.25	0.05	66.41	0.45	24.41

Annex 2. Untransformed site level data for field based and remotely sensed co-variates

Site	Term	Frut	Dist	Tcov	EVI	TRI
26	0.00	0.15	0.30	40.40	0.34	18.87
27	0.20	0.25	0.00	58.55	0.40	7.89
28	0.10	0.85	0.00	42.95	0.41	29.69
29	0.30	0.55	0.03	54.50	0.42	25.69
30	0.40	0.10	0.00	68.65	0.43	18.55
31	0.40	0.00	0.00	70.30	0.45	6.42
32	0.15	0.00	0.10	63.24	0.46	9.24
33	0.25	0.15	0.02	60.09	0.46	15.23
34	0.00	0.05	0.00	66.20	0.46	11.84
35	0.05	0.00	0.00	65.70	0.44	32.53
36	0.00	0.10	0.20	29.15	0.39	69.81
37	0.05	0.45	0.00	49.90	0.44	42.37
38	0.10	0.80	0.05	48.30	0.45	26.05
39	0.15	0.40	0.08	31.05	0.45	14.82
40	0.20	0.40	0.00	17.65	0.43	28.70
41	0.00	0.00	0.17	34.66	0.42	37.84
42	0.00	0.25	0.10	62.40	0.45	9.34
43	0.00	0.10	0.00	44.50	0.45	68.26
44	0.00	0.00	0.08	33.55	0.46	12.64
45	0.00	0.00	0.00	58.55	0.46	16.43

Annex 3. Database of 37 sloth bears for 12 microsatellite loci in three locations from Nepal

SN	Location	ID	G10La	G10Lb	G1Aa	G1Ab	G10Ba	G10Bb	G10Ja	G10Jb	CXX203	CXX203	UMAR2a	UMAR2b	G10Ha	G10Hb	Cxx20a	Cxx20b	MU05a	MU05b	MU09a	MU09b	MU59a	MU59b	G10Ma	G10Mb	Sex
1	CNP	C322	128	128	186	186	148	148	100	106	146	150	198	198	236	242	141	147	138	138	126	130	114	116	214	214	F
2	CNP	C344	128	128	186	186	148	156	100	108	144	150	198	198	228	236	141	147	136	138	126	126	114	114	214	214	M
3	CNP	C345	128	128	186	186	148	156	100	108	146	150	198	198	228	236	141	147	136	138	126	126	114	114	214	214	M
4	CNP	C353	128	130	186	186	148	156	100	108	144	146	198	198	236	242	141	143	134	136	126	130	114	114	214	214	M
5	TJF	U006	128	130	186	186	148	148	100	110	146	146	198	198	236	242	143	143	138	138	126	126	116	116	214	214	UK
6	CNP	C346	128	128	186	198	148	148	100	114	144	144	198	198	236	242	141	141	136	138	126	126	114	114	214	214	M
7	TJF	H001	128	128	186	190	148	148	100	114	144	146	198	198	234	234	141	143	138	138	126	130	114	116	208	214	M
8	CNP	C029	128	128	186	186	148	148	100	114	146	150	198	198	236	236	143	147	136	136	126	130	116	116	210	214	M
9	CNP	C339	128	132	186	186	148	148	100	114	144	146	198	198	228	236	141	143	138	138	126	130	114	116	210	214	UK
10	CNP	C352	132	132	186	186	148	156	100	114	144	150	198	198	236	242	141	147	136	142	126	126	114	116	210	214	F
11	CNP	C015	128	128	186	186	148	148	108	108	144	146	198	206	228	234	141	143	136	136	126	126	114	116	214	214	M
12	CNP	CH010	128	128	186	186	148	156	108	108	144	144	198	198	234	236	141	141	136	138	126	126	114	114	214	214	M
13	CNP	C022	130	132	186	186	148	148	108	112	144	146	198	198	228	236	141	143	136	138	126	130	114	116	214	214	UK
14	CNP	H003	128	128	186	186	148	148	108	114	144	146	198	198	228	234	141	143	136	138	126	130	114	116	214	214	M
15	BNP	B009	128	132	186	186	148	148	112	112	144	144	198	198	236	236	141	141	134	138	126	126	114	116	208	208	M
16	CNP	C325	128	132	186	186	148	148	112	114	144	144	198	198	228	236	141	141	138	138	130	130	116	116	210	210	UK
17	BNP	B004	128	128	186	186	148	148	112	116	144	144	198	198	228	242	141	141	134	138	126	126	114	116	214	214	F
18	CNP	C341	128	128	186	186	148	148	114	114	144	146	198	198	228	228	141	143	136	138	126	130	114	114	214	214	F
19	CNP	C112	128	132	186	190	148	148	100	100	144	144	198	206	236	236	141	141	136	138	126	130	114	116	210	214	M
20	CNP	C108	128	132	186	190	148	148	100	100	144	144	206	206	236	236	141	141	136	138	126	130	114	116	210	214	M
21	TJF	H011	128	128	186	194	148	148	100	112	144	146	198	198	236	242	141	143	138	138	130	130	116	116	210	214	UK
22	CNP	C355	128	128	186	194	148	148	108	108	144	144	198	198	228	236	141	141	136	138	126	130	114	114	210	214	UK
23	CNP	C123	128	128	186	198	148	148	100	112	144	144	198	198	228	236	141	141	136	138	126	130	114	116	0	0	UK
24	CNP	C028	128	132	186	198	148	148	100	114	146	150	198	198	234	234	143	147	136	138	126	130	116	116	208	210	M
25	CNP	C300	130	132	186	186	148	148	100	114	144	144	198	198	228	228	141	141	136	136	130	130	116	116	208	214	UK
26	CNP	C336	128	128	186	198	148	148	100	116	146	148	198	198	234	234	143	145	136	138	126	126	114	114	214	214	UK
27	CNP	C001	132	132	186	198	148	148	108	108	146	146	198	200	234	242	143	143	138	138	126	126	114	116	214	214	M
28	CNP	C117	128	128	186	198	148	148	108	114	144	144	198	198	228	228	141	141	136	136	130	130	114	116	214	214	M
29	CNP	C347	128	128	186	198	148	148	114	114	146	146	198	198	228	228	143	143	138	138	130	130	114	116	214	214	F
30	CNP	C343	128	132	186	198	148	148	114	114	144	144	198	198	228	236	141	141	136	138	130	130	114	114	214	214	M
31	CNP	C335	128	128	190	198	148	148	100	114	144	146	198	206	234	242	141	143	138	138	126	126	116	116	210	214	M
32	CNP	C311	128	128	190	198	148	148	100	114	144	144	198	198	236	236	141	141	136	136	130	130	114	114	210	214	F
33	CNP	C354	128	128	190	198	148	148	114	114	144	144	198	198	234	242	141	141	138	138	126	126	116	116	214	214	UK
34	CNP	H006	128	130	190	198	148	148	114	114	144	150	198	198	228	242	141	147	136	138	126	130	114	116	208	214	UK
35	CNP	C122	128	132	186	198	148	148	108	114	144	144	198	198	228	242	141	141	136	138	126	130	114	116	214	214	F
36	CNP	C320	128	128	198	198	148	148	114	116	144	144	198	206	228	228	141	141	136	138	126	130	116	116	210	214	M
37	CNP	C120	128	128	198	220	148	148	114	116	144	144	198	206	228	228	141	141	136	138	126	130	116	116	210	214	UK

Annex 4. Primers used for mitochondrial analysis of sloth bears

No	Primer Name	Primer sequence (5'-3')	Remarks
1	Mt_SB_R3	TACGCCGCTTGATTCGGT	
2	Mt_SB_R4	TAGGAGGGAAGCAGAGCAGA	
3	Mt_SB_F1	ATGAATCGGAGGACAACCAG	
4	Mt_SB_F2	TCTGCCCTCCTAAGACTCA	
5	Mt_SB_R2	AGTCACTCAGGGCAAGGATG	
6	Mt_SB_R1	ACTCGGGTCAATCGCATAAC	
7	D4	GCAAGGCACTGAAAATGCCT	

Annex 5. Primers used for microsatellite analysis and sex identification of sloth bears

Locus		Primer sequence (5'-3')	References
G1A	F:	GACCCTGCATACTCTCCTCTGATG	Paetkau et al., 1995
	R:	GCACTGTCCTTGCGTAGAAGTGAC	
G10B	F:	GCCTTTTAATGTTCTGTTGAATTTG	Paetkau et al., 1995
	R:	GACAAATCACAGAAACCTCCATCC	
G10C	F:	AAAGCAGAAGGCCTTGATTTCCTG	Paetkau et al., 1995
	R:	GGGGACATAAACACCGAGACAGC	
G1D	F:	GATCTGTGGGTTTATAGGTTACA	Paetkau et al., 1995
	R:	CTACTCTTCCTACTCTTTAAGAG	
G10J	F:	GATCAGATATTTTCAGCTTT	Paetkau et al., 1998
	R:	AACCCCTCACACTCCACTTC	
G10L	F:	GTACTGATTTAATTCACATTTCCC	Paetkau et al., 1995
	R:	GAAGATACAGAAACCTACCCATGC	
MU09	F:	TTGAAGTTCAGGGTAAATGC	Taberlet et al., 1997
	R:	ATATAGCAGCATATTTTTGGCT	
MU26	F:	GCCTCAAATGACAAGATTTC	Taberlet et al., 1997
	R:	TCAATTAAAATAGGAAGCAGC	
MU59	F:	GCTCCTTTGGGACATTGTAA	Taberlet et al., 1997
	R:	GACTGTCACCAGCAGGAG	
CXX203	F:	TTGATCTGAATAGTCCTCTGCG	Cronin et al., 2009
	R:	AGCAACCCCTCCCATTTACT	
CXX20	F:	AGCAACCCCTCCCATTTACT	Ostander et al., 1993
	R:	TTGTCTGAATAGTCCTCTGCG	
UMAR2	F:	TCACGGGTTTGTAGTAAACA	Poissant & Davis, 2011
	R:	CACAAAGTGGATGCTAAGAA	
G10M	F:	TTCCCCTCATCGTAGGTTGTA	Paetkau et al., 1995
	R:	TTTCCAAATAATTTAAATGCATCC	
MU05	F:	GTGATTTTTCTTGTAGCCTAGG	Taberlet et al., 1997
	R:	GAAACTTGTTATGGGAACCA	
Y-chromosome (SMCY)	F:	GTCTTCCTCCTTAGAGGGTAATTAGG	Bidon et al., 2013
	R:	TTCGTTTGATAATGGCCTAAAACTG	
Y-chromosome -318.2	F:	AAGAAAAGTCATGCAACAGATACAG	Bidon et al., 2013
	R:	TGATGCTTTGTGATCCTAATGTG	
X-chromosome (ZFX)	F:	AAAGAAATCCCTCAAACACGTTAC	Bidon et al., 2013
	R:	TCGCCACCCRCAAATAG	

Annex 6: List of insects reported in the diet of sloth bear

S.N	Species	Family	Order	Reference
1	*Reticulitermes spp.*	Rhinotermitidae	Blattodea	Joshi et al., 1997
2	*Hypotermes spp.*	Termitidae	Blattodea	Joshi et al., 1997
3	*Macrotermes spp*	Termitidae	Blattodea	Joshi et al., 1997
4	*Microcerotermes spp.*	Termitidae	Blattodea	Joshi et al., 1997
5	*Odontotermes obesus*	Termitidae	Blattodea	Laurie & Seidensticker,1977
6	*Onthophagus spp.*	Scarabaeidae	Coleoptera	Laurie & Seidensticker,1977
7	*Phyleophaga rugosa*	Scarabaeidae	Coleoptera	Joshi et al., 1997
8	*Scaretes spp.*	Scarabaeidae	Coleoptera	Laurie & Seidensticker,1977
9	*Tenebrionid beetle*	Tenebrionidae	Coleoptera	Rather et al., 2020
10	*Cicida spp.*	Cicadidae	Hemiptera	Philip et al., 2021
11	*Apis dorsata*	Apidae	Hymenoptera	Chhangani, 2002
12	*Camponotus compressus*	Formicidae	Hymenoptera	Laurie & Seidensticker,1977
13	*Camponotus irritans*	Formicidae	Hymenoptera	Seidensticker et al., 2021
14	*Dorylus labiatus*	Formicidae	Hymenoptera	Maewada et al., 2019
15	*Solenopsis spp.*	Formicidae	Hymenoptera	Laurie & Seidensticker, 1977
16	*Gryllotalpa africana*	Gryllotalpidaae	Orthoptera	Laurie & Seidensticker, 1977

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SN	Species	Family	Order	Reference
1	*Phoenix acaulis*	Arecaceae	Arecales	Joshi et al., 1997
2	*Phoenix dactylifera*	Arecaceae	Arecales	Philip et al., 2021
3	*Phoenix humilis*	Arecaceae	Arecales	Sreekumar & Balakrishnan, 2002
4	*Phoenix sylvestris*	Arecaceae	Arecales	Maewada et al., 2019
5	*Tagetes erecta*	Asteraceae	Asterales	Chhangani, 2002
6	*Cordia domestica*	Boraginaceae	Boraginales	Philip et al., 2021
7	*Cordia gharaf/sinensis*	Boraginaceae	Boraginales	Philip et al., 2021
8	*Cordia oblique*	Boraginaceae	Boraginales	Ramesh et al., 2009
9	*Ehretia aspera*	Boraginaceae	Boraginales	Philip et al., 2021
10	*Ehretia laevis*	Boraginaceae	Boraginales	Laurie & Seidensticker,1977
11	*Brassica oleracea*	Brassicaceae	Brassicales	Chhangani, 2002
12	*Careya papaya*	Caricaceae	Brassicales	Laurie & Seidensticker, 1977
13	*Capparis zeylanica*	Capparaceae	capparales	Samad & Hosetti, 2018
14	*Alangium salvifolium*	Cornaceae	Cornales	Maewada et al., 2019
15	*Citrullus lanatus*	Cucurbitaceae	Cucurbitales	Chhangani, 2002
16	*Cucumis sativus*	Cucurbitaceae	Cucurbitales	Mewada & Dharaiya, 2010
17	*Melothria maderaspatana*	Cucurbitaceae	Cucurbitales	Philip et al., 2021
18	*Dillenia indica*	Dilleniaceae	Dilleniales	Laurie & Seidensticker, 1977
19	*Impatiens balsamina*	Balsaminaceae	Ericales	Philip et al., 2021
20	*Diospyros melanoxylon*	Ebenaceae	Ericales	Philip et al., 2021
21	*Diospyros montana*	Ebenaceae	Ericales	Baskaran et al., 2015
22	*Diospyros embryopteri*	Ebenaceae	Ericales	Palei et al., 2019
23	*Careya arborea*	Lecythidaceae	Ericales	Laurie & Seidensticker, 1977
24	*Madhuca indica/longifolia*	Sapotaceae	Ericales	Maewada et al., 2019

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SN	Species	Family	Order	Reference
25	*Acacia leucophloea*	Fabaceae	Fabales	Philip et al., 2021
26	*Alibizzia odoratissma*	Fabaceae	Fabales	Ramesh et al., 2009
27	*Arachis hypogaea*	Fabaceae	Fabales	Palei et al., 2019
28	*Bauhinia racemosa*	Fabaceae	Fabales	Philip et al., 2021
29	*Butea monosperma*	Fabaceae	Fabales	Philip et al., 2021
30	*Casia fistula*	Fabaceae	Fabales	Joshi et al., 1997
31	*Cassia tora*	Fabaceae	Fabales	Sukhadiya et al., 2013
32	*Cicer arietinum*	Fabaceae	Fabales	Chhangani, 2002
33	*Cyamopsis tetragonoloba*	Fabaceae	Fabales	Chhangani, 2002
34	*Dichrostachys cinerea*	Fabaceae	Fabales	Chhangani, 2002
35	*Medicago sativa*	Fabaceae	Fabales	Chhangani, 2002
36	*Phaseolus radiatus*	Fabaceae	Fabales	Chhangani, 2002
37	*Pithecellobium dulce*	Fabaceae	Fabales	Philip et al., 2021
38	*Pueraria tuberosa*	Fabaceae	Fabales	Philip et al., 2021
39	*Saraca indica*	Fabaceae	Fabales	Philip et al., 2021
40	*Tamarindus indica*	Fabaceae	Fabales	Chhangani, 2002
41	*Vigna aconitifolia*	Fabaceae	Fabales	Chhangani, 2002
42	*Carissa spinarum*	Apocynaceae	Gentianales	Philip et al., 2021
43	*Carrisa congesta/carandas*	Apocynaceae	Gentianales	Philip et al., 2021
44	*Xemenia americana*	Oleaceae	Gentianales	Samad & Hosetti, 2018
45	*Canthium parviflorum*	Rubiaceae	Gentianales	Kumar & Paul 2021
46	*Catunaregam spinosa*	Rubiaceae	Gentianales	Maewada et al., 2019
47	*Gardenia latifolia*	Rubiaceae	Gentianales	Samad & Hosetti, 2018
48	*Ixora coccinea*	Rubiaceae	Gentianales	Sreekumar & Balakrishnan, 2002

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SN	Species	Family	Order	Reference
49	*Ixora pavetta/parviflora*	Rubiaceae	Gentianales	Samad & Hosetti, 2018
50	*Morinda pubescens*	Rubiaceae	Gentianales	Maewada et al., 2019
51	*Callicarpa macrophylla*	Lamiaceae	Lamiales	Laurie & Seidensticker, 1977
52	*Gmelina arborea*	Lamiaceae	Lamiales	Philip et al., 2021
53	*Lantana camara*	Verbenaceae	Lamiales	Philip et al., 2021
54	*Annona squamosa*	Annonaceae	Magnoliales	Philip et al., 2021
55	*Miliusa tomentosa*	Annonaceae	Magnoliales	Maewada et al., 2019
56	*Miliusa velutina*	Annonaceae	Magnoliales	Joshi et al., 1997
57	*Aporosa lindleyana*	Phyllanthaceae	Malpighiales	Sreekumar & Balakrishnan, 2002
58	*Baccaurea courtallensis*	Phyllanthaceae	Malpighiales	Sreekumar & Balakrishnan, 2002
59	*Bridelia retusa*	Phyllanthaceae	Malpighiales	Joshi et al., 1997
60	*Putranjiva roxburghii*	Putranjivaceae	Malpighiales	Maewada et al., 2019
61	*Flacourtia indica*	Salicaceae	Malpighiales	Philip et al., 2021
62	*Flacourtia jangomas*	Salicaceae	Malpighiales	Philip et al., 2021
63	*Flacourtia sepiaria*	Salicaceae	Malpighiales	Philip et al., 2021
64	*Bombax ceiba*	Malvaceae	Malvales	Joshi et al., 1997
65	*Grewia asiatica*	Malvaceae	Malvales	Philip et al., 2021
66	*Grewia flavescens*	Malvaceae	Malvales	Philip et al., 2021
67	*Grewia hirsuta*	Malvaceae	Malvales	Maewada et al., 2019
68	*Grewia schlerophylla*	Malvaceae	Malvales	Joshi et al., 1997
69	*Grewia tenax*	Malvaceae	Malvales	Samad & Hosetti, 2018
70	*Grewia villosa / orbiculata*	Malvaceae	Malvales	Samad & Hosetti, 2018
71	*Anogeissus latifolia*	Combretaceae	Myrtales	Philip et al., 2021
72	*Terminalia arjuna*	Combretaceae	Myrtales	Chhangani, 2002

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SN	Species	Family	Order	Reference
73	*Lagerstromia microcarpa*	Lythraceae	Myrtales	Ramesh et al., 2009
74	*Punica granatum*	Lythraceae	Myrtales	Chhangani, 2002
75	*Eugenia spp*	Myrtaceae	Myrtales	Laurie & Seidensticker, 1977
76	*Psidium guajava*	Myrtaceae	Myrtales	Chhangani, 2002
77	*Syzygium cerasoides*	Myrtaceae	Myrtales	Palei et al., 2019
78	*Syzygium cumini*	Myrtaceae	Myrtales	Joshi et al., 1997
79	*Syzygium heyneanum*	Myrtaceae	Myrtales	Maewada et al., 2019
80	*Olea glandulifera*	Oleaceae	Olea	Ramesh et al., 2009
81	*Bambusa vulgaris*	Poaceae	Poales	Philip et al., 2021
82	*Dendrocalamus strictus*	Poaceae	Poales	Kumar & Paul, 2021
83	*Heteropogon contortus*	Poaceae	Poales	Ramesh et al., 2009
84	*Saccharum officinarum*	Poaceae	Poales	Chhangani, 2002
85	*Seteria intermedia*	Poaceae	Poales	Ramesh et al., 2009
86	*Sporobolus spp.*	Poaceae	Poales	Baskaran et al., 1997
87	*Triticum aestivum*	Poaceae	Poales	Chhangani, 2002
88	*Zea mays*	Poaceae	Poales	Chhangani, 2002
89	*Artocarpus heterophyllus*	Moraceae	Rosales	Palei et al., 2019
90	*Artocarpus hirusta*	Moraceae	Rosales	Sreekumar & Balakrishnan, 2002
91	*Artocarpus integrifolia*	Moraceae	Rosales	Sreekumar & Balakrishnan, 2002
92	*Ficus arnottiana*	Moraceae	Rosales	Philip et al., 2021;
93	*Ficus benghalensis*	Moraceae	Rosales	Khanal & Thapa, 2014
94	*Ficus cunia / semicordata*	Moraceae	Rosales	Joshi et al., 1997
95	*Ficus glomerata / racemosa*	Moraceae	Rosales	Joshi et al., 1997
96	*Ficus religiosa*	Moraceae	Rosales	Philip et al., 2021

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97	*Ficus rumphii / cordifolia*	Moraceae	Rosales	Philip et al., 2021
98	*Ficus virens / infectoria*	Moraceae	Rosales	Philip et al., 2021
99	*Ziziphus mauritiana / jujuba*	Rhamnaceae	Rosales	Joshi et al., 1997
100	*Ziziphus nummularia*	Rhamnaceae	Rosales	Philip et al., 2021
101	*Ziziphus oenoplia*	Rhamnaceae	Rosales	Philip et al., 2021
102	*Ziziphus rugosa / glabra*	Rhamnaceae	Rosales	Palei et al., 2019
103	*Ziziphus xylopyrus*	Rhamnaceae	Rosales	Maewada et al., 2019
104	*Prunus persica*	Rosaceae	Rosales	Philip et al., 2021
105	*Rosa indica*	Rosaceae	Rosales	Chhangani, 2002
106	*Anacardium occidentale*	Anacardiaceae	Sapindales	Palei et al., 2019
107	*Buchanania lanzan*	Anacardiaceae	Sapindales	Rather et al., 2020
108	*Magnifera indica*	Anacardiaceae	Sapindales	Joshi et al., 1997
109	*Rhus semialata / chinensis*	Anacardiaceae	Sapindales	Laurie & Seidensticker, 1977
110	*Semecarpus anacardium*	Anacardiaceae	Sapindales	Philip et al., 2021
111	*Boswellia serrata*	Burseraceae	Sapindales	Philip et al., 2021
112	*Agele marmelos*	Rutaceae	Sapindales	Joshi et al., 1997
113	*Murraya koenigii*	Rutaceae	Sapindales	Joshi et al., 1997
114	*Zanthoxylum asiaticum*	Rutaceae	Sapindales	Baskaran et al., 1997
115	*Schleichera oleosa*	Sapindaceae	Sapindales	Bargali et al., 2004
116	*Schleichera trijuga*	Sapindaceae	Sapindales	Laurie & Seidensticker, 1977
117	*Ipomoea batatas*	Convolvulaceae	Solanales	Palei et al., 2019
118	*Capsicum annum*	Sollanaceae	Solanales	Mewada, 2015
119	*Lycopersicon lycopersicum*	Sollanaceae	Solanales	Chhangani, 2002
120	*Solanum indicum*	Sollanaceae	Solanales	Laurie & Seidensticker, 1977