

Project Update: January 2017

A field study on "genetic variation and ecology of Asiatic black bears (*Ursus thibetanus*)" in Annapurna Conservation Area of Nepal was conducted from June 27th 2016 to January 3rd 2017. The non-invasive samples of bears were collected from different sites of Annapurna Conservation Area whereas DNA was extracted in genetic lab of GENETUP, Kathmandu and diet of bears was analysed in the zoological lab of Institute of Forestry, Pokhara. A series of meetings were organised with related stakeholders for research permission, field coordination, logistic arrangement and lab work. First, a meeting was organised with Dr Maheshwar Dhakal and Mr Gopal Prakash Bhattarai, Deputy Director General and Mr Laxman Prasad Paudel, Ecologist of Department of National Park (DNPWC) to obtain legal permission of study. Similar type of meeting was also organised with Mr Ganga Jung Thapa, Executive Director and Dr Naresh Subedi, Coordinator, National Trust for Nature Conservation (NTNC) for seeking field support as NTNC has located field stations in different parts of Annapurna Conservation Area. Similarly, meeting was organised with Dr Bhagawan Maharjhan and Dr Bhawana Shrestha of GENETUP for seeking support of lab facility to extract DNA and organise PCR. After getting research permission from DNPWC, I travelled to Pokhara where I got permission from Annapurna Conservation Area Project (ACAP) for fieldwork. Mr Lal Prasad Gurung, Project Director and his staff from ACAP had fully supported me for the organisation of fieldwork in different sites of conservation area. Without support of ACAP central and field team, it was almost impossible to organise field work in remote mountain terrain. Fieldwork was organised after first discussion with staff of field station and discussion with community leaders (**Photo 1**).

Annapurna conservation area (**Photo 2**) is the largest conservation area of Nepal and a well-known tourist destination for mountain trekking and cultural tourism. Mixed evergreen forest and conifer forest of southern part provides habitat for Asiatic black bears. Field visits were organised in seven different sites of Annapurna conservation to represent variation in sample after the confirmation of bear existence from stakeholders, field staff, local people and final confirmation with shepherd to save our energy, money and time. Five and two units were selected from southern and trans-Himalayan region of conservation area respectively. Non-invasive samples especially faecal were searched in summer (July-September) and pre-winter (October-December) season. First, my team searched bear trail, footprint, climbing scratches, ground/log digging sites, bear food platforms (**Photo 3**) to find the existence of bears in the particular sites following the recent information from livestock herders and villagers (**Photo 4**) who frequently visit the forest. If the above-mentioned signs observed, we intensified searching for fresh faeces (≤ 4 days) for genetics and any age of faeces for diet of bears (**Photo 5**). The age of the faeces were estimated based on freshness of faeces, other bear sign in surroundings and local villagers knowledge. The surfaces of fresh faeces were rubbed using a sterile cotton swab and preserved in tube having 99.5% ethanol

(Photo 6). 99 fresh fecal samples were collected from seven different sites within Annapurna Conservation. Of which 46 were collected in July-September and 53 were collected in November-December period. Similarly, 46 and 53 faeces were found in agricultural land and wild habitat respectively (**Table 1**).

I also expected to collect hair sample that is non-invasive too but DNA quality is always good so I set up hair trap (**Photo 7**) in four different places of Mustang district with the aim to extend in other sites by observing its effectiveness. A hair trap consisted of a single barbed wired strand approximately 20 m long wrapped around at least four trees, 40-50 cm above the ground. Rotten flesh meat was baited in the center of trap at 3 m above ground level to attract bears. The trap was closely monitored by automatic camera capture (**Photo 8**). Unfortunately, I could not get any bears signs from the trap located in four locations. Fortunately, 11 hair samples were collected from barbed fences (**Photo 9**) which was set up to protect maize land and one hair sample was collected from broken branches. The GPS location, sample characteristics, and habitat descriptions were noted in detail.

Genomic DNA was extracted using Qiagen's QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) for faecal samples and Wako DNA Extractor FM Kit (Wako, Osaka, Japan) for hair samples following manufacturer's protocol with slight modification by considering equipment available in the genetic lab of GENETUP (**Photo 10**). Microsatellite and mitochondrial DNA will be analysed in future to know the genetic diversity and phylogenetic relationship of Asiatic black bears in Nepal.

Similarly, all faecal samples were soften in water and washed them through 2 mm sieves with the help of under graduate students form Institute of Forestry to separate individual food items (**Photo 11**). All items were identified to the finest taxonomic resolution possible. I visually estimated the relative volume of each food item and assigned an exact volume. Items found in trace amounts were given an arbitrary volume of 1% or only noted, but bear hairs, dry leave, and wood particles were excluded before estimating volume. I identified wild fruits, bamboo shoots, grasses, agriculture crop (maize, rice and apple), mammal's hair and insects in the bear faeces. I will calculate percent frequency of occurrence (FO) and percent volume (PV) to identify contribution of each food item of bear.



Photo 1: Interaction with ACAP field staff and



Community meeting in Mustang

community members in Sikes.



Photo 2: Map of Annapurna Conservation Area



Photo 3: Claw mark of bear on tree



Photo 4: A livestock owner who spent his whole life in forest (also attack by bear)



Photo 5: Bear faeces found in forest



Photo 6: Collection of faecal sample for genetic study from maize land



Photo 7: Hair trap set up



Photo 8: Camera for monitoring trap site



Photo 9: Bear hair in barbed wire fence



Photo 10: Extraction of DNA from bear faeces



Photo 11: a) Reference of bear food items



Photo11: b) washing of bear faeces to identify food items



Photo 12: Bear habitat landscape in Annapurna Conservation area

Table 1: Details of sample collection

S.N.	Site (place)	July - September	October - December	Total	Remarks (Agriculture land /Forest)
1	Mustang (6)	30 (11)*	3	33 (11)*	31/2
2	Mygdi (2)	6	6	12	6/6
3	Ghandruk (2)	(1)*	2	2 (1)*	0/2
4	Siding (3)	3	11	14	3/11
5	Karuwa (2)	1	7	8	0/8
6	Siklesh (3)	1	24	25	1/24
7	Manag (3)	5		5	5/0
Total		46 (12)*	53	99 (12)*	46/53

*hair samples