Project Update: November 2017

In each study site, transects line with the length of 0.5 km was laid in different altitudes at an interval of 300 m starting from 3000 m to 4500 m. Each transect visited three times at an interval of 3 days to collect pellet samples for DNA analysis. To maximise the rate of finding pellets, adaptive cluster sampling (Thompson 1991) was applied.

Here is lab method:
Ten pellets from each sample were placed in a petri dish and left for 5 minutes at room temperature to dry ethanol. The outer layer of each pellet was peeled into smaller pieces using sterile scissors. Then, DNA extractions from the pellet samples were performed as per the manufacturer's instructions for both pellet and tissue samples using a DNeasy Stool and Tissue Kit (QIAGEN). Mitochondrial Cytochrome b (Cytb) gene was amp gene was amplified with primers (L14724; 5'CGAAGCTTGATATGAAAAACCATCGTTG3', H15149; 5'AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3') and polymerase chain reaction (PCR). All PCR applications follows; initial denaturation at 95°C for 10 minutes followed by 40 cycles at 95°C for 45 seconds, 50°C for 45 seconds and 72°C for 1.5 minutes, and a final extension was held at 72°C for 5 minutes. PCR products were tested in 1.5% agarose gel. Positive samples were sequenced following bi-directional sequencing. To compare the species, sequences of the cytb gene of species of *Moschus* were downloaded from the NCBI GenBank.

Result:
The aligned dataset of cytb sequence contained 380-branch point's sequence (bps), including 375 variable sites and 58 parsimony informative sites.

Molecular data analysis suggested that the populations of musk deer from Manang of ACA were genetically similar to *M. leucogaster* (Tibet, China) and clustered together in a BI tree. Therefore, the species of musk deer in Manang is *M.leucogaster.*

Order:	Artiodactyla	Owen, 1848
Family:	Moschidae	Gray, 1821
Genus:	Moschus	Linnaeus, 1758
Specie:	Moschus leucogaster	

Regarding the population count of the deer, the budget was highly limited to form microsatellite loci test. Therefore, primers were tested and fixed. The population of the deer can be counted using different loci. Twelve primers of varying length (Table 1) were fixed for individual identity. These primers can be used for the individual count and this research is the first research testing such primer for different species (Forest musk deer) of same genus. Before this research, such primer were never tired for *M.lecucogaster*. The process to be followed is that the extracted DNA could be amplified using Multiplex amplification. Before that the primer of different length should be grouped in to one group and given different color using dyes (HEX, FAM etc.) before performing PCR. Each DNA sample should be amplified at least 4 times in each kind of primer. That means, one sample must go through 9 times

(if nine primers are used) multiply by 4 replication. For one sample, 36 times PCR reactions must be performed. Therefore, based on the budget, the species in the Nyeshyang valley was identified and the extracted DNA are deposited in – 25 °C for future use when budget would be available.

Table 1. Twelve polymorphic microsatellite loci

Locus	Repeat motif	Primer sequence(5'-3')	Ta (°C)	Product size(bp)	Accession No (GenBank)
Mb06	(AC)12	F:GATAAGCAGGCAGCAACG R:TGTCCAGGAAGAGGAGGG	58.0	294-302	EF599335
Mb10*	(AC)50	F:GTGGGAAGGCAGCACAGA R:AAGGCTCAGGTACAGTCAAGAA	60.5	290-340	EF599336
Mb18	(GT)15	F:CTCCAGGCAAGAACACTG R:GCAAGAAGTTATGCAATCAA	55.2	256-286	EF599337
Mb30	(CA)22	F:TAGACCATGACGCCAGAT R:GCTACACTGAGCCACCTAA	59.3	150-168	EF599338
Mb32	(GT)54CT(GT)	F:GCAAACACGACCAGAAAC R:CAGAAGGGAATGGCAGTA	57.9	181-197	EF599339
Mb33	(GT)26	F:TCCTCGCTGATTATTTGG R:CGGATTCGTAAAGTGGGT	55.2	226-260	EF599340
Mb34	(GT)16...(GT)13 CT(GT)6	F:CAACATTTGGGAGGAGGAT R:GTGAGGGCTTCTGGTGAT	57.9	339-365	EF599341
Mb37	(GT)9ATGG (GT)13ATGG(GT)9	F:TGTGGGTGAACTCAATCT R:ATGGTATCTGACTCCAATAT	58.2	238-256	EF599342
Mb38	(AC)14...(AC)14	F:AGTGAGGCGAGTCTGTGAG R:TCCCGTGTCCAAGAAAGT	60.0	259-285	EF599343
Mb39*	(GT)34	F:ATCAAACCCACATCTCCT R:TGCCCTGGTTAGAACTCC	56.9	257-305	EF599344
Mb40*	(GT) 5GC (GT) 7... (GT)9GC(GT)5	F:CACCTAGTGGCGATTTCA R:AACAGAGGGCGGTTGGAT	56.9	327-369	EF599345
Mb41*	(AC)3A(AC)13 A(AC)9	F:GGACTATCAGCCCACCTCT R:TTCTTAACCACTGGACCACC	53.3	292-326	EF599346

Source: (Zhao et.al, 2008)

From the proposed budget, we were able to perform DNA extraction and Polymerase Chain Reaction (PCR), Electrophoresis and visualization, and DNA sequencing for species identification. The cost for isolation kits, chemicals and consumables were covered from this project, for example, the cost of purchasing DNA isolation kit (pellets), consumables (tubes/tips/glove/scalpels/petri dishes), chemicals and enzymes (Hot start master mix, Bovine Serum albumin-BSA, nuclease free water, Agarose, loading dyes, Ethidium Bromide).