

SNOW LEOPARD FOUNDATION

# **RESEARCH UPDATE 2008-2014**

## SNOW LEOPARD PROGRAM, PAKISTAN

Draft Report

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## **1 INTRODUCTION**

### 1.1 BACKGROUND

In Pakistan, snow leopard is found throughout the mountain ranges of the north, in Chitral, Dir, Swat and Kohistan districts of Khyber Pakhtunkhwa and in the northern part of Neelum Valley, District of Azad Jammu and Kashmir (Malik, 1997). The total snow leopard habitat available in Pakistan is about 80,000 km<sup>2</sup> of which about 40,000 km<sup>2</sup> is considered to be its prime habitat (Fox, 1989). It occurs in the Hindu Kush range in the Chitral District (Khyber Pakhtunkhwa), and in the Karakorum Range of the Gilgit-Baltistan in the Gilgit, Ghizer, Hunza-Nagar, Skardu and Ghanche districts. A good population of snow leopard is also reported from Shimshal area in Hunza-Nagar, but no density estimate is available (Wegge, 1988).

Reliable information on snow leopard population in Pakistan is lacking. There are certain limitations in available population estimates as most of these estimates are based on anecdotal information or interviews and expert judgments. Based on surveys undertaken in the early 1970s, Schaller (1976) estimated the total population of snow leopards in Pakistan to be around 150 to 200 animals. Malik (1997) reported that the number could be around 400 animals. Later, based on sign surveys carried out in Skardu and Ghanche Districts of the Gilgit-Baltistan, (Hussain, 2003) estimated of 90-120 in Baltistan and 300-420 animals throughout Pakistan.

The Snow Leopard Survival Strategy (SLSS) (McCarthy and Chapron, 2003) and Pakistan's strategic plan for snow leopard conservation in 2008 (Khan, 2008), identify information gaps in snow leopard ecology as one of the major limitations in formulating and implementing an effective conservation strategy. To address this issue, the Snow Leopard Foundation (SLF) Pakistan embarked on an ambitious data collection program in the snow leopard range in 2008. We employed four techniques (conflict- surveys, sign-based occupancy, camera trapping, and molecular genetics) to gather information on snow leopard population, habitat occupancy, resource selection, and conflicts with humans, throughout the range of the species in Pakistan.

### 1.2 OBJECTIVES

The SLF research program aimed at answering following questions:

- Snow leopard population and its spatial structure in Pakistan
- Densities of natural prey in snow leopard range
- Resource selection by snow leopard and its main prey
- Nature, magnitude and spatial pattern of human-cat conflicts in snow leopard range in Pakistan

## 2 METHODOLOGY

### 2.1 STUDY AREA

Utilizing three methods; camera trapping, sign-based occupancy, and intensive search for fecal samples, we targeted entire snow leopard range in Pakistan. We started with most potential habitats, and so far we sampled 15 study areas spanning over 19000 sq.km (Figure 1). In this effort we covered major protected areas in the snow leopard range and most of areas in Pakistan that have been reported to have good snow leopard numbers (except for central and eastern part of Central Karakoram National Park). Our sampled area covers about 24% of reported snow leopard habitat (80,000 sq.km, McCarthy and Chapron, 2003), but approximately 70-80% of good quality habitat assessed by us through Maximum Entropy Modeling recently.

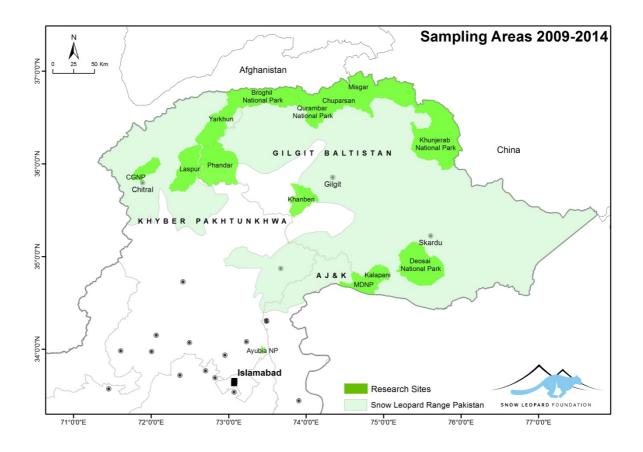


Figure 1: Map showing snow leopard range in Pakistan and our study areas, surveyed during 2008-2014.

## 2.2 DATA COLLECTION

#### 2.2.1 Site Occupancy Surveys

Estimating abundance or density is one of the primary roles of a wildlife monitoring program to identify the major issues to be managed. But it is always difficult and expensive to measure the abundance of many species. As an alternative state variable, site occupancy can be considered as an appropriate index for monitoring abundance. The reasoning here is that at an appropriate scale, the two state variables (population and proportion of sites occupied) are positively

correlated (MacKenzie and Nichols 2004). Occupancy is defined as the proportion of area, patches or sites occupied by a species (MacKenzie et al., 2002, 2006). Site occupancy is being used widely as a reliable surrogate index (MacKenzie et. al. 2005) for monitoring wild populations (e.g. Bailey et. al. 2004; Crossland et. al. 2005; Possingham et. al. 2006; McCarthy et al., 2010; Long et al., 2010; Sarmento et al., 2011; Karanth et al., 2011; Hines et al., 2010; Midlane et al., 2014).

Site occupancy surveys were conducted by Snow Leopard Foundation in different areas in Northern Pakistan from 2010 to 2012 to assess the occupancy of snow leopard. Each study area was divided into small grids cells (either 10×10 km or 5×5 km) on GIS maps. Each grid cell (site) was accessed by GPS and multiple visits (repeat surveys or points) (McKenzie et. al. 2002) were conducted to search the signs of the snow leopard. Only fresh; less than 10 days, scrape and pugmark are used as detections of snow leopard for occupancy while older signs; less than 30 days were used for 'site used' estimation. Analyses were carried out in Program PRESENCE (Hines, 2007). The best fitting model was determined using the Akaike Information Criteria (AIC). The model that has the best fit (likelihood) and minimum number of parameters obtains the minimum value of AIC value (Akaike 1985; Burnham and Anderson 2002). Model averaging was used to obtain most appropriate estimates of site occupancy for a particular landscape.

Total 167 sites with 1507 repeat surveys points were searched for snow leopard signs in different landscapes of snow leopard range in Pakistan. Survey areas included;

- ► Khunjerab National Park-KVO-Shimshal
- Broghil-Qurumber National Parks
- Phandar valley, Ghizer
- Misgar-Chapursan

#### 2.2.2 Camera Trapping

Camera trapping is being increasingly deployed for the monitoring of shy and uncommon wildlife (Karanth and Nicholes, 1998; Jackson et al., 2006). Camera trapping was conducted in all three provinces of snow leopard range in Pakistan; Gilgit-Baltistan, Khyber Pakhtunkhwa and Azad Jammu & Kashmir. Total 708 (+ more than 20 stations, for a few days, at different areas of Chitral for lynx) camera stations were set in different areas from 2006 to 2015 which were active for more than 20,000 trap days in the field. Trail cameras used for capturing snow leopard were consisting of; CamTrakker™ (Ranger, Wattkinsville, GA, USA) and Reconyx<sup>TM</sup> (HC500 Hyperfire<sup>TM</sup> and PC900 Hyperfire<sup>TM</sup>; Reconyx, Holmen, Wisconsin, USA).

The site for camera installation was selected near to track, scats, hairs or other wild animal's sign. Minimum aerial distance was kept 1 km between the two nearest camera stations. A camera station either consisted of a pair of cameras, in opposite directions, or single camera.

All necessary measures and precautions; about camera height, front view, its sensor, etc., were taken care off while setting up a cameras following standard protocols (Jackson et al., 2006). Majority of camera stations were also supplied with different types of lure at station; castor, skunk and fish oil, to increase the capture probability of animal in front of camera.

Areas where camera trapping was conducted include; Chitral Gol National Park (CGNP), Tooshi Game Reserve (TGR), Buffer areas of CGNP & TGR, Laspur valley, Khujerab National Park, Khujerab Villigers Organization, Shimshal, Broghil National Park, Qurumber National Park, Deosai National Park, Yarkhun valley, Misgar, Astor, Musk Deer National Park and Khanberi.

#### 2.2.3 Genetic Analysis

Fecal samples were collected by experienced researchers from 2009 to 2013 from different parts of snow leopard range. Staff was experienced in identifying snow leopard scats. The age of all samples collected was estimated based on their physical appearance. We collected over 1000 fecal samples of all carnivore species encountered, and were run for species and individual IDs.

All DNA extraction was performed in a laboratory dedicated to the extraction of degraded DNA. Total DNA was extracted from c. 15 mg of faeces, using the DNeasy Blood and Tissue Kit (QIAgen GmbH, Hilden, Germany), following the manufacturer's instructions, with a slight modification as described by Shehzad et al. (2012b). Blank extractions were performed to monitor any contamination.

Species identification was performed through NGS, by amplifying DNA extract using primers for vertebrates targeting about 100 bp of the mitochondrial 12S rRNA gene, as described in Shahzad et al. (2012b). Genetically identified snow leopard scats were genotyped at 7 microsatellite loci (PUN082, PUN100, PUN124, PUN132, PUN225, PUN229, and PUN327—Janecka et al. 2008).

Snow leopard diet was analyzed by characterizing the prey DNA present in fecal samples after amplification of a diagnostic fragment and sequencing of polymerase chain reaction (PCR) products, using next-generation sequencing. This provides diet information without any a priori knowledge about the prey and is a cost-effective method as millions of readings can be generated from a single sequencing run. This method has several advantages over classical microscopy, which requires substantial skill and time and is prone to misidentification in the case of closely related species (Pompanon et al., 2012).

The sequence analysis and taxon assignation were done using OBITools, as described in Shehzad et al. (2012a, b).

Genetic analysis was carried out in Laboratory of Alpine Ecology, University Joseph Fourier France, and spygen (a private lab in France).

## **3 PRELIMINARY RESULTS**

### 3.1 SITE OCCUPANCY OF SNOW LEOPARD

Site occupancy surveys were conducted in Khunjerab National Park, including buffer area and Shimshal in November-December 2010 by dividing the area into  $10 \times 10$  km grid cells. Total 14 sites were accessed and 4 to 20 points were searched in a site for signs of the snow leopard. An occupancy estimate at 0.8820 ± 0.0954SE was calculated which is highest among all the areas surveyed in Pakistan. Probability of detection was influenced by topography and number of observers.

During May-August 2011, site occupancy surveys were conducted in the Broghil National Park and Qurumber National Park by dividing the area into 5×5 km grid cells. Multiple visits (4-16) were conducted in each site. During occupancy surveys 47 sites were surveyed. Due to tiny presence of snow leopard in the area, sign detection was also lower resulting instead of site occupancy, habitat used or site used by snow leopard in a month period was estimated which ranged from 0.0001 to 0.9630 at site level. Camera trapping, conducted in June-July 2012, confirmed the presence of snow leopard at one out of 80 sites.

In May 2012, to assess the occupancy of snow leopard in Phandar valley of Ghizer district, 51 sites (5×5 km) were accessed and 2-18 points were explored in different sites. Unfortunately no fresh or confirmed sign of snow leopard was detected in the area which limited further analysis. No camera was installed in this area.

In Misgar and Chapursan, site occupancy survey was conducted in June-July 2012 and total 55 sites (5×5 km grid cells) were surveyed with 2 to 12 points in a site. Proportion of site occupied by snow leopard, was at  $0.6021 \pm 0.1511$ SE. Sign detections were influenced by habitat type and terrain brokenness as well as distance of settlements from site also affected occupancy of snow leopard. Camera trapping, conducted in May-June 2013 confirmed the presence of snow leopard at 14 out of 59 camera trap stations.

## 3.2 PHOTO-CAPTURE SUCCESS

In all studies combined, more that 700 trail camera stations were established, in prime habitats of Chitral, Gilgit Baltistan, and Azad Jammu and Kashmir. These cameras remained active for over 20,000 trap days.

Snow leopard detection was not great with trail cameras, as it was photo-captured in 63 capture events at only 45 stations (in over 700 stations). For most of study areas there was either single capture (Qurumber NP, Musk Deer NP, Laspur valley) or no capture (Broghil NP, Hundrab NP, Deosai NP, Yarkhun valley, etc). Multiple captures occurred only in Shimshal, Khunjerab, and Misgar. Unique individuals identified from photo-captured data in Shimshal and Khunjerab are nine only.

## 3.3 GENETIC BASED INDIVIDUAL IDENTIFICATION

Out of over 1000 fecal samples analyzed, 113 belonged to snow leopard. Genotyping indicate, in total there were 23 different individuals. These samples originated primarily from Khunjerab NP, Shimshal, Misgar, and Chapurson, collected from 2010-2013.

## 4 COMMENTS IN CONTEXT OF SL RED LIST ASSESSMENT

Both camera trap and genetics data indicate that two family groups were quite mobile among study areas and increased detection as well. There are various complications in our data, and our colleagues are investigating various possibilities of Capture Mark Recapture (CMR) models to address these challenges. We cannot estimate population at this stage, nevertheless two elements are quite obvious; our estimates are likely to have large CI, and much smaller that prevailing snow leopard population guestimates. The available guestimates for the snow leopard population for Pakistan are 300-420 (Hussain 2003, SLSS 2003 &14). Hussain (2003) did interviews and sign surveys in selected areas in Baltistan and based on that he suggested these numbers, while earlier versions of SLSS did estimation based on available habitat. In view of new information these guestimates seems to be incorrect, and actual population may be much smaller than the lower range of these guesstimates.

We believe our genetics results are helpful to understand uncertainties around prevailing population estimates not only for Pakistan but also for rest of the range. In order to understand basis of available estimates, we revisited Snow Leopard Survival Strategy (SLSS), 2003. Page 15 & 18 while acknowledging difficulties in surveying snow leopard populations indicate estimates for most of the range area were based on available habitat or in combination of signs and area. Snow Leopard Information Management System (SLIMS), which was used in past on assumption of having correlation between signs and snow leopard population, has already be proven incorrect. Interestingly, SLSS 2003 mentions an older estimate of snow leopard population to be 2000 for the entire range, but rejects it on the grounds that ungulates population was intact in 70s. However, in context of recent genetic and camera trap results from Pakistan, guestimates of 70s appear close to the reality as compared to recent ones. Given the situation we are nervous to accept that >6000 snow leopards are available in wild, to ensure >2500 MI, as estimated by Chapron (2015).

Arguments of range area have also been used in discussion in context of snow leopard Red List Assessment, to suggest that there must be more snow leopards than are needed for MI to be more than 2500. However, as we are seeing in Pakistan, large parts of snow leopard potential habitat may have no or very low occupancy.

We also did Maximum Entropy Modeling, using 332 snow leopard locations (genetics + camera + occupancy confirmed sites). Initial results indicate suitable habitat to be much smaller as compared to known distribution range. There is good concurrence among good habitat and higher detection of snow leopards. Snow leopard population seems to be concentrated in smaller patches of habitat in relation to their distribution.

We also would like to share that, IUCN Pakistan and Ministry of Environment facilitated a country-level assessment of mammal species in Pakistan. All stakeholders and researchers with a larger consensus placed snow leopard under critically endangered category in Pakistan (Sheikh and Malour, 2003: Status and RedList of Pakistan's mammals). Our new data have left us quite worried about the status of snow leopards.

## **5 ACKNOWLEDGEMENTS**

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