

Snow Leopard Conservation Grants, Snow Leopard Network

FINAL REPORTS FOR 2010 PROJECTS

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Non-invasive approach for the diet analysis of snow leopard (*Uncia uncia*) by using DNA barcoding

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1. Executive Summary

Please describe the original goals and the final results of your project. This may be used in press releases and other publicity material about the Grants Program, so please write it for the general public who may not have scientific background

Snow Leopard (*Uncia uncia*) classified as an endangered species by the IUCN, is disappearing from many parts of its formally vast range, an area in excess of 2.5million square kilometers. This beautiful yet mysterious cat is found sporadically through the mountains of central and south Asia. Snow leopards are one of the most endangered of all the large cats and may number few as 3500 in the wild, despite inhabiting 12 countries in central and south Asia. Snow leopard is a single species at the top of the food chain, which is considered an indicator of healthy mountain ecosystems. Unfortunately, not only its historical range has become increasingly fragmented, but also its population has declined significantly due to widespread poaching for pelt and bones, retribution from pastoralist and rapidly dwindling natural prey base.

To study the composition of animal diets, several methods have been developed. All of these methods have certain limitations, and their results are generally not comparable (Shrestha and Wegge 2006). In collaboration with Snow Leopard Trust (SLT) USA, a collection of 202 fecal samples from Tost Mountains, South Gobi, Mongolia, was included in this study. This was the first ever study on the diet of snow leopard using DNA based techniques. The main aim of this study was to evaluate the main prey taxa of snow leopard and the real magnitude of wild & livestock prey items present in the diet. Wild prey comprised about 80% diet of the snow leopard while 20% of the diet is shared by domestic livestock (Figure1).

2. Objectives

What was the purpose of the project? How was it expected to contribute to the knowledge or conservation of snow leopards, their prey, or habitat?

Identification of DNA sequence diversity is a powerful means for assessing the species present in environmental samples. The main aim of this study was to understand feeding ecology of snow leopards, their main prey species, and real magnitude of livestock depredations. This information will help to understand the level of conflict with human communities and design appropriate management strategy.

3. Methods

Describe the methods you used in detail, so that someone else could repeat the work, or, avoid the problems that you encountered

Following is the outline of the methods used from fecal collection to genetic analysis;

- (i) In collaboration with Snow Leopard Trust (SLT) USA, a collection of 202 samples from Tost Mountains, South Gobi, Mongolia, was also included in this study.
- (ii) DNA extraction from the above fecal samples was carried out at Laboratoire d'Ecologie Alpine (LECA), University Joseph Fourier, Grenoble, France.
- (iii) DNA-based identification of the predator species that produced from these feces (amplification of a fragment of mt-DNA using primers that are specific to snow leopard) (Ficetola *et al.* 2010).
- (iv) DNA amplification from the feces extracts using universal primers from mitochondrial DNA that amplify a short fragment of about 100 bp (unpublished universal primers);
- (v) Blocking of predator DNA by using internal blocking primers (Vestheim & Jarman 2008) specific to predator (*Uncia uncia*) sequences;
- (vi) Sequencing of the PCR product using the Solexa / Illumina sequencing platform.
- (vii) Identification of the different prey based on the sequences produced by the Solexa / Illumina sequencing, using the DNA barcoding concept (using mitochondrial sequences in public databases);
- (ix) Frequency of occurrence of different prey species, and prey selection analysis.

4. Summary of the results

Out of 202 samples received from Mongolia for the diet analysis. Sample identification for snow leopard samples was done using species-specific primers (Figure 2; Ficetola *et al.* 2010). Eighty-eight identified samples, as snow leopard using snow leopard specific primers were further analyzed for the diet analysis.

Using feces as a source of DNA we were able to identify 5 different prey items in the diet of snow leopard. Seventy nine percent of the diet comprising of wild prey taxa and 19% of the diet was composed of livestock. *Capra sibirica* was at the top of prey items consumed (69%), followed by *Capra hircus* (18%), *Ovis ammon* (9%), *Ovis aries* (3%) and *Alectoris chukar* (1%) (Figure 1).

5. Photographs

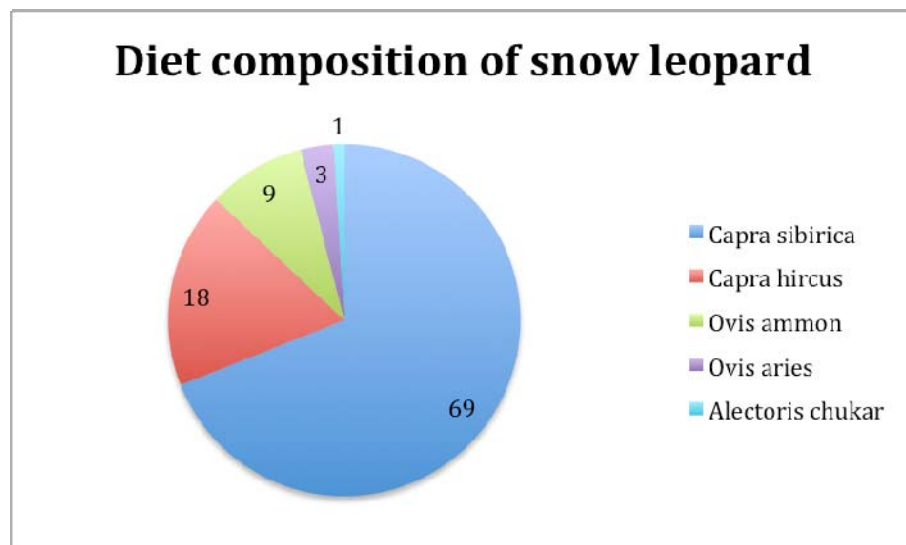


Figure 1



Figure 2

References:

Ficetola GF, Coissac E, Zundel S, Riaz T, **Shehzad W**, Bessière J, Taberlet P, Pompanon F (2010) An *In silico* approach for the evaluation of DNA barcodes. *BMC Genomics* **11**, 434.

Shrestha R. and Wegge P. 2006. Determining the composition of herbivore diets in the Trans-Himalayan rangelands: a comparison of field methods. *Rangeland Ecology and Management*, **59**, 512-518.

Vestheim H, Jarman SN (2008) Blocking primers to enhance PCR amplification of rare sequences in mixed samples- a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology* **5**, 11.