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Implication of snow leopard distribution, population dynamics and landscape genetics, and prey preference for its conservation in Nepal

Vliv rozšíření, populační dynamiky a krajinné genetiky, a preference druhů kořisti na ochranu levharta sněžného v Nepálu

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ABSTRACT

This thesis deals with an endangered large mammal species - snow leopard, its distribution, population dynamics, landscape genetics and connectivity, trophic ecology and human-snow leopard conflicts in the Nepalese Himalaya (Sagarmatha National Park (SNP), Lower Mustang (LM) and Upper Manang (UM) in the Annapurna Conservation Area, during Wet and Dry seasons in 2014–2016. In the case of snow leopard study, we used data obtained from camera traps, scat's genetic analysis and monitoring of fresh pugmarks and scrapes while direct count method was used to study for its main prey, blue sheep and Himalayan tahr.

In **study 1**, we assessed the determinants of habitat suitability of snow leopards using MaxEnt model and mapped the distribution of suitable habitat for snow leopards in Nepal. Altitude and Annual mean temperature are important common factors contributing to snow leopard habitat suitability within the area studied, which is indicated by both the percentage contribution of environmental variables and Jackknife test from MaxEnt model. Some other uncommon factors also seem to play a role, as they were important in at least one of the analyses. These were: distance from road, and precipitation of driest month but their importance has to be considered with caution.

In **study 2**, we present our observations along with other published data on population abundance and trend in changes of population sizes of snow leopard and its main prey, Himalayan tahr and blue sheep in the three study areas. Additionally, population data of the main leopard prey (sex ratio, female to cub ratio), wherever these values were available, are also presented. The basic analyses of these data performed yield predictions useful for developing of effective snow leopard management strategies.

In **study 3**, we use our data collected in Nepal to determine the areas suitable for snow leopards, by using habitat suitability maps, and describe the genetic structure of the snow leopard within and between these areas. We also determine the influence of landscape features on the genetic structure of its populations and reveal corridors connecting suitable areas. We conclude that it is necessary to protect these natural corridors to maintain the possibility of snow leopards' migration between suitable areas, which will enable gene flow between the diminishing populations and thus maintain a viable metapopulation of snow leopards.

In **study 4**, we studied diet and prey preference of snow leopards in the three studied areas. We collected 268 scats along 139.3 km linear transects, of which 122 were genetically confirmed to belong to snow leopards. Their diet was identified by comparing hairs in scats with our reference collection of the hairs of potential prey. In the SNP, the most frequent prey in snow leopard faeces was the Himalayan tahr in both winter and summer. In LM and UM, its main prey was blue sheep in winter, but yak and goat in summer. In terms of relative biomass consumed, yak was the main prey everywhere in both seasons. Snow leopards preferred large prey and avoided small prey in summer but not in winter, with regional differences. It preferred domestic to wild prey only in winter, and in SNP. We show that snow leopards consume a diverse range of prey, which varies both regionally and seasonally. We conclude that in order to conserve snow leopards it is also necessary to conserve its main wild species of prey, which will reduce the incidence of losses of livestock.

In **study 5**, we assess how the knowledge and perception of local people of snow leopard depredation has changed over time and its correlation with livestock losses in the central and north-eastern Himalayas in Nepal. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

ABSTRAKT

Tato práce se zabývá ohroženým velkým druhem savců - sněžným levhartem, jeho rozšířením, populační dynamikou, genetikou a konektivitou krajiny, trofickou ekologií a konflikty sněžným levhartem a člověkem v nepálském Himálaji: Národní park Sagarmatha (SNP), Dolní Mustang (LM) a Upper Manang (UM) v Annapurna Conservation Area, během let 2014–2016. V případě studie levhartů sněžných jsme použili údaje získané z fotopastí, genetické analýzy trusu a sledování čerstvých pobytových známek a škrábanců, zatímco přímé odpočty byly použity ke studiu hlavní kořisti, nahura modrého a tahra himálajského.

Ve **studii 1** jsme pomocí modelu MaxEnt hodnotili determinanty vhodnosti stanovišť levhartů sněžných a mapovali rozšíření vhodného prostředí pro levharty sněžné v Nepálu. Nadmořská výška a roční průměrná teplota jsou důležitými společnými faktory, které přispívají k vhodnosti stanoviště levharta sněžného ve studované oblasti, což je indikováno jak procentním příspěvkem proměnných prostředí, tak testem Jackknife z modelu MaxEnt. Zdá se, že tu hrají roli i některé další neobvyklé faktory, které byly důležité alespoň v jedné z analýz. Byly to: vzdálenost od silnice a srážky v nejsušším měsíci, ale jejich důležitost je třeba zvažovat opatrně.

Ve **studii 2** prezentujeme naše pozorování spolu s dalšími publikovanými údaji o početnosti populace a trendu ve změnách populačních velikostí sněžného levharta a jeho hlavní kořisti, tahra himálajského a nahura modrého ve třech studovaných oblastech. Kromě toho jsou také prezentovány údaje o populaci hlavní kořisti levharta (poměr pohlaví, poměr počtů samic k počtu mláďat), pokud byly tyto hodnoty k dispozici. Základní analýzy těchto dat nám poskytly predikce užitečné pro vývoj efektivních strategií řízení levhartů sněžných.

Ve **studii 3** používáme naše údaje shromážděné v Nepálu k určení oblastí vhodných pro levharty sněžné pomocí map vhodnosti stanovišť a k popisu genetické struktury sněžného levharta v těchto oblastech a mezi nimi. Rovněž určujeme vliv krajinných prvků na genetickou strukturu jeho populací a odhalujeme koridory spojující vhodné oblasti. Dospěli jsme k závěru, že je nutné chránit tyto přirozené koridory, aby byla zachována možnost migrace levhartů sněžných mezi vhodnými oblastmi, což umožní tok genů mezi ubývajícími populacemi a tím bude udržována životaschopná metapopulace levhartů sněžných.

Ve **studii 4** jsme studovali potravní a kořistní preference levhartů sněžných ve třech studovaných oblastech. Shromáždili jsme 268 vzorků trusu podél 139,3 km lineárních transektů, z nichž 122 bylo geneticky potvrzeno, že patří k levhartům sněžným. Potrava levharta byla identifikována porovnáním chlupů v trusu s naší referenční sbírkou chlupů potenciální kořisti. V SNP byl nejčastější kořistí ve výkalech levharta sněžného himálajský tahr v zimě i v létě. V LM a UM byl jeho hlavní kořistí v zimě nahur modrý, v létě však jak a koza. Pokud jde o relativní spotřebovanou biomasu, jak byl hlavní kořistí všude v obou ročních obdobích. Sněžní levharti upřednostňovali velkou kořist a malé kořisti se vyhýbali v létě, ale ne v zimě, s regionálními rozdíly. Levhart dával přednost domácí divoké kořisti pouze v zimě a na SNP. Ukazujeme, že sněžní levharti konzumují rozmanitou škálu kořisti, která se liší regionálně i sezónně. Dospěli jsme k závěru, že za účelem ochrany levhartů sněžných je také nutné chránit jejich hlavní divoký druh kořisti, což sníží výskyt ztrát hospodářských zvířat.

Ve **studii 5** hodnotíme, jak se v průběhu času měnily znalosti a vnímání místních lidí o plenění levhartů sněžných a jejich korelace se ztrátami hospodářských zvířat ve středním a severovýchodním Himálaji v Nepálu. Dospěli jsme k závěru, že ve studovaných oblastech stále existuje velká hrozba pro dlouhodobé přežití levhartů sněžných a jejich přirozené kořisti. Měla by být použita zmírňující opatření zjištěná během diskusí s místními lidmi, aby se vytvořila situace prospěšná pro místní obyvatele i pro dlouhodobé přežití levhartů sněžných.

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1. INTRODUCTION

Snow leopard, *Panthera uncia* (Schreber 1775), *syn. Uncia uncia* is a member of the genus *Panthera* in the family *Felidae*. Snow leopards are restricted to subalpine and alpine regions in South and Central Asia in 12 countries: China, Bhutan, Nepal, India, Pakistan, Afghanistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, Russia and Mongolia (McCarthy et al. 2017). It is whitish-grey (tinged with yellow), and patterned with dark grey rosettes and spots (McCarthy and Chapron 2003). In general, snow leopards live solitarily, though small groups of up to six snow leopards are reported (McCarthy and Chapron 2003; Valentová et al. in press).

In the 1972 IUCN's Red List of Threatened Animals, it is listed as an "endangered" species (EN) due to its small population worldwide. In 2017, its categorization was changed to "vulnerable". This down-listing in the IUCN Red List is not a demotion, but it tells us that it is still endangered and in need of wide-range population monitoring and conservation (Mallon and Jackson 2017). Snow leopard is also included in Appendix I of CITES, with a minimum global population of 4,000, with 2,710–3,386 mature individuals (McCarthy et al. 2017; Valentová et al. in press). Based on the recent review of metadata (Valentová et al. in press), the total size of its current potential range is approximately 1,738,700 km² and the total population is between 4,501 to 6,430.

Distribution and habitat suitability

Habitat suitability is defined in terms of a habitat's potential to support a particular species and habitat suitability index is a numerical index, which ranges from a completely unsuitable habitat to an optimal habitat (Kellener 1992). This reflects spatial variability in the probability of species occurrence (Bacon et al. 2017). Habitat suitability models are used to predict species occurrence through modelling of proper environmental variables and are increasingly applied to wildlife management issues (Ottaviani et al 2004; Elith et al. 2006; Hirzelet al. 2006). Spatial structure and configuration of landscapes may have profound impacts on population distribution of a species (Collinge 2010) and habitat-based conservation measures help to keep populations at viable sizes for a longer period (Paper – I).

So far, variety of techniques and statistical methods have been used for the species distribution modelling (Corsi et al. 2000; Elith et al. 2006; Guisan and Zimmerman 2000; Scott et al. 2002; Franklin 2009). They are based on resource selection function (Boyce and McDonald 1999; Manly et al. 2002), generalized linear models (McCullagh and Nelder 1989), algorithmic modelling based on machine learning (Ripley 1996) and maximum entropy model called MaxEnt (Phillips et al. 2006). Among them, currently the most often used method is MaxEnt, because of its efficiency to handle complex interactions between response and predictor variables, and possibility to be integrated with GIS techniques (Elith et al. 2006; Yi et al. 2016) ((Paper – I).

The first preliminary habitat suitability study of snow leopard in Nepal was done by Hunter and Jackson (1997). It was based on polygon digitized expert-based model. Another suitability map was prepared using occurrence points of scats, pugmarks and scrapes, and the model was based on resource selection factor available by GIS techniques (WWF-Nepal 2009). However, molecular analyses revealed that as much as 50% of the scats collected, which look

like snow leopard scats, in fact belong to other carnivores like wolf, common leopard, red fox and golden jackal (Shrestha et al. 2018). The third study was done by Aryal et al. (2016), who estimated habitat suitability using MaxEnt model with 364 occurrences obtained mainly from few areas (Paper – I).

Habitat suitability area and spatial pattern proposed by these studies are strongly different. For example, in the Sagarmatha National Park, neither its northern part, nor its high mountains, nor its deep river gorges were reported as suitable habitats by WWF-Nepal (2009), but the whole Sagarmatha National Park, including large areas outside the park, were reported as suitable habitats by Aryal et al. (2016). Generally, one has to be very cautious in determining the suitable habitat, as even tiny parts of unsuitable habitat (high mountain peaks, deep gorges etc.) within a large area of suitable habitat can negatively affect the movement of snow leopard between patches of suitable habitat (Paper – I). Bearing in mind all these discrepancies, one has to conclude that the area of the suitability habitat of snow leopard in Nepal has still to be determined and refined (Paper – I).

Abundance, population size and population dynamics of snow leopard and its prey species

Because of the rugged and practically inaccessible terrain inhabited by snow leopards, its elusive nature and low population density, very little information is available on its distribution and population status (Jackson et al. 2006). Thus, an effective, scientific and standardized method is needed to supplement conventional techniques like surveys of signs of their presence, and so ensure the long-term survival of this endangered felid. Promising scientific techniques such as non-invasive genetic sampling and molecular scatology may yield realistic population estimates of snow leopards when complemented with information obtained using conventional techniques (Waits and Paetkau 2005; Waits et al. 2007; Broquet et al. 2007; Janecka et al. 2008). The benefit of non-invasive genetic sampling is that the target species never has to be directly observed or handled, as the most common samples used in this case are hairs and scats (Caragiulo et al. 2016). However, when collecting scat of a focal species like snow leopard in the field it is likely it will be misidentified as being scat of another species. Therefore, genetic analysis of DNA, extracted from scat, is needed to accurately identify whether it is scat of snow leopard or its co-predators (Paper – II).

The Sagarmatha National Park (SNP) is one of the most pressing regions regarding snow leopard survival. Because of adequate protection measures, snow leopards have returned to the SNP after forty years gap of its occurrence (Shrestha 2004 and 2006; Ale and Boesi 2005). For assuring its survival there, a number of questions raises. In this thesis, we will address the following ones: (i) What are the expected trends in population size and demographic structure of both snow leopard and its prey – Himalayan tahr – in the Sagarmatha region? (ii) Is snow leopard population in the SNP viable over a long run? (iii) Will the existing Himalayan tahr population be able to provide enough food for the snow leopard population of sufficient size to survive? (iv) What are the answers to these questions, when other areas of Nepal are considered? (Paper – III).

Data on population size of Himalayan tahr from 1989 and 2010 in SNP have already been presented (Shrestha 2004 and 2006; Ale 2007; Lovari et al. 2009; Ferretti et al. 2014).

However, the variability in mortality rate of different sex age classes of Himalayan tahr, and relation between snow leopard density and reproductive rate of Himalayan tahr are still missing. Better understanding of seasonal population dynamics is vital for predicting the impact of snow leopard density on Himalayan tahr or relationship between predator and prey (Paper – III).

Similarly, snow leopards have been reported in Lower Mustang and Upper Manang of ACA in several studies (Oli 1994; Ale et al. 2014) and in other areas of ACA (Aryal et al. 2014; Wegge et al. 2012), but a detailed knowledge of population trends in successive years is still lacking. We know virtually nothing about the perspectives of this species long-term survival. Long-term monitoring of snow leopard distribution patterns and demographic parameters, such as abundance, survival and recruitment, is fundamental for answering these questions. However, until now, such estimates have been based on indirect index methods, such as sign encounter rates, because of snow leopard's highly elusive behaviour, rugged landscapes forming its territory, and because of limited resources and lack of manpower necessary for collection of such data. Therefore, data available on snow leopard biology are scant, biased, and outdated and generally lack the scientific rigor (Fox et al. 1991; Jackson and Hunter 1996; Jackson et al. 2006) ((Paper – III).

Landscape genetics and connectivity

Snow leopard tends to inhabit areas with a particular type of habitat. Within these areas, adult snow leopards generally occur solitarily (Jackson and Ahlborn 1989; McCarthy et al. 2005). Between such areas, snow leopards are recorded only occasionally and are then moving between suitable areas. Based on data on its occurrence, a good and reliable map of the snow leopard distribution and migration can be produced by using habitat suitability models. Such maps act as the main components for good management plans. Migration between suitable areas may result in inbreeding (Laikre 1999), fragmentation of populations (Randi 2003), reduction in genetic variation due to habitat fragmentation, loss of connectivity (Dixon et al. 2007), bottlenecks or genetic drift (Fauvergue et al. 2012). Conservation genetics then reveals the key factors, which may cause snow leopard extinction in such situations (Caragiulo et al. 2016). Thus both population genetics and habitat suitability models are crucial for designing proper management plans for its conservation (Li et al. 2016; Riordan et al. 2015; Li et al. 2020) (Paper – IV).

After the snow leopard-specific primers have been developed that enable to amplify mitochondrial DNA and polymorphic microsatellite loci (Zhang et al. 2007; Janecka et al. 2008), it became popular to identify many characteristics based on genetic studies. This included determination of species, sex, and individuals, and estimation of population size and density (McCarthy et al. 2008; Lovari et al. 2009; Janecka et al. 2011; Karmacharya et al. 2011; Wegge et al. 2012; Chetri et al. 2019), determination of genetic diversity at microsatellite loci (Janecka et al. 2008; Karmacharya et al. 2011), identification of phylogeography and genetic structure in the global range (Janecka et al. 2017) etc. However, studying only population genetic structure by itself is not sufficient. In Nepal, only one study (Karmacharya et al. 2011) analysed descriptive genetic diversity, and none exists on spatial population genetic structure

based on Bayesian clustering, which is crucial for management plans aiming at snow leopard long-term persistence (Caragiulo et al. 2016) (Paper – IV).

The decline in the area of suitable habitat due to human activities and global change will make migration between such areas increasingly difficult (McCarthy and Mallon 2016; McCarthy et al. 2003; McCarthy et al. 2017). To assure the future of snow leopards, it is necessary to safeguard the survival of this species as a metapopulation, which includes maintaining the connectivity between such areas (Li et al. 2016; Riordan et al. 2015; Li et al. 2020), as only this will preserve its genetic variability (Janecka et al. 2017). Although some studies on habitat suitability were performed in Nepal (Hunter and Jackson 1997; WWF-Nepal 2009; Aryal et al. 2016) and in Tibet (Li 2013; Bai et al. 2018), the connectivity between them has never been analysed (Paper – IV).

We also need to know, how the populations differ genetically within and between such areas, in order to determine, which areas host rare genotypes and are therefore especially worthy of preservation (Randi 2003; Dixon et al. 2007; Fauvergue et al. 2012) (Paper – IV).

Diet and prey preference

Large carnivores (lion, tiger, common and snow leopard etc.) are often at risk of extinction. This, together with their visual attractiveness for people, makes them flagship species in conservation biology. Their hunting behaviour and patterns in their selection of prey may profoundly affect the population dynamics of their prey (Sih et al 1998; Miller et al. 2001; Ripple et al. 2014), which in turn affects the population dynamics of these large carnivores. In addition, if large carnivores attack domestic animals, the local people turn against them and retaliate, which causes a mixture of both positive and negative indirect interactions between wild prey on one side and cattle and other domestic animals on the other side, all sharing a common predator (Sundararaj et al. 2012; Dorresteijn et al. 2015; Ogutu et al. 2017). Therefore, an understanding of the trophic ecology and foraging strategies of large carnivores is important for predicting their population dynamics and developing effective conservation programmes (Paper – V).

A single snow leopard requires about 1.5 kg of meat per day, which is equivalent to 20–30 adult blue sheep per year (Schaller et al. 1988; Fox et al. 1989; Jackson et al. 1998). Snow leopard diet is determined by analysing the remnants of prey in their scats (Chundawat et al. 1992; Oli et al. 1993; Bagchi et al. 2006; Shrestha 2008; Lovari et al. 2009; Shehzad et al. 2012; Wegge et al. 2012; Prasad et al. 2013; Jumabay-Uulu et al. 2013; Aryal et al. 2014; Lyngdoh et al. 2014). These analyses indicate that snow leopards mainly eat ungulates (*Ovis* spp. and *Capra* spp.), but will also eat smaller prey such as marmot (*Marmota* spp.), hare (*Lepus* spp.) and/or pika (*Ochotona* spp.). According to these analyses, wild ungulates constitute the main part of the diet of snow leopard (25–90%), followed by livestock (0–67%) and smaller prey such as rodents and birds (1–40%) ((Paper – V).

However, most of the data used in these analyses are not reliable for two reasons, as is explained below. The first reason is that scats of sympatric carnivores, like wolf, common leopard, red fox, golden jackal etc., may be very similar and therefore prone to misidentification. Actually, DNA analyses of snow leopard scats confirms the visual

identification in only 40–60% of the cases (Anwar et al. 2011; Janecka et al. 2011; Shehzad et al. 2012; Jumabay-Uulu et al. 2013), which suggests that the results of diet analyses obtained using conventional methods may be strongly biased. When the scats are not genetically identified, the results are biased towards other species such as marmots (*Marmota* spp.) or bharal (*Pseudois nayaur*) (Lyngdoh et al. 2014; Lovari et al. 2013), but when identified using genetic markers, a much larger percentage of large-bodied ungulate prey is recorded (Anwar et al. 2011; Shehzad et al. 2012; Jumabay-Uulu et al. 2013) (Paper – V).

The second reason is that of the studies on snow leopard foraging behaviour, only those of Lyngdoh et al. (2014) and Chetri et al. (2017) compare snow leopard diet with prey availability, and many other studies either lack information on prey abundance (Oli et al. 1993; Chundawat et al. 1994; Prasad et al. 2013; Aryal et al. 2014) or provide data for only the largest prey available or data from a single season (Bagchi et al. 2006; Shrestha 2008; Wegge et al. 2012; Prasad et al. 2013; Jumabay-Uulu et al. 2013). However, as the gut contents reflects both availability and the predator's preference for different species of prey, these two factors must be strictly distinguished (Hayward et al. 2006). Here we take into account both of the above-mentioned reasons for possible bias (Paper – V).

Human-snow leopard conflict

In the Himalayas above 3,000 m, where the dominant vegetation is grass and alpine shrubs the majority of the people there are still dependent on animal husbandry for their livelihood (Miller 1995; Richard et al. 2000; Gurung and McVeigh 2002; McVeigh 2004), especially in areas away from trekking routes. Livestock is important in their day-to-day life and culture and livestock herding is their main economic activity, as they have limited livelihood opportunities due to shortness of the summer season, which limits them to one crop per year (MOAC 2011). However, livestock and snow leopard share the same habitat. Because of this, snow leopards frequently encounter and kill livestock and this can have a substantial effect on the local economy (Oli et al. 1994; Jackson et al. 1996; Bagchi and Mishra 2006; Shrestha 2006; Shrestha et al. 2012; Li et al. 2013; Suryawanshi et al. 2013; Ale et al. 2014; Alexander et al. 2015). Depredation of livestock is a major problem in the Himalayan region and is often reported in the local press (Fig. 8.1). The loss of livestock due to attacks by snow leopards has resulted in them being viewed as vermin that need to be eradicated (Din et al. 2017). Understanding the predation pressure on livestock and the existing ecological and social issues associated with human-snow leopard conflicts is important for developing effective means of managing and conserving large carnivores in habitats used for livestock grazing (Bagchi and Mishra 2006) (Paper – VII).

The incidence of killing, however, is highly site dependent, differs from site to site, between years, with changes in the pattern of livestock herding, and the density and behaviour of snow leopards (Jackson 2010; Suryawanshi et al. 2013). It is therefore urgent to study, how the conflict between local people and snow leopards is changing over time, and determine the major factor responsible for heightening this problem (Paper – VII).

2. AIMS OF THE STUDY

- To determine the distribution and habitat suitability of snow leopard in Nepal using MaxEnt Modelling (Paper – I)
- To determine abundance, population size and population dynamics of snow leopard and its prey species in the Nepalese Himalayas (Papers – II and III)
- To assess the landscape genetics and connectivity of snow leopard in the Nepalese Himalayas (Paper – IV)
- To determine the diet and prey selection by snow leopards in the Nepalese Himalayas (Paper – V and VI)
- To assess the snow leopard-human conflict, threat reduction assessment and effectiveness of mitigation measures in the Nepalese Himalayas (Paper – VII)

3. MATERIALS AND METHODS

In **paper I**, we apply MaxEnt to the distribution data on snow leopard from Nepal based on a large set of occurrence data (450 observations) collected from a much wider range of areas (9 districts) than the previous studies. We used camera traps, scat collections and monitoring of fresh pugmarks and scrapes for snow leopard presence locations. All our data based on scats were consistently genotyped, to avoid misidentification of the species that produced them. All fresh pugmarks and scrapes were verified, whether or not they originate from snow leopard by using movement pattern of snow leopard from camera trap data.

In **paper II**, we studied the distribution and population size of snow leopards based on a genetic analysis of scat. In **paper III**, it is focusing on trends in changes of population sizes of snow leopard and its prey in Nepal in the three study areas in ACA and SNP. Scrape marks and camera traps were used for monitoring snow leopard while direct count method applied for monitoring main prey species. Transects characterized by elevation and several topographic parameters were established to cover the most of the typical biotope of snow leopard in study areas. Snow leopard and its prey were repeatedly monitored in SNP during dry seasons 2006, 2007, 2009 and 2015 and in ACA during dry season 2014 and wet season 2016. Encounter rate (sings/km) of scrapes marks are considered as the most reliable determinants for abundance indices. In all study areas, we walked and searched for prey within each valley, divided into several polygons based on physical barrier such as river, deep gorge or high mountain. We perform basic analyses of the data and derive predictions useful for developing effective snow leopard management strategies.

In **paper IV**, after performing habitat suitability modelling by using MaxEnt, we used circuit-scape theory model for the connectivity analysis. We collected 268 putative snow leopard samples of faeces, hairs and urine. From the collected samples, 6 microsatellite loci from different 6 chromosomes were selected for microsatellite-DNA sequence. After filtering positive samples, we obtained only 63 microsat data to analysis genetic structure or genetic variability.

In **paper V**, hair identification key of Himalayan mammals of Nepal as a tool to study food habits of snow leopard was developed by preparing microscopical hair slides to identify and examine cuticular scale cast or impression, medulla and cross-section. In **paper VI**, we studied the prey of snow leopard in three Himalayan regions in Nepal (Sagarmatha National Park (SNP), Lower Mustang (LM) and Upper Manang (UM) in the Annapurna Conservation Area, during winter and summer in 2014–2016. We collected 268 scats along 139.3 km linear transects, of which 122 were genetically confirmed to belong to snow leopard. Their diet was identified by comparing hairs in scats with our reference collection of the hairs of potential prey. We determined prey availability using 32–48 camera-traps and 4,567 trap nights.

In **paper VII**, we assess the knowledge and perception of local people of livestock losses due to snow leopards in the central and north-eastern Himalayas in Nepal. In nine settlements in three protected areas (Annapurna Conservation Area – ACA, Manaslu Conservation Area – MCA and Sagarmatha National Park – SNP) we studied, how the perception of local people of snow leopard depredation has changed over time and its correlation with livestock losses. We carried out questionnaire-based interviews of 1015 households from 2004 to 2016, which included 26.45% to 100% of all households in the settlements.

4. RESULTS AND DISCUSSION

In **paper I**, altitude and annual mean temperature are important common factors contributing to snow leopard habitat suitability within the area studied indicated by both the percentage contribution of environmental variables and Jackknife test from MaxEnt model. Some other uncommon factors also seem to play a role, as they were important in at least one of the analyses. These were: distance from road, distance from roads and precipitation of driest month but their importance has to be considered with caution. To conclude: the habitat suitability models indicate that the main danger for snow leopard survival may be climate warming and human expansion. Both these phenomena will push the lower limit of its distribution upwards to higher elevations, which will entail two negative effects.

In **paper II**, after data quality filtering, 63 microsatellite genotypes were obtained, corresponding to 22 individuals according to the identity analysis. In **paper III**, our study showed that number of scrapes/km was positively correlated with snow leopard density obtained by camera trapping. With data from ACA, we found that number of scrapes recorded during dry period 2014 were significantly higher than during wet period 2016. In total 19 individuals of snow leopards were identified by camera trap in three studied areas. There was no significant trend in population density of blue sheep either in LM or in UM. In SNP, there were some fluctuations and differences among several valleys but overall the tahr population has decreased during 1989–2015.

In **paper IV**, the connectivity maps reveal the Central and Eastern Nepal are interconnected by rather narrow corridors, which are very vulnerable, because they contain bottlenecks. Because of high mountain barrier between the protected areas, the corridor goes partially through the Tibetan Qomolangma National Nature Reserve. Observed heterozygosity

shows leopard populations have moderate genetic diversity. According to the genetic results, the genetic structures between Manang and Mustang are more similar and sufficient to prevent the effects of genetic drift in these two regions, as there are three possible corridors between them. In contrast, between Sagarmatha north (N-S) and Sagarmatha south (SW-S), there is a weak narrow belt of corridor, high deep gorge, two big river meeting between them. There is also one route between them with high tourist visitors. Because of this, snow leopard population fragmented into two sub-cluster., i.e., they do not share too much genetic diversity because of disturbance and barrier. The results of the landscape genetic analysis indicate only a fraction degree of gene flow between the two areas studied (LM & UM vs. N-S & SW-S) which is approximately 300 km apart, however snow leopard can cover even 27-40 km per day and that the average home range is 200-500 km².

In **paper V**, we provide a detailed microstructure characteristics and micrometres measurements from the hairs of Himalayan mammals sampled within the snow leopard habitats in Nepal. Moreover, digital photographs reference key of the medulla, cuticle (scales) and cross-sections of guard hairs with the description of hair characteristics are provided as a tool for hair identification.

In **paper VI**, in the SNP, the most frequent prey in snow leopard faeces was the Himalayan tahr in both winter and summer. In LM and UM, its main prey was blue sheep in winter, but yak and goat in summer. In terms of relative biomass consumed, yak was the main prey everywhere in both seasons. Snow leopard preferred large prey and avoided small prey in summer but not in winter, with regional differences. It preferred domestic to wild prey only in winter, and in SNP. Unlike most other studies carried out in the same area, our study uses genetic methods for identifying the source of the scat. Studies solely based on visual identification of samples may be strongly biased. Diet studies based on frequency of occurrence of prey tend to overestimate the importance of small prey, which may be consumed more often, but contribute less energy than large prey. However, even assessments based on prey biomass are unlikely to be accurate, as we do not know whether the actual size of the prey consumed corresponds to the average size used to calculate the biomass eaten. For example, large adults may be too difficult to catch and therefore mostly young animals are consumed, whose weight is much lower. We show that snow leopard consumes a diverse range of prey, which varies both regionally and seasonally. We conclude that in order to conserve snow leopards it is also necessary to conserve its main wild species of prey, which will reduce the incidence of losses of livestock.

In **paper VII**, herding of yak/nak (nak is female yak), sheep/goats and cattle (cows, oxen and horses) were found to be the main sources of livelihood for all households in the villages. Herders reported losses of livestock mainly due to attacks by snow leopard and two other carnivores (wolf and lynx). Most (1.5% to 14.3%) losses were attributed to snow leopard, while the other predators accounted for a meagre 0.16% to 5.3%. Predator-induced loss was substantial for the local families and reached \$349 per household per year. However, livestock mortality due to other causes (disease or natural disasters) was higher than that attributed to predators. We also evaluated the effectiveness of existing mitigating programmes, described community-based local mitigation measures and assessed the subsequent reduction in the level

of conflict. This revealed that the number of conflicts was lower than in 1990 and 50% of the respondents had changed their mind about snow leopard conservation. Surprisingly many respondents (15%) were against the conservation of snow leopards and even considered retaliatory killing of this predator as the best solution. Of the five snow leopard-human conflict mitigation measures, compensation from a community-based livestock insurance scheme and the improving of animal husbandry were the most popular in all the regions studied. Altogether, 15 human-induced threats to the future survival of snow leopards and its wild prey were identified in two protected areas. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

5. CONCLUSIONS

In the studies outline above, we attempt to gain insights into snow leopard distribution, population dynamics, landscape genetics and connectivity, trophic ecology and human-snow leopard conflicts in the Nepalese Himalaya.

The results presented in **paper I** revealed that annual mean temperature and altitude were the two main factors influencing snow leopard habitat suitability in Nepal using the MaxEnt model. As these two factors are strongly correlated, one can conclude that the main factor driving the presence of snow leopard is just one of them – biologically more meaningful is altitude. The response curve of different predictor variables of our study revealed that the probability of snow leopard presence in Nepal was highest in the altitude of around 4,000 m a.s.l. with a relatively cold and dry climate, shrubs, rocks and open grassland.

In **paper II**, we studied the distribution and population size of snow leopards based on a genetic analysis of scat. **Paper III** is focusing on trends in changes of population sizes of snow leopard and its prey in Nepal in the three study areas, Lower Mustang, Upper Manang and Sagarmatha National Park. Here we present our observations along with other published data on population abundance and trend of snow leopard and its main prey, Himalayan tahr and blue sheep. Additionally, population data of the main leopard prey (sex ratio, female to cub ratio) wherever these values were available are also presented. We perform basic analyses of these data and derive predictions useful for developing effective snow leopard management strategies.

The main message in **paper IV** is the delimitation of the areas with suitable habitat for snow leopards in Nepal and identification of the main corridors connecting these areas. We show that the genetic structure of snow leopard populations is mainly influenced by the proximity of people and trekking routes used by tourists, which pose a barrier to the dispersal of snow leopards. Topography of the terrain also plays a major role, as it determines the occurrence of suitable habitat for snow leopards in Nepal.

In **paper V**, we provide the hair identification key of Himalaya mammals of Nepal as a tool to study food habits of snow leopard. During the study for **paper VI**, we found snow leopard consumes a diverse range of prey, which varies both regionally and seasonally. In the

SNP, the most frequent species of prey was Himalayan tahr in both winter and summer, followed by cow and musk deer in winter, and cow and yak in summer; weasel spp. and dog were also consistently recorded in both seasons and some small prey occurred only in summer, whereas in the ACA, the main prey was blue sheep in winter and yak and goat in summer. Snow leopard prefers large prey and avoids small prey in summer but not in winter. In SNP, wild prey was eaten only in winter. A higher percentage of wild animals was consumed in winter (69%) and domestic animals in summer (54%). In overall (in term of frequency of occurrence of prey in the diet), snow leopard consumed 57% of wild prey and 43 % of domestic animals. In term of prey preference/selection, interestingly, livestock was preferred only in SNP throughout the year and in winter in the whole study area, precisely where and when wildlife is more frequently available.

In **paper VII**, we assess the knowledge and perception of local people of livestock losses due to snow leopards in the central and north-eastern Himalayas in Nepal. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

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5. CURRICULUM VITAE

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Research Interests

Population ecology, evolutionary biology and animal behaviour, landscape and conservation biology, wildlife education and conservation.

Education

2002-2004: M.Sc. Central Department of Zoology (Ecology Group), Tribhuvan University, Kathmandu, Nepal.

1997-2001: B.Sc. (Zoology), Prithivee Narayan Campus, Tribhuvan University, Pokhara, Nepal.

Professional experience (Appointments)

2017-Present: PhD Researcher, Department of Biodiversity Research, Global Change Research Institute (CzechGlobe), Czech Academy of Sciences, Brno, Czech Republic.

2010-2014: Snow Leopard Scouts and Science Co-coordinator, Snow Leopard Conservancy (SLC)-Nepal Program in collaboration with National Trust for Nature Conservation (NTNC), Nepal.

2011-2014: Adjunct Lecturer/Course Module Coordinator for BSc and MSc (Wildlife Biology) Institute of Forestry (IOF), Pokhara Campus, Tribhuvan University.

2008-2009: Lecture/Coordinator of XI and XII of Biology course of Science Colleges, Pokhara, Nepal.

2008: Consultant, WWF-NEPAL. Project: Presence/Absence and Relative Abundance Survey of Snow Leopard in Makalu Barun National Park and Rolwaling of Dolakha District of SHL.

2007: Field Research Officer in Sagarmatha National park, Nepal, Ev-K2-CNR, Italy. Project: VANISHING TRACKS on the Roof of the World.

2006: Researcher, Forum of Natural Resource Managers (FONAREM) Kathmandu Nepal. Project: Snow leopard and Himalayan tahr project.

2004: Research Assistant, Tourism for Rural Poverty Alleviation Program (TRPAP)-UNDP/ Department of National Parks and Wildlife Conservation. Project: Status, distribution of Himalayan tahr and predator impact in Sagarmatha National Park.

Research Grants (acting as PI)

2019: Integrating snow leopard and co-predators (common leopard, wolf and lynx) conservation with livelihood initiatives in Nepal funded by 2nd Rufford Small Grant (UK) (GBP 6000) (ID: 28596-2).

2016: Interaction between sympatric species (snow leopard, common leopard, wolf and lynx) and their conservation in Annapurna Conservation Area, Nepal funded by 1st Rufford Small Grant (GBP 5,000) (UK) (ID: 16363-1).

2015-2016: Population dynamics and habitat corridor model of snow leopard in Annapurna and Sagarmatha (Mt. Everest) regions of Nepal funded by Sabin Snow Leopard Conservation Grant/Panthera (USA) (\$ 20,000.00).

2014-2016: Nepal snow leopard project funded by Czech Globe-Global Change Research Institute, Czech Academy of Sciences, Czech Republic (grant GB14-36098G of GACR) (\$ 18,000.00).

Workshop/Conference

2020: First National Conference on Zoology: Biodiversity in a Changing World, virtually from November 28-30, 2020 organized by Central Department Zoology and Alumni Association of Central Department of Zoology, Kirtipur, Nepal. (Oral presentation: *Implications of landscape genetics and connectivity of snow leopard in the Nepalese Himalayas for its conservation*).

2018: First Pallas's cat global action planning meeting, Nordens Ark, Sweden (November 12-16, 2018) organized by Pallas's Cat International Conservation Alliance (PICA) and Nordens Ark and facilitated by the IUCN SSC Cat Specialist Group chairs (Contributor to action planning).

2016: Capacity building workshop for snow leopard landscape management planning and mapping in Kathmandu Nepal (April 20-27, 2016) organized by Government of Nepal & Global Snow Leopard and Ecosystem Protection Program (GSLEP) (Participant).

2012: The 2nd Asia Regional Conference of the Society for Conservation Biology 7-10 August 2012, Bangalore, India. Organized by Ashoka Trust for Research in Ecology and the Environment, Wildlife Conservation Society and others. Oral presentation. Abstract: *Snow leopard Panthera uncia in Himalaya of Nepal: a survey of status, distribution, prey species and livestock depredation*.

6. PUBLICATIONS (No. of citations based on Google Scholar: 91, h-index 7)

Shrestha B, Kindlmann P (2020) Implications of landscape genetics and connectivity of snow leopard in the Nepalese Himalayas for its conservation. *Scientific Reports* 10, 19853. <https://doi.org/10.1038/s41598-020-76912-7>. (IF= 4.12) (2020)

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Timsina B, Rokaya MB, Munzbergova Z, Kindlmann P, **Shrestha B**, Bhattarai B, Raskoti BB (2016) Diversity, distribution and host-species associations of epiphytic orchids in Nepal. *Diversity and Distribution* 25:2803-2819. IF: 4.092 (2018). Number of citations: 13

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Shrestha B, Rana BD, Karki BJ, Khatri BT, Kindlmann P (accepted) Snow leopard-human conflict and effectiveness of its mitigation measures. In: Kindlmann P. (ed.) *Population dynamics of snow leopard*, Springer, Dordrecht, in press.

Valentová KA, **Shrestha B**, Kindlmann P (accepted). Distribution, threats and conservation of snow leopard throughout the World. In: Kindlmann P. (ed.) *Population dynamics of snow leopard*, Springer, Dordrecht, in press.

Valentová KA, **Shrestha B**, Kindlmann P. (accepted) Methods of estimating snow leopard abundance. In: Kindlmann P. (ed.) *Population dynamics of snow leopard*, Springer, Dordrecht, in press.