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UNDERSTANDING SNOW LEOPARD (*PANTHERA UNCIA*) POPULATION  
STRUCTURE, DIET, AND HUMAN-WILDLIFE DIMENSIONS USING  
NONINVASIVE GENETIC APPROACHES

A Dissertation

Submitted to the Bayer School of Natural and Environmental Sciences

Duquesne University

In partial fulfillment of the requirements for  
the degree of Doctor of Philosophy

By

Charlotte E. Hacker

December 2021

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2021

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STRUCTURE, DIET, AND HUMAN-WILDLIFE DIMENSIONS USING  
NONINVASIVE GENETIC APPROACHES

By

Charlotte Hacker

Approved July 12, 2021

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## ABSTRACT

# UNDERSTANDING SNOW LEOPARD (*PANTHERA UNCIA*) POPULATION STRUCTURE, DIET, AND HUMAN-WILDLIFE DIMENSIONS USING NONINVASIVE GENETIC APPROACHES

By

Charlotte Hacker

December 2021

Dissertation supervised by Dr. Jan E. Janecka

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Hacker CE, Jevit M, Hussain S, Muhammad G, Munkhtsog B, Munkhtsog B, Zhang Y, Li D, Liu Y, Farrington J, Balbakova F, Alamanov A, Kurmanaliev O, Buyanaa C, Bavandonoj G, Ochirjay M, Liang X, Xiao X, Weckworth B, Jackson R, Janecka J. 2021. Regional comparison of snow leopard diet using DNA metabarcoding. *Biodiversity and Conservation*. DOI: 10.1007/210531-021-02118-6.

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Hacker CE, Li Y, Li J, Li D, Janecka J, Zhang Y, Jackson R. The functional and perceived effectiveness of Foxlights at preventing livestock loss on the Qinghai-Tibetan Plateau. *Wildlife Society Bulletin*. In Review.

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Hacker CE, Cong W, Dai Y, Xue Y, Bao R, Li D, Janecka JE, Zhang Y. Seasonal differences in dietary composition of a predator guild in China. In Prep.

Snow leopards (*Panthera uncia*) are a large cat endemic to the mountains of Central Asia. Despite their status as a flagship species, there are numerous knowledge gaps surrounding their population status, dietary ecology, and relationship with humans. Such knowledge gaps are prohibitive for effective conservation action for the species. Snow leopards are threatened by habitat loss, decreased prey availability, retaliatory killings,

and large-scale negative impacts associated with climate change. In order to help bridge these gaps, noninvasive genetic methodologies were deployed in this work across several disciplines. Population genetic metrics were garnered at local, regional, and range-wide scales, data surrounding dietary ecology were acquired through the use of DNA metabarcoding, and information surrounding human-snow leopard dimensions was gained via structured interviews and assessments of frameworks for herder involvement in research efforts. Range-wide, snow leopards were found to vary in both their population status, genetics, and prey use. Analyses regarding snow leopard populations at local scales in China and Mongolia suggest that some populations are thriving, while others will require additional data to determine the need for potential conservation interventions. On a range-wide scale, genetic profiles broadly grouped into three clusters (Central, Northern, and Western) that may support subspecies status. The divergence between snow leopards belonging to the Northern and Central subspecies is particularly stark with very little evidence of gene flow across the Gobi Desert. Snow leopards possessed low levels of genetic diversity regardless of examination at range-wide, regional, or local scales. Across their range, snow leopards predominately consumed larger-bodied wild hoof stock species, though these varied based on availability. Livestock constituted anywhere from 0% to 33% of dietary repertoire, speaking to the need of herder support in reducing the potential for negative attitudes and retaliatory killings. In general, snow leopards displayed a wide breadth of prey items consumed, including within the realm of domestic animals. In China, snow leopards most overlapped in diet with Tibetan wolves (*Canis lupus*) regardless of location or time of year, with both species showing a high dependence on blue sheep (*Pseudois nayaur*) and pika (*Ochotona*



spp.). Occurrences of livestock among the snow leopard and its sympatric counterparts were highest in the Tibetan wolf, which could contribute to the snow leopard being afforded more tolerance among herders. Herders in the Sanjiangyuan region of China had positive attitudes towards snow leopards overall, with religion and years of formal education playing an important role in shaping these attitudes. However, the role of livestock loss in shaping attitudes revealed itself to be much more complicated. One way of reducing livestock loss is through the deployment of non-lethal deterrents, such as flashing lights. The effectiveness of flashing lights at deterring predators from livestock was perceived by herders to be high, but functional effectiveness was limited in interpretation due to the majority of domestic animal attacks occurring during the day by wolves. Results from this indicate that herders approve of and will deploy user-friendly, small, and mobile devices with familiar characteristics, but that complications in designing research studies can limit further analytic assessments. Future snow leopard research should consider the value of tailored research efforts based on knowledge surrounding genetics, prey availability, and the factors that impact human attitudes towards the species within a particular area. However, the expansive distribution of the snow leopard also mandates that trans-boundary cooperation and communication is prioritized. Local communities within snow leopard habitat play a crucial role in the conservation success of the species and can serve as allies in protection efforts. Greater incorporation of their local ecological knowledge and skillsets can provide the additional resources necessary to continue advancing current understanding of snow leopards.

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# Chapter I.

## Introduction

Adapted from Janecka et al. (Hacker CE) (2020) Noninvasive genetics and genomics sheds light on the status, phylogeography, and evolution of elusive carnivores: The case of the snow leopard. In: *Conservation Genomics of Mammals – Integrative Research Using Novel Approaches*. J Ortega and JE Maldonado, Eds. Springer, Switzerland. Pp. 83-120.

### Snow leopard range and habitat

The snow leopard (*Panthera uncia*) is one of five big cat species belonging to the genus *Panthera* (Kitchener et al., 2016) (Figure 1). It is endemic to the mountains of Central Asia, including the Altai, Tian Shan, Kunlun Shan, Pamir, Hindu Kush, Karakorum, and Himalayan mountain ranges. The species is found in 12 countries across approximately 1.8 million km<sup>2</sup> of habitat - Afghanistan, Bhutan, China, India, Kazakhstan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia, Tajikistan, and Uzbekistan (Jackson et al., 2010; Kitchener et al., 2016; McCarthy et al., 2016) (Figure 2). Snow leopards commonly occupy steppe habitat, and are found at altitudes ranging from 1800 to 5800m (McCarthy et al., 2005; Stroganov, 1962).



Figure 1. Camera trap image of a snow leopard.  
(Camera trap image from the Chinese Academy of Forestry)

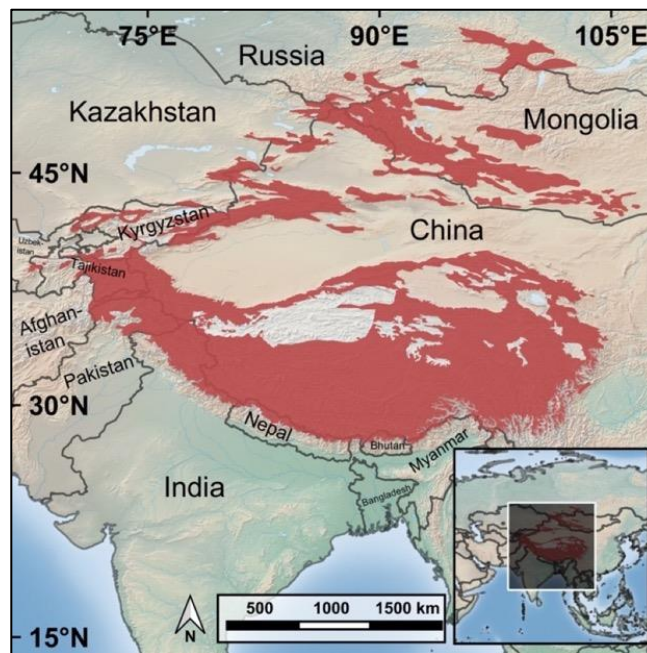


Figure 2. The species distribution of the snow leopard in Central Asia.  
(Snow Leopard Range data taken from the 2008 International Conference on Rangewide Conservation Planning for Snow Leopards)

### Specialized adaptations of the snow leopard to extreme environments

Snow leopards are able to thrive in these environments due to a suite of unique morphological adaptations. First, their skulls are smaller than that of the lion (*Panthera leo*), tiger (*Panthera tigris*), leopard (*Panthera pardus*), and jaguar (*Panthera onca*), but are more highly vaulted (Haltcnorth, 1937). Similar to the cheetah (*Acinonyx jubatus*),

this extra vertical space holds a large nasal cavity thought to assist in extracting the maximum amount of oxygen possible with each breath (Hemmer, 1972). Their canines are also more robust than would be expected for a species that commonly kills by biting their prey at the throat. This has been speculated to be advantageous for chasing prey down steep and unpredictable terrain whereby bite location may vary based on the angle of attack possible (Kitchener et al., 2016). During these pursuits of wild prey along mountainsides, the tail of the snow leopard acts as a steering rudder and assists with balance (Rieger, 1984). They also serve as an added layer of warmth when wrapped around the body (Rieger, 1984). Snow leopards have the densest fur of any species in the *Panthera* genus at 4,000 hairs per square centimeter (Heptner & Sludskii, 1972). These hairs comprise a pelage that forms a pattern of rosettes dotted against a light to dark grey, creamy, background with shades of brown intermittently dispersed throughout and dark black spots on the head, neck, and lower limbs (Hemmer, 1972). These markings and their coloring allow the snow leopard to seamlessly blend in with their surrounding environment (Kitchener et al., 2016), and have largely attributed to their recognizability among the public.

#### Difficulties in studying the species

The snow leopard was first described in 1761 by Buffon who named it l'Once (Buffon, 1761). Over 250 years later, it has become a recognizable flagship species serving to inspire conservation for the fragile landscapes it occupies (Blomqvist & Sliwa, 2016). Despite their charisma, wild snow leopards remain widely understudied in comparison to other big cats (Jan E. Janecka et al., 2020). This is due to a myriad of

reasons. First, the species occupies rugged and remote habitat that can be inhospitable to humans (Fox & Chundawat, 2016). Second, researchers must navigate countries that may be politically unstable, require extensive permitting processes, or lack access to accurate maps (Hunter et al., 2016). Third, the snow leopard is remarkably well camouflaged with its surrounding environment, making it extremely difficult to spot in its native habitat. Lastly, snow leopard specimens from museums are rare, as hunting them for sport was never popular, and very few accurate fossil records for the species exist (Jan E. Janecka et al., 2017, 2020; Kitchener et al., 2016). The first scientific snow leopard study was not until 1970 (Hunter et al., 2016) and the general global public had little knowledge of its existence until popular novels and photographs of the animal were published via mass media (Jackson & Hillard, 1986; Matthiessen, 1978). Since that time, studies on the species have remained relatively steady, leading to a large increase in understanding of this elusive cat in the last 25 years (Fox & Chundawat, 2016). One of the key contributions to this success has been the introduction of noninvasive genetics (Jan E. Janecka et al., 2020).

#### The role of noninvasive genetic sampling in understanding snow leopards

Noninvasive genetic sampling allows for the procurement of DNA from an animal without the need to handle or observe it in the wild (Waits & Paetkau, 2005). It is of particular value for rare and elusive species, like the snow leopard. Sources of DNA can include scat, urine, sloughed skin, regurgitates, and saliva (Waits & Paetkau, 2005). Scat has become the most commonly used source of DNA for noninvasive genetic studies surrounding carnivores (Rodgers and Janecka 2013), but the first published work using

noninvasive sampling for snow leopards used hair and skin samples to design genetic markers that would amplify a section of the control region of the mitochondrial genome (F. Zhang et al., 2007). Shortly thereafter, noninvasively collected scat from three areas of Central Asia successfully provided genetic information on snow leopards with species specific molecular markers (Janečka et al., 2008).

Genetic information that can be obtained from scat include both mitochondrial DNA (mtDNA) and nuclear DNA. DNA from the mitochondria is most commonly used for species identification, while nuclear DNA is most commonly used for individual identification (Rastogi et al., 2007). Markers for individual identification are typically short, repetitive sequences called microsatellites or short tandem repeats (STRs).

Microsatellites serve as neutral, non-coding markers with high-mutation rates that follow patterns traditional Mendelian inheritance (Jarne & Lagoda, 1996), allowing for the examination of several facets of populations genetics. Species specific microsatellite primers have been developed for snow leopards to reduce genotyping errors that may otherwise be caused using microsatellite primers for the domestic cat (*Felis catus*) (Janečka et al., 2008; Jan E. Janecka et al., 2014). This has greatly helped to facilitate population studies of snow leopards using noninvasive genetics. Unfortunately, these studies remain few, and are greatly lacking in comparison to other big cat species. To date, less than ten published studies exist (Aruge et al., 2019; Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011; Karmacharya et al., 2011; Korablev et al., 2021; Y. Zhang et al., 2019; Y. Zhou et al., 2014).

## Snow leopard phylogeography

At a larger scale, the use of species-specific microsatellites can elucidate species phylogeography. Phylogeography was a term coined in 1987 as a field of study under the umbrella of biogeography (Avice, 2009; Avice et al., 1987). It examines the processes and drivers of current spatial distributions of gene lineages to link geography with genealogy (Avice, 2009). Phylogeography incorporates time and space to investigate evolutionary processes at micro- and macro-scales both past and present (Avice, 2000). Its study allows for interpretation of the influence of historical processes on the evolutionary mechanisms that have led to the contemporary geographic distributions of species and their features (Avice, 2000), informing the drivers that shape populations at a genetic level. The snow leopard was the last of the five big cat species to have a range-wide genetic assessment completed (Jan E. Janecka et al., 2017). Results were used to draw inferences on past demographic processes and how they shaped the species, to clarify taxonomy, and to provide insight for snow leopard conservation decision making. Overall, genetic diversity for the species was found to be low, but there was strong molecular and geographic support for three evolutionarily significant units that may warrant subspecies status - a Northern (*Panthera uncia irbis*), Central (*P. u. uncioides*), and Western (*P. u. uncia*). Unfortunately, sampling gaps, lack of clear phylogenetic breaks in some regions of the species' range, and number of samples from particular locations, casted doubt on the validity of snow leopard subspecies (Senn et al., 2018). Thus, knowledge gaps in snow leopard taxonomy and phylogeography remain.

## Snow leopard landscape genetics

There also remain questions at smaller landscape scales. Landscape genetics is the integration of population genetics with landscape ecology (Manel et al., 2003). It is an interdisciplinary approach that examines how geography impacts gene flow, genetic drift, adaptation, and population structure by combining genetic and spatial data (Manel et al., 2003; Robinson & Weckworth, 2016; Shirk et al., 2017). Genetic structure is primarily shaped by population size and the intensity of migration between populations (i.e., the movement of alleles during dispersal followed by successful mating in the new population) (Montgelard et al., 2014). Thus, genetic structure provides an empirical estimate of population connectivity. Populations that maintain greater levels of movement between them will have more similarity in their allele frequencies, whereas those where dispersal has been prevented or limited will experience genetic divergence and develop population structure. Landscape genetics has been nearly absent from published snow leopard literature with the exception of two studies (Korablev et al., 2021; Shrestha & Kindlmann, 2020). One notable area of interest moving forward is the study of landscape connectivity between the central and northern portions of the species' range. The need for molecular studies between the two areas has been labeled as "urgent" (Kitchener et al., 2017). The Gobi Desert separates the two areas with hundreds of kilometers of unsuitable habitat and serves as a barrier to gene exchange between snow leopard populations in Mongolia and northwestern China (Jan E. Janecka et al., 2017; Riordan et al., 2016). However, the degree of restriction to snow leopard movement, as well as how landscape features on the opposing sides of the Gobi Desert restrict

connectivity, is under explored despite the stark differences in habitat available for snow leopards in northwestern China versus Mongolia.

#### The study of snow leopard diet

Snow leopards are found in a wide variety of habitats that have been uniquely modified by glacial cycles in the Pleistocene (Yang et al., 2009). These habitats often harbor their own unique fauna, which serve as the basis of the snow leopard's dietary repertoire. Snow leopard diet has been relatively extensively studied in comparison to other facets of its ecology, but has historically used the method of microhistology (Anwar et al., 2011; Bagchi & Mishra, 2006; Chetri et al., 2017; Fox & Chundawat, 1988; Jackson, 1996; Khatoon et al., 2017; Lhagvasuren & Munkhtsog, 2000; Lovari & Mishra, 2016; Oli et al., 1994; Suryawanshi et al., 2017; Wegge et al., 2012). With this technique, scats are collected and dried, and digested hair or bone is compared to a reference database of known prey species. Unfortunately, this method is time consuming, dependent on the availability of a reference hair or bone from all potential prey species, prone to bias based on perceived expectations of diet, and can suffer from inter-observer bias. Additionally, intra-phenotypic variation within prey species can cause misidentifications (Pompanon et al., 2012). Fortunately, more recent advances in genetic methodologies have allowed for the examination of prey DNA from noninvasively collected scat samples via DNA metabarcoding.



## Using DNA metabarcoding to study snow leopard diet

In DNA metabarcoding, a pair of universal primers amplify a gene segment conserved enough that all species possess it but divergent enough to be different among all possible prey items (Valentini et al., 2009). This segment is then sequenced, and the resulting reads are matched to a reference database to identify organisms within each sample. The advent of next-generation sequencing (NGS) has streamlined this process, allowing for rapid sequencing of multiple samples in parallel (Shokralla et al., 2012). It serves as a promising tool for better understanding snow leopard feeding ecology (Shehzad et al., 2012).

Previous diet research supports the notion that snow leopards prefer larger-bodied wild hoof stock species (Bagchi & Mishra, 2006; Bagchi et al., 2020; R.M. Jackson, 1996; Lhagvasuren & Munkhtsog, 2000; Shehzad et al., 2012). However, livestock predation by snow leopards does occur (Bagchi et al., 2020; Chetri et al., 2017; J.D. Farrington & Tsering, 2019; Johansson et al., 2015; C. Mishra et al., 2016; Shrestha et al., 2018; Suryawanshi et al., 2013; Wegge et al., 2012). This is problematic as livestock loss causes financial burdens that promote negative attitudes and can lead to retaliatory killings (Hussain, 2003; Jackson & Wangchuk, 2001). Knowledge of dependency on domestic animals through space and time is imperative, but largely unknown. Other less understood facets of snow leopard diet further include the contribution of small mammals and birds to their diets (Jan E. Janecka et al., 2020) as well as dietary niche overlap between snow leopards and other sympatric carnivore species. DNA metabarcoding has the capacity to accurately and reliably determine the prey items of hundreds of scat samples, and thus its use shows great potential in answering these questions.

Furthermore, knowledge of livestock in snow leopard diet using DNA metabarcoding can assist in determining where studies examining attitudes of herders towards snow leopards are best suited, as well as where mitigation measures may be needed.

#### The role of snow leopards in livestock loss

Humans, livestock, predators, and prey share common resources, which can lead to conflict (Aryal et al., 2014). However, long-term conservation success hinges on local community support and participation (Jackson & Wangchuk, 2004). In order to gauge behavioral outcomes and likelihood of support, research on attitudes is first needed because attitudes precede and direct behavior (Dickman, 2010; Kansky & Knight, 2014; Vaske & Manfredi, 2012). Previous studies on attitudes towards snow leopards have been completed (Alexander, Chen, et al., 2015; C. Li et al., 2015; Oli et al., 1994; Samelius et al., 2020; Suryawanshi et al., 2014; A. Xu et al., 2008). However, attitudes are influenced by many factors, including knowledge, social norms, economic constraints, and religious affiliation (Dickman, 2010) and therefore are likely inconsistent across snow leopard range (Alexander, Chen, et al., 2015). While some areas may show tolerance despite livestock loss, others may display more negative attitudes towards the species, which may be indicative of an increased threat of retaliatory killings (J. Li et al., 2013; Oli et al., 1994). Regardless, any loss of livestock, which leads to lethal removal of snow leopards or not, is a burden on families, the community, insurance companies, and agencies seeking to protect wildlife (Treves & Karanth, 2003).

Livestock loss can be largely prevented using non-lethal strategies (Ogada et al., 2003; Shivik et al., 2003). However, rigorous testing on their effectiveness is lacking

despite their wide-spread use and importance in mitigating conflicts between humans and wildlife (J. R. B. Miller et al., 2016; Treves et al., 2016; van Eeden et al., 2018). The snow leopard to date has only one publication examining the effectiveness of a non-lethal deterrent (fences), but only functional effectiveness was measured (Samelius et al., 2020). In order for herders to use interventions, they must perceive that the intervention works (Ohrens, Santiago-Ávila, et al., 2019). Otherwise, what might be a seemingly functional deterrent will never get used (Cavalcanti et al., 2012; Ohrens, Santiago-Ávila, et al., 2019).

#### Harnessing herder knowledge and involvement in snow leopard research

Herders living in snow leopard habitat have adapted and thrived for millennia (Petousi & Robbins, 2014), developing strong environmental ties to the land as necessitated by pastoralism (R. M. Jackson, 1998; R. M. Jackson & Wangchuk, 2001; Kis et al., 2016). Unfortunately, lack of quantitative measures provided by herders in support of their statements has led to questioning of knowledge legitimacy. Herders are not considered as part of the ‘academic elite’ and their expertise has often been dismissed as unscientific or uninformed (Cencetti, 2011). The misconception by outside entities that rural pastoralists are ignorant and lack innovation has proven difficult to overcome (Brockington & Homewood, 1999). “Facts” observed between western scientists and study subjects are often prioritized over the “human-nature relationships” perceived by indigenous peoples and the environments they live in (Cochran et al., 2013). Such misunderstanding is unfortunate since policies shaped using information gathered by scientists and shared with government officials both directly and indirectly impacts local

people (J.M. Foggin, 2012), while often excluding them from planning or decision making (Roturier & Roué, 2009). Fortunately, the value of local knowledge is becoming more widely accepted and recognized on international scales (Gadgil et al., 1993). However, published examples, perspectives, and potential outcomes of how such research may involve or engage these communities is lacking (David-Chavez & Gavin, 2018).

#### The urgent need for snow leopard research

The above gaps in snow leopard taxonomy, genetic structure, population metrics, diet, and human-wildlife dimensions represent a dearth in understanding of snow leopards, which requires accurate understanding now more than ever. The upheaval within the research community surrounding the 2017 IUCN delisting of the snow leopard from Endangered to Vulnerable served to demonstrate how little is really known about the species (Ale & Mishra, 2018). This is concerning, as the mountainous regions in which snow leopards reside are currently undergoing extreme shifts brought on by climate change (M. Zhang et al., 2019). One study examining weather data from 1957 to 2005 found that Central Asia was warming at a higher rate than any other area globally, with both warmer and colder temperature extremes observed (M. Zhang et al., 2019). The ability to preemptively formulate effective conservation solutions for snow leopards will be dependent on adequate understanding of how the species may adapt to these large-scale changes, which will require knowledge of how many individuals are in an area, their genetic diversity and heterozygosity, dietary plasticity, and current corridors to movement that may be impeded. Because people will also be experiencing these changes

alongside snow leopards, it is further imperative that conservation action consider how disruptions in snow leopard populations due to climate change may impact herder livelihoods via increased livestock depredation or via alterations in ecosystem functions that they indirectly depend on.

Loss of snow leopards would have drastic consequences. They are considered an umbrella species with spill-over benefits for numerous other wildlife (Alexander et al., 2016). They shape prey communities and are essential to ecosystem equilibrium (Karanth & Nichols, 1998). Loss of the species would also remove one of Central Asia's most iconic flagship animals, as well as a species steeped in rich history, folklore, and religious practices for minority communities (Blomqvist & Sliwa, 2016; J. Li et al., 2014).

#### Dissertation goals and subgoals

The goal of this dissertation was to use noninvasive genetic approaches to add meaningful and reliable information to current snow leopard literature. This included three major research goals with nine subgoals – Goal 1) the study of population parameters and genetic structure: Goal 1 - Subgoal 1) the study of local population metrics in China and Mongolia; Goal 1 - Subgoal 2) range-wide genetic structure; Goal 1 - Subgoal 3) landscape connectivity between snow leopard habitat in China and Mongolia. Goal 2) the study of snow leopard diet: Goal 2 - Subgoal 1) improving molecular dietary protocols and determining snow leopard diet range-wide; Goal 2 - Subgoal 2) examining the diets of predator guilds in three areas of China; Goal 2 - Subgoal 3) examining seasonal differences in the diets of a predator guild in one area over the course of one year. Goal 3) the study of human-carnivore dimensions: Goal 3 -

Subgoal 1) understanding the drivers of negative versus positive attitudes towards snow leopards; Goal 3 - Subgoal 2) evaluating the perceived versus functional effectiveness of a non-lethal predator deterrent in preventing livestock loss; Goal 3 - Subgoal 3) the creation of a framework to be used in efforts for including local communities in snow leopard research. Ultimately, the information obtained from these collective works will help inform conservation management decision across a wide area of a conservation concerns and challenges facing snow leopards.

Dissertation chapter summaries

#### Chapter 2.1 - Genetic metrics of local populations in China and Mongolia

Noninvasive genetic analyses were conducted for five snow leopard populations in China (Qilian Shan – Qinghai, Qilian Shan – Gansu, East Burhanbuda Mountain, Yushu, and the Chang Tang region) and one snow leopard population in Mongolia (Southwestern Mongolia). Species, sex, and individual identification assays were performed and population metrics calculated. Abundance estimates ranged from six individual snow leopards in East Burhanbuda Mountain to 21 in Southwestern Mongolia. The average number of unique alleles ranged from 2.824 in East Burhanbuda Mountain to 5.848 in the Chang Tang region. Observed heterozygosity was lower than expected heterozygosity in all areas, suggesting that forces such as inbreeding are present. The low levels of genetic diversity found in this study are in alignment with previous work examining snow leopard genetics. While microsatellites remain the most plausible tool to assess population genetics due to availability of lab equipment and relatively low cost, future

work using single nucleotide polymorphisms (SNPs) will likely play a large role in better elucidating snow leopard population metrics and structure. This work helps to inform conservation managers at their respective local levels in the studied areas, and provides a comparison point for other research efforts surveying snow leopard populations across their range.

## Chapter 2.2 – Range-wide phylogeography and taxonomy resolution

A 2017 publication by Janecka et al. investigated snow leopard phylogeography by genotyping 33 microsatellites in 70 snow leopard individuals across 21 localities. Results showed molecular support for three primary genetic clusters (Central, Western, and Northern) that closely aligned with known geographic barriers, potentially adhering to subspecies status within the metapopulation. To further investigate these genetic clusters, the initial dataset used in Janecka et al. (2017) was improved upon via the addition of samples from the Central and Western genetic clusters, as well as in between them. Analyses completed in Janecka et al. (2017) were then redone with this updated dataset. Results continued to provide support for three primary genetic clusters with additional population structuring evident within each. The phylogeographic break in-between the Western and Central genetic cluster was similarly suggested to be the Himalayas as opposed to the Qinghai-Tibetan Plateau itself. As in Janecka et al. (2017), the greatest observed genetic differentiation remained between the Central and Northern genetic clusters. Results herein align with previous metrics used to delineate and assign subspecies status in felid species. Recognized subspecies status within the currently

considered monotypic snow leopard population could have large-scale outcomes for species management, funding, and awareness.

### Chapter 2.3 – Landscape genetics of snow leopard populations in Mongolia and China

Landscape genetics can help inform how geography and other landscape variables shape snow leopard genetic structure, and can elucidate barriers to gene flow and connectivity between populations. Previous work has shown relatively high levels of genetic differentiation between snow leopard populations in Mongolia and northwestern China. To further investigate and discern movement within and between these areas, a total of 97 individual snow leopard genetic profiles belonging to Mongolia and northwestern China were investigated. Analyses supporting two genetic clusters split individual snow leopards starkly between those from China and those from Mongolia. Groupings based on geography were then defined *a priori* for four genetic clusters (Middle Qinghai, Northern Qinghai/Gansu, Southern Mongolia, and Western Mongolia). Genetic clusters for snow leopards in Mongolia divided individuals from Western and Southern Mongolia into two clusters, with some degree of admixture. Snow leopards from China clustered together with the exception of six individuals from Yanchiwan, who divided out into their own cluster. Movement of snow leopards in Mongolia was eastward, while movement northward and southward in Northwestern China was equal. Snow leopards in Mongolia had less genetic structuring than snow leopards in China, indicating greater landscape connectivity. Genetic diversity metrics were low across all populations. The Gobi Desert appears to serve as a large barrier to gene flow between snow leopard populations in



Mongolia and Northwestern China, and observed connectivity between the two is likely not sufficient to maintain gene flow. Conservation efforts in these areas should focus on maintaining historical corridors for snow leopard movement, and populations appearing to serve as “sinks”, such as those in Mongolia, may warrant increased genetic monitoring for conservation efforts.

### Chapter 3.1 – Snow leopard diet composition range-wide

Classic methods surrounding the study of snow leopard diet have largely focused on microhistology. The advent of next-generation sequencing (NGS) has made it possible to rapidly and accurately determine dietary items from scat while removing many of the challenges associated with microhistology. However, investigated gene segments must be both conserved and divergent among all possible prey species. Lack of divergence in mitochondrially encoded 12S ribosomal RNA (MT-RNR1) in discerning caprid species has been a previous limitation in snow leopard diet analysis. A segment of mitochondrial cytochrome *c* oxidase subunit 1 (MT-CO1) was identified and used to supplement MT-RNR1 in holistically determining snow leopard diet from 165 scat samples collected in four range countries. Larger-bodied hoof stock species were the most commonly consumed prey item, with the predominate species varying by country examined. A wide variety of livestock was identified in snow leopard diet, along with small mammals and birds. This may indicate an opportunistic approach to foraging that snow leopards take, increasing dietary plasticity. Nearly 1/3 of larger bodied prey items in snow leopard diet were livestock, with relatively larger percentages in Pakistan and Mongolia. Dependency

on livestock as a dietary resource may challenge snow leopard conservation, as it may be indicative of a depleting wild prey base or increased competition with sympatric carnivores, and could increase the risk of retaliatory killings and negative attitudes towards the species among herders.

### Chapter 3.2 – Carnivore diet on the Qinghai-Tibetan Plateau of China

Carnivores play important roles in shaping the ecosystems they inhabit. Understanding predator guild diet can elucidate how sympatric carnivores coexist within a landscape, and how they contribute to ecosystem function processes. In this study, molecular diet analyses for predator guilds in three regions on the Qinghai-Tibetan Plateau (Qilian Shan, East Burhanbuda Mountain, and Yushu) were completed from 760 noninvasively collected scat samples. A total of 33 unique prey species were identified among nine carnivore host species. Blue sheep and pika were dominant in the diets of nearly all carnivores examined. Livestock presence in diet was relatively low at 4 to 7% of dietary occurrences. Livestock in diet significantly varied across carnivore species, with wolves having the highest occurrence. However, livestock in diet did not vary across the three regions examined. Dietary breadth was greatest for the red fox, a generalist mesocarnivore, and highest overall in Qilian Shan. Dietary similarity between any two given pairs of carnivores was highest between the Tibetan wolf and snow leopard. Dietary niche overlap was greater than expected overall, indicating that other mechanisms of niche partitioning, such as spatial and temporal, are responsible for sympatric species coexistence in these landscapes.

### Chapter 3.3 – Seasonal variation of carnivore diet in Gouli Nature Reserve, China

Carnivores may change foraging strategies based on seasonal shifts in prey availability, abundance, and accessibility. Such shifts can have large-scale conservation ramifications, as carnivores play an important role in shaping the habitats they live in. Seasonal changes in predator guild diet on the Qinghai-Tibetan Plateau are largely unknown, despite the area being a biodiversity hotspot of global geographic importance heavily affected by the negative impacts of climate change. In this study, ten fixed transects measuring 22.5km in total length were sampled during the months of March, July, September, and December to investigate seasonal variations in prey frequency and biomass for carnivores in Gouli Nature Reserve, Dulan County, Qinghai Province, China. A total of 581 scats were collected, with 511 representing eight carnivore host species and 26 unique prey species. Sampling completeness averaged 86.9% of potential dietary items captured (range = 33.3% to 100%). There was no significant difference in metrics obtained via prey frequency versus prey biomass. Pika and blue sheep dominated the dataset of recorded prey items. Livestock was present in the diets of four of the eight studied carnivores and during all four sampling seasons. Intraguild predation was noted, but minimal. Diet did not significantly vary across seasons overall for either frequency or biomass. The only significant difference in diet where sample sizes allowed comparison was for the red fox between the months of March and December. Dietary diversity was highest for the red fox, Tibetan wolf, or Eurasian lynx depending on the metric used (richness, Shannon-Wiener Index, Simpson's Index). Dietary niche overlap was greater

than expected overall and for most pairs of investigated carnivores. Pianka's Index values were highest between the Tibetan wolf and snow leopard, and the red fox and Tibetan fox. Other mechanisms of niche partitioning, whether temporal, spatial, or a combination thereof, are likely responsible for the successful coexistence of these species. Results suggest a low diversity of highly abundant prey year round, indicating that large-scale changes in the availability of these select prey may have dire consequences. Conservation measures that deter carnivores, particularly Tibetan wolves, from predating livestock may increase herder livelihoods. However, calculated biomass of species such as yak indicate that livestock may play an important role in sustaining predator guilds. Lack of accessibility to livestock may therefore negatively impact the predator guild at-large. Future studies should focus on more fine-scale differences in space and time, with attempts to increase the number of scats collected.

#### Chapter 4.1 – Assessment of herder attitudes towards snow leopards

Attitudes precede and direct behavior. Knowledge of herder attitudes towards snow leopards is therefore imperative in gauging support for conservation initiatives and wildlife protection. This study used an unbiased balanced survey to determine the drivers of positive versus negative attitudes towards snow leopards and their conservation. A total of 73 herders in Zhiduo County, Yushu Prefecture, Qinghai Province, China were interviewed in July 2018. Agreement with positive statements was significantly higher than agreement with negative statements ( $p < 0.001$ ) despite 45.8% of respondents losing livestock to what they believed to be a snow leopard in the last five years. Predation was

considered the greatest threat to livestock, followed by disease and then grassland degradation. More years of formal education and fewer livestock lost were the most important factors for shaping positive attitudes, while fewer livestock lost, less importance of snow leopards attributed to religion, and fewer years of formal education were the most important factors in shaping negative attitudes. The role of livestock loss in shaping attitudes is likely more complex than what this survey could discern, as the age, condition, and sex of the animal lost may contribute differently to frustration levels experienced. At least one dog was owned by 70% of interviewees, and were more common among younger herders. Increased use of large dog breeds under loose ownership practices may have negative outcomes for wildlife and livestock and warrant further study in the area. Herders provided relative abundancies of three key mammal species in the landscape (blue sheep, argali, and snow leopard) that matched with previous work in the study area assessing population numbers via rigorous scientific methods, further showcasing the value of blending local ecological knowledge and western science. Future study efforts should focus on untangling the complexities of livestock loss in shaping attitudes towards snow leopards.

#### Chapter 4.2 – The effectiveness of Foxlights in deterring predators from livestock

Conflicts between herders and predators in the form of livestock predation remains challenging for carnivore conservation. Livestock loss can lead to large financial burdens on pastoralists and their families, negative attitudes towards at-risk species, disagreements with entities seeking to protect carnivores, and is emotionally

traumatizing. Non-lethal strategies that deter carnivores from preying livestock have gained momentum, yet few studies have tested their perceived and functional effectiveness. This study sought to examine the perceived (herders think the device works at preventing livestock loss) and functional (device leads to a reduction in livestock loss) of Foxlights in Zhiduo County, Yushu Prefecture, Qinghai Province, China. Foxlights are small, portable, and solar powered. They intermittently flash a series of three colored lights upon night fall, with the goal of keeping carnivores from approaching livestock corrals. A pre-test / post-test approach over a period of three months was used. A total of ten herders received two Foxlights each (experimental group), while ten herders did not (control group). Each herder was interviewed before study start to obtain information on carnivores present, livestock holdings, demographics, husbandry practices, and previous livestock loss. Two separate follow up interviews after the study period, one for herders with Foxlights and one for herders without, were completed to discern livestock loss during the study period, any changes in livestock holdings, and questions surrounding the efficacy of Foxlights via herder opinion using a Likert type scale. Generalized estimating equations (GEEs) were used to determine the most important factors contributing to livestock loss. During the study period, a total of 12 loss events occurred, resulting in the depredation of two adult female sheep, one young female sheep, two adult female yak, seven young male yak, eight young female yak, and four subadult yak of unknown sex. Female yak were disproportionately preyed relative to the total number of yak available. Wolves were implicated in 11 of the reported loss events, while snow leopard was implicated in one. All herders in the experimental group perceived Foxlights as effective and found them easy to implement and herders with Foxlights experienced

significantly less livestock loss than those without. A greater number of people living in the household and possession of Foxlights contributed most to fewer livestock loss events, while Foxlight possession contributed most to fewer livestock lost. Taken together, these results suggest a high degree of functional effectiveness. However, 83% of livestock loss events occurred during the day, when Foxlights would presumably not be working. Foxlights may have longer-lasting effects that carry into the following day or may serve as deterrents by their mere presence for neophobic species. Given that most livestock loss was attributed to diurnal Tibetan wolves, it is not surprising that loss events occurred during the day, though why these significantly differed between those with and without Foxlights warrants further investigation. Future studies should be planned for longer time periods with a cross-over design and larger sample size. However, this may require upwards of two years to account for variables introduced by semi-nomadic practices. Future work should also incorporate more rigorous testing of potential habituation of carnivores to Foxlight presence.

#### Chapter 4.3 – A framework for increased inclusion of local communities in snow leopard research

Local residents living in study areas can be valuable resources for executing research objectives and conservation initiatives. The use of local ecological knowledge (LEK) and traditional ecological knowledge (TEK) in western science has increased in recent years, but stereotypes surrounding the expertise and validity of information provided by indigenous communities persist. Understanding of the natural history and ecology of the

snow leopard has greatly improved in the last quarter century, in part due to the inclusion of local communities living in snow leopard habitat. However, a constructive framework for expert elicitation and engagement is lacking. The goal of this work was to provide such a framework, with outlined considerations and acknowledgement of ethical practices, using two case studies. Facets of expert elicitation of local residents included efforts for study goal identification, study design, and study execution. The various skillsets and LEK possessed by residents can make them valuable field guides, drivers, translators, and scientific assistants. In addition, individuals themselves can serve as a source for raw data points. Broad outcomes for inclusive efforts include determination of locally relevant conservation concerns, integration of western science with LEK, additional income sources for local peoples, increased communication and trust, preservation of local culture, greater research flexibility, and increased community conservation capacity building. Considerations in implementing this framework include the proper use of ethical review boards, understanding the political ecology of an area, adequate dissemination of results to the local community and other entities, adhering to guidelines in identifying experts among residents, and awareness of potential bias that may be introduced through social, political, or demographic means.



## Chapter 2

### Goal 1

## Snow leopard population parameters at local and range-wide scales

### Chapter 2.1

#### Goal 1 – Subgoal 1

### Genetic metrics of local populations in China and Mongolia

Adapted from Zhang *et al.* (Hacker CE) (2017) *The genetic structure of snow leopard populations in Sanjiangyuan and Qilianshan National Parks*. *Acta Theriologica Sinica* 39: 442-449.

#### Introduction

#### Introduction to the family Felidae

Studies examining the population genetics of species are imperative to designing effective conservation management plans. This is particularly pertinent for threatened species with a reduced number of individuals. Small population sizes that are isolated are more at risk for genetic drift, or the random loss of genetic diversity, leading to lowered ability to adapt to evolutionary changes (Azizan & Paradis, 2021). Inbreeding is also more likely, causing increased risk of mortality and disease susceptibility as well as decreased breeding success (O'Brien *et al.*, 1985; Ralls *et al.*, 1979; Wildt *et al.*, 1987). The calculation of genetic metrics allows for the realization of the processes contributing to currently threatened species, such as inbreeding, using comparable quantitative numbers (DeSalle & Amato, 2004). Such information is incredibly important for

mammalian families that have a number of species listed as Threatened or Endangered, such as members of the family *Felidae* (Azizan & Paradis, 2021).

*Felidae* consists of 41 extant species, making it the second largest family in the Order Carnivora (Kitchener et al., 2017). However, geographic bias in studies examining genetic diversity in *Felidae* is evident in that published studies on felids in Asia are disproportionately lacking in comparison to the number present (Azizan & Paradis, 2021). In addition, a meta-analysis investigating the global patterns of genetic diversity for felids using 147 studies published between 1999 and 2019 found that species requiring large home range sizes tended to have lower genetic diversity, perhaps due to increased likelihood of encountering a suite of environmental pressures, including human infrastructure. Furthermore, species in Asia had lower genetic diversity and allelic richness than those in South America or Africa, likely due to the long-term consequences of Pleistocene extinction events. Genetic diversity was also lower in species that were threatened in comparison to those that were low risk. The snow leopard (*Panthera uncia*) is a threatened felid species that occupies large home ranges in Asia, and thus is likely to have low genetic diversity based on the criteria above. Unfortunately, few studies have examined snow leopard genetics despite the fact that the advent of noninvasive sampling has helped to circumvent many of the previous challenges associated with studying the species.

#### Gaps in knowledge surrounding snow leopard populations in China

China holds 1.1 million km<sup>2</sup> of the snow leopard's range, with one-third to one-half of the total species population residing there (Riordan & Shi, 2016). Population

estimates of the species in China are predominately guess work, with only a few studies determining population size based on rigorous methods (Riordan & Shi, 2016). Various numbers for population estimates and distribution size of snow leopards in China have been published. One of the first estimates suggested 2,000 individuals (Schaller, 1990). Another estimated total habitat area in the country to be 1.1 million km<sup>2</sup>, with a population of 2,000 to 2,500 individuals (Fox, 1994). A 2008 range-wide meeting held in Beijing resulted in a total habitat area estimate of 0.65 million km<sup>2</sup> (Riordan & Shi, 2016). However, scientists based in China were skeptical of the metrics that resulted from the meeting, and reconvened in November 2012 to develop a National Action Plan (Riordan & Shi, 2016). Using a range map produced by the Snow Leopard Species Survival Plan, experts at the 2012 meeting estimated the range of snow leopard habitat in China to be 2.08 million km<sup>2</sup>, housing 4,500 individuals (Riordan & Shi, 2016). Snow leopards are found in eight provinces in China, including Gansu, Qinghai, and the Tibet Autonomous Region (Riordan & Shi, 2016).

#### Snow leopard populations in Gansu and Qinghai Provinces

Gansu Province lies on the northeastern edge of the Qinghai-Tibetan Plateau. Snow leopards have previously been noted to be largely absent from the area aside from signs of snow leopard presence in Subei County (D. O. Hunter & Jackson, 1995). However, Gansu is now thought to support a seemingly healthy population of 168 snow leopards across 77,585km<sup>2</sup> of habitat (Riordan & Shi, 2016). Qinghai Province shares a border to the west of Gansu and houses a mixture of high-elevation grasslands, rangelands, wet-lands, and mountainous outcrops (Riordan & Shi, 2016). Wildlife in

Qinghai Province were hit hard during China's cultural revolution (McCarthy & Chapron, 2003). Since then, species have largely recovered. A population of approximately 650 snow leopards over a range of 65,000km<sup>2</sup> was estimated in the late 1980s (Schaller, Junrang, et al., 1988). A 2012 estimate of snow leopards living in the province was calculated at 1,039 individuals across a habitat area of 479,620km<sup>2</sup> (Riordan & Shi, 2016).

### The geography, habitat, and wildlife of Qilian Shan in Qinghai and Gansu Provinces

The Qilian Shan is a fold-thrust belt that spans Qinghai and Gansu from east to west (H. Zhang et al., 2017). It is composed of smaller parallel ranges, the Tulai Nanshan, Shule Nanshan, and Danghe Shan (Schaller, Junrang, et al., 1988). These mountains are characterized by eroded slopes and narrow passages (Schaller, Junrang, et al., 1988). Elevations below 3,300m consist mostly of desert, while higher altitude shrubs and grasses transfer to alpine meadow at 3,800m (Schaller, Junrang, et al., 1988). It is located north of the Kunlun Shan and Qaidam Basin and south of the Hexi corridor and Altyn Tagh fault, where its northern slopes (4,000 – 5,000m) give way to the much lower Mongolian Plateau (1,000 – 2,000m) (G. Wang et al., 2003; H. Zhang et al., 2017). The area plays important ecological roles that impact all of northwestern China (Yan & Ding, 2020). The Qilian Shan hosts rich biodiversity (Qian et al., 2019). Local faunal species include white lipped deer (*Cervus albirostris*), musk deer (*Moschus* sp.), wild yak (*Bos mutus*), Tibetan wild donkey (*Equus kiang*), black necked crane (*Grus nigricollis*), blue sheep (*Pseudois nayaur*), Tibetan brown bear (*Ursus arctos pruinosus*), Chinese desert cat (*Felis bieti*), Tibetan gazelle (*Procapra picticaudata*), beech marten (*Martes*

*foina*), Eurasian lynx (*Lynx lynx*), and snow leopard, among others (Yan & Ding, 2020). In an effort to protect this rich biodiversity, the Qilian Mountain National Nature Reserve (QNNR) was established as a 26,531km<sup>2</sup> county-level run conventional protected area (CPA) in 1988 (J. H. Z. Wang, 2019).

### History of the Qilian Shan National Nature Reserve

QNNR was part of a larger network of CPAs established to meet the public's interest in nature-based tourism and recreation as well as their concern for the environment (Sheng et al., 2020; G. Wang et al., 2011). Such protected areas have become an important source of tourism and revenue (G. Wang et al., 2011). Unfortunately, the management of CPAs across China has been uncoordinated and ineffective, creating worry that the focus of CPAs is economic gain rather than environmental preservation (Sheng et al., 2020). Indeed, CPAs have continued to face a number of environmental issues. Previous literature has defined water pollution, habitat alteration and loss, vegetation loss, and soil deterioration as problematic since the establishment of QNNR. For example, bountiful water resources in QNNR for hydroelectricity resulted in the construction of 42 power stations within it, many of which were built without concern for downstream water shortages, drainage basin degradation, or potential for regional biodiversity losses (Sheng et al., 2020).

### Introduction of the pilot park program in China

To counteract issues stemming from mismanagement, China instituted a pilot national park system in 2015, of which the QNNR and an additional 25,469km<sup>2</sup>

surrounding it were absorbed in June 2017 (Sheng et al., 2020). With this system, negative environmental outcomes associated with lack of environmental legislation, funding, conservation professionals, and law enforcement are anticipated to decrease due to a singular administrative entity within the central government of China overseeing the parks (Sheng et al., 2020). Careful evaluation of science-based criteria and indicators are necessary for identifying key areas and species of protection. With this in mind, one of the primary initiatives of the pilot park program was to make management decisions based on science (Sheng et al., 2020). Efforts were to include an integrated ecological monitoring network in Qinghai and Gansu provinces with cooperative relationships between the Qilian Mountain Park Administration and universities, research institutions, protection units, and monitoring stations (Yan & Ding, 2020). Within this framework, the snow leopard was prioritized as a focus species of interest (Yan & Ding, 2020).

#### Previous snow leopard research efforts in QNNR

At the 2008 snow leopard range-wide assessment meeting in Beijing, six Snow Leopard Conservation Units (SLCUs) in Qilian Shan were noted. At that time, information surrounding habitat quality, prey availability, population estimates, population trends, and the status of killing snow leopards were unknown (McCarthy et al., 2016). A total of 68.5% of the QNNR is in Gansu Province, while the remaining 31.5% is in Qinghai Province (Yan & Ding, 2020). The disparity in area on either side appears to be similarly reflected in targeted research efforts. While researchers in recent years have established the presence of healthy breeding populations of snow leopards in QNNR, with an estimated 168 individuals across a habitat area of 77,585 km<sup>2</sup>, these

estimates have been focused on the Gansu side of the park (Riordan & Shi, 2016). News of thriving snow leopard populations in QNNR in Gansu Province is promising, given that very few snow leopards or blue sheep inhabited the area in the mid 1990's (D. O. Hunter & Jackson, 1995). Studies done in this area include occupancy modeling to determine the influence of environmental and anthropogenic variables on snow leopard site use (Alexander, Shi, et al., 2016), population and density estimates using camera trapping (Alexander, Gopaldaswamy, et al., 2015; Alexander, Zhang, et al., 2016a), snow leopard involvement in conflict with humans using herder interviews (Alexander, Chen, et al., 2015), as well as determination of the spill-over benefits of snow leopard conservation for other species (Alexander et al., 2016). A species distribution model (SDM) for snow leopards that adopted a multi-scale and meta-replicated approach was constructed using species presence data collected from camera trapping and genetically confirmed snow leopard scat samples in Gansu but only camera trapping in Qinghai (Atzeni et al., 2020). Published information surrounding the genetics of snow leopards in the QNNR outside of presence confirmation is grossly lacking aside from 11 samples from Akesai County, Gansu Province and 2 samples from Tianjun, Qinghai Province used in a snow leopard phylogeography study (Jan E. Janecka et al., 2017).

#### The Kunlun Shan in Qinghai Province and past conservation efforts

To the south west of Qilian Shan lies the Kunlun Shan, a mountain range that stretches westward along the southern edge of the Tarim Basin (Riordan & Shi, 2016). Towards its eastern end is East Burhanbuda Mountain in Dulan County, Qinghai Province (Y. Liu, 1993). The area consists primarily of rugged grassland and rock slopes

(Y. Liu, 1993). Vegetation includes alpine steppe, alpine meadow, and shrublands (Liu et al. 2010). The climate is characterized by long, dry, and cold winters with strong winds and solar radiation (A. Xu et al., 2008). Dulan County has a history of successful conservation endeavors, including the establishment of the Dulan International Hunting Area in 1985 by the Qinghai Wildlife Management and Protection Bureau. At that time, there were no hunting areas in Qinghai Province (Y. Liu, 1993). These were established in Balong and Gouli township and went on to become one of the most successful international hunting areas in Qinghai Province, and has played a vital role in encouraging residents to value and preserve wildlife due to its potential to draw in tourism and support for the local economy. Local peoples made money off of international hunting by serving as hunting guides, renting out their horses for expeditions, serving as game guards to counteract poaching, by constructing and cleaning hunting camp sites, and by cooking for tourists and their affiliated guides (Y. Liu, 1993). The international hunting club also played a huge role in improving road conditions and supplied medical staff during hunting season, who were also available to the community at-large (Y. Liu, 1993). Further, locals found it exciting to interact with foreigners, an unexpected and indirect benefit of the hunting conservation program (Y. Liu, 1993). The success of the hunting program hinges on healthy wildlife populations, encouraging locals to conserve habitats and follow wildlife protection laws, including those that protect snow leopards. The 2008 snow leopard meeting in Beijing identified Dulan County as a SLCU with an area of high quality habitat covering 4,619km<sup>2</sup> with a medium degree of prey availability supporting 1 to 49 snow leopard individuals with an increasing population (McCarthy et al., 2016). Dulan County was considered to be a Type I SLCI,



meaning that the area had enough resident snow leopards to be self-sustaining over the next 100 years (McCarthy et al., 2016).

#### Previous snow leopard studies in East Burhanbuda Mountain

Past work examining snow leopards in East Burhandbuda Mountain in Dulan County include a study by A. Xu et al. (2008) and Janecka et al. (2008). Janecka et al. was the first study to apply noninvasive genetics with snow leopard specific microsatellite primers. They collected 50 scats along 11.1km of transects. A total of three of these scats were genetically identified as snow leopard, confirming the presence of the species in the area. However, no complete microsatellite genotypes could be garnered. Genotyping was attempted again for a range-wide phylogeography study (Jan E. Janecka et al., 2017), producing viable genotypes representative of three individuals. A. Xu et al. documented snow leopard presence via camera trapping, resident interviews, and sign surveys along 29 transects between March and May of 2006. Snow leopards were found to be present in the area, along with a seemingly healthy population of prey species. The study also interviewed 27 residents using questionnaires to assess attitudes towards the species. All but one individual had seen a snow leopard, with 22% having seen more than one in the previous three years. However, respondents indicated that the snow leopard population was decreasing due to hunting and trapping, habitat fragmentation, and competition with wolves. Attitudes towards snow leopards were found to be positive, with no instances of retaliatory killings noted in the previous three years. Future threats were revealed by the study, including an increase in fenced pastures for livestock, a growing number of livestock, and iron mining in the region. The authors called for the

establishment of a large protected area, citing it as the most effective way to conserve snow leopards and their habitat.

### The creation of the Gouli Nature Reserve

Calls for the creation of a protected area and the ability of the region to support healthy snow leopard populations led to the establishment of the Gouli Nature Reserve in 2009. The reserve has no set size, but is within the 2,559.4 km<sup>2</sup> of Gouli township (Rou Bao, personal communication) and has human inhabitants. Residents in the area are predominately Tibetan semi-nomadic pastoralists keeping livestock such as yak (*Bos grunniens*), sheep (*Ovis aries*), goats (*Capra aegagrus hircus*), and horses (*Equus caballus*) in summer pastures at approximately 4,600m with winter and spring pastures at lower altitudes of around 4,000m (A. Xu et al., 2008). To the west of the Gouli Nature Reserve is the township of Balong. Residents in Balong are primarily of Mongolian descent and agriculturalists (Y. Liu, 1993). Those who are pastoralists keep similar livestock as herders residing in the Gouli Nature Reserve with the exception of Bactrian camel (*Camelus bactrianus*) being common in Balong (personal observation). The religious beliefs of both cultures are similar, though sustainable hunting is a long-standing tradition among the Mongolian people who reside in Dulan, whereas residents of Tibetan descent are less inclined to hunt due to their religious beliefs surrounding the sacredness of wildlife (Y. Liu, 1993). Despite the call to action which resulted in the formation of a protected area in 2009, little research has been conducted outside of a camera trapping effort that started in 2016. In addition, aside from genetic identification of snow leopard presence in Janecka et al. (2008) and the genotypes of three individuals

used for a phylogeography study (Jan E. Janecka et al., 2017), no published research has examined snow leopard population genetics.

### Snow leopard habitat and conservation success in the Sanjiangyuan region of China

In contrast to Dulan County, the Sanjiangyuan region has been much more extensively studied. The area extends over 360,000km<sup>2</sup> and has an estimated 65,000km<sup>2</sup> of suitable snow leopard habitat (Dai et al., 2019; J. Li et al., 2014). It provides some of the largest known stretches of snow leopard habitat (Y. Liu et al., 2016), and has long been noted as a snow leopard “hot spot” (Schaller, Junrang, et al., 1988). Of the estimated 650 snow leopards estimated by Schaller et al. (1988) in all of Qinghai Province, two-thirds of them were estimated to be in the Sanjiangyuan region. Other wildlife in the area include Przewalski's gazelle (*Procapra przewalskii*), Tibetan antelope (*Pantholops hodgsonii*), Tibetan gazelle, wild yak, Tibetan wild donkey, blue sheep (*Pseudois nayaur*), Tibetan brown bear, Tibetan wolf (*Canis lupus*), Tibetan fox (*Vulpes ferrilata*), and dhole (*Cuon alpinus*) (data provided by the Sanjiangyuan National Park administration; <http://sjy.qinghai.gov.cn/>). The Sanjiangyuan region includes the Sanjiangyuan National Park (Mandarin Chinese for “Source of the Three Rivers” – the three rivers being the Yangtze, Yellow, and Mekong). Established in 2003, it was China’s first national pilot park (Y. Liu et al., 2016). The park spans an area of 152,000km<sup>2</sup> (Y. Liu et al., 2016) with estimates of suitable snow leopard habitat in the reserve being anywhere from 10,000km<sup>2</sup> (Riordan & Shi, 2016) to 38,000km<sup>2</sup> (J. Li, 2012). Tibetans account for 90% of the 1 million people that reside there (J. Li et al., 2014; Y. Liu et al., 2016). In addition to the 21 conservation stations built throughout the park, cooperation

among park officials, local residents, NGOs, universities, and research institutes has lent itself to the formation of successful conservation initiatives since the park's conception (Y. Liu et al., 2016; Shen & Tan, 2012).

#### Yushu Prefecture as a conservation and ecotourism hub

Indirectly contributing to an uptake in conservation research in the area was the devastating 2010 earthquake along the Garzê-Yushu Fault measuring 7.1 on the Richter scale and leveling 80% of the buildings in the nearby city of Yushu (L. C. Chen et al., 2010). Official tolls counted 2,698 people killed, 12,135 injured, and 270 missing (Z. Zhang et al., 2012). While the aftermath of the earthquake incited debilitating mental health disorders for many residents (Dongling et al., 2016; Z. Zhang et al., 2012), much of the city and the surrounding environment benefited from post-disaster reconstruction policies. Yushu rebranded itself as an ecotourism city and targeted efforts in the surrounding area maximized opportunities for local residents and their communities to benefit financially while also supporting conservation development (Ding, 2013; J.M. Foggin & Yuan, 2020).

#### Previous snow leopard studies in the Sanjiangyuan region

Such efforts facilitated ease of access for researchers and emphasized protection of the wide degree of biodiversity in the area, resulting in several studies surrounding snow leopards. One doctoral thesis estimated 40 individuals across 1,500km<sup>2</sup> of habitat in Suojia township (J. Li, 2012). Continuous camera trapping work by the NGO, Shan Shui, has determined the number of individuals in numerous areas of the Sanjiangyuan region

(Y. Liu et al., 2016). A total of 6 to 8 individuals were observed in 2013 across an area of 300km<sup>2</sup> in the village of Yunta. A 2014 survey found 20 individuals across 950km<sup>2</sup> in the township of Zhaqing while 40 individuals were estimated in 2010 in Nyanpo Yutze (Y. Liu et al., 2016). Habitat availability that is contiguous along with the establishment of the Sanjiangyuan National Park, and the overall positive attitudes towards snow leopards in the area make Yushu Prefecture a strong hold for species persistence. Consistent monitoring efforts will be vital to maintaining current snow leopard protection efforts. Knowledge therein can also aid in assisting other areas in formulating successful snow leopard conservation action plans across China and particularly in Qinghai Province.

#### Previous research in the Chang Tang region of the Tibetan Plateau

To its southwest, Qinghai Province shares a border with the Tibet Autonomous Region (TAR). The 2012 consensus in China suggested that the TAR had 1,797 snow leopard individuals across 402,683km<sup>2</sup> of habitat (Riordan & Shi, 2016). The Chang Tang region (Tibetan for “northern plains” (D. J. Miller & Schaller, 1996)) is a substantial land mass within this habitat network of which snow leopard population status is largely unknown. It is one the last, largely undisrupted rangelands in the world and is characterized by a harsh environment with high altitudes and scarce vegetation (J.D. Farrington & Tsering, 2019; Fox & Tsering, 2005; D. J. Miller & Schaller, 1996). Its remoteness left it largely uninhabited until the second half of the 20<sup>th</sup> century (Schaller, 1998), only used by semi-nomadic herders in the summer months as grazing range for their sheep, goats, and yak (Fox & Tsering, 2005; Schaller, 1998). Wildlife in the area include Tibetan antelope, gazelle, Tibetan wild donkey, blue sheep, argali (*Ovis ammon*),

pika (*Ochontona* sp.), marmot (*Marmota himalayana*), Tibetan brown bear, Tibetan fox, Eurasian lynx (*Lynx lynx*), wolf (*Canis lupus*), and snow leopard (Schaller, 1998).

Wildlife in the area, particularly the Tibetan antelope, suffered heavily in the 1980s and early 1990s due to poaching (D. J. Miller & Schaller, 1996; Schaller, 1998). Mass media campaigns focused on the plight of the Tibetan antelope and a volunteer anti-poaching team helped draw in public awareness and support for wildlife protection in the area, resulting in the establishment of several nature reserves within the Chang Tang area (Y. Liu et al., 2016; Wright & Kumar, 1998). At present, the Chang Tang reserve covers 290,000km<sup>2</sup> (J.D. Farrington & Tsering, 2019).

Research in the TAR of China has numerous challenges due to its harsh environment and political sensitivities that restrict access (Riordan & Shi, 2016). As a result, there are few studies surrounding snow leopards from the area. Previous work has indicated that snow leopards were found sporadically across the TAR, with populations localized to prime habitat. However, overall consensus was that snow leopards were rare, even when observations of a high number of prey were noted (Schaller, 1998). A distribution map was created based on 270 snow leopard location records collected from 2006 to 2007 via sightings from observers, occurrences of conflict with humans, and observations of snow leopard signs to construct a distribution map of the species in the Chang Tang region (John D. Farrington & Tsering, 2020). The intensity of conflicts between humans and snow leopards as well as snow leopard presence was determined by surveying 234 residents in Nagchu Prefecture in conjunction with sign surveys along 15 transects (J.D. Farrington & Tsering, 2019). To date, the only published genetics study of snow leopards in the TAR is published in Mandarin Chinese (Y. Zhou et al., 2014). That

study examined 56 scat samples from the Qiangtang National Nature Reserve in Shenza and Bange County. Knowledge of snow leopard genetic structure, diversity, and abundance remains unknown for the Chang Tang Nature Reserve and language barriers remove access to current genetic literature. Such challenges are unfortunate, as the area remains one of the most mysterious in regards to its contribution to China's status as holding the largest number of snow leopards of the species' 12 range countries.

### Snow leopard populations in Mongolia

After China, Mongolia holds the second largest population of snow leopards, though population estimates range from 1,000 to 1,700 (Munkhtsog et al., 2016; Schaller et al., 1994). Snow leopard range in the country was most recently suggested to be 103,000km<sup>2</sup>, though estimates of 90,000km<sup>2</sup> and 130,000km<sup>2</sup> have also been reported (Mallon, 1984; McCarthy, 2000a; Schaller et al., 1994). Mongolia has the lower elevational limits of the species' range, with altitudes ranging from 600m to 4,200m (Munkhtsog et al., 2016). The species is primarily confined along the Altai, Trans Altai-Gobi, Gobi-Altai, Khangai, and Khovsgol mountains with movement across less rugged areas being relatively common (Munkhtsog et al., 2016).

### The history of snow leopard research and legislative protection in Mongolia

Mongolia has historically taken a strong stance on snow leopard protection. At least 10 protected areas that cover 20% of the snow leopard's range in Mongolia have been established (McCarthy & Chapron, 2014). It has been illegal to kill snow leopards in the country since 1972 and the inception of the snow leopard conservation project with

the Mongolian Association for Conservation of Nature (MACNE) in 1994 led to the first radio-collaring study of the species beginning in 1993 and the first community based conservation program in species range (Munkhtsog et al., 2016). These efforts have expanded into a network of collaborative initiatives that have directly led to seminal studies on the species. Some of the more recent work includes genetic metrics using noninvasive approaches (Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011; Korablev et al., 2021), molecular diet analysis (C.E. Hacker et al., 2021; Shehzad, McCarthy, et al., 2012), range size and movement (Johansson et al., 2016; McCarthy et al., 2005), population trends (K. Sharma et al., 2014), human-snow leopard dimensions (Augugliaro et al., 2020; Johansson et al., 2015; Charudutt Mishra et al., 2003), co-occurrence with sympatric species (Lukarevskiy et al., 2020; Rovero et al., 2020), disease (Esson et al., 2019; Johansson, Ullman, et al., 2020), and cub rearing (Johansson, Ausilio, et al., 2020). Coverage of snow leopard habitat for noninvasive studies examining population genetics are largely uniform with the exception of samples representative of southwestern Mongolia in between the Gobi Desert and Gobi-Altai Mountain range.

#### Importance and research goals of this study

Collectively, the range countries of China and Mongolia contribute 0.74 to 2.21 million km<sup>2</sup> of snow leopard range (D. O. Hunter & Jackson, 1995; Mallon, 1984; McCarthy, 2000b; McCarthy & Chapron, 2003; Riordan & Shi, 2016; Schaller et al., 1994), making them strong holds for species persistence. Given the importance of genetic information in shaping effective conservation action plans, systematic noninvasive genetic sampling efforts were undertaken to close knowledge gaps surrounding snow



leopard population genetics in the Qilian Shan range of Qinghai Province, the Chang Tang region of the TAR, the Gouli region of Dulan County, China, and southwestern Mongolia. In these areas, it was sought to 1) validate the presence of snow leopards , 2) determine the number of unique individuals, 3) examine sex ratio, 4) investigate metrics of genetic diversity and structure, and 5) make suggestions for future conservation actions based on scientific findings. Efforts were also undertaken in the Qilian Shan range of Gansu Province and the Sanjiangyuan region in Yushu, Qinghai Province to contribute towards continued monitoring efforts that are imperative for maintenance of current species survival plans. In these areas, it was sought to 1) contribute to metrics of snow leopard populations, 2) provide more fine scale data for previously broadly assessed regions, and 3) make suggestions for future conservation actions based on findings and assessment of current species protection strategies.

## Materials and Methods

### Study areas

#### *Chang Tang, TAR*

The Chang Tang area of the northwestern Tibet Autonomous Region (TAR) (30°N, 88°E) is an 800 mile east to west contiguous stretch of land on the western Tibetan Plateau that sits at an average elevation of 4,500m (J.D. Farrington & Tsering, 2020; Fox et al., 2008; D. J. Miller & Schaller, 1996). It consists of steppe arid grasslands with occasional glaciated peaks rising over 6,000m in height (J.D. Farrington & Tsering, 2020). The climate is harsh, with no frost free seasons and an average annual precipitation of 10.16 to 30.48cm (D. J. Miller & Schaller, 1996). Samples were collected

in Shainza County, Nagchu Prefecture, approximately 52km southwest of Siling Lake (31.66496°N, 88.56158°E) (Figure 3).

#### *Qilian Shan, Qinghai*

The Qinghai Province side of Qilian Shan hosts smaller parallel mountain ranges adjacent to the main range (Schaller, Junrang, et al., 1988). The Kangze'Gyai peak at 5,808m in the Shule Nanshan range sits higher than the main range's Qilian Shan peak in Gansu Province. Samples were collected in two broad areas of the Shule Nanshan range approximately 60km apart (38.812219°N, 97.5787°E; 38.680298°N, 38.731976°E) with Hala Lake to the south and southwest of each area, respectively (Figure 3).

#### *Qilian Shan, Gansu*

The Gansu Province side of Qilian Shan houses the Qilian Shan peak at 5,547m approximately 60km south of the city center of Jiuquan in Suzhou District. To the west of the Suzhou District is the southern portion of Subei County. Subei County is a non-contiguous autonomous county broken into two parts, separated by Guazhou County and the city of Yumen. Samples were collected in the lower half of Subei County in the Yanchiwan National Nature Reserve (a part of QNNR) in the Yanchiwan township area (39.2097°N, 97.1811°E). Yanchiwan National Nature Reserve is approximately 13,600km<sup>2</sup> with an average altitude of 4,800m (Atzeni et al., 2020) (Figure 3).

### *East Burhanbuda Mountain, Qinghai*

The Gouli Region is located in Dulan County, Qinghai Province on the eastern end of the Kunlun Shan. The Gouli Nature Reserve (35.659528°N, 98.499298°E) is approximately 60km southeast of Dulan, the county seat of Dulan County. Balong Township (36.006323°N, 97.486185°E) is approximately 125km from Dulan (Y. Liu, 1993). Both reside south of the Xining-Golmud highway (Harris, 2000). The topography of both areas is similar, with altitudes ranging between 3,400m to 5,000m (Y. Liu, 1993) (Figure 3).

### *Yushu, Qinghai*

The villages of Suojia and Duocai are located in Zhiduo County, Yushu Prefecture, in the Sanjiangyuan region of Qinghai Province. Zhiduo County covers an area of 38,793km<sup>2</sup> (excluding Hoh Xil National Nature Reserve) and has an average elevation of 4,500m. Suojia (34.329444°N, 94.2466667°E) is located in the Yangtze River Zone of Sanjiangyuan National Park. Duocai (33.507226°N, 95.527888°E) lies adjacent to the Sanjiangyuan National Park, sharing an eastern border with Suojia (Figure 3).

### *Southwestern Mongolia*

The Govi-Altay (45.2534°N, 97.2574°E), Bayankhongor (44.945°N, 100.244°E), and Ovorhangay (44.8231°N, 101.5846°E) provinces occupy southwestern Mongolia from west to east. Bisecting through them is the southern flank of the Govi-Altai

Mountain range. The province of Govi-Altay is predominately characterized by rolling hills and plateaus, though steep ridgelines and cliffs are also present (McCarthy et al., 2005), Bayankhongor Province is one of the largest provinces in the country, and houses the country's most densely populated city, which shares the same name (Ferreira Guerreiro, 2015; Oyuntsetseg et al., 2014). The southern portion of the province is characterized as steppe and desert steppe, leading into the Gobi Desert towards the southeast (Ferreira Guerreiro, 2015; Oyuntsetseg et al., 2014). Ovorhangay Province houses Myangan Yamaat, the highest mountain peak in Baga Bogd at 3,600m. Baga Bogd is a massif that is part of the Gurvan Bogd Mountain Range, the eastern terminus of the Govi-Altai range (Carretier et al., 2002; Demoux et al., 2009) (Figure 3).

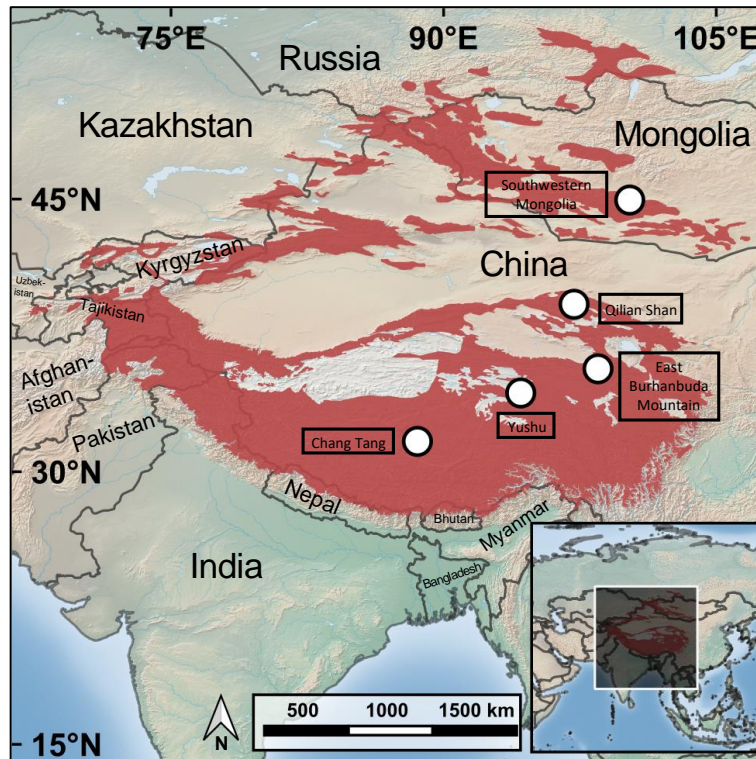


Figure 3. Sample collection sites for local snow leopard population estimates with snow leopard distribution range denoted in red. (Snow Leopard Range data taken from the 2008 International Conference on Rangewide Conservation Planning for Snow Leopards)

### Sample collection and storage

Sampling methods followed those described in (Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011).

#### *Chang Tang, TAR*

Sampling took place between October 2016 and May 2017 along transects over an area of approximately 415km<sup>2</sup>. Date, name of collector, GPS coordinates, signs of snow leopard presence, sample identification field name, and other remarks were recorded (Appendix I: Supplemental Material 1). Each scat was stored in a 50mL conical tube with silica desiccant and 95% ethanol.

#### *Qilian Shan, Qinghai*

Sample collection took place over two time periods. The first was in May 2017 along transects covering a cumulative area of approximately 1,500m<sup>2</sup> (600km<sup>2</sup> in the western half, 900km<sup>2</sup> in the eastern half). Number, sample identification field name, location, GPS coordinates, time of collection, altitude, name of collector, and photograph confirmation were recorded (Appendix I: Supplemental Material 2). Each scat was stored in a 15mL conical tube with silica desiccant. The second was in September 2017 in close proximity to the areas where samples were collected in May 2017. Number, sample identification field name, location, GPS coordinates, time of collection, altitude, and photograph confirmation were recorded (Appendix I: Supplemental Material 3). Each scat was stored in a 15mL conical tube with silica desiccant.

*Qilian Shan, Gansu*

Sample collection took place in July 2018 across 15.5km of transects over an area of approximately 541km<sup>2</sup>. Date, site name, transect number, sample identification field name, GPS coordinates, altitude, and any associated comments were recorded (Appendix I: Supplemental Material 4). Each scat was stored in a 15mL conical tube with silica desiccant or in a 10.16cm by 7.62cm Ziploc bag.

*East Burhanbuda Mountain, Qinghai*

Sample collection occurred in July 2018 in Gouli Nature Reserve and the township of Balong across 20km of transects over an area of approximately 521km<sup>2</sup>. Date, site name, transect number, sample identification field name, GPS coordinates, altitude, and any associated comments were recorded (Appendix I: Supplemental Material 5). Each scat was stored in a 15mL conical tube with silica desiccant or in a 10.16cm by 7.62cm Ziploc bag.

*Yushu, Qinghai*

Sample collection took place in two different areas in two different time periods. The first was in Suojia Village in April through July 2016. Herders were asked to collect ad libitum. Date, GPS coordinates, and suspected carnivore origin were recorded (Appendix I: Supplemental Material 6). Each scat was stored in a 6.35cm by 10.80cm coin envelope. The second collection event in Yushu was approximately 200km from the first in Duocai Village in July 2018 and covered 13km of transects. Date, site name, transect number, sample identification field name, GPS coordinates, altitude, and any

associated comments were recorded (Appendix I: Supplemental Material 7). Each scat was stored in a 15mL conical tube with silica desiccant.

### *Southwestern Mongolia*

Sample collection took place in August 2015 across three different areas – eastern Govi-Altay Province, central Bayanhangor Province, and the eastern border of Overhangay Province. A total of 17 transects were completed by foot with a total area coverage of approximately 324km<sup>2</sup> (Appendix I: Supplemental Material 8). Each scat was stored in a 15mL conical tube with silica desiccant.

### DNA extraction

DNA from each sample was extracted using the Qiagen QIAamp DNA Stool Mini Kits [QIAGEN, Hilden, Germany] following manufacturer's protocols with the exception of two additional washing steps to remove residual AW buffer (Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011). DNA quality and quantity was assessed using a Nanodrop Lite Spectrophotometer and ran on a 1.5% agarose gel.

### Species, individual, and sex identification

To determine scats of snow leopard origin, a 148-bp segment of the mitochondrial cytochrome *b* gene (CYTB) specific to the species was amplified in triplicate using PCR (Jan E. Janečka et al., 2014). Amplifications were performed at a 10uL volume with 1.5uL of DNA extract, 5uL of Type-it Multiplex PCR Master Mix 2x [QIAGEN, Hilden,

Germany], 0.2uL of the CYTB-SCT-PUN forward primer, 0.2uL of the CYTB-SCT-PUN reverse primer, and 3.1uL of PCR grade water. Samples that failed to amplify or produced faint bands were subjected to a second species identification assay using the same protocol above.

Samples that were snow leopard positive were subject to an individual identification assay at four loci (Multiplex 1: PUN1157FAM, PUN132NED, PUN834PET, PUN229VIC) for samples from Chang Tang and Qilian Shan – Qinghai May 2017, seven loci for samples from Qilian Shan – Qinghai September 2017, Qilian Shan – Gansu (Multiplex 1: PUN1157FAM, PUN132NED, PUN834PET, PUN229VIC; Multiplex 2: PUN935 FAM, PUN124 NED, PUN894 VIC), and eight loci for southwestern Mongolia (Multiplex 1: PUN1157FAM, PUN132NED, PUN834PET, PUN229VIC; Multiplex 2: PUN935FAM, PUN124NED, PUN272PET, PUN894VIC) (Janečka et al., 2008). Amplifications were carried out in a 10uL reaction with 1.5uL of DNA extract, 5uL of Type-it Multiplex PCR Master Mix 2x [QIAGEN, Hilden, Germany], 0.1uL of each of the four forward primers for a total of 0.4uL, 0.1uL of each of the four corresponding reverse primers for a total of 0.4uL, and 2.7uL of PCR grade water. Microsatellite PCR amplicons were shipped to the Genomics Core at Cornell University and fractionated on a 3730xl DNA analyzer [Applied Biosystems, Foster City, CA, USA]. Two positive snow leopard controls of which all microsatellites allele calls were known were included. A negative control of PCR grade water with no DNA was included to screen for contamination. The returned raw data were analyzed and genotypes scored using GeneMarker® [SoftGenetics, State College, PA, USA]. Heterozygous alleles were defined as those showing two peaks on the resulting electropherogram, and



homozygous alleles defined as those showing only one peak. Heterozygous and homozygous alleles had to be observed in two or more of the replicates in the triplicate. An individual was identified via the unique composite of alleles across microsatellite loci. These were summed to determine abundance for each investigated population. Crude estimates of density were calculated by taking the approximate sampling area and dividing it by the number of unique individual snow leopards.

A sex identification assay was completed for each individually identified snow leopard by amplifying a 200bp segment on the AMELY gene found only on the Y chromosome in triplicate. Amplifications were performed at a 10uL volume with 1.5uL of DNA extract, 5uL of Type-it Multiplex PCR Master Mix 2x [QIAGEN, Hilden, Germany], 0.3uL of the AMELY forward primer, 0.3uL of the AMELY reverse primer, and 2.9uL of PCR grade water. Samples that amplified at least two of three replicates were called as male. Samples that showed no amplification were called as female. Samples that had one replicate amplify were subject to second round of the sex identification assay. Two positive snow leopard controls of known sex and a negative control of PCR grade water with no DNA were included to screen for PCR failure and contamination.

### Data Analysis

The highest quality sample for each individual from Chang Tang - TAR, Qilian Shan - Qinghai, Qilian Shan - Gansu, and Yushu was further genotyped at 30 loci resulting in 34 loci in total (Table 1). The highest quality sample for each individual from Southwestern Mongolia was further genotyped at 27 loci resulting in 36 loci in total.

Protocols were identical to those for individual identification. Summary statistics of genetic metrics including allele frequencies, observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and relatedness using Grafen’s relatedness coefficient (Queller & Goodnight, 1989) were completed using GenAEx 6.503 (Peakall & Smouse, 2012). Grafen’s relatedness coefficient provides a measure representative of the proportion of alleles shared between individuals by common descent while also considering population frequencies of the alleles. Coefficients measure from -1 to 1, with values of 0 to 1 indicating more shared alleles than expected and -1 to 0 indicating fewer alleles than expected. Two randomly chosen individuals of no relation would be expected to have a value of 0, second degree relatives a value of 0.25, and first-degree relatives consisting of parent-offspring or full siblings a value of 0.5.

A chi-square goodness of fit test ( $\chi^2$ ) (Snedecor & Cochran, 1989) was used to determine if the expected ratio of males versus females in a population was significantly different than expected (1:1). Analyses were performed in SPSS statistics 26.0 (SPSS, Chicago, USA) with significance set  $p \leq 0.05$ .

Table 1. The 34 loci examined for the snow leopard population surveys.

Multiplex 1	PUN1157F	PUN229V	PUN132	PUN843P
Multiplex 2	PUN 935F	PUN124N	PUN272P*	PUN894V
Multiplex 3	PUN82F	PUN327V	PUN1262N	PUN225P
Multiplex 4	PUN100F	PUN8972V	PUN1262N (1283N)	PUN668P
Multiplex 5	PUN80F	PUN 894V	PUN1270N	PUN249P
Multiplex 6	PUN1030F	PUN9013D1V	PUN1275N	PUN272P
Multiplex 8	PUN1047F	PUN917V	PUN1289N	PUN347P
Multiplex 9	PUN1131F	PUN924V	PUN1293N	PUN44P
Multiplex 10	PUN1138F	PUN1307N	PUN664P	PUN928V

\* Locus not always included in Multiplex 2 due to it not being polymorphic across snow leopard range (i.e. the locus is polymorphic in Mongolia but not in China)

## Results

### Species, individual, and sex identification, and genetic diversity

#### *Chang Tang*

A total of 22 scat samples were collected. Of those, 19 (86.4%) were confirmed to be of snow leopard origin while three samples failed to amplify. Of the 19 confirmed snow leopard samples, 11 produced viable genotypes that could be used for individual identification (57.9% success rate; 42.1% failure rate). A total of seven unique individuals were identified with two individuals being capture twice and one individual being capture three times. The seven individuals comprised four males and three females ( $X^2(1, N = 7) = 0.143, p = 0.705$ ) (Table 2). Genotyping success rate of the additional 30 loci was 81.9% (18.1% failure), bringing the genotyping success rate across all 34 loci to 84.1% (15.9% failure).

The number of alleles was 5.848. Observed heterozygosity was 0.393 while expected heterozygosity was 0.440. The mean relatedness for all samples considered was -0.181 (Table 3). Four pairs of individuals had Grafen's coefficients greater than 0. Three pairs were considered second degree relatives and one pair of individuals was considered first degree.

#### *Qilian Shan, Qinghai*

A total of 110 samples were collected. Of those, 26 (23.6%) were confirmed to be of snow leopard origin while the remaining 66 failed to amplify. A total of 11 samples produced viable genotypes that agreed across triplicates while the remaining 15 failed to

do so (42.3% success rate; 57.7% failure rate). A total of ten unique individuals were identified, with one individual being captured twice. The ten individuals comprised three males and seven females ( $X^2(1, N = 10) = 1.600, p = 0.206$ ) (Table 2). Genotyping success rate at the additional 27 to 30 loci was 69.5% (30.5% failure). This brought the genotyping success rate across all samples to 73.5% (26.5% failure rate). Of note, six additional unique genetic profiles were determined for samples collected in May 2017, which would have brought the total number of individual snow leopards to 16. However, Bayesian clustering analysis revealed that ten of these samples were Pallas's cat (*Otocolobus manul*) and thus they were removed from further analysis (Figure 4).

The average number of alleles per locus was found to be 3.294. Observed heterozygosity was 0.350 while expected heterozygosity was 0.496. The mean relatedness for all samples considered was -0.142 (Table 3). A total of ten pairs had Grafen's coefficients greater than 0. Of these ten pairs, eight were considered lower than second degree relatives, one pair was considered second degree relatives, and one pair was considered first degree.

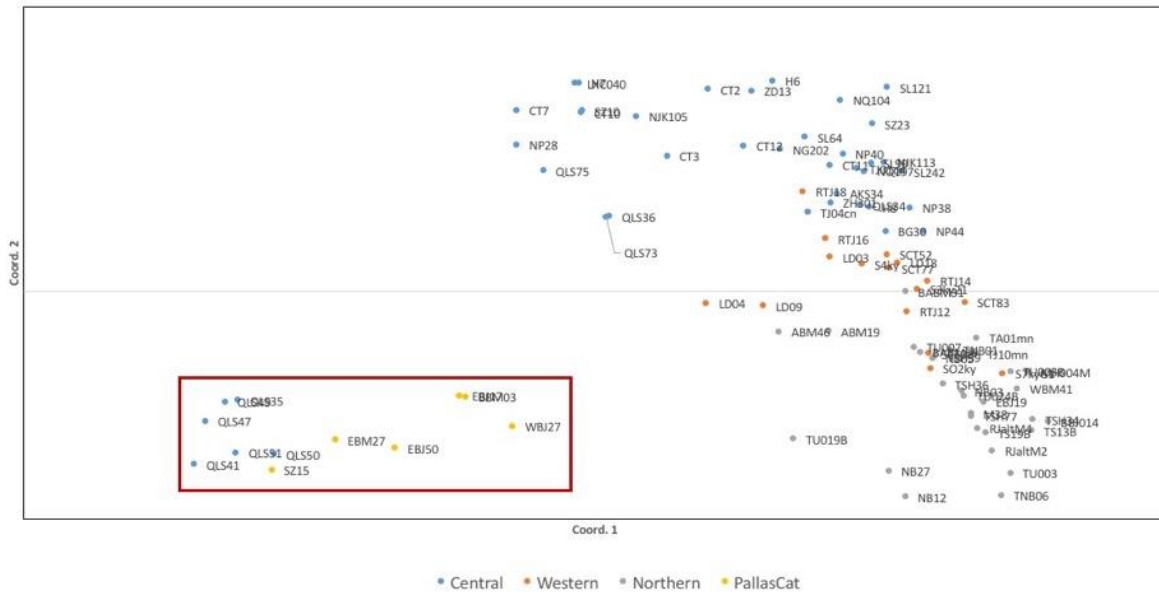


Figure 4. PCoA plot showing genetic relationships between snow leopards and Pallas’s cats from the Qilian Shan (QLS) dataset. The clustering of the six Qilian Shan (QLS) presumed snow leopard individuals clustering with known Pallas’ cat individuals indicate that the six QLS individuals are Pallas’ cat and not snow leopard.

#### *Qilian Shan, Gansu*

A total of 142 samples were collected. Of those, 54 (38%) were confirmed to be of snow leopard origin while 88 failed to amplify. A total of 28 produced viable consensus genotypes (51.9% success rate; 48.1% failure rate). In total, 20 unique individuals were identified with one individual captured four times, one individual capture three times, and two individuals capture twice. The 20 individuals comprised 13 males and 7 females ( $X^2(1, N = 20) = 1.800, p = 0.180$ ) (Table 2). Genotyping success at the additional 27 loci was 75.9% (24.1% failure rate), bringing the genotyping success rate across all samples to 80.3% (19.7% failure rate).

The average number of alleles per locus was 3.588. The number of effective alleles was 2.191. Observed heterozygosity was 0.278 while expected heterozygosity was 0.486. The mean relatedness considering all samples was -0.080 (Table 3). A total of 53 pairs of individuals had Grafen’s coefficients greater than 0. Of these, 29 pairs were

lower than second degree relatives, nine pairs were second degree relatives, and 15 were first parents/offspring or siblings.

#### *East Burhanbuda Mountain, Qinghai*

A total of 137 samples were collected. Of those, 22 (16.1%) were confirmed to be snow leopard origin while 115 failed to amplify. A total of nine produced viable consensus genotypes (40.9% success rate; 59.1% failure rate). A total of six individuals were identified with one individual being capture three times and one individual being capture twice. The six individuals comprised one male and five females ( $X^2(1, N = 6) = 2.6667, p = 0.102$ ) (Table 2). Genotyping success at the additional 27 loci was 78.3% (21.7% failure rate). This brought the genotyping success rate across all samples to 82.8% (17.2% failure rate).

The average number of alleles per locus was 2.824. Observed heterozygosity was 0.294 while expected heterozygosity was 0.451. The mean relatedness for all samples considered was -0.218 (Table 3). Three pairs of individuals had Grafen's coefficients greater than 0. They comprised two pairs that were lower than second degree relatives, and one pair that was considered second degree relatives.

#### *Yushu, Qinghai*

A total of 28 samples were collected in Suojia and 78 in Duocai, for a total of 106 samples. Of these, 22 (12 from Suojia, 10 from Zhiduo) were samples were confirmed to be of snow leopard origin, while the remaining 84 failed to amplify. A total of 19 produced viable consensus genotypes (86.4% success rate; 13.6% failure rate). A total of

15 unique individuals were captured, with two individuals captured three times, and the remaining individuals being captured once. The 15 individuals comprised a significantly different ratio of sexes with 13 females and two males ( $X^2(1, N = 15) = 8.067, p = 0.005$ ) (Table 2). Genotyping success at the additional 27 loci was 88.6% (11.4% failure rate). This brought the genotyping success rate across all samples to 91.9% (8.1% failure rate).

Of the 28 samples from Suojia in which herders were asked to identify the host predator species that deposited the scat, four samples were stated to be brown bear, five samples were stated to be wolf, 16 were said to be snow leopard, and three were of carnivore origin but undiscernible. All 12 samples that were identified as snow leopard via PCR were all identified as snow leopard by the herders. Five samples identified as snow leopard by herders failed to amplify during the species identification assay.

The average number of alleles per locus was 3.441. Observed heterozygosity was 0.303 while expected heterozygosity was 0.495. The mean relatedness for all samples considered was -0.082 (Table 3). A total of 25 pairs of individuals had Grafen's coefficients greater than 0. In total, 18 pairs were considered lower than second degree relatives, four pairs were considered second degree relatives, and three pairs were first degree relatives.

### *Southwestern Mongolia*

Collection efforts resulted in 190 scat samples. Of those, 47 (24.7%) were determined to be of snow leopard origin while 143 failed to amplify. Of those, 30 produced viable genotypes (63.8% success rate, 36.2% failure rate) resulting in the

determination of 21 unique individuals comprised of 12 males and nine females ( $X^2 (1, N = 21) = 0.429, p = 0.513$ ). Two individuals were captured two times, one individual was captured four times, and one individual was captured five times (Table 2). Genotyping success rate across the additional 27 loci was 70% (30% failure), bringing the genotyping success rate for all samples to 66.9% (33.1% failure).

The average number of alleles per locus was 4.176. Observed heterozygosity was 0.374 while expected heterozygosity was 0.533. The mean relatedness when all samples were considered was -0.098 (Table 3). A total of 71 individual pairs had Grafen's coefficients above 0. In total, 51 pairs were considered lower than second degree relatives, 15 were considered secondary relatives, and five were considered first degree relatives.



Table 2. Snow leopard individuals detected across study sites in China and Mongolia.

Snow Leopard	No. of Detections	Sample ID	Sex ID
<i>Snow leopards detected in Chang Tang Reserve, Tibet Autonomous Region</i>			
Individual 1	1	CT2	M
Individual 2	3	CT10, CT15, CT18	M
Individual 3	2	CT3, CT6	F
Individual 4	1	CT7	F
Individual 5	2	CT11, CT17	M
Individual 6	1	CT12	M
Individual 7	1	LXC040	F
<i>Snow leopards detected in Qilian Shan, Qinghai May 2017</i>			
Individual 1	1	QLS34	M
Individual 2	1	QLS36	M
Individual 3	1	QLS73	F
Individual 4	1	QLS75	F
Individual 5	1	QS37	M
Individual 6	1	QS08	F
Individual 7	1	QS31	F
Individual 8	1	QS16	F
Individual 9	2	QS17, QS06	F
Individual 10	1	QS20	F
<i>Snow leopards detected in Qilian Shan, Gansu July 2018</i>			
Individual 1	1	SBC61	F
Individual 2	4	SBC54, SBC57, SBC59, SBC67	F
Individual 3	1	SBC74	M
Individual 4	1	SBC30	M
Individual 5	1	SBC75	F
Individual 6	1	SBC83	F
Individual 7	1	SBC66	F
Individual 8	1	SBC42	F
Individual 9	3	SBC55, SBC58, SBC70	M
Individual 10	1	SBC29	F
Individual 11	2	SBC63	F
Individual 12	1	SBC27, SBC28	F
Individual 13	1	SBC08	M
Individual 14	1	SBC90	F

Individual 15	1	SBC68	M
Individual 16	1	SBC11	F
Individual 17	1	SBC31	F
Individual 18	2	SBC07, SBC45	M
Individual 19	1	SBC64	M
Individual 20	1	SBC26	F

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*Snow leopards detected in Yushu – 2016/July 2018*

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Individual 1	3	SUJ12, SUJ17, SUJ22	M
Individual 2	1	SUJ20	F
Individual 3	1	SUJ11	F
Individual 4	1	SUJ26	F
Individual 5	1	SUJ15	M
Individual 6	1	SUJ27	F
Individual 7	1	SUJ28	F
Individual 8	1	SUJ24	F
Individual 9	3	SUJ25, SUJ13, SUJ23	F
Individual 10	1	YUS01	F
Individual 11	1	YUS34	F
Individual 12	1	YUS18	F
Individual 13	1	YUS04	F
Individual 14	1	YUS02	F
Individual 15	1	YUS13	F

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*Snow leopards detected in Southwestern Mongolia August 2015*

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Individual 1	1	MnMJ128	M
Individual 2	1	MnMJ150	M
Individual 3	1	MnMJ157	F
Individual 4	1	MnMJ158	F
Individual 5	1	MnMJ186	M
Individual 6	1	MnMJ182	M
Individual 7	1	MnMJ13	M
Individual 8	1	MnMJ14	F
Individual 9	1	MnMJ15	F
Individual 10	2	MnMJ174, MnMJ130	M
Individual 11	1	MnMJ156	F
Individual 12	1	MnMJ140	M
Individual 13	1	MnMJ163	M

Individual 14	5	MnMJ112, MnMJ96, MnMJ94, MnMJ157, MnMJ158	F
Individual 15	4	MnMJ95, MnMJ92, MnMJ91, MnMJ114	M
Individual 16	1	MnMJ113	M
Individual 17	1	MnMJ22	F
Individual 18	1	MnMJ137	F
Individual 19	1	MnMJ184	M
Individual 20	1	MnMJ187	F
Individual 21	2	MnMJ120, MnMJ125	M

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Table 3. Genetic diversity values for local snow leopard populations examined. N is the number of total samples collected while n is the number of snow leopard positive samples.

	Transect Length and Approx. Area	Abundance Sex Ratio Density	No. of Alleles ( $A_N$ )	No. of Effective Alleles ( $N_E$ )	Observed Heterozygosity ( $H_O$ )	Expected Heterozygosity ( $H_E$ )	Fixation Index (F)	Mean Relatedness (Queller & Goodnight 1989)
Chang Tang (N=22, n=19)	415km <sup>2*</sup>	7 (4M, 3F) 0.59/100km <sup>2</sup>	5.848	2.697	0.393	0.440	0.085	-0.181
Qilian Shan, Qinghai (N=110, n=26)	1,500km <sup>2*</sup>	10 (3M, 7F) 0.67/100km <sup>2</sup>	3.294	2.464	0.350	0.496	0.303	-0.142
Qilian Shan, Gansu (N=142, n=54)	15km 541km <sup>2</sup>	20 (13M, 7F) 3.7/100km <sup>2</sup>	3.588	2.191	0.278	0.486	0.381	-0.080
East Burhanbuda Mountain (N=137, n=22)	20km 521km <sup>2</sup>	6 (1M, 5F) 0.86/100km <sup>2</sup>	2.824	2.073	0.294	0.451	0.336	-0.218
Yushu (N=106, n=22)	13km <sup>+</sup> 200km <sup>2+</sup>	15 (2M, 13F) 7.5/100km <sup>2</sup>	3.441	2.331	0.303	0.495	0.386	-0.082
Southwestern Mongolia (N=190, n=47)	324km <sup>2*</sup>	21 (12M, 9F) 6.5/100km <sup>2</sup>	4.176	2.512	0.374	0.533	0.323	-0.098

\* Data reflective of total transect length unavailable.

+ Does not include total transect length and area of first sampling session in Yushu.

## Discussion

### Number of snow leopard positive samples versus number of scats collected

Population genetics is at the forefront of making management decisions for threatened wildlife (O'Brien, 1994). The snow leopard is a species with an expansive distribution that requires localized population assessments for conservation planning and protection. Of the six populations examined, the Chang Tang region had the greatest percentage of snow leopard positive samples relative to the total number collected at 86.4%. All others were considerably lower, with 16.1% in East Burhanbuda Mountain, 20.8% in Yushu, 23.6% in Qilian Shan, Qinghai, 24.7% in Southwestern Mongolia, and 38.0% in Qilian Shan, Gansu. Previous noninvasive genetics work found snow leopards to comprise 68% of carnivore scats collected in Ladakh, India, 8.3% in Dulan County, Qinghai, China, 45.8% in South Gobi, Mongolia (Janečka et al. 2008), 41% in South Gobi, Mongolia (Janečka, Munkhtsog, et al., 2011), 66% in Pakistan (Aruge et al. 2019), and 27% in Nepal (Karmacharya et al. 2011). A number of other sympatric carnivores may co-exist with snow leopards depending on region (Schaller et al. 1988). Collecting scat that appears to be of carnivore origin thereby casts a wide-net of possible host species that dilute the number of snow leopard positive samples. The number of snow leopard positive samples may be indicative of their abundance in the environment, though the results in this study do not suggest so. It is more likely that sampling scheme, collector bias, or lab protocols greater impact the percentage of snow leopard positive scats in a dataset. For example, researchers collecting scats in the Chang Tang region for this study were specifically targeting samples they believed to be of snow leopard origin.

The samples in Qilian Shan, Gansu were collected on routes where camera traps had previously shown snow leopards to be present by another research group. In addition, the use of a species specific PCR identification assay versus the amplification and subsequent sequencing of cytochrome *b* (Aruge et al., 2019; Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011), may further hinder the legitimacy of comparisons to other studies.

### Snow leopard abundance

The greatest number of snow leopard individuals was identified in southwestern Mongolia at 21 while the lowest was in East Burhanbuda Mountain at six. Given Mongolia's decades of conservation efforts for the species, it is not surprising that numbers would reflect larger populations. Similarly, the Sanjiangyuan region has benefited from swift conservation action since the beginning of the 21<sup>st</sup> century, likely aiding in the perseverance of snow leopards in Yushu with the capture of 15 unique individuals in this study. Furthermore, the Sanjiangyuan region is known for its continuous snow leopard habitat, which connects individuals across a large portion of their distribution in China (Y. Liu et al., 2016). This is in contrast to other areas of the Qinghai-Tibetan Plateau that are more isolated, such as East Burhanbuda Mountain.

The number of snow leopards captured on the Qinghai Province side of the Qilian Shan range was half the number of those captured on the Gansu Province side at 10 versus 20 unique genetic profiles, respectively. Despite the QNNR being a long-term established protected area dating back to 1988, it remained plagued with environmental issues and corruption up through the 2010s. Snow leopards were largely absent from the

area in the mid-1990s with the exception of a small population in Gansu's Subei County (D. O. Hunter & Jackson, 1995). The larger numbers observed in Gansu likely reflect an established snow leopard population, while the smaller numbers observed in Qilian Shan's Qinghai Province may represent a newer snow leopard population that has only recently been able to migrate westward due to an increase in suitable habitat with better environmental protection laws and enforcement. Snow leopards on the Gansu side of the range may also have benefited from longer-term research efforts. However, the Chang Tang region lacks almost any anthropogenic disturbance and only seven individuals were identified.

The added perspective of density can much better detail to the true status of snow leopard populations. Unfortunately, there are a number of difficulties in estimating density with noninvasive scat surveys. Density can be derived from population estimates by calculating the area of buffered transects (Janečka, Munkhtsog, et al., 2011). Due to the multiple data sources of the populations examined in this study, a standardized density estimate cannot be formulated. However, crude estimates of area interpreted with caution can help to elucidate the scale at which relative densities vary between the six populations. The number of individuals per 100km<sup>2</sup> was highest in Yushu at 7.5 snow leopards and lowest in the Chang Tang region at 0.59 snow leopards. Values in between consisted of Qilian Shan, Qinghai (0.67/100km<sup>2</sup>), East Burhanbuda Mountain (0.86/100km<sup>2</sup>), Qilian Shan, Gansu (3.7/100km<sup>2</sup>), and Southwestern Mongolia (6.5/100km<sup>2</sup>). Previous density estimates in Yushu by Schaller et al. 1988 were 0.3 to 1 individual per 100km<sup>2</sup>. Our estimate of 7.5 was much larger. Similarly, although the density estimates in this study for the Chang Tang region were low, at only 0.59 snow

leopards per 100km<sup>2</sup>, they were much higher than the previously reported value of 0.024 snow leopards per 100km<sup>2</sup> (J.D. Farrington & Tsering, 2019). The density estimate for East Burhanbuda Mountain was only slightly higher in this study than a value of 0.37 derived from previous work (G. Wu, 2009) while the density estimate for Qilian Shan, Gansu was similar to one previous study (Alexander, Gopaldaswamy, et al., 2015), but higher when compared to another (Alexander, Zhang, et al., 2016a). Comparison of density for Southwestern Mongolia to previous studies is difficult, but densities of 0.7 to 1.4 have been previously published (Janečka, Munkhtsog, et al., 2011; McCarthy et al., 2016), values which are much smaller than the 6.5 individual per 100km<sup>2</sup> reported in this study. There is no previously published literature on the density of snow leopards in Qilian Shan on the Qinghai Province side of the range. The values herein are a very rough estimate, and only serve as a proxy metric for snow leopard populations in the six areas examined. Previous range-wide assessments have shown that smaller, more intensively studied areas typically reveal higher density estimates than larger study areas (Fox & Chundawat, 2016). Ideally, estimates from Yushu, Southwestern Mongolia, the Chang Tang region, and East Burhanbuda Mountain are reflective of increasing snow leopard populations, which would be intuitive for Yushu and East Burhanbuda Mountain given the establishment of nature reserves in the area. However, methodological differences and introduced biases in density estimates can greatly impact data interpretation (Janečka, Munkhtsog, et al., 2011; Kachel, 2014). The majority of previous studies have relied on sign surveys (Hussain, 2003; Schaller, Junrang, et al., 1988; G. Wu, 2009; A. Xu et al., 2008) or camera trapping (Alexander et al., 2016, 2016; Buzzard



et al., 2017; Jackson et al., 2006; Janečka, Munkhtsog, et al., 2011; Kachel, 2014; Rovero et al., 2020), making comparisons to genetic studies difficult.

#### Potential impacts of PCR failure and genotyping errors

Amplification failure during PCR, genotyping errors, and differences in success rates may have impacted the number of snow leopard positive samples, the total number of individuals detected, sex ratio, and density. Typically, less than 80% of samples will have DNA that is suitable enough for species identification, and fewer than 70% of those will be suitable for individual identification (Rodgers & Janečka, 2013). Samples which do not amplify during the snow leopard specific identification assay could either truly not be of snow leopard origin, or may have been snow leopard samples that failed to amplify. Similarly, snow leopard positive samples that did not amplify during the sex identification assay could have either been females or male samples that failed to amplify. In this study, the success of individual identification ranged from 40.9 to 86.4%, with an average of 57.2%. Scat is a notoriously difficult medium to work with in noninvasive genetic studies (Broquet & Petit, 2004). DNA yield and quality is often low due to degradation from passing through the digestive tract and exposure to outside elements (Janečka, Munkhtsog, et al., 2011). Suboptimal collection and storage of scat samples can lead to further degradation. In this study, all samples were treated uniformly using protocols from Janecka et al. (2008) with the exception of those from the Chang Tang region. These were stored in 95% ethanol as opposed to silica desiccant. The genotyping success rate was highest for samples from the Chang Tang region, which may suggest that ethanol is a superior preservative. However, ethanol is more difficult to

transport and may be subject to travel restrictions. In this study, the ethanol leaked from the sample tubes and removed all writing in permanent marker, eliminating all identifying information from each collected scat with the exception of one sample. Regardless of these associated challenges, it remains that information for snow leopard populations is desperately lacking, and thus information collected in a rigorous and transparent manner is helpful to conservation practitioners, particularly since what little current research exists indicates that snow leopards have low levels of genetic diversity, a finding echoed in this work.

#### Comparison of genetic diversity metrics

Janecka et al. (2017) sampled 70 individuals across species range and reported low values of genetic diversity, similar to those found in this study ( $A_N = 5.8$ ,  $H_O = 0.433$ ,  $H_E = 0.568$ ). Karmacharya et al. (2011) examined ten snow leopard positive samples across six microsatellites in Nepal and reported the higher levels of genetic diversity ( $H_O = 0.579$ ,  $H_E = 0.631$ ). Caragiulo et al. (unpublished) used DNA from the blood of five snow leopards anesthetized for GPS collaring in the Tost Mountains of southern Mongolia across 12 microsatellite loci and reported low genetic diversity ( $A_N = 2.92$ ,  $H_O = 0.536$ ,  $H_E = 0.544$ ). This pattern was also observed in SNP analysis using the same samples genotyped by Caragiulo et al. ( $A_N = 1.12$ ,  $H_O = 0.047$ ,  $H_E = 0.042$ , Montgelgard et al. 2014).

Metrics obtained for genetic diversity of the six populations in this study were highest in the Chang Tang region despite having only seven individuals as a source of data. Interestingly, its average number of alleles per locus was the same as Janecka et al.

(2017), which used data representative of the species range-wide from 70 individuals. The sex ratio for the Chang Tang region was also the most even, with a 4:3 split of males versus females. Higher levels of genetic diversity observed in the Chang Tang region may be due to increased gene flow across the landscape coupled with low anthropogenic disturbances. Continued work in the Chang Tang region is much needed, as diversity estimates found in this study suggest that snow leopards in the region may possess a greater adaptability to environmental changes. This is paramount, as climate change will undoubtedly cause drastic changes in snow leopard habitat (J. Li, McCarthy, et al., 2016). The snow leopard metapopulation range-wide may become dependent on a few isolated populations to carry them through the negative outcomes associated with their quickly changing landscapes. Unfortunately, the Chang Tang region of the Tibetan Plateau is anticipated to become unsuitable habitat by 2070 (J. Li, McCarthy, et al., 2016). Thus, some form of adaptation or migration will likely have to occur to ensure persistence.

In contrast, snow leopard populations in the Qilian Shan range lie in an area of climate refugia for the species under various climate change scenarios (J. Li, McCarthy, et al., 2016). Overall, the number of individuals was double on the Gansu Province side of the range than on the Qinghai Province side at 20 versus ten unique genetic profiles detected, respectively. However, diversity metrics on either side were comparable. South of the Qilian Shan range, the snow leopard population in East Burhanbuda Mountain had some of the lowest metrics for genetic diversity. However, the success of individual identification was also lowest in East Burhanbuda Mountain, which may have biased results in the form of lost information and possible identification of unique individuals.

#### Unexpected amplification of non-target species DNA

The amplification of Pallas's cat DNA using snow leopard specific identification primers speaks to the potential for species specific primers to attach to binding sites of non-target species. The snow leopard species identification primers designed by Janecka et al. (2014) used snow leopards scat samples from Mongolia. However, snow leopard distribution is large, and nucleotide substitutions in cytochrome *b* sequences may overlap with other felids in different regions of Asia. This was the only occurrence of such observed false positives. Despite its low likelihood, researchers should be cognizant that unusual patterns observed in genetic datasets may be a result of species misidentification.

#### Conclusions and future directions

As of 2020, less than 3% of snow leopard habitat has been rigorously surveyed (R. K. Sharma & Singh, 2020). The need for studies surrounding local population estimates of the species is dire. The analysis of six populations herein adds substantially to the current coverage of rigorously surveyed habitat and helps to elucidate the current status of snow leopard populations across the Qinghai-Tibetan Plateau and in Southwestern Mongolia, providing information for targeted action for the species. Future work should focus on further exploring snow leopard populations in the far western and northwestern reaches of China, beyond the Chang Tang region examined in this study. It would also be of interest to reassess areas of studied snow leopard range using next-generation sequencing (NGS) technologies to investigate genetic diversity metrics revealed through the analysis of single nucleotide polymorphisms (Janjua et al. 2019; Janecka et al. 2020).

## Author Attributions

Charlotte Hacker, Yuguang Zhang, Yu Zhang, Xue Yadong, Bayaraa Munkhtsog, Matthew Jevit, Liji Wu, Xieranima, Xuchang Liang, and Xiaoxing Bian collected scat samples. Charlotte Hacker, Yu Zhang, Yunrui Ji, Benita Jebeckumar, and Cong Wei extracted DNA from scat samples. Charlotte Hacker genotyped samples. Charlotte Hacker and Jan Janecka performed data analyses. Charlotte Hacker and Jan Janecka interpreted data. Yuguang Zhang translated results and interpretations into Mandarin Chinese for publication.

## Chapter 2.2

### Goal 1 – Subgoal 2

### Range-wide phylogeography and taxonomy resolution

Adapted from Janecka *et al.* (Hacker CE) (2017) *Range-wide snow leopard phylogeography supports three subspecies*. Journal of Heredity. 108:597-607.

#### Introduction

#### The evolution of the Panthera genus

All extant cat species can be traced back to several ancestral cat species, collectively called *Pseudaelurus*, that resided in South East Asia 11 million years ago during the late Miocene (Johnson et al., 2006; O'Brien & Johnson, 2007). In total, eight lineages have diverged from this ancestor, including bay cat, caracal, ocelot, lynx, puma, Asian leopard cat, domestic cat, and panthera (O'Brien & Johnson, 2007). The panthera lineage was the first to diverge from its ancestral species approximately 10.8 million years ago, and then split into two groups approximately 4.8 million years later (Hemmer, 1979; O'Brien & Johnson, 2007). The first group consisted of the clouded leopards and the second consisted of those species now known in the genus *Panthera* (Buckley-Beason et al., 2006; Johnson et al., 2006).

Species of the panthera lineage experienced rapid movement and radiation during the late Pliocene 3 to 4 million years ago, though divergence times are difficult to assess due to introgression and heterogeneous gene histories (O'Brien & Johnson, 2007). Previous work supports two distinct clades – one consisting of the lions, leopards, and jaguars and the other consisting of the snow leopard and tiger (B. W. Davis et al., 2010; Johnson et al., 1996). Relationships between the lion, leopard, and jaguar have been difficult to resolve, though studies indicate that the leopard and lion are the closest related

of the trio (Barnett et al., 2009; B. W. Davis et al., 2010; Johnson et al., 1996). The jaguar most likely split from the group at the very end of the Pliocene, while the leopard and lion split later during the Pleistocene (B. W. Davis et al., 2010). On the opposing end, the relationship between the snow leopard and tiger is more clearly defined, with snow leopards having diverged from tigers between 2.7 to 3.7 million (B. W. Davis et al., 2010) or 4.86 to 5.13 million years ago during the Pliocene (Tseng et al., 2013).

The Pleistocene was a time period met with mass extinctions whereby species of the panthera lineage were locally extirpated in some areas and confined in others. For example, what are now modern day jaguars and lions were eliminated from North America, but were able to thrive in South America and Africa, respectively (O'Brien & Johnson, 2007). Many species only narrowly escaped extinction. For example, the modern day tiger was almost eliminated 73,000 years ago due to volcanic eruptions in Borneo (O'Brien & Johnson, 2007). Biogeographic events such as these have had large-scale impacts on species in the genus *Panthera*, particularly in the arena of species wide genetic structure and diversity, both of which play large roles in species taxonomy and thus conservation management decisions.

#### The importance of phylogeography to taxonomy

Taxonomy and conservation are two interconnected concepts. In order to conserve wildlife, it must first be described and categorized (Mace, 2004). Species are the natural taxonomic rank for conservation assessments and management given that gene flow is largely restricted within species (Hey et al., 2003; Mace, 2004). Although the “species” problem poses surmounting challenges for biologist and systematists, it is

widely accepted that species have special properties that set them apart from the taxa of other ranks (Hey et al., 2003). Within species are what were first called “varieties”, now known to be subspecies (Mayr, 1982). Prior to the advent of molecular genetics, species and subspecies designations were based on an organism’s geographic origin and morphological characteristics such as pelage and body size (Kitchener & Dugmore, 2000). However, morphological diagnostic characters can be unreliable (Driscoll et al., 2009). In addition, designations were often made based on one individual determined to have unique features from their previously described counterparts (Kitchener & Dugmore, 2000). In the early use of the term “subspecies”, researchers often applied it as if it were a well-defined entity for any distinct natural population that was not considered sufficiently different to be considered a separate species (Mayr, 1982). These determinations have led to wide taxonomic debate across the animal kingdom (Kitchener, 1999). One necessary measure to resolve taxonomy is the study of range-wide species phylogeography (Jan E. Janecka et al., 2020).

Effective conservation action plans are dependent on a reliable understanding of a species’ population structure and history, the presence of demographic partitions throughout its range, and characterization on the distribution of its genetic diversity (Avice, 1989; O’Brien, 1994). For example, environmental shifts that caused differences in vegetation and habitat types in Africa spurred the genetic divergence observed in modern lions (Bertola et al., 2016). Lack of genetic diversity in Caspian tigers (*Panthera tigris virgata*) was driven by demographic expansion through narrow corridors that restricted gene flow, making the species susceptible to its anthropogenic-caused collapse in the early 20<sup>th</sup> century (Driscoll et al., 2009).



The importance of molecular data in making taxonomic decisions cannot be understated, as it allows conservationists to identify populations of the greatest value that warrant protection, and thus where to allocate funding and efforts to conserve endangered populations. This is vital for species in the order Carnivora, which are the least genetically diverse clade among mammals (Azizan & Paradis, 2021). The situation is particularly dire for members of the genus *Panthera*, all of which possess some form of IUCN protected status and are globally in decline.

#### Previous phylogeography studies of *Panthera* species

Previous phylogeography studies of member species in the *Panthera* genus used microsatellites and mtDNA as investigated genetic markers (Bertola et al., 2016; Driscoll et al., 2009; Eizirik et al., 2001; Luo et al., 2004; Uphyrkina et al., 2001). These markers can be extremely helpful in determining population genetic metrics for a species. For example, mtDNA has many copies of maternally inherited circular DNA that evolves rapidly and does not undergo recombination (Barr et al., 2005; Hebert et al., 2004). Nuclear DNA possess microsatellites, also called short-tandem repeats (STRs), that are neutral, non-coding, and non-adaptive loci (Jarne & Lagoda, 1996). When used both separately and in tandem, mtDNA and microsatellites have allowed for the examination of phylogeography for numerous species all with conservation action application.

A study on the population history and genetic diversity of the jaguar (*Panthera onca*) found low to moderate levels of mtDNA diversity and medium to high levels of microsatellite diversity with no strong geographical genetic structure observed (Eizirik et al., 2001). An analysis of the phylogeographic patterns and molecular genetic diversity in

modern day leopards (*Panthera pardus*) found a high level of genetic diversity and support for a minimum of nine subspecies (Uphyrkina et al., 2001). A study on the genetic ancestry of tigers (*Panthera tigris*) found relatively low genetic diversity, but suggested that six subspecies of tiger be recognized based on population structure analyses (Luo et al., 2004). Investigation of lion (*Panthera leo*) phylogeography revealed that modern day lions were one of three distinct clades which also included the extinct American lion and Pleistocene cave lions (Barnett et al., 2009). Another study suggested splitting African lions into a northern and southern subspecies (Bertola et al., 2016). The snow leopard was the last species from the *Panthera* genus to have a comprehensive analysis on its phylogeography, population structure, and taxonomy completed (Jan E. Janecka et al., 2017).

#### Impacts of historic biogeographic events in Central Asia

Since the Pleistocene, Central Asia has experienced various climatic changes and differences in uplift (Joshi et al., 2020). Such processes can lead to distinct paleo-ecological niches. Sitting centrally in snow leopard range is the Tibetan Plateau, a 2.5 million km<sup>2</sup> immense massif known as the “roof of the world” (Yang et al., 2009; B. Zhang et al., 2002). It’s relatively recent and rapid uplifting after the India-Eurasian collision has led to a high variety of ecosystems and ecological processes, making it a biodiversity hotspot (B. Li, 1994; Yang et al., 2009; B. Zhang et al., 2002). All organisms found therein can be traced to the Early Pliocene to the Late Miocene with rapid speciation continuing during the Pleistocene due to periodic glacial cycles and geographic isolation (Yang et al., 2009). These rapid changes also led to massive

extinction events, whose negative impacts on genetic fitness and diversity remain evident for Felidae species in non-tropic regions of Asia (Azizan & Paradis, 2021).

### Potential impacts of current environmental changes

Genetic analyses of species both historic and current can hold key insights into how megafaunal carnivore populations may respond to environmental changes both in the short and long term (Azizan and Paradis 2021, Barnett et al. 2009). The mountainous regions of Central Asia are experiencing large-scale shifts due to climate change (M. Zhang et al., 2019). Humans have spurred land use changes that, in conjunction with climate change, serve as one of the largest threats to biodiversity (Pimm & Raven, 2000). Indeed, most predictive models indicate losses of biodiversity that would qualify the present time period as the sixth mass extinction (Bellard et al., 2012). However, some researchers have suggested that climate change impacts on species will not be as severe as anticipated (Hof et al., 2011). Previous wide-spread and rapid climate changes 2.5 million years ago and onward did not cause the noticeable level of species extinctions that present-day scientists typically associate with the Anthropocene (Hof et al., 2011). The notion that large mammals were the primarily impacted group of previous climate change events (Koch & Barnosky, 2006) may in fact be due to a biased fossil record (Hof et al., 2011). Many species survived the glacial cycles that occurred during the Pleistocene, indicating that other strategies allow species to keep pace with rapidly changing climate (Hof et al., 2011). For example, intraspecific variation in physiological, phenological, behavioral, and morphological traits as well as phenotypic plasticity may allow species to avoid microevolutionary processes all together (Hof et al., 2011).

However, this would likely require a large degree of genetic diversity to allow individuals or populations capable of surviving in novel environments caused by rapid climate changes to reproduce. Thus, knowledge of current range-wide species diversity as well as an understanding of the impacts of previous mass biogeographic alternations may provide critical insight into how snow leopards may adapt to climate change. Unfortunately, a number of previous challenges have made this a difficult endeavor.

#### Difficulties in studying snow leopard phylogeography

Snow leopards occupy harsh inhospitable habitats that are often inaccessible to humans in regions of the world that can be politically unstable, making the obtainment of DNA from wild individuals across species range challenging (Jan E. Janecka et al., 2020). Studies examining the phylogeography of other large felids, such as the African lion and Caspian tiger have benefited from the availability of zoological animals with known pedigrees or museum specimens, both current and ancient, of decisive ancestry (Bertola et al., 2016; Driscoll et al., 2009; Luo et al., 2004). These resources for snow leopards are lacking as the species was never popular to hunt for sport, origins of individuals constituting the founder population in zoological facilities are largely unknown, and paleontological records are nearly non-existent (Jan E. Janecka et al., 2020; McCarthy et al., 2016). Misidentification of the species with common leopard (*Panthera pardus*) and Eurasian lynx (*Lynx lynx*) prior to modern records was also high and those historical accounts of the species and their range cannot be trusted (Kitchener et al., 2016; McCarthy et al., 2016).

#### Previous snow leopard subspecies designations

Differences between snow leopard populations based on notable gaps in species distribution, particularly between the southern and northern portions of snow leopard range, have previously been noted (Fox, 1994). Previous attempts at distinguishing subspecies have largely been based only on coat color differences and, in some instances, with one observed individual. One subspecies was proposed for an individual snow leopard spotted in north eastern India (Zukowsky, 1950). Two subspecies comprising the central Asiatic snow leopard (*Panthera uncia uncia*) and the snow leopard of Tibet and northwestern China (*Panthera uncia uncioides*) were proposed (Stroganov, 1962), and the Transbaikal snow leopard subspecies (*Panthera uncia baikalensis-romanii*) was proposed (Medvedev, 2000).

#### Synopsis of the first published snow leopard phylogeography study

Janecka et al. (2017) examined the phylogenetic relationships among current snow leopard populations throughout their geographic range. The study examined the genetic information from 33 microsatellites and 683-bp of mtDNA for 70 snow leopard individuals across 21 localities within species range. Metrics of genetic diversity showed that overall variation was low, but results surrounding population structure using nuclear DNA provided strong molecular and geographic evidence for the recognition of three genetic clusters that may constitute separate subspecies, a Northern (*Panthera uncia irbis*), Central (*P. u. uncioides*), and Western (*P. u. uncia*). These proposed subspecies corresponded with modern geography and Pleistocene geological events, as suggested in phylogeographic studies on other carnivore species found in the mountains of Central Asia (D. K. Sharma et al., 2004).

### Examples of past calls for taxonomic revision based on molecular evidence

Subspecies ambiguity is common in widely distributed species with large home ranges. Results from previous work examining the range-wide genetic structure of species have found conflicting data with current taxonomic standings and have called for revision. Jaguars had eight recognized subspecies at the time of the study completed by Eizirk et al. (2001) (Seymour, 1989). However, Eizeirk et al. showed that jaguars were monotypic, lacking any evidence of subspecies or Evolutionary Significant Units. The authors called for broad, range-wide conservation initiatives and taxonomic revision. This request was granted in the 2017 Felidae taxonomic revision (Kitchener et al., 2017). As another example, the modern common leopard once had 27 subspecies described which were later re-classified into eight (Miththapala et al., 1996). Other studies for species that share habitat with snow leopards have called for even higher recognition beyond subspecies. For example, one study found that the Himalayan (Tibetan) wolf (*Canis lupus*) warranted its own species status separate from the grey wolf (Werhahn et al., 2020). Given the molecular evidence and congruency of genetic differences with geographic features, the call for subspecies status by Janecka et al. (2017) was not unwarranted. However, some facets of the study warranted further investigation.

### Dataset improvement to the Janecka et al. (2017) phylogeography study

Although the most complete range-wide sampling effort to date, spatial gaps in genetic information in several areas of snow leopard range made boundaries between genetic clusters unclear. This was particularly evident in snow leopard habitat between the Western and Central genetic clusters, inciting caution in the interpretation of the

results (Senn et al., 2018). Geography of the landscape between the Western and Central genetic clusters further added complexity in the midst of missing genetic information, as the Qinghai-Tibetan Plateau is regarded as an open landscape that then meets the Pamir Knot to the west, a junction point with some of the world's highest mountains. A better understanding of where the boundary between the proposed Central and Western subspecies lies, as well as the upholding of the integrity of the molecular evidence for the three genetic clusters with the addition of more samples into the phylogeographic dataset, would provide greater confidence in applying subspecies status to snow leopards. The application of such would have momentous outcomes for snow leopard conservation.

#### The IUCN downlisting of snow leopards

The ICUN Red List of Threatened Species is the globally accepted standard framework for assessing species status and extinction risk (Mallon & Jackson, 2017). Regular assessments are completed to make decisions based on the most recent information available. For the snow leopard, recent population estimates generated from a 2008 range-wide conference held in Beijing, China suggested that numbers were much higher than previously thought (McCarthy et al., 2017). This subsequently led to downlisting the snow leopard from Endangered to Vulnerable in 2017 (McCarthy et al., 2017). This decision was not met without resistance, and was contentious among researchers and in-country biologists (Ale & Mishra, 2018; Mallon & Jackson, 2017). The removal of an Endangered status reduces funding from donors, government programs, and non-profits (Mallon & Jackson, 2017), and diminishes conservation efforts focused on endangered species. Experts supportive of the delisting pointed to inadequate

evidence of a declining meta-population and recent surveys revealing higher numbers (McCarthy et al., 2017). However, population estimates were extrapolated from data obtained in only few areas, while status and threats to snow leopard populations are known to vary regionally (Mallon & Jackson, 2017). For example, snow leopards in Mongolia are generally accepted to be thriving, in large part due to the early efforts for snow leopard conservation in the 1970s (Munkhtsog et al., 2016). However, the snow leopard in countries like Kazakhstan is one of their most endangered animals (Maheshwari & Niraj, 2016). Further still, some areas of snow leopard habitat are simply data deficient. For example, snow leopards in Afghanistan have likely been greatly impacted by civil war, and the current status is little known (Maheshwari & Niraj, 2016). Even vast areas of snow leopard habitat in Tibet remain largely untouched due to political sensitivities (Riordan & Shi, 2016).

The IUCN recognizes subspecies as individual units of protection. Therefore, recognizing snow leopard subspecies would allow for funding and efforts towards protection of regionally declining populations. Generating further evidence for listing declining subspecies would have positive impacts for snow leopards, their ecosystems, and local human communities. Because snow leopards are an apex predator, their protection benefits all area wildlife (Alexander et al., 2016). Snow leopard conservation efforts are designed to produce jobs (Y. Liu et al., 2016), offer alternative income sources (Charudutt Mishra et al., 2003), implement livestock insurance (Hussain, 2000), and promote conservation education (Jackson & Wangchuk, 2001; Y. Liu et al., 2016). These efforts have led to reduced poaching and retaliatory killings, and more positive attitudes (Bagchi & Mishra, 2006; McCarthy et al., 2016). Range-wide reductions of these efforts



as a result of the recent IUCN delisting will be detrimental, but may be counteracted in areas where most needed if they are focused and applied to endangered subspecies.

### Study goals

In an effort to increase confidence in the subspecies status as determined by Janecka et al. (2017), the goals of this study were to a) increase the sample size of snow leopards in the initial phylogeography dataset from the Western and Central genetic cluster, b) include samples from in between the Western and Central genetic cluster to determine the phylogeographic break between the two, and c) rerun analyses as done in Janecka et al. 2017 to determine if patterns first observed in Janecka et al. 2017 remained.

## Materials and Methods

### Study site, sample collection, DNA extraction, genotyping

#### *Original phylogeography samples*

A total of 70 samples were collected across 21 localities throughout snow leopard range from 2005 to 2009. For more information, see Janecka et al. (2017).

Samples that geographically aligned with the proposed Central and Western genetic cluster from Janecka et al. (2017) were added to the dataset for continued analyses. Samples were chosen based on the potential utility of the sample's geographic origin in discerning the phylogeographic break between the proposed Central and Western subspecies. Representative individual snow leopards from each area were selected based on previous genotyping success. Samples representative of the Central

genetic cluster included three from Dulan County, Qinghai Province, China, four from Yanchiwan Nature Reserve, Subei County, Gansu Province, China, five from Suojia Township, Yushu Prefecture, Qinghai Province, China, and seven from the Chang Tang region, Tibet Autonomous Region, China. Samples representative of the Western genetic cluster included five from Kyrgyzstan (Figure 5). For information on DNA extraction and genotyping of samples, see Chapter 2.1. See Chapter 2.1 for information surrounding study sites in China.

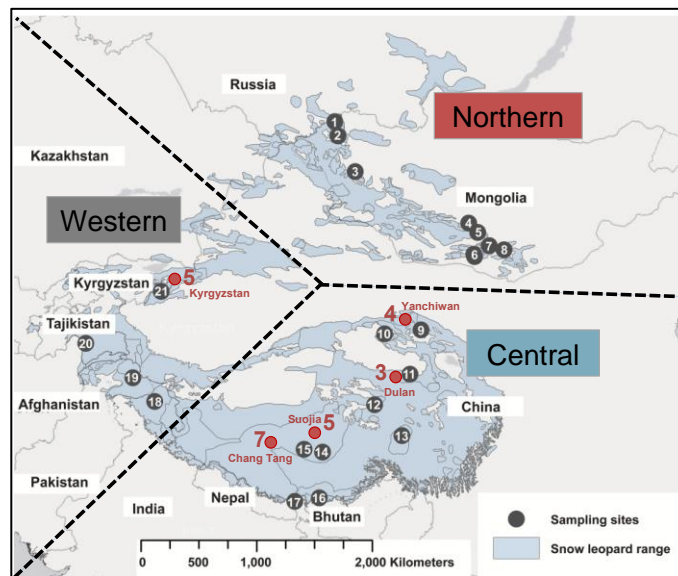


Figure 5. Locations and number of samples added to the snow leopard phylogeography dataset within each determined genetic cluster denoted in red. Modified from Figure 1 of Janecka et al. 2017.

### Data Analysis

Selected data analysis approaches used were kept identical to those in Janecka et al. (2017) for direct comparison. See Janecka et al (2017) or Chapter 2.1 of this dissertation for methods surrounding estimates of genetic diversity. Population structure was assessed using the pairwise fixation index ( $F_{ST}$ , Weir and Cockerham 1984) to examine gene flow between the three previously defined genetic clusters.  $F_{ST}$  values and

their significance were estimated through an analysis of molecular variance (AMOVA) in GenAlEx 6.503 using genetic clusters and sampling regions as populations (Peakall and Smouse 2012). Population assignment tests as well as isolation-by-distance patterns (IBD) were performed in GenAlEx and a Principal Component Analysis (PCA) in *adegenet* 1.4.2 (Jombart 2008) using R Studio 1.2.1335 (R Core Team, 2013). A Bayesian clustering approach to determine the number of unique genetic clusters (K) range-wide was implemented in STRUCTURE 2.3.4 (Prichard et al. 2000). The following parameters were used: admixture, *alpha* inferred from the data (initial value 1.0), correlated allele frequencies, sampling locations as prior (LOCPRIOR), and 2,000,000 MCMC iterations after a burn in of 200,000 with genetic clusters varying from 1 to 10. Data from the STRUCTURE run were uploaded into STRUCTURE HARVESTER 0.694 (Earl & vonHoldt 2011) to identify the most probable K using the estimator  $\Delta K$  (Evanno et al., 2005). CLUMPAK (Cluster Markov Packager Across K) (Kopelman et al., 2015) was used to provide a graphical summation of the ancestry plots from iterative STRUCTURE runs at the most informative K-values. The same approach was also used for analysis of hierarchal genetic structure with the proposed Central and Western subspecies because additional samples were added to their respective geographic locations.

## Results

Genetic diversity estimates are summarized in Table 4. Congruent with Janecka et al. (2017), the Northern genetic cluster had the overall lowest metrics of genetic diversity while the Central genetic cluster had the highest. When all samples were pooled, 28 loci were out of HWE, but this number decreased to 10, 1, and 3 loci when broken up into the

Central, Western, and Northern clusters, respectively. When considered among the seven regions, only Tibet/Nepal/Bhutan and Southern Mongolia had loci out of HWE with two and one loci, respectively (Table 5).

Overall  $F_{ST}$  among the three populations was 0.128 ( $p < 0.001$ ), indicating that the allele frequencies between them showed more differences than expected. Genetic dissimilarity based on  $F_{ST}$  among the three genetic clusters was greatest between the Northern and Central ( $F_{ST} = 0.152$ ). The next highest  $F_{ST}$  value was between the Western and Northern ( $F_{ST} = 0.111$ ) with Central and Western following third ( $F_{ST} = 0.104$ ). AMOVA tests indicated that 13% of observed molecular variance was between populations, 28% among individuals, and 59% within individuals. The  $F_{ST}$  values among the seven investigated regions revealed that genetic differentiation was highest between Southern Mongolia and Southern Qinghai and lowest between Southern Qinghai and Northern Qinghai (Table 6).

The PCA revealed three major groups of clustered samples, which corresponded to the Central, Western, and Northern genetic clusters (Figure 6A). Further division into the seven geographic regions showed the three genetic clusters were retained within the confines of the PCA plot (Figure 6B).

Population assignment tests showed no misassignment for the samples in the Central, Northern, and Western genetic clusters (Appendix II: Supplemental Material 1). Among the seven sampling regions, three samples from Northern Qinghai misassigned to Southern Qinghai, one sample from Southern Qinghai misassigned to Northern Qinghai, one sample from India/Pakistan misassigned to Tajikistan/Kyrgyzstan, two samples from Tajikistan/Kyrgyzstan misassigned to India/Pakistan, one sample from Western Mongolia

assigned to Southern Mongolia and one sample from Southern Mongolia misassigned to Western Mongolia (Appendix II: Supplemental Material 2).

When all samples were considered range-wide, the ad-hoc Evanno method supported the division of two genetic clusters ( $K = 2$ ) (Figure 7A). The summarized STRUCTURE plot showed the added samples from Kyrgyzstan as sharing genetic information with samples from Mongolia (Figure 8A). Upon the consideration of three genetic clusters ( $K = 3$ ), the three genetic clusters were recovered, with the additional samples from China clustering into the Central genetic cluster and the additional samples from Kyrgyzstan clustering into the Western genetic cluster (Figure 8B). The compoplot resulting from the PCA was used as an additional visual aid. It similarly showed differentiation of individuals pre-assigned to the three genetic clusters which also corresponded to the seven geographic areas where samples were collected (Figure 9A,B).

When only the 43 samples indicative of the Central genetic cluster, the 21 samples from the Westerns genetic cluster, and the 30 samples representative of the Northern genetic cluster were assessed, the ad-hoc Evanno method supported the division of 2 genetic clusters for each (Figure 7B,C,D). The additional samples added to the Central genetic cluster from Dulan County, Yanchiwan, and Suojia grouped into one cluster, while samples from the Chang Tang region grouped into the second (Figure 8C). The Kyrgyzstan samples added to the Western genetic cluster clustered with the second of the two genetic clusters identified (Figure 8D).

Table 4. Comparison of genetic diversity values overall and for the three proposed snow leopard subspecies in Janecka et al. 2017 versus addition of samples from Central and Western genetic clusters. N is the number of total samples collected, while n is the number of snow leopard positive samples.

	No. of Alleles ( $A_N$ )	No. of Effective Alleles ( $N_E$ )	Observed Heterozygosity ( $H_O$ )	Expected Heterozygosity ( $H_E$ )	Fixation Index (F)
<b>Overall</b>					
Janecka et al. (N = 70)	5.8	2.8	0.433	0.568	0.246
This Study (N = 94)	6.2	2.9	0.413	0.581	0.288
<b>Central Subspecies</b>					
Janecka et al. (n = 24)	4.2	2.8	0.446	0.521	0.138
This Study (n = 43)	4.9	2.7	0.413	0.545	0.225
<i>Northern Qinghai</i>					
Janecka et al. (n = 6)	3.1	2.3	0.457	0.484	0.053
This Study (n = 14)	4	2.5	0.429	0.531	0.170
<i>Southern Qinghai</i>					
Janecka et al. (n = 5)	2.6	2.1	0.450	0.434	-0.019
This Study (n = 10)	3.3	2.3	0.391	0.479	0.203
<i>Tibet/Nepal/Bhutan</i>					
Janecka et al. (n = 13)	3.3	2.2	0.441	0.452	0.012
This Study (n = 19)	3.6	2.3	0.412	0.482	0.121
<b>Western Subspecies</b>					
Janecka et al. (n = 16)	4.0	2.6	0.461	0.522	0.126
This Study (n = 21)	4.3	2.7	0.430	0.520	0.176
<i>India/Pakistan</i>					
Janecka et al. (n = 8)	3.2	2.3	0.508	0.473	-0.082
This Study (n = 8)	3.2	2.25	0.505	0.458	-0.087
<i>Tajikistan/Kyrgyzstan</i>					
Janecka et al. (n = 8)	3.3	2.4	0.415	0.485	0.174
This Study (n = 13)	3.6	2.5	0.385	0.485	0.228
<b>Northern Subspecies</b>					
Janecka et al. (n = 30)	3.9	2.3	0.408	0.481	0.152
This Study (n = 30)	3.9	2.3	0.403	0.476	0.154
<i>Western Mongolia</i>					
Janecka et al. (n = 15)	3.1	2.2	0.416	0.442	0.058
This Study (n = 15)	3.1	2.2	0.413	0.439	0.059
<i>Southern Mongolia</i>					
Janecka et al. (n = 15)	3.3	2.1	0.399	0.450	0.110
This Study (n = 15)	3.3	2.1	0.391	0.445	0.115

Table 5. The number of loci out of Hardy-Weinberg equilibrium (HWE) within the dataset overall, partitioned into three genetic clusters, and then into seven regions.

	Number of Loci out of HWE	Percentage of loci out of HWE
One Population		
Range-Wide (N = 94)	28	84.8%
Three Populations		
Central (n = 43)	10	30.3%
Western (n = 21)	1	3.0%
Northern (n = 30)	3	9.1%
Seven Populations		
Northern Qinghai	0	0%
Southern Qinghai	0	0%
Tibet/Nepal/Bhutan	2	6.1%
India/Pakistan	0	0%
Tajikistan/Kyrgyzstan	0	0%
Western Mongolia	0	0%
Southern Mongolia	1	3.0%

Table 6. Pairwise  $F_{ST}$  values between the seven geographic regions within the dataset.

	Northern Qinghai	Southern Qinghai	Tibet/Nepal/Bhutan	India/Pakistan	Tajikistan/Kyrgyzstan	Western Mongolia	Southern Mongolia
Northern Qinghai	-						
Southern Qinghai	0.053	-					
Tibet/Nepal/Bhutan	0.066	0.073	-				
India/Pakistan	0.106	0.125	0.099	-			
Tajikistan/Kyrgyzstan	0.105	0.136	0.107	0.073	-		
Western Mongolia	0.130	0.157	0.132	0.114	0.087	-	
Southern Mongolia	0.122	0.168	0.144	0.137	0.106	0.070	-

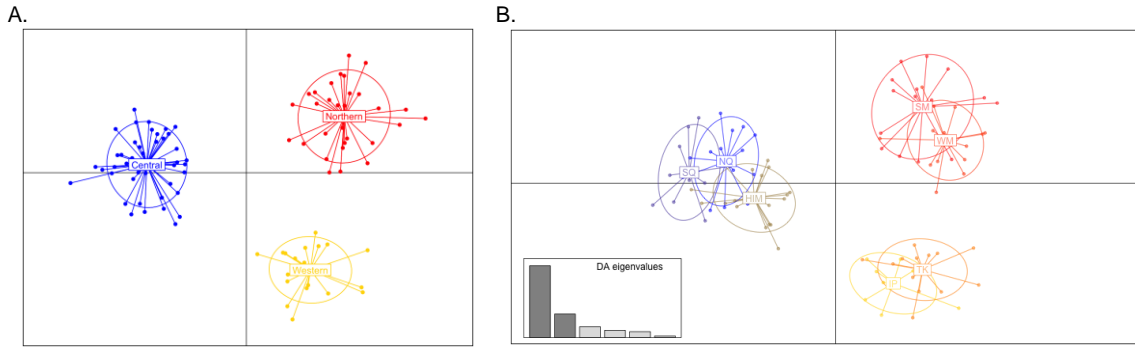


Figure 6. Genetic relationships between genetic clusters along x and y coordinates for A) the three proposed subspecies populations; and B) the seven geographic regions where samples were collected (SQ – Southern Qinghai, NQ – Northern Qinghai, HIM – Tibet/Nepal/Bhutan, IP – India/Pakistan, TK – Tajikistan/Kyrgyzstan, WM – Western Mongolia, SM – Southern Mongolia). Each node represents an individual snow leopard.

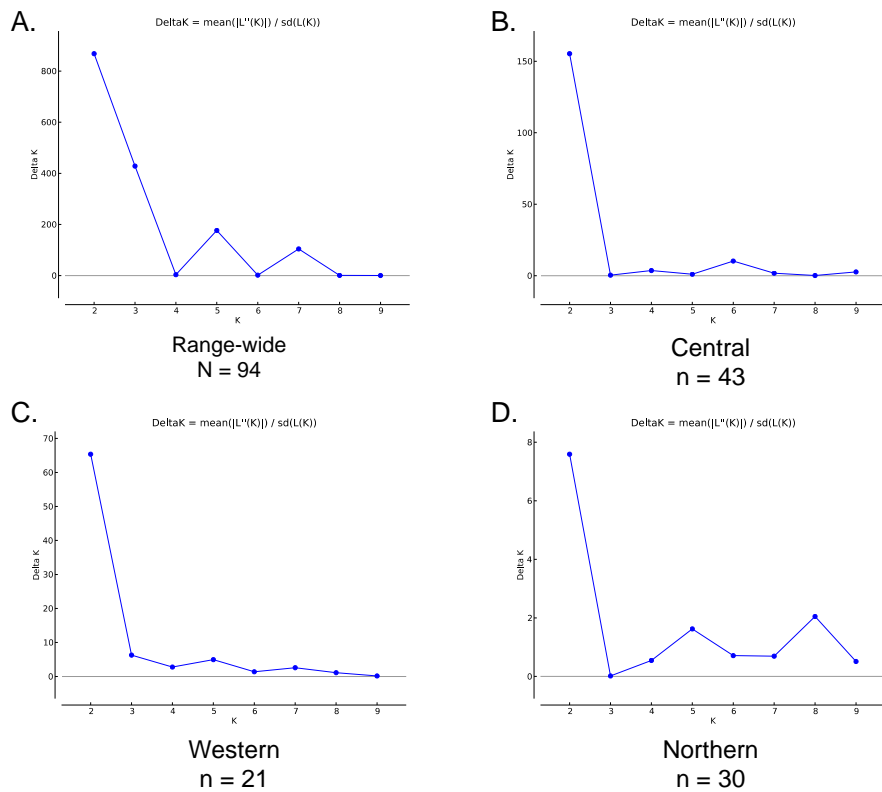


Figure 7.  $\Delta K$  values for the appropriate number of unique genetic clusters A) range-wide; B) the Central genetic cluster; C) the Western genetic cluster; and D) the Northern genetic cluster.



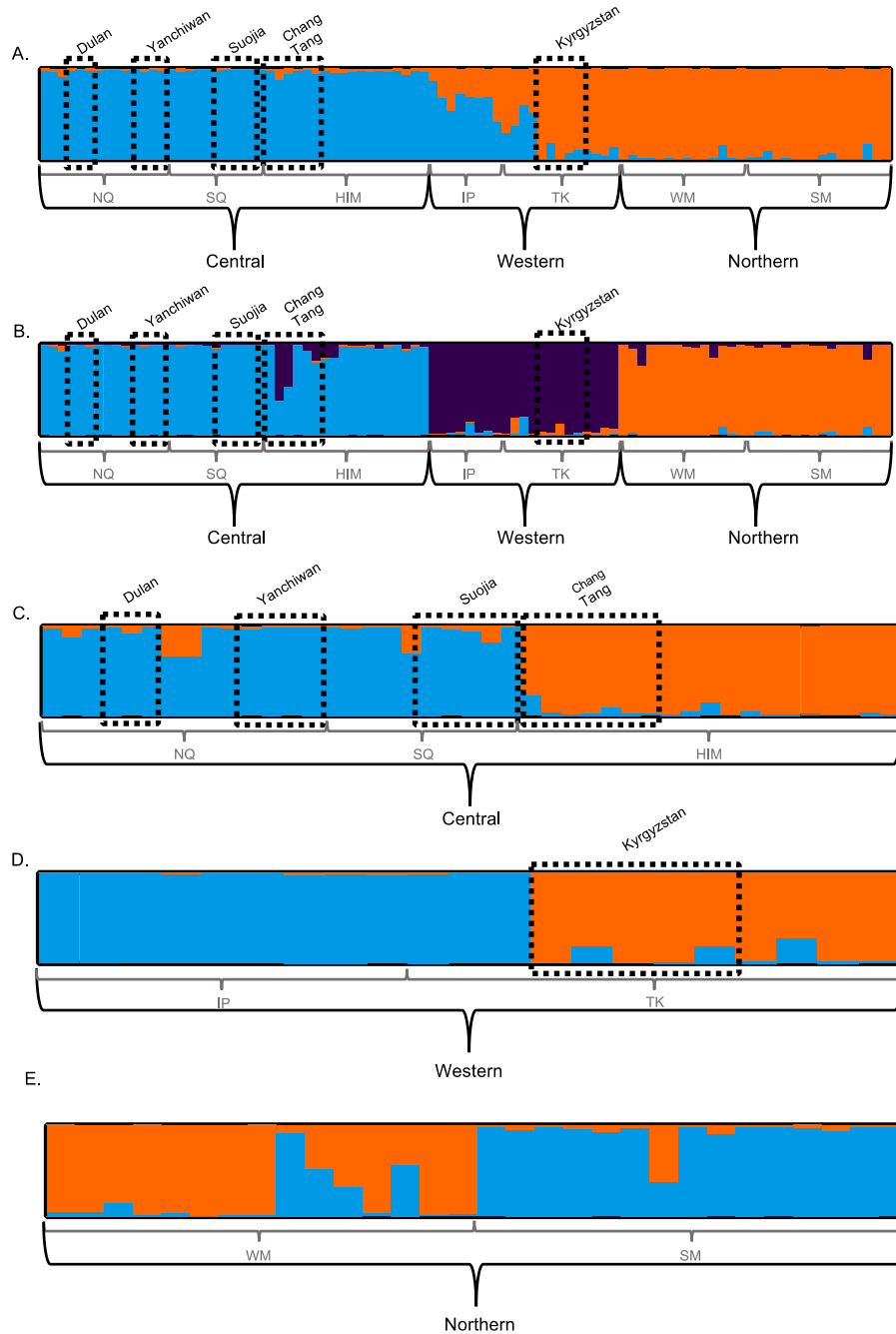


Figure 8. Consensus STRUCTURE plots of genetic clusters with sampling regions outlined range-wide and by proposed subspecies with A) range-wide genetic data at  $K = 2$ ; B) range-wide genetic data at  $K = 3$ ; C) for genetic clusters outlined for the Central subspecies at  $K = 2$ ; D) for genetic clusters outlined for the Western subspecies at  $K = 2$ ; and E) for genetic clusters outlined for the Northern subspecies at  $K = 2$ . Region abbreviations key as follows: SQ – Southern Qinghai, NQ – Northern Qinghai, HIM – Tibet/Nepal/Bhutan, IP – India/Pakistan, TK – Tajikistan/Kyrgyzstan, WM – Western Mongolia, SM – Southern Mongolia. Each bar represents one individual snow leopard genetic profile and each color is indicative of a unique genetic cluster.

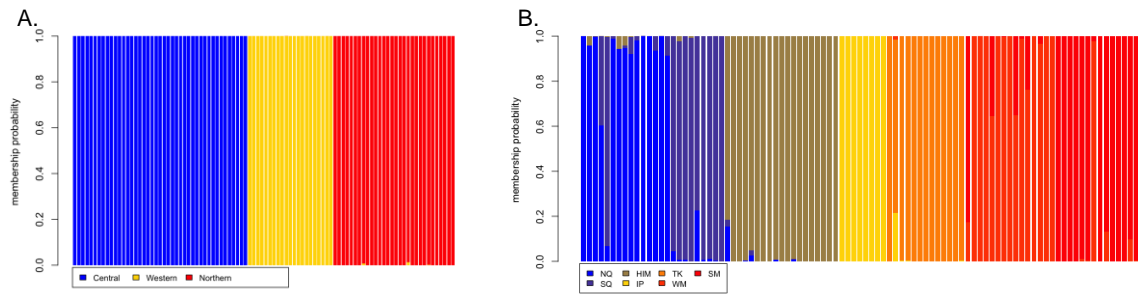


Figure 9. Complots for A) the three proposed snow leopard subspecies; B) the seven geographic areas from which samples were collected from. Region abbreviations key as follows: SQ – Southern Qinghai, NQ – Northern Qinghai, HIM – Tibet/Nepal/Bhutan, IP – India/Pakistan, TK – Tajikistan/Kyrgyzstan, WM – Western Mongolia, SM – Southern Mongolia. Each vertical line represents an individual snow leopard, with each color showcasing the assigned population.

## Discussion

Subspecies designations have historically been arbitrary (Ryder, 1986). The snow leopard is no exception, with previous proposals often justified by the sighting of one aberrant individual (Zukowsky, 1950). It was not until more recent range-wide phylogenetic analyses that snow leopard taxonomy could be adequately and scientifically assessed (Jan E. Janecka et al., 2017). Based on the discreteness of nuclear loci that resulted in three genetic clusters supported by known geographical boundaries, three subspecies were proposed. However, sampling gaps and sample size numbers within the three proposed subspecies limited support for the conclusion, particularly for the Western and Central genetic clusters. In this work, additional samples anticipated to cluster with the proposed Western and Central subspecies, as well as samples from a region of snow leopard range previously unexamined, were added to the preexisting phylogeography dataset used in Janecka et al. (2017) in an effort to better understand the range-wide genetic structure of the species.

### Continued support for three primary genetic clusters

The addition of samples and re-running of analyses used in Janecka et al. (2017) continued to support the notion of three subspecies within the snow leopard metapopulation, corresponding to the western, central, and northern regions of their range. Results from the Evanno method indicated two genetic clusters in the dataset. However, visual analyses showed a high degree of introgression for most snow leopard individuals from the western and northern portions of species range. The consideration of three genetic clusters, which was the next most appropriate according to the Evanno method, showed clearly defined genetic breaks among snow leopards belonging the central, western, and northern portions of their range. As expected, the additional samples from Dulan and Yanchiwan grouped with the Northern Qinghai region, the Suojia samples clustered with the Southern Qinghai region, and the Chang Tang region samples clustered to the Tibet/Nepal/Bhutan region of the Central cluster. The Kyrgyzstan samples also adhered to the initial prediction of clustering with the samples from the Tajikistan/Kyrgyzstan samples from the proposed Western subspecies.

### Determination of the phylogeographic breaks between genetic clusters

Of particular interest in this study was the determination of the phylogeographic break between the Western and Central genetic clusters. Janecka et al. 2017 postulated that the Himalayas were the likely landscape barrier due to their height and distance coupled with numerous rivers and potentially unsuitable habitat, though this point has been argued against with the notion that the Himalayas would serve as a nominal barrier due to the vastness of the Qinghai-Tibetan Plateau to the north (Senn et al., 2018). Our

results found that samples from the Chang Tang region clustered with the Tibet/Nepal/Bhutan region of the Central subspecies. However, four of the Chang Tang samples had admixture with the Western subspecies. This helps to support the notion that the Himalayas do serve as the primary biogeographic boundary, likely limiting gene flow between the Pamir-Karakorum mountains and the Qinghai-Tibetan Plateau itself. However, the presence of admixture indicates that snow leopards may successfully make their way from range countries belonging to the Western genetic cluster to the Qinghai-Tibetan Plateau, though the current dataset suggests that movement in the opposite direction occurs less frequently. Admixture could also indicate that the severity of current biogeographical boundaries may have historically differed through time.

#### Potential historical influence on current genetic patterns in the Chang Tang region

The meeting point of the Himalaya, Karakoram, Hindukush, Tien Shan, and Pamir ranges (the “Pamir Knot”) is one of the most heavily glaciated parts of the world (Khan et al., 2016). These glacial fields have likely served as a substantial barrier to snow leopard movement and will continue to do so. In contrast to other parts of the world, glacial fields in the Karakorum mountains have remained largely stagnant over the previous century (Iturrizaga, 2011). Instances of glaciers surging are widespread and glaciers that did retreat did not do so significantly (Gardelle et al., 2012; Iturrizaga, 2011). The first glacial advance in the region occurred in the early Quaternary 2.6 million years ago when snow leopards were diverging from their most closely related species, the tiger (B. W. Davis et al., 2010; Seong et al., 2007). This was followed by a series of progressively smaller glacial extensions up through the Holocene marked with glacial-

interglacial cycles through the late Quaternary (Seong et al., 2007). Modeling of snow leopard habitat between the Central and Western genetic clusters 6,000 years ago (6 kyr ago), a warm period during the mid-Holocene, showed snow leopard habitat in the nearby western Kunlun Shan that would not be considered suitable at present (J. Li, McCarthy, et al., 2016). Reduced glacial mass, coupled with habitat linking the “Pamir Knot” to the Qinghai-Tibetan Plateau via the western Kunlun Shan may have facilitated gene exchange, whose genetic markers remain in snow leopard populations in the Chang Tang region today.

#### Genetic differences between the Central and Northern genetic clusters

The genetic break between the Northern and Central clusters is far clearer than that between the Western and Central. Even prior to phylogenetic analyses, unofficial Northern and Central subspecies designations were largely accepted due to the obvious geographic barrier separating snow leopard populations in Mongolia and China (Fox, 1994). The greatest observed dissimilarity among the three genetic clusters in both Janecka et al. (2017) and this study was between the Northern and Central. Previous research has found that snow leopards can travel long distances between isolated massifs in steppe habitat and thus distance between snow leopard populations may not be genetically disconnected (McCarthy & Chapron, 2014). However, the Gobi Desert has over 400km of unsuitable snow leopard habitat (Jan E. Janecka et al., 2017; J. Li, McCarthy, et al., 2016; Riordan et al., 2016). Movement from the Tost Uul in Mongolia to Qilian Shan in China may be possible via the Ala Shan and Yin Shan Mountains of China (Riordan et al., 2016). Inability to cross open deserts has previously been

suggested as a mechanism lending itself to genetic differentiation in large felids such as the tiger (Driscoll et al., 2009). Local evidence, previous accounts, and research studies have supported the notion that snow leopards can traverse this terrain (McCarthy, 2000b; McCarthy et al., 2005; Riordan et al., 2016; Schaller, 1998). However, movement in this region is likely not substantial enough to maintain connectivity. Further fine-scale analyses surrounding gene flow and potential barriers to movement in this area are investigated in Chapter 2.3 of this dissertation.

#### Genetic differences between the Western and Northern genetic clusters

Genetic discordance between the proposed Western and Northern clusters in this study is clearer than in Janecka et al. 2017. The previous phylogeography dataset showed introgression between the Western and Northern genetic clusters. The addition of more samples from Kyrgyzstan from the Western cluster in this study appears to have absolved most of the visual evidence of introgression, demonstrating a potentially starker break in historical gene flow than previously thought. However, the  $F_{ST}$  value between the geographic areas corresponding to this division, Western Mongolia and Tajikistan/Kyrgyzstan, did not greatly differ between Janecka et al. 2017 (0.092) and this study (0.087) and no misassignments between the two regions were observed in either.

#### Genetic patterns within the three identified clusters

In accordance to the findings in Janecka et al. 2017, analyses of the proposed subspecies themselves revealed similar patterns in genetic structuring. Each proposed subspecies split into two genetic clusters. Within these, further genetic structuring was

evident. For the proposed Central subspecies, Northern and Southern Qinghai in China clumped into one genetic cluster separate from Tibet/Nepal/Bhutan. For the proposed Western subspecies, the two genetic clusters comprised one cluster exclusively of samples from Kyrgyzstan, including those added in this study, and another with samples from Tajikistan, India, and Pakistan. With this additional information, the original delineation of management units (MUs) in Janecka et al. 2017 is also further supported.

#### Range-wide snow leopard genetic diversity

Low microsatellite diversity was observed for the species even with additional samples. However, the addition of snow leopard individuals to the Central and Northern genetic clusters increased some genetic diversity estimates, such as the average number of alleles per locus. The higher number of samples added to the Central genetic cluster appeared to correspond to a larger increase in the number of different alleles observed (4.2 versus 4.9) while the limited number of individuals added to the Western subspecies only increased the number of different alleles observed by 0.3 (4.0 versus 4.3). This may indicate that low genetic diversity estimates could be a result of smaller sample sizes. However, similar studies examining felid genetics have found higher metrics with a similar or fewer number of individuals (Eizirik et al., 2001; Uphyrkina et al., 2001). Under range-wide consideration, the number of alleles increased from 5.8 to 6.2. The number of effective alleles, as well as observed and expected heterozygosity, displayed only marginal differences from Janecka et al. 2017 and the fixation index in all three cases increased.

#### Lack of mitochondrial DNA diversity

Evidence of mitochondrial divergence has been previously recommended as a requirement for subspecies delineation (Moritz, 1994). However, different mtDNA haplotypes were not observed in Janecka et al. 2017 and subspecies diagnosability based on mtDNA has proven to be questionable (Driscoll et al., 2009; Toews & Brelsford, 2012). Therefore, the assessment of mtDNA for the samples added to this work was not done. Discordance of phylogeographic patterns and genetic diversity between mtDNA and nuclear DNA have been previously reported (Eizirik et al., 2001; Toews & Brelsford, 2012; Zink, 2004). While some species from the genus *Panthera*, such as lions (Antunes et al., 2008; Bertola et al., 2015) and common leopards (Uphyrkina et al., 2001) have shown agreement in the genetic diversity and phylogenies produced between mtDNA and nuclear loci, others, such as jaguars (Eizirik et al., 2001), have not. Janecka et al. (2017) found no diversity in the mitochondrial gene segments examined. However, a previous study found nine haplotypes among 48 snow leopard mtDNA fragments across three regions of China's Qinghai-Tibetan Plateau (Y. Zhou et al., 2014). Lack of mtDNA diversity in the cumulative 663-bp investigated in Janecka et al. (2017) could be due to many reasons. First, it may infer a recent population expansion, as has been previously suggested for jaguars (Eizirik et al., 2001). However, this is unlikely given the evident lack of gene flow observed in the nuclear loci examined. Second, it could be an artifact of the sequences selected or the number of base pairs analyzed. Third, it is possible that the mitogenome has undergone a selective sweep via selective environmental pressures related to hypoxia adaptation (da Fonseca et al., 2008; Hassanin et al., 2009) or mitochondrial replacement after hybridization with the African lion lineage (G. Li et al., 2016). Fourth, diversity in mtDNA may have been lost during a population genetic



bottleneck estimated to have occurred about 8,000 years ago (Jan E. Janecka et al., 2017). Also of prospect is a combination of these coupled with species behavioral strategies.

Mitochondrial DNA is maternally inherited. Snow leopards are tied to the mountains, primarily moving through corridors and avoidant of open steppe (Jackson et al., 2010; Johansson et al., 2016). Intra-sex home range overlap is also low even in the absence of geographical barriers, indicating territoriality (Johansson et al., 2016). However, related mother-daughter pairs exhibit high overlap, suggesting that daughters do not disperse far from their natal home ranges (Johansson et al., 2016). Movement data from GPS collared mother-offspring pairs found that snow leopard cubs did not display separation behavior until 20 to 22 months of age (Johansson, Ausilio, et al., 2020). While the male cub in the study fully dispersed within a month, the two female cubs had not dispersed within the four and seven months they were monitored post initiation of separation behavior and likely established home ranges that overlapped with their mothers as large felids are known to do (Gour et al., 2013; Johansson, Ausilio, et al., 2020; Kitchener, 1991; Samelius et al., 2012). At their respective ages of approximately three years old upon the establishment of their home range, they would presumably soon after be breeding. Snow leopard reproductive age is not well understood. Individuals in zoological collections typically breed at just under five years old (Lynch et al., 2015), though occurrences of progeny *ex-situ* by age two have been recorded (Blomqvist, 2008). The IUCN Red List assessment for the species denotes reproductive maturity at two years of age with the caveat that actual breeding does not occur until three years of age (McCarthy et al., 2017). Narrow mountainous environments, coupled with overlapping mother-daughter home ranges and initial home range establishment close to reproductive

age may lend itself to the dominance of a single family matriline mitochondrial haplotype. Such hypotheses for lack of mtDNA diversity rooted in large felid female behavior have been raised in other studies. For example, both the Caspian tiger (Driscoll et al., 2009) and the American puma (*Puma concolor*) (Culver et al., 2000) are species that exhibit low mtDNA diversity and have occupied narrow corridors during their natural histories. The only visible genetic structure observed in jaguars was found in mtDNA, but not in nuclear DNA, and corresponded geographically with the Amazon River, suggesting that the river itself is more of an impediment for females than for males (Eizirik et al., 2001).

#### Outcomes for the elevation of genetic clusters to subspecies status

We contend that the current recognized taxonomy of the snow leopard does not reflect the species' phylogenetic data, and that the three genetic clusters observed in this study and in Janecka et al. 2017 may warrant subspecies status (Western – *Panthera uncia uncia*, Central – *P. u. uncioides*, and Northern– *P. u. irbis*). Similar discrepancies have been voiced surrounding the African lion (Antunes et al., 2008; Barnett et al., 2006b, 2006a, 2014; Bertola et al., 2011, 2016; Bruche et al., 2013; Dubach et al., 2005). Changes to the current monotypic status of the snow leopard could have major outcomes for its conservation and protection. Previous work has suggested that an animal's charisma is more likely to garner public support and funds than their IUCN status (Colléony et al., 2017). While snow leopards have been touted and used as such (Hotham et al., 2016), this does not negate the fact that research partnerships, legislation, funding, and government support are largely based on IUCN status (Vié et al., 2008). Researchers

studying these species are also eligible for a wider variety of grants, whereby an Endangered status of the study organism is a requirement.

As a whole, the snow leopard population has greatly benefit from increased awareness and research efforts over the last 25 years (Alexander, Zhang, et al., 2016b; Fox & Chundawat, 2016; Riordan & Shi, 2016). Unfortunately, the population is expected to be decreasing, conservation successes are not uniform across species range, and challenges vary regionally (McCarthy et al., 2017). For example, populations of snow leopards belonging to the Western subspecies may be at great risk. Populations in Pakistan may be lower than originally thought (Nawaz & Hameed, 2019), local extinction events have been documented in Kyrgyzstan (Taubmann et al., 2016), and the China-Pakistan Economic Corridor poses perhaps the first major roadway bisecting through prime snow leopard habitat, increasing pollution, habitat loss, and likelihood of wildlife-vehicle collisions (Ullah Khan et al., n.d.). The small population of snow leopards in northern Afghanistan appear to be at higher risk of retaliatory killings and use in the illegal wildlife trade than in other parts of their range, with pelts still widely available in markets (Moheb & Paley, 2016). In Tajikistan, local extinctions and continued poaching of preferred prey items, such as urial (*Ovis vignei*) and ibex (*Capra sibirica*), have reduced much of the nutritional value available to snow leopards (Saidov et al., 2016). In Uzbekistan, the practice of capturing snow leopard cubs and adults for captive facilities was common practice well into the 2000s, minefields left over from the civil war in nearby Tajikistan are occasionally triggered by wildlife, and avalanches are a relatively high cause of natural mortality for the species (Esipov et al., 2016). Meanwhile, snow leopard populations belonging to the Northern genetic cluster appear to be stable or

increasing in large part to early conservation interventions as well as more recent conservation activism efforts in Mongolia (Munkhtsog et al., 2016). In addition, climate change models suggest that snow leopard habitat will increase 8% to 29% in Mongolia and 21% to 67% in Russia, suggesting that there will be greater areas suitable habitat by the year 2070 (J. Li, McCarthy, et al., 2016). This is in contrast to populations from the Central genetic cluster, where all range countries are expected to lose habitat (J. Li, McCarthy, et al., 2016). The majority of snow leopards from the Central genetic cluster are found in China, which has made great strides in snow leopard conservation (Alexander, Zhang, et al., 2016b; Riordan & Shi, 2016). However, much of the area remains data deficient, with areas in the Tibet Autonomous Region grossly understudied (Riordan & Shi, 2016). Formal acceptance of snow leopard subspecies would allow for the Western and Central subspecies to retain the formal benefits associated with an Endangered status, which are needed, while all three subspecies would continue to additionally procure the benefits of being a charismatic mammal.

#### Future directions and continued improvements to current dataset

Though the genetic information presented herein remains the most comprehensive and accessible dataset to date, future work remains much needed. First, while the Central and Western genetic clusters warrant status as separate subspecies, this study was only marginally able to determine the phylogeographic break between the two. A greater number of samples from the Tibet Autonomous Region and western Nepal would be helpful in further solidifying the location of this boundary, but what is of more desperate need is samples from the Xinjiang Uygur Autonomous Region, China. Both the Tian

Shan and Kunlun Shan transect Xinjiang to its north and south, respectively (Schaller, Li, et al., 1988). This makes the area a potentially crucial connection point between all three proposed subspecies. Very little information is known about snow leopard populations in Xinjiang, and thus sampling in this area would be helpful at both local and range-wide scales. Alternative methods that investigate snow leopard genetic structure would also be helpful in species conservation efforts. Single nucleotide polymorphisms (SNPs) offer greater insight into genetic variability and are less prone to the errors commonly associated with microsatellite genotyping (Caragiulo et al., 2016). At the time of the initial phylogeography study, the application of this technique was hindered by the species not having a developed SNP panel. However, one has since been published (Janjua et al., 2020), making one of the only remaining hurdles the ability to successfully extract intact DNA from snow leopard scat that is suitable for SNP analysis.

#### Author Attributions

Charlotte Hacker, Yuguang Zhang, Yu Zhang, Xue Yadong, Bayaraa Munkhtsog, Liji Wu, Xierannima, Xuchang Liang, and Xiaoxing Bian collected scat samples. Charlotte Hacker, Yu Zhang, Yunrui Ji, and Cong Wei extracted DNA from scat samples. Jan Janecka modified DNA extraction protocol. Charlotte Hacker genotyped samples. Jan Janecka provided initial phylogeography dataset from Janecka et al. (2017). Charlotte Hacker and Jan Janecka performed data analyses. Charlotte Hacker and Jan Janecka interpreted data. Charlotte Hacker wrote the manuscript.

## Chapter 2.3

### Goal 1 – Subgoal 3

# Landscape genetics of snow leopard populations in Mongolia and China

Adapted from Hacker *et al.* (In Prep) *Genetic diversity, structure, and connectivity of snow leopards (Panthera uncia) across Mongolia and northwestern China.*

#### Introduction

Snow leopards occupy naturally fragmented mountain habitats with corridors that are increasingly disrupted by anthropogenic factors that impact dispersal routes important to migration (Robinson & Weckworth, 2016). Migration is a mechanism that can maintain diversity, panmixia, demographic stability, and potential for evolutionary adaptation; while isolation leads to divergence, structure, and population instability (Slatkin, 1987). In severely fragmented habitats, species consist of small, isolated populations that are subject to genetic erosion, inbreeding, and increased extinction risk (McNeely *et al.*, 1990; Slatkin, 1987). The maintenance of both migration and genetic diversity is important for long-term species persistence (McNeely *et al.*, 1990). Thus, understanding landscape connectivity is imperative for designing effective conservation plans. A 2008 range-wide assessment in Beijing brought together 22 experts to identify snow leopard conservation units (SLCUs) (McCarthy *et al.*, 2016). Of the 69 units identified, there was complete lack of information on connectivity in 54% of them. Within the remaining SLCUs, connectivity was considered to be infrequent or absent in 47%, suggesting that in most areas snow leopard movement is hindered, which may have consequences on both demographics and genetic fitness of the populations (McCarthy *et al.*, 2016).

#### Identification of three primary genetic clusters in the snow leopard species

One work examining snow leopard phylogeography using microsatellites found support for three distinct and significant genetic clusters (i.e., Western – *Panthera uncia uncia*, Central – *P. u. uncioides*, and Northern– *P. u. irbis*), potentially warranting subspecies status (Jan E. Janecka et al., 2017). The greatest genetic differentiation observed was between the proposed Northern and Central subspecies (Jan E. Janecka et al., 2017). Additional sampling and fine-scale analyses are needed to better understand barriers to snow leopard movement both across Mongolia and into northern China so that natural corridors and stepping stones between larger habitat patches can be identified, maintained, and integrated into predictions surrounding large-scale shifts caused by climate change (Jan E. Janecka et al., 2017, 2020; J. Li, McCarthy, et al., 2016).

#### The impact of climate change on Central Asia

The Tibetan Plateau and its surrounding mountain chains are warming at two-times the rate of the Northern Hemisphere (F. Chen et al., 2009). Climate change is expected to have variable effects on snow leopard range countries. For example, snow leopard habitat projected into 2070 using a Representative Conservation Pathway scenario of RCP8.5 predicts that while 42% of current snow leopard habitat area in China will be lost, 29% will be gained in Mongolia (J. Li, McCarthy, et al., 2016). Understanding whether snow leopards in China may be able to overcome current landscape barriers and disperse to this new habitat northward will be of importance in designing future conservation management strategies.

#### The Gobi Desert as a barrier to gene exchange

Among the greatest landscape barrier to the movement of snow leopards between Mongolia and China is the Gobi Desert (Jan E. Janecka et al., 2017; Riordan et al., 2016). Previous research investigating connectivity between Mongolia and China and its implications for conservation used various methodological approaches. Riordan et al. (2015) used resistant kernel modeling with lifetime dispersal scenarios of 100, 500, and 1,000km, and only found connectivity across the Gobi Desert at 1,000km. The maximum entropy model developed by Li et al. (2016) using 2,111 locations and geographic features showed no connectivity in the area. In the phylogeographic study of Janecka et al. (2017), the Gobi region was found to have the highest genetic divergence observed range-wide. Over 400km of unsuitable habitat is present in the Gobi Desert separating southern Mongolia and northern China (Jan E. Janecka et al., 2017; J. Li, McCarthy, et al., 2016; Riordan et al., 2016). The only possible corridors facilitating movement from the Tost Uul in Mongolia to Qilian Shan in China are the Alashan and Yin Shan Mountains of China (Riordan et al., 2016). Local evidence, previous accounts, and research studies have attested to snow leopards occasionally navigating this terrain (McCarthy, 2000a; McCarthy et al., 2005; Riordan et al., 2016; Riordan & Shi, 2016; Schaller, 1998). However, arguments remain regarding the level of snow leopard movement in this region and if it is substantial enough to maintain connectivity. In addition, the level of connectivity between peripheral habitat patches in southern Mongolia and the larger core habitat in the Altai of western Mongolia is not well understood. Understanding these processes can be attained by in depth analysis of genetic structure coupled with habitat modeling.

The coupling of genetics and landscape data for understanding connectivity



Microsatellites have been successfully used to understand the fine-scale structure and landscape connectivity of numerous felids, including the African lion (*P. leo*), Asian leopard (*P. pardus orientalis*), tiger (*P. tigris*), mountain lion (*Puma concolor*), Canadian lynx (*Lynx canadensis*), ocelot (*Leopardus pardalis*), and bobcat (*L. rufus*) (Dures et al., 2020; Haag et al., 2010; Janečka, Tewes, et al., 2011). The comparison of discontinuities in genetic variation with environmental variables, such as mountains, roadways, temperature, and humidity gradients can then assist in identifying causes of population subdivision and microevolutionary processes (Manel et al., 2003; Montgelard et al., 2014). This powerful tool can be applied hierarchically through space, enabling resolution of population substructure across differing geographical scales and understanding how the landscape facilitates or impedes movement among resource patches (Manel et al., 2003). It can additionally aid in identifying breaks in gene flow that do not appear to have any obvious cause (Manel et al., 2003), making it an important tool for identifying cryptic patterns in landscape connectivity.

#### Research goals and hypotheses of this study

In this study, the contemporary genetic variation among snow leopards in Mongolia and northwestern China was examined to understand landscape connectivity and barriers to dispersal using 26 polymorphic microsatellite loci genotyped in 97 individuals sampled noninvasively via scat collection. We hypothesized that 1) two distinct genetic clusters would arise, with one belonging to snow leopards in Mongolia and one belonging to snow leopards in China, with little to no admixture between the two, that 2) metrics of genetic diversity would be lowest in snow leopards from Southern Mongolia, due its non-

continuous stepping stone habitat and highest in Western Mongolia due to the presence of more continuous habitat, and that 3) snow leopards in Northern Qinghai, China would show introgression with snow leopards in Middle Qinghai, China, due to higher quality habitat availability in Northern Qinghai. Our results provide information critical for the design and implementation of conservation actions aimed at maintaining stable snow leopard populations and protecting the ecosystems they occur in.

## Materials and Methods

### Sample Collection

Scat samples presumed to be of carnivore origin were collected between 2007 and 2015 in Mongolia, and between 2009 and 2018 in northwestern China during numerous noninvasive genetics surveys (Appendix I: Supplemental Material 9). Sampling methods followed those described in (Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011). Elevation of collected samples ranged from 1,606m in Arts Bogd Uul, Övörhangay, Mongolia to 3,954m in Qilian Shan, Qinghai Province, China (Figure 10).

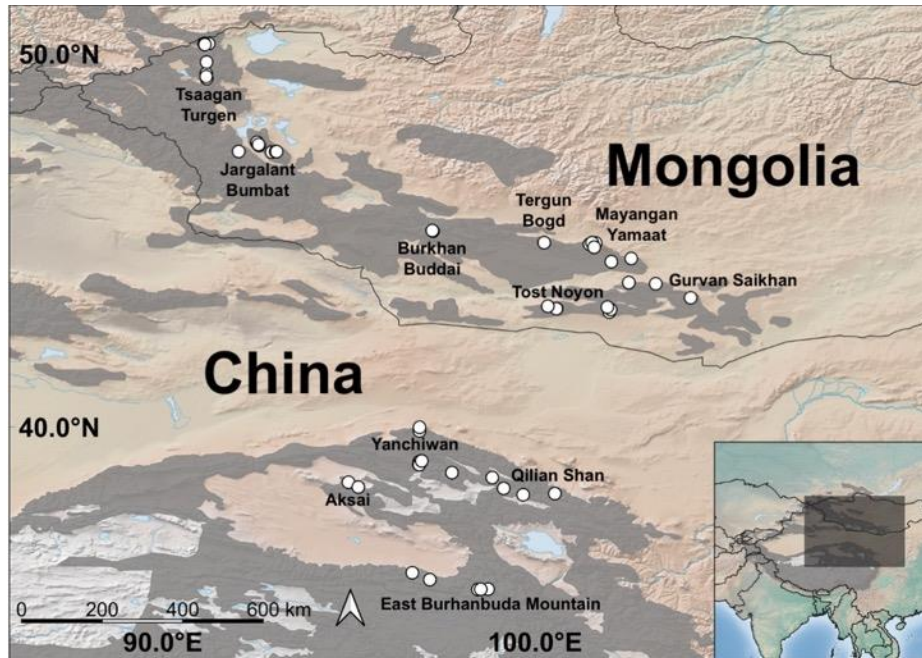


Figure 10. The spatial distribution of scat samples genetically confirmed to be of snow leopard origin (N = 97) used for microsatellite genotyping. (Snow Leopard Range data taken from the 2008 International Conference on Rangewide Conservation Planning for Snow Leopards)

### DNA Extraction

See Chapter 2.1 of this dissertation.

### Genotyping

Individuals were initially identified by genotyping between four to eight polymorphic microsatellite loci in triplicate using fluorescently labeled primers (Multiplex 1 and Multiplex 2 – see Chapter 2.1 of this dissertation for more information). (Janečka, Munkhtsog, et al., 2011; Jan E. Janecka et al., 2014, 2017). The highest quality sample from individuals with sufficient genotyping success were genotyped at additional microsatellites for a total of 32 loci (Jan E. Janecka et al., 2017). Fragment analyses and allele scoring were identical to methods outlined in Chapter 2.1 of this dissertation. Samples with missing data for >40% of their loci (>12 of the 32 loci genotyped) were

removed from the dataset. Loci with data missing for >15% of individuals were also removed from the data set (six of 32 loci removed - PUN132, PUN225, PUN668, PUN917, PUN1289, PUN347). Allele dropout was estimated by comparing allele peaks across each triplicate for the polymorphic loci used for individual identification. Given that only high-quality samples were used for further genotyping (i.e., samples that provided robust genotypes at a minimum of four loci), and that the primers used were designed specifically for snow leopards, this provides a reasonable estimate of allele dropout across the data set.

#### Population structure using *a priori* defined groups

Samples were initially grouped into populations based on habitat distribution and known biogeographic boundaries. Estimated parameters including number of alleles ( $A_N$ ), effective number of alleles ( $A_E$ ), observed heterozygosity ( $H_O$ ), and expected heterozygosity ( $H_E$ ) using GenAlEx 6.503 (Peakall and Smouse 2012). GENEPOP 4.7.2 (Raymond and Rousset 1995, Rousset 2008) was used to test for the presence of null alleles, estimate linkage disequilibrium (LD), and deviations from Hardy-Weinberg Equilibrium (HWE). Null alleles were examined using an iterative expectation and maximization approach to find the maximum likelihood estimate of null allele frequency (Dempster et al. 1977, Weir 1996). Parameters for testing LD using the Markov chain method were set to a dememorization number of 10000 with 100 batches of 5000 iterations. HWE was tested using the Markov chain method with a dememorization number of 10000 with 20 batches of 5000 iterations. The Bonferroni correction was applied to both analyses surrounding LD and HWE to correct for multiple comparisons.

Population structure using the *a priori* groups was examined by estimating pairwise  $F_{ST}$  values between putative subpopulations, isolation-by-distance (IBD), Principal Component Analysis (PCA), and population assignment using two methods. The first assignment test was completed using GenAIEx 6.503 (Peakall and Smouse 2012) with the following parameters – Assign All Pops and Leave One Out for Frequency Estimates. The second was implemented in STRUCTURE (Pritchard et al. 2000). PCA was completed using the package *adegenet* (Jombart 2008, Jombart et al. 2010). PCA allows for visualization how an individual’s genetic variation is related to another in space (Engelhardt and Stephens, 2010). The number of DA axes retained was set at  $(K - 1)$  and the number of PCA axes to be retained was determined via cross-validation with 90% of the data used as a training set and the remaining 10% used as a validation set with the number of replicates set to 20. A Mantel test with 9999 permutations was used to test for IBD with a non-significant or negative correlation, to examine if the potential effect of IBD on observed genetic structure (Mantel 1967). Significant correlations between geographic distance and genetic distance among samples do to IBD can bias tests that use hierarchical structuring.

#### Population structure without regard to geographic origin

A Discriminant Analysis of Principal Components (DAPC) was done using the package *adegenet* (Jombart 2008, Jombart et al. 2010) in RStudio version 1.2.1335 (R Core Team, 2013) to find the appropriate number of genetic clusters and to examine genetic variability and membership probabilities of individuals. DAPC is a flexible multivariate method that identifies and describes clusters of genetically related

individuals using sequential K-means and model selection. DAPC performs faster and generally better than STRUCTURE at characterizing population subdivision, particularly for those populations which may be more complex in nature (Jombart et al. 2010). The number of appropriate genetic clusters for the dataset were found using a K-means procedure as implemented using the function *find.clusters*. The appropriate value of K was determined by examining graphical output of Bayesian information criterion (BIC) values, with the smallest BIC value indicating the most appropriate number of clusters for the dataset.

The program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used as an additional tool to examine genetic structure in the dataset without regard for sampling locations. STRUCTURE uses maximum likelihood estimation to determine the appropriate number of clusters and implements a model-based Bayesian clustering method to assign individuals to a genetic cluster, or population, based on allele frequencies and HWE expectations. The number of potential subpopulations (K) evaluated ranged from 1 through 10 with 5 independent runs for each value of K. Each run used a Markov chain Monte Carlo (MCMC) burn-in period of 400,000 iterations followed by a posterior distribution sampling space of 800,000 iterations with no *a priori* assumptions of population origin. Data from the STRUCTURE run were uploaded into STRUCTURE HARVESTER 0.694 (Earl & vonHoldt 2011) to identify the most probable K using the estimator  $\Delta K$  described in Evanno et al. (2005). CLUMPAK (Cluster Markov Packager Across K, Kopelman et al. 2015) was used to provide a graphical summation of the ancestry plots from iterative STRUCTURE runs at the most informative K-values.

## Results

### Genetic Diversity

A total of 32 loci were genotyped for 97 samples. For 14 samples the allele dropout was estimated for four loci. For 22 samples the allele dropout was calculated for 7 loci. For 39 samples the allele dropout was estimated for 8 loci. Based on these 75 samples the allele dropout rate was estimated to be 6%. Null allele frequencies ranged from 0.069 to 0.558. The average frequency was  $0.206 \pm 0.116$ . Two loci (PUN1047 and PUN664) exhibited null allele frequencies  $> 0.5$ . Samples with  $>40\%$  missing data and loci with  $>15\%$  missing data, as well as the two loci with null frequencies  $> 0.5$ , were removed, resulting in a final microsatellite data set with 24 loci genotyped for 97 individuals.

For the entire data set,  $A_N$  was 5.958 with an  $A_E$  of 2.604,  $H_O$  was 0.374, and  $H_E$  was 0.562. China and Mongolia had a comparable number of alleles, effective number of alleles, as well as observed and expected heterozygosity. When four populations were considered, the number of alleles ranged from 3.250 in Western Mongolia to 4.583 in Southern Mongolia. The number of effective alleles ranged from 2.029 in Western Mongolia to 2.364 in Northern Qinghai/Gansu. Observed heterozygosity was lowest in Northern Qinghai/Gansu (0.336) and highest in Southern Mongolia (0.403). Expected heterozygosity was lowest in Western Mongolia (0.442) and highest in Middle Qinghai (0.494) with Northern Qinghai/Gansu closely following (0.493) (Table 7).

Table 7. Descriptive statistics across 24 microsatellite loci for hierarchally grouped putative populations.

	Number of Alleles ( $A_N$ )	Number of Effective Alleles ( $A_E$ )	Observed Heterozygosity ( $H_O$ )	Expected Heterozygosity ( $H_E$ )
One Population Mongolia/China (n = 97)	5.958	2.604	0.374	0.562
Two Populations Mongolia (n = 63)	4.875	2.197	0.388	0.493
China (n = 34)	4.542	2.504	0.351	0.530
Four Populations Western Mongolia (n = 25)	3.250	2.029	0.362	0.442
Southern Mongolia (n = 38)	4.583	2.176	0.403	0.486
Northern Qinghai/Gansu (n = 25)	4.167	2.364	0.336	0.493
Middle Qinghai (n = 9)	3.250	2.243	0.393	0.494

With the 97 samples pooled together, 24 loci showed significant deviation from HWE. When split into two populations, 11 loci from China and nine loci from Mongolia deviated from HWE. When split into four populations, one locus from Western Mongolia, five loci from Southern Mongolia, seven loci from Northern Qinghai/Gansu, and no loci from Middle Qinghai were out of HWE. Lastly, when broken into 11 populations, the only locations with loci out of HWE were Jargalant Bumbat and Yanchiwan with one loci each (Table 2). When considered as one population across 24 loci with 325 combinations of any two given loci possible, 118 (36.3%) pairs showed significant LD.



Table 8. Results of Hardy-Weinberg Equilibrium (HWE) tests for 24 microsatellites.

	Number of Loci out of HWE	Percentage of loci out of HWE
One Population		
Mongolia/China (n = 97)	24	100%
Two Populations		
Mongolia (n = 63)	9	37.5%
China (n = 34)	11	45.8%
Four Populations		
Western Mongolia (n = 25)	1	4.2%
Southern Mongolia (n = 38)	5	20.1%
Northern Qinghai/Gansu (n = 25)	7	25.0%
Middle Qinghai (n = 9)	0	0%
Eleven Populations		
Tsaagan Turgen (n = 12)	0	0%
Jargalant Bumbat (n = 13)	1	4.2%
Burkhan Buudai (n = 6)	0	0%
Tergun Bogd (n = 5)	0	0%
Mayangan Yamaat (n = 12)	0	0%
Gurvan Saikhan (n = 7)	0	0%
Tost Noyon (n = 8)	0	0%
Yanchiwan (n = 15)	1	4.2%
Qilian Shan (n = 8)	0	0%
Akesai (n = 2)	0	0%
East Burhanbuda Mountain (n = 9)	0	0%

#### Population structure with *a priori* groups

The overall  $F_{ST}$  estimated between China and Mongolia snow leopard populations was 0.098 ( $p < 0.001$ ).  $F_{ST}$  was also significant ( $p < 0.001$ ) when sampled sites were divided into four main subpopulations, indicating that differences in alleles are significantly more different than would be expected by chance. The greatest  $F_{ST}$  was between Western Mongolia and Middle Qinghai (0.132). Southern Mongolia had comparable pairwise  $F_{ST}$  with Middle Qinghai (0.131). In contrast the lowest  $F_{ST}$  was between Western and Southern Mongolia (0.047) (Table 9).

Table 9. Pairwise  $F_{ST}$  values between four putative snow leopard subpopulations (Western Mongolia, Southern Mongolia, Northern Qinghai/Gansu, Middle Qinghai) derived from 24 microsatellite loci.

	Western Mongolia	Southern Mongolia	Northern Qinghai/Gansu	Middle Qinghai
Western Mongolia	0.000	-	-	-
Southern Mongolia	0.047	0.000	-	-
Northern Qinghai/Gansu	0.129	0.126	0.000	-
Middle Qinghai	0.132	0.131	0.077	0.000

The paired Mantel test between genetic distance and geographic distance of samples revealed a negative correlation across China. There was no correlation found when all samples were considered, nor when samples from Western and Southern Mongolia tested separately, reducing concern for spatial autocorrelation (Figure 11).

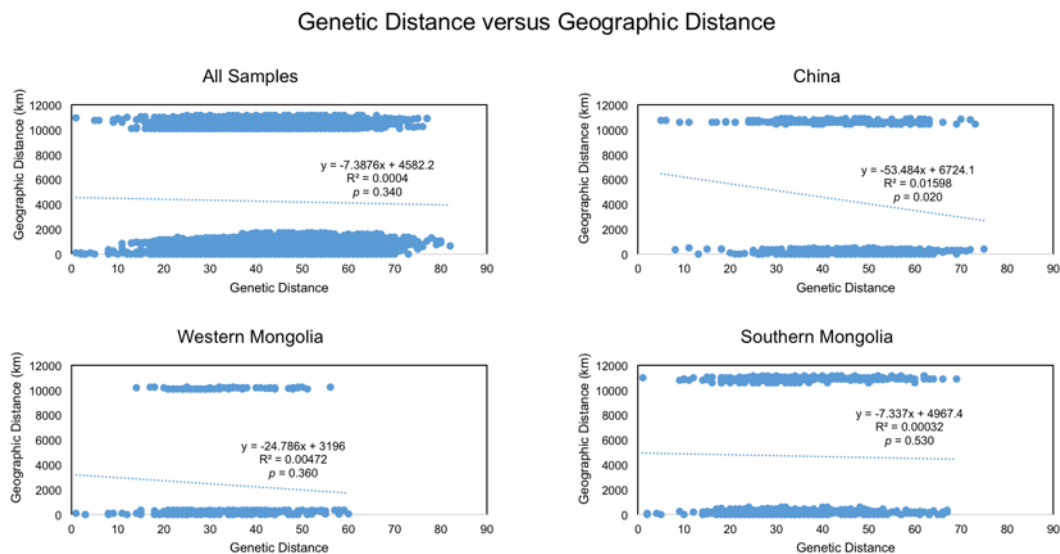


Figure 11. Genetic distance versus geographic distance as determined by the Mantel test for Isolation by Distance (IBD). Results indicate lack of correlation between the linear genetic distance between to samples and their geographic coordinates except for samples originating from China.

When samples were divided into China and Mongolia, population assignment tests resulted in one individual sampled in China misassigning to Mongolia (Table 4).

When samples were divided into four putative populations, misassignments were found

between Western and Southern Mongolia as well as between Northern and Middle Qinghai. Some differences were noted between software program used. For example, no misassignments between Southern Mongolia and Middle Qinghai were detected in GenAlex, whereas four misassignments were detected in STRUCTURE. Of note is that there were no misassignments between Southern Mongolia and Northern Qinghai, an area that would have to be crossed to get to Middle Qinghai (Table 10; Figure 12).

Table 10. Information surrounding population misassignment using genotyping data across 24 microsatellite data for hierarchally grouped putative populations.

Sampling Location Population	Assigned Population	Dispersal Direction	Number of Misassignments (GenAlex)	Number of Misassignments (STRUCTURE)
<b>Two Populations</b>				
Mongolia	China	north	0	0
China	Mongolia	south	1	1
<b>Four Populations</b>				
Western Mongolia	Southern Mongolia	west	2	4
Western Mongolia	Northern Qinghai/Gansu	northwest	0	0
Western Mongolia	Middle Qinghai	northwest	0	0
Southern Mongolia	Western Mongolia	east	7	6
Southern Mongolia	Northern Qinghai/Gansu	north	0	0
Southern Mongolia	Middle Qinghai	north	0	4
Northern Qinghai/Gansu	Southern Mongolia	south	0	0
Northern Qinghai/Gansu	Western Mongolia	southeast	0	0
Northern Qinghai/Gansu	Middle Qinghai	south	3	8
Middle Qinghai	Northern Qinghai/Gansu	south	2	7
Middle Qinghai	Western Mongolia	southeast	0	0
Middle Qinghai	Southern Mongolia	north	0	0

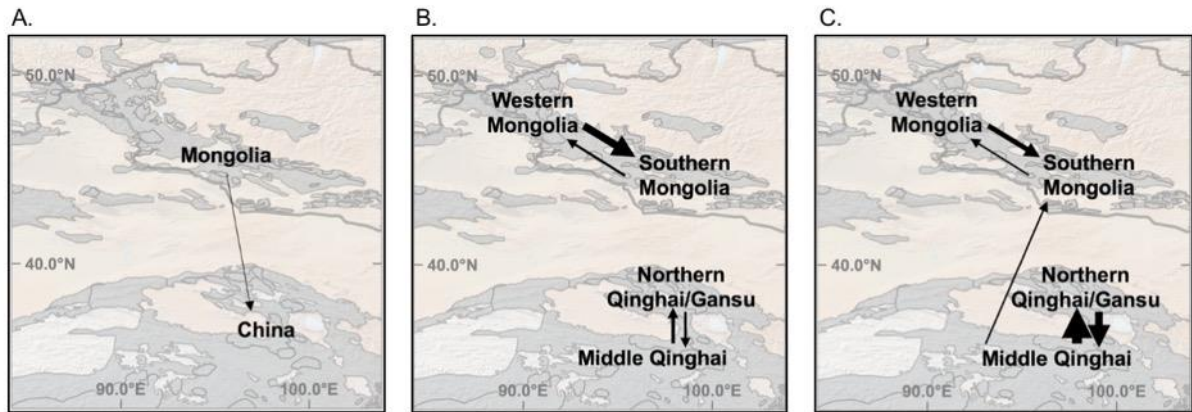


Figure 12. The relative number of genetic misassignments ranging from 0 (no arrow) to 8 (boldest arrow) between any two given populations at two hierarchal tiers with two data analysis methods used with arrow point indicative of genetic dispersal direction A) evidence of recent genetic dispersal from Mongolia to China as found in both GenAIEx and STRUCTURE analysis; B) evidence of recent genetic dispersal between the four populations as found using GenAIEx; and C) evidence of recent genetic dispersal between the four populations as found using STRUCTURE.

#### Population structure without regard to sampling

The K-means algorithm run with increasing values of K revealed the lowest BIC score to be at four clusters (Figure 13) that corresponded to the groups initially defined *a priori*. The PCoA plot of the four population clusters done in GenAIEx showed broad separation of samples from China and Mongolia with marginal overlap between the two (Figure 14A). A PCA scatterplot created in *adegenet* examining how the four clusters related to one another through space revealed clear differentiation between Mongolia and China, with substantially more overlap for the two clusters within each country (Figure 14B). These plots also revealed the slightly higher similarity between Southern Mongolia and the two Chinese populations.

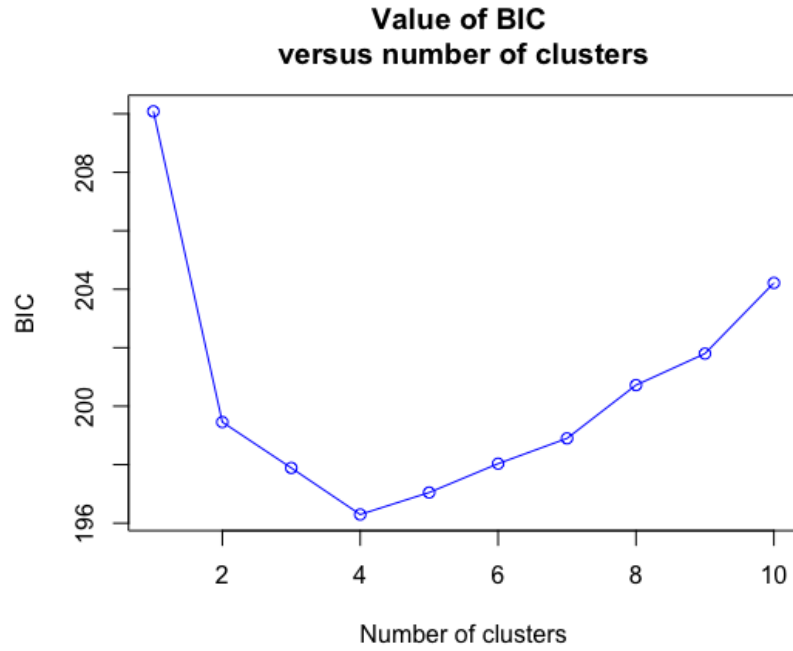


Figure 13. The Bayesian information criterion (BIC) value plotted against the number of genetic clusters examined in the dataset. The smallest BIC value indicates the appropriate number of clusters to be retained.

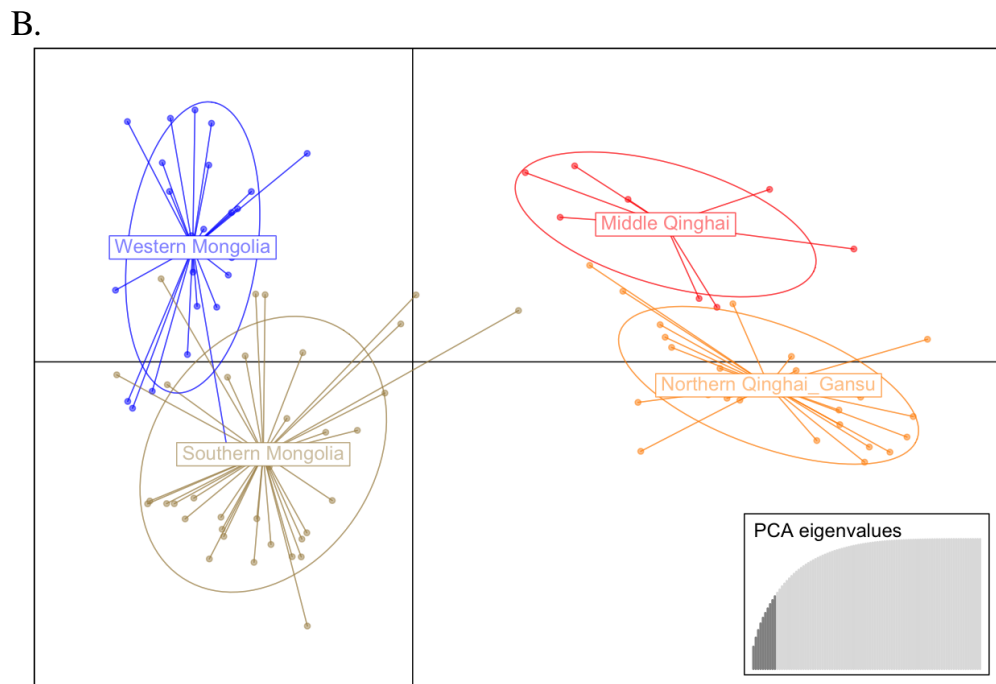
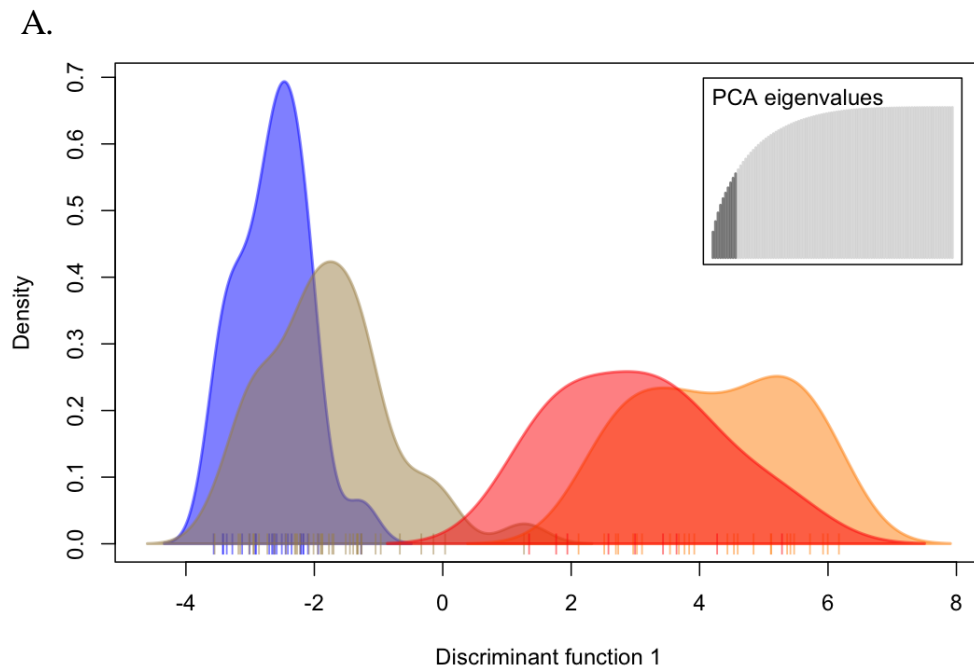


Figure 14. A) Genetic relationships between Western Mongolia, Southern Mongolia, Middle Qinghai, and Northern Qinghai/Gansu for one discriminant function; and B) the PCA scatter plot showing the relationships between samples and their respective genetic clusters with the x axis showing relative genetic differences between populations in China and Mongolia and the y axis showing relative genetic differences between the two genetic clusters in Mongolia as well as the two genetic clusters in northwestern China.

Compared to the DPAC analysis, results from STRUCTURE supported that the

most significant differentiation occurred at a higher scale, with the greatest support for division into two genetic clusters (Figure 15). Samples in Cluster 1 (blue) consisted of individuals from Mongolia and Cluster 2 (orange) consisted of snow leopards from Mongolia (Figure 7A). Ancestry plots were also examined at  $K = 4$  (Figure 16A) to evaluate whether this supported the same populations structure signals as the  $F_{ST}$ , population assignment, and DAPC analysis. When STRUCTURE was forced to divide into 4 genetic clusters the patterns were as follows: Cluster 1 (purple) consisted of primarily individuals from Western Mongolia, but included four individuals with a greater degree of genetic information associated with Southern Mongolia. Cluster 2 (blue) consisted almost exclusively of individuals from Southern Mongolia with snow leopards in Burkhan Buudai to the west to Gurvan Saikhan to the east having a large amount of the genetic information associated with Western Mongolia present. Cluster 3 (orange) consisted of about half the individuals sampled in Northern Qinghai and all the individuals sampled in Middle Qinghai indicating more connectivity in this area than across Mongolia. Cluster 4 (green) only consisted of individuals from Yanchiwan (the most westerly site from Northern Qinghai/Gansu). In Southern Mongolia, there were some individuals that had small levels of ancestry from the Cluster 3 distributed in China ( $0.05 < q < 0.2$ ). Similarly, there were a few snow leopards in China that had limited levels of ancestry from the two clusters distributed in Mongolia. The ancestry plots from the DAPC analysis at  $K = 4$  showed similar patterns with the exception of Northern Qinghai/Gansu and Middle Qinghai, which split into the two identified groups based on geographic origin as opposed to assigning a fourth genetic cluster to the seven individuals from Yanchiwan in the STRUCTURE plot (Figure 16B).

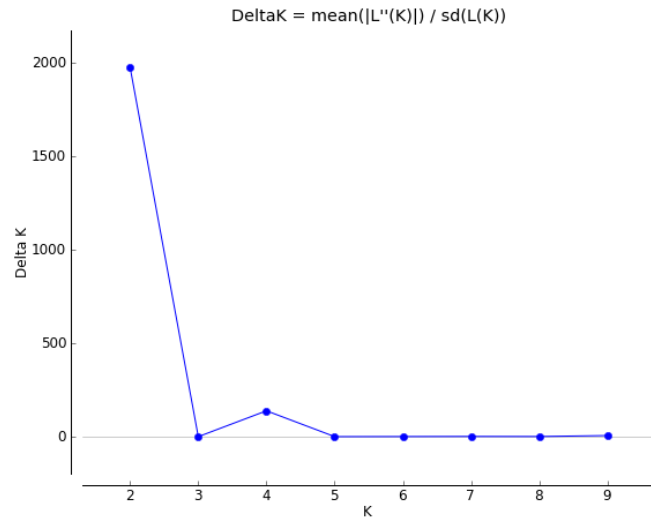


Figure 15. Peaks for  $\Delta K$  for snow leopard samples collected from China and Mongolia. The highest peak is indicative of the most appropriate number of genetic clusters for the microsatellite dataset of 97 snow leopards living in Mongolia and northwestern China.



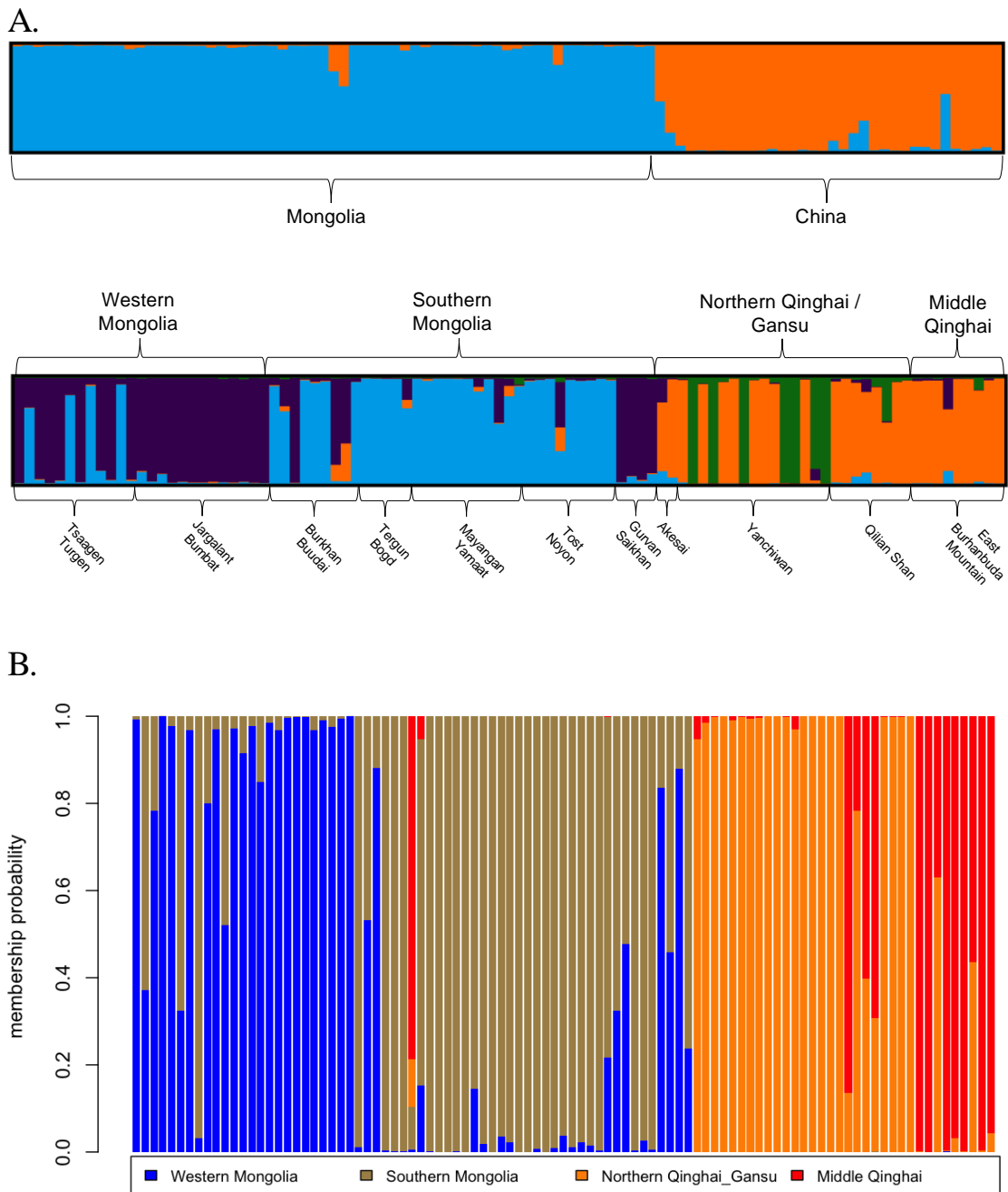


Figure 16. Genetic clusters of snow leopard genetic profiles from China and Mongolia with A) Bar plots of STRUCTURE results with maximum statistical support as determined by  $\Delta K$  of two genetic clusters and maximum statistical support as determined by Bayesian information criterion (BIC) values of four genetic clusters; and B) a compplot resulting from DAPC showing membership probabilities of each individual with the four genetic clusters defined in the dataset.

## Discussion

Understanding landscape connectivity is critical for designing effective conservation action plans, yet it is lacking for the threatened snow leopard. Landscape genetics provides empirically derived data for understanding connectivity, the impact of biogeographical barriers, dispersal, and diversity across habitat patches distributed within a larger metapopulation (Levins, 1969). Analysis of 24 microsatellite loci revealed snow leopards in Mongolia and northwestern China have low levels of genetic diversity, and substantial genetic differentiation between the countries, supporting previous phylogeography work (Jan E. Janecka et al., 2017). Although there was some limited evidence of occasional dispersal and low-level admixture, the main landscape connectivity occurs within the Altai and Gobi-Altai of Mongolia and in China within the mountain chains of Qinghai and Gansu Provinces.

### Hardy-Weinberg equilibrium and null alleles

When samples were pooled together, all loci were found to be out of HWE. When divided into smaller populations, the number of loci out of HWE decreased, indicative of the Wahlund effect. The Wahlund effect results in lower observed heterozygosity relative to expected heterozygosity when individuals from multiple breeding populations are mixed (Waples & Allendorf, 2015). This is intuitive in our dataset, as evident by the population structure observed in subsequent analyses within snow leopards in this region.

Null alleles have a potential for impacting results, causing  $H_E$  to be higher than  $H_0$  and leading to departures from HWE. The primary cause of null alleles is PCR amplification failure due to mutations at primer binding sites, which is more likely when

using microsatellites from other species (Waples & Allendorf, 2015). The primers used in this study were derived from microsatellite loci previously isolated in the domestic cat (*Felis catus*). However, the flanking regions for all loci were sequenced in the snow leopard and primers were redesigned using the snow leopard sequence (Janečka et al., 2008; Jan E. Janecka et al., 2014, 2017). In addition, the primers were moved closer to repeats to reduce amplicon length. The loci were renamed by changing the FCA prefix to PUN. These strategies substantially reduce the likelihood of null alleles. The PUN microsatellites have been successfully used in numerous studies examining snow leopard genetics (Aruge et al., 2019; Jan E. Janecka et al., 2017; Karmacharya et al., 2011; Y. Zhang et al., 2019). Nonetheless, we detected null alleles in several loci. To avoid any potential bias, we excluded two loci for which the null alleles were estimated to be over  $p > 0.5$  for analyses pertaining to population assignment, IBD,  $F_{ST}$ , population structure, and genetic diversity metrics (Carlsson, 2008; Dakin & Avise, 2004; Huang et al., 2016; Waples & Allendorf, 2015).

#### Comparison of genetic diversity to other snow leopard studies and to other felid species

Previous microsatellite studies on the jaguar (*P. onca*) (29 loci,  $A_N = 8.31$ ,  $H_E = 0.739$ ,  $N = 42$ ) (Eizirik et al., 2001), tiger (*P. tigris*, 30 loci  $A_N = 7.30$ ,  $H_O = 0.537$ ,  $H_E = 0.739$ ,  $N = 113$ ) (Luo et al., 2004), and common leopard (*P. pardus*) (25 loci,  $A_N = 11.08$ ,  $H_E = 0.793$ ,  $N = 75$ ) (Uphyrkina et al., 2001) observed higher genetic diversity than the estimates for snow leopards analyzed in this study ( $A_N = 6.29$ ,  $H_O = 0.374$ ,  $H_E = 0.567$ ). The lower diversity in this felid is consistent with previous studies. Janecka et al. (2017) sampled 70 individuals across species range and reported similarly low values ( $A_N = 5.8$ ,

$H_O = 0.433$ ,  $H_E = 0.568$ ). Zhang et al. (2019) reported genetic diversity for 26 snow leopards in Qilian Shan National Park in China ( $A_N = 3.47$ ,  $H_O = 0.371$ ,  $H_E = 0.541$ ) and 15 snow leopards in Sanjiangyuan National Park in China across 15 microsatellites ( $A_N = 3.03$ ,  $H_O = 0.379$ ,  $H_E = 0.541$ ). Karmacharya et al. (2011) examined ten snow leopard positive samples across six microsatellites in Nepal and reported the highest levels of genetic diversity ( $H_O = 0.579$ ,  $H_E = 0.631$ ). Caragiulo et al. (unpublished) used DNA from the blood of five snow leopards anesthetized for GPS collaring in the Tost Mountains of southern Mongolia across 12 microsatellite loci and reported low genetic diversity ( $A_N = 2.92$ ,  $H_O = 0.536$ ,  $H_E = 0.544$ ). This pattern was also observed in SNP analysis using the same samples genotyped by Caragiulo et al. ( $A_N = 1.12$ ,  $H_O = 0.047$ ,  $H_E = 0.042$ ).

#### Modern day molecular signature of a genetic bottleneck.

Taken together these results are consistent with the modeling done in Janecka et al. (2017) that detected a major genetic bottleneck in this species approximately 8,000 years ago. It is likely that the low diversity in snow leopards is a result of this more ancient bottleneck caused by fluctuating habitat in the mid Pleistocene, similar to that observed in North American populations of mountain lions (*Puma concolor*) and range-wide in cheetahs (*Acinonyx jubatus*). However, the already low diversity potentially makes snow leopards more sensitive to further loss in genetic diversity that would be caused by contemporary population reductions and isolation. These reductions would have the potential to further reduce adaptability in snow leopards and cause inbreeding depression.

## Genetic differentiation and inferred connectivity between *a priori* geographic areas

Genetic differentiation estimated from pairwise  $F_{ST}$  was highest between Middle Qinghai and Western Mongolia and lowest between Southern Mongolia and Western Mongolia. Because IBD was not significant, the greatest amount of genetic differentiation among the two most geographically distant populations is more reflective of the landscape features between them. These include a series of small “stepping stones” that serve as suitable snow leopard habitat moving eastward from western to southern Mongolia, followed by the Gobi Desert in northwestern China in which there is very little to no suitable habitat and a gap in snow leopard range. Substantially reduced connectivity in the boundary region of the two countries is indicated by the 3-fold higher  $F_{ST}$  between Southern Mongolia and Northern Qinghai compared to that between Southern and Western Mongolia, despite being approximately the same distance apart (~500km).

The smaller degree of genetic differentiation across Mongolia suggests that habitat blocks running from the Altai Mountain range in the west towards Gurvan Saikan and the Tost and Noyon Mountains in the south act as “stepping stones” and are able to provide connectivity for individual snow leopard movement to peripheral habitat patches. In a similar fashion, in Northern Qinghai/Gansu where the Tibetan Plateau suddenly rises >3,000m above from the desert, the Qilian Shan mountain range run east-west and then connects south until reaching the eastern end of the Kunlun Shan in the middle of Qinghai leading to connectivity within China. Middle Qinghai and Northern Qinghai/Gansu had slightly higher genetic differentiation compared to Mongolia, reflective of more barriers to movement. Another factor leading to slightly lower connectivity is that there may be more high-quality habitat and resource availability in

Qinghai compared to parts of Mongolia. For example, Yanchiwan (Subei County, Gansu Province, China) is considered a snow leopard “hotspot” where the home ranges of multiple snow leopard individuals overlap (Y. Zhang et al., 2019). Such high-quality habitat may be able to support more individuals and reduce the need to disperse out of the area. However, this needs to be interpreted with caution as samples sizes were smaller in China.

#### Individual snow leopard assignment to population of origin

Demographic connectivity is important to consider when shaping conservation action plans for populations with relatively low genetic diversity (Lowe & Allendorf, 2010). Genetic approaches including the assignment of individuals to their most likely population of origin based on multi-locus genotypes has become insightful for estimating dispersal because misassignments and dispersal are correlated and thus, higher misassignment rates indicate more movement across a landscape (Berry et al., 2004; Paetkau et al., 2004). Two methods were used to determine population origin at two spatial scales: (1) Mongolia and northwestern China, (2) Western Mongolia, Southern Mongolia, Northern Qinghai/Gansu, and Middle Qinghai. When analyzed across a larger region, only one misassignment was observed; a snow leopard sampled in China was assigned to Mongolia. This lack of misassignment is consistent with the pairwise  $F_{ST}$  values that indicate limited movement in this region.

When divided into four populations, a greater number of misassignments were identified, with more dispersal events detected in STRUCTURE as opposed to GenAIEx. In both analyses, consistent with the low  $F_{ST}$ , the most misassignments occurred between

Western and Southern Mongolia, with a greater number of individuals sampled in Southern Mongolia assigning to Western Mongolia, suggestive of more movement eastward. This is consistent with potential source-sink dynamics in the region. There is more contiguous habitat in Western Mongolia. Snow leopards may be dispersing out of these core areas to smaller habitat patches to the southeast. Within China, misassignments were observed between Northern Qinghai/Gansu and Middle Qinghai in approximately equally numbers in either direction. Analyses in STRUCTURE revealed more dispersal events overall in China than with GenAIEx.

#### Connectivity within China and Mongolia versus between China and Mongolia

Snow leopard habitat becomes more fragmented moving from the Altai Mountains in Western Mongolia down to the Gobi-Altai in Southern Mongolia. In Gurvan Saikhan and Tost and Noyon Uul the habitat patches are small. Despite that, snow leopard densities are high. There is ongoing debate whether these populations are connected down to Northern Qinghai/Gansu. The  $F_{ST}$  and misassignment test suggests that snow leopards in search of suitable habitat would be more likely to move northwest as there are larger patches of habitat (Arts Bogd and Baga Bogd) that are only 100km apart. In contrast, to the south, snow leopards have to cross >400km of unsuitable habitat that represents substantial “high-cost” distances between even smaller stepping-stone patches before they get to higher quality habitat on the northern Tibetan Plateau (Riordan et al., 2016). Such movement could occasionally occur, resulting in the introgression observed in the STRUCTURE analysis. However, this introgression is likely a result of sweepstakes dispersal events that lead to small levels of admixture, rather than frequent

dispersal. In a study of white-footed mice (*Peromyscus leucopus*), emigration rates were found to be higher from smaller patches than from larger patches, presumably because density was higher in the smaller patches, encouraging individuals to vacate (Anderson & Meikle, 2010). It is possible that the smaller patches in Southern Mongolia may occasionally experience migration out of the areas when snow leopard density increases beyond carrying capacity, providing a pulse of migrants moving out of the area, but these would still be more likely move in a northwest direction.

#### Patterns in population structuring with two genetic clusters and four genetic clusters

We used two approaches to determine the most significant number genetic clusters (K). The DAPC approach identified  $K = 4$  as most significant, which were largely consistent with the above division of samples into the 4 *a priori* populations. In contrast, in the STRUCTURE analysis although at  $K = 4$  the composition of the genetic clusters was similar to DAPC, the most significant division was  $K = 2$ . The rationale for deciding on the most ecologically meaningful populations divisions for conservation and management purposes need consider the scale at which the genetic variation is viewed, the levels of admixture, and the levels of genetic divergence.

The DAPC analysis found genetic structuring across the four populations examined (Western Mongolia, Southern Mongolia, Middle Qinghai, Northern Qinghai/Gansu). However, the break between samples from Mongolia and samples from China is blunt and obvious, with only one individual from Mongolia harboring genetic variation from China, and only two individuals from China harboring genetic variation from Mongolia. In contrast, samples in the genetic clusters within each reveal much



higher levels of introgression. Similarly, the PCA scatter plot resulting from DAPC analysis indicates that the greatest difference in genetic differentiation is between Mongolia and China, rather than between clusters that occur within each respective country.

At  $K = 2$  for the STRUCTURE plot, the genetic clusters correspond to the Mongolia and China population with only six out of 97 individuals showing low-to-moderate levels of introgression and no admixture. This is the most significant division in STRUCTURE indicating the greatest levels of divergence at this grouping, consistent with the PCA plots,  $F_{ST}$ , and misassignment tests that indicate lack of connectivity between China and Mongolia. When STRUCTURE was forced to divide the samples into four clusters, the results showed one predominant genetic cluster in Southern Mongolia, one in Western Mongolia, and one in China. Substantial admixture was evident between Southern Mongolia and Western Mongolia. Within China, the STRUCTURE plot showed an unexpected clustering pattern in which seven individuals sampled in Yanchiwan were almost exclusively comprised of their own genetic cluster. This may be an artifact of a higher number of samples collected from the area, forcing the STRUCTURE plot to adopt one of the four clusters within that more heavily sampled region. However, it could also be that unique alleles are present in this particular population.

#### Contribution of findings to snow leopard taxonomy

This finer-scale analysis of genetic structure provides inferences on landscape connectivity between China and Mongolia consistent with the taxonomic recognition of the proposed Northern (*P. uncia irbis*) and Central subspecies (*P. uncia uncioides*) in

China, of which subspecies status recognition would have numerous conservation outcomes for snow leopards (see Chapter 2.2 of this dissertation).

### Future needs for snow leopard landscape genetics

The field of landscape genetics is increasingly broadening in scope and can play an instrumental role in shaping conservation and management decisions (Montgelard et al., 2014). Unfortunately, the application of genetics for snow leopards has been hesitantly embraced by NGOs and range country government entities, despite its ability to provide valuable information on elusive and cryptic species, and increasing opportunities for including the local communities in wildlife monitoring efforts (Jan E. Janecka et al., 2020; Rodgers & Janečka, 2013). Wider use of genetics is imperative to realistically assess the snow leopard population range-wide (Jan E. Janecka et al., 2017). However, this will require coordinated efforts for training and standardization across range countries. Microsatellites have historically been difficult to work with due to genotyping errors and difficulty faced when combining data from different studies and labs (MCKELVEY & SCHWARTZ, 2004). As next-generation sequencing (NGS) technologies continue to develop, the use of single nucleotide polymorphisms (SNPs) will likely outpace microsatellites in application to landscape genetic studies (Montgelard et al., 2014). For snow leopards, the recent design of a hypervariable SNP panel and additional SNPs identified throughout the snow leopard genome provides a meaningful step forward in using SNPs for addressing knowledge gaps for this elusive species (Jan E. Janecka et al., 2020; Janjua et al., 2020). Unfortunately, sequencing and analysis remains prohibitively expensive and genotyping SNPs in scat has yet to be done on a large-scale,

and thus microsatellites will likely continue to play a large role in our understanding of snow leopard genetics for some time to come.

### Conclusions and conservation action plan suggestions

The Gobi Desert serves as a substantial barrier to snow leopard movement and gene flow between China and Mongolia (Jan E. Janecka et al., 2017; Riordan et al., 2016). However, admixture and introgression reveal that the stepping-stone habitat within Mongolia and China serves as an important mechanism in maintaining landscape connectivity in each of these regions. With current information, management actions should focus on maintaining populations and corridors where populations are naturally connected (i.e., across Mongolia and within Northern and Middle Qinghai), instead of trying to establish corridors where there is little evidence for historical dispersal (i.e., between China and Mongolia). Such efforts would not only provide the benefit of maintaining genetic diversity, but also demographic benefits that would stabilize local populations through maintenance of metapopulation dynamics.

### Author Attributions

Charlotte Hacker, Yuguang Zhang, Yu Zhang, Matt Jevit, Bariushaa Munkhtsog, Bayaraa Munkhtsog, Jan Janecka, Naranbaatar Galsandorj, Chimedoorj Buyanaa, Gantulga Bayandonoi, Munkhtogokh Orchijav, and Liji Wu collected scat samples. Bariushaa Munkhtsog, John D. Farrington, and Diqiang Li provided administrative support. Charlotte Hacker, Yu Zhang, Yunrui Ji, Cong Wei, Benita Jebeckumar and Jan Janecka extracted DNA from scat samples. Jan Janecka modified DNA extraction protocol.

Charlotte Hacker and Jan Janecka genotyped samples. Charlotte Hacker and Jan Janecka performed data analyses. Charlotte Hacker, Jan Janecka, and Rodney Jackson interpreted data. Charlotte Hacker wrote manuscript. Jan Janecka, Rodney Jackson, and Charleen Gavette edited manuscript

## Chapter 3

### Goal 2

## Investigation of carnivore diet in snow leopard habitat using DNA metabarcoding

### Chapter 3.1

#### Goal 2 – Subgoal 1

#### Snow leopard diet composition range-wide

Adapted from Hacker *et al.* (2021) *Regional comparison of snow leopard diet using DNA metabarcoding*. Biodiversity and Conservation. DOI:10.1007/210531-021-02118-6

#### Introduction

#### Predicted changes in snow leopard habitat

Models based on occurrence records from 1983 to 2015 predict a net reduction of between 8 to 23% of currently identified snow leopard range (~2.2 million km ) by 2070 (J. Li, McCarthy, et al., 2016). Within the remaining area, connectivity will be reduced with a greater number of isolated habitat patches (J. Li, McCarthy, et al., 2016). Many of these losses are predicted to be disproportionately greater in some range countries. For example, China holds roughly 50% of known snow leopard range and is expected to lose between 14 to 42% (J. Li, McCarthy, et al., 2016). Such large-scale changes will likely cause reductions and distribution shifts of snow leopard, their prey, and biodiversity, heavily influencing predator-prey dynamics. Diet analysis provides information related to

animal ecology and evolution (Symondson, 2002). Thus, knowledge of snow leopard diet is especially needed in the context of climate change.

### Snow leopard livestock depredation and its role in conflicts with humans

While snow leopards are thought to prefer wild prey, they often consume livestock (Aryal et al., 2014; Bagchi et al., 2020; Chetri et al., 2017; Johansson et al., 2015; C. Mishra et al., 2016; Wegge et al., 2012), which leads to one of the largest sources of conflict between snow leopards and humans. Pastoralism is the primary source of income across the snow leopard range (McCarthy & Chapron, 2014). Even families that work in different sectors, such as harvesting caterpillar fungus (*Ophiocordyceps sinensis*) maintain livestock for their own sustenance and status (John D Farrington, 2016; Winkler, 2020). Livestock depredation causes financial burdens that promote negative attitudes and can lead to retaliatory killing (Hussain, 2003; Jackson & Wangchuk, 2001). Retaliation eliminates specific individuals, and herders sometimes attempt to sell body parts in illegal wildlife markets in order to recover some of their financial losses (Nowell et al., 2016). Understanding snow leopard dependency on livestock is therefore key for mitigating conflict and wildlife crime.

### Introduction to DNA barcoding

Molecular analysis via DNA barcoding provides a method that overcomes challenges many of the challenges associated with more traditional methods such as microhistology and local interviews (Bohmann et al., 2011; Valentini et al., 2009). NGS DNA metabarcoding approach has been used with varying success in carnivores (Deagle

et al., 2005; Shehzad, McCarthy, et al., 2012; Shehzad, Riaz, et al., 2012; Xiong et al., 2017). The first application for snow leopards was from the Tost Uul of southern Mongolia using MT-RNR1 (Shehzad, McCarthy, et al., 2012). Lack of divergence in this marker between domestic sheep (*Ovis aries*) and argali (*O. ammon*) necessitated sequencing a second marker, cytochrome *b* (MT-CYB). However, the distinction between these two species was based on a single 1-bp substitution, which is not a sufficient number of differences to reliably discriminate taxa, and may not be a fixed difference in other regions of Asia. Similar issues arise when using these gene segments for domestic goat (*Capra aegagrus hircus*), Siberian ibex (*C. sibirica*), and Himalayan tahr (*Hemitragus jemlahicus*). Thus, a more informative marker is needed to differentiate wild versus domestic ungulates across snow leopard range.

#### The potential utility of cytochrome *c* oxidase subunit 1

Cytochrome *c* oxidase subunit 1 (MT-CO1) is widely used for DNA metabarcoding because it exhibits substantial divergence between closely related species (Hebert et al., 2003). This gene has fewer indels for a more robust alignment, as compared to MT-RNR1 (King et al., 2008), and the 3rd codon position substitution rate is three-fold higher than in rRNA genes (Bucklin et al., 2011). MT-CO1 has been used to study diet in African free-tailed bats (*Chaerephon pumilus*) (Bohmann et al., 2011), pigs (*Sus scrofa*) (Robeson et al., 2018), warblers (King et al., 2015), and a variety of fish (Leray et al., 2015), but has not yet been applied to large carnivores.

#### Study goals and overall impacts

We embarked on an effort to expand the potential for molecular barcoding to study snow leopard prey use. Our objectives were to: 1) validate MT-CO1 suitability for discerning wild versus domestic goats and sheep; 2) determine diet composition from scat in four range countries; and 3) compare diet and livestock dependency in the two areas where we had sufficient sample sizes (i.e., Mongolia versus Pakistan). Our findings further elucidate snow leopard diet and expand molecular toolkits for assessing factors contributing to livestock depredation, understanding snow leopards prey utilization in changing landscapes, and efficacy of interventions.

## Materials and Methods

### Study areas, sample collection, DNA extraction, and molecular species identification

A total of 165 high quality scats of snow leopard originating from four countries were collected (79 samples from Pakistan (central coordinates: 36.57067° N, 74.78634° E), 64 from Mongolia (central coordinates: 44.89448° N, 101.57734° E and 43.638609° N, 103843341° E), 11 from China (central coordinates: 38.25599° N, 99.15692° E and 31.50613° N, 88.16854° E), 11 from Kyrgyzstan (central coordinates: 41.92028° N, 78.53944° E) (Figure 17; Table 11). Sampling methods followed those described in (Janečka et al., 2008; Janečka, Munkhtsog, et al., 2011). See Chapter 2.1 for DNA extraction and molecular species identification.



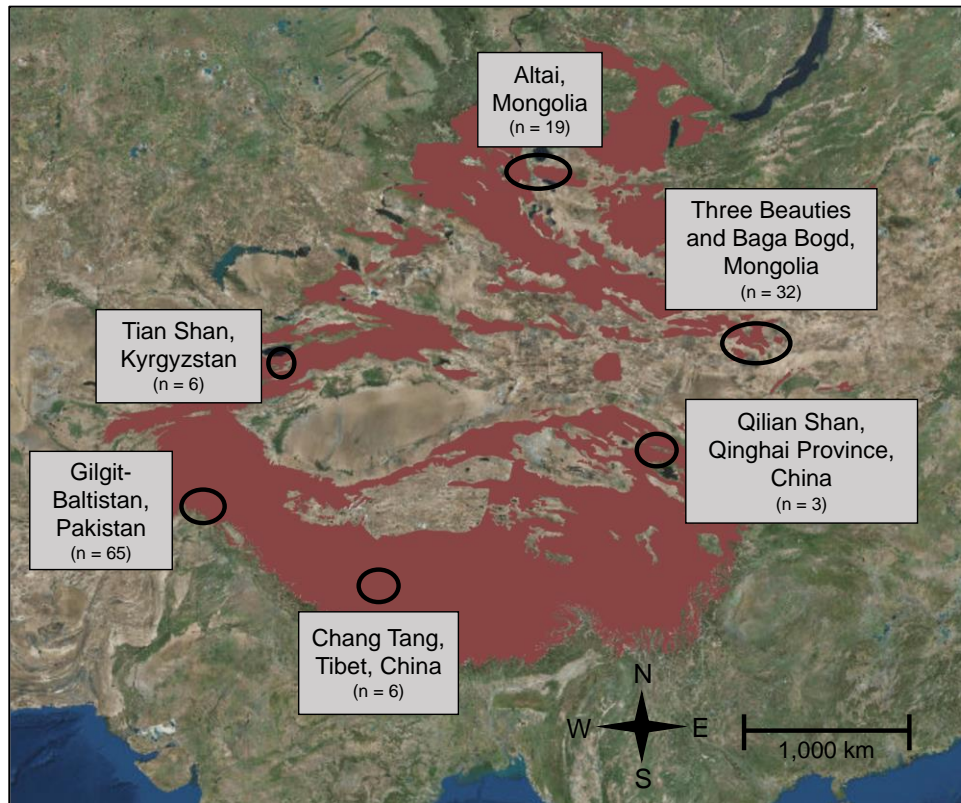


Figure 17. Snow leopard scat collection sites for molecular diet analysis with their respective number of analyzed samples. Spatial data adapted from the IUCN Red List of Threatened Species Version 2019-1.

Table 11. Further description of the study sites presented in Figure 1 of the manuscript where scat samples used for dietary analysis were collected.

Study Site and number of samples examined	Central Coordinates	Geographic Features	Primary Potential Snow Leopard Wild Prey Items Present
Altai, Mongolia (n = 19)	44.89189° N, 101.58006° E	Jagged mountains with flat summits separated by basins. Arid with limited vegetation (Cunningham, 2005)	Siberian ibex ( <i>Capra sibirica</i> ), marmot ( <i>Marmota</i> spp.), argali ( <i>Ovis ammon</i> ), snowcock ( <i>Tetraogallus</i> spp.) (Munkhtsog et al., 2016)
Three Beauties and Baga Bogd, Mongolia (n =32)	43.638609° N, 103.843341° E	Three Beauties: Three lower-altitude linear massifs with dry stream beds. Arid with semi-shrubs, shrubs, and grasses (R. P. Reading et al., 1999)	Siberian ibex, argali, marmot (only in Baga Bogd), chukar partridge ( <i>Alectoris chukar</i> ), pika ( <i>Ochotona</i> spp.) (R. P. Reading et al., 1999; Retzer & Nadrowski, 2002)
	44.89448° N, 101.57734° E	Baga Bogd: One longer massif with steep minimally eroded rock faces and deep river bed cuts (Vassallo et al., 2007)	
Qilian Shan, Qinghai Province, China (n = 3)	38.25599° N, 99.15692° E	A series of three parallel mountain ranges characterized by eroded slopes and rocky narrow passages. Shrubs and grasses lead into alpine meadow at higher elevations (Schaller, Junrang, et al., 1988)	Blue sheep ( <i>Pseudois nayaur</i> ), red deer ( <i>Cervus elaphus</i> ), white lipped deer ( <i>Cervus albirostris</i> ), marmot (Sullivan et al., 2018)
Chang Tang, Tibet, China (n = 6)	31.50613° N, 88.16854° E	Arid and cold with sparse vegetation dominated by cold-desert grasslands and saline lakes. A vast undisturbed continuous landscape with broad rolling steppes interrupted by mountains and large basins (D. J. Miller & Schaller, 1996; Schaller, Junrang, et al., 1988)	Tibetan gazelle ( <i>Procapra picticaudata</i> ), Tibetan antelope ( <i>Pantholops hodgsonii</i> ), blue sheep, argali, kiang ( <i>Equus kiang</i> ), wild yak ( <i>Bos mutus</i> ), marmot, pika (D. J. Miller & Schaller, 1996)
Tian Shan, Kyrgyzstan (n = 6)	41.92028° N, 78.53944° E	Characterized by long, parallel mountains and valleys with extensive ice fields and water ways with harsh winters (J.D. Farrington, 2005)	Siberian ibex, argali, marmot, red deer (J.D. Farrington, 2005; C. L. Zhou et al., 2015)
Gilgit-Baltistan, Pakistan (n = 65)	36.57067° N, 74.78634° E	Cold winters with short warmer summers. Characterized by steep peaks and slopes (Ali et al., 2017)	Siberian ibex, markhor ( <i>Capra falconeri</i> ), musk deer, marmot, pika (Anwar et al., 2011)

MT-CO1 *Caprinae* marker design

A total of 241 reference MT-CO1 sequences of wild and domestic ungulates that overlap snow leopard range were downloaded from GENBANK and imported into MEGA7.0 (Kumar et al., 2016). Additional taxa were added to root the phylogenetic tree and resolve species relationships. Multiple sequence alignment was performed using MUSCLE with default parameters (Edgar, 2004). Pairwise nucleotide differences were estimated using Distance Estimation Analysis and the Maximum Composite Likelihood model with uniform rates among sites. Only unique species haplotypes were retained. A phylogenetic tree was constructed in MEGA7.0 using Maximum Likelihood (GTR + Gamma + Invariant sites model) with 1,000 bootstrap replicates. The reconstructed phylogeny was evaluated, over-represented haplotypes removed, and clades checked against an accepted Bovidae phylogeny (Bibi, 2013) to ensure that there were no mislabeled or erroneous sequences.

Nucleotide substitution rates were estimated for each aligned site using Maximum Composite Likelihood under the Tamura-Nei model to determine positions with the highest levels of differentiation. The site-specific substitution rate was imported into R (version 3.5.3) and a sliding window analysis completed (runmed, 11-bp window size) (Proutski & Holmes, 1998). The running median was plotted to identify segments with the greatest number of substitutions. The total number of fixed differences between each species pair was calculated for MT-CO1. Species that exhibited high differentiation (>10% divergence) were removed to focus on finding a segment between 200 and 250-bp that could differentiate closely related taxa. These aligned segments were extracted and evaluated by reconstructing a Maximum Likelihood phylogenetic tree with 100 bootstrap replicates.

MT-CO1 primers were designed in more conserved sites flanking the most variable segments using Primer3 (Untergasser et al., 2012). Primer annealing sites were examined in the full alignment and only those with less than 2 mismatches with other taxa were retained to ensure primers amplified in other ungulates (Murphy & O'Brien, 2007). Degenerate primers were designed to account for variable positions in annealing sites (Murphy & O'Brien, 2007). Illumina adapters were added to the 5' end of primers. A total of two forward and three reverse primers with 34-bp Illumina adapters were designed and tested as three different pairs: MT-CO1-379F & MT-CO1-466Rd (107-bp product), MT-CO1-379F & MT-CO1-604Rd (262-bp product), and 716F & COI-897Rd (201-bp product). The Illumina forward overhang was 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG and the reverse overhang was 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG.

#### PCR for diet analysis

Eight samples were run in duplicate with and without snow leopard-specific MT-RNR1 oligoblockers to assess reduction in amplification of snow leopard MT-RNR1 sequences. The three pairs of MT-CO1 primer candidates were tested and amplicons visualized on a 1.5% agarose gel. The most robust MT-CO1 primer pair (MT-CO1-379F & MT-CO1-604Rd) and MT-RNR1 primers (12SV5F/12SV5R, Riaz et al. 2011) were used to amplify the molecular markers in separate PCR reactions containing 1.5µL of DNA template, 7.94µL of KAPA HiFi HotStart ReadyMix (2X) [Kapa Biosystems, Wilmington, MA, USA], 0.16µL of 20µM forward primer, 0.16µL of 20µM reverse primer, and 5.2µL of PCR grade water. PCR conditions consisted of 95°C for 3 min, 35

cycles of 95°C for 30 sec, 60°C for 30 sec, and 72 °C for 30 sec, followed by 5 min extension step at 72°C and 4 °C hold.

### Next-Generation Sequencing

#### *Nextera XT amplicon library preparation*

Amplicons were purified with Agencourt AMPure XP beads [Beckman Coulter, Inc., Brea, CA, USA]. The Nextera<sup>®</sup> XT Index Kit [Illumina, Inc., San Diego, CA, USA] was used to incorporate unique barcodes to amplicons from different samples via a second indexing PCR. A total of 1.5µL of each forward and reverse index primer and 1.5µL of the bead-cleaned amplicons from the first PCR were pipetted into the appropriate well. To each well, 3µL of PCR grade water and 7.5µL of KAPA HiFi HotStart ReadyMix (2X) were added and a second PCR was run at 95°C for 3 min, followed by 8 cycles of 95°C for 30 sec, 55 °C for 30 sec, and 72 °C for 30 sec, followed a 72°C extension step for 5 min and 4 °C hold.

Gel excision was used to extract and purify the indexed amplicons. A total of 2µL of barcoded amplicons from 96 reactions in each PCR plate were pooled. A 20µL aliquot of these pooled amplicons was combined with 6µL 3X DNA loading dye and electrophoresed at 90 volts for 120 min on a 2% TAE agarose gel stained with GelRed [Phenix Research Products, Thomas Scientific, Swedesboro, NJ, USA]. Gels were visualized under blue light and from each pooled sample lane the region between 90-bp and 350-bp was excised. The barcoded pooled amplicons were recovered from the agarose excision using the MinElute Gel Extraction Kit [QIAGEN, Hilden, Germany] yielding the final Illumina library ready for sequencing. A duplicate subset of 18 samples

were also cleaned using Agencourt AMPure XP PCR purification beads to compare the two purification methods.

#### *DNA quantification, normalization, and sequencing*

A total of 1µL of the purified barcoded ampliconic library was quantified using an Invitrogen Qubit® 2.0 Fluorometer [Thermo Fisher Scientific, Waltham, MA, USA].

Pooled ampliconic libraries were normalized to 2nm. Paired-end sequencing was performed using the Mid Output 300-cycle kit on an Illumina NextSeq 500 by the Health Sciences Sequencing Core (Children’s Hospital of Pittsburgh). For a subset of 18 samples, a second ampliconic library was prepared and sequenced on an Illumina MiSeq with a paired-end run using a V2 Micro Kit at the Janecka Genomics Laboratory (Duquesne University) as a quality control.

#### Data analysis

##### *Reference file construction*

Wild and domestic snow leopard prey items were identified via a literature search (Anwar et al., 2011; Jumabay-Uulu et al., 2014; Khatoon et al., 2017; Lhagvasuren & Munkhtsog, 2000; Shehzad, McCarthy, et al., 2012) and consultation with local experts. All available MT-RNR1 and MT-CO1 sequences for potential prey species were downloaded from NCBI GenBank and BOLD (Barcode of Life Data Systems) and representative haplotypes used to create a reference file that included MT-CO1 from 9 species and MT-RNR1 for 26 other species (Appendix II: Supplemental Material 3; Appendix II: Supplemental Material 4).

### *Diet analysis*

After sequencing, FASTQ sequences were demultiplexed, adapters removed, and reads imported into CLC Genomics Workbench v7.5.1 [CLC bio, QIAGEN, Aarhus, Denmark]. Raw reads were trimmed using a quality score limit of 0.1 and mapped to the reference FASTA file using local alignment with the following parameters - mismatch cost: 2; insertion cost: 3; deletion cost: 3; length fraction: 0.9; similarity: 0.94; non-specific matches mapped randomly. Prey identification for each sample was made based on reference taxa that had the highest number of reads mapped with the fewest mismatches. Species identification was made when reads mapped with >98% similarity. To ensure prey were correctly identified, the consensus sequence for the dominant prey item was extracted and blasted against the nucleotide databases with blastn. Species that had greatest similarity to the consensus sequence were compared to the result from our mapping-to-reference method. In addition, the scat collection sites were compared with the distribution of identified prey species based on CITES Red List range maps to verify they occurred in the region.

Samples in which prey could not be discerned were subjected to a second round of read mapping and analyses at 0.85 length fraction and 0.90 similarity. If still unsuccessful, unmapped reads were further analyzed to rule out a potentially incomplete reference file. This was done by performing a de novo assembly with the following parameters - minimum contig length of 100; mismatch cost: 2; insertion cost: 3; deletion cost: 3; length fraction: 0.9; similarity: 0.98. Consensus sequences were extracted for contigs with the highest number of mapped reads. Low coverage definition threshold was set at 10,000. Nucleotides in sites with conflicting reads were resolved via majority rule

and ambiguous sites were coded with an “N”. The efficacy of oligoblockers towards reducing the number of MT-RNR1 reads mapping to snow leopard, as well as the number of reads and mapped reads between two NGS platforms (NextSeq 500 vs MiSeq) and PCR clean-up methods (gel excision vs magnetic beads) were analyzed using a paired t-test with significance set at  $p < 0.05$  [R studio, version 1.1.442].

Percentages of prey species in diet was calculated by dividing the number of occurrences of any given prey species in the diet by the total number of all occurrences of cumulative prey species. Percentages were presented overall and by country. The country level was selected as most conservation management decisions and legislation are made at the country level. The Simpson’s Diversity Index was used to assess dietary diversity (Simpson 1949).

$$D = 1 \left( \frac{\sum n(n-1)}{N(N-1)} \right)$$

Where  $n$  is the number of each dietary item consumed and  $N$  is the total number of all dietary items. The index ranges from 0 (lowest diversity) to 1 (greatest diversity).

## Results

### Determination of MT-CO1 suitability and primer selection

A total of 241 sequences for seven sheep and goat species were downloaded and redundant sequences removed. The final *Caprinae* MT-CO1 matrix had 104 unique haplotypes, with the number for each species ranging from one to 44. One to 4 representative sequences of each taxa were used to reconstruct a *Caprinae* phylogeny and to create a reduced matrix to identify the most variable gene regions (Figure 18). A



sliding window analysis of the site-specific substitution rate was performed to identify gene segments with the highest number of nucleotide differences among the aligned species. The following seven segments of the MT-COI gene were selected as having the highest potential to differentiate domestic and wild goats and sheep – nucleotide positions 303 to 531 (258-bp), 354 to 636 (282-bp), 404 to 636 (232-bp), 515 to 713 (198-bp), 750 to 950 (200-bp), 841 to 1,100 (258-bp), and 515 to 713 (198-bp). Of the three primers pairs designed to amplify the most variable regions, MT-CO1-379F and MT-CO1-604Rd (MT-CO1-379F – GGAGCAGGAACAGGTTGAA, MT-CO1-604Rd - ATTGTGATRCCAGCTGCYAA) had the most robust PCR amplification with caprine positive controls and did not amplify snow leopard, making the use of blockers unnecessary. With the two 33-bp Illumina adapters on each primer, the final amplicon length was 328-bp. Within this segment of MT-CO1, there are 9 nucleotide differences separating domestic sheep and argali, 3 differentiating domestic goat and markhor (*C. falconeri*), and 12 fixed substitutions between domestic goat and Siberian ibex, resulting in sufficient resolution for species identification.

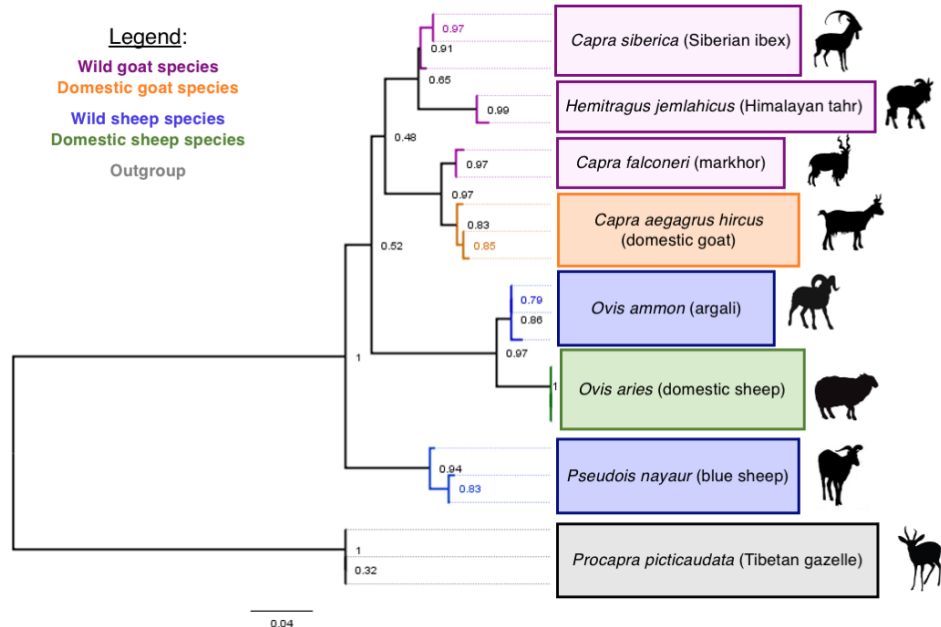


Figure 18. The MT-CO1 maximum likelihood phylogeny of *Caprinae* prey species consumed by snow leopards and the outgroup Tibetan gazelle *Procapra picticaudata* based on 262-bp of a MT-CO1 segment used to differentiate domestic and wild goat and sheep. The region corresponds to 361-bp to 623-bp positions of the *Capra siberica* MT-CO1 reference sequence. Bootstrap values from 100 replicates are denoted on each node.

### Diet composition using MT-CO1 and MT-RNR1

The MT-CO1 primers only amplified goat and sheep, and did not yield MT-CO1 segments for other ungulate families (e.g., *Bovidae*) or mammalian Orders, such as Rodentia (i.e., marmots) and Lagomorpha (i.e., hares and pika). Therefore, the goat and sheep species consumed were identified using reads mapped to MT-CO1, while remaining taxa were identified using MT-RNR1. Using these barcoding markers, dietary items were determined for 131 (79.4%) of 165 scats. This included 65 samples from Pakistan, 51 from Mongolia, 9 from China, and 6 from Kyrgyzstan. No prey sequences were observed in 33 samples (20%). One sample failed (0.6%). No ambiguity was observed in the mapped reads using MT-CO1 (Figure 19).

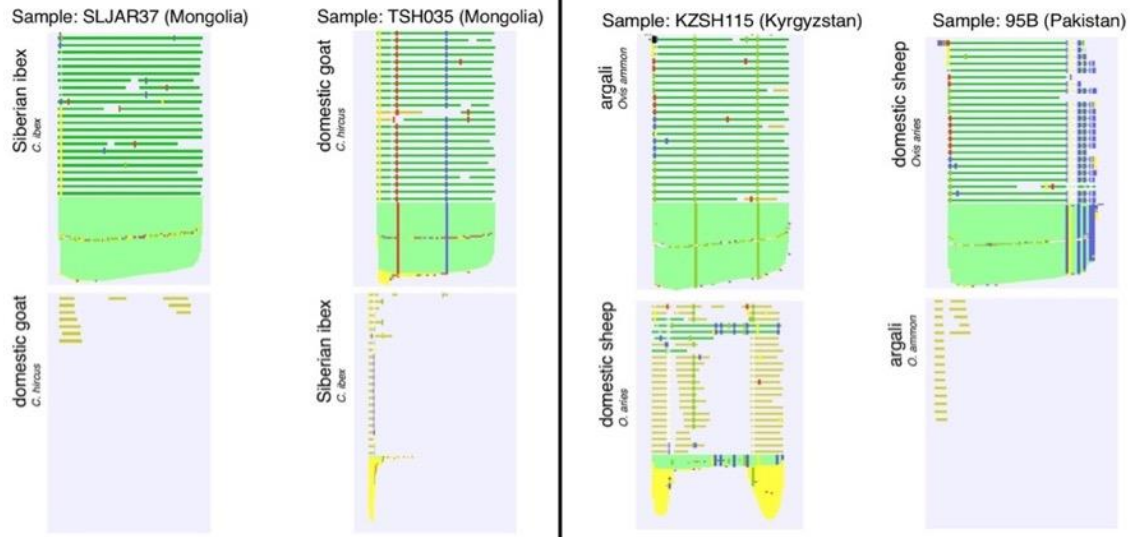


Figure 19. Illumina reads mapped to *Caprinae* reference sequences in CLC Genomics Workbench were unambiguous between goat species (domestic goat [*Capra aegagrus hircus*] and Siberian ibex [*C. ibex*]) species and sheep species (domestic sheep [*Ovis aries*]) and argali ([*O. ammon*]), enabling this marker to be used to identify livestock in scats.

### Total diet composition

The overall composition of snow leopard diet revealed 133 occurrences of 15 different species: 13 domestic goat, 29 Siberian ibex, 38 markhor, 9 blue sheep (*Pseudois nayaur*), 9 domestic sheep, 2 argali, 1 yak (*Bos grunniens*), 4 cow (*B. taurus*), 1 horse (*Equus caballus*), 17 marmot (*Marmota* sp.), 3 pika (*Ochotona* sp.), 1 hare (*Lepus* sp.), 3 Himalayan snowcock (*Tetraogallus himalayensis*), 2 Altai snowcock (*T. altaicus*), and 1 pig (*Sus spp.*) (Table 12; Figure 20). Secondary prey items were found in 2 scat samples. Of the 131 samples whose prey could be determined, 107 (81.6%) consisted of medium- to large-bodied prey, with 78 being wild (73%) and 29 domestic (27%) (Figure 21).

### Diet composition in Pakistan

Nearly half of the 165 samples originated from Pakistan (47.8%). Of these 79 samples, 65 (82.3%) had prey items discerned. Diet included 9 different species: 11

domestic goat, 1 blue sheep, 1 Siberian ibex, 38 markhor, 6 domestic sheep, 3 cow, 1 marmot, 1 hare, and 3 Himalayan snowcock (Table 12; Figure 20). A total of 60 of 65 samples contained DNA belonging to caprines. Among medium- and large-bodied prey items in Pakistan, 33% comprised domestic, while 67% were wild (Figure 21).

#### *Diet composition in Mongolia*

A total of 64 scat samples were from Mongolia (38.8%), 51 (78.5%) had prey items identified. The diet composition of snow leopards revealed 52 observations of 11 species: 2 domestic goat, 23 Siberian ibex, 3 domestic sheep, 1 argali, 1 yak, 1 cow, 1 horse, 15 marmot, 3 pika, 2 Altai snowcock, and 1 pig (Table 12; Figure 20). Of these, 29 contained DNA belonging to caprines (54.7%). Livestock species comprised 27% of medium- and large-bodied prey items, while wild species 73%. (Figure 21).

#### *Diet composition in China*

Of the samples analyzed, 11 were from China (6.7%). Prey items were determined in 9 (81.8%). Snow leopard diet composition in China included only 2 species: 8 blue sheep and 1 marmot (Table 12; Figure 20). Of these 9, 8 contained DNA belonging to caprines (88.9%). No livestock were found (Figure 21).

#### *Diet composition in Kyrgyzstan*

Kyrgyzstan had 11 (6.7%) samples and 6 (54.5%) of these had dietary items resolved. Diet composition revealed 6 occurrences of 2 species: 5 Siberian ibex, and 1

argali (Table 12; Figure 20). All contained medium- to large-bodied prey that were caprines (100%) and no livestock was found (Figure 21).

Table 12. The diet composition in snow leopard scat based on total occurrences of each prey species using MT-CO1 and MT-RNRI metabarcoding, as well as the percentage of caprines, medium- to large- and small-bodied prey, and livestock across four different countries.

Prey Item	All Four Countries (N = 131)	Pakistan (n = 65)	Mongolia (n = 51)	China (n = 9)	Kyrgyzstan (n = 6)
Domestic Goat	13	11	2	0	0
Siberian Ibex	29	1	23	0	5
Markhor	38	38	0	0	0
Blue Sheep	9	1	0	8	0
Domestic Sheep	9	6	3	0	0
Argali	2	0	1	0	1
Yak	1	0	1	0	0
Cow	4	3	1	0	0
Horse	1	0	1	0	0
Marmot	17	1	15	1	0
Pika	3	0	3	0	0
Hare	1	1	0	0	0
Himalayan snowcock	3	3	0	0	0
Altai snowcock	2	0	2	0	0
Pig	1	0	1	0	0
Total Occurrences	133	65	53	9	6
# of Species	14	9	11	2	2
% Wild Species Occurrence	79%	69%	85%	100%	100%
% Livestock Occurrence	21%	31%	15%	0%	0%
% of Caprines	75%	88%	55%	89%	100%
Total Small Body	26	5	20	1	0
Total Medium to Large Body	107	60	33	8	6
% of Medium to Large Body					
Domestic	27%	33%	24%	0%	0%
Wild	73%	67%	76%	100%	100%

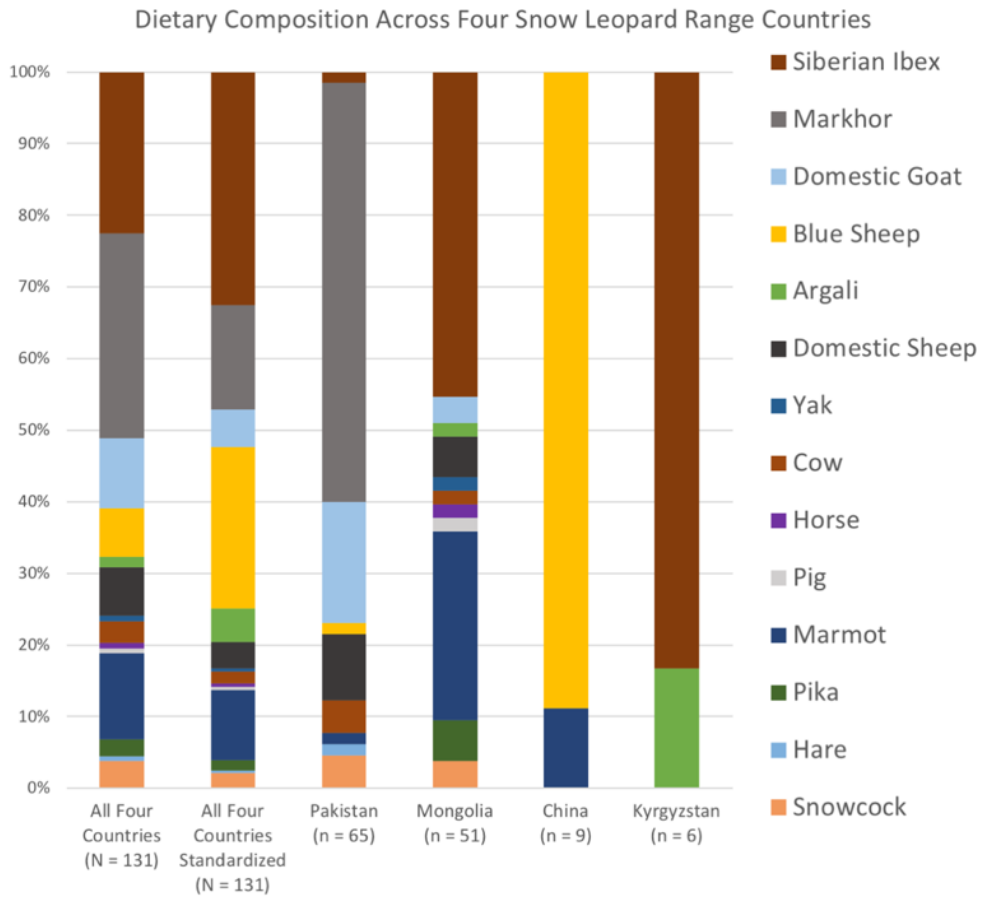


Figure 20. The overall diet composition among snow leopard scat samples derived from the sum of all occurrences for each prey species cumulatively overall and among four range countries.

## Snow Leopard Medium to Large Bodied Prey Consumption

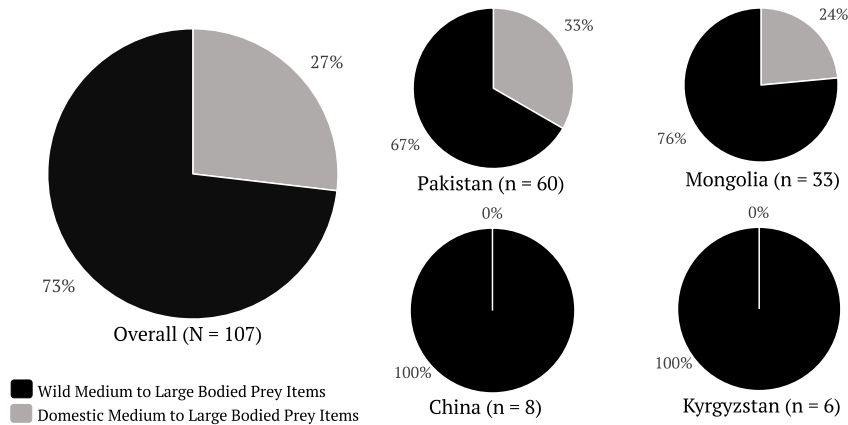


Figure 21. Percentages of wild versus domestic prey consumed by snow leopards with A) The percentage of wild and domestic medium- to large-bodied prey items (sheep [*Ovis* spp.], goats [*Capra* spp., *Pseudois nayaur*], and cattle [*Bos* spp.]) detected in scat samples cumulatively; and B) The percentage of domestic and wild medium to large-bodied prey items (sheep [*Ovis* spp.], goats [*Capra* spp., *Pseudois nayaur*], and cattle [*Bos* spp.]) detected in scat samples by country.

### Simpson's Diversity Index

When considering all samples, the Simpson's Diversity Index (D) of prey diversity was 0.84. Diet diversity was then second highest in western Mongolia followed by southern Mongolia and then Pakistan (D = 0.73, 0.66, and 0.63, respectively).

### Comparison of oligoblockers, sequencing instruments, and amplicon clean-up techniques

Oligoblockers were ineffective at significantly reducing the number of mapped snow leopard reads to MT-RNR1 (87.08% of reads/sample mapped to snow leopard without blockers compared to 84.92% with blockers,  $p = 0.06$  (

Table 13). For the 18 samples done using two different NGS approaches, the difference in average number of sequences produced per sample using the NextSeq500 with gel excision versus the MiSeq with bead purification was not significant (124,035



versus 145,169 mean reads/samples, respectively,  $p = 0.302$ ). However, the average number of reads mapped to reference sequences between the two methods was significantly different (93,688 mean reads/sample for NextSeq500 versus 49,513 for MiSeq, respectively,  $p = 0.001$ ). Three of the 18 samples had house mouse (*Mus musculus domesticus*) reads in both sequencing runs (Appendix II: Supplemental Material 5).

Table 13. The percentage of reads mapped to snow leopard using *Panthera uncia* oligoblocker and not using *P. uncia* oligoblocker. Use of the oligonucleotide blocker did not significantly reduce the number of reads which mapped to snow leopard ( $p = 0.06$ ).

Samples	% of reads mapped to <i>P. uncia</i> without oligoblocker	% of reads mapped to <i>P. uncia</i> with oligoblocker
MJ3	81.81	74.53
MJ4	88.93	83.66
MJ5	88.50	85.75
MJ6	43.20	42.29
MJ7	97.59	96.93
MJ8	98.36	98.21
MJ10	99.12	98.73
MJ11	99.18	99.26

## Discussion

Molecular diet analyses have shed light on numerous trophic relationships (Bohmann et al., 2011; King et al., 2015; Leray et al., 2015; Shehzad, McCarthy, et al., 2012; Shehzad, Riaz, et al., 2012; Xiong et al., 2017). In a previous study, the MT-RNR1 marker performed well for identifying a broad diversity of prey, but could not discern wild and domestic sheep and was ambiguous among goats (Shehzad, McCarthy, et al., 2012). The additional marker applied to snow leopard diet thus far, a 100-bp segment of MT-CYB, previously displayed only a 1-bp difference between domestic and wild sheep in Mongolia (Shehzad, McCarthy, et al., 2012). Relying on a 1-bp difference can lead to misinterpretation due to sequencing errors, and uncertainty whether this single difference

is fixed range-wide. To date, this has limited snow leopard diet analysis and assessments of livestock depredation using DNA metabarcoding (Jan E. Janecka et al., 2020). In our study, MT-CO1 served as a suitable marker for differentiating caprines in snow leopard scats from four countries. Parallel sequencing of MT-CO1 and MT-RNR1 amplicons yielded information important for understanding prey use and informing conservation management decisions.

#### Dietary composition and livestock presence in diet

We found the diet comprised of 15 unique species among 131 genetically-verified snow leopard scats. Although sample numbers from China and Kyrgyzstan were few, the prey identified matched known ranges ([www.iucnredlist.org](http://www.iucnredlist.org)), corroborating marker efficacy, and providing preliminary insights into snow leopard diets in those countries. Blue sheep occurred in 8 of the 9 samples from China, a significant range country enveloping most of the blue sheep distribution (Harris, 2014). The ninth sample contained marmot, which are common and have previously been found in a substantial portion of snow leopard diet on the Tibetan Plateau (Schaller, Junrang, et al., 1988). Among the six samples from Kyrgyzstan, Siberian ibex was found in five and argali in one. Consistent with this pattern, Siberian ibex occurs throughout Kyrgyzstan, while argali distribution is more fragmented (Harris, n.d.). The lack of domestic animals in these scats may be an artifact of sampling location, low sample size, and limited seasons. Our results in Kyrgyzstan are consistent with previous work (Jumabay-Uulu et al., 2014), but strong recent circumstantial evidence suggests that livestock loss by snow leopards in the Chang Tang is known to occur (J.D. Farrington & Tsering, 2019). Additional studies

will further elucidate prey composition, how often livestock are consumed by snow leopards, and whether snow leopard depredations reported by herders might be instead due to wolves (*Canis lupus*) or free-ranging dogs (*C. lupus familiaris*) (Home et al., 2017; Suryawanshi et al., 2013).

#### Snow leopard dietary composition in Pakistan

The majority of scat samples from Pakistan contained the endangered markhor (58%), whose habitat overlaps with snow leopards in northern Pakistan (i.e., the Gilgit-Baltistan region). Only one Siberian ibex (2%) and one blue sheep (2%) were recorded. This area is the very western most edge of blue sheep range, consistent with its low frequency in the diet. Siberian ibex is common in Gilgit-Baltistan, but they typically occupy elevations higher than markhor. Seasonality can play a large role in prey availability and diet (Johansson et al., 2015; Khatoon et al., 2017). The samples from Pakistan were collected during the winter months, when snow leopards move down in elevation, presumably closer to markhor as compared to Siberian ibex. This, coupled with primarily winter sample collection, when marmots are hibernating, may partially explain the higher occurrence of both markhor and livestock (31%) (Table 14). It is possible that in the summer, when marmots are readily available and snow leopards move to higher elevation, there is greater reliance on Siberian ibex and marmots.

#### Comparison of this study to previous snow leopard diet studies in Pakistan

Previous dietary studies in Pakistan using microhistology indicated livestock predation was common, similar to our results (Table 14), whereas we noted higher

consumption of domestic goat relative to sheep. In addition, our study had lower Siberian ibex and higher markhor occurrence (previously 20.4% Siberian ibex and 7.4% markhor, Anwar et al 2011; 2% Siberian ibex and 5% markhor, Khatoon et al. 2017). Several factors such as location, season, year, and methods could contribute to these differences. The Khatoon et al. (2017) samples were collected west of the area sampled in Anwar et al (2011) and this study; prey availability may be variable across these spatial scales. Seasonal differences may also play a role, as the samples in Khatoon et al (2017) were collected in winter and summer, compared to our primarily winter collections. The differences between our results and Anwar et al. (2011) may also be in part due to differences in microhistology compared to DNA sequencing. Potentially, hair misidentification may be more prevalent in markhor compared to other caprine species, causing incorrect classifications. In molecular studies, primer bias could lead to varying PCR drop-out errors affecting the proportions of particular species detected in a scat. In order to discern feeding ecology trends it is important that future studies sample adequately through time and space, and apply standardized analyses of scats.

### Snow leopard diet in Mongolia

Similar to Pakistan, the diet of snow leopards in Mongolia was skewed towards one of the wild caprines; Siberian ibex comprised 45% of dietary occurrences. Siberian ibex is abundant in Mongolia (R. Reading et al., 2020), and along with marmot availability during spring and summer, may contribute to reduced livestock consumption (15%). Snow leopards in Mongolia consumed a variety of livestock, indicating that all are at risk regardless of size or disposition. Pig (*Sus* spp.) was identified in one scat from

the Altai region of Mongolia, but the MT-RNR1 genetic marker was unable to discern it as wild (*Sus scrofa*) or domestic (*S. s. domesticus*). A total of 27,819 domestic pigs were reported in Mongolia in 2018 ((NSO), 2019), 90% of which were found in Ulaanbaatar and neighboring provinces (Heilmann et al., 2020), distant from the Altai region. This, coupled with the presence of wild boar in western Mongolia (Keuling & Leus, 2019), suggests the scat item in question is not of domestic origin. Regardless, the consumption of wild boar in this study and one previous one from Kazakhstan (Zhiryakov & Baidavletov RZ, 2002) demonstrates snow leopards may consume pigs when available. Our data also suggest that in some areas marmot is a commonly utilized food source, a finding in contrast to other studies (Table 14). The samples containing marmot in our study were from Baga Bogd, Mongolia, an area that has the endangered Mongolian marmot (*Marmota sibirica*) (Clayton, 2016). The role of other smaller mammals (i.e., pika and hare) and birds (i.e., snowcock) in snow leopard diet is not well understood. Their consumption occurred in two of four sites, demonstrating snow leopards may opportunistically consume a variety of small prey, which may help sustain them between larger kills (Khatoon et al., 2017).

#### Comparison of snow leopard diet from Mongolia in this study to others

Mongolia has been the site of several snow leopard diet studies, including those using microhistology (Lhagvasuren & Munkhtsog, 2000), GPS coordinates of kill sites (Johansson et al., 2015), and DNA metabarcoding (Shehzad, McCarthy, et al., 2012) (Table II). Over half of our samples in Mongolia (32 of 51) were from the same region as Shehzad et al., enabling a more direct comparison. While comparable domestic sheep

consumption was noted (3%), we found no instances of domestic goat, while Shehzad et al. detected domestic goat in 17% of scats. In both studies, Siberian ibex was the dominant prey species. We recorded consumption of smaller mammals (i.e., pika and marmot), which were not observed in Shehzad et al. (2012a). This could be the result of differences in sampling methodology, or variations caused by seasonal and fine-scale geographic differences.

#### Number of unique prey items per scat sample

Only two of the 131 scat samples had more than one prey item identified. Both samples were from Mongolia, with one containing Siberian ibex and marmot, and the second containing Siberian ibex and Altai snowcock. This suggests snow leopards typically consume one prey item at a time (Shehzad, McCarthy, et al., 2012). However, this stands in contrast to Lhagvasuren and Munkhtsog (2000) in which 58 of 168 samples had more than one prey item present. This discrepancy may be partly due to a combination of uneven distribution of prey DNA in scat and PCR preferentially amplifying the most abundant DNA template. Typically, in a microhistology analysis the entire scat is analyzed, whereas in a genetic analysis a small piece of the scat is used in the DNA extraction. If this leads to bias in the DNA approach, this could be reduced by sub-sampling scats during extractions. However, one other consideration is that scats in Lhagvasuren and Munkhtsog (2000) were not genetically identified. Misidentification of carnivore scats, including snow leopards, is common and without genetic confirmation they are often confused with other carnivore, such as wolves and foxes (Janečka et al., 2008).

### Addressing limited detection of argali

Although argali is considered an important prey species, we detected it in only 2% of all scats in two countries (i.e., Mongolia and Kyrgyzstan). Argali was also not commonly observed in the diet by several previous studies (Table II). The primary threats to argali are poaching, overharvesting, and competition with livestock leading to reduced populations (Harris, n.d.). In addition, unlike the more abundant Siberian ibex, argali prefer open alpine grasslands with moderate slope (Harris, n.d.). Snow leopards are highly adapted for steep rocky terrain (Jackson, 1996) and use broken features to ambush and chase down prey. As a consequence, they may be less effective at capturing argali on more gentle hillsides and grasslands. Therefore, utilization of argali may be more dependent on local site topographic characteristics, and less significant for snow leopard diet across their distribution.

### Snow leopard prey preference and specialization

Our results corroborate snow leopard preference of medium to large ungulates, with only 19% of scats containing small mammals or birds. The effective management and protection of regional prey populations, such as Siberian ibex in Mongolia, Kyrgyzstan, and Pakistan, markhor in northern Pakistan, and blue sheep in China is imperative for minimizing livestock depredation and sustaining carnivores (Bagchi et al., 2020). Snow leopard dependence on one particular caprine at a given site may indicate local diet specialization that could vary across regions and by season (Lyngdoh et al., 2014). The ability of predators to adapt to a shifting prey base is paramount for reducing extinction risk (Lovari & Mishra, 2016). More ecologically flexible species (i.e.,

generalist) have lower extinction risk than those exploiting a narrower environmental niche (i.e., specialist) (Lovari & Mishra, 2016). Snow leopards have previously been categorized as near-specialists (Lovari et al., 2013). The dietary breadth we observed increased when all four sampled regions were considered. Although locally snow leopards may act as specialists with adaptive hunting behaviors specific to the local prey species, our results suggest that they are able to exploit a wider range of prey.

Characterizing dietary plasticity and overlap across different regions will elucidate the breadth of snow leopard prey use, and its adaptive potential for shifting habitat and prey distribution that are expected with climate change (J. Li, McCarthy, et al., 2016). In depth understanding of feeding ecology will require long-term monitoring, research, and conservation efforts under the changing conditions facing High Asia.

#### Shortcomings and challenges of DNA barcoding in understanding dietary ecology

There are numerous challenges to making inferences on feeding ecology from molecular analysis of scat. The occurrence of a species in scat does not necessarily reflect the number of individuals consumed. A snow leopard may feed on a larger ungulate kill for up to one week based on reported kill intervals (Johansson et al., 2015). Therefore, nearby scats collected over several days could be associated with the same predation event or a kill shared by multiple snow leopards (e.g., female and cubs). In addition, the consumed prey could be an adult, sub-adult, or juvenile, thus leading to uncertain biomass conversions. Observations of kill sites by GPS-collared individuals (Johansson et al. 2015), concurrently with DNA metabarcoding of scat would provide important details on predation to better understand feeding ecology.



The lack of reference sequences for diverse populations of many prey species, both domestic and wild, limits interpretation of sequence data. Sequencing DNA barcoding markers of species underrepresented in NCBI and BOLD databases is needed, especially small mammals. For example, MT-RNR1 sequences were available for only four (*O. erythrotis*, *O. curzoniae*, *O. hyperborean*, and *O. dauurica*) of the ten pika species in our study areas and therefore we only identified these to genus. Similar issues limited marmot and hare identification. Fine resolution of trophic interactions will require more phylogenetically and phylogeographically comprehensive reference databases. Researchers need to carefully curate their final reference alignments due to the occasionally mislabeled sequences in GenBank (Beaz-Hidalgo et al., 2015). Three deposited cattle sequences from the Chang Tang region (Tibet) in GenBank had 99% similarity with the blue sheep consensus sequence, providing strong evidence that when archived these sequences were mislabeled as cattle. To recognize potential errors, we reconstructed a maximum likelihood tree, compared it to the Bovidae phylogeny (Bibi, 2013), and only used reference sequences that were in well-accepted and expected topological positions within the constructed *Caprinae* phylogeny. In addition, to avoid confusion with gene names all studies should use the official HUGO gene nomenclature (HGNC, 2019)

Since Illumina generates millions of reads it is highly sensitive to low-level contamination that would not be detected using Sanger sequencing (Pompanon et al., 2012). In the first NextSeq500 run, we found an unexpectedly high frequency of scats (14%) across all four countries with the same MT-RNR1 haplotype identical to a house mouse reference sequence. Re-sequencing the 18 samples on a MiSeq in a different lab

that did not handle mouse DNA reduced the scats in which mouse reads were detected by ~90%, indicating low-level contamination at the sequencing core. To avoid any potential bias, these 18 samples were excluded from the diet analysis.

#### Comparison of NGS methodology

We compared different instruments (NextSeq500 vs MiSeq), blocker oligonucleotides, and final amplicon clean-up methods (magnetic bead vs gel excision) using the 18 samples that were re-sequenced. The NextSeq500 was able to generate more reads for similar cost, but the two clean-up methods were comparable. In contrast to Shehzad et al. (2012a), blocker oligonucleotides did not significantly reduce amplification of snow leopard MT-RNR1 in our study. However, we do not necessarily advise against using blocker oligos; instead we recommend trial sequencing be done before embarking on a specific metabarcoding project in order to optimize methods in the particular lab where work will be done, prior to sequencing all samples. One benefit of the MT-CO1 primers we designed for caprines is that they do not amplify carnivore DNA, eliminating the need for blocker oligonucleotides.

#### Limitations of DNA barcoding in snow leopard range countries

Despite the vast amount of information generated by NGS, this is a laboratory intensive procedure that is expensive, necessitates access to Illumina instruments as well as computers with greater capacity than standard desktops, and requires extensive training in molecular techniques and bioinformatics. Adhering to laboratory best practices, establishment of appropriate research space, and increased funding is necessary

to facilitate widespread use of NGS in the wildlife field. Its role in snow leopard research conservation will become more substantial with increased access to this approach in snow leopard range countries.

### Conclusions and future directions

Caprines were found to be an important food source for snow leopards in all four countries examined. Reliable differentiation of these species in scat samples is imperative for accurate understanding of snow leopard predation, predicting changes in prey use as a consequence of environmental alterations, and mitigating conflict between snow leopards and humans. Our findings show that mitochondrially encoded cytochrome *c* oxidase subunit I (MT-CO1) can reliably discern domestic and wild goats and sheep, thus providing a more comprehensive assessment of diet when used in conjunction with mitochondrially encoded 12S ribosomal RNA (MT-RNR1). These data contribute to the understanding of snow leopard feeding ecology at larger geographic scales, and provide additional tools for diet studies that can yield information applicable to conservation planning. Future efforts should focus on sampling across seasons, years, regions, and areas with different levels of conflict between snow leopards and humans. Application of MT-RNR1 and MT-CO1 sequencing to other carnivores, such as wolves, red fox (*Vulpes vulpes*), Eurasian lynx (*Lynx lynx*), and Pallas's cat (*Otocolobus manul*), and feral domestic dogs offers the potential for understanding predator-prey relationships among the communities inhabiting high-altitude regions of Asia.

Table 14. Previous work conducted on snow leopard diet in Pakistan and Mongolia compared to findings in this study. The number of scats examined (n) is specified. \*Johansson et al. (2015) did not differentiate domestic sheep and goat kills. Note: Bold text highlights important differences between this and other studies, while *italicized* text highlights the similarities among studies as mentioned in the text.

	Pakistan			Mongolia				
	This study (n=65)	Anwar et al. 2011 (n=49)	Khatoon et al. 2017 (n=56)	This study (n=51) all samples	This study (n=32) Three Beauties and Baga Bogd only	Shehzad et al. 2012a (n=81)	Lhagvasuren & Munkhtsog 2000 (n=168)	Johansson et al. 2015 (n=249 kills)
Domestic Goat	<i>17%</i>	<i>12%</i>	<i>9%</i>	4%	0%	17%	4%	20%*
Siberian Ibex	2%	10%	2%	45%	53%	70%	39%	65%
Markhor	58%	3%	5%	0%	0%	0%	0%	0%
Blue Sheep	2%	0%	0%	0%	0%	0%	0%	0%
Domestic Sheep	9%	<i>16%</i>	<i>12%</i>	6%	3%	3%	17%	20%*
Argali	0%	0%	0%	2%	3%	9%	0%	8%
Yak	0%	1%	6%	2%	0%	0%	0%	0%
Cow	5%	8%	0%	2%	3%	0%	5%	0%
Horse	0%	0%	0%	2%	0%	0%	5%	0%
Marmot	2%	0%	9%	26%	21%	0%	3%	0%
Pika	0%	0%	3%	6%	9%	0%	4%	0%
Rabbit/Hare	2%	0%	4%	0%	0%	0%	1%	0%
Birds	5%	2%	0%	4%	6%	1%	2%	0%
	(snowcock)	(species unknown)	(species unknown)	(snowcock)	(snowcock)	(chukar)	(species unknown)	(species unknown)
Pig	0%	0%	0%	2%	0%	0%	0%	0%
Other	0%	48%	50%	0%	0%	0%	20%	7%

## Author Attributions

Yuguang Zhang, Yanlin Liu, Yu Zhang, Matthew Jevit, Ghulam Muhammad, Bariushaa Munkhtsog, Bayaraa Munkhtsog, Farida Balbakova, Azat Alamanov, Omurbek Kurmanaliev, Chimeddorj Buyanaa, Gantulga Bayandonoi, Munkhtogtokh Ochirjav, Xuchang Liang, and Xiaoxing Bian collected scat samples. Charlotte Hacker and Jan Janecka extracted scat samples. John D. Farrington, Diqiang Li, Yuguang Zhang, Bariushaa Munkhtsog, and Shafqat Hussain provided administrative support. Matthew Jevit conducted preliminary analyses. Jan Janecka and Rodney Jackson acquired funding for sample analysis. Charlotte Hacker and Jan Janecka designed primers, prepared sample library, and conducted data analyses. Charlotte Hacker, Jan Janecka, Byron Weckworth, Rodney Jackson, John D. Farrington, and Shafqat Hussain assisted with data interpretation. Charlotte Hacker wrote the manuscript. Charlotte Hacker and Jan Janecka designed figures and tables. Jan Janecka, Rodney Jackson, Byron Weckworth, and John D. Farrington contributed substantially to manuscript edits.

## Chapter 3.2

### Goal 2 – Subgoal 2

# Carnivore diet on the Qinghai-Tibetan Plateau of China

Adapted from Hacker *et al.* (In Review). *Dietary diversity and niche-partitioning of predator species across the Qinghai-Tibetan Plateau of China using DNA metabarcoding*. Biological Conservation.

#### Introduction

The maintenance of viable carnivore populations is essential to ecosystem equilibrium but cannot be done without understanding how carnivores effect prey populations and coexist with each other. Carnivores control prey densities which in turn affect vegetation (Ripple *et al.*, 2014), provide resources for scavengers (Wilmers *et al.*, 2003), and influence disease dynamics and parasite transmission (Terborgh & Estes, 2010). Knowledge of carnivore diet can elucidate presence of prey, predator-prey dynamics, niche differentiation (Foster *et al.*, 2013), and extent of livestock loss (C.E. Hacker *et al.*, 2021). Apex carnivores are larger-bodied, occupy higher trophic positions, are typically specialized hunters and consume vertebrates (Ritchie & Johnson, 2009). Mesocarnivores occupy mid-trophic positions, are smaller bodied with a more generalist diet, and also consume non-vertebrates (Newsome *et al.*, 2017). Multiple carnivores in these groups can be found sympatrically in a “predator guild” (Menge & Sutherland, 1987; Paine, 1980).

#### Niche-partitioning strategies for sympatric carnivores

Niche-partitioning is a strategy that allows predator guilds coexist. This can be done through dietary (Schoener, 1974), spatial (Durant, 2000), or temporal means

(Hayward & Slotow, 2009). Partitioning in carnivores is complex, fluid, and entails interspecies interactions (Beschta & Ripple, 2009). Studies investigating how carnivores coexist in most ecosystems are desperately lacking due to inherent challenges. First, assessing carnivore impact necessitates large spatial scales that can require copious amounts of research effort (Beschta & Ripple, 2009). Second, few systems host the diversity necessary to answer questions surrounding interspecific relationships. One of the world's exceptions to this is the Qinghai-Tibetan Plateau (QTP) in China (Ripple et al., 2014).

#### The geography, wildlife, and importance of the Qinghai-Tibetan Plateau

The QTP is a 2.5 million km<sup>2</sup> landscape that remains grossly understudied despite its geographic significance (B. Zhang et al., 2002). It holds the headwaters for three major rivers, the Yangtze, the Yellow, and the Mekong River, on which 40% of the world's population depends on or is influenced by (J.M. Foggin, 2012). In addition, it strongly influences global weather patterns (Duan et al., 2012; B. Zhang et al., 2002). Ecosystem stability in this region will be vital worldwide. The relatively recent rapid uplift of the plateau has led to numerous ecosystem types (Chang, 1981). It has undergone mass alterations in the last few decades due to anthropogenic and economic development, leading to unprecedented pressure on its wildlife (J.M. Foggin, 2008; J. Liu et al., 2003; Schaller, 1998). Fortunately, conservation policies and national parks have worked to recover species once threatened with extinction and their habitats (Bleisch et al., 2009; Frank, 2020).

Apex carnivores on the QTP include the Tibetan wolf (*Canis lupus*), Tibetan brown bear (*Ursus arctos pruinosus*), snow leopard (*Panthera uncia*), common leopard (*P. pardus*), and Eurasian lynx (*Lynx lynx*) (A. Xu et al., 2008). Tibetan fox (*Vulpes ferrilata*), red fox (*V. vulpes*), Pallas's cat (*Otocolobus manul*), and Asian badger (*Meles leucurus*), among others, round out the mesocarnivores. Native prey species include blue sheep (*Pseudois nayaur*), Tibetan gazelle (*Procapra picticaudata*), argali (*Ovis ammon*), white-lipped deer (*Cervus albirostris*), marmot (*Marmota* sp.), chukar partridge (*Alectoris chukar*), and pika (*Ochotona* sp.), among others (Jackson, 2012; Schaller, Junrang, et al., 1988). This rangeland system has been used by pastoralists for thousands of years (D. J. Miller & Bedunah, 1994). Livestock, including domestic yak (*Bos grunniens*), goat (*Capra aegagrus hircus*), sheep (*Ovis aries*), and horse (*Equus caballus*), are also present and may serve as food for wild carnivores.

#### Challenges of livestock depredation for carnivore conservation

Livestock can play an important role in sustaining carnivore populations (Ahmed et al., 2012; Hacker et al., 2021; Wang et al., 2014). However, livestock depredation creates financial burdens (Mkonyi et al., 2017), and can create tension between local residents and entities seeking to protect wildlife (Wang & Macdonald, 2006). Knowledge of livestock in diet is necessary to construct tailored mitigation strategies and for designing assistance programs, such as insurance, that relieve associated financial burdens (Hussain, 2000). Existing evidence shows that these increase populations of predators and reduce retaliatory killings (Hazzah et al., 2014; Maclellan et al., 2009). Such action is prudent, as the mountainous interior of Asia is warming at twice the rate of non-arctic



regions in the Northern Hemisphere (Chen et al., 2009). Climate change will likely initiate large-scale weather events that cause reductions and distribution shifts of carnivores and their prey. Changes in the dynamics of one predator species can impact others (Post et al., 1999). Carnivores in shifting landscapes may find themselves overlapping with novel species, whereby they may be unsuccessful at competing for resources, and may increasingly pursue livestock. Carnivore diets can be an informative indicator of ecosystem change in species distribution and abundance and understanding potential adaptability to future changes.

#### Previous predator guild diet studies on the Qinghai-Tibetan Plateau

Although there have been previous studies of carnivore diet on the QTP (Aichun et al., 2006; B. Liu & Jiang, 2003; Schaller, Junrang, et al., 1988; Tsukada et al., 2014; Weidong, 2010; Werhahn et al., 2019), information for more than two sympatric carnivores within the same time frame across multiple areas is lacking. In addition, most previous studies did not genetically confirm scats and host misidentification was likely high (Weiskopf et al., 2016). Lastly, the majority of these works relied on microhistology, an error-prone method in which scats are collected and dried, and digested hair is compared to a reference database of known species (Pompanon et al., 2012). The advent of next-generation sequencing and DNA metabarcoding is quickly changing the capacity for large-scale comprehensive analysis of diet, overcoming previous limitations (Bohmann et al., 2011; Valentini et al., 2009). Identification of the carnivore and prey consumed in each sample is completed by PCR amplification and sequencing of a diagnostic gene segment. The derived sequence is then matched to a

reference sequence in NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) or BOLD database (<http://www.boldsystems.org/>).

### Study purpose and objectives

Knowledge of feeding ecology among carnivores is critical not only from an ecological perspective but also from an economics and conservation perspective (Ciucci et al., 1996). Here, we sought to use DNA metabarcoding to study carnivore diets across three sites on China's QTP to: 1) determine carnivore diversity; 2) investigate carnivore food habits and prey selection; 3) understand potential mechanisms of niche-partitioning, and 4) broadly compare carnivore and prey composition to provide information for designing effective conservation action.

### Materials and Methods

#### Study sites

A total of 760 scats of putative carnivore origin were collected across three sampling sites on the QTP of China (Qilian Shan, East Burhanbuda Mountain, and Yushu). Carnivore scats were identified by the presence of hair and bones, as well as nearby accompanying signs such as pug marks and scrapes.

Yushu Prefecture is located in the southwestern corner of Qinghai Province, and consists primarily of alpine meadow vegetation with limestone massifs and small rugged ranges surrounded by rolling grassland, with juniper forests along mountainsides (Schaller, Junrang, et al., 1988). Qilian Shan (up to 5,800m in elevation) runs across Gansu and Qinghai Provinces along the very northeastern corner of the QTP. It is

comprised of three parallel subsidiary ranges, the Tuali Nanshan, Shule Nanshan, and Danghe Nanshan (Schaller, Junrang, et al., 1988). At lower elevations, Qilian Shan is composed of deserts giving way at 3,800m to shrubs, grasses, and alpine meadows (Schaller, Junrang, et al., 1988). The Kunlun Shan range sits southwest of Qilian Shan and is the longest mountain system in Asia (D. J. Miller & Bedunah, 1994). East Burhanbuda Mountain is on the eastern end of this mountain chain and consists of rugged grassland with rock slopes (Y. Liu, 1993).

### Sample collection

Samples were collected over nine different sampling trips from September 2017 to December 2019 (Table 15). Sampling methods followed those described in Janečka et al., (2008) and Janečka, Munkhtsog, et al., (2011). A total of 75 samples were collected from Yushu Prefecture (Zhiduo County), 135 samples from East Burhanbuda Mountain (Dulan County), and 550 samples from Qilian Shan (Qilian, Tianjun, Menyuan, and Subei Counties).

Table 15. Information surrounding scat sampling sessions.

Region	Collection Time Period	Number of Scats Analyzed
Qilian Shan	September 2017	38
	July 2018	136
	October 2018	39
	April 2019	113
	May 2019	93
	July 2019	131
	East Burhanbuda Mountain	July 2018
Yushu	July 2018	75

### DNA extraction

See Chapter 2.1 of this dissertation.

### PCR for species and diet analysis

Both Cytochrome *c* oxidase subunit 1 (MT-CO1) and MT-RNR1 were sequenced to determine carnivore identity and diet. See Chapter 3.1 for methodology.

### Next-generation sequencing

Amplicons were normalized by measuring gel band brightness using GeneTools Version 4.03.05.0 [SynGene, Frederick, MD, USA]. The E.Z.N.A.® Gel Extraction Kit [Omega Bio-Tek, Inc., Norcross, GA, USA] was used to extract and purify MT-RNR1 and MT-CO1 products. The NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® [New England Biolabs, MA, USA] was used to prepare sequencing libraries following manufacturer's recommendations. Index primers were used to incorporate unique barcodes to amplicons from different samples. The ampliconic library was quantified using an Invitrogen Qubit® 2.0 Fluorometer [Thermo Fisher Scientific, Waltham, MA, USA]. Paired end 250 cycle - sequencing was completed on an Illumina NovaSeq 6000 by Guangdong Magigene Biotechnology Co., Ltd. [Guangzhou, China].

### Diet analysis

See Chapter 3.1 for methodology.

## Data analysis

All statistical analyses were performed in Microsoft Office Excel for Mac Version 16.43, SPSS statistics 26.0 (SPSS, Chicago, USA), and R version 3.5.2 (R Core Team 2018) using base R functions, as well as the *EcoSimR* packages (Gotelli et al., 2015). Percentage of host species composition overall and by region was examined by summing the number of scats belonging to each host (s) and dividing it by the total number of scats observed (S) ( $s/S*100$ ). The frequency of occurrence of prey species in scats were summed overall and by region for both individual host species and by the categories of apex carnivore or mesocarnivore. The number of prey items per scat were assigned to each host species and averaged. Overall differences in livestock dependency across host species and region were calculated using a Friedman's test followed by a Wilcoxon Signed Rank Test. Dietary diversity overall and by region for each carnivore host species was assessed by calculating richness, Shannon's Index (Shannon & Weaver, 1949) and Simpson's Index (Marchesi et al., 2002; Simpson, 1949). Interspecific dietary niche overlap was evaluated using Pianka's Index (value = 0 indicating no dietary overlap and value = 1 indicating complete dietary overlap) for host species with sufficient sample sizes. Values of Pianka's Index were calculated relative to 999 simulated null models of randomized dietary item frequency of occurrence data using the 'niche\_null\_model' function in the *EcoSimR* package (Gotelli et al., 2015). Jaccard's Similarity Index (based on binary presence absence data of prey) between any two given carnivore host species was calculated to assess dissimilarity between diets, then subtracted from 1 to obtain values for Jaccard's Distances and visualized using multidimensional scaling via Principal Coordinates Analysis (PCoA).

## Results

### Carnivore and prey species identification

Of the 760 samples sequenced, 46 were regurgitated bird pellets from birds of prey (steppe eagle (*Aquila nipalensis*), upland buzzard (*Buteo hemilasius*), Eurasian eagle owl (*Bubo bubo*), saker falcon (*Falco cherrug*)), 11 samples were not species of interest (human (*Homo sapiens*), yak (*Bos grunniens*), and marmot (*Marmota himalayana*)), 13 were inconclusive, and four were undetermined (Figure 22). These samples were removed from analysis. The remaining 686 samples were composed of the following carnivores: 147 red fox, 25 Tibetan fox, 153 Tibetan wolf, 191 snow leopard, 72 Eurasian lynx, seven Tibetan brown bear, 88 Pallas's cat, two Asian badger, and one beech marten (*Martes foina*) (Figure 23; Figure 24). Dietary items for Asian badger and beech marten were reported but were removed due to low sample sizes. Tibetan brown bear, Tibetan wolf, snow leopard, and Eurasian lynx were termed apex carnivores while Tibetan fox, red fox, and Pallas's cat were termed mesocarnivores.

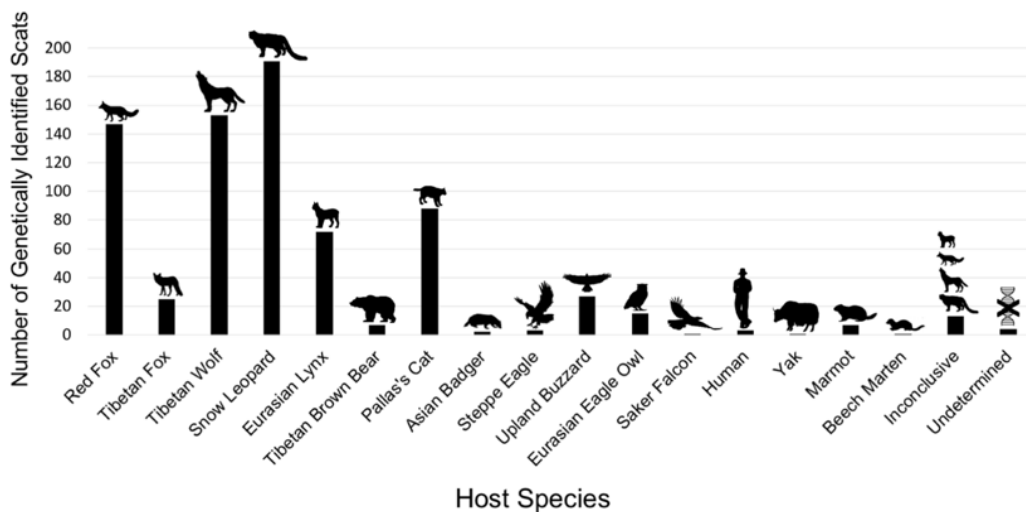


Figure 22. The number of scats identified to host species among the 760 scat samples collected on the Qinghai-Tibetan Plateau. The x-axis shows each host identified by

common name with Inconclusive denoting that host could not be determined due to possible contamination and Undetermined denoting that there was not enough DNA in the sample to determine the host species. The y-axis shows the number of genetically verified scats for each host along the x-axis.

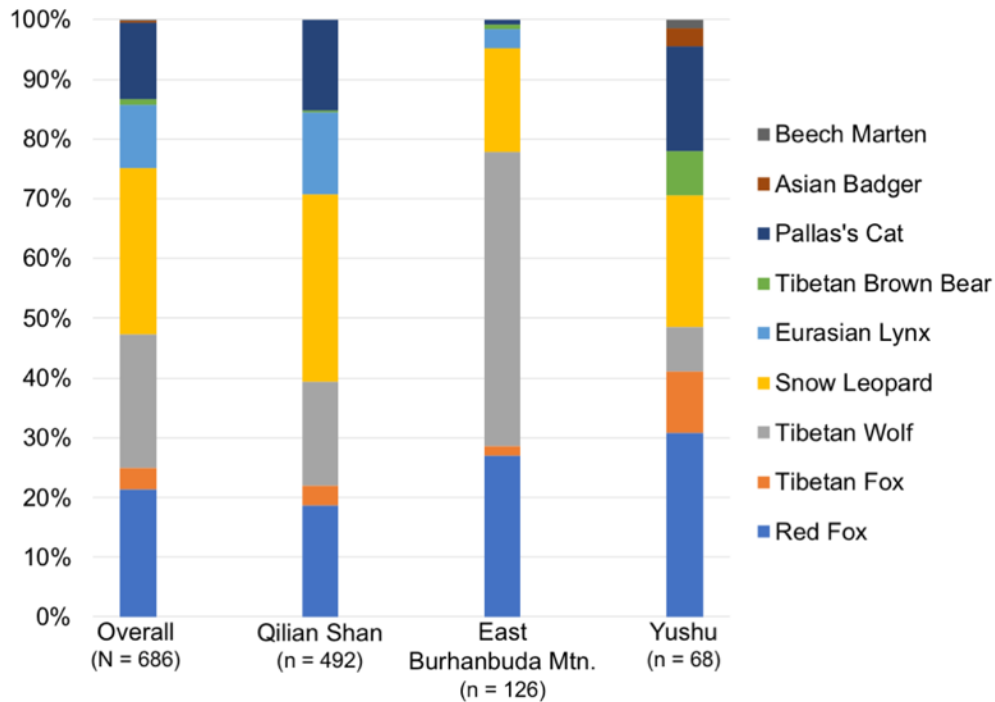


Figure 23. The composition of genetically determined carnivore species from scat samples noninvasively collected across the Qinghai-Tibetan Plateau of China. The x-axis has the names of each region as well as the number of scat samples analyzed from each. “Overall” refers to all three regions combined. The y-axis has the percentages of each carnivore host out of a total of 100%.

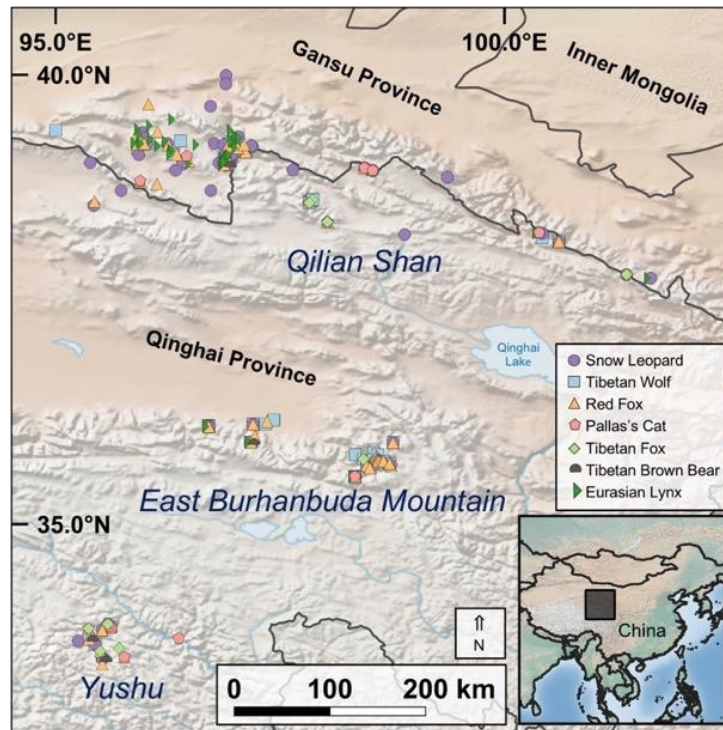


Figure 24. The three study regions with scat collection locations matched to carnivore host identification for dietary analysis.

There were 33 unique prey species identified from three animal classes (Mammalia, Aves, Actinopterygii). Small mammals were found in the diets of all carnivores and made up 80.7% of counts in mesocarnivore diet and 41% of apex carnivore diet. Livestock were present at 7.5% in the diets of three apex carnivores (Tibetan wolf, snow leopard, Eurasian lynx) and in two mesocarnivores (Tibetan fox, red fox) at 1.8%. These made up 4.3% of diet across all host species. Medium to large ungulates comprised nearly half the dietary counts of apex carnivores at 48.2%. Intraguild predation of mesocarnivores was identified by Tibetan wolves and snow leopards at 5.9% and 1%, respectively. One prey item per scat was identified in 87.6% of samples. Occurrences of multiples included three prey items in two wolf scats, one lynx scat, and six red fox scats and two prey items in 18 wolf scats, ten lynx scats, one Asian badger scat, one beech marten scat, three Pallas's cat scat, 16 snow leopard scats, and 27



red fox scats. Prey identification, counts, and relative frequencies can be found in Table 16, Figure 25, and Figure 26.

#### *Carnivore and prey species identification in Qilian Shan*

A total of 492 samples within the 686 sample set originated from Qilian Shan. They comprised 92 red fox, 16 Tibetan fox, 86 Tibetan wolf, 154 snow leopard, 68 Eurasian lynx, one Tibetan brown bear, and 75 Pallas's cat. Small mammals were found in the diets of all host species and made up 73.3% of total prey counts. Livestock occurrence was 4%, with its contribution to apex carnivore diet at 6.2% and mesocarnivore diet 0.9%. Qilian Shan had the highest diversity of livestock taken, and the single occurrence of domestic pig (*Sus scrofa*) and domestic horse (*Equus caballus*) found in snow leopard diet. Prey identification, counts, and relative frequencies can be found in Table 16, Figure 25, and Figure 26.

#### *Carnivore and Prey Species Identification in East Burhanbuda Mountain*

Among the 686 samples, 126 were from East Burhanbuda Mountain. A total of 34 were red fox, two Tibetan fox, 62 Tibetan wolf, 22 snow leopard, four Eurasian lynx, one Tibetan brown bear, and one Pallas's cat. Livestock made up 7% of prey. Livestock presence in diet was driven by apex carnivores at 11.4% of dietary items. Blue sheep made up over a third of prey counts at 38.3%. The single occurrence of perch fish was from a red fox. Prey identification, counts, and relative frequencies can be found in Table 16, Figure 25, and Figure 26.

*Carnivore and prey species identification in Yushu Prefecture*

A subset of 68 samples of the 686 sample set were from Yushu Prefecture. These were 21 red fox, seven Tibetan fox, five Tibetan wolf, 15 snow leopard, five Tibetan brown bear, 12 Pallas's cat, two Asian badger, and one beech marten. A total of 6.3% of counts were livestock, with contributions of apex carnivores or mesocarnivores at 8.3% and 7%, respectively. The two Asian badger scats contained pika and plateau vole while the beech marten scat contained blue sheep and pika. Prey identification, counts, and relative frequencies can be found in Table 16, Figure 25, and Figure 26.

Table 16. A heat map showing counts of prey species in percentages (%) found in carnivore diet as well as relative frequencies of detection by color ranging from no occurrence of prey species in diet (white) to high occurrence of prey species in diet (dark gray) on the Qinghai-Tibetan Plateau.

		<i>Apex Carnivores</i>												<i>Mesocarnivores</i>																		
		Tibetan Brown Bear				Tibetan Wolf				Snow Leopard				Eurasian Lynx				Tibetan Fox				Red Fox				Pallas's Cat						
<i>Carnivore Host Species</i>		O	QS	EB	Y	O	QS	EB	Y	O	QS	EB	Y	O	QS	EB	O	QS	EB	Y	O	QS	EB	Y	O	QS	EB	Y				
<i>Region</i>																																
<i>Number of Scats</i>		7	1	1	5	153	86	62	5	191	154	22	15	72	68	4	25	16	2	7	147	92	34	21	88	75	1	12				
Domestic Ungulates	Domestic Goat	0.0	0.0	0.0	0.0	4.1	6.1	1.5	0.0	2.4	3.0	0.0	0.0	1.2	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
	Domestic Sheep	0.0	0.0	0.0	0.0	5.3	5.1	6.2	0.0	1.5	1.8	0.0	0.0	1.2	1.3	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
	Domestic Yak	0.0	0.0	0.0	0.0	12.3	5.1	21.5	25.0	0.0	0.0	0.0	0.0	1.2	0.0	14.3	4.0	0.0	0.0	14.3	4.4	2.6	5.3	11.1	0.0	0.0	0.0	0.0				
	Domestic Horse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
	Domestic Pig	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
Wild Ungulates	Blue Sheep	71.4	0.0	100.0	80.0	44.4	40.8	50.8	37.5	59.7	58.4	62.5	68.8	13.1	13.0	14.3	4.0	6.3	0.0	0.0	14.4	13.0	26.3	3.7	2.2	2.6	0.0	0.0				
	Musk Deer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
Carnivores	Pallas's Cat	0.0	0.0	0.0	0.0	0.6	1.0	0.0	0.0	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
	Red Fox	0.0	0.0	0.0	0.0	4.1	2.0	7.7	0.0	0.5	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
	Tibetan Fox	0.0	0.0	0.0	0.0	1.2	1.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
Small Mammals	Himalayan Marmot	14.3	100.0	0.0	0.0	5.8	4.1	7.7	12.5	7.8	6.0	20.8	6.3	7.1	6.5	14.3	0.0	0.0	0.0	0.0	5.0	4.3	13.2	0.0	0.0	0.0	0.0	0.0				
	Woolly Hare	0.0	0.0	0.0	0.0	4.7	8.2	0.0	0.0	7.3	8.4	4.2	0.0	53.6	58.4	0.0	0.0	0.0	0.0	0.0	10.0	7.8	23.7	0.0	1.1	1.3	100.0	0.0				
	Pika	14.3	0.0	0.0	20.0	14.6	21.4	3.1	25.0	14.1	15.7	4.2	12.5	13.1	11.7	28.6	92.0	93.8	100.0	85.7	43.3	49.6	15.8	55.6	91.2	93.5	0.0	76.9				
	Chinese Scrub Vole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	3.9	0.0	0.0	0.0	0.0	0.0	3.3	4.3	2.6	0.0	2.2	1.3	0.0	7.7				
	Grey Red-Backed Vole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	2.6	2.6	0.0	0.0	0.0	0.0	15.4				

	Plateau Vole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.6	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0	25.9	2.2	0.0	0.0	0.0
	Sikkim Mountain Vole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0
	Long-Tailed Dwarf Hamster	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0
	Chinese Birch Mouse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	House Mouse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0
	Zokor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	14.3	0.0	0.0	0.0	0.0	2.8	4.3	0.0	0.0	0.0	0.0	0.0	0.0
Birds	Birds of Prey	0.0	0.0	0.0	0.0	0.6	1.0	0.0	0.0	1.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	1.7	2.6	0.0	0.0	0.0	0.0	0.0
	Ground Feeding Birds	0.0	0.0	0.0	0.0	1.2	2.0	0.0	0.0	1.5	1.8	0.0	0.0	2.4	2.6	0.0	0.0	0.0	0.0	0.0	2.8	2.6	5.3	0.0	0.0	0.0	0.0	0.0
	Perching Birds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	4.3	0.0	3.7	1.1	1.3	0.0	0.0
Fish	Perch	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	2.6	0.0	0.0	0.0	0.0	0.0
Un-known	Inconclusive	0.0	0.0	0.0	0.0	1.2	2.0	0.0	0.0	1.9	1.2	4.2	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

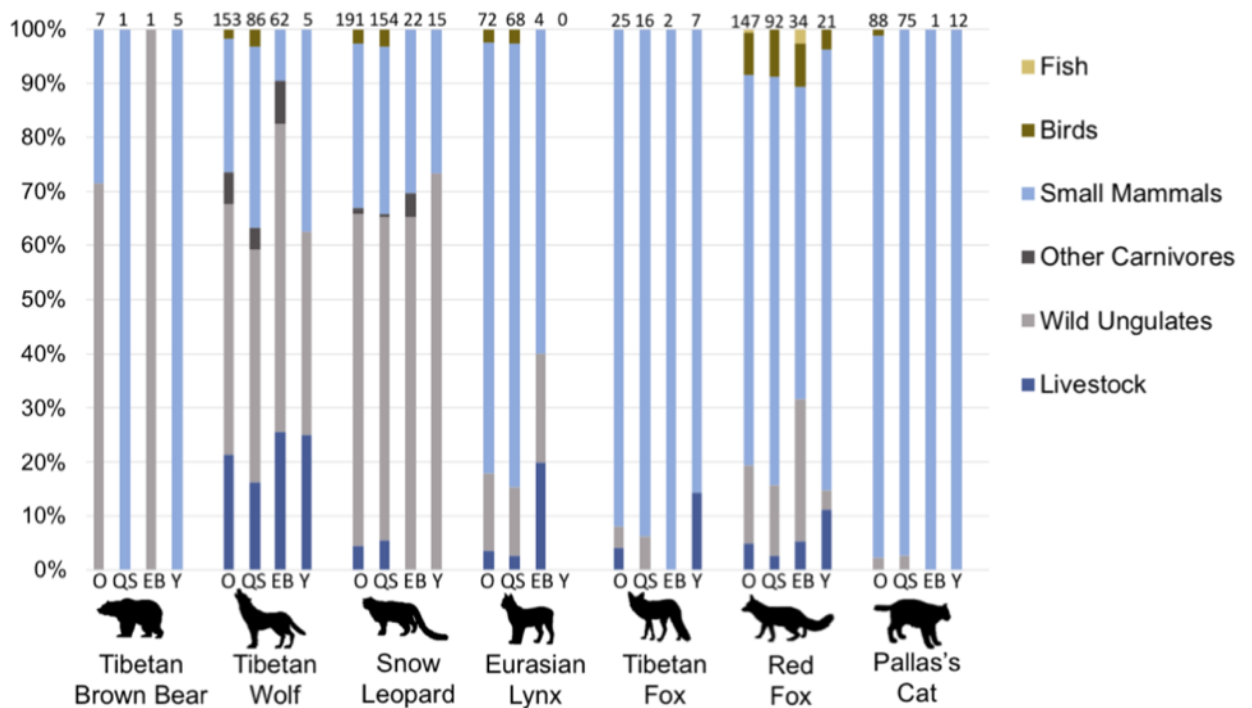


Figure 25. The percentage of each prey category by host species overall (O) and for the regions of Qilian Shan (QS), East Burhanbuda Mountain (EB), and Yushu (Y) with scat number collected for each species at the top of its respective bar. Perch (*Percidae*) comprised all occurrences of “Fish”. “Bird” were made up of perching birds, ground dwelling birds, and raptor species. Species from the orders Rodentia and Lagomorpha were clustered into “Small Mammals”. Carnivores determined to be consumed as a result of intraguild predation were combined into “Other Carnivores”. “Wild Ungulates” consists of all wild hoof stock species. “Livestock” consists of all domestic animals observed as prey combined.

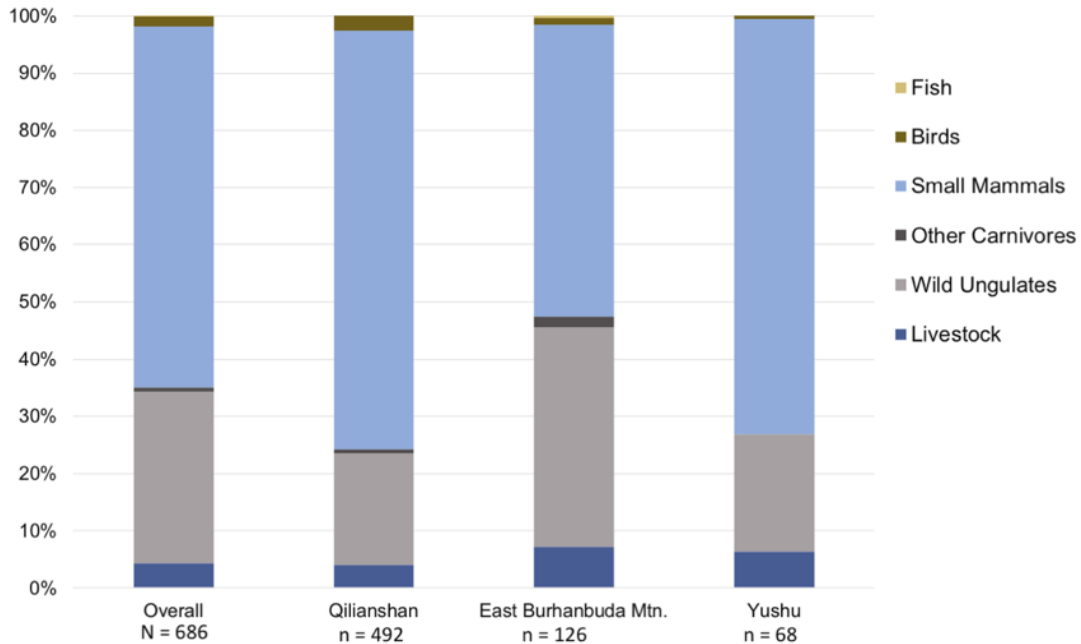


Figure 26. The percentage of each prey category by region with number of scats examined. Perch (*Percidae*) comprised all occurrence of “Fish”. “Bird” were made up of perching birds, ground dwelling birds, and raptor species. Species from the orders Rodentia and Lagomorpha were clustered into “Small Mammals”. Carnivores determined to be consumed as a result of intraguild predation were combined into “Other Carnivores”. “Wild Ungulates” consists of all wild hoof stock species. “Livestock” consists of all domestic animals observed as prey combined.

### Livestock dependency

Tibetan wolf had the highest percentage of livestock at 21.7% with domestic yak being most common followed by sheep and goat. Red fox was second at 5% with yak and sheep, followed closely by snow leopard at 4.9% with goat, sheep, pig and, horse. A total of 4% of Tibetan fox diet was domestic yak and 3.6% of Eurasian lynx diet had domestic goat, sheep, and yak. Apex carnivores as a group had 6.25 times the frequency of livestock in their scat than mesocarnivores at 50 and 8 counts, respectively. Qilian Shan had 31 counts of livestock in carnivore diet, followed by East Burhanbuda Mountain at 22, and Yushu at 6. Statistically significant differences were found for livestock presence in diet across species ( $p = 0.017$ ) but not across regions ( $p = 0.350$ ). There were no

statistically significant differences in livestock consumption between any two carnivores or apex versus mesocarnivores overall or between regions.

#### Dietary diversity and diet similarity

Prey diversity was greatest in Qilian Shan, followed by East Burhanbuda Mountain, and Yushu (Table 17). Red fox had the greatest dietary breadth while the closely related Tibetan fox had the lowest. Richness was similar for Tibetan wolf, snow leopard, and Eurasian lynx, but the two other measures of diversity that took into account evenness varied, with Tibetan wolf showing the highest values, followed by Eurasian lynx, and snow leopard. Jaccard's Distance revealed that diets were most similar between Tibetan wolf and snow leopard (0.56) and least similar between Tibetan fox and snow leopard (0.07). All other values fell between 0.13 and 0.39 (Table 18, Figure 27).

Table 17. A series of diversity indices to describe dietary breadth for each carnivore species detected overall and by region. Richness corresponds to the number of unique prey items in the diet. The Shannon-Wiener Index (H) combines species richness (number of unique prey species) and evenness (abundance of each prey species in diet) to provide an output of diversity with 0 implying no diversity in diet and higher numbers reflecting more diversity in diet. The Simpson's index of diversity measures the probability that two randomly selected dietary items would be the same species with a lower value reflective of greater biodiversity.

Carnivore Host Species	<i>Richness</i>				<i>Shannon-Wiener Index</i>				<i>Simpson's Index</i>			
	Overall	Qilian Shan	E. Burhanbuda Mountain	Yushu	Overall	Qilian Shan	E. Burhanbuda Mtn.	Yushu	Overall	Qilian Shan	E. Burhanbuda Mtn.	Yushu
Tibetan Brown Bear	3	1	1	2	0.8	-	-	0.5	0.48	-	-	0.6
Tibetan Wolf	13	13	8	4	1.78	1.8	1.37	1.32	0.26	0.24	0.36	0.18
Snow Leopard	14	13	6	5	1.34	1.41	1.02	0.86	0.4	0.38	0.45	0.53
Eurasian Lynx	13	11	4	-	1.6	1.47	1.33	-	0.32	0.36	0.1	-
Tibetan Fox	3	2	1	2	0.33	0.23	-	0.41	0.84	0.88	1	0.71
Red Fox	23	19	10	5	2.09	1.91	1.94	1.16	0.22	0.27	0.15	0.37
Pallas's Cat	6	5	1	3	0.43	0.33	-	0.69	0.83	0.87	-	0.59
All Predator Species	33	31	14	9	2.01	1.84	1.76	1.39	0.21	0.25	0.27	0.3



Table 18. A matrix of calculated inversed Jaccard Coefficient based on binary presence absence data of prey items in diet.

Carnivore	Region and Sample Sizes	Tibetan Wolf	Snow Leopard	Tibetan Brown Bear	Eurasian Lynx	Tibetan Fox	Red Fox	Pallas's Cat
Tibetan Wolf	O (N = 153)							
	QS (n = 86)	-						
	EB (n = 62)							
Snow Leopard	O (N = 191)	0.56						
	QS (n = 154)	0.50	-					
	EB (n = 22)	0.56						
	Y (n = 15)	IS						
Tibetan Brown Bear	O (N = 7)	0.25	0.23	-				
Eurasian Lynx	O (N = 72)	0.39	0.30	0.23	-			
	QS (n = 68)	0.75	0.75	IS				
Tibetan Fox	O (N = 25)	0.15	0.07	0.20	0.23	-		
	QS (n = 16)	0.83	0.83	IS	0.90			
Red Fox	O (N = 147)	0.25	0.29	0.13	0.38	0.13		
	QS (n = 92)	0.81	0.81	IS	0.80	0.90	-	
	EB (n = 34)	0.71	0.64	IS	IS	IS		
	Y (n = 21)	IS	0.50	IS	IS	IS		
Pallas's Cat	O (N = 88)	0.20	0.27	0.29	0.27	0.29	0.21	
	QS (n = 75)	0.79	0.79	IS	0.80	0.60	0.80	-
	Y (n = 12)	IS	0.60	IS	IS	IS	0.67	

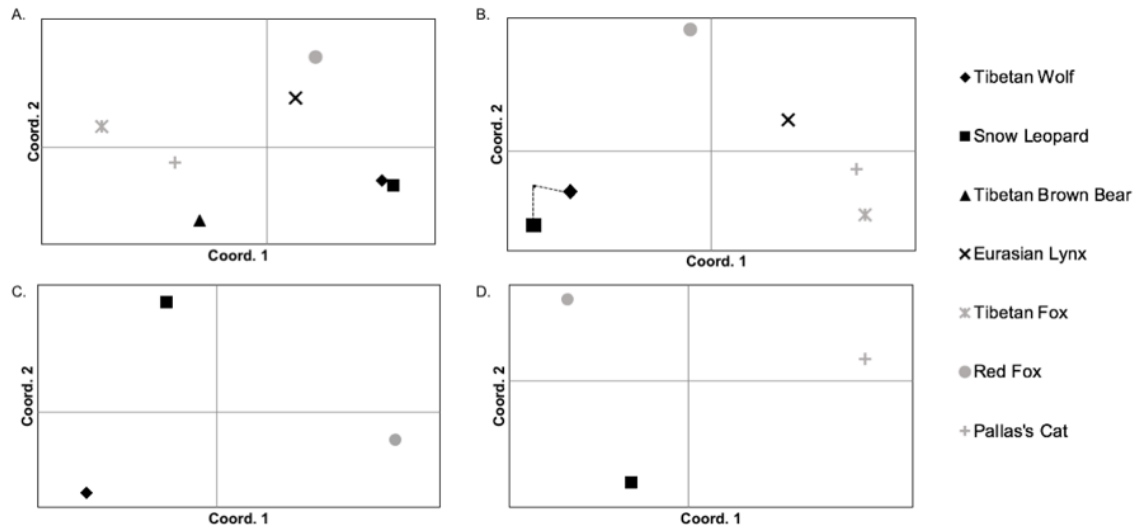


Figure 27. Similarity in dietary composition among carnivore species examined using Principal Coordinates Analysis (PCoA) plots of Jaccard's Coefficients representing A) across all areas, B) in Qilian Shan, C) in East Burhanbuda Mountain, and D) in Yushu. Points in black represent apex carnivores and points in gray represent mesocarnivores.

### Niche overlap

Dietary niche overlap among carnivores overall, in Qilian Shan, and in East Burhanbuda Mountain was significantly greater than expected, but not in Yushu (Table 19, Figure 28A). Pairwise comparisons revealed no significant difference in observed versus expected dietary overlap between apex carnivores and mesocarnivores (Table 19, Figure 28). Red fox had the highest number of greater than expected niche overlap occurrences, with the exception of the Tibetan wolf in Qilian Shan (Table 19, Figure 28). No significant differences in observed versus expected dietary overlap was found for any two carnivores in East Burhanbuda Mountain or Yushu (Table 19, Figure 28C,D).

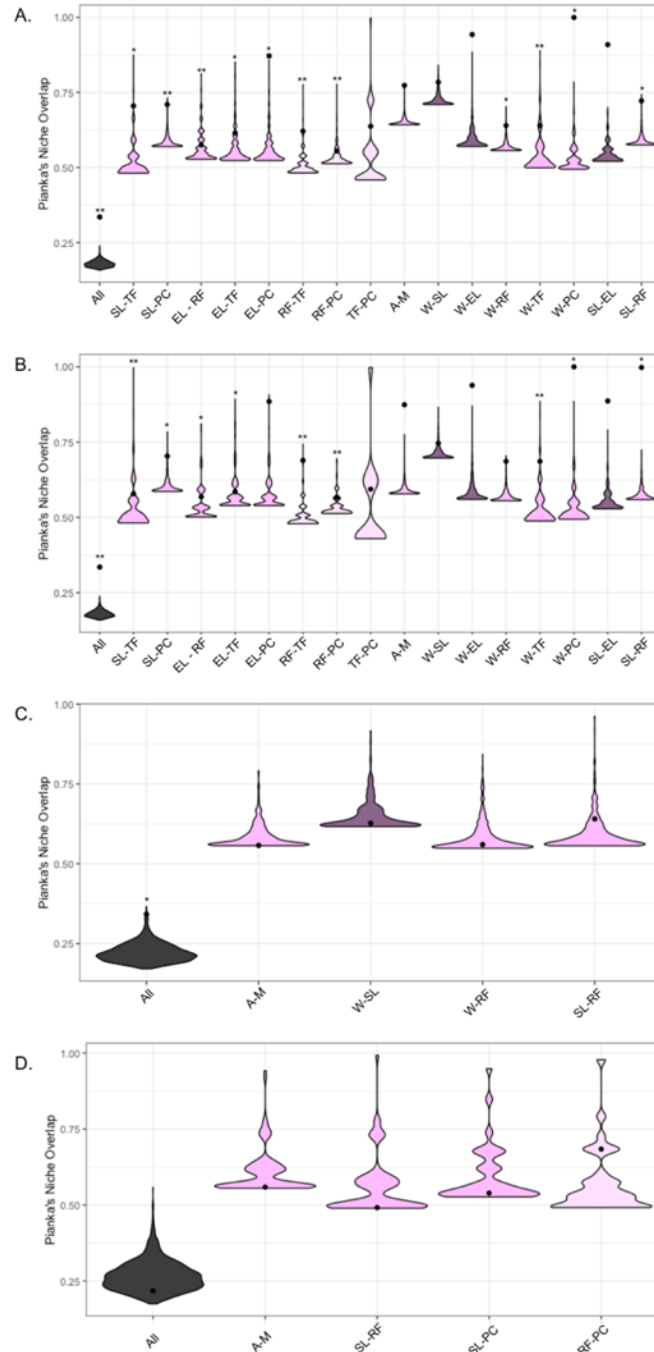


Figure 28. Pianka's Index of dietary niche overlap for carnivore species A) across all areas, B) in Qilian Shan, C) in East Burhanbuda Mountain, and D) in Yushu. Dietary niche overlap was investigated among all carnivores (All), between apex and mesocarnivores (A – apex, M - mesocarnivore), and between any two given carnivore species when sample sizes allowed (SL – snow leopard, W – Tibetan W, TB – Tibetan brown bear, EL – Eurasian lynx, RF – Red Fox, TF – Tibetan Fox, PC – Pallas's cat). The comparison of two apex carnivores is denoted by dark purple, two mesocarnivores by light pink, and one apex carnivore with one mesocarnivore by fuchsia. Density plots represent Pianka's Index values from randomized frequency of occurrence data. Points indicate the observed dietary niche overlap between species. (\*\* $p < 0.0001$ ; \* $p < 0.05$ )

Table 19. Pianka's Index values overall and by region. Values closer to 0 indicate no niche overlap while values closer to 1 indicate complete overlap. Significant values indicate higher or lower niche overlap than expected. IS indicates insufficient sample size.

Carnivore	Region / Sample Sizes	Tibetan Wolf	Snow Leopard	Eurasian Lynx	Tibetan Fox	Red Fox	Pallas's Cat	All	Meso-carnivore
Tibetan Wolf	O (N=153)								
	QS (n=86)	-							
	EB (n=62)								
Snow Leopard	O (N=191)	0.71 (0.975)							
	QS (n=154)	0.7 (0.535)	-						
	EB (n=22)	0.63 (0.578)							
	Y (n=15)	IS							
Eurasian Lynx	O (N=72)	0.58 (0.603)	0.56 (0.276)						
	QS (n=68)	0.57 (0.586)	0.57 (0.220)	-					
Tibetan Fox	O (N=25)	0.87 (0.001**)	0.77 (0.002*)	0.64 (0.046*)					
	QS (n=16)	0.86 (0.001**)	0.87 (0.001**)	0.69 (0.048*)	-				
Red Fox	O (N=147)	0.62 (0.044*)	0.64 (0.043*)	0.94 (0.001**)	1 (0.001**)				
	QS (n=92)	0.58 (0.17)	0.59 (0.155)	0.94 (0.003*)	1 (0.001**)				
	EB (n=34)	0.56 (0.579)	0.64 (0.125)	IS	IS	-			
	Y (n=21)	IS	0.49 (0.946)	IS	IS				
	O (N=88)	0.62 (0.049*)	0.78 (0.001**)	0.68 (0.045*)	0.72 (0.132)	0.91 (0.001**)	-		

Pallas's Cat	QS (n=75)	0.69 (0.009*)	0.75 (0.002*)	0.74 (0.052)	1 (0.062)	0.89 (0.001**)	
	Y (n=12)	IS	0.75 (0.825)	IS	IS	IS	
All	O (N=686)						0.33 (0.001**)
	QS (n=492)						0.33 (0.001**)
	EB (n=126)						0.34 (0.005*)
	Y (n=68)						0.22 (0.857)
Apex Carni- vore	O (N=423)						0.71 (0.012*)
	QS (n=309)						0.58 (0.836)
	EB (n=89)						0.56 (0.843)
	Y (n=29)						0.56 (0.842)

## Discussion

We identified nine carnivores out of 686 scat samples (range: 7 to 8 per region) with a total of 33 unique prey items observed (range: 9 to 31 per region). Carnivore species had greater dietary overlap than expected overall, in Qilian Shan, and in East Burhanbuda Mountain. This may indicate a high availability and low diversity of prey that allows for a convergence of resource use despite differing natural histories (Trevelline et al., 2018). Blue sheep and pika appear to be the primary drivers of observed overlap. Other mechanisms of niche partitioning such as spatial (Durant, 2000), temporal (Chesson, 1985; Hayward & Slotow, 2009), or a combination of such (Crooks & Van Vuren, 1995; Hayward & Kerley, 2008), are likely responsible for the successful coexistence of these species.

### The importance of blue sheep and pika in sustaining predator guilds

Blue sheep were detected from all three regions and were more common in apex carnivore diet, although occurrences in red fox were higher than in Eurasian lynx. Given the size of blue sheep, it is more likely that mesocarnivores scavenge or kill very young individuals. On the opposing size scale, small mammals contributed to the diets of every carnivore examined with most occurrences being pika, though the number of marmot and pika in East Burhanbuda Mountain samples were similar. Pika were considered important prey in previous studies (Q. X. Liu et al., 2010; Schaller, 2012). Their role in apex carnivore diets remains less understood, but are at minimum an important supplement to ungulates. Pika were previously heavily poisoned due to concerns of their role in grassland degradation (A. T. Smith et al., 1990; A. T. Smith & Foggin, 1999) but its

application in recent years has decreased. The presence of other smaller mammals such as woolly hare, marmot, vole, and zokor in diet suggest that carnivores can exploit a variety of smaller species, which may have reduced pressure when pika poisoning was at its peak. However, such shifts may be detrimental for the Eurasian lynx. Woolly hare in their diet was 0.2 to 0.5 times higher than any other species. This agrees with past studies that categorized Eurasian lynx as specialists in some regions (Mengüllüoğlu et al., 2018; Soyumert et al., 2019).

### Snow leopard and Tibetan wolf dietary ecology and comparison

Specialists are considered less adaptable than generalists. This is concerning for threatened species like the snow leopard, which have previously been characterized as having specialized dietary needs (Lyngdoh et al., 2014). Our study found that they had the second greatest dietary breadth, but the lowest diversity among apex carnivores. While the number of different prey items for snow leopards was high (richness), the number of each was skewed towards blue sheep (evenness). Snow leopards had a nearly 15% higher frequency for this ungulate than any other apex carnivore. Previous work has supported blue sheep as important prey for snow leopards in China (C.E. Hacker et al., 2021), and our results echo this finding. Also akin to previous studies is the observed similarity between snow leopard and Tibetan wolf diet. Previous studies in Kyrgyzstan (Jumabay-Uulu et al., 2014) and the Pamir Mountains of China (J. Wang et al., 2014) have found dietary similarity among snow leopards and wolves. However, other work has contrasted this (Chetri et al., 2017). Our Pianka's Index of 0.71 was not significantly

higher than expected, but this value coupled with the Jaccard's Distance between the two speaks to the shared resources between Tibetan wolf and snow leopard.

### Mesocarnivore diets and prey diversity

Mesocarnivores did not have greater dietary breadth than apex carnivores with the exception of the red fox, which had 23 unique dietary species. Other mesocarnivores, like the Pallas's cat and Tibetan fox appear to be specialists. Little published literature on Pallas's cat diet exists. A total of 96.7% of their diet were small mammals with most of those being pika, consistent with preferred Pallas's cat habitat (Ross et al., 2020). An occurrence of Eurasian collared dove demonstrated that they exploit birds. These findings align with a previous study that identified pika in 79%, and birds in 50%, of 14 Pallas's cat scat samples (Zhao et al., 2020).

Red foxes also had the highest counts of greater than expected niche overlap overall and in Qilian Shan with the exception of the Tibetan wolf. This is expected given that their high dietary diversity would include prey consumed by other carnivores, while simultaneously decreasing similarity. This was particularly notable between red fox and Tibetan fox, which opposes a previous study that found while prey from 30 red fox and 15 Tibetan fox scat samples did not significantly differ, the two had low dietary overlap due to prevalence of insects in red fox diet (Tsukada et al., 2014). The genetic markers used in this study were not designed to amplify or discern DNA from Insectivora, and thus some components of red fox diet may be missing. Similarly, diet items for omnivores such as Tibetan brown bear were likely missed.

### Livestock presence in diet



Livestock in carnivore diet was found in all three regions but was low. While lacking statistical significance between region and with any one particular carnivore species, conclusions regarding ecological significance and species management can be made. Apex carnivores had higher counts of livestock than mesocarnivores. This was driven by wolves with contributions from snow leopard and, infrequently, Eurasian lynx. Snow leopards had the most diverse livestock diet with four species represented, two of which, domestic pig and horse, were isolated occurrences. Snow leopards do consume yak (C.E. Hacker et al., 2021), but our study aligns with past studies in that they typically avoid them (Chetri et al., 2017). Red fox contributed most to livestock counts in mesocarnivore diet, but this is likely scavenging or kills of newborns given their smaller body sizes. Protecting livestock may require a more holistic approach to deter these many carnivores, particularly snow leopards. However, herders with domestic yak should focus on deterring wolves. Such strategies should be non-lethal to protect carnivore populations and ecosystem function (Marker et al., 2003; McManus et al., 2015; J. R. B. Miller et al., 2016; Treves et al., 2016).

#### Evidence of intraguild predation

Carnivore coexistence can foster extreme forms of inter-species competition like intraguild predation (Palomares & Caro, 1999). We identified mesocarnivores in Tibetan wolf and snow leopard diets in Qilian Shan and East Burhumbuda Mountain among 12 scat samples. Identifying these as a diet item versus contamination was based on lack of other carnivore DNA in the sample, successful read mapping to host and prey, and no other dietary item in the sample. Wolves are known to eat red fox, badger, and Pallas's

cat (Ross et al., 2020) and snow leopard to consume red fox and beech marten (Lyngdoh et al., 2014) with consumption of Pallas's cat likely (Ross et al., 2020).

#### Caution towards the impact of spatial and temporal partitioning

Though this study contributes substantially to current understanding of carnivore guilds on the QTP, facets of this research should be cautiously interpreted. Samples were not collected in winter to avoid introducing variation, but small temporal shifts can impact resource use. Similarly, fine-scale spatial differences may alter prey availability. A study that samples along the same transects through time will be necessary to better discern carnivore-prey dynamics in a particular system. Also concerning are sample size differences. Sample sizes for the majority of carnivores in this work are higher than in previous studies (Reshamwala et al., 2018; Tsukada et al., 2014) and have been genetically identified to ensure accuracy. Thus, information presented herein contributes substantially to preexisting knowledge. Future work that examines resource use by individual and sex to identify intraspecies differences would be valuable. For example, livestock kills may be more likely males (Johansson et al., 2015), or the result of competitive exclusion (Soberón, 2007).

#### Limitation of DNA barcoding

There is also an inability to determine if prey items were a result of predation or scavenging. This holds importance to livestock loss as Tibetan wolves may scavenge, rather than kill, domestic animals (B. Liu & Jiang, 2003). It is also unknown how many prey were killed. Carnivores may return multiple times to feed off of or share one

carcass. Age, size, or condition of prey can also not be determined. Further, species presence in diet is not necessarily reflective of environmental abundance. However, these shortcomings are also present in traditional methods. With DNA metabarcoding, marker selection may limit data interpretation if it is unable to discern species or does not amplify DNA from all possible prey. Contamination is also of concern as next-generation sequencing is sensitive (Pompanon et al., 2012).

### Conclusions and suggested conservation action

This study provided a glimpse into difficult-to-study carnivore guild dynamics in an area that has global impacts. Based on our findings and cautious interpretation of results, we suggest the following actions. First, the carnivore species overlapped in resource use significantly more than expected. This may indicate that diets have converged due to low prey diversity but sufficient availability, which can make the loss of an abundant prey species even more dire. Blue sheep and pika should be managed as part of carnivore species survival plans. Second, prey that are crucial for a specific carnivore should be safe-guarded. For example, pika for the Tibetan fox and Pallas's cat, as well as woolly hare for the Eurasian lynx. Third, carnivores with preference for one prey item should be monitored, as loss of this resource may have negative outcomes. Fourth, mechanisms that protect herder livelihoods should be expanded with focus on non-lethal strategies for deterring Tibetan wolves. Fifth, studies that investigate the diets of species with wide dietary breadths should consider multiple genetic markers that span all possible species consumed. Finally, future efforts should include sampling across one

site through time. Such efforts would shed additional knowledge of the ecological communities on the QTP and provide information for conservation efforts.

#### Author Attributions

Charlotte Hacker and Yuguang Zhang conceived study. Charlotte Hacker, Jan Janecka, and Yuguang Zhang acquired funding. Charlotte Hacker, Wei Cong, Yu Zhang, Yadong Xue, Jia Li, Yunchuan Dai, Liji Wu collected scat samples. Charlotte Hacker, Wei Cong, and Yunrui Ji extracted samples. Charlotte Hacker prepared samples for sequencing. Jan Janecka, Diqiang Li, and Yuguang Zhang provided administrative support. Charlotte Hacker, Cong Wei, and Jingjing Yu performed data analysis. Charlotte Hacker, Jan Janecka, and Yuguang Zhang interpreted data. Charlotte Hacker designed figures and tables, and wrote manuscript. Jan Janecka and Yuguang Zhang contributed to manuscript edits.

## Chapter 3.3

### Goal 2 – Subgoal 3

#### Seasonal variation of carnivore diet in Gouli Nature Reserve, China

Adapted from Hacker *et al.* (In Prep.) *Seasonal differences in dietary composition of a predator guild in China.*

##### Introduction

Optimal foraging theory outlines that predators choose prey based on cost-benefit. However, benefit may seasonally change as shifts in prey availability and abundance increases the associated costs of their pursuit (Krebs & Davies, 1993). For example generalist predators would presumably “switch” between prey species based on abundance or availability (Begg *et al.*, 2003; Pyke *et al.*, 1977). Classical optimal diet theory also predicts that diets will be more diverse when preferred food types are not as abundant or available (Perry & Pianka, 1997).

##### Lack of literature surrounding the dietary ecology of high altitude predator guilds

High altitude environments are under mounting pressure from climate change and increasing human activities (Öztürk *et al.*, 2015). Species living in these environments will be required to withstand potential reduction in habitat area, increased habitat fragmentation, loss of dispersal corridors, and increasing frequency of conflicts with humans (McRae *et al.*, 2008). Unfortunately, research in these landscapes to better understand species status and ecology in an effort to design effective conservation action plans is difficult due to rough terrain, harsh climates, and political sensitivities (Riordan & Shi, 2016). This, coupled with the fact that carnivores naturally live at low densities and occupy large home ranges (Beschta & Ripple, 2009), has grossly limited available literature surrounding predator guilds residing at high altitudes relative to information available from other ecosystem types (Boitani, 2012; Cupples *et*

al., 2011; Ray & Sunquist, 2001). This is despite the extreme biodiversity in predator species in some high altitude landscapes, such as that of the Qinghai-Tibetan Plateau (QTP) of China.

#### Habitat characteristics and wildlife on the Qinghai-Tibetan Plateau

The QTP is a 2.5 million km<sup>2</sup> landscape that undergone mass alterations in the last few decades due to anthropogenic and economic development across western China, leading to unprecedented pressure on area wildlife (J.M. Foggin, 2008; J. Liu et al., 2003; Schaller, 1990). Since that time, conservation policies and national park systems have worked to protect landscapes and encourage the repopulation of animals once threatened with extinction such as the Tibetan antelope (chiru, *Pantholops hodgsoni*) (Bleisch et al., 2009) and wild yak (*Bos mutus*) (Q. Shi et al., 2016). Prey species include blue sheep (*Pseudois nayaur*), Tibetan gazelle (*Procapra picticaudata*), argali (*Ovis ammon*), white-lipped deer (*Cervus albirostris*), marmot (*Marmota* sp.), chukar partridge (*Alectoris chukar*), and pika (*Ochotona* sp.), among others (Jackson, 2012; Schaller, 1998). Apex (top) carnivores found on the QTP include the Tibetan wolf (*Canis lupus*), Tibetan brown bear (*Ursus arctos pruinosus*), snow leopard (*Panthera uncia*) (A. Xu et al., 2008), and Eurasian lynx (*Lynx lynx*). Mesocarnivores include the red fox (*Vulpes vulpes*), Tibetan fox (*V. ferrilata*), Pallas's cat (*Otocolobus manul*), Eurasian badger (*Meles meles*), and beech marten (*Martes foina*).

#### Conflicts between carnivores and herders on the Qinghai-Tibetan Plateau

The QTP is a rangeland system that has been used by pastoralists for thousands of years (D. J. Miller & Bedunah, 1994), and is touted as housing the best known grasslands in Asia (D. J. Miller & Bedunah, 1994). A large number of livestock, including domestic yak (*B. grunniens*),

goat (*Capra aegagrus hircus*), sheep (*O. aries*), and horse (*Equus caballus*), are also present and may serve as food items that wild predators consume. Livestock can play an important role in predator diet and in sustaining carnivore populations, but their predation creates financial burdens on herders, their families, and the local economy (Mkonyi et al., 2017), is emotionally traumatizing (J. R. B. Miller et al., 2017), and can create tension between local residents and the conservation and government agencies seeking to protect at-risk wildlife (S. W. Wang & Macdonald, 2006). Knowledge of livestock frequency in diet by species is necessary to construct tailored mitigation strategies that prevent predators from taking livestock and for designing assistance programs that relieve the financial burdens associated with domestic animal loss, such as insurance schemes (Jackson, 2012).

#### The role of climate change

Such action is prudent, as the mountainous interior of Asia is warming at twice the rate of non-arctic regions in the Northern Hemisphere (D. Chen et al., 2009), creating unpredictability for herders in managing their livestock based on traditional ecological knowledge. Climate change will likely initiate large-scale weather events that cause reductions and distribution shifts of carnivores, their prey, and biodiversity, heavily influencing animal behavior and ecosystem function. Changes in the dynamics of one carnivore species has previously been shown to have large-scale impacts on other species. For example, snow fall has been found to correlate with grey wolf pack size and pack size to correlate with moose predation (Post et al., 1999). Predators under very quickly shifting landscapes may also find themselves overlapping in novel space and time with another species, whereby they are unsuccessful at procuring the resources necessary for survival, and may pursue livestock as an alternative. Knowledge of carnivore diet can be an

informative indicator of ecosystem changes in species distribution and abundance of both predators and prey as well as predicting how species may adapt to future changes.

#### Methodology used in study carnivore diet

The majority of previous studies surrounding predator diet on the QTP did not genetically identify host scat, relying only on morphological assessments, which are commonly inaccurate (Weiskopf et al., 2016). In addition, these studies relied on microhistology, a tedious and error-prone method in which scats are collected and dried, and digested hair is compared to a reference database of known species (Pompanon et al., 2012). The advent of next-generation sequencing and DNA metabarcoding is quickly changing the knowledge base and capacity for accurately assessing animal diet, overcoming its previous challenges (Valentini et al., 2009).

In determining diet with DNA metabarcoding, the organisms within each scat are identified by PCR amplification and sequencing of a diagnostic gene segment with the derived sequence bioinformatically matched to a reference database and thus its sample origin (Schnell et al., 2015; Valentini et al., 2009). Mitochondrially encoded 12S rRNA (MT-RNR1) serves as an ideal genetic marker for this purpose and has shown utility in past studies (Bohmann et al., 2011; Robeson et al., 2018; Shehzad, McCarthy, et al., 2012), including on the QTP (C.E. Hacker et al., 2021).

Most dietary studies on the QTP have been focused on one or two species (Aichun et al., 2006; B. Liu & Jiang, 2003; Q. X. Liu et al., 2010; Schaller, Junrang, et al., 1988; Tsukada et al., 2014; Weidong, 2010; Werhahn et al., 2019). One previous study used DNA metabarcoding to examine the diets of nine predator species living in three regions of the QTP (Hacker et al. In



Review). However, these samples were collected and analyzed without respect to seasonal differences, leaving a substantial knowledge gap in how diet changes through time.

### The need for studies examining seasonal changes in diet

Climate change is now constantly modifying the temporal structure of species interactions (Kharouba et al., 2018; Nakazawa & Doi, 2012; Wolkovich & Cleland, 2014). This is challenging as accounting for temporal fluctuations is imperative to understanding and predicting species community structure (Chesson, 1985). More fine-scale analyses that study seasonality can aid in accounting for changes that go against core assumptions and fundamental modeling concepts in ecology due to climate change. Seasonality plays a large role in shaping predator ecology, phenology, and resource use. Resource availability may change (Scharf & Schlicht, 2000; Woodward & Hildrew, 2002), behaviors of predator or prey may be altered (Dutour et al., 2017), use of habitat may shift (Florke et al., 2020), and species equilibrium of ecosystems may be altered (Osman, 1978).

### Study purpose and goals

To help close the knowledge gap surrounding how prey use, livestock dependency, and resource overlap changes seasonally, we noninvasively sampled 10 fixed transects across four seasons for one year in the Gouli Nature Reserve in Dulan County, Qinghai Province, China. The goals of this study were to (1) detail the diet of carnivore species in the Gouli Nature Reserve; (2) assess the extent to which dietary frequency and biomass seasonally changes; and (3) quantify dietary diversity, similarity, and niche overlap for identified carnivores host species.

## Materials and Methods

Necessary permits and permissions for sampling were obtained from the Qinghai Wildlife Management and Protection Bureau and the Dulan County Forestry Reserve prior to study start.

### Study site

Gouli Township is located in Dulan County, Haixi Mongolian-Tibetan Autonomous Prefecture, Qinghai Province, China and holds the Gouli Nature Reserve (35.659528°E, 98.499298°N), a protected area established in 2009. The reserve has no set size, but is within the 2,559.4 km<sup>2</sup> of Gouli Township (Rou Bao, personal communication) and has human inhabitants. Residents in the area are predominately Tibetan and are semi-nomadic pastoralists keeping livestock such as yak, sheep, goats, and horses in summer pastures of approximately 4,600m with winter and spring pastures at lower altitudes of around 4,000m (A. Xu et al., 2008). The climate is characterized by long, dry, and cold winters with strong winds and solar radiation (A. Xu et al., 2008). During the period of this study, the mean temperature ranged from -8.17°C in December 2019 to 15.78°C in July 2020. The greatest amount of precipitation took place in June 2020 with 73.15mm and the lowest in January 2020 with 1.78mm (NOAA, <https://www.ncdc.noaa.gov/>). Topography predominately consists of grassland with bare rock slopes (Y. Liu, 1993).

### Pilot study

Conducting pilot experiments is helpful for determining appropriate sampling designs for studies involving the use of environmental DNA (Dickie et al., 2018). A total of 137 scat samples believed to be of carnivore origin were collected along 20 km of transects as part of a separate study examining predator guild diet across China (see Chapter 3.2) These results both confirmed the presence of an active predator guild in the area as well as a seemingly abundant prey base.

### Transect selection

Transects were selected in September 2019 based on predator presence confirmed either by camera trap or herder report within the previous 4 months. Transects were then further narrowed down by accessibility year-round (i.e. not prone to flooding, reachable in winter), seasonal grazing status, and then by relative location, so as not to bias sampling to exclusion of livestock presence in summer or winter, and to space out transects into four respective areas within Gouli Township – Ren Long Cun of Gouli, Duo Jiao Hu, Delong Guo, and Re Long Guo. This resulted in a total of ten transects totaling 22.5km (Table 20, Figure 29).

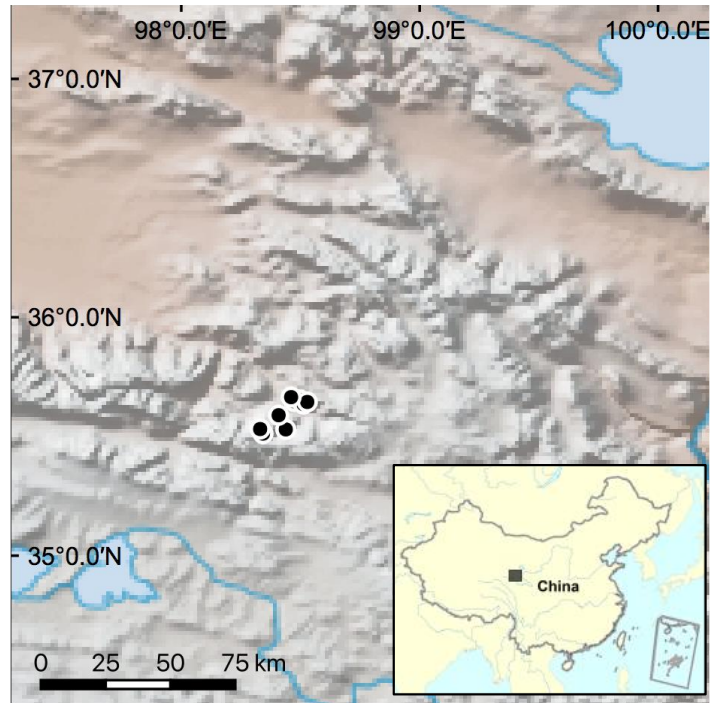


Figure 29. The fixed transects sampled in Gouli Nature Reserve, Dulan County, Qinghai Province, China.

Table 20. Detailed information on the ten transects sampled for molecular dietary analysis of carnivore species.

Transect Number	Sampling Area	GPS Location Start	GPS Location End	Elevation Range (m)	Distance (km)	Dominant Topographic Features	Landform Ruggedness	Grazing Status
1	Ren Long Cun of Gouli	35.64816°E, 98.47176°N	35.64738°E, 98.49340°N	3,938 – 4,429	2	Ridgeline; Hillside	Very Broken/Steep	Seasonal - Livestock kept in winter
2	Ren Long Cun of Gouli	35.64819°E, 98.471706°N	35.6467°E, 98.47106°N	3,921 – 4,200	2.3	Hillside	Very Broken/Steep	Seasonal - Livestock kept in winter
3	Ren Long Cun of Gouli	35.65337°E, 98.49767°N	35.65968°E, 98.50040°N	4,311 – 4,449	2	Ridgeline; Hillside	Moderately Steep	Seasonal - Livestock kept in winter
4	Delong Gou	35.51239°E, 98.34261°N	35.53093°E, 98.43856°N	4,458 – 4,679	3.5	Ridgeline	Moderately Steep	Seasonal - Livestock kept in summer
5	Delong Gou	35.5315°E, 98.3306°N	35.5245°E, 98.3334°N	4,531 – 4,700	2.2	Hillside	Moderately Steep	Seasonal - Livestock kept in summer
6	Delong Gou (Xia La Wen)	35.52952°E, 98.843827°N	35.5300°E, 98.4381°N	4,048 – 4,172	1.7	Ridgeline; River/Drainage Bottom	Moderately Steep	Seasonal - Livestock kept in summer
7	Duo Jiao Hu	35.63624°E, 98.51250°N	same as start - transect followed in oblong loop	4,040 - 4,189	2.7	River/Drainage Bottom	Flat	Seasonal - Livestock kept in winter
8	Duo Jiao Hu	35.64507°E, 98.52818°N	same as start - transect followed in oblong loop	4,152 - 4,259	1.6	Ridgeline; Hillside; River/Drainage Bottom	Moderately Steep	Seasonal - Livestock kept in winter
9	Ren Long Cun of Gouli	35.66530°E, 98.46046°N	35.674134°E, 98.459936°N	4,122 - 4,275	1.4	Hillside; River/Drainage Bottom	Rolling	Seasonal - Livestock kept in winter
10	Re Long Guo	35.58954°E, 98.40818°N	35.58211°E, 98.40253°N	4,016 - 4,174	3.1	Ridgeline; Hillside; River/Drainage Bottom	Very Broken/Steep	Year-Round - Livestock kept in winter and summer

### Sample collection

Sample collection followed methods outlined in Chapter 3.2 of this dissertation, with care to leave approximately  $\frac{1}{2}$  to  $\frac{3}{4}$  of the scat so as not to disrupt the role scat plays in chemical communication to other predators (Chetri et al., 2017), and to help determine the degradation timeline of scat in the environment. Colored nail polish coordinating with sampling session was used to mark any remaining scat that was not collected so that it would not be sampled in future efforts. It was not anticipated that the nail polish would impact wildlife given that a small amount was used and that volatile organic compounds evaporate quickly. Because the ageing and degradation process of scats on the QTP is not well understood, only scats that were “fresh” (outside casing largely intact, little to no white discoloration) were collected in the first sampling month of September 2019, so as to avoid collecting samples that were not representative of the targeted investigated time frame. A total of 188 scat samples were collected in September 2019, 130 in December 2019, 133 in March 2020, and 130 in July 2020 resulting in 581 scat samples total.

### DNA extraction, PCR for species and diet analysis, sequencing, diet analysis

See Chapter 2.1 for DNA extraction, Chapter 3.2 for PCR for species and diet analysis as well as sequencing, and Chapter 3.1 for data analysis.

### Data analysis

All statistical analyses were performed in Microsoft Office Excel for Mac Version 16.43 and R version 3.5.2 (R Core Team 2018) using base R functions, as well as the *iNEXT* (Hsieh et

al., 2016) and *EcoSimR* packages (Gotelli et al., 2015). Percentage of host species composition overall and by season was examined by summing the number of scats belonging to each host ( $s$ ) and dividing it by the total number of scats observed ( $S$ ). Percentage of the relative frequency of occurrence ( $r/R*100$ ) of identified prey species ( $r$ ) to the number of prey items found ( $R$ ) was calculated overall and by season and for each individual host species and among all host species combined. Effective taxonomic richness and dietary diversity of each carnivore overall and by month was calculated using Hill numbers (Hill, 1973) and sampling completeness for each carnivore overall and by month was calculated using the 'iNEXT' function (Hsieh et al., 2016). This enabled comparison of dietary diversity between species with varying sample sizes (Heck et al., 1975; Hurlbert, 1971) and allowed for evaluation of sampling completeness. The number of scat samples with more than one prey item present were summed and assigned to host species. Prey species were grouped hierarchally into broader categories for various applications of data interpretation (Table 21).

Table 21. The name of each prey species and its grouping by secondary and tertiary naming for data analysis and interpretation.

Primary Tier Prey Name	Secondary Tier Prey Name	Tertiary Tier Prey Name
Domestic Yak	Domestic Yak	
Domestic Camel	Domestic Camel	Livestock
Domestic Goat	Domestic Goat	
Domestic Sheep	Domestic Sheep	
Blue Sheep	Blue Sheep	Wild Ungulates
White-lipped Deer	White-lipped Deer	
Tibetan Fox	Tibetan Fox	Carnivores
Red Fox	Red Fox	
Himalayan Marmot	Himalayan Marmot	
Woolly Hare	Woolly Hare	
Zokor	Zokor	
Mountain Weasel	Mountain Weasel	
Pika	Pika	
Long-tailed Dwarf Hamster	Long-tailed Dwarf Hamster	Small Mammals
Narrow Headed Vole		
Grey Red-Backed Vole	Vole species	
Chinese Scrub Vole		
Sikkim Vole		
Common Shrew	Common Shrew	
Upland Buzzard	Birds of Prey	
Falcon		
Common Raven		
Brown Accentor	Perching Birds	Birds
Pine Bunting		
Himalayan Snowcock	Ground Feeding Birds	
Chukar Partridge		
Undetermined	Undetermined	Undetermined

It is impossible to discern biomass from scat, as the age, sex, and condition of the animal is unknown. However, frequency estimates can be biased given that prey items in a given diet can be of difference sizes (Floyd et al., 1978; Weaver, 1993). Thus, we attempted to calculate the relative biomass consumed by each host predator species overall and across each season using a linear regression model designed for the wolf (Floyd et al., 1978) with slight modifications



(Weaver, 1993) as deployed in previous carnivore diet analysis studies similar to this one (N. E. Davis et al., 2015; Werhahn et al., 2019).

$$Y = 0.439 + 0.008X$$

Where X is the mean mass of any given prey item and Y is the mass of the food item per scat. Y was then multiplied by the number of occurrences of each respective food item to estimate its relative total mass contribution to the diets of Tibetan wolves, snow leopards, Eurasian lynx, Tibetan fox, red fox, Pallas's cat, and beech marten overall and by season where sample sizes allowed.

Significant seasonal differences in the frequency of each carnivore species detected as well as seasonal differences in dietary frequency and biomass were calculated using a Friedman's Test using the four sampling seasons as independent repeated measures for species and species as independent repeated measures for season. A Wilcoxon signed rank test was used to compare the carnivores detected as well as their diets between any two given seasons. The percentages calculated for frequency of dietary items and biomass were also assessed using a Wilcoxon signed rank test to determine if they were significantly different from one another for future applications.

For calculations of species diversity metrics, dietary similarity, and niche overlap, see Chapter 3.2.

## Results

### Carnivore and prey species identification

Of the 581 scat samples examined, seven were regurgitated bird pellets from raptor species (upland buzzard (*Buteo hemilasius*), Eurasian eagle owl (*Bubo bubo*), falcon (*Falco* sp.)). One sample was from a non-target host (white-lipped deer (*Cervus albirostris*)), eight samples were inconclusive, and 54 samples did not have sufficient DNA for analysis. These were removed from further analysis, leaving 511 scat samples of carnivore origin from eight carnivore species. Of these, one sample belonged to Tibetan brown bear, 252 samples to Tibetan wolf, 46 to snow leopard, five to Eurasian lynx, 56 to Tibetan fox, 135 to red fox, ten to Pallas's cat, and seven to beech marten. In March, 57 scats were from Tibetan wolf, 23 from snow leopard, three from Eurasian lynx, 23 from Tibetan fox, and 20 from red fox. In July, 98 scats belonged to Tibetan wolf, two were from snow leopard, three from Eurasian lynx, 13 from Tibetan fox, nine from red fox, and one from Tibetan brown bear. For September, there were 55 scats identified as belonging to Tibetan wolf, 15 to snow leopard, three to Tibetan fox, 63 to red fox, five to Pallas's cat, and seven to beech marten. December had 42 scats belonging to Tibetan wolf, five to snow leopard, 16 to Tibetan fox, 43 to red fox, and five to Pallas's cat (Figure 30). There was no statistically significant difference in the number of scats belonging to carnivores detected across seasons ( $\chi^2 (3)=1.174, p = 0.759$ ).

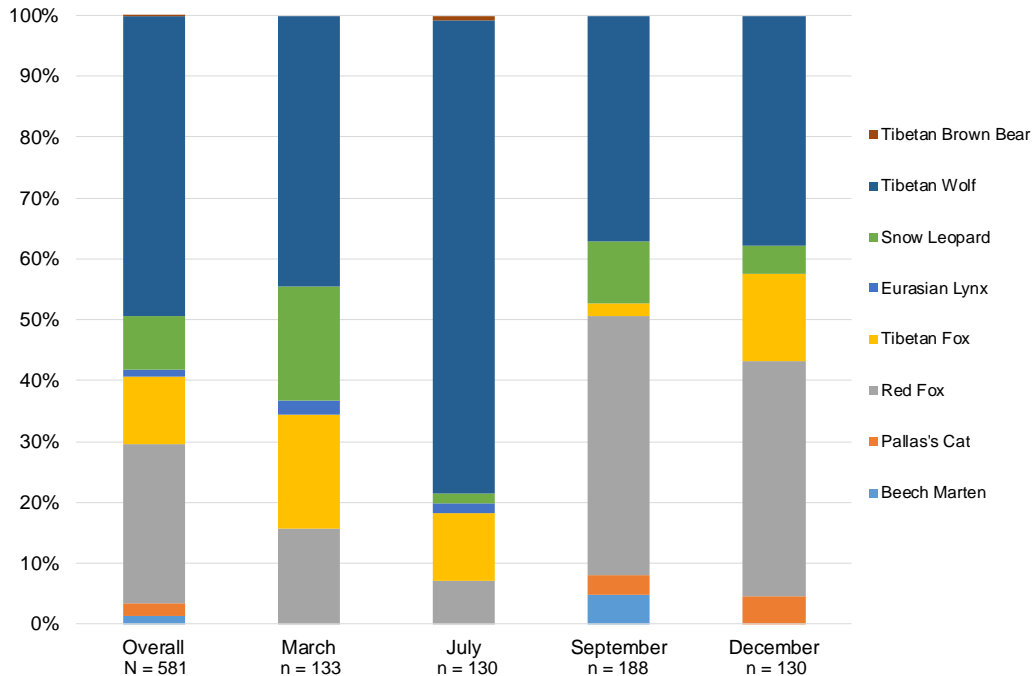


Figure 30. The relative percentage of frequency counts of scats genetically determined to belong to each identified carnivore host species overall and by month.

There were 26 unique prey species identified from two animal classes (Mammalia, Aves). Biomass of identified prey were determined from peer-reviewed literature (Table 22). Of the 26 identified prey, domestic ungulates comprised 12.4% of diet occurrences and 32.2% of total biomass consumed. Wild ungulates constituted 31.7% of occurrences and a biomass of 35.4%. Small mammals made up 52.1% of occurrences and 29.5.8% of calculated biomass while 3.4% of counts were bird species that comprised 0.8% of biomass. Carnivores were 0.5% of prey counts and 2.2% of prey biomass. A total of 6.6% of frequency counts were undetermined prey species. Pika as a small mammal dominated dietary frequency at 43.1% of counts, but only 19% of biomass. Alternatively, the blue sheep dominated dietary biomass at 35.1% with 31.5% of dietary counts. Proportion of dietary items by frequency and biomass overall can be found in Figure 3. Prey counts and biomass per species can be found in Table 23, Table 24, and Figure 31. Metrics of sampling completeness can be found in Table 25.

The one Tibetan brown bear scat had one prey item. A mean of 1.42 prey/scat ( $\pm 0.85$ ) was found for Tibetan wolf, 0.98 prey/scat ( $\pm 0.54$ ) for snow leopard, 1.40 prey/scat ( $\pm 0.89$ ) for Eurasian lynx, 1.18 prey/scat ( $\pm 0.43$ ) for Tibetan fox, 1.51 prey/scat ( $\pm 0.81$ ) for red fox, 0.90 prey/scat ( $\pm 0.32$ ) for Pallas's cat, and 1.57 prey/scat ( $\pm 0.79$ ) for beech marten.

Table 22. The determined mass of an average adult sized individual for each prey item and the source of the metric.

Prey Species	Mean Average Mass (kg)	Source
Domestic Yak (plateau)	304	(Committee, 1983)
Domestic Camel	495	<a href="https://animaldiversity.org/">https://animaldiversity.org/</a>
Domestic Goat (cashmere)	28.5	(Bai et al., 2006)
Domestic Sheep (Tibetan)	36.81	(T. Xu et al., 2017)
Blue Sheep	57.25	(Lovari, 2020)
White-lipped Deer	135	<a href="https://animaldiversity.org/">https://animaldiversity.org/</a>
Tibetan Fox	4.75	(Harris et al., 2008)
Red Fox	8.1	Walkers Mammals of the World
Himalayan Marmot	6.6	(Nikol'skii & Ulak, 2006)
Woolly Hare	2.3	<a href="https://animaldiversity.org/">https://animaldiversity.org/</a>
Zokor	0.38	(A. T. Smith et al., 2010)
Mountain Weasel	0.23	(Holmes, 2007)
Pika	0.16	(A. T. Smith, 2019)
Long-tailed Dwarf Hamster	0.032	(A. T. Smith et al., 2010) (pg. 243)
Narrow Headed Vole	0.035	(Sheftel & Henttonen, 2016)
Grey Red-Backed Vole	0.035	(Sheftel & Henttonen, 2016)
Chinese Scrub Vole	0.035	(Sheftel & Henttonen, 2016)
Sikkim Vole	0.035	(Sheftel & Henttonen, 2016)
Common Shrew	0.008	(Hutterer & Kryštufek, 2017)
Upland Buzzard	1.31	(Q. Cui et al., 2008)
Falcon	1	(Shobrak, 2015)
Common Raven	1.2	(Birdlife International, 2012)
Brown Accentor	0.03	(McClure, 1991)
Pine Bunting	0.03	(Birdlife International, 2017)
Himalayan Snowcock	3.1	(Chardine & Dunning, 1994)
Chukar Partridge	0.61	<a href="https://animaldiversity.org/">https://animaldiversity.org/</a>

### *March*

There were 14 unique prey species identified in the scats of five carnivore hosts. Small mammals made up the majority of dietary counts at 34.8% but were second in terms of contributed biomass at 17.6%. Blue sheep was 44.5% of prey counts and constituted a similar value of biomass at 44.9%. Livestock constituted 15.9% of prey items identified and 35.9% of calculated biomass, while carnivores were 1.2% of counts and 0.7% of biomass. Birds were 3.7% of prey counts and 0.9% biomass. A total of 2.9% of prey counts were undetermined.

Proportion of dietary items for March can be found in Figure 3. Prey counts and biomass per species for the month of March can be found in Table 23, Table 24, and Figure 31. Metrics of sampling completeness can be found in Table 25.

### *July*

A total of nine unique prey species were captured in scats from six different carnivores. Under half of dietary counts were composed of small mammals at 40.2% and collectively the group contributed 20.4% of total biomass. Blue sheep made up 40.2% of counts and the majority of biomass at 38.0%. Livestock constituted 18.7% of prey counts but was 41.2% of biomass, while carnivores were 0.9% and 0.5% for prey counts and biomass, respectively. No birds were observed in diet. Only 0.1% of counts were prey that could not be determined. The only count of Tibetan brown bear was observed in July with Himalayan marmot identified (100% biomass). Proportion of dietary items for July can be found in Figure 3. Prey counts and biomass per species for the month of July can be found in Table 23, Table 24, and Figure 31. Metrics of sampling completeness can be found in Table 25.

### *September*

Samples collected in September returned 18 different prey species amongst six various carnivore species. Small mammals comprised 77.8% of prey counts and, 64.3% of biomass. Birds were at 9.4% and 0.5% of prey counts and biomass, respectively. Wild ungulates consisted of blue sheep and white-lipped deer to total 9.4% of diet occurrences and 16.0% of biomass. Carnivores constituted 0.9% of counts and 0.7% of biomass. A total of 22.7% of counts were undetermined. The only counts of beech marten were observed in September with seven dietary items observed comprised of five counts of pika (19.2% biomass), one mountain weasel (5.5%

biomass), one pine bunting (0.7% biomass), and one Himalayan snowcock (74.5% biomass).

Proportion of dietary items for September can be found in Figure 3. Prey counts and biomass per species for the month of September can be found in Table 23, Table 24, and Figure 31. Metrics of sampling completeness can be found in Table 25.

### *December*

Samples contained 15 different prey species from five different carnivore species. Among these, small mammals made up 67.2% of diet item counts and 44.5% of consumed biomass. Blue sheep constituted 20.1% of counts and 27.9% of biomass while carnivores comprised 3.7% of counts and 9.7% of biomass. Domestic ungulates were 6.0% of diet counts and 17.3% of biomass. Birds were 3.0% of counts and 0.5% of biomass. No dietary items were undetermined for the month of December. Proportion of dietary items for December can be found in Figure 3. Prey counts and biomass per species for the month of December can be found in Table 23, Table 24, and Figure 31. Metrics of sampling completeness can be found in Table 25.

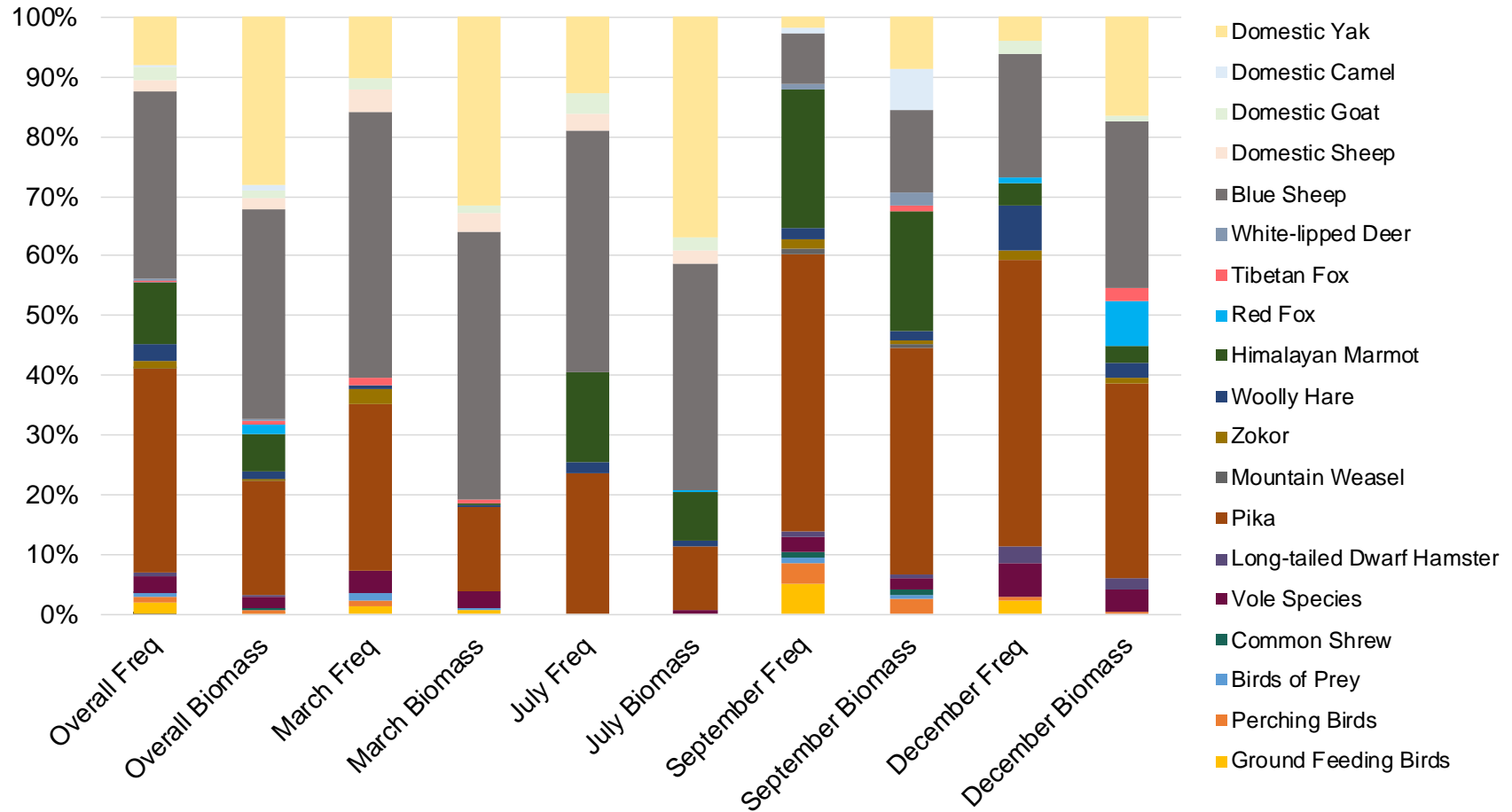


Figure 31. The relative percentages for dietary biomass and frequency overall and by month for all eight carnivore species identified in the study combined.



Table 23. The percentages (%) of frequency of occurrence for prey items in the diets of eight carnivores species overall and across four seasons. (O = Overall, M = March, J = July, S = September, D = December).

		Tibetan Wolf					Snow Leopard					Eurasian Lynx			Tibetan Fox					Red Fox					Pallas's Cat		
		252	57	98	55	42	46	24	2	15	5	5	3	2	56	23	14	3	16	135	20	9	63	43	10	5	5
		O	M	J	S	D	O	M	J	S	D	O	M	J	O	M	J	S	D	O	M	J	S	D	O	S	D
Domestic Ungulates	Domestic Yak	11.0	15.9	14.1	0.0	5.7	4.3	7.7	0.0	0.0	0.0	0.0	0.0	0.0	2.9	3.4	5.6	0.0	0.0	4.2	8.0	5.9	3.0	3.3	0.0	0.0	0.0
	Domestic Camel	0.3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Domestic Goat	3.2	3.7	4.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	1.7	0.0	0.0	0.0
	Domestic Sheep	3.2	7.3	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wild Ungulates	Blue Sheep	39.2	57.3	44.1	5.0	34.0	59.6	73.1	100.0	28.6	60.0	28.6	33.3	25.0	4.3	3.4	11.1	0.0	0.0	10.1	20.0	17.6	4.5	10.0	0.0	0.0	0.0
	White-lipped Deer	0.3	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carnivores	Tibetan Fox	1.9	2.4	0.0	1.7	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Red Fox	3.8	0.0	1.1	0.0	22.6	2.1	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Smaller Mammals	Himalayan Marmot	13.7	0.0	15.3	33.3	7.5	4.3	0.0	0.0	14.3	0.0	14.3	0.0	25.0	4.3	3.4	5.6	0.0	5.6	4.8	0.0	17.6	7.6	0.0	0.0	0.0	0.0
	Woolly Hare	1.6	0.0	1.7	1.7	3.8	0.0	0.0	0.0	0.0	0.0	14.3	33.3	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	5.9	1.5	5.0	0.0	0.0	0.0
	Zokor	0.3	0.0	0.0	0.0	1.9	2.1	0.0	0.0	7.1	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	5.6	0.6	0.0	0.0	1.5	0.0	0.0	0.0	0.0
	Mountain Weasel	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pika	11.8	9.8	14.1	8.3	11.3	12.8	7.7	0.0	21.4	20.0	42.9	33.3	50.0	79.7	79.3	77.8	50.0	88.9	53.6	48.0	52.9	53.0	56.7	90.0	80.0	100.0
	Long-tailed Dwarf Hamster	0.3	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.0	6.7	0.0	0.0	0.0
	Vole Species	1.3	2.4	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9	6.9	0.0	0.0	0.0	9.5	24.0	0.0	4.5	11.7	0.0	0.0	0.0
	Common Shrew	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	1.5	0.0	0.0	0.0	0.0
Birds	Birds of Prey	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	3.4	0.0	0.0	0.0	0.6	0.0	0.0	1.5	0.0	0.0	0.0	0.0
	Perching Birds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	25.0	0.0	1.8	0.0	0.0	3.0	1.7	0.0	0.0	0.0
	Ground Feeding Birds	1.1	1.2	0.0	3.3	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	0.0	0.0	7.6	3.3	0.0	0.0	0.0
Undetermined		7.3	0.0	0.6	41.0	0.0	14.9	11.5	0.0	28.6	0.0	0.0	0.0	0.0	1.4	0.0	0.0	25.0	0.0	4.2	0.0	0.0	10.6	0.0	10.0	20.0	0.0

Table 24. The percentages (%) of biomass contribution for prey items in the diets of eight carnivores species overall and across four seasons. (O = Overall, M = March, J = July, S = September, D = December).

		Tibetan Wolf					Snow Leopard					Eurasian Lynx			Tibetan Fox					Red Fox					Pallas's Cat		
		252	57	98	55	42	46	24	2	15	5	5	3	2	56	23	14	3	16	135	20	9	63	43	10	5	5
		O	M	J	S	D	O	M	J	S	D	O	M	J	O	M	J	S	D	O	M	J	S	D	O	S	D
Domestic Ungulates	Domestic Yak	34.1	38.8	39.1	0.0	0.0	16.2	24.3	0.0	0.0	0.0	0.0	0.0	0.0	15.8	18.3	25.4	0.0	0.0	18.4	18.3	25.1	17.9	16.7	0.0	0.0	0.0
	Domestic Camel	1.3	0.0	0.0	19.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Domestic Goat	2.0	2.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.9	0.0	0.0	0.0
	Domestic Sheep	2.7	5.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wild Ungulates	Blue Sheep	39.9	47.5	39.7	11.7	43.0	70.9	72.0	100.0	56.7	74.0	44.1	50.0	39.5	7.4	5.7	15.8	0.0	0.0	16.3	28.5	23.5	8.4	15.7	0.0	0.0	0.0
	White-lipped Deer	0.5	0.0	0.0	6.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carnivores	Tibetan Fox	1.0	1.1	0.0	2.1	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Red Fox	2.1	0.0	0.6	0.0	0.0	1.4	0.0	0.0	0.0	13.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Smaller Mammals	Himalayan Marmot	7.8	0.0	7.8	42.9	35.0	2.8	0.0	0.0	15.5	0.0	12.1	0.0	21.7	4.1	3.1	4.3	0.0	6.2	4.2	0.0	12.9	7.7	0.0	0.0	0.0	0.0
	Woolly Hare	0.8	0.0	0.8	2.0	0.7	0.0	0.0	0.0	0.0	0.0	11.3	25.5	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.0	4.0	1.4	4.0	0.0	0.0	0.0
	Zokor	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	5.5	0.5	0.0	0.0	1.4	0.0	0.0	0.0	0.0
	Mountain Weasel	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pika	6.3	4.0	6.2	11.5	19.0	8.7	3.7	0.0	27.8	12.1	32.5	24.5	38.8	66.6	64.4	54.4	66.7	88.3	42.3	33.6	34.6	47.9	43.6	100.0	100.0	100.0
	Long-tailed Dwarf Hamster	0.1	0.0	0.0	1.9	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	5.1	0.0	0.0	0.0
	Vole Species	0.7	1.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	5.6	0.0	0.0	0.0	7.5	16.8	0.0	4.1	9.0	0.0	0.0	0.0
	Common Shrew	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	1.4	0.0	0.0	0.0	0.0
Birds	Birds of Prey	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	2.9	0.0	0.0	0.0	0.5	0.0	0.0	1.4	0.0	0.0	0.0	0.0
	Perching Birds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	33.3	0.0	1.4	0.0	0.0	2.7	1.3	0.0	0.0	0.0
	Ground Feeding Birds	0.4	0.5	0.0	2.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	2.8	0.0	5.8	2.7	0.0	0.0	0.0

Table 25. Observed as well as lower and upper confidence limits of sampling completeness overall and by month for carnivore species with at least one month containing sufficient sample numbers. Values closer to 1 are indicative of higher sampling completeness. The symbol (-) indicates that sample sizes were not sufficient to determine sampling completeness.

	Overall	March	July	September	December
Tibetan Wolf	0.962 (0.947 - 0.977)	0.953 (0.916 - 0.989)	0.971 (0.946 - 0.995)	0.759 (0.653 - 0.864)	0.919 (0.843 - 0.996)
Snow Leopard	0.935 (0.879 - 0.992)	1.000 (0.938 - 1.000)	-	0.782 (0.532 - 1.000)	0.733 (0.280 - 1.000)
Eurasian Lynx	0.817 (0.438 - 1.000)	0.333 (0.000 - 0.750)	0.750 (0.559 - 0.941)	-	-
Tibetan Fox	0.940 (0.889 - 0.992)	0.831 (0.704 - 0.957)	0.891 (0.785 - 0.996)	-	0.896 (0.844 - 0.949)
Red Fox	0.922 (0.891 - 0.953)	0.915 (0.828 - 1.000)	0.706 (0.517 - 0.895)	0.904 (0.847 - 0.962)	0.935 (0.890 - 0.979)
Beech Marten	0.608 (0.356 - 0.860)	-	-	0.608 (0.338 - 0.877)	-

#### Dietary frequency versus biomass

There were no significant differences detected in the percentages calculated using frequency counts for diet versus biomass overall ( $Z = -1.108$ ,  $p = 0.268$ ), for March ( $Z = -1.924$ ,  $p = 0.054$ ), July ( $Z = -1.305$ ,  $p = 0.192$ ), September ( $Z = -0.616$ ,  $p = 0.538$ ), or December ( $Z = -0.874$ ,  $p = 0.382$ ).

#### Seasonal changes in diet

When considering all observed carnivores, diets across months were not significantly different in frequency ( $\chi^2 (3) = 2.893, p = 0.408$ ) or biomass ( $\chi^2 (3) = 3.663, p = 0.300$ ). The Tibetan wolf was the only carnivore with sufficient sample sizes to investigate dietary differences across all four months, differences of which were found to be insignificant for frequency ( $\chi^2 (3) = 3.643, p = 0.303$ ) and biomass ( $\chi^2 (3) = 2.023, p = 0.568$ ). The months of March, July, and December were compared for the Tibetan fox with no significant differences in diet found in frequency ( $\chi^2 (2) = 2.800, p = 0.247$ ) or biomass ( $\chi^2 (2) = 0.080, p = 0.961$ ). The months of March, September, and December were compared for the red fox, with differences in dietary frequency being statistically significant ( $\chi^2 (2) = 7.048, p = 0.029$ ). Follow up Wilcoxon signed rank tests revealed significant differences in red fox diet between March and December ( $Z = 2.585, p = 0.010$ ) but not between March and September ( $Z = 1.490, p = 0.136$ ), or between September and December ( $Z = -0.159, p = 0.874$ ). A statistically significant difference in red fox dietary biomass across months was not detected ( $\chi^2 (2) = 2.261, p = 0.323$ ). Scat sample sizes for snow leopard were only large enough for comparison in the months of March and September, and these were not found to be significantly different in terms of frequency ( $Z = -0.272, p = 0.785$ ) or biomass ( $Z = 0.000, p = 1$ ).

#### Dietary diversity and similarity

Dietary richness ranged from one prey item in Pallas's cat to 18 prey items for the red fox. The Tibetan wolf had the highest dietary diversity according to the Shannon-Wiener Index and the Eurasian lynx for the Simpson's Index (Table 26). Similarity based

on Jaccard's Distances were calculated for carnivore hosts overall and for months where sufficient samples sizes allowed, with limited dissimilarity in diets observed (Table 27, Figure 32).

Table 26. Dietary diversity metrics surrounding carnivore diet overall and by season. (Shannon-Wiener Index; higher value equates to higher diversity; Simpson's Index; lower value equates to higher diversity; The symbol (-) indicates lack of dietary information for that host species. (O = Overall, M = March, J = July, S = September, D = December).

	<i>Richness</i>					<i>Shannon-Wiener Index</i>					<i>Simpson's Index</i>				
	O	M	J	S	D	O	M	J	S	D	O	M	J	S	D
Tibetan Wolf	16	9	9	9	9	1.846	1.385	1.636	1.431	1.899	0.234	0.363	0.26	0.362	0.182
Snow Leopard	5	3	1	3	3	1.018	0.583	0	1.28	0.95	0.506	0.684	1	0.222	0.3
Eurasian Lynx	4	3	3	3	-	1.277	1.099	1.04	-	-	0.19	0.166	0.667	-	-
Tibetan Fox	8	6	4	2	3	0.851	0.833	0.761	0.637	0.426	0.656	0.626	0.601	0.333	0.784
Red Fox	18	6	5	12	11	1.614	1.335	1.282	1.519	1.496	0.338	0.283	0.309	0.374	0.344
Pallas's Cat	1	-	-	1	1	0	0	0	-	-	1	-	-	1	1
Beech Marten	4	-	-	4	-	1.074	-	-	1.074	-	0.357	-	-	0.357	0
All Carnivores	26	14	9	17	14	1.889	1.528	1.633	1.138	1.863	0.224	0.294	0.246	0.273	0.242

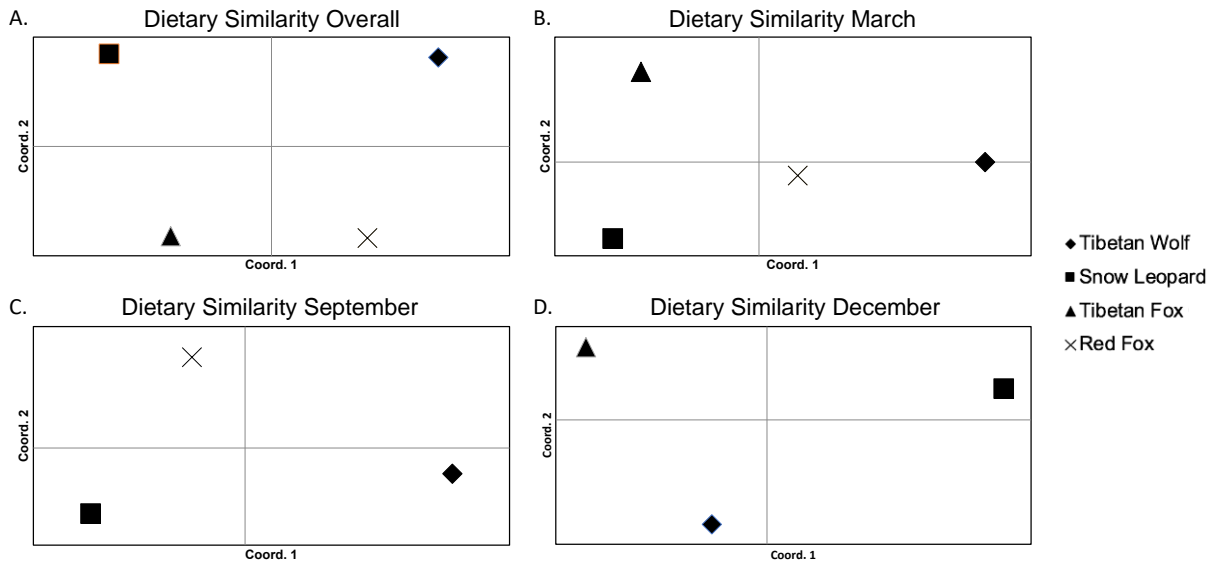


Figure 32. Dietary similarity between host carnivore species as represented by PCoA plots constructed from Jaccard's coefficients where sample sizes allowed for data interpretation A) all data points regardless of month; B) for March; C) for September; and D) for December.

Table 27. Jaccard coefficients for dietary similarity overall and across seasons for carnivore species with appropriate sample sizes for comparisons. (TW = Tibetan Wolf, SL = Snow Leopard, TF = Tibetan Fox, RF = Red Fox, PC = Pallas's Cat).

		Overall					March				July		September			December		
		TW	SL	TF	RF	PC	TW	SL	TF	RF	TW	TF	TW	SL	RF	TW	TF	RF
Overall	TW	-																
	SL	0.400	-															
	TF	0.353	0.556	-														
	RF	0.556	0.357	0.615	-													
	PC	0.067	0.167	0.125	0.077	-												
March	TW	0.533	0.273	0.333	0.400	0.125	-											
	SL	0.200	0.500	0.375	0.231	0.333	0.375	-										
	TF	0.313	0.500	0.750	0.462	0.167	0.400	0.500	-									
	RF	0.333	0.375	0.444	0.385	0.200	0.625	0.600	0.571	-								
July	TW	0.600	0.500	0.417	0.467	0.111	0.545	0.333	0.500	0.400	-							
	TF	0.267	0.667	0.500	0.308	0.250	0.333	0.750	0.667	0.500	0.444	-						
September	TW	0.600	0.250	0.214	0.375	0.111	0.308	0.200	0.250	0.273	0.286	0.300	-					
	SL	0.267	0.667	0.500	0.308	0.250	0.200	0.400	0.429	0.286	0.300	0.600	0.300	-				
	RF	0.444	0.417	0.727	0.846	0.091	0.357	0.273	0.545	0.455	0.429	0.364	0.333	0.364	-			
December	TW	0.667	0.600	0.385	0.533	0.100	0.500	0.300	0.333	0.364	0.583	0.400	0.462	0.400	0.500	-		
	TF	0.200	0.500	0.375	0.231	0.333	0.100	0.200	0.286	0.143	0.200	0.400	0.200	0.750	0.273	0.300	-	
	RF	0.500	0.250	0.417	0.692	0.111	0.545	0.333	0.364	0.556	0.500	0.300	0.385	0.182	0.538	0.462	0.091	-



### Dietary niche overlap

Dietary niche overlap was greater than expected among all carnivores when all samples were considered regardless of collection month (Pianka's Index = 0.523,  $p < 0.001$ ), and subsequently between Tibetan wolf and snow leopard (Pianka's Index = 0.815,  $p < 0.001$ ), Tibetan wolf and red fox (Pianka's Index = 0.747,  $p = 0.005$ ), Tibetan wolf and Tibetan fox (Pianka's Index = 0.697,  $p = 0.012$ ), snow leopard and red fox (Pianka's Index = 0.747,  $p = 0.005$ ), as well as red fox and Tibetan fox (Pianka's Index = 0.823,  $p < 0.001$ ), but not for snow leopard and Tibetan fox (Pianka's Index = 0.611,  $p = 0.958$ ) (Figure 33A, Table 28A). Statistical significance for all predators considered and for any two given pairs was lost for the month of March with the exception of Tibetan wolf and snow leopard (Pianka's Index = 0.799,  $p = 0.007$ ) (Figure 33B, Table 28B). Only the Tibetan wolf and red fox could be compared for the months of September and December, both of which showed very similar, but insignificant, dietary overlap (Figure 33C,D, Table 28C,D).

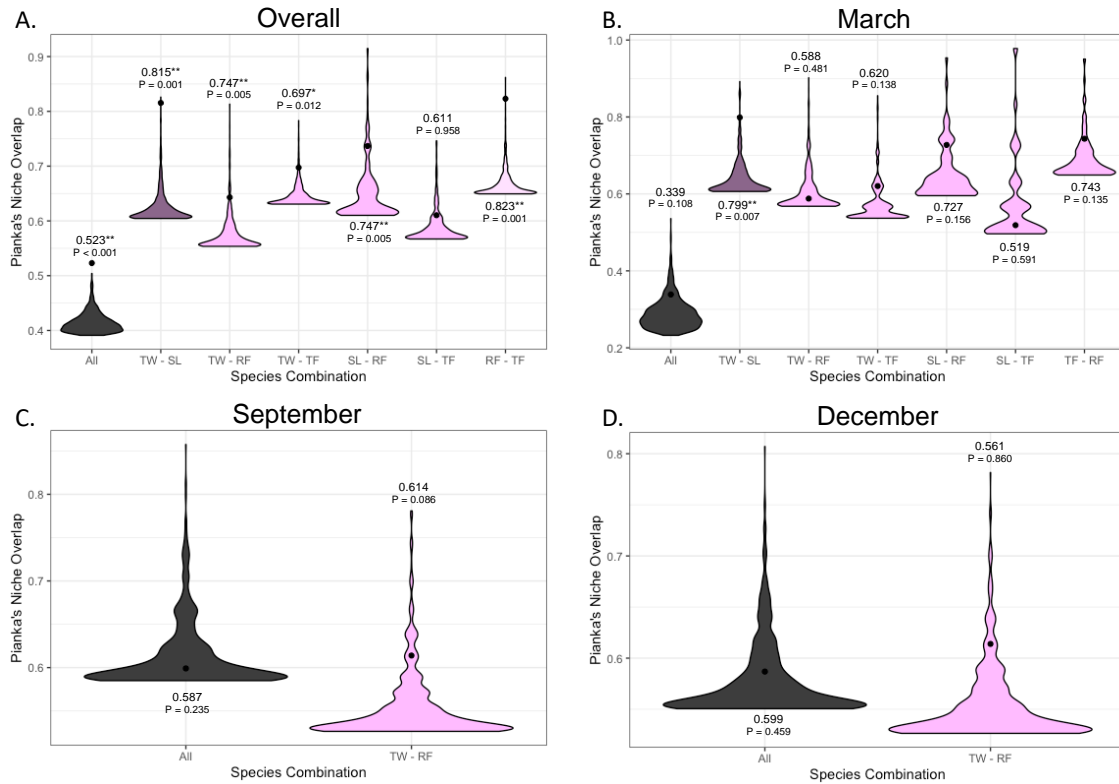


Figure 33. Violin plots representative of dietary niche overlap derived from Pianka's index values for host carnivores samples (All) as well as pairwise comparisons where sample sizes allowed for statistical significance to be determined. (TW – Tibetan wolf; SL – snow leopard; RF – red fox; TF – Tibetan fox). All carnivores under consideration are denoted by black coloration, pairwise comparisons between two apex predators are denoted by purple, and pairwise comparisons between two mesocarnivores are denoted by pink for A) all data points regardless of month; B) prey determined in scats collected in March; C) prey determined in scats collected in September; and D) prey determined in scats collected in December.

Table 28. Pianka's index values representing dietary overlap between predator species where comparisons were permitted by sufficient sample sizes for A) all data points regardless of month; B) samples collected in March; C) samples collected in September; and D) samples collected in December.

A.

	All Carnivores Overall	Tibetan Wolf Overall	Snow Leopard Overall	Tibetan Fox Overall	Red Fox Overall
All Carnivores Overall	0.523** $p < 0.001$				
Tibetan Wolf Overall		-			
Snow Leopard Overall		0.815** $p < 0.001$	-		
Tibetan Fox Overall		0.697* $p = 0.012$	0.611 $p = 0.958$	-	
Red Fox Overall		0.643 $p = 0.389$	0.747** $p = 0.005$	0.823** $p < 0.001$	-

B.

	All Carnivores March	Tibetan Wolf March	Snow Leopard March	Tibetan Fox March	Red Fox March
All Carnivores March	0.339 $p = 0.108$				
Tibetan Wolf March		-			
Snow Leopard March		0.799** $p = 0.007$	-		
Tibetan Fox March		0.620 $p = 0.138$	0.519 $p = 0.591$	-	
Red Fox March		0.588 $p = 0.481$	0.727 $p = 0.156$	0.743 $p = 0.135$	-

C.

	All Carnivores September	Tibetan Wolf September	Red Fox September
All Carnivores September	0.587 $p = 0.235$		
Tibetan Wolf September		-	
Red Fox September		0.614 $p = 0.086$	-

D.

	All Carnivores December	Tibetan Wolf December	Red Fox December
All Carnivores December	0.599 $p = 0.459$		
Tibetan Wolf December		-	
Red Fox December		0.561 $p = 0.860$	-

## Discussion

The collection of 581 samples revealed eight carnivore host species ranging in size from the beech marten to the Tibetan brown bear. Just under half of these were Tibetan wolves at 49.3%. Next were red fox at 26.4%, Tibetan fox at 11%, snow leopard at 9%, Pallas's cat at 2%, beech marten at 1.4%, Eurasian lynx at 1%, and Tibetan brown bear at 0.2%. The number of unique host species determined ranged from five in March and December to six in July and September. Though not statistically significant, the percentage of scats from each identified carnivore varied by collection month. Tibetan wolves were consistent apart from increased detection in July, which appeared to displace fox scats, given their relatively high percentages in September and December. March showed the most even detection of carnivores with snow leopard and Tibetan fox scats displacing the otherwise high percentages of Tibetan wolf and red fox. Differences by sampling month of carnivores observed along fixed transects could be a result of sampling scheme, or may reflect differences in seasonal spatial use. For example, carnivores in Italy were shown to shift their space use in winter when competitive stress for food was higher (Torretta et al., 2016). Regardless of variable and sometimes smaller sample sizes, the number of scats collected from each carnivore, barring the Tibetan brown bear and Pallas's scat, showed sampling completeness ranging in observed values of 33.3 to 100% with an average of 85.9%.

### Dietary composition across seasons

A total of 26 different prey items from two animal classes were observed. The number of unique prey ranged from nine in March to 18 in September. Small mammals dominated dietary frequency. This was largely driven by pika, especially in September. Pika are an abundant and year-round species exploited by both apex and mesocarnivores (see Chapter 3.2). Wild ungulates were second for counts of dietary frequency but first for biomass, and were exclusively comprised of blue sheep with the exception of white-lipped deer from September in one Tibetan wolf scat. Blue sheep are a medium bodied ungulate that are ubiquitous and abundant across the Gouli Nature Reserve. They have been previously shown as an important dietary item for many carnivores in China (see Chapter 3.2). Livestock made up much more in biomass at 32.2% than it did in frequency at 12.4%. This is likely because of the share of heavier yak. Both birds and carnivores had minimal contributions to diet.

Differences in diet across season for identified carnivores were not statistically significant for frequency or biomass. This also held true for the Tibetan wolf, the only carnivore who had a sufficient number of scats from each season to make such an assessment. This is in alignment with previous work studying wolf diet in the United States (Y. Shi et al., 2021), but contrasts work in Nepal (Chetri et al., 2017). Unfortunately, DNA metabarcoding is unable to determine the number of individual prey items killed, and thus estimates of frequency and biomass may be erroneous. Regardless, some of the results herein constitute biological importance and significance.

#### Dietary composition of the Tibetan wolf across seasons

The Tibetan wolf exploited livestock year round. Yak, goats, and sheep were identified in the months of March and July while domestic camel was observed in September and yak and goats in December. Blue sheep constituted a relatively large proportion of diet year round, except for September, when Tibetan wolves appeared to take advantage of the high availability of Himalayan marmot. Marmots typically give birth in late spring and early summer with a weaning period of 15 days (Hoffmann & Smith, 2008). Thus, an influx of marmots would be evident in scat samples representative of late July, August, and September, while samples from March that represented the months of January, February, and early to mid-March would expectedly lack marmot given their hibernation status over the colder winter months.

#### Dietary composition of the snow leopard across seasons

Sample sizes for some identified carnivore hosts limited analyses of dietary changes through time. Nonetheless, data were able to provide some insight into dietary ecology. For example, sample sizes from snow leopards were able to show that the species' prey use was not significantly different between March and September. This is surprising as changes in climate between these two months are stark. However, this finding agrees with previous work that did not find season to be a significant predictor in snow leopard diet composition in Nepal (Chetri et al., 2017). Nearly three-quarters of snow leopard diet in this study was comprised of the blue sheep. Otherwise, snow leopard supplemented their diets with pika, and exploited Himalayan marmot similarly to the Tibetan wolf. The only record of domestic animals occurred in March samples, which may reflect the lowered productivity and prey base in winter.

### Dietary composition for the Tibetan fox across seasons

The Tibetan fox also showed no significant differences in diet for the months of March, July, and December. This is likely driven by the strong dependence of the Tibetan fox on pika, which are in abundance year-round (Badingqiuying et al., 2016). A pika poisoning program launched in 1958 was meant to curtail populations and thus reduce the grassland erosion they were perceived to cause (A. T. Smith et al., 1990; A. T. Smith & Foggin, 1999). However, pika are now recognized as important and necessary environmental engineers for alpine grassland ecosystems and poisoning efforts have largely subsided (A. T. Smith & Foggin, 1999; Wei et al., 2020). Nonetheless, poisoning remains in practice in isolated areas and may impact carnivores dependent on them. Pika have previously been shown to constitute the majority of Tibetan fox diet (Hacker et al. In Review, Q. X. Liu et al., 2010). Pallas's cat produced a similar finding in this study, though scat sample sizes for the species were small.

Birds were also present in relatively high amounts in Tibetan fox diet compared to other carnivores. Only the beech marten had a higher occurrence, though this carnivore's sample size is small and only from September. Interestingly, marmot were present in every month for Tibetan fox except for September when they would be considered most abundant. This may be due to increased competition with snow leopards and Tibetan wolves for marmot, causing the Tibetan fox to exploit other prey.

### Dietary composition of the red fox across seasons

Species exploited all year round at high occurrences for the red fox included domestic yak, blue sheep, and pika. Red foxes were the only carnivore to show

significant differences in seasonal diet, which was found between the months of March and December. This is in contrast to the snow leopard, but is an intuitive conclusion given environmental differences in late summer versus winter. However, significance in dietary differences for red fox was only found when considering dietary frequency and not biomass.

Despite differences in conclusions drawn between the two for the red fox, frequency counts of diet and biomass were not significantly different from one another overall. While this suggests that dietary studies may be able to use either in drawing conclusions, information surrounding biomass may yield differing interpretations of results. For example, livestock in this study only comprised 12.4% of dietary occurrences, but equated to 32.2% in biomass. In this case, it could be concluded that livestock, and specifically yak, play an important role in ecosystem function as a food item of substance. This was particularly apparent in the sampling months of March and July, and may suggest shifts in the wild prey base or ease of access to livestock due to changes in herder practices. Despite their large size, yak were found in the diets of both apex and mesocarnivores. It is most likely that yak in the diets of Tibetan and red foxes were either kills of very young individuals or a result of scavenging. Unfortunately, presence of carrion in diet is impossible to discern without visualization of the feeding event.

#### The potential role of carrion in contribution to diet

Carrion from yak at the study site is likely abundant, as Tibetan belief systems do not promote burial of dead livestock (Ye, 2003) and thus may have inflated the interpretation



of livestock in diet as the result of a direct kill. Carrion can add to the dietary repertoire of smaller carnivores as it gives them the opportunity to feed on an animal much larger than themselves (DeVault et al., 2003; Selva et al., 2005). This was also likely the cause of the presence of blue sheep in mesocarnivore diet in this study.

#### Prey size and sustainability for larger carnivore species

Typically, competitive relationships between predators of different sizes is asymmetric because apex predators can feed on larger prey that are not accessible to mesopredators (Sinclair et al., 2003). However, mesopredators may take advantage of recent kills made by larger carnivores of larger prey items (Torretta et al., 2016). If larger prey species are unavailable, competition with mesocarnivores will increase but will ultimately be unsustainable because predators over 21.5kg will be unable to meet their energetic needs on smaller prey alone (Carbone et al., 1999). In this study, the average adult sized snow leopard, Tibetan brown bear, Tibetan wolf, and larger adult male Eurasian lynx would be unable to survive should larger prey deplete and reliance of livestock would be necessary. Though very small adult snow leopard females may be able to subsist on smaller prey (Britannica, 2020). Thus, loss of blue sheep in the Gouli Nature Reserve would have disastrous consequences for larger carnivores. This may also extend to mesocarnivores, as the evidence of intraguild predation indicates that larger carnivores would exploit species such as the Pallas's cat, Tibetan fox, and red fox if necessary.

#### Dietary diversity across carnivore species

Red foxes had the highest metric for richness at 18 different prey items identified in the diet ranging from five in July to 12 in September. The Tibetan wolf was close behind with 16 different prey items, with nine unique diet items for each month examined. Other carnivores in the dataset had lower numbers of unique prey species determined but this may be due to sample size, as was likely the case for the Pallas's cat. A previous study investigating 88 Pallas's cat scat samples found pika to comprise 91% of dietary occurrences, with woolly hare, vole species, smaller bodied birds, and blue sheep making up the remaining 9% for a total of six unique prey species identified (Hacker et al. In Review). However, those findings and the findings herein are in stark contrast to a study that found 18 different prey items in 14 Pallas's cat scats from the Gongga Mountain Nature Reserve in southwestern China (Zhao et al., 2020). This could suggest that Pallas's cats in the Gouli Nature Reserve have fewer dietary options in comparison to other areas of their range. Differences could also be due to methodology, whereby identified items vary based solely on microhistology versus DNA metabarcoding (Khanam et al., 2016; Nichols et al., 2016).

Both the beech marten and Eurasian lynx had four unique prey items present with seven and five scats collected for each, respectively. Previous work done over a larger area overlapping the Gouli Nature Reserve collected four Eurasian lynx scats and found six different prey items (Hacker et al. In Review). Three of the six - blue sheep, pika, and Himalayan marmot, overlapped with the prey species identified in this study. Only one beech marten scat sample was identified in the aforementioned study. It contained blue sheep and pika, neither of which were identified in this study. Both of these studies combined, which culminate in six unique prey species among eight beech marten scats,

insinuates that the beech marten may be a generalist with a wide dietary breadth. This notion is supported by a 2002 study from Luxembourg, which found 84 different prey items among 53 beech marten scats (Baghli et al., 2002).

Only five unique prey items were determined from the 46 snow leopard scats collected. This aligns with molecular diet work in Dulan County from 2018 that found five prey items among 22 snow leopard positive scats (Hacker et al. In Review). Previous work has shown that snow leopard diet varies regionally with preference towards medium to large-bodied hoof stock (Hacker et al. 2021). This observed skew towards a particular ungulate species based upon region has categorized snow leopards as dietary “near-specialists” (Lyndogh et al. 2014). Such unevenness in diet has driven lower dietary diversity metrics, masking the otherwise relatively large breadth of prey observed in snow leopard diet both locally (Hacker et al. In Review) and range-wide (C.E. Hacker et al., 2021). Findings from this much smaller spatially scaled study speak to the impact of study size location and area in determining dietary breadth and potential plasticity for species that have large distribution ranges.

#### Dietary similarity between species

Plots showcasing dietary similarity where sample sizes allowed showed a generally even appearance among any two given species overall and by season. This suggests overlapping dietary resource use, which is further echoed in the results examining niche overlap. Dietary niche overlap among the Tibetan wolf, snow leopard, Tibetan fox, and red fox was greater than expected. This is likely driven by the heavy consumption of blue sheep and pika within the dataset. Pairwise, dietary overlap was greater than expected

between the Tibetan wolf and snow leopard, Tibetan wolf and Tibetan fox, snow leopard and red fox, and red fox and Tibetan fox. Overlap between the Tibetan wolf and snow leopard with mesopredators is not unsurprising as the dietary niche of mesocarnivores is often nestled within the dietary niche breadth of apex carnivores (Gittleman, 1985; Sinclair et al., 2003) unless larger predators have become specialized to a specific larger prey item (Van Valkenburgh et al., 2004). Diversity of larger prey is limited in the Gouli Nature Reserve. Snow leopards may be considered the most specialized for hunting blue sheep, and the skew in their diet towards the species may have been responsible for the only pair of carnivores to not show greater than expected dietary overlap overall – the snow leopard and the Tibetan fox. Regardless, the data in this study broadly supports the notion that blue sheep in diet is relatively common. Thus, dietary overlap between mesopredators and apex predators in the Gouli Nature Reserve is not unsurprising. However, the Tibetan wolf and the red fox did not overlap in diet more than expected by month. This may be due to the species' generalist diets and wide dietary breadths. The red fox may reduce competition with wolves by selecting differing food items based upon season, while ultimately overlapping in diet when assessing prey use over the larger temporal period of one year.

#### Dietary niche overlap among carnivore species

The only month with sufficient sample sizes to compare dietary niche overlap was March. However, its smaller sample size appeared to lower statistical significance. The only dietary overlap that was greater than expected was between the Tibetan wolf and snow leopard. The Tibetan wolf and snow leopard also had the highest Pianka's index

values overall and for March at 0.815 and 0.799, respectively. Previous work has shown that snow leopards and Tibetan wolves have high dietary overlap (J. Wang et al., 2014), especially in winter (Bocci et al., 2017). Yet, other studies have concluded the opposite and found that wolves preferred plains-dwelling species while snow leopards preferred cliff-dwelling species (Chetri et al., 2017; Werhahn et al., 2019). Our results may align more with the former because the Gouli Nature Reserve lacks diverse medium to large bodied prey that are in abundance. Other strategies such as foraging behavior, habitat use, and activity patterns are likely used to minimize competition for resources between the two species (Bianchi et al., 2014).

#### Sample collection timing and recovery of DNA

Scat as a source of DNA is challenged by rapid degradation (Vynne et al., 2012). The QTP is cool and dry, buying some time for researchers. Prey items that were undetermined in this study either did not have any prey items present or lacked a sufficient number of DNA reads to determine the prey item consumed. Previous studies have found scat without prey present, as grooming and other metabolic processes can result in defecation (Shehzad et al. 2012). This phenomenon would lend itself to an expected baseline measure of scat samples anticipated to not have prey present, and would presumably be low. However, the number of undetermined scat samples was much higher in September than in any other month. This may be due to more rapid DNA degradation caused by increased temperatures and rain in the summer months. It appears evident that time of sampling can impact information acquisition with DNA metabarcoding. Because September was the first month of collection, it could also be that

collected scats were older than thought and thus more degraded. However, only scats that were deemed “fresh” or “near fresh” were collected in September to avoid collecting samples not pertinent to the time frame of interest.

#### Recaptured samples and aging scat

Methods for determining scat age for studies on the QTP are largely ambiguous, as little is known about the timing and contributing factors to degradation. Researchers have postulated that scat could be months old (Janečka, Munkhtsog, et al., 2011). Previous work deemed scats “new” or “recent” if they fell within 2 months of aged appearance (Janečka, Munkhtsog, et al., 2011; Kachel, 2014), but there is currently no standardized visual of what a scat fitting into this category looks like. In this study, all remaining portions of collected scat were marked with colored nail polish. Only two instances of recaptures occurred. Both were in December for scats that had been originally sampled in September. This suggests that scat samples in the Gouli Nature Reserve either do not last as long as previously thought, or that they may travel long distances. Such findings are promising and suggest that estimates for metrics derived from noninvasive genetics, such as population abundance or density, may be more valid as they would not violate the two month population closure assumption (Karanth et al., 2004). Regardless, scat remains a difficult medium, and the collection of fresher scat is encouraged for extracting high quality and quantity DNA for applications such as DNA metabarcoding.

#### Conclusions and recommended conservation actions

This study offers insight into the dietary composition and temporal differences for a predator guild at a small spatial scale in a sensitive high altitude environment. Based on study findings and heedful interpretations of results, the following conservation recommendations are made. First, limited differences in resource use and high niche overlap suggest that other mechanisms such as spatial and daily temporal differences are responsible for successful coexistence of species with the exception of the Tibetan wolf and red fox. However, this also insinuates that changes in prey availability will have large-scale impacts on the entire predator guild. Future research in the area should focus on discerning micro-spatial and micro-temporal differences to ensure that these avenues of coexistence are maintained, particularly for the Tibetan wolf and snow leopard and for the months of February and March when environmental productivity is low.

Second, the majority of dietary items observed were blue sheep and pika. These contributed heavily to both frequency counts and biomass. It is imperative that these species be monitored and protected. While small mammals supplemented diet, the blue sheep appears to be a vital sustaining component to apex carnivores in this study, especially the snow leopard. Similarly, pika were a dietary staple for every mesocarnivore species investigated.

Third, livestock appear to be an important component to predator diet, but may not be crucial to sustaining larger carnivore populations so long as blue sheep are available. Regardless, domestic animal presence in diet indicates that solutions are needed to protect the interests of both herders and carnivores. Many of these solutions call for the prevention of predators from accessing livestock, for increased vocational opportunities for residents that do not include herding, and for the burying of dead

livestock to prevent attracting carnivores close to homesteads (Aryal et al., 2014; Dai et al., 2021; Dai, Hacker, et al., 2020; van Eeden et al., 2018). Such changes should be done gradually to allow carnivores to adapt to the decrease of livestock availability, and for researchers to monitor its impacts. For the time being, herders should focus on preventing livestock loss in the months preceding March and July. Mechanisms such as livestock insurance can assist in relieving financial burdens experienced by livestock loss while still providing an “ecosystem service” to the area’s predator guild.

Fourth, studies of this kind would benefit from improvements in methodology - specifically those which increase sample sizes. However, scaling up field studies can be cost and time prohibitive. Greater involvement from the on-site local community in sample collection may assist in overcoming these challenges (Charlotte E. Hacker et al., 2020). Furthermore, patterns of seasonal dietary changes can be complicated by sexual differences in diet. Future work that identifies males versus females would help elucidate mechanisms which reduce intersexual resource competition (Davidson et al., 2013). More accurate counts of prey and livestock would further be beneficial to determine if prey use corresponds directly with availability, which our study was unable to obtain for all months examined. Despite its shortcomings, this study provides insight into the dietary composition of several carnivore species as well as a series of general seasonal trends for the Tibetan wolf, snow leopard, Tibetan fox, and red fox.

#### Author Attributions

Charlotte Hacker, Jan Janecka, and Yuguang Zhang conceived study. Charlotte Hacker and Yuguang Zhang acquired funding. Charlotte Hacker, Yunchuan Dai, Wei Cong, Jia



Li, Ye Li, and Rou Bao collected scat samples. Charlotte Hacker and Wei Cong extracted scat samples and prepared samples for sequencing. Yuguang Zhang, Diqiang Li, and Jan Janecka provided administrative support. Charlotte Hacker performed data analyses, designed figures and tables, and wrote the manuscript. Yuguang Zhang contributed edits to the manuscript.

# Chapter 4

## Goal 3

### Understanding human-carnivore dimensions

#### Chapter 4.1

##### Goal 3 – Subgoal 1

#### Assessment of herder attitudes towards snow leopards

Adapted from Hacker *et al.* (2020) *Determinants of herder attitudes towards the threatened snow leopard (Panthera uncia) in Yushu Prefecture, China*. *Oryx*. DOI:10.1017/S0030605319001315

#### Introduction

The overlap between humans, livestock, and predators can often have negative outcomes, such as economic loss (Pettigrew *et al.*, 2012), retaliatory killings from livestock predation (Oli *et al.*, 1994), human mortality (Treves & Karanth, 2003), and disease (Thirgood *et al.*, 2005). Such outcomes threaten large mammals and are anticipated to increase in severity (Kansky & Knight, 2014). Felids are often engaged in conflict despite low densities because they occupy large home ranges and attack domestic animals and humans (Treves & Karanth, 2003). Because many felids are threatened, killings can be detrimental to their populations (Woodroffe & Ginsberg, 1998). Such loss can have macroecological impacts as large felids influence trophic cascades in a downward manner and increase biodiversity (B. Miller *et al.*, 2001).

The role of livestock depredation in challenging carnivore conservation initiatives

Many countries have adopted preservation strategies that focus on protecting threatened carnivore populations (Treves & Karanth, 2003). However, effective implementation of conservation laws and policies protecting the natural world hinge on the attitudes of local residents towards the protected wildlife in question (Suryawanshi et al., 2014). In areas dependent on agro-pastoralism, predators can cause large financial burdens on herders via livestock depredation, ultimately leading to negative attitudes, the encouragement of retaliatory killings, and disagreements between community members and agencies seeking to protect at-risk animals (Treves & Karanth, 2003).

Carnivores place financial burden on herders via livestock depredation, leading to negative attitudes, retaliatory killing, and disagreements between community members and agencies seeking to protect at-risk wildlife (Treves & Karanth, 2003). Livestock loss is preventable with non-lethal means (Ogada et al., 2003). Predator proof corrals have been successful in India (Jackson & Wangchuk, 2004), Africa (Lichtenfeld et al., 2015), and North America (Cluff & Murray, 1995). Increased human activity was effective in Kenya (Ogada et al., 2003), while shifting campsites decreased loss in Mongolia (Mijiddorj et al., 2018). Guard dogs (*Canis familiaris*) have been used to alert herders and ward off predators (Ogada et al., 2003). In addition, post-predation interventions, such as livestock insurance, can reduce financial loss and negative perceptions. Unfortunately, these programs often do not cover the full value of animal, take a long time to process, can be area dependent (i.e. only in the confines of a national park), are subject to fraudulent claims, and overall ineffective action can promote animosity between villages and park authorities (Hemson et al., 2009; Jackson & Wangchuk, 2004).

#### Importance of local community support

The success of mitigation strategies largely depends on community participation (Jackson & Wangchuk 2004). Effective implementation requires research on local stakeholder attitudes (Dickman, 2010; Kansky & Knight, 2014). While values direct attitudes, attitudes precede and direct behavior (Vaske & Manfredo, 2012). Thus, knowledge of herder attitudes is necessary for inferring actions towards carnivores. Attitudes are influenced by many factors, including knowledge, social norms, economic constraints, and religious affiliation (Dickman, 2010). Few studies have examined the attitudes of pastoralists in Central Asia despite overlap of people with carnivores, including the threatened snow leopard (*Panthera uncia*) (Green & Zhimbiev, 1997; Suryawanshi et al., 2014).

#### Snow leopards and previous studies examining pastoralist attitudes

Snow leopards influence surrounding ecosystems through predation on wild ungulates and competition with sympatric carnivores (J. Li et al., 2014). Reduced wild prey populations combined with higher abundance of domestic animals leads to livestock predation by snow leopards (Schaller, Junrang, et al., 1988). Depredation events are costly to herders; they foster negative attitudes towards snow leopards and motivate calls for their elimination. Previous attitude studies towards snow leopards have been done in China (Alexander, Chen, et al., 2015; C. Li et al., 2015; J. Li et al., 2013; A. Xu et al., 2008). However, findings cannot be generalized to other regions (Alexander, Chen, et al., 2015). Additionally, repeated assessments are needed to examine temporal changes, particularly on the Tibetan Plateau where rapid societal and environmental changes are occurring (Bauer, 2015).

## Study goals and hypotheses

This study sought to identify variables impacting herder attitudes towards snow leopards and their conservation. Associations between herding practices, animal loss, and demographics were explored to understand the most important factors influencing attitudes. We hypothesized that negative attitudes would be increase with greater livestock loss and fewer years of formal education, whereas positive attitudes would increase with fewer losses, more years of formal education, livestock insurance, and Tibetan beliefs. We further predicted that herders would consider depredation most threatening risk to livestock and that herders with predator proof corrals would report fewer losses.

## Materials and Methods

### Study area

Interviews were conducted in Suojia Village, Zhiduo County, Yushu Prefecture, Qinghai Province, China (Figure 34). Zhiduo County is 38,793.4 km<sup>2</sup> (excluding Hoh Xil National Nature Reserve) with 22,394.8km<sup>2</sup> of this area overlapping with Sanjiangyuan National Park (Dai, Hacker, et al., 2020). Sanjiangyuan National Park is 152,000 km<sup>2</sup> and is the largest stretch of continuous snow leopard habitat in China (Y. Liu et al., 2016). The area has alpine meadow vegetation with limestone massifs and smaller mountain ranges (Schaller, Junrang, et al., 1988). The climate is windy and dry with temperatures from -20°C in January to 8°C in July (Mallon, 2004). Interview site elevations averaged 4,429 m, ranging from 4,109 m to 4,670 m.

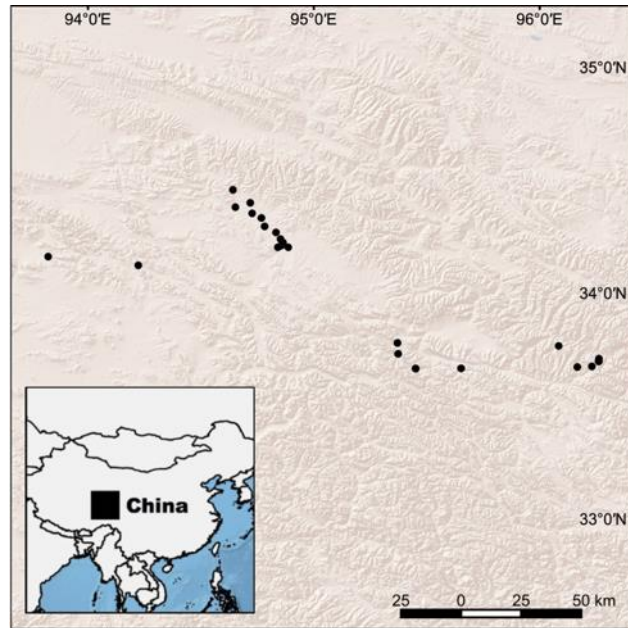


Figure 34. The interview study site location in Suojia Village, Yushu Prefecture, Qinghai Province, China.

### Questionnaire development

Preliminary interviews were completed with 5 herders to identify problems with clarity (Hemson et al., 2009). The revised questionnaire contained attitude statements regarding snow leopards, importance of snow leopards to religion, and perceived wildlife abundance ranked on a Likert type scale (Likert, 1932) followed by questions surrounding animal ownership and management, livestock loss to snow leopards, knowledge of retaliatory killing, threats to livestock, and demographics (Appendix III: Supplemental Material 1). A 5-year recollection period was chosen to avoid recall bias (Bernard, 2013). Retaliatory killing questions were carefully worded to reduce social desirability bias (Fisher, 1993). The questionnaire was limited to one page to avoid interviewee fatigue and disinterest (De Vaus & de Vaus, 2013).

### Data collection

An Institutional Review Board exemption was granted prior to the study. Herders were interviewed in July 2018. Verbal interviews were conducted to avoid misunderstanding stemming from illiteracy and lasted approximately 15 minutes. Local guides served as translators. The content, objectives, and anonymity were explained to the potential interview subject. Respondents could cease at any time. The snowball sampling strategy was used to maximize sample size and engender participant trust (Goodman, 1961; Sadler et al., 2010).

### Data analysis

Reported scores from each herder were summed and divided by the total number of interviews to calculate the percentage of herders who had positive, neutral, or negative attitudes towards a specific statement. The Likert type scale values of 1, 2, and 3 were clustered into a “Disagree” category, 4 assigned to a “Neutral” category, and 5, 6, and 7 were clustered into an “Agree” category.

Cronbach’s alpha was used to ensure internal consistency within all five attitude statements (Cronbach, 1951). A Principal Component Analysis (PCA) with varimax rotation and pairwise exclusion of cases was used to identify variables most heavily contributing to attitude variation (Jolliffe, 2002; Kaiser, 1958). Components with eigenvalues  $>1$  were selected for interpretation (Kaiser, 1960) and internal consistency of statements in separate components assessed. Likert type scores for statements in each component were averaged to give one overall agreement score. A Wilcoxon Signed-Rank

Test was completed to assess statistical differences between PCA components (Wilcoxon, 1945).

Relationships among variables were examined using Spearman Rho (Spearman, 1904). Those exhibiting multi-collinearity with high significance ( $p < 0.01$ ) had one variable removed based on author expertise and number of other highly significant correlations (Dormann et al., 2013). Generalized estimating equations (GEEs) were used to determine influential predictors of positive and negative attitudes. GEEs are appropriate for datasets with non-normal distributions without having to make data corrections (Kowalski & Tu, 2007; Tang et al., 2012). A series of general models were constructed (Table 1). The first model was built considering all variables. The least impactful variable to the model was then removed in a stepwise fashion for each sequential model. The quasiliikelihood under the independence model criterion (QIC) was used to rank models, with the lowest QIC value deemed most appropriate (J. Cui, 2007; Hardin & Hilbe, 2003). SPSS (version 25.0 [Chicago, IL]) was used with significance set at  $p < 0.05$ .

## Results

### Interview responses

Interviews of 73 herders were completed. The study's geographic area spanned approximately 476 km<sup>2</sup>. The greatest Euclidian distance between sites was 231 km. Among herders, 94.5% reported pastoralism and 5.5% reported civil servant jobs as their



primary income source. No knowledge of retaliatory killing of snow leopards were reported. Results of remaining interview questions are presented in Table 29.

Table 29. Interview survey results of demographics, perceptions of wild animal abundance, livestock holdings, herding practices, animal loss, retaliatory killings, and threats to livestock.

Demographics				
	<i>Mean (<math>\bar{x}</math>)</i>	<i>SD</i>	<i>Range</i>	
Age:	37.4	10.7	21 - 73	
Years of formal education:	2	3.3	0 - 12	
Number of children:	1.4	1.9	0 - 8	
Perceptions of Wild Animal Abundance				
	<i>Mean (<math>\bar{x}</math>)</i>	<i>Range</i> <i>1 (none) - 7 (many)</i>		
blue sheep:	6.2	1 - 7		
argali:	5.6	1 - 7		
snow leopard:	5	2 - 7		
Animal Ownership				
	<i>Yak</i>	<i>Sheep</i>	<i>Dog</i>	
% of herders who own:	98.8%	3%	71.2%	
Average no. owned:	72.7	0.6	1.2	
Range:	0 - 250	0 - 20	0 - 5	
Total # of animals:	4,723	40	81	
Management Practices				
	<i>Possess Insurance</i>	<i>Guard Livestock</i>	<i>Predator-Proof Corrals</i>	
% of herders who use practice:	56.2%	33.8%	4.2%	
Animal Loss				
% of herders who lost livestock to snow leopards in previous 5 years:	45.8%			
	<i>Yak</i>	<i>Horse</i>	<i>Total</i>	
No. of animals lost:	75	4	79	
	<i>Mean (<math>\bar{x}</math>)</i>	<i>SD</i>	<i>Range</i>	
Animal loss per household:	2.5	2.2	0 - 12	
Threats to Livestock Survival				
	<i>Disease</i>	<i>Predation</i>	<i>Grassland Degradation</i>	<i>Drought</i>
% of herders who consider threat as greatest to livestock livelihood:	36.7%	43.3%	20%	0%

## Factor analysis and correlation coefficients

Reliability statistics showed internal consistency ( $\hat{\alpha} = 0.72$ ). There were 2 components with eigenvalues  $>1$ , accounting for 67.3% of variance observed (Table 30). The highest scores for component 1 were associated with positive attitudes towards snow leopards and named “Snow Leopard Positive” (statements: “We need snow leopards in the wild.”; “I enjoy seeing snow leopards.”; and “We need to keep snow leopards safe.”;  $\hat{\alpha} = 0.70$ ). The highest scores for component 2 were associated with negative attitudes and named “Snow Leopard Negative” (statements: “I am concerned that snow leopards will kill my animals.” and “I am afraid of snow leopards.”;  $\hat{\alpha} = 0.53$ ). Agreement was significantly higher for positive than for negative statements ( $p < 0.001$ ). The mean scores for each attitude statement and percentages of agreement, neutrality, and disagreement were evaluated and predictor variable relationships assessed (Table 31, Figure 35).

Table 30. The two major components that had eigenvalues  $>1$  based on herder interviews regarding attitudes towards snow leopards and their conservation.

	Snow Leopard Positive	Snow Leopard Negative
We need snow leopards in the wild.	0.811	-
I enjoy seeing snow leopards.	0.756	-
We need to keep snow leopards safe.	0.767	-
I am concerned that snow leopards will kill my animals. (inversed)	-	0.755
I am afraid of snow leopards. (inversed)	-	0.886

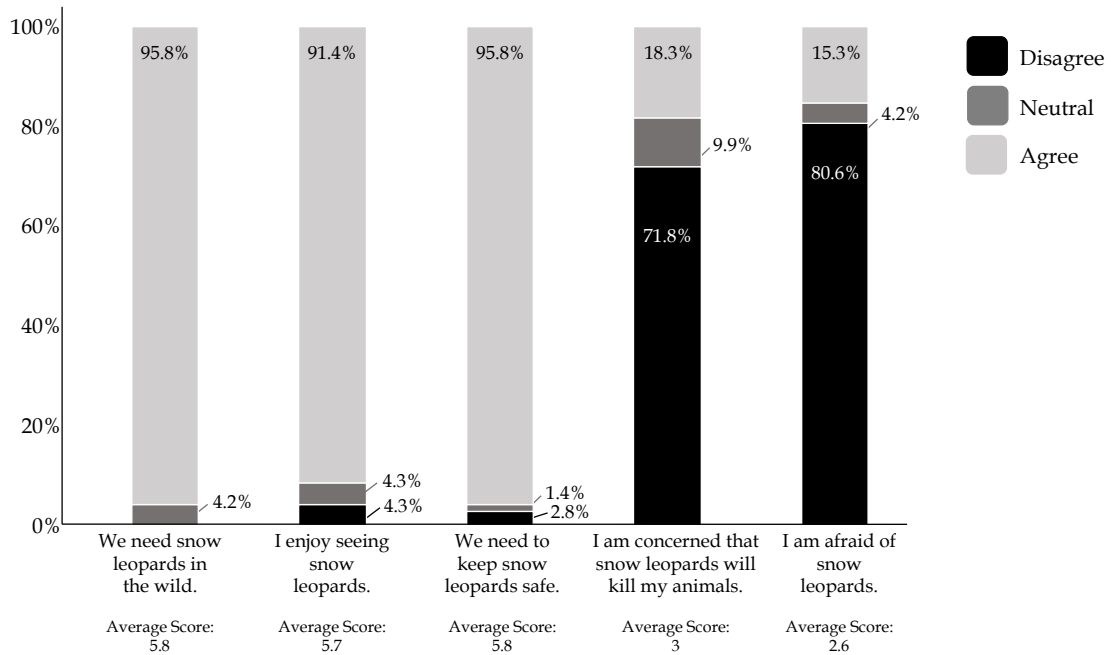


Figure 35. Overall percentage of agreement, disagreement, or neutral standing with statements surrounding snow leopards. Overall average scores were ranked from 1 (strongly disagree with statement) to 7 (strongly agree with statement).

Table 31. The Spearman rank-order Correlation Coefficients of relationships between predictor variables.

		Important to Religion	SL Abundance	Total Livestock Owned †	Dog Ownership	Guard Livestock	Predator Proof Corral Use †	Possess Insurance	No. of Animals Lost	Age †	Years of Education	No. of Children †
Important to Religion	Correlation Coeff. Sig. (2-tailed)	-										
Snow Leopard Abundance	Correlation Coeff. Sig. (2-tailed)	-0.156 0.203	-									
Total Livestock Owned †	Correlation Coeff. Sig. (2-tailed)	0.094 0.439	.301*	-								
Dog Ownership	Correlation Coeff. Sig. (2-tailed)	0.233 0.066	0.085 0.505	.317** 0.009	-							
Guard Livestock	Correlation Coeff. Sig. (2-tailed)	0.201 0.100	0.103 0.401	0.061 0.615	0.144 0.248	-						
Predator Proof Corral Use †	Correlation Coeff. Sig. (2-tailed)	-0.284* 0.019	0.119 0.333	-0.103 0.392	-0.356** 0.003	0.146 0.225	-					
Possess Insurance	Correlation Coeff. Sig. (2-tailed)	-0.015 0.903	-0.167 0.174	-0.042 0.728	0.006 0.960	-0.233 0.051	0.180 0.134	-				
No. of Animals Lost	Correlation Coeff. Sig. (2-tailed)	-0.052 0.669	0.083 0.496	.264* 0.024	0.075 0.550	-0.158 0.188	-0.015 0.902	0.053 0.659	-			
Age †	Correlation Coeff. Sig. (2-tailed)	-0.054 0.661	0.164 0.182	-0.068 0.571	-0.260* 0.038	0.032 0.793	.334** 0.005	-0.225 0.063	0.147 0.221	-		
Years of Formal Education	Correlation Coeff. Sig. (2-tailed)	-0.158 0.223	-0.209 0.103	0.049 0.706	-0.026 0.848	-0.191 0.140	0.106 0.418	.285* 0.026	.274* 0.030	-0.499** 0.000	-	
No. of Children †	Correlation Coeff. Sig. (2-tailed)	-0.027 0.837	0.020 0.879	-0.037 0.771	-0.262* 0.047	0.003 0.982	0.188 0.143	0.047 0.719	0.200 0.113	.619** 0.000	-0.301* 0.018	-

\* Correlation is significant at the 0.05 level (2-tailed); \*\* Correlation is significant at the 0.01 level (2-tailed).

† Denotes removal of variable from consideration in general model construction.

### Attitude correlates

The model with the lowest QIC score for Snow Leopard Positive included number of animals lost (-0.125, 95% CI [-0.221, -0.028],  $p = 0.012$ ) and years of education (0.077, 95% CI [0.18, 0.135],  $p = 0.011$ ). The model with the lowest QIC score for Snow Leopard Negative included number of animals lost (-0.154, 95% CI [-0.244, -0.063],  $p = 0.001$ ), years of education (-0.099, 95% CI [-0.174, -0.024],  $p = 0.010$ ), and importance of snow leopards to religion (-1.119, 95% CI [-2.175, -0.63],  $p = 0.038$ ) (Table 32).

Table 32. The Generalized Linear Model outcomes using Generalized Estimating Equations of factors affecting positive versus negative attitudes towards snow leopards.

<i>Component 1: Snow Leopard Positive</i>					<i>Component 2: Snow Leopard Negative</i>				
Predictor Variables	QIC	ΔQIC	QICc	ΔQICc	Predictor Variables	QIC	ΔQIC	QICc	ΔQICc
Positive Attitudes ~ SL Abundance + Important to Religion + Dog Ownership + Guard Livestock + Posses Insurance + Loss to Snow Leopards + No. of Animals Lost + Years of Education	71.634	-	66.219	-	Negative Attitudes ~ SL Abundance + Important to Religion + Dog Ownership + Guard Livestock + Posses Insurance + Loss to Snow Leopards + No. of Animals Lost + Years of Education	57.789	-	56.308	-
Positive Attitudes ~ SL Abundance + Important to Religion + Dog Ownership + Guard Livestock + Posses Insurance + No. of Animals Lost + Years of Education	70.006	-1.628	64.253	-1.966	Negative Attitudes ~ Important to Religion + Dog Ownership + Guard Livestock + Posses Insurance + Loss to Snow Leopards + No. of Animals Lost + Years of Education	56.214	-1.575	54.310	-1.998
Positive Attitudes ~ SL Abundance + Important to Religion + Guard Livestock + Posses Insurance + No. of Animals Lost + Years of Education	69.033	-0.973	63.074	-1.179	Negative Attitudes ~ Important to Religion + Dog Ownership + Guard Livestock + Loss to Snow Leopards + No. of Animals Lost + Years of Education	54.085	-2.129	52.343	-1.967
Positive Attitudes ~ SL Abundance + Important to Religion + Guard Livestock + No. of Animals Lost + Years of Education	68.468	-0.565	62.356	-0.718	Negative Attitudes ~ Important to Religion + Guard Livestock + Loss to Snow Leopards + No. of Animals Lost + Years of Education	52.197	-1.888	50.402	-1.941
Positive Attitudes ~ SL Abundance + Guard Livestock + No. of Animals Lost + Years of Education	66.024	-2.444	68.488	+6.132	Negative Attitudes ~ Important to Religion + Loss to Snow Leopards + No. of Animals Lost + Years of Education	50.088	-2.109	48.459	-1.943
Positive Attitudes ~ Guard Livestock + No. of Animals Lost + Years of Education	64.992	-1.032	66.949	-1.539	Negative Attitudes ~ Important to Religion + No. of Animals Lost + Years of Education	48.867	-1.221	46.833	-1.626
Positive Attitudes ~ No. of Animals Lost + Years of Education	64.458	-0.534	65.983	-0.966					
<i>Snow Leopard Positive Final Model: No. of Animals Lost (-0.125, 95% CI [-0.221, -0.028] p = 0.012); Years of Education (0.77, 95% CI [0.018, 0.135], p = 0.011)</i>					<i>Snow Leopard Negative Final Model: Important to Religion (-1.119, 95% CI [-2.175, -0.63], p = 0.038); No. of Animals Lost (-0.154, 95% CI [-0.244, -0.063], p = 0.001); Years of Education (-0.099, 95% CI [-0.174, -0.024], p = 0.010)</i>				

## Discussion

Similar to previous studies from China, herders had positive attitudes towards snow leopards (Alexander, Chen, et al., 2015; C. Li et al., 2015; J. Li et al., 2014). The most important factors for positive attitudes were more years of formal education and fewer animals lost. Education has previously been linked to positive attitudes towards snow leopards (Suryawanshi et al., 2014). In our study, age and education was also negatively correlated, suggesting that younger herders are receiving more schooling. Reduced nomadism among Tibetans has decreased the income generated from traditional sources such as pastoralism, lessening the assistance families need from children and allowing time for schooling (Bauer, 2015). Today's students have more conservation education opportunities in school and through organizations that inspire environmental protection (Shen & Tan, 2012). In addition, students learn to read and write, promoting lifetime awareness for nature, wildlife laws, and the benefits of carnivores (Suryawanshi et al., 2014).

### The role of livestock loss in shaping attitudes

Previous research found that livestock depredation did not substantially influence negative attitudes (Suryawanshi et al., 2014). In contrast, this study found that the number of animals lost contributed to both greater positive and negative attitudes towards snow leopards. Intuitively for positive attitudes, those who lost less livestock would be more likely to agree with positive statements. For negative attitudes, it is possible they may have been instilled prior to loss, or that the herder experienced greater predation more than five years prior, and still agreed with negative statements during the interview.



Another possibility is that herders with more negative attitudes exercise greater caution and take protective measures not outlined in this survey (e.g., fenced pastures; housing newborns), thus reducing loss. The number of animals owned positively correlated with livestock loss, indicating that herders experienced equal rates of predation. However, the frustration of livestock predation is likely dependent on the animal's size, age, and condition.

### The role of religion and belief systems

Tibetan religious beliefs that mandate respect for wildlife may contribute to decreasing negative attitudes. Tibetan Buddhism influences approximately 80% of geographic regions overlapping with snow leopard habitat (J. Li et al., 2014). Snow leopards were considered religiously important by 93% of herders. Such beliefs likely contributed to the absence of retaliatory killing, although previous interviews done in our study area from 2009 to 2011 reported their occurrence (J. Li et al., 2013, 2014). Although promising that no killings were reported in our 5-year window, it is also possible there would have been reports with more interviews, or that herders adopted a “universal silence” in our study (Oli et al., 1994).

### Herder concern for Tibetan wolves

Other sympatric carnivores, such as wolves (*Canis lupus*), likely face a greater risk for retaliatory killing despite Buddhist teachings (Charudutt Mishra, 1997). While attitudes towards wolves were not addressed, 6 herders expressed unsolicited concern. Wolves are viewed more negatively because herders presume they play a larger role in

livestock predation (Suryawanshi et al., 2013). Herders are considered knowledgeable in discerning the species responsible for kills via resulting wounds (Aryal et al., 2014). However, some deaths in this study may have been misidentified and caused by wolves or domestic dogs.

### Dog ownership

Over 70% of herders interviewed owned at least one dog. Predator proof corrals were positively correlated with herder age while dog ownership was negatively correlated, demonstrating that dogs are more popular with younger herders. Neither predator proof corrals, guarding, nor dog ownership were correlated with number of animals lost, suggesting these practices alone may not be sufficient to stop animal loss, are not deployed effectively, or are implemented post-predation. However, livestock guarding was closely competitive in the final model shaping positive attitudes, and therefore may be playing the largest role among the deterrent methods assessed in this study.

Dogs can spread disease (Mamaev et al., 1995), stress, harass, and kill wildlife (Lenth et al., 2008; Young et al., 2011), and compete for resources (Vanak et al., 2009). The number of dogs reported in our study is likely a small portion of the total population because ownership is informal with limited spaying/neutering, leading to free-ranging and feral individuals (Home et al., 2017). In trans-Himalayan India, dogs were responsible for 40% more livestock mortalities than snow leopards (Suryawanshi et al., 2013). Research examining how dogs may be impacting wildlife and livestock is needed.

### The status of livestock insurance among respondents

Nearly half of the herders who reported livestock loss possessed insurance. In China, compensation is targeted towards losses caused by protected animals (Pettigrew et al., 2012). Herders did not comment on insurance, but it is widely accepted that the programs are typically insufficient (Madhusudan, 2003). Conservation performance payments that compensate herders for achieving goals that benefit carnivores may be an alternative in areas with inadequate coverage (Kunkel et al., 2016; Zabel & Holm-Müller, 2008).

### Local ecological knowledge

Herders indicated relatively high numbers of snow leopards, blue sheep (*Pseudois nayaur*), and argali (*Ovis ammon*). Although reported wildlife abundance may show limited consistency across herders, it provides useful information utilizing local knowledge (A. Davis & Wagner, 2003). Prey counts were planned to coincide with interviews to substantiate herder responses. However, much of the wild ungulate population moved to higher elevations due to warmer temperatures and increased human activity (Xie Ran, personal communication), making this infeasible. For snow leopard abundance, scat surveys as part of a separate study examining snow leopard population genetics revealed 6 individuals (0M:6F) (Y. Zhang et al., 2019). Pastoralists depend on their environment and can serve as strong conservation allies (J. Marc Foggin & Torrance-Foggin, 2011). Past studies found agreement in the status of wildlife populations between local perceptions and scientific studies (Danielsen et al., 2014; van der Hoeven et al., 2004), though more work is needed in our study area.

Predation was considered the primary threat to livestock by 43.3% of herders. Disease was second at 36.7%. Predation has been reported of largest concern in prior studies, even when disease caused more mortality (Dar et al., 2009). Grassland degradation was third at 20%, with herders implicating pika (*Ochotona curzoniae*) as a pest that kills grass roots and competes with livestock for vegetation (H. Zhou et al., 2005). Drought and other weather conditions were not reported as threatening, likely because major climatic events are relatively infrequent.

Our study contributes to previous work studying herder attitudes towards snow leopards in China, and provides data from a single point in time (2018) seven years after a previous survey (J. Li et al., 2013). Herders in Suojia had overall positive views towards snow leopards. Formal education played a significant role in increasing positive attitudes and decreasing negative attitudes. It is unlikely that older adults harboring negative views will change their views through conservation education alone and compensation for actions that protect apex predators may be required (Conforti & De Azevedo, 2003). How livestock loss shapes attitudes towards snow leopards is unclear; more research is needed to determine livestock factors impacting tolerance, including species, (i.e., sheep, goat, or yak), age, and quality of animal lost. Predator proof corrals, guarding, and dogs do not seem to significantly reduce loss, but this finding could be influenced by inconsistent implementation and temporal shifts in management practices. Herders may be effective sources of information on relative wildlife abundancies, but this should be corroborated with population surveys. Our results also support the notion that Tibetan beliefs play an important role in protecting snow leopards (J. Li et al., 2013),

exemplifying the role of societal and cultural norms in wildlife perceptions (Dickman, 2010).

#### Author Attributions

Charlotte Hacker conceived study. Charlotte Hacker, Jan Janecka, and Yuguang Zhang acquired funding. Charlotte Hacker, Lance Miller, and Jan Janecka designed interview survey, Yunchuan Dai, Yifan Cheng, Yu Zhang, and Yuguang Zhang administered interviews. Yunchuan Dai translated interviews from Mandarin Chinese to English. Charlotte Hacker scored interview data. Charlotte Hacker and Lance Miller performed data analyses. Charlotte Hacker designed figures and tables. Charlotte Hacker, Jan Janecka, and Lance Miller interpreted data. Charlotte Hacker wrote the manuscript. Lance Miller, Yuguang Zhang, and Jan Janecka contributed edits to the manuscript.

## Chapter 4.2

### Goal 3 – Subgoal 2

# The effectiveness of Foxlights in deterring predators from livestock

Adapted from Hacker *et al.* (In Review) *The perceived and functional effectiveness of Foxlights at preventing livestock loss on the Qinghai-Tibetan Plateau*. Wildlife Society Bulletin.

#### Introduction

Conflicts between animals and humans have existed for millennia (Treves *et al.*, 2006). These conflicts contribute greatly to the difficulties surrounding the conservation and management of large carnivores in modern times (Quigley *et al.*, 2015). Livestock depredation creates financial burdens on herders, their families, and the local economy (Mkonyi *et al.*, 2017), is emotionally traumatizing (J. R. B. Miller *et al.*, 2017), and can create tension between local residents and the conservation and government agencies seeking to protect at risk wildlife (S. W. Wang & Macdonald, 2006). However, livestock loss can largely be prevented (Ogada *et al.*, 2003).

#### Methods used to prevent livestock depredation

Lethal methods which result in the complete removal of the predator via its death include culling and hunting (Bradley, 2004; Jaeger, 2004) or poisoning via chemical bombs (Griffiths *et al.*, 1978). However, such methods have largely lost public support and favor given that they are less effective (McManus *et al.*, 2015; J. R. B. Miller *et al.*, 2016; Treves *et al.*, 2016), inhumane and impractical (Cavalcanti *et al.*, 2012), do not always align with the perceived damage that predators cause (Marker *et al.*, 2003), and can have large-scale ecosystem effects that disrupt important ecological processes (Estes *et al.*, 2011). Non-lethal mechanisms of livestock depredation control have increased in use and application worldwide (Shivik *et al.*, 2003), and

are most likely to promote positive coexistence between humans and carnivores (Treves et al., 2016). These include noise via firecrackers (Beckmann et al., 2004), rubber buckshot and slugs (Beckmann et al., 2004), taste aversion (Griffiths et al., 1978), electric fencing (Cavalcanti et al., 2012), fladry (Davidson-Nelson & Gehring, 2010), flashing lights (Ohrens, Bonacic, et al., 2019), human guarding (Ogada et al., 2003), and guard dogs (Rigg & Avenue, 2001), among others. Despite their wide-spread use and importance to mitigating conflicts between humans and wildlife, rigorous testing surrounding the effectiveness of these interventions is lacking (J. R. B. Miller et al., 2016; Treves et al., 2016; van Eeden et al., 2018). Those that do exist are based on narrative review, evaluated for only one predator species amongst a landscape that houses many (J. R. B. Miller et al., 2016), or are only discussed in the context of one criteria of effectiveness as opposed to both functional and perceived effectiveness (Ohrens, Santiago-Ávila, et al., 2019).

#### Perceived versus functional effectiveness

Perceived effectiveness is the perception of how well an intervention worked as reported by humans (Ohrens, Santiago-Ávila, et al., 2019). Functional effectiveness is whether the intervention reduces future attacks by wildlife and can be difficult to evaluate rigorously (Ohrens, Santiago-Ávila, et al., 2019; Treves et al., 2016). However, knowledge of functional effectiveness is imperative to provide evidence for preventing future attacks on livestock, not just suggestions of ideal deterrents as determined by assumptions and beliefs (Treves et al., 2019). Perceived and functional effectiveness may not always align, sometimes leading to negative outcomes including wasted resources and harm to animals (Ohrens, Santiago-Ávila, et al., 2019). For example, a functionally effective intervention method may still receive push-back if the perceived effectiveness of the method is lower than its actual utility (Cavalcanti et al., 2012;

Ohrens, Santiago-Ávila, et al., 2019). Alternatively, an ineffective method may be chosen if its perceived effectiveness is greater than its functional effectiveness (Ohrens, Santiago-Ávila, et al., 2019).

#### Need for predator deterrent studies on the Qinghai-Tibetan Plateau

For its landmass and percentage of people dependent on pastoralism, studies testing the functional effectiveness of interventions that prevent livestock loss are lacking in Asia, with the exception of India, in comparison to North America, Europe, and Africa (J. R. B. Miller et al., 2016; van Eeden et al., 2018). Knowledge of effectiveness of an intervention from both angles is imperative to promoting more peaceful coexistence between humans and wildlife, particularly in fragile landscapes with a high number of large carnivores and livestock, like that of the Qinghai-Tibetan Plateau (QTP). Large carnivores require extensive areas with low anthropogenic impact, and thus they commonly occupy and thrive in inhospitable areas with low human densities (Cavalcanti et al., 2012). The QTP is 2.5 million km<sup>2</sup> with an average elevation of 4,500m (B. Zhang et al., 2002). It is one of the world's largest pastoral ecosystems (Miehe et al., 2009) and holds the Sanjiangyuan region, an area of approximately 395,000km<sup>2</sup> that houses the headwaters of three major rivers in China - the Yangtze, Yellow, and Mekong (Y. Liu et al., 2016). The region's distinctive landscapes, endemic species, relevance to humans, and history of pastoralism spanning two millennia (Y. Liu et al., 2016) make it a target area for conservation research. Livestock kept here include yak (*Bos grunniens*), domestic goat (*Capra aegagrus hircus*), domestic sheep (*Ovis aries*), and domestic horse (*Equus caballus*) (Blench, 2001; D. J. Miller, 1999; Q. Qiu et al., 2012). A myriad of scientific publications, studies, and conservation education efforts have stemmed from work done in the Sanjiangyuan region, including those



showing the intensity of conflicts between humans and wildlife (Dai, Xue, et al., 2020; C.E. Hacker et al., 2020; J. Li et al., 2013). However, studies testing the effectiveness of livestock predation deterrents are to date non-existent.

### Objectives of this study

We aimed to help close this gap and contribute to the call for more rigorous studies surrounding the effectiveness of deterrents by testing the perceived and functional effectiveness of Foxlights [Caloola, New South Wales, Australia] in Duocai, Yushu Prefecture, Qinghai Province, China in preventing livestock loss. Foxlights are a non-lethal deterrent that emit a series of intermittent colored flashing lights at 360° in the absence of sunlight ([www.Foxlightsaustralia.com.au](http://www.Foxlightsaustralia.com.au)) (Figure 36). These flashing lights are meant to deter predators from livestock via aversive conditioning, in which the predator associates the light with human presence (M. E. Smith et al., 2000), thus reducing motivation to enter areas where livestock are kept (Bomford & O'Brien, 1990). Foxlights are easy to install, portable, operate via electrical charge or sunlight, and have little effect on livestock behavior (Ohrens, Bonacic, et al., 2019).

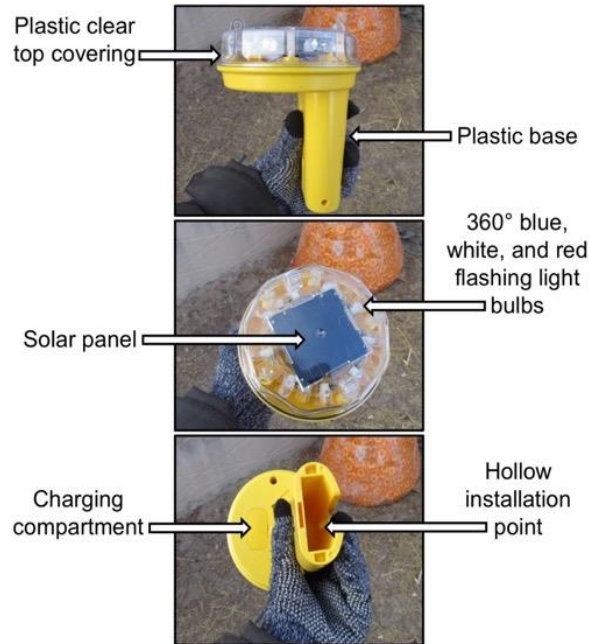


Figure 36. Characteristics of the Foxlight, a solar-powered predator deterrent that flashes nine small light bulbs randomly and intermittently in red, blue, and white color in the absence of sunlight.

## Materials and Methods

### Permits and permissions

Appropriate permits were obtained from the Sanjiangyuan National Nature Reserve, the Qinghai Forestry Bureau, and the Zhiduo County Government. Exemption from the Institutional Review Board (IRB) at Duquesne University was granted as information collected would not cause harm or embarrassment to the herders interviewed.

### Study area

The study was conducted in the township of Duocai, located on the QTP in Zhiduo County, Yushu Prefecture, Qinghai Province, China (33.8100°N, 95.4219°E). It is dominated by

alpine meadow grassland and has a continental climate, with a maximum temperature of 28°C, minimum of -42°C, and average annual temperature of 2.9°C (R. Wu et al., 2015). Duocai borders the Changjiang River Zone of the Sanjiangyuan National Park (Dai et al., 2019), and houses a number of wildlife species, including the snow leopard (*Panthera uncia*), Tibetan brown bear (*Ursus arctos pruinosus*), Tibetan wolf (*Canis lupus*), Tibetan fox (*Vulpes ferrilata*), red fox (*Vulpes vulpes*), Eurasian lynx (*Lynx lynx*), Tibetan gazelle (*Procapra picticaudata*), and blue sheep (*Pseudois nayaur*) (Sanjiangyuan National Park Administration; [sjy.qinghai.gov.cn/](http://sjy.qinghai.gov.cn/)). Interview sites were a mean altitude of 4,504m (range: 4,299m to 4,831m). Residents are predominately Tibetan and rely on rearing livestock for income or subsistence (Government of Zhiduo County, [www.zhiduo.gov.cn](http://www.zhiduo.gov.cn)).

### Experimental design

All potential participants had the study content, objectives, and expectations explained to them prior to their consent to participate and were informed that they could cease at any time. No incentives were provided to herders for their data (Ohrens, Bonacic, et al., 2019). Verbal interviews, as opposed to written, were selected due to concerns in misunderstandings stemming from illiteracy (J.M. Foggin, 2012). Interviews were provided in Mandarin Chinese to a local Tibetan translator who was used for all interviews to ensure clarity and consistency.

A randomized control-experimental pre-test / post-test group was used (Bonate, 2000) with one set of ten interviewed herders receiving two Foxlights each (experimental group) and one set of ten herders receiving no Foxlights (control group). All 20 herders were given the same interview prior to Foxlight installation in December 2019 consisting of information pertaining to demographics, livestock holdings, insurance, herding practices, livestock losses, current predator

deterrents in place, predator retaliatory killings, perceived effectiveness of properly positioned or executed deterrents, and predator presence (Appendix III: Supplemental Material 2, Appendix III: Supplemental Material 3). A 2-year recollection period was chosen to avoid recall bias (van Arsdale, 1996) and questions surrounding retaliatory killings were placed towards the end of the interview and carefully worded so that herders would not implicate themselves or others when providing answers (Fisher, 1993; Treves et al., 2006). The same 20 herders interviewed in December 2019 were re-interviewed three months later in March 2020. The post-Foxlight interview consisted of two versions, one for herders provided with Foxlights and one for herders who did not deploy this deterrent (Appendix III: Supplemental Material 4, Appendix III: Supplemental Material 5, Appendix III: Supplemental Material 6, Appendix III: Supplemental Material 7). Both interviews requested information surrounding livestock holdings and husbandry practices, livestock loss since December 2019, and comparison to perceived loss and reasoning to the previous year. The interview given to herders with Foxlights had additional questions surrounding ease, implementation, and perceived effectiveness. Each interview lasted approximately 30 minutes.

It was attempted to keep a minimum of 1-km Euclidian distance from each participant household. Each herder was trained on how to use the Foxlight and was assisted in installation to ensure all were installed in a similar fashion at 1.7 to 2.5m above the ground, and within eye line of an advancing predator (Figure 37). Anecdotal evidence of predator presence was collected from local herders.



Figure 37. Examples of congruency in Foxlight installation among herders in the experimental group. Foxlights were installed in either corners or entry points of night time pastures at heights ranging from 1.7 to 2.5m from the ground to remain visible to wild predators.

### Data Analysis

#### *Differences in livestock ownership and loss pre- and post-Foxlight*

Differences in predation of livestock may not be related to Foxlight use but rather to one group having more livestock than the other. A Mann-Whitney U Test with samples treated independently was used to test for statistically significant differences in the number of livestock owned in total and by species before Foxlights were provided. The successful deterrence of predators from locations using an intervention may increase their likelihood and pursuit of killing livestock at a location without the intervention, thus inflating the apparent effect of the intervention (Treves et al., 2006). To help account for this possibility, a Mann-Whitney U Test was used to determine differences in the number of livestock loss events or the number of total

livestock lost in the previous two years. A Mann-Whitney U Test was also used to test for significant differences in livestock lost and number of livestock loss events between the two groups.

### *Visibility analysis*

To assess visibility of Foxlights, a viewshed was constructed in ArcGIS Pro (Esri [Redlands, CA]) by applying a raster file of the terrain of Zhiduo County at 30m resolution. A 1km buffer was applied to each Foxlight placement point to determine if any locations with Foxlights may have emitted light which could be seen by predators at control points without Foxlights.

### *Generalized estimating equations*

A master table for all investigated variables was created (Table 33). A Pearson correlation was conducted and all correlated variables  $\geq 0.7$  (Dormann et al., 2013; Naha et al., 2020) had one variable of the pair omitted from further analysis. The variable removed depended on expert opinion on relevance to generate the final list of variables for the dataset. Generalized estimating equations (GEEs) were used to determine influential predictors of livestock loss events as well as number of livestock lost. GEEs are appropriate for small datasets with non-normal distributions without having to make data corrections (Kowalski & Tu, 2007). The total number of livestock killed was set as the response variable. “Herder” was set as the subject variable. A linear scale response variable with main effects examined was selected. A series of general models were constructed (Table 34). The first model was built considering all variables. The least impactful variable to the model was then removed in a stepwise fashion for each

sequential model. The quasilielihood under the independence model criterion (QIC) was used to rank models, with the lowest QIC value deemed most appropriate (J. Cui, 2007). SPSS (version 25.0 [Chicago, IL]) was used with significance set at  $p < 0.05$ .

Table 33. The variables used in the Pearson correlation to determine multicollinearity.

Type of Variable	Variable Name	SPSS Name	Unit	Notes
Demographics	Household Size	Household_Size	Numeric	
	Herder	Herder	Nominal	
	Village	Village	Nominal	
Environmental	Altitude	Altitude	m	
	No. of Predator Species in Area	Num_Predators_Present	Numeric	
	Visibility	Visibility	km <sup>2</sup>	
	Foxlight Use	Foxlight_Status	Binary	1 (Yes) / 2 (No)
Livestock Ownership	No. of Yak	Num_Yak	Numeric	
	No. of Sheep/Goat	Num_SheepGoat	Numeric	
	No. of Horse	Horse_Total	Numeric	
	No. of Total Livestock	Num_Total	Numeric	
Deterrent Use	Human Guarding All Day	Human_Guard_All_Day	Binary	1 (Yes) / 2 (No)
	Human Guarding All Night	Human_Guard_All_Night	Binary	1 (Yes) / 2 (No)
	Human Guarding Sometimes Day	Human_Guard_Sometimes_Day	Binary	1 (Yes) / 2 (No)
	Human Guarding Sometimes Night	Human_Guard_Sometimes_Night	Binary	1 (Yes) / 2 (No)
	Light Use	Light_Use	Binary	1 (Yes) / 2 (No)
	Noise Use	Noise_Use	Binary	1 (Yes) / 2 (No)
	Scarecrow Use	Scarecrow_Use	Binary	1 (Yes) / 2 (No)
Livestock Loss	No. of Yak Lost	Num_Yak_Lost	Numeric	
	No. of Sheep/Goat Lost	Num_SheepGoat_Lost	Numeric	
	No. of Total Livestock Lost	Num_Total_Lost	Numeric	All potential response variables for the model.
	No. of Total Loss Events	Num_Loss_Events	Numeric	
	No. of Diurnal Loss Events	Diurnal_Loss	Numeric	
	No. of Nocturnal Loss Events	Nocturnal_Loss	Numeric	

Table 34. Variables used as predictors for analysis using Generalized Estimating Equations.

Type	Predictor	Explanation	Variable Level
Co-variate	Altitude	Meters above sea level.	Numeric (m)
	Household Size	The number of people living in the home of the herder.	Numeric (Number of people)
	Number of Yak	The number of yak owned.	Numeric (Number of yak)
	Number of Sheep/Goats	The number of sheep and goats owned.	Numeric (Number of goats and sheep)
	Number of Horses	The number of horses owned.	Numeric (Number of horses)
	Foxlight Visibility	The radial distance at which the Foxlight could be observed.	Numeric (km <sup>2</sup> )
Factor	Number of Predator Species Present	The number of different carnivore species in proximity to the herder's home.	Numeric (Number of species)
	Village	The village name of the herder's home.	Nominal. The name of the village.
	Noise Use	Use or no use of noise makers to deter predators.	Ordinal. Coded as 0 if herders do not use noise. Coded as 1 if herders do.
	Scarecrow Use	Use or no use of scarecrows to deter predators.	Ordinal. Coded as 0 if herders do not use scarecrows. Coded as 1 if herders do.
	Human Guard All Night	Herders actively guard their livestock for the entirety of the night.	Ordinal. Coded as 0 if herders do not guard their livestock all night. Coded as 1 if herders do.
	Human Guard Sometimes Night	Herders actively guard their livestock for a portion of the night.	Ordinal. Coded as 0 if herders do not sometimes guard their livestock throughout the night. Coded as 1 if herders do.
	Foxlight Status	Herder possession of a Foxlight or not.	Ordinal. Coded as 0 for not having a Foxlight. Coded as 1 for having a Foxlight.

## Results

### Demographics

No herders declined to participate in the study. All 20 participants reported livestock rearing as their primary income source. The average family size was 4.2 individuals (range 2 to 6 individuals). Average years of formal education was 1.5 years (range 0 to 15 years).



### Livestock holdings and loss prevention

Prior to the implementation of Foxlights, 95% of herders owned yak, with an average of 82.5 yak (range: 0 to 200) per herder and a total of 1,650 yak among the twenty herders interviewed. A total of 10% of herders owned goats with an average of 2.25 individuals (range: 0 to 40) per herder and a total of 45 goats for all herders interviewed combined. Sheep were owned by 20% of herders with an average of 70.45 individuals per herder (range: 0 to 440) with 1,415 sheep in total. Horses were reported to be owned by 35% of herders with an average of 1 horse per herder interviewed (range: 0 to 6) and a total of 20 horses. Herd sex composition could not be determined at the time of the initial interview as most herders were not confident in their recollection, and thus they were asked to count the number of males and females more accurately for the follow-up interview. Those who were provided Foxlights had significantly more livestock overall than those who were not provided Foxlights ( $p = 0.019$ ), but statistical differences were not found in the number yak owned ( $P = 0.143$ ), number sheep owned ( $p = 0.143$ ), number of goats owned ( $p = 0.481$ ), or number of horses owned ( $p = 0.529$ ).

On average, herders moved their livestock 2.6 times per year (range: 0 to 4). The majority of herders stated they moved because of low forage availability at 66% while the remaining 33% noted that both low forage availability or the quality of forage available were reasons for moving.

A total of 90% of herders used guard dogs to protect livestock from predators. Dogs were considered as the most effective means to protect livestock by 15% of herders and the average Likert scale score of dog effectiveness on a scale of 1 (not effective at all) to 7 (highly effective)

was a 5. The vast majority of herders practiced human guarding to protect their livestock at 90% of those interviewed with 80% of interviewees reporting it as the most effective method to deter predators. A total of 60% guarded the entire day while 40% only sometimes guarded during the day. A total of 80% of herders sometimes guarded at night while 20% always guarded at night. Just over half of those interviewed housed newborn animals inside at 55%, while 15% used predator proof corrals, 55% used scarecrows, 55% used noise, 10% used light, and 5% used firecrackers. Of these, 60% claimed scarecrows were the least effective method at deterring livestock while 25% reported noise, followed by light at 5%, and predator proof corrals at 5%.

All 20 herders interviewed possessed livestock insurance. All domestic species were covered with the exception of horses, of which only 14% of the herders who owned them had insurance policies against predation. The vast majority of herders were satisfied overall with their insurance at 95% with 0% of herders agreeing that insurance was expensive, 30% agreeing that payment was sufficient, 0% agreeing that payment was timely, and 45% agreeing that evidence collection was difficult.

#### Livestock loss in previous two years

All twenty herders interviewed experienced livestock loss in the previous two years. A total of 38 loss events were recorded with an average of 1.9 events per herder (range: 1 to 4). In total, 212 yak and 149 sheep were predated. Those who owned yak lost an average of 10.60 yak (range: 0 to 21) over the course of two years and those who owned sheep lost an average of 37.25 sheep (range: 20 to 62). A total of 42% of loss events herders attributed to wolves, 29% to bears, and 24% to snow leopards. Identification of the predator responsible was primarily by sight with 85% of herders stating that they witnessed attacks or the immediate aftermath post-

predation. The remaining 10% and 5% stated they attributed predator identification via puncture wounds or hearsay from someone else claiming to have witnessed the event, respectively. The majority of loss events occurred over 1km from the herder's home at 63%. A total of 24% of events occurred within 100m of the herder's home. The number of livestock loss events between the control and experimental group pre-deployment of Foxlights were not significantly different ( $p = 0.353$ ). However, the number of livestock lost between the control and experimental group pre-deployment of Foxlights were significantly different between the two groups ( $p = 0.035$ ) in that herders who were given Foxlights lost significantly more livestock in the previous two years than herders who were not given Foxlights.

#### Predator presence and perceptions of threat

Wolves, Tibetan brown bears, and foxes were reported by all herders to be in the nearby vicinity of their homes. A total of 75% of herders reported snow leopard in close proximity and 70% reported Eurasian lynx in close proximity. Wolves were reported by 100% of herders to be the most threatening to livestock. However, one herder noted that they would consider snow leopards equally as dangerous and another herder stated that they would consider Tibetan brown bears equally as dangerous.

#### Retaliatory killings

Two of the twenty herders interviewed had knowledge of wolves being killed in the previous two years.

#### Livestock loss during 3-month study period

A total of twelve loss events were reported over the three-month study period resulting in the depredation of two adult female sheep, one young female sheep, two adult female yak, seven young male yak, eight young female yak, and four subadult yak of unknown sex. Female yak were disproportionately predated relative to the total number of yak available. Two loss events occurred at night while the remaining ten occurred during the day. Three livestock loss events occurred among two herders with Foxlights and nine loss events among nine herders without Foxlights, although one of these was within 1km of a herder home in possession of Foxlights. This difference was found to be statistically significant ( $p = 0.019$ ). Nine animals in total were lost for herders with Foxlights and 15 lost for herders without Foxlights. This was also found to be statistically significant ( $p = 0.043$ ). On average, depredation occurred 1,200m from the herder's home (range: 800 to 1,500m). Wolves were deemed the responsible predator in 11 of the 12 loss events (92%), with snow leopard attributed to one (8%) (Table 35, Figure 38).

Table 35. Details surrounding livestock loss over the three-month study period.

Study Group	Loss Date	Predator	Distance to House	Time of Day	Deterrents in Place	Livestock Loss
Foxlight	Mar. 2020	Wolf	1500m	Day	Foxlight	One adult female yak and one young female yak
Foxlight	Mar. 2020	Wolf	1000m	Day	Foxlight	Two adult female sheep and one young female sheep
Foxlight	Jan. 2020	Wolf	1000m	Day	Dogs, Foxlight	Four subadult yak
Control*	Jan. 2020	Wolf	800m	Day	None	One young male yak
Control	Jan. 2020	Wolf	1300m	Day	None	One young male and one young female yak
Control	Jan. 2020	Wolf	1000m	Day	None	One subadult female and one young male
Control	Dec. 2019	Wolf	1000m	Day	None	One young male
Control	Jan. 2020	Wolf	1200m	Day	Dogs	One young female
Control	Dec. 2019	Wolf	1000m	Day	Dogs	One young male
Control	Dec. 2019	Wolf	800m	Night	None	One young male
Control	Dec. 2019	Wolf	1200m	Day	None	Two young males
Control	Feb. 2020	Snow Leopard	800m	Night	Dogs	One adult female and three young females

\*Herder home in control group which was within 1km of a herder home in the experimental group.

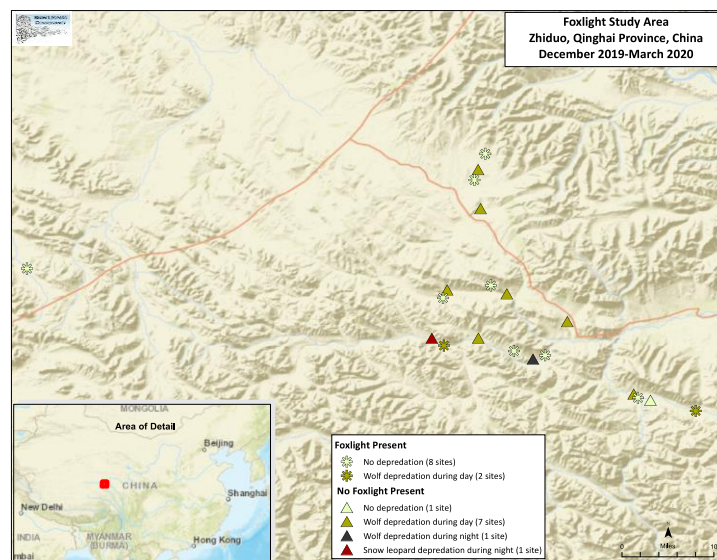


Figure 38. Sites of depredation over of the three-month study period.

Visibility analysis

Placement of the 1km buffer around each Foxlight implementation location and the output viewshed revealed that the light emitted from the Foxlights installed for herder 2 may have been visible to predators in the same area as herder 3, who was not provided Foxlights (Figure 39).

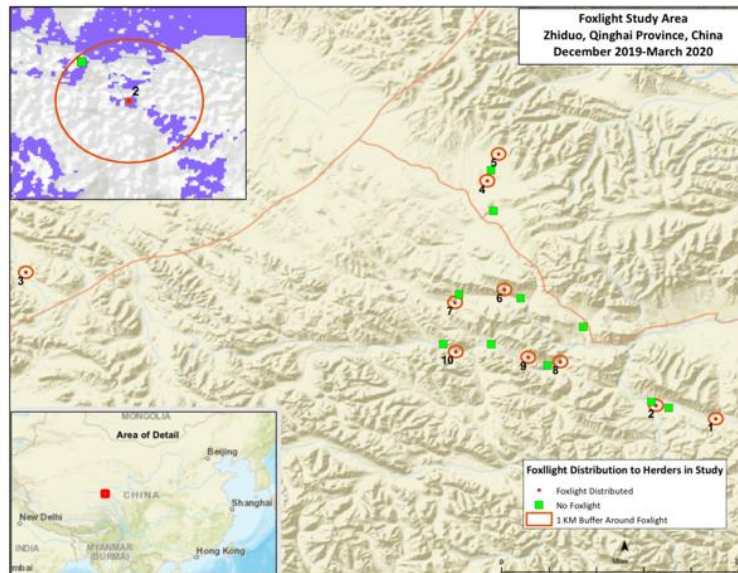


Figure 39. The location of sites with Foxlights with a 1km buffer to determine visibility overlap with study sites that did not have Foxlights. The inset in the top left corner shows the overlap of a Foxlight site with a site that was not provided with a Foxlight, with its potential visibility to predators denoted in purple.

### Perceived Foxlight effectiveness

Of the ten herders with Foxlights, eight stated that livestock loss was less than the same time the previous year, one said it was the same, and one said it was more. All herders attributed less loss to having the Foxlight. Of those herders in the control group, one herder said it was less, eight said it was more, and one said it was the same. Those who said it was more pointed to an increasing wolf population, and the one herder who said it was less attributed it to use of guard dogs. Nine of the ten herders in the control group asked for Foxlights from the interviewer based on communication of their effectiveness from peers who had the Foxlight. No device issues were

reported aside from one herder stating that the Foxlights were not bright enough. All Foxlights were in good physical condition after the three month study period. Herders scored ease of use on a Likert scale of 1 (difficult to use) to 7 (very easy to use) as a 7. There was little evidence of social pressure to implement or not implement the Foxlight. On a Likert scale of 1 (no social pressure to implement) and 7 (high social pressure to implement), the average response value was 1.7. On a Likert scale of 1 (Foxlights are not at all effective) to 7 (Foxlights are very effective), the average response value was a 6.8. All ten herders in the experimental group wanted to keep using the Foxlights, though 80% of them suggested adding a noise component, and 100% of herders said that the implementation of at least three Foxlights would be better than two.

#### Functional Foxlight effectiveness

The Pearson correlation identified significant variable relationships that led to the removal of seven variables from the dataset (Table 36, Table 37). Predictive variables that were removed included “Light Use”, “Human Guard All Day”, “Human Guard Sometimes During the Day”, and “Number of Total Livestock Owned”. Response variables that were removed included “Number of Yak Lost”, “Number of Goat and Sheep Lost”, “Number of Diurnal Loss Events”, and “Number of Nocturnal Loss Events”. Generalized estimating equations were used for the response variables, “Number of Livestock Lost” and “Number of Livestock Loss Events”. The final model for “Number of Livestock Lost” included “Household Size” and “Foxlight Status” as predictors that decreased the number of livestock lost. The final model for “Number of Livestock Lost” had “Foxlight Status” as the predictor that decreased the number of livestock loss events (Table 38).

Table 36. Results of the Pearson Correlation with all possible variable relationships considered. Those highlighted in yellow represent statistically significant relationships. Those highlighted in blue represent statistically significant relationships whose correlation R value is  $\geq 0.7$ .

		Herder	Attitude	Household_Size	Num_Yak	Num_SheepGoat	Num_Horse	Num_Total	Num_Yak_Lost	Num_SheepGoat_Lost	Num_Total_Lost	Num_Loss_Events	Diurnal_Loss	Nocturnal_Loss	Viability	Num_Predators_Present	Noise_Use	Scarecrow_Use	Light_Use	Human_Guard_AI_Day	Human_Guard_AI_Night	Human_Guard_So_metimes_Day	Human_Guard_So_metimes_Night	Foalight_Status
Herder	Pearson Correlation	1																						
	Sig. (2-tailed)																							
	N	20																						
Attitude	Pearson Correlation	-0.056	1																					
	Sig. (2-tailed)	0.813																						
	N	20	20																					
Household_Size	Pearson Correlation	-0.293	0.083	1																				
	Sig. (2-tailed)	0.210	0.729																					
	N	20	20	20																				
Num_Yak	Pearson Correlation	-0.283	0.006	0.087	1																			
	Sig. (2-tailed)	0.226	0.981	0.714																				
	N	20	20	20	20																			
Num_SheepGoat	Pearson Correlation	-0.314	0.017	0.315	0.453	1																		
	Sig. (2-tailed)	0.178	0.067	0.176	0.048																			
	N	20	20	20	20	20																		
Num_Horse	Pearson Correlation	-0.087	0.414	-0.014	0.117	0.514	1																	
	Sig. (2-tailed)	0.715	0.068	0.941	0.004	0.013																		
	N	20	20	20	20	20	20																	
Num_Total	Pearson Correlation	-0.344	0.347	0.284	0.685	0.611	0.423	1																
	Sig. (2-tailed)	0.137	0.134	0.225	0.001	0.003	0.003																	
	N	20	20	20	20	20	20	20																
Num_Yak_Lost	Pearson Correlation	0.401	-0.109	0.354	-0.236	-0.181	-0.337	-0.223	1															
	Sig. (2-tailed)	0.080	0.646	0.126	0.316	0.446	0.146	0.344																
	N	20	20	20	20	20	20	20	20															
Num_SheepGoat_Lost	Pearson Correlation	-0.318	-0.112	0.508	0.310	0.507	-0.128	0.844	0.175	1														
	Sig. (2-tailed)	0.100	0.638	0.011	0.163	0.012	0.590	0.013	0.460															
	N	20	20	20	20	20	20	20	20	20														
Num_Total_Lost	Pearson Correlation	0.167	-0.139	0.534	-0.060	0.090	-0.335	0.052	0.864	0.540	1													
	Sig. (2-tailed)	0.481	0.558	0.018	0.800	0.706	0.149	0.829	0.000	0.007														
	N	20	20	20	20	20	20	20	20	20	20													
Num_Loss_Events	Pearson Correlation	-0.039	-0.338	0.346	-0.090	-0.036	-0.429	-0.061	0.794	0.595	0.429	1												
	Sig. (2-tailed)	0.876	0.156	0.147	0.715	0.885	0.067	0.805	0.001	0.014	0.000													
	N	19	19	19	19	19	19	19	19	19	19	19												
Diurnal_Loss	Pearson Correlation	0.167	-0.139	0.534	-0.060	0.090	-0.335	0.052	0.864	0.540	1.000	0.007	1											
	Sig. (2-tailed)	0.481	0.558	0.018	0.800	0.706	0.149	0.829	0.000	0.007	0.000													
	N	20	20	20	20	20	20	20	20	20	20	20	20											
Nocturnal_Loss	Pearson Correlation	0.425	-0.080	0.323	-0.122	-0.138	-0.157	-0.152	0.532	-0.065	0.412	0.184	0.412	1										
	Sig. (2-tailed)	0.062	0.737	0.164	0.607	0.562	0.507	0.523	0.016	0.787	0.071	0.452	0.071											
	N	20	20	20	20	20	20	20	20	20	20	20	20	20										
Viability	Pearson Correlation	0.072	-0.021	0.376	-0.112	0.206	-0.235	0.132	0.414	0.310	0.482	0.314	0.482	-0.052	1									
	Sig. (2-tailed)	0.763	0.930	0.103	0.639	0.383	0.318	0.580	0.069	0.169	0.031	0.191	0.031	0.828										
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20									
Num_Predators_Present	Pearson Correlation	0.273	-0.069	-0.301	-0.283	0.047	-0.472	-0.053	-0.095	0.232	0.022	0.073	0.022	-0.222	0.239	1								
	Sig. (2-tailed)	0.244	0.771	0.197	0.227	0.848	0.036	0.825	0.660	0.304	0.925	0.768	0.925	0.347	0.310									
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20								
Noise_Use	Pearson Correlation	0.050	-0.009	-0.221	-0.126	0.180	0.065	0.109	-0.116	-0.132	-0.154	-0.444	-0.154	-0.163	0.285	0.195	1							
	Sig. (2-tailed)	0.834	0.971	0.349	0.597	0.448	0.787	0.648	0.826	0.578	0.518	0.057	0.518	0.493	0.223	0.410								
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20							
Scarecrow_Use	Pearson Correlation	-0.357	-0.139	0.013	-0.139	0.250	-0.169	0.160	-0.198	-0.053	0.174	-0.093	-0.142	0.281	0.237	0.174	1							
	Sig. (2-tailed)	0.122	0.559	0.957	0.558	0.287	0.477	0.500	0.403	0.281	0.823	0.477	0.823	0.552	0.230	0.313	0.483							
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20						
Light_Use	Pearson Correlation	0.009	0.064	0.013	0.485	0.359	0.353	0.446	-0.279	0.254	-0.120	-0.487	-0.120	-0.255	0.060	0.124	0.408	-0.212	1					
	Sig. (2-tailed)	0.971	0.788	0.957	0.030	0.120	0.086	0.049	0.234	0.281	0.613	0.049	0.613	0.278	0.803	0.601	0.076	0.389						
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20				
Human_Guard_AI_Day	Pearson Correlation	0.071	-0.116	-0.026	0.493	0.215	0.228	0.329	-0.049	0.187	0.041	-0.079	0.041	0.230	-0.174	-0.138	-0.236	-0.082	0.123	1				
	Sig. (2-tailed)	0.787	0.619	0.913	0.027	0.362	0.333	0.156	0.837	0.429	0.865	0.748	0.865	0.329	0.462	0.562	0.317	0.731	0.605					
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20			
Human_Guard_AI_Night	Pearson Correlation	-0.134	-0.422	-0.054	0.055	-0.205	-0.157	-0.152	0.208	-0.096	0.130	0.026	0.130	-0.118	0.168	-0.205	0.454	-0.099	0.183	0.057	1			
	Sig. (2-tailed)	0.575	0.064	0.823	0.818	0.385	0.510	0.521	0.378	0.686	0.584	0.915	0.584	0.619	0.480	0.386	0.077	0.679	0.440	0.811				
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20		
Human_Guard_So_metimes_Day	Pearson Correlation	-0.227	0.151	0.040	0.447	-0.170	-0.176	-0.278	-0.198	-0.168	-0.237	-0.154	-0.237	-0.207	-0.014	0.035	0.061	0.179	-0.242	0.099	-0.308	1		
	Sig. (2-tailed)	0.335	0.524	0.861	0.048	0.476	0.458	0.236	0.403	0.478	0.314	0.530	0.314	0.362	0.954	0.862	0.800	0.450	0.303	0.000	0.166			
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	
Human_Guard_So_metimes_Night	Pearson Correlation	0.242</																						



Table 37. Significant relationships between considered variables ( $p \geq 0.05$ ), the direction of the correlation between the two variables, and their status of meeting the cut off status for variable removal ( $R \geq 0.7$ ). Bolded text indicates the variables removed from further analysis.

Variable 1	Variable 2	Direction	Over 0.7?
Num_Yak	Num_Total	Positive	Yes
Num_Yak_Lost	Num_Total_Lost	Positive	Yes
Num_Yak_Lost	Num_Loss_Events	Positive	Yes
Num_Yak_Lost	Diurnal_Loss	Positive	Yes
Num_Total_Lost	Num_Loss_Events	Positive	Yes
Num_Loss_Events	Diurnal_Loss	Positive	Yes
Light_Use	Foxlight_Status	Negative	Yes
Human_Guard_All_Day	Human_Guard_Sometimes_Day	Negative	Yes
Household_Size	Num_SheepGoat_Lost	Positive	No
Household_Size	Num_Total_Lost	Positive	No
Household_Size	Diurnal_Loss	Positive	No
Num_Yak	Num_SheepGoat	Positive	No
Num_Yak	Num_Horse	Positive	No
Num_Yak	Light_Use	Positive	No
Num_Yak	Human_Guard_All_Day	Positive	No
Num_Yak	Human_Guard_Sometimes_Day	Negative	No
Num_SheepGoat	Num_Horse	Positive	No
Num_SheepGoat	Num_Total	Positive	No
Num_SheepGoat	Num_SheepGoat_Lost	Positive	No
Num_SheepGoat	Foxlight_Status	Negative	No
Num_Horse	Num_Total	Positive	No
Num_Horse	Num_Predators_Present	Negative	No
Num_Total	Num_SheepGoat_Lost	Positive	No
Num_Total	Light_Use	Positive	No
Num_Total	Foxlight_Status	Negative	No
Num_Yak_Lost	Nocturnal_Loss	Positive	No
Num_SheepGoat_Lost	Num_Total_Lost	Positive	No
Num_SheepGoat_Lost	Num_Loss_Events	Positive	No
Num_SheepGoat_Lost	Diurnal_Loss	Positive	No
Num_Total_Lost	Diurnal_Loss	Positive	No
Num_Total_Lost	Visibility	Positive	No
Num_Loss_Events	Light_Use	Negative	No
Num_Loss_Events	Foxlight_Status	Positive	No
Diurnal_Loss	Visibility	Positive	No
Num_Predators_Present	Human_Guard_Sometimes_Night	Positive	No
Noise_Use	Foxlight_Status	Negative	No
Human_Guard_All_Night	Human_Guard_Sometimes_Night	Negative	No

Table 38. Model outcomes using Generalized Estimating Equations of predictors for number of livestock lost and number of livestock loss events.

<i>Number of Livestock Lost</i>					<i>Number of Livestock Loss Events</i>				
Predictor Variables	QIC	ΔQIC	QICc	ΔQICc	Predictor Variables	QIC	ΔQIC	QICc	ΔQICc
Num Livestock Lost ~ Noise Use + Scarecrow Use + Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility + Num Predator Species Present	43.327	-	42.830	-	Num Livestock Loss Events ~ Noise Use + Scarecrow Use + Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility + Num Predator Species Present	31.991	-	32.756	-
Num Livestock Lost ~ Noise Use + Scarecrow Use + Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility	41.498	-1.829	40.830	-2	Num Livestock Loss Events ~ Scarecrow Use + Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility + Num Predator Species Present	30.471	-1.520	30.756	-2
Num Livestock Lost ~ Scarecrow Use + Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility	39.805	-1.693	39.002	-1.828	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility + Num Predator Species Present	28.529	-1.942	28.760	-1.996
Num Livestock Lost ~ Scarecrow Use + Human Guard All Night + Foxlight Status + Village + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility	38.540	-1.265	37.257	-1.745	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Foxlight Visibility + Num Predator Species Present	23.245	-5.284	22.859	-5.901
Num Livestock Lost ~ Scarecrow Use + Human Guard All Night + Foxlight Status + Village + Altitude + Household Size + Num of Goat Sheep + Num Horse + Foxlight Visibility	37.632	-0.908	35.505	-1.752	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse + Num Predator Species Present	21.922	-1.323	20.863	-1.996

Num Livestock Lost ~ Scarecrow Use + Human Guard All Night + Foxlight Status + Village + Altitude + Household Size + Num of Goat Sheep + Num Horse	37.254	-0.378	32.096	-3.409	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Altitude + Household Size + Num Yak + Num of Goat Sheep + Num Horse	20.247	-1.675	18.88 1	-1.982
Num Livestock Lost ~ Human Guard All Night + Foxlight Status + Village + Altitude + Household Size + Num of Goat Sheep + Num Horse	36.632	-0.622	33.326	+1.230	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Household Size + Num Yak + Num of Goat Sheep + Num Horse	20.144	-0.103	16.98 4	-1.897
Num Livestock Lost ~ Human Guard All Night + Foxlight Status + Village + Household Size + Num of Goat Sheep	38.932	+2.300	35.674	+2.348	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Household Size + Num Yak + Num Horse	18.268	-1.876	15.39 8	-1.586
Num Livestock Lost ~ Human Guard All Night + Foxlight Status + Village + Household Size	40.155	+1.223	36.517	+0.843	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Household Size + Num Yak	16.585	-1.683	14.14 8	-1.250
Num Livestock Lost ~ Foxlight Status + Village + Household Size	37.396	-2.759	36.891	+0.374	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status + Household Size	14.563	-2.022	12.28 5	-1.863
Num Livestock Lost ~ Foxlight Status + Household Size	36.141	-1.255	35.816	-1.075	Num Livestock Loss Events ~ Human Guard All Night + Human Guard Sometimes Night + Foxlight Status	10.325	-4.238	12.09 5	-0.190
Num Livestock Lost ~ Household Size	36.410	+0.269	36.293	+0.477	Num Livestock Loss Events ~ Human Guard Sometimes Night + Foxlight Status	9.714	-0.611	10.66 7	-1.428
					Num Livestock Loss Events ~ Foxlight Status	9	-0.714	9	-1.667
<i>Final Model: Num Livestock Lost ~ Foxlight Status + Household Size</i>					<i>Final Model: Num Livestock Loss Events ~ Foxlight Status</i>				

## Discussion

Despite the availability and use of non-lethal methods aimed at keeping predators from attacking livestock, studies surrounding the perceived and functional effectiveness of these methods are lacking, or isolated to one geographic area. Methods that keep predators from pursuing livestock from dawn to dusk are of particular interest given that predator attacks at night-time can be devastating. Such attacks often occur in corral areas where multiple livestock are killed simultaneously, increasing herder frustration, economic burden, emotional trauma, and risk of retaliatory killings (Jackson and Wangchuk 2004). Intermittent and randomly flashing lights may serve as a promising method to deter predators from nighttime attacks. Here, we tested both the functional and perceived effectiveness of solar-powered Foxlights on the Qinghai-Tibetan Plateau (QTP) of China.

### Perceived effectiveness of Foxlights

In this study, the perceived effectiveness of Foxlights amongst herders who were provided them was high. Uncertainty and novelty of methods can dampen adoption (Ohrens, Santiago-Ávila, et al., 2019). Foxlights are beneficial in that light in and of itself is not a novel deterrent, which may have contributed to the more widespread acceptance of the technique from the study's start. Skepticism regarding the application of non-lethal predator deterrents can strain relationships between herders and researchers, and may be linked to a wider need for conservation education (Cavalcanti et al., 2012). Ranchers in Brazil opposed jaguar conservation because they viewed it as incompatible with livestock operations. Their reasoning was founded in the idea that a greater number of cattle would

increase the jaguar population that would eventually be undeterred by non-lethal anti-predation measures. In reality, many more factors surrounding jaguar ecology play into their population size and pursuit of livestock. Foxlights were well received by herders in this study, and the device itself worked consistently and continuously for the three months. When asked what improvements could be made to the Foxlights, 80% of herders stated that adding a noise component would be beneficial. Biosonic repellents can cause animal distress by inciting pain or discomfort (Bomford & O'Brien, 1990; M. E. Smith et al., 2000). While perhaps effective for predators, harsh sounds would also likely distress livestock. Future research on how these sounds impact domestic animal behavior, stress hormones, and other measures of welfare such as mass and body condition, would be helpful in detailing the practicality of adding such a feature to Foxlights.

#### Functional effectiveness of Foxlights

The functional effectiveness in this study upon initial observation appeared to be high. Herders with Foxlights lost significantly fewer livestock and experienced significantly fewer livestock loss events. However, 83% of livestock loss events happened during the day, when Foxlights would presumably not be working and the control versus experimental groups would be considered “equal”. Previous studies that have tested Foxlights have also displayed a pattern that exhibited functional effectiveness of Foxlights with the caveat of livestock loss events occurring during the day while other studies did not differentiate (Ohrens, Bonacic, et al., 2019). For example, Foxlights were functionally effective at deterring leopards (*Panthera pardus*) from killing livestock in the western Himalayas, but 60% of livestock kills occurred during the day (Naha et al.,

2020). The authors go on to attribute this to a diurnal activity peak that predators take advantage of due to poor herding practices and ease of access to livestock. However, this does not address the difference between the experimental versus the control groups, as both would presumably experience the same loss during the day due to poor herding practices. In our study, all loss events, with the exception of one to a snow leopard, were attributed to wolves. Knowledge of diurnal attacks is in and of itself valuable, as most current mitigation schemes, including the one presented in this study, focus on preventing nighttime attacks when livestock are herded into corrals (Jackson & Wangchuk, 2001; Samelius et al., 2020). Livestock are also killed while on the range (Atickem et al., 2017; Iliopoulos et al., 2009) but tools preventing this loss are seldom discussed in mitigation plans. Foxlights may have some sort of longer lasting impact than previously anticipated. For example, wolves may see the Foxlights at night, associate them with a particular home, and continue to avoid that home the following day. Similarly, Foxlights may push wolves out of the surrounding area of the home at night, leaving them closer to homes without Foxlights the following day. Neophobia during the day by the mere presence of the Foxlight may also be contributing to avoidance behavior.

#### Influential factors in determining livestock loss

Regardless of diurnal versus nocturnal losses, the model generated for number of livestock loss events and number of livestock lost both attributed the presence of the Foxlight as being one of the primary predictors. Use of Foxlights as well as a larger household size predicted less livestock loss, while use of Foxlights predicted fewer livestock loss events. These findings are intuitive. Predators are deterred by human

activity (Ogada et al., 2003). Having a large household would presumably lend itself to the presence of more bodies and human vocalizations. In regards to fewer numbers of livestock loss events, the model with the second lowest QIC score also included the practice of guarding livestock sometimes throughout the night and was only 0.611 higher than the lowest model that had only Foxlight status as a predictor. Night guarding during optimal surveillance hours when predators are active has been suggested as a way to reduce predation in previous studies (Cavalcanti et al., 2012). A total of 80% of interviewed herders in the study stated that human guarding was the most effective means to keep their livestock safe. Similar to the role of household size in reducing the number of livestock lost, livestock guarding at night would prevent nocturnal losses of livestock due to the presence of a human.

#### Role of Tibetan wolves in livestock loss

Wolves were ubiquitous across the study area and were considered as the most threatening to livestock by all of the herders interviewed. This risk perception was echoed in herder reports of depredation in the two years prior to the study with wolves being reported as responsible for 68% of livestock loss events in the previous two years, with snow leopard following at 21%, and brown bear at 11%. During the three-month study period, wolves were responsible for 92% of the 12 livestock loss events and snow leopard for the remaining 8%. This may indicate that Foxlights are more effective at deterring snow leopards and brown bears, but the short study period makes this an unsupported conclusion.

#### Self-reporting of losses and potential bias

In addition, livestock losses were self-reported by herders. Discrepancies between researchers and herders in the number of livestock lost to predation have been noted in previous studies (Cavalcanti et al., 2012) and disproportionate concern assigned to a certain species warrants investigation if present (Treves et al., 2006). However, we suggest that livestock losses reported herein are relatively accurate given that most losses in the area are inspected and reported to insurance programs for compensation (Samelius et al., 2012). Previous reviews and meta-analyses of deterrent effectiveness have excluded studies lacking external field verification of livestock loss (Treves et al., 2016). We cautiously warrant against this as it excludes a wide number of studies that may hold important information for examining the effectiveness of interventions across a wider scale. A third-party confirmation of livestock loss is often infeasible in most studies as it would require someone to be continuously present at the site (Cavalcanti et al., 2012) who is knowledgeable of discerning livestock kills. Connection to a government resource or livestock insurance agency which monitors and confirms losses is also possible, but must be in a manner congruent with the study's guidelines of livestock loss number and predator responsible without the risk of insurance fraud or untimeliness of entities investigating the kill site. Further, exclusion of herder reports of livestock loss and responsible predator perpetuates the unfounded bias by outside scientists and conservationists that rural pastoralists are unknowledgeable (Brockington & Homewood, 1999).

#### Status of livestock insurance



Livestock insurance is an important mechanism for reducing financial burden on herders (Hussain, 2000). Livestock insurance was common and generally deemed to be satisfactory for 95% of herders interviewed in this study. The most frequently reported shortcoming was that livestock insurance was not timely, a regular issue in remote mountainous environments (Paltsyn et al., 2016). All livestock were insured with exception of horses, of which only one of the seven herders who owned had an insurance policy for. Horses are large in stature and may not be at as great of a risk for predation by predators. However, horse has previously been found in snow leopard diet (C.E. Hacker et al., 2021). While livestock insurance can play an important role in securing herder financial security post-livestock loss, methods that protect herder financial security via the halting of livestock depredation in the first place are preferred, especially since insurance only offsets the economic impact of livestock loss, and not its associated negative perceptions and fear (C. Mishra et al., 2016). While this can include large-scale conservation efforts like increasing wild prey numbers so that wild predators do not have a reason to pursue livestock, most rely on the herders themselves taking active steps to make livestock unprofitable as a food item within the context of optimal foraging theory (MacArthur & Pianka, 1966).

#### Key suggested measures for conservation

Foxlights require little change in human behavior and are based off of simple technology that is understandable, making it more likely to succeed as a self-sustaining intervention (Ogada et al., 2003; Osborn & Parker, 2003). However, reducing the dependence of residents on livestock herding all together is likely key for reducing

negative outcomes for large carnivore populations (Dai, Xue, et al., 2020). Better ability to cope with livestock loss via additional sources of food or income can reduce herder vulnerability at both individual and communal levels (Treves et al. 2006), thus potentially reducing negative attitudes toward wildlife and lowering the risk of retaliatory killings. However, fewer livestock or inability to access livestock due to a high degree of functional effectiveness of a deterrent may reduce carrying capacity of carnivores in an area (Spencer et al., 2020), which could drastically impact ecosystem function.

### Study standards and rigor

Functional effectiveness of predator deterrents is difficult to test and requires rigorous study designs that meet a series of standards (Ohrens, Santiago-Ávila, et al., 2019; Treves et al., 2019). Treves et al. (2019) made suggestions in an effort to formulate more rigorous assessments of intervention effectiveness broken down in study designs categorized as “Bronze Standard”, “Silver Standard”, “Gold Standard”, and “Platinum Standard”. Where possible, our study implemented recommended steps for reducing bias and increasing inference. Participants in this study were randomly selected with experimental and control groups occurring simultaneously, and the researcher interpreting results was blinded as to which subject received the treatment or control during the initial phases of data analysis. However, we were unable to reach “Platinum Standard” due to lack of cross-over design and due to herder awareness of the treatment they were receiving because of the obvious nature of Foxlights being present on their properties (Ohrens, Bonacic, et al., 2019; Treves et al., 2019). For herders practicing nomadism that move several times a year, implementation of a cross-over design may be

impossible as time for testing the deterrent is limited before the herder relocates and introduces a myriad of other environmental variables. The herders in our study moved on average three times in a 12 month calendar year, with some moving up to four times. This limited our study duration period to three months, and the addition of a crossing-over piece to the study would have allowed for only a month and a half of data collection in each condition, likely not a sufficient time period to untangle drivers of livestock loss and functional effectiveness of the Foxlights. Future studies working with nomadic herders would need to be a long-term, likely multi-year study, to incorporate a crossing-over design, in which all herder homesteads and movement patterns are accounted for both with and without the presence of the Foxlight. This brings about its own unique challenges, but would provide all herders with a chance to be a part of the experimental group, and would assist in answering other questions that are greatly lacking in what little literature testing functional effectiveness of deterrents exists, including long-term alterations in animal behavior and habituation.

#### Role of *ex situ* experiments

More studies that test how predator deterrents alter animal behavior are needed, though *in situ* implementation poses numerous challenges. Partnerships with *ex situ* facilities may be a productive avenue for the completion of these kinds of studies. Researchers should keep in mind that these are more “laboratory conditions” as opposed to experimental trials under real-life *in-situ* working conditions (Ohrens, Santiago-Ávila, et al., 2019). However, such studies may still be able to highlight potential variation in how different species react to deterrents (Cavalcanti et al., 2012), how individuals within

a species vary (Gustavson et al., 1974), or timelines of habituation to novel stimuli (Goodyear & Schulte, 2015).

### Predator habituation

Carnivores are often sensitive to changes in their environment, and thus the introduction of novel stimuli may keep them away from an area (M. E. Smith et al., 2000). However, these effects can be temporary (M. E. Smith et al., 2000). Concrete scientific research surrounding habituation would be particularly helpful to support or ease herder concerns over the long-term success of deterrents such as noise-making, scare crows, and shouting, as previous work has shown that livestock owners warn that animals become aware and then unafraid of these methods (Ghimire, 2019). Habituation to noise and lighting has been previously found for the jaguar after a period of time (Cavalcanti et al., 2012). Habituation to Foxlights in this study is not clear.

### Economic feasibility of Foxlights

Economic feasibility is an important component to the long-term success of interventions (Treves et al., 2006). In 2019 when the Foxlights were acquired, they retailed for \$129.00USD each. Method effectiveness is necessary for policy making or large-scale funding (van Eeden et al., 2018), and thus studies like this one may aid in garnering financial assistance for distribution of Foxlights. Otherwise, herders would have to carefully consider the monetary benefit of purchasing a Foxlight. Foxlights require minimal upkeep and take very little time to install, thus drastically reducing costs associated with time and physical labor to implement, unlike other methods such as

fencing with fladry (approximately \$4,392USD per year of operation including time costs) (Davidson-Nelson & Gehring, 2010) and tall fences (approximately \$1,000USD not including time costs) (Samelius et al., 2020). Livestock prices in Qinghai Province vary depending on supply and demand but typical values for one head of yak are anywhere from \$583USD to \$667USD, and one sheep head for \$100USD to \$167USD (Lucock & Woodford, 2018). By those numbers, herders implementing two Foxlights would have to halt the depredation of one yak or two sheep to get the return on the devices purchases. However, this number of necessary livestock loss may vary based on livestock insurance policies and payouts, making the loss of livestock less expensive.

#### Herder participation in study

All herders who were asked to participate agreed to do so regardless of whether they were put into the control or experimental group, participated through the end of the study period as directed, and did not ask for compensation. More intensive interventions may be met with resistance. Research that is minimally intrusive with clear understandable objectives and outcomes are afforded more tolerance from local community members, though participation of community members in control groups may still be difficult (Treves et al., 2006). In this case, the ease of using Foxlights, relatively short interview time-spans, and previous presence of the research group in the area for past studies (Dai, Xue, et al., 2020; C.E. Hacker et al., 2020; Y. Zhang et al., 2019) may have afforded increased flexibility and willingness to participate with the research group as a third party that could provide the skills to test interventions and supplement the resources currently available to local stakeholders (Treves et al., 2006). That being said,

response bias may also impact herder responses and study conclusions in that respondents will tell the interviewer what they think they want to hear.

### Conclusions and future directions

Studies into how non-lethal interventions effect predators and other wildlife remain greatly needed (Ohrens, Santiago-Ávila, et al., 2019). Our study helped to close this gap by testing Foxlights using a rigorous research design appropriate for the study area and the herding practices of its inhabitants. Willingness of herders to implement the device, positive perceptions towards the device's efficacy in deterring predators, its ease of use, and relatively low cost suggest that Foxlights could be a useful addition to the repertoire of methods used by herder to deter predators from taking livestock. However, while functional effectiveness was also high, future study is needed to determine how diurnal versus nocturnal livestock losses are impacted by Foxlight use. A long-term study that covers a full calendar year of nomadic movements would be helpful. Because single interventions are rarely effective for long and vary by context and application (Ogada et al., 2003; Shivik et al., 2003), the use of multiple deterrents is encouraged. For example these could consist of loud sound-based deterrents (e.g., shouting herders, gunshot) triggered by motion-sensors, set to play by day or night, and placed near Foxlights with the objective of scaring off predators that more closely approaching the resting livestock.

### Author Attributions

Rodney Jackson conceived study. Rodney Jackson and Charleen Gavette provided Foxlights. Charlotte Hacker designed study methodology. Rodney Jackson, Charlotte

Hacker, Jan Janecka, and Yuguang Zhang designed interviews. Yunchuan Dai translated interviews from English to Mandarin Chinese. Charlotte Hacker, Jia Li, and Ye Li administered interviews. Charleen Gavette created map figures. Charlotte Hacker created other figures. Yuguang Zhang and Jan Janecka provided administrative support. Charlotte Hacker and Charleen Gavette performed data analyses. Charlotte Hacker wrote manuscript. Yuguang Zhang, Rodney Jackson, and Jan Janecka contributed edits to manuscript.

## Chapter 4.3

### Goal 3 – Subgoal 3

# A framework for increased inclusion of local communities in snow leopard research

Adapted from Hacker *et al.* (2020) *Pastoralism partnerships: recognizing the value of herder involvement in China's snow leopard conservation efforts*. Sustainability.  
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#### Introduction

The Qinghai-Tibetan Plateau (QTP) is one of the world's largest pastoral ecosystems (Miehe *et al.*, 2009). It has experienced rapid environmental and societal changes under the context of nearly two millennia of human-wildlife coexistence (J.M. Foggin, 2008; Schaller, 1988). The area is significant geographically, providing the headwaters for the Three Rivers, on which 40% of the world's population depends or is significantly influenced by (J.M. Foggin, 2008; Y. Liu *et al.*, 2016). The QTP has a rich diversity of unique mammalian species including kiang (*Equus kiang*), argali (*Ovis ammon*), Siberian ibex (*Capra siberica*), blue sheep (*Pseudois nayaur*), Tibetan wolf (*Canis lupus*), pika (*Ochotona* sp.), zokor (*Myospalax* sp.), woolly hare (*Lepus oiostolus*), Himalayan marmot (*Marmota himalyana*), Pallas's cat (*Otocolobus manul*), Tibetan fox (*Vulpes ferrilata*), Eurasian lynx (*Lynx lynx*), and snow leopard (*Panthera uncia*) (Jackson, 2012; Schaller, 1988). In addition to these there are a number of livestock species occupying rangelands located in extremely harsh environments (D. J. Miller, 1999). Domestic yak (*Bos grunniens*) are one of the predominant livestock as they are adapted to withstanding extremely low temperatures and high winds, and low levels of atmospheric oxygen (Q. Qiu *et al.*, 2012). Other domestics commonly kept include domestic goat (*Capra aegagrus hircus*), domestic sheep (*Ovis aries*) (Blench, 2001; D. J. Miller, 1999), horse (*Equus caballus*) (C.E. Hacker *et al.*, 2020), and camel (*Camelus bactrianus*). The presence of the pastoralist



communities who subsist on these livestock has been traced using ecological evidence as far back as 8,800 years ago (Miehe et al., 2009).

### Background information on the residents of the Qinghai-Tibetan Plateau

Most inhabitants of the QTP are Tibetans that practice Tibetan Buddhism, a religion that combines Bon and Buddhism (Shen & Tan, 2012) and views humans as part of an interactive network of living entities where kindness to all creatures earns one good karma (Feng, 2005). This even extends to protection of the soil itself, so as not to disrupt the deities housed there (Stein, 1986). The residents on the QTP have adapted and thrived for millennia (Petousi & Robbins, 2014) and developed strong environmental ties to the land as necessitated by pastoralism (Jackson, 1998; Jackson & Wangchuk, 2001; Kis et al., 2016; Schaller, 1988). Livestock owners, whose animals depend on the range, possess a broad and intricate relationship with nature that reflects intimate understanding of complex ecological processes (Coughenour, 1991; Kis et al., 2016; Scoones & Graham, 1994; Walker & Steffen, 1993). Herders are dependent on such understanding to reliably provide their livestock with forage and water, through husbandry practices passed down from generation to generation, and adapted as necessary to shifting cultural and societal norms, which communities in this region have increasingly faced in recent years (Cencetti, 2011; Kis et al., 2016).

### History of protected areas in China

Given the environmental, societal, and cultural importance of the QTP, the Chinese government has implemented policies aimed at ecological preservation following two large-scale communal farming periods in which land was tended as a collective effort, the first in 1949 and

the second in the early 1980s. This was followed by widespread development that places unprecedented pressure on China's ecosystems (J. Liu et al., 2003; Riordan et al., 2016), with particular concern toward the alarming increase in grassland degradation (Cencetti, 2011; Harris, 2010). Subsequently, policies and projects aimed at environmental protection, like establishment of the National Parks Network and grassland zoning regulations were formulated (J.M. Foggin, 2008; He, 2009; Yeh, 2005). However, these necessitated large economic investments (Yeh, 2005) that radically impacted lives of Tibetans residing in these landscapes (Cencetti, 2011). One well known example is the establishment of the 152,300km<sup>2</sup> Three Rivers Sources Nature Reserve (*Sanjiangyuan*) in May 2000, an area home to nearly 200,000 Tibetans (J.M. Foggin, 2012). *Sanjiangyuan* consists of three sectors – a 31,200km<sup>2</sup> core area where all flora and fauna are protected and no husbandry is permitted; a 39,200km<sup>2</sup> buffer area in which husbandry is permitted in limited amounts with relocation of Tibetans in only seriously degraded areas; and a 81,900km<sup>2</sup> area development zone not substantially protected from human disturbance, and where activities like ecotourism and conservation research are encouraged (Cencetti, 2011).

### The uptake of conservation science efforts in China

Funding allocation for conservation research in China has rapidly expanded since 2000; for example, National Science Foundation of China funds increased 34% annually from 2001 to 2008 with the Ecology Section seeing a four-fold increase in their budget (He, 2009). In 2014, biodiversity studies consumed large portions of the foundation's \$3.1 billion USD budget (J. Qiu, 2014). In addition, China's invitations targeting foreign biologists, starting in the 1980s, prompted fruitful collaborative efforts. While notable barriers to conservation science in China remain (Edward Grumbine & Xu, 2011; J. Li, Xiao, et al., 2016), this push for enhanced

biodiversity knowledge has proven especially beneficial toward shaping policies and protection for at-risk species.

### Importance of community involvement in conservation initiatives

Community involvement, consultation, and requests for assistance from local residents have been largely lacking in QTP research and conservation initiatives (Shen & Tan, 2012). Historically, most involvement has taken the form of “passive participation” via government mandates surrounding resettlement and “ecological migrations” (Cencetti, 2011; Patrick Bixler et al., 2015). The misconception by outside entities that rural pastoralists are ignorant and lack innovation has proven difficult to overcome (Brockington & Homewood, 1999). Because herders do not fall within the category of an ‘academic elite’, their expertise has often been dismissed as unscientific or uninformed (Cencetti, 2011). Local knowledge and informational legitimacy is often called into question due to lack of quantitative measures provided by herders in support of their statements. This is particularly relevant in relation to researchers trained in western science, whereby “facts” observed between scientists and study subjects are prioritized over the “human-nature relationships” perceived by indigenous peoples and the environments they live in (Cochran et al., 2013). However, while many Tibetans may “live off the land” utilizing native plants and animals, they may in fact possess expansive knowledge about wildlife in the area, and potentially play an important role in its protection. Such misunderstanding is unfortunate since policies shaped using information gathered by scientists and shared with government officials both directly and indirectly impacts local people (J.M. Foggin, 2012), while often excluding them from planning or decision making (Roturier & Roué, 2009). Fortunately, the value of local knowledge is becoming more widely accepted and recognized on international scales (Gadgil et

al., 1993). These relationships are particularly important for at-risk species, like the snow leopard.

### The role of local ecological knowledge in strengthening conservation science

Local residents can hold a plethora of knowledge surrounding the species and its habitat given their overlap, importance as a symbol associated with Tibetan religion (C.E. Hacker et al., 2020; J. Li et al., 2013), and intensity of conflicts surrounding livestock depredation (J. Li et al., 2013). This knowledge is applicable and can complement scientific snow leopard research efforts through the participatory approach of expert elicitation of traditional or local ecological knowledge. We use local ecological knowledge (LEK), which differs from traditional ecological knowledge (TEK), as defined by Olsson and Folke (2001)). “LEK is knowledge held by a specific group of people about their local ecosystems...it concerns the interplay among organisms and between organisms and their environment. LEK may be a mix of scientific and practical knowledge; it is site specific and often involves a belief component”(Olsson & Folke, 2001). Expert elicitation refers to structured discussions with experts concerning areas of uncertainty and are valuable in fields with limited data availability (Morgan, 2014; Schmidt et al., 2017). Indigenous people with long standing knowledge of a specific geographic area should be considered as “experts” for their possession of LEK and its value for strengthening conservation science. Successful examples include studies in Cameroon (van der Hoeven et al., 2004), Greenland (Danielsen et al., 2014), India (Singh et al., 2020), and Spain (Anadón et al., 2009).

### Goals and objectives of this paper

Although the knowledge possessed by local communities may be seen as helpful to conservation efforts, published examples and perspectives of how such research may involve or engage these communities is lacking (David-Chavez & Gavin, 2018). To help bridge this gap, and by drawing on our perspectives and experiences from our own two original snow leopard studies undertaken in the QTP, we highlight how local community residents and those who are not academically trained (1) can be involved in snow leopard research efforts (2) through a logical framework that (3) supports local capacity for environmental management in ways that draw in diverse stakeholders. Specifically, we believe the targeted elicitation of local resident knowledge can assist in goal formulation, study design, execution, and identification of representative study types. This refines sampling, helps integrate western science with indigenous knowledge, offers supplementary income to local people, increases levels of trust and communication between researchers and local stakeholders, enables greater research flexibility, and increases community-based conservation capacities.

#### Case Studies of Local Involvement in Snow Leopard Research

Cumulatively, the authors of this manuscript have conducted numerous studies surrounding snow leopards which have depended on local participation, support, and involvement. In the following two sub-sections, two specific studies are highlighted to later demonstrate how local herders and residents were involved in research efforts, as well as how their involvement was integral to the completion of each study.

##### Study 1: Temporal changes in snow leopard diet

Determination of diet is necessary for understanding roles snow leopards play as apex predators in their habitats, their dependence on livestock, and how this species adapts to large-scale landscape and distributional changes caused by climate change. New techniques harnessing the power of next-generation sequencing (NGS) have the ability to reliably discern prey DNA extracted from scat down to multispecies levels (Bohmann et al., 2011; Valentini et al., 2009). This method has been applied to snow leopards previously at local (Shehzad, McCarthy, et al., 2012) and range-wide scales (C.E. Hacker et al., 2021). One pressing question involves how snow leopard diet may change seasonally, and thus we initiated a study to repeatedly sample a site in Dulan County, Qinghai Province, China across seasons beginning in September of 2019.

#### Study 2: Determination of the effectiveness of Foxlights® at deterring predators from livestock

Livestock depredation imposes often severe financial burdens on herders, their families, and the local economy (Mkonyi et al., 2017), leading to tension between local residents and conservation and government agencies seeking to protect “at risk wildlife” (S. W. Wang & Macdonald, 2006). The killing of carnivores is neither an effective solution (McManus et al., 2015; J. R. B. Miller et al., 2016; Treves et al., 2016) nor does it necessarily align with the actual damage caused by predators (Marker et al., 2003). Unfortunately, widespread testing of the effectiveness of non-lethal deterrents is lacking (J. R. B. Miller et al., 2016; van Eeden et al., 2018), and thus we initiated a study seeking to test the functional and perceived effectiveness of the solar-powered Foxlight® [Caloola, New South Wales, Australia] as one example of a cost-effective non-lethal predator deterrent that emits a series of bright intermittent flashing colored lights, starting at dusk and continuing through the night until dawn. The device projects lights in an irregular circular fashion that are visible up to about one kilometer, depending upon the local

terrain. Foxlights® have proven effective at deterring large predators (Ohrens, Bonacic, et al., 2019), and thus the authors decided to examine their effectiveness in the QTP, particularly for deterring snow leopards from attacking livestock as a food source in Yushu Prefecture, Qinghai Province, China.

### Framework for Community Engagement via Expert Elicitation in Snow Leopard Research

There are many opportunities for local residents to be involved in research and conservation efforts, related to a wide array of skills and knowledge typically possessed by residents of remote rural areas. Figure 40 offers a framework for involving local residents in snow leopard research on the QTP, under such themes as problem identification, design, and execution as well as the outcomes associated with these efforts.

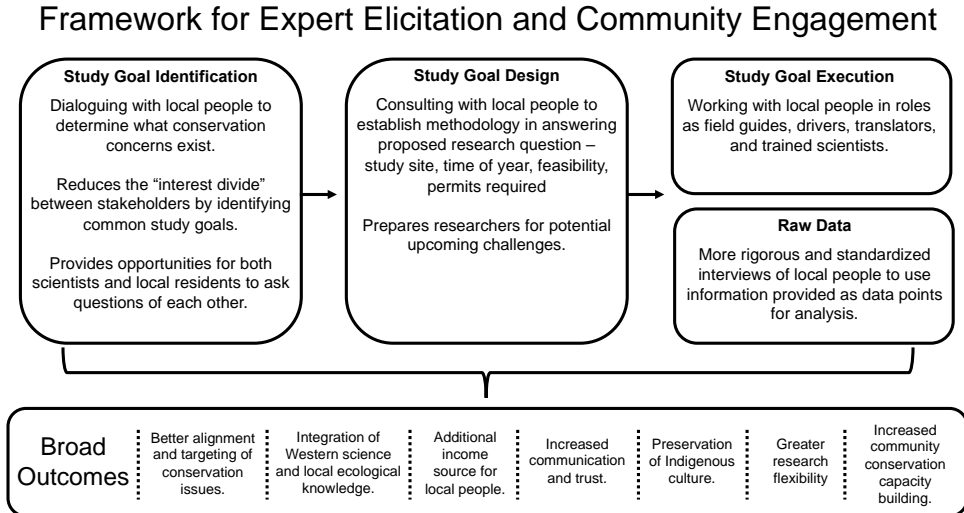


Figure 40. A framework for expert elicitation and community engagement in snow leopard research and its associated broad outcomes to stakeholders.

#### Expert elicitation in study goal identification

Communities are more likely to actively participate in environmental research if they perceive the potential for resource management providing benefits which address concerns

relating to their own livelihoods (Högger, 2000). Indeed, some advocate strongly that outside researchers should find ways of contributing to local needs in an effort to remove the “interest divide” between them (Louis, 2007; Schulman, 2007). In addition, participation in conservation efforts is likely to be greatest when a high level of engagement with the local community is present from the beginning of the proposed initiative (Evely et al., 2011). At minimum, researchers should first interact with area residents to determine the most pressing conservation challenges that they and local ecosystems or species may be facing. This approach was followed in Study 2, where local interviews revealed loss of livestock to snow leopards was high, and that depredation generally comprised the greatest concern to livestock owners compared to other cause of death. The herders’ desire to mitigate such conflicts resulting from domestic animal losses using non-lethal measures naturally following, and with local participation, the research team designed mitigation measures around testing the effectiveness of Foxlights® for deterring predators from attacking livestock. It should be noted that while much of this process involved asking local herders questions, it also offered a platform for them to question researchers regarding the study, scope, intent, and outcomes (Baumgartner et al., 2004).

### Expert elicitation in study design

The QTP consists of four broad ecosystem types – montane forest, alpine shrub/meadow, alpine steppe, and alpine desert (S. Wu & Feng, 1992; B. Zhang et al., 2002). Within these are several ecoregions that are unique to only the QTP and hold a rich biodiversity of flora and fauna (B. Zhang et al., 2002). Extensive LEK of these smaller ecoregions is helpful for selecting sampling locations that are feasible to access, as well as the best times of year for undertaking research activities along with potential logistical and other research constraints. For example, in



Study 1, answering questions surrounding dietary changes over time required continuous scat sampling along fixed transect lines over all seasons. This requires that transect lines be accessible on a year-round basis, and not be subject to flooding or high levels of snowfall that would prohibit scat sample collection. Thus, local residents were consulted to help determine which transect lines should be selected. With the exception of some heavy snowfall during certain winter months, the transects selected remained largely accessible during the winter, enabling scale collection across a temporal gradient while controlling for spatial sampling requirements.

#### Expert elicitation for study execution

##### *Field guides*

The QTP covers 2.5 million km<sup>2</sup> (B. Zhang et al., 2002); thus LEK from area residents is vital for successfully navigating this vast landscape. LEK regarding the surrounding geographic features, local flora and fauna identification, or how to reach specific GPS coordinates and study sites plays a critical role in ensuring both study success and safety. Beyond the traditional field guiding role, local people are usually knowledgeable about nearby towns and can assist in many tasks, from procuring food to locating the nearest medical facility in case of an emergency. During Study 1, we found field guides were essential for finding suitable sampling areas and then navigating the transects. They were well informed on routes through the harsh terrain. They were also key during prey counts and the collection of carnivore sign data, species identification of pugmarks and bird calls, often spotting wildlife from long distances. For Study 2, field guide knowledge greatly facilitated locating herders suitable for the deployment of the Foxlight® devices while also maintaining the planned spatial requirements of sampling.

### *Drivers*

The QTP has undergone rapid societal, socioeconomic, and infrastructural development changes starting in 2000 with China's campaign to "Open Up the West" (J.M. Foggin, 2008). This has included the construction of extensive road networks (X. Xu et al., 2006), with greatly decreased travel times from population centers (Gao et al., 2019). Local people are familiar with all types of roadways and their conditions within reach of their homes, vital information for accessing field sites. Some were familiar with the greater QTP landscape, places accessibly only by all-terrain vehicles. Those holding a valid driver's license and with proven driving skills are widely sought after by both national and international researchers. Driving conditions in the QTP's remote mountains require experienced drivers to ensure the wellbeing of the entire field crew. We used the same experienced driver for both studies, who was integral for navigating complex and often unmarked roadways encompassing a combination of pavement, dry river beds, dirt tracks, and fragile grasslands amongst a backdrop of steep terrain and, at times, unpredictable weather conditions. The driver proved his worth in an emergency by quickly getting a field team member suffering from severe altitude sickness to the nearest hospital, where this person was able to recover from a potentially life-threatening condition.

### *Translators*

One of the greatest challenges researchers face is recruiting suitable translators for ensuring interviews are accurate and largely free of misunderstandings when involving different language speakers (Mercer et al., 2008). The majority of QTP pastoralists are Tibetan, although smaller ethnic groups of Kazak and Mongol nomads are also present (D. J. Miller, 1999). Local

dialects may differ, but Tibetan is the ubiquitous spoken language (D. J. Miller, 1999). Mandarin Chinese has been promoted as China's national language since 1956 (Rohsenow, 2004; M. Zhou, 1999). Children living in minority regions of the country start learning Mandarin in state schools from the third grade onward (M. Zhou, 2004); however, many Tibetans have not received a formal education, nor do all children attend state schools (Y. Wang & Phillion, 2009). This scenario is changing as the current generation has greater opportunities for education, given an increased emphasis on fluency in Mandarin Chinese for improving their job prospects (Nima, 2001). Thus, while many QTP native residents are not proficient in Mandarin, there are skilled community members able to bridge this gap and communicate seamlessly between these two languages, and thus better positioned to assist researchers in different ways: First, they assist in communication with other residents along with translating information in Tibetan records and historical documents. Second, they are able to gather information specifically related to study execution, such as directions to field sites, or arranging hotel accommodations and food purchases. More importantly, they may translate information from scientists, sharing knowledge with local people on diverse topics from conservation education to study outcomes and scientific results. However, researchers need to be aware that the "local elite" status of some translators may introduce their own bias or fail to fully disclose information from respondents (Brittain et al., 2020). Study 2 mentioned above would not have been possible without a reliable local translator, for it required the understanding and consent of participants, LEK on surrounding area carnivores, and instructions for the proper placement and use of solar-powered Foxlights®.

### *Scientific assistants*

With proper training, area residents can play very useful and active roles in research. This may include wildlife observation, site monitoring, data recording, and camera trap servicing and noninvasive scat sampling collection for genetics and dietary studies (Janečka et al., 2008). In Study 1, local residents and the driver were trained in scat sample collection techniques. Such training was taken as part of an effort to establish a long term study field site whereby community participation was perceived more as “Collegial” and “Indigenous” rather than simply “Collaborative” (David-Chavez & Gavin, 2018). A trip in September and December of 2019 yielded summer and fall data. With researchers outside of the QTP facing restrictions because of COVID-19 starting in January 2020, and the low priority given to field work because of the outbreak, scat collection and monitoring was suddenly dependent upon local community members. These trained project participants were able to complete collection of scats from all sampling locations by June 2020. This was only possible because of close relationships built between the scientific researchers and local partners over the preceding two years, as well as the project’s investment in field training and sharing of knowledge leading up to the COVID-19 event.

### Expert elicitation for raw data

While discussion of local residents to gain knowledge about conservation concerns, terrain, and the like are typically informal and constitute open dialogue, other interviews are systematically designed to gain targeted information to better understand habitat status and human-wildlife coexistence. The responses from the interviewees themselves serve as raw data for subsequent analysis. Several previous studies have interviewed local QTP residents to gather

baseline information surrounding conflict between animals and humans, and how local attitudes and perceptions towards wildlife are shaped (Alexander, Chen, et al., 2015; C.E. Hacker et al., 2020; C. Li et al., 2015; J. Li et al., 2013; A. Xu et al., 2008). This kind of approach was taken in Study 2 during the initial phase of identifying areas of conservation concern held by the local community. In addition to open dialogue and the opportunity for questions, a one page interview was administered to identify key factors driving positive versus negative attitudes towards snow leopards (C.E. Hacker et al., 2020). In addition, herders ranked snow leopard abundance on a Likert-type scale a 5 out of 7. Scat collections followed by genetic analysis done in the same area identified six snow leopard individuals in an approximately 30 km<sup>2</sup> area (Y. Zhang et al., 2019), helping to corroborate herder responses and demonstrating their important role in providing supporting information on the species' distribution and relative abundance. Similarly, herders interviewed by Farrington and Tsering (2019) (J.D. Farrington & Tsering, 2019) in the Chang Tang region of Tibet, China claimed snow leopards were wide-spread, a contention supported by sign surveys conducted along transects in the same area. Given this past corroboration, it was elected in Study 2 to rely on herder reports of carnivore presence within a 2km radius of their homes to identify potential species responsible for livestock loss in the upcoming three month study period testing the solar powered Foxlights<sup>®</sup>. Continued data point collection from herders via monitoring, signs surveys, and observations over time will be of great value in examining long-term species population trends.

### Outcomes of Local Participation and Inclusion

It is only fair and ethical that community members living in these habitats become more substantial stakeholders for outcomes resulting from wildlife management and economic

development. Figure 41 shows how local herders and residents are intrinsically linked to a wider system of multiple key players who must work in concert for the persistence of fragile landscapes, rare species and unique cultures found within them. The inclusion of local residents in research and conservation is imperative to the success of environmental protection efforts, and provides multi-pronged benefits for all stakeholders involved.

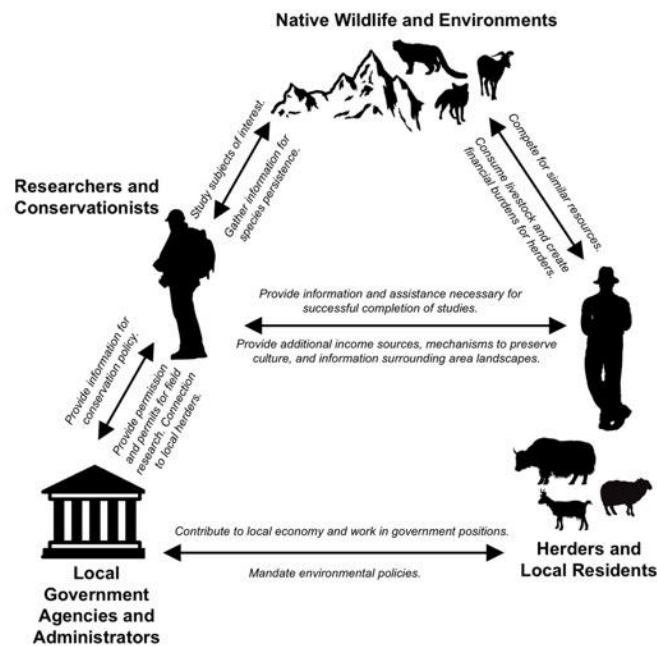


Figure 41. Relationships between stakeholders involved in conservation science and practice.

### *Identifying locally relevant concerns*

Outside scientists are not always sufficiently aware or cognizant of all conservation issues within an environment. Further, what an outside scientist may perceive as a threat to the ecosystem or livelihoods of local people, the residents may view as a “way of life” or beneficial activity (Mercer et al., 2008). Dialogue, including back and forth consulting with local residents will help delineate urgent areas of concern. Such process is vital to building the trust needed to achieve mutual understanding and consensus for reaching a common goal.

### *The integration of western science and LEK*

Western conservationists have been criticized for ignoring the more circular holistic approach to biodiversity protection that is deeply imbedded in many traditional cultures. Just because scientific knowledge has held a long-standing centralized role in advanced societies does not mean other knowledge systems cannot contribute in a meaningful way (Mazzocchi, 2006). Modern science has much to gain from the more holistic, qualitative, and geographically-focused approaches taken by local communities in regards to environmental preservation (Mazzocchi, 2006; Mercer et al., 2008). Unfortunately, LEK has historically been repressed (Laws, 1994). Integrating the two avenues of understanding is complex, but frameworks for integrating western science and indigenous knowledge have been previously proposed (Mercer et al., 2008). Such integration has been met with continued resistance because of the notion that these two knowledge systems are “too different” from one another (Nadasdy, 1999). For this and other reasons, western science has remained dominant and overwhelmingly the main force in wildlife conservation.

### *Additional income sources*

Most researchers employ local residents on an informal basis. Nonetheless, even modest and temporary economic incentives induce a positive response for conservation and research from local people (Y. Liu, 1993). Residents who assist in the scientific process by contributing their LEK should be fairly compensated for their involvement, with payments adjusted based on the amount of time and the provision of in-kind, unique training. There is an urgent need to diversify sources of income, especially for remote mountain communities. One successful example of supplemental alternative income is that associated with the international trophy

hunting program in Dulan County, Qinghai Province, China (Y. Liu, 1993). Herders are provided with opportunities to work as hunting guides, game guards, and were able to lease their horses to trophy hunters. These prospects helped reduce poaching and increase local support for wildlife protection measures (Y. Liu, 1993). Lowering financial dependence on animal husbandry is thought to help reduce the number of livestock owned and resulting grassland degradation (Du, 2012), and the frustrations stemming from conflicts between humans and predators (Dai, Xue, et al., 2020), and limit the pursuit of local wildlife for illegal trade. Expert elicitation of LEK as a common practice in research efforts may be able to provide similar benefits.

#### *Increased communication and trust*

The wide disconnect between scientific research and management is all too common in wildlife studies (Edward Grumbine & Xu, 2011). All stakeholders need to come together to meet mutual needs; however, combining interests that are sometimes very diverse or even conflicting, often complicates the implementation of wildlife policies and efforts across an otherwise cohesive geographic area (Taub, 2018). In addition, distrust as a result of historical repression and exploitation by outsiders on indigenous communities can pose a large barrier (Burnette & Sanders, 2014). Designing and carrying out studies that engage area residents provides opportunities for communication, bridging such gaps with ongoing dialogue supporting social cohabitation, and enhancing knowledge exchange (Mazzocchi, 2006). As researchers and local entities work together, an understanding of findings and trust amongst members of both parties will usually surface through conversation, cooperation, and cultural exchange. This leads to a research project that is better integrated for supporting conservation that harmonizes the aspirations of local communities as well as ecological sensitivities (Jackson & Lama, 2016). It



should be noted that trust building with indigenous people in conservation is complex, with prominent factors such as power asymmetry, risk, historical oppression, predictability, commitment, social distance, and reciprocity playing large roles in the formation of collaborative partnerships (Burnette & Sanders, 2014). However, trust in conservation governance is paramount. Previous work has shown that compliance with conservation policy is more likely if local people living under the policy framework trust the personnel involved than if they were provided with social or economic incentives (Viteri & Chávez, 2007).

### *Preservation of Tibetan culture*

Cultural exchange coupled with open communication between scientists and local herders may also aid in preserving the practices, history, and beliefs of Tibetan and other ethnic minority traditions while simultaneously cementing cultural identity and pride for younger generations (Shen & Tan, 2012). Researchers are expected to take diligent field notes, which may include records of local flora and fauna natural history, weather patterns, and the anthropology of the community they are working in. A written record of the Tibetan traditional knowledge and practices in a more accessible and widely used language, like English or Mandarin Chinese, is imperative given that Tibetan traditions are rapidly eroding as the younger generation forgoes herding for urban-based vocations (Riordan & Shi, 2016). These pressures and societal shifts experienced by younger Tibetans can create an identity paradigm, whereby individuals incrementally replace their unique cultural identify with ones conforming to changing societal pressures in order to be “ordinary” and to succeed (Yi, 2008). Exchange of information between local stakeholders and researchers may instill a sense of pride when local residents discuss their culture and daily herding practices for dissemination via documentaries (Shen & Tan, 2012) as

well as through peer reviewed literature, pamphlets, photography, and other literary or visual means.

Snow leopard conflict studies typically involve interviews addressing current and historical herding, religious, and cultural practices, as well as community demographics (Alexander, Chen, et al., 2015; C.E. Hacker et al., 2020; C. Li et al., 2015; J. Li et al., 2013; A. Xu et al., 2008). These surveys also provide snapshots of Tibetan society for cultural preservation and informing future generations. One important challenge is that most studies typically depend on the “heads of the household,” and thus predominantly represent the perspectives of men. For example, 91.8% of the respondents in Hacker et al. (2020) (C.E. Hacker et al., 2020) were males between ages 21 and 73. Men similarly dominated analyses at 86% of those interviewed in Li et al. (2013) (J. Li et al., 2013), and Li et al. (2015) (C. Li et al., 2015) opted to remove 9 of the 295 interviews completed because the respondents were females. Interviewing females and younger individuals aged 16 to 30 would be highly beneficial for documenting more holistic views of Tibetan lifestyles. Their inclusion would help further advance snow leopard–habitat–human understanding by uncovering potential differences between the sexes along with changing attitudes within the up-and-coming generation (Alexander, Chen, et al., 2015). Expanding the diversity that LEK is based upon would address important knowledge gaps surrounding local perceptions of conflict between humans and snow leopards, while ensuring more complete buy-in for any proposed conservation actions.

#### *Greater research flexibility*

As noted, researchers face numerous problems while working in remote regions, including study or sampling permit acquisition, visas and travel logistics, unpredictable weather

patterns, periodic vehicle breakdowns, theft of supplies, safety of the field team, obtaining food and lodging, among others. Offering local community members basic training and employment as valued team members generally brings immeasurable benefits, including enabling the study to continue after researchers have left. Local people are able to support vital research tasks on more flexible time scales given their residency and proximity to the study area, thus lowering project costs while expanding the study window.

#### *Increased community conservation capacity building*

Tibetans harbor special appreciation for the natural world emanating from their long-standing Buddhist beliefs (Shen & Tan, 2012). However, willingness to tolerate predators often declines as conflicts between humans and wildlife grow (Tsering et al., 2013), a trend problematic for the success of conservation efforts (Jackson & Wangchuk, 2001). Herder involvement in ecological research, sustainable economic endeavors like well-managed trophy hunting, and stronger protection of endangered taxa offers avenues for personal investments. If appropriately designed, such initiatives engender positive attitudes towards wildlife, hopefully leading to lower instances of behaviors detrimental to conservation efforts, such as retaliatory killing. Education is an important force for promoting positive attitudes towards wildlife. For example, Hacker et al. (2020) (C.E. Hacker et al., 2020) found that herders with additional years of formal education were more likely to have positive attitudes towards snow leopards. Participation in research studies provides valuable opportunities for gaining deeper understanding of ecosystem functions and basic biological principles that local people may be unfamiliar with. It will also likely lead to the recruitment from local communities of the next generation of conservationists and wildlife biologists.

## Other Considerations

### *Ethical Review Boards and IRBs*

Studies involving LEK may be subject to laws aimed at protecting local people's rights from poorly designed or even unscrupulous research endeavors. Institutional Review Boards (IRBs, also known as Ethical Review Boards) serve to help ensure compliance and protection for these individuals and their communities. Therefore, avenues available for obtaining IRB exemptions and/or permissions should be carefully considered by all researchers in order to abide by these important and necessary regulations. It is paramount that researchers be engaged with IRBs and not view the obtainment of IRB permission for research as a simple matter of bureaucracy for which consent from study participants can be easily secured (Brittain et al., 2020). Understanding of consent varies by culture, and should be recognized as a continuous negotiation with either party able to cease if they wish to do so (Freeman et al., 2009). Forms or contracts should be provided in the native language, shared or distributed with all participants; scientists should take it upon themselves to conduct their own research risk assessments to ensure that ethical, physical, and mental implications of all stakeholders are considered (Brittain et al., 2020).

### *Political ecology*

The term "political ecology" has a number of definitions (Forsyth, 2003). Here, the phrase is used in reference to the combination of ecological concerns with political economy (i.e. society-environment relationships). Indigenous people have faced resettlement as the government of China expands infrastructure and its suite of protected lands (Du, 2012). The

rationale for resettlement has been largely driven by government goals surrounding grassland recovery and poverty alleviation (Shiyuan, 2009). However, permanent herder relocation can lead to reduced quality of life due to novel urban, social, and financial stresses (Du, 2012). In areas where local residents are not heavily dependent on livestock the provision of alternative income sources such as scientific and conservation initiatives may not require resettlement on economic or environmental management grounds. The removal of local people from an ecosystem can greatly hinder both restoration and conservation efforts. For example, successful operation of the Bird Island Waterfowl Refuge in Qinghai Province was heavily contingent upon participation by local community members, but after people were resettled the program failed. This was at least partially attributed to the loss of their vital partnership (Y. Liu, 1993).

#### *Dissemination of results*

Sharing results from scientific studies, especially involving LEK, is in the best interest of environmental protection and long-term stewardship. Negative extraction, in which knowledge from local people is taken but not shared with them (Barber et al., 2014), breaks the relationship of participation and trust. Dissemination of findings promotes transparency along with educating local residents in aspects of environmental management, helping illustrate how those involved can bring added value to their communities. However, research findings should be shared appropriately and in a considerate manner, particularly if they may cause embarrassment, create hostility toward other stakeholders, or threaten reputations (Brittain et al., 2020). Research findings that are written for publication should adequately recognize local residents and other non-academic collaborator, preferably via co-authorship (Sarna-Wojcicki et al., 2017), or at minimum, highlighted in the acknowledgements section of the published document. Information

also needs to be distributed in non-scientific articles, news, pamphlets, and in lectures as most locals will not have access to scientific publications.

### *Considerations for identifying experts*

Expert elicitation can be highly informative and valuable when done correctly, however, if it does not involve truly knowledgeable “experts”, it can lead to maligned policies and research findings (Morgan, 2014). Some studies harnessing LEK may be stricter than others in this definition. For example, researchers in Pima County, Arizona studying the pygmy owl (*Glaucidium brasilianum*) only considered indigenous persons as “experts” if they possessed LEK and an understanding of scientific language. The caveat of requiring previous scientific training and literacy left out a number of local people (Schulman, 2007). In another example, some studies may only consider elders as holding enough LEK expertise giving their perceived wisdom and experience, thus excluding the LEK that younger generations may possess (Mercer et al., 2008). We echo that in any case, experts should be carefully selected by individuals leading the study effort with transparency in study methodology as to how experts were selected (A. Davis & Wagner, 2003). As a general note of caution, research studies should not be solely rely on LEK and/or herder information as key data sources for solving complex challenges related to ecosystem management but should integrate it with their research approach (Fernández-Giménez & Estaque, 2012).

### *Addressing bias*

Scientists need to take necessary precautions to prevent respondents from unduly influencing, socially biasing or bringing other perceptual data errors into play (Fisher, 1993).

For example, interview respondents may perceive the need to provide information that supports the project's expected outcomes or is in alignment with the political interests of local leaders. Gender and age are pervasive sources of potential bias, making it important to consult all segments of the targeted society, however, this may be difficult to achieve.

## Conclusions

Coordinated efforts to include local community members in research and their value to the realization of conservation goals is now widely accepted (DeCaro & Stokes, 2008). Our understanding of the snow leopard has greatly increased over the last 25 years (Fox & Chundawat, 2016), yet many aspects of this remarkable felid remain understudied and data deficient (Alexander, Zhang, et al., 2016b). Pressing conservation issues that require attention include habitat distribution, population assessments, predator-prey relationships, human interactions, and conservation policy impact (Alexander, Zhang, et al., 2016b). Addressing these knowledge gaps and range coverage for snow leopard surveys in China is a particularly massive endeavor that requires large-scale coordinated efforts by scientists, government officials, and herders working cooperatively and in tandem. One important way of fostering such relationships is to deploy a framework that includes local residents as active, employed participants in research and conservation efforts. Such interweaving of stakeholders could also provide alternative and additional income sources not directly related to animal husbandry. In addition, if young community members are engaged it may be the spark that will inspire some of them to become the next generation of conservation biologists. Such initiatives serve to highlight the added values of natural resources affected by pastoralists, to provide the needed open communication and information sharing avenues for improving conservation outcomes, findings,

and applications in accordance with traditional values – and to cement cost-effective opportunities for expanding knowledge on snow leopards.

#### Author Attributions

Charlotte Hacker conceived paper concept. Charlotte Hacker wrote manuscript and designed figures. Yuguang Zhang, Diqiang Li, Jan Janecka, and Rodney Jackson provided edits to manuscript. Diqiang Li provided administrative support.



## Chapter 5

### Concluding remarks and future directions

Conservation biology has been described as a “crisis discipline” (DeSalle & Amato, 2004). Biologists have to make rapid decisions with limited amounts of information. Thus, it is imperative that the information used is accurate and that tools are formulated to help quickly address issues reliably (DeSalle & Amato, 2004). This has been challenging for the snow leopard (*Panthera uncia*), as its elusive nature, occupancy of harsh environments, and extraordinary camouflage have proven difficult obstacles for scientists (Fox & Chundawat, 2016). Fortunately, the advent of noninvasive molecular genetic approaches coupled with the greater inclusion of local communities and their knowledge across the conservation science discipline, has elucidated a large proportion of previously unknown information. This dissertation sought to expand on current knowledge by applying noninvasive genetic approaches to a wide breadth of scientific questions, and by using information garnered from those approaches to further study the co-existence between snow leopards and humans.

#### Local snow leopard population estimates

Local population estimates for snow leopards are lacking, particularly with regard to their nearly 2 million km<sup>2</sup> range and in comparison to other big cat species (Jackson et al., 2010; Jan E. Janecka et al., 2020). The examination of five snow leopard populations in China and one in Southwestern Mongolia in this work allowed for greater understanding of the current status of the species in their respective areas. However, it also elucidated how methodologies may impact results and supported the notion that direct comparisons between snow leopard populations are

difficult. Regardless, knowledge of fine-scale metrics can greatly benefit the formulation of conservation action plans that are tailored to smaller regions and provide a starting comparison point for the assessment of snow leopard populations through time. At present, population trends of the species are largely unknown (McCarthy et al., 2017). The ability of local population estimates derived from noninvasive surveys to contribute towards this gap makes them an important continual effort, in addition to increased accuracy in the number of individuals range-wide.

### Snow leopard phylogeography

Just as noninvasive genetics can be applied to smaller regional areas, it can similarly be applied to larger geographic areas. Range-wide standardized efforts for snow leopards are a massive undertaking, but necessary for understanding top listed research needs, such as taxonomy (McCarthy et al., 2017). The phylogeographic assessment of snow leopards can offer important insight into population structure and history, the presence of demographic barriers, and characterization of overall genetic diversity (Avice, 1989; O'Brien, 1994). Snow leopards were lacking such an assessment until recently, which found evidence that the species was not monotypic, but rather consisted of three primary genetic clusters that could be elevated to subspecies status (Jan E. Janecka et al., 2017). However, difficulty in collecting scat in specific parts of the snow leopard's range led to sampling gaps that left barriers between the Western and Central genetic clusters unresolved (J. E. Janecka et al., 2018; Senn et al., 2018). Efforts in this work to address those shortcomings included the addition of noninvasively collected samples from the proposed Central and Western genetic clusters, as well as an area in between the two. Upon repetition of the analyses used in Janecka et al. (2017), conclusions from the original work

remained largely unchanged, though insights into the break between the Western genetic cluster with respect to the Northern and Central genetic cluster were gained. The addition of samples to the Western genetic cluster removed the previously seen admixture between the Western and Northern genetic clusters, indicating greater divergence than originally thought. The gap in between the Western and Central genetic clusters continued to support the Himalayas as being the primary barrier to gene flow. However, observed admixture between the Western and Central genetic clusters in individuals belonging to the newly added samples indicates that genetic exchange in some capacity occurs. The break between the Northern and Central genetic clusters also remained stark, further supporting the intensity of the Gobi Desert as a barrier to gene flow. This prompted additional investigation using genetic information collected noninvasively from scats belonging to snow leopards in Mongolia and northwestern China.

#### Snow leopard landscape genetics

Landscape genetics allows for the understanding of landscape connectivity, ultimately informing barriers to gene flow. Snow leopards occupy fragmented habitats owing to the mountain landscapes they live in (Robinson & Weckworth, 2016) and possess low levels of genetic diversity (Chapters 2.1 and 2.2). This bolsters potential for negative outcomes associated with isolated populations (Slatkin, 1987). In this research, the assessment of microsatellite data for 64 individual snow leopards from Mongolia and 35 from China revealed little to no connectivity between the two populations. This supports the established notion that the Gobi Desert serves as a substantial barrier to gene flow (Chapter 2.2). Within each country, genetic diversity was greatest in Southern Mongolia and lowest in Western Mongolia. This is surprising given the isolated habitat patches in Southern Mongolia. However, snow leopards are able to

traverse long distances, and these patches may not be as much of an impediment as initially rendered. While Western Mongolia has more continuous habitat, data suggests that it may be more geographically separated from the western regions of the snow leopard's range than initially proposed. Thus, snow leopards in Western Mongolia may not benefit from genetic exchange with other populations in western range countries. This is further supported by higher misassignment rates which indicate that movement is more often southeast, rather than northwest. Within China, Middle Qinghai had lower genetic diversity than Northern Qinghai/Gansu. Genetic structure analyses showed mixed results. One showed a unique pattern in which seven snow leopard samples from China comprised their own unique genetic cluster. Another analysis showed two separate genetic clusters, with admixture evident and in alignment with misassignment results. The pattern in the first analysis could be due to temporal differences in sample collection, which spanned nearly ten years, too many samples from one specific region, or a unique set of alleles in one population. Regardless, it appears that corridors between Middle and Northern Qinghai/Gansu allow for gene flow. These natural corridors should be protected, as should those observed for snow leopards in Mongolia. It is not recommended that efforts for connectivity between these two populations be made, as snow leopards from China and Mongolia show little to no genetic exchange in either this or the phylogeography dataset. In addition, such efforts would impact other species, including snow leopard prey.

#### Snow leopard molecular dietary analysis

DNA metabarcoding to determine the prey items of predators showcases another application of noninvasive genetics that has the potential to transform our knowledge of species like the snow leopard. Snow leopard diet has been relatively well studied, though it has been

hindered by tedious error-prone techniques and lack of genetic confirmation of scat origin. In regards to DNA metabarcoding, it has been hindered by lack of divergence between wild versus domestic goat and sheep species with the traditionally used genetic marker, MT-RNR1 (Shehzad, McCarthy, et al., 2012). This study was able to design primers to amplify a segment of MT-CO1 which was able to discern wild versus domestic goat and sheep species across six regions of snow leopard range. This helped elucidate differences in snow leopard diet using one comprehensive method for direct comparison between areas. Results revealed that snow leopards prefer medium to larger-bodied hoof stock, but that these species vary in accordance to regional availability. The presence of birds and small mammals help support the idea that snow leopards opportunistically feed on these species in between larger kills. A wide breadth of livestock in diet was also observed. The degree to which livestock was found in snow leopard diet in Pakistan and Mongolia eluded to a high dependency on domestic animals, potentially indicating an increased risk for retaliatory killings, negative attitudes towards snow leopards, and increased pressure on insurance companies to mitigate burdens associated with livestock loss for herders.

#### Predator guild diet across the Qinghai-Tibetan Plateau

While snow leopards show dietary differences across their range due to prey availability and accessibility, they may also be influenced by competition with other sympatric carnivores. One method by which to avoid this competition is through dietary niche-partitioning (Schoener, 1974). In this study, the diets of carnivores sympatric with the snow leopard were investigated across three different regions on the Qinghai-Tibetan Plateau in China. Irrespective of region, blue sheep made up the majority of snow leopard diet. However, the snow leopard showed a wide dietary breadth, negating the notion that they are dietary specialists per say. In general, pika

and blue sheep were collectively important prey items, warranting special attention to their protection. Livestock dependency did not differ among the three sites, and was relatively low. Tibetan wolves were responsible for more livestock loss than any other carnivore examined. This suggests that snow leopards may not be a primary target of conflicts between herders and wildlife, and rather that wolves may require more active attention. Snow leopards had the greatest dietary overlap with Tibetan wolves, though this overlap was not significantly higher than expected. It is likely that other mechanisms such as spatial or temporal niche partitioning allow for the coexistence of the carnivore species examined in this study, particularly for Tibetan wolves and snow leopards. However, landscape wide shifts brought on by climate change may drastically change this dynamic and thus continued efforts within study sites are of grave importance. Smaller temporal changes, such as season, can also shift predator guild functioning.

#### Predator guild diet analysis across seasons

A one-year study in one of the three aforementioned study sites was conducted in an effort to investigate temporal changes in predator guild diet, similarity, and niche overlap. These encompassed four seasons (March, July, September, and December). Diet was found to not significantly differ across months when considered overall, though some key differences were evident. The only predator which could be assessed for all four seasons was the Tibetan wolf, speaking to the difficulty in amassing the sample sizes necessary for studying carnivore diet. However, sampling completeness analyses indicated that anywhere from 33.3% to 100% of possible dietary items were captured for each species. Thus, limited sampling may be able to provide reliable information about diet in some cases. The only detectable difference was found for the red fox between the months of March and December. The Tibetan wolf showed the

highest degree of niche overlap with the greatest number of other carnivore species, including the snow leopard, red fox, and Tibetan fox. The highest degree of diet overlap was between the snow leopard and Tibetan wolf in March. Blue sheep and pika were found to be an important food source year round, though the emergence of Himalayan marmot post-hibernation showed an influx in detection frequency. Livestock were found in diet year round, but were highest in July. Increased depredation of livestock during a particular season may require an increase in herder support. However, the loss of livestock during all four seasons showcases that herders must regularly protect their animals to avoid the burdens associated with losing domestic animals.

#### Assessing drivers of herder attitudes towards snow leopards

Livestock depredation can have poor outcomes, and may prompt negative attitudes towards species like the snow leopard. This work aimed to better understand how these attitudes are shaped via an interview format of local herders. Findings aligned with previous work in that attitudes towards snow leopards were generally positive. Fewer animals lost coupled with more years of formal education were found to drive positive attitudes while fewer animals lost, fewer years of formal education, and less importance of snow leopards attributed to religion were found to drive negative attitudes. These findings are intuitive with the exception of fewer number of animals lost contributing to negative attitudes. However, this is likely due to an oversimplification of the degree of burden experienced when an animal is predated. Frustration can greatly vary depending on the age, sex, and condition of the animal. Thus, untangling the role of livestock loss in shaping attitudes will require much more detailed assessments of herder experiences. However, the knowledge of livestock loss impacting negative attitudes, and the

degree of livestock loss experienced in the previous five years warrants efforts to deploy and test the effectiveness of non-lethal predator deterrents.

### The functional and perceived effectiveness of Foxlights

Predator deterrents are an important component to building and maintaining positive coexistence between herders and carnivores. In this study, the functional and perceived effectiveness of the Foxlight were determined. A total of 12 loss events were recorded across the three month study, resulting in the deaths of 24 animals. Livestock loss events and the number of animals lost were found to be significantly lower for herders with Foxlights than for those without. However, functional effectiveness does not appear to be as straight-forward as it would seem. Herders without Foxlights experienced significantly more livestock loss events those with Foxlights. However, ten of the 12 loss events occurred during the day when the Foxlights were presumably not working to deter predators. Overall, it is not surprising that most loss events occurred during the day as 11 of the 12 loss events were caused by wolves, who are diurnal. Foxlights in and of themselves as a novel item may be deterring wolves during daylight hours, or Foxlights may have longer lasting impacts on avoidance behavior that continues into the following day from the night before. Longer term research with higher sample sizes will be necessary to better pinpoint potential reasons behind the findings herein. Only one loss event was from a snow leopard, showing that they play a much smaller role in livestock loss. While functional effectiveness of the deterrent had difficulties in interpretation, perceived effectiveness as reported by herders was high. All ten participants stated that they would continue using the Foxlight and eight of them attributed a decrease in livestock loss in comparison to the same time period the previous year due to having them. Herders were further consulted for feedback on



improvement, which included adding more Foxlights, making the Foxlight brighter, and providing a noise component.

#### The importance of local involvement in snow leopard research efforts

Consulting herders is one avenue whereby the conservation of snow leopards has greatly benefitted, though there is room for expansion. The aforementioned studies in this dissertation were all dependent on the deployment of a constructed framework with outlined opportunities for the involvement of local communities. This ensured that local residents were interwoven into the network of conservation stakeholders. There are numerous benefits to such planned efforts, and the future success of snow leopard protection efforts will depend on the local and traditional ecological knowledge harbored by communities sharing habitat with them. This will be especially necessary given the differences in genetics, diet, co-existence with sympatric carnivores, and belief systems within the snow leopard metapopulation across their range as revealed by this dissertation.

#### The future of noninvasive genetics in studying snow leopards

Though the species remains largely outpaced in relation to other big cats of the *Panthera* genus, noninvasive genetic studies surrounding the snow leopard are gaining momentum. As demonstrated in these collective works, use of noninvasive genetics can cascade into several studies by providing necessary information for targeted research efforts and initiatives. Future work will require continued sampling efforts, especially in data deficient areas of the species' range. Sampling of carnivores living in snow leopard habitat would also be of continued value given their role in shaping the ecosystems that snow leopards reside in. Long term research

projects should be more regularly established, with the notion that local communities are of great value in making such efforts successful.

The hindrance of low quality and quantity DNA in scat will continue to be a large barrier in gathering information *via* noninvasive genetics, and methodological advancements that prevent such errors are needed. Another barrier includes the inability to combine microsatellite datasets. These short-comings have led to resistance in more fully adopting noninvasive genetics as a standard practice in snow leopard research despite the wealth of information it has provided. Next-generation sequencing methods that examine single nucleotide polymorphisms (SNPs) will provide more concrete datasets that have greater capacity for inter-research group collaboration. Unfortunately, scat remains a challenging medium to work with for SNP analysis, and future research efforts will have to overcome this.

Lastly, noninvasive genetics work on this species will require the immersive involvement of in-country scientists as laboratories and research stations are established. However, some methodologies may take longer to come to fruition. For example, while DNA metabarcoding is useful tool in determining diet, it is prohibitively expensive and requires extensive laboratory training. In-country labs and scientists may not be able to apply this technique without the assistance of funders and trained researchers. In the interim, microsatellite analysis remains a useful and more accessible method for using genetic data to inform conservation management decisions.

## Appendix I: Sampling Sheets for Local Population Metrics

Appendix I: Supplemental Material 1. Information for samples collected in the Chang Tang region of the Tibet Autonomous Region, China.

Date	Collected By	GPS Model	WayPointID	Latitude	Longitude	Scrape	Sample ID	Remarks
10/31/16	万志康 梁旭昶 塔杰	62s	22	31.55028	88.09113	No	20161031-WZK-022	
10/31/16	万志康 梁旭昶 塔杰	62s	25	31.52279	88.10650	Yes	20161031-WZK-025	
10/31/16	万志康 梁旭昶 塔杰	62s	28	31.52144	88.11179	Yes	20161031-WZK-028	
10/31/16	万志康 梁旭昶 塔杰	62s	29	31.52091	88.11267	No	20161031-WZK-029	
10/31/16	卞晓星 多杰加 徐雯靓	62sc	1025	31.54502	88.10008	Yes	20161031-BXX-1025	
11/2/16	多杰加 徐雯靓 塔杰 伦珠	60	444	31.53711	88.02727	Yes	20161102-DJ-444	
11/2/16	梁旭昶 曲美罗布	62s	40	31.50613	88.16854	No	20161102-LXC-040	
11/1/16	卞晓星 徐雯靓 多杰加 伦珠	62sc	1028	31.56733	88.05275	Yes	20161101-BXX-1028	
11/1/16	卞晓星 徐雯靓 多杰加 伦珠	62sc	1029	31.56813	88.05278	No	20161101-BXX-1029	
11/1/16	卞晓星 徐雯靓 多杰加 伦珠	62sc	1031	31.57218	88.04715	Yes	20161101-BXX-1031	
11/5/16	多杰加	60	460	31.57361	88.17920	Yes	DJ-20161105-460	
11/5/16	卞晓星 曲美罗布	62sc	1046	31.58178	88.16984	Yes	20161105-BXX-1046	
11/6/16	多杰加 伦珠	62sc	1047	31.54383	88.16808	No	DJ-20161106-1047	
11/7/16	卞晓星 徐雯靓 塔杰	60	467	31.50499	88.23782	Yes	20161107-BXX-467	
11/16/16	卞晓星	60	474	31.50225	88.03237	Yes	20161115-BXX-474	
11/15/16	多杰加	62sc	1068	31.49925	88.03691	No	20161115-DJ-1068	not snow leopard
1/13/17	卞晓星	62sc	1189	31.52154	88.11096	No	20170113-BXX-1189	
1/8/17	卞晓星	62s	248	31.48360	88.08313	Yes	20170108-BXX-248	
5/12/17	卞晓星	62sc	1213	31.59056	88.02548	Yes	20170513-BXX-1213	
11/23/16	梁旭昶	62s	76	31.66496	88.56158	No	20161123-LXC-076	village 5
5/14/17	塔杰	62s	961	31.45880	88.19190	No	20170514 塔杰 961	
5/20/17	塔杰	62s	997	31.58180	88.16988	No	20170520 塔杰 997	
5/16/17	卞晓星	62sc	1234	31.57466	88.00222	No	20170515-BXX-1234	
5/20/17	卞晓星	62sc	1238	31.57565	88.04559	No	20170520-BXX-1238	
5/12/17	卞晓星	62sc	1213	31.59056	88.02548	No	20170513-BXX-1213	
11/16/16	多杰加	62sc	1072	31.48652	88.07601	No	20161116-DJ-1072	
11/7/16	多杰加	62sc	1059	31.57294	87.92318	No	20161107-DJ-1059	
5/14/17	卞晓星	62sc	1216	31.49987	88.03524	Yes	20170514-BXX-1216	

Appendix I: Supplemental Material 2. Information for samples collected in Qilian Shan, Qinghai May 2017.

Num	Sample ID	Collecting Time	Location	Longitude	Latitude	Height	Collectors	Photo
1	qhqls-yhl-20170518-gyy-01		油葫芦	99.75217	38.22341	3509	gyy	T
2	qhqls-yhl-20170518-gyy-02		油葫芦	99.75105	38.22294	3586	gyy	F
3	qhqls-yhl-20170518-gyy-03		油葫芦	99.746933	38.22213	3663	gyy	F
4	qhqls-shy-20170519-gyy-01		三河源	99.15693	38.35599	3906	gyy	T
5	qhqlxyl-20170520-gyy-01	16:27	沙塘沟	98.587895	38.779427	3767	gyy	F
6	qhqlxyl-20170521-gyy-02	9:16	沙塘沟	98.620006	38.825544	3948	gyy	T
7	qhqlxyl-20170521-gyy-03	10:47	沙塘沟	98.614756	38.830447	4054	gyy	T
8	qhqlxyl-20170522-gyy-04	12:07	下才日洼	98.646222	38.568972	3916	gyy	T
9	qhtjx-20170523-GYY-01	14:59	在茫	97.5787	38.812219	3607	gyy	F
10	qhtjx-20170524-gyy-06		花儿地	97.212524	38.882481		gyy	T
11	qhqtjx-20170525-gyy-05	13:57	花儿地	97.2816	38.888226	3974	gyy	F
12	qhqls-20170517-hy-01	17:53	油葫芦	99.6017	38.28298	3932	hy	T
13	qhqls-20170518-hy-02	11:17	油葫芦	99.6547	38.2515	3829	hy	F
14	qhqls-20170520-hy-03	13:45	央隆	98.6789	38.7889	3955	hy	T
15	qhqls-20170520-hy-04	14:00	央隆	98.6786	38.7881	3980	hy	F
16	qhqls-20170520-hy-05	14:11	央隆	98.6784	38.7876	3958	hy	T
17	qhqls-20170521-hy-06	10:04	黑刺沟	98.5604	38.8794	4021	hy	T
18	qhqls-20170521-hy-07	10:10	黑刺沟	98.5604	38.8794	4024	hy	T
19	qhqls-20170521-hy-08		黑刺沟	98.5606	38.8794	4029	hy	T
20	qhqls-20170521-hy-09		黑刺沟	98.5606	38.8794	4029	hy	T
21	qhqls-20170521-hy-10		黑刺沟	98.5606	38.8794	4029	hy	T
22	qhqls-20170521-hy-11	11:25	黑刺沟	98.5838	38.8738	3946	hy	T
23	qhqls-20170521-hy-12	11:25		98.5838	38.8738	3946	hy	T
24	qhqls-20170521-hy-13	12:10	黑刺沟	98.5843	38.8731	4019	hy	T
25	qhqls-20170521-hy-14			98.5989	38.8769		hy	T
26	qhqls-20170523-hy-15	16:15	五社	97.5071	38.8033	3633	hy	T
27	qhqls-20170523-hy-16	16:45	五社	97.5066	38.8036	3651	hy	T
28	qhqls-20170524-hy-17	13:29	深沟	97.3782	38.9633	3922	hy	T
29	qhqls-20170524-hy-18	15:36	深沟	97.3762	38.9772	3675	hy	T
30	qhqls-20170525-hy-19	9:41	登龙沟	97.8596	38.6082	3991	hy	T
31	qhqls-20170518-lgl-01	12:50	油葫芦 休龙	99.791301	38.15557	3753	lgl	T
32	qhqls-20170518-lgl-02	13:01	油葫芦 休龙	99.79095	38.155572	3762	lgl	T
33	qhqls-20170518-lgl-03	13:11	油葫芦 休龙	99.798952	38.155569	3768	lgl	T
34	qhqls-20170518-lgl-04	12:43	央隆 大那吾卡	98.594554	38.661118	3787	lgl	F
35	qhqls-20170524-lgl-05	10:23	苏里 菜和村	97.622035	38.858574	3812	lgl	T
36	qhqls-20170524-lgl-06	11:21	苏里 菜和村	97.625628	38.858194	3833	lgl	T

Num	Sample ID	Collecting Time	Location	Longitude	Latitude	Height	Collectors	Photo
37	qhqls-20170524-lgl-07	11:31	苏里 梁和村	97.625659	38.858196	3831	lgl	T
38	qhqls-20170525-lgl-08	10:35	苏里 阿达沟	97.809996	38.6855	4052	lgl	T
39	qhqls-20170525-lgl-09	10:50	苏里 阿达沟	97.809238	38.685533	4054	lgl	T
40	qhqls-20170525-lgl-10	13:58	苏里 阿达沟	97.823203	38.720816	3851	lgl	T
41	qhqls-20170525-lgl-11	14:35	苏里 阿达沟	97.827263	38.720053	3795	lgl	T
42	QHQLS-20170517-LJ-01	15:52	西鼻口	99.63077	38.26813	3865	LJ	T
43	QHQLS-20170519-LJ-01	15:33	一道口	99.15678	38.34678	3971	LJ	F
44	QHQLS-20170519-LJ-02	16:42	一道沟	99.15668	38.34821	3948	LJ	T
45	qhqls-20170521-lj-01	14:25		98.55095	38.6328	4042	lj	T
46	QHQLS-20170523-LJ-01	17:40	苏里	97.50732	38.79073	3650	LJ	T
47	QHQLS-20170523-LJ-02	17:58		97.50495	38.7942	3659	LJ	T
48	QHQLS-20170523-LJ-03	18:04		97.50458	38.79469	3657	LJ	T
49	QHQLS-20170525-LJ-01	12:01		97.27779	38.87535	4007	LJ	F
50	QHQLS-20170525-LJ-02	12:10		97.27747	38.8754	4041	LJ	F
51	QHQLS-20170525-LJ-03	12:40		97.27824	38.87531	4050	LJ	T
52	QHQLS-20170525-LJ-04	14:05		97.28208	38.88916	4010	LJ	T
53	QHQLS-20170525-LJ-05	14:26		97.28793	38.89609	3961	LJ	F
54	QHQLS-20170525-LJ-06	14:31		97.28846	38.89617	3958	LJ	F
55	QHQLS-20170525-LJ-07	15:40		97.28916	38.89916	3938	LJ	F
56	QHQLS-20170527-LJ-01	14:28		100.76367	38.15247	3209	LJ	F
57	QHQLS-20170527-LJ-02	14:29		100.76367	38.15287	3209	LJ	F
58	QHQLS-20170519-XYD-01	12:56	一道沟西	99.132821	38.373998	3807	XYD	T
59	QHQLS-20170519-XYD-02	14:04	二道沟西	99.108506	38.376049	3809	XYD	T
60	QHQLS-20170519-XYD-03	14:30	二道沟西	99.105733	38.377534	3860	XYD	T
61	QHQLS-20170519-XYD-04	16:33	二道沟口北	99.082853	38.373581	3892	XYD	T
62	QHQLS-20170521-XYD-05	13:06	大东沟	98.680282	38.731976	3947	XYD	T
63	QHQLS-20170521-XYD-06	13:35	大东沟	98.678884	38.731462	3891	XYD	T
64	QHQLS-20170518-ZJ-01	13:10	东沟	99.77028	38.15377	3872	ZJ	T
65	QHQLS-20170518-ZJ-02	13:27	东沟	99.770453	38.15436	3891	ZJ	T
66	QHQLS-20170518-ZJ-03	13:30	东沟	99.770453	38.15436	3891	ZJ	T
67	qhqls-20170519-zj-04	14:25	狗熊峡	99.044307	38.379494	3996	zj	T
68	qhqls-20170519-zj-05	16:07	狗熊峡	99.059359	38.37715	3899	zj	T
69	qhqls-20170521-zj-06	9:18	热水沟	98.65148	38.824137	4040	zj	T
70	qhqls-20170521-zj-07	9:30	热水沟	98.651136	38.824287	4051	zj	T
71	qhqls-20170521-zj-08	10:37	兔儿沟	98.715687	38.707006	4009	zj	T
72	qhqls-20170524-zj-09	10:45	深沟	97.567927	38.869593	3729	zj	T
73	qhqls-20170524-zj-10	13:10	深沟	97.552962	38.888864	4005	zj	T

Num	Sample ID	Collecting Time	Location	Longitude	Latitude	Height	Collectors	Photo
74	QHQLS-20170520-ZY-01	15:58	热水埡口	98.73598	38.79382	4131	ZY	T
75	qhqls-20170522-zy-01			98.899865	38.624697	4053	zy	T
76	qhqls-20170524-zy-01		天骏	97.471355	38.925556	3537	zy	T
77	qhqls-20170524-zy-02		天骏	97.419827	38.94613	3938	zy	T
78	qhqls-20170525-zy-01		天骏	97.474342	38.737002	3826	zy	T
79	qhqls-20170525-zy-02		天骏	97.474342	38.737002	3826	zy	T
80	qhqls-20170525-zy-03		大西沟	97.47989	38.735793	3866	zy	T
81	qhqls-20170525-zy-04		大西沟	97.483947	38.734887	3897	zy	T
82	QHQLS-20170518-LYL-01		油葫芦哈熊沟	99.67938	38.18485	3635	LYL	F
83	QHQLS-20170518-LYL-02		油葫芦哈熊沟	99.67938	38.18485	3635	LYL	F
84	QHQLS-20170521-LYL-01		大龙空	98.50888	38.92808	3968	LYL	F
85	QHQLS-20170521-LYL-02		大龙空	98.508878	38.928085	3967	LYL	F
86	QHQLS-20170522-LYL-01		石头沟	98.792231	38.602709	4033	LYL	F
87	QHQLS-20170523-LYL-01	16:11		97.769261	38.7764	3572	LYL	F
88	QHQLS-20170527-LYL-01	10:30	大红沟	100.52988	38.21628	3588	LYL	F

Appendix I: Supplemental Material 3. Information for samples collected in Qilian Shan, Qinghai September 2017.

Num	SampleID	Collecting time	Location	Longitude	Latitude	Height	Photograph
1	qhqls-20170914-hy-01			97.3761	38.9777	3704	T
2	qhqls-20170915-hy-02	13:26	登龙沟	97.8698	38.595	4281	T
3	qhqls-20170918-hy-03	11:20	黑刺沟	98.599	38.8769	4146	T
4	qhqls-20170918-hy-04	11:30	黑刺沟	98.5987	38.877	4130	T
5	qhqls-20170918-hy-05	12:50	黑刺沟	98.5904	38.8727	4022	T
6	qhqls-20170918-hy-06	13:11	黑刺沟	98.5907	38.8712	4069	T
7	qhqls-20170918-hy-07	13:57	黑刺沟	98.5856	38.8725	3992	T
8	qhqls-20170918-hy-08	14:05	黑刺沟	98.5849	38.873	4019	T
9	qhqls-20170918-hy-09	14:30	黑刺沟	98.5843	38.8731	4019	T
10	qhqls-20170918-hy-10	14:33	黑刺沟	98.5845	38.8731	4017	T
11	qhqls-20170923-hy-11	11:55	西鼻	99.62789	38.26937	3886	T
12	qhqls-20170923-hy-12	1:37	西鼻	99.6017	38.2831		T
13	qhqls-20170923-hy-13	13:39	西鼻	99.6017	38.2832	3928	T
14	qhqls-20170923-hy-14	13:41	西鼻	99.6017	38.2832	3929	T
15	qhqls-20170924-hy-17	12:03	芒扎	100.7167	38.2147	3391	T
16	qhqls-20170917-zy-01	16:40	央隆	98.84902	38.64463	3954	
17	qhqls-20170913-xyd-01		团结峰 3 号栅格	97.467419	38.802563	4014	T
18	qhqls-20170914-xyd-02		苏里 34 号栅格	97.42073	38.9466	3859	T
19	qhqls-20170913-lj-01	12:14		97.49767	38.78539	3785	T
20	qhqls-20170913-lj-03	15:41		97.50616	38.79329	3684	T
21	qhqls-20170913-lj-04	15:50		97.50513	38.79421	3677	
22	qhqls-20170913-lj-05	15:54		97.50513	38.79424	3679	T
23	qhqls-20170914-lj-01	12:50		97.50525	38.92998	3638	T
24	qhqls-20170914-lj-02	13:00		97.50534	38.9301	3640	T
25	qhqls-20170915-lj-01	11:33		97.28134	38.88018	4137	

Appendix I: Supplemental Material 4. Information for samples collected in Qilian Shan, Gansu.

Date	Transect	Sample ID	Latitude	Longitude	Altitude	Comments
7/30/18	1	gssb20180730-zy-01	39.037653	96.482266		
7/30/18	1	gssb20180730-zy-02	39.037653	96.482266		
7/30/18	1	gssb20180730-CEH-01	39.092837	96.952762		
7/30/18	1	gssb20180730-CEH-02	39.092837	96.952762		
7/30/18	1	gssb20180730-CEH-03	39.092119	96.956465		
7/30/18	1	gssb20180730-CEH-04	39.092119	96.956465		
7/30/18	1	gssb20180730-CEH-05	39.091812	96.956743		
7/30/18	1	gssb20180730-CEH-06	39.091812	96.956743		
7/30/18	1	gssb20180730-CEH-07	39.090343	96.957723		
7/30/18	1	gssb20180730-CEH-08	39.090209	96.957599		
7/30/18	1	gssb20180730-CEH-09	39.090209	96.957599		
7/30/18	1	gssb20180730-CEH-10	39.091166	96.956956		
7/30/18	1	gssb20180730-CEH-11	39.091205	96.956886		
7/30/18	1	gssb20180730-CEH-12	39.09263	96.954798		
7/30/18	1	gssb20180730-CEH-13	39.092865	96.952751		
7/30/18	1	gssb20180730-CEH-14	39.093785	96.94506		
7/30/18	1	gssb20180730-CEH-15	39.093785	96.94506		
7/30/18	1	gssb20180730-CEH-16	39.093785	96.94506		
7/30/18	1	gssb20180730-CEH-17	39.093785	96.94506		
7/30/18	1	gssb20180730-CEH-18	39.090382	96.966257		
7/30/18	1	gssb20180730-CEH-19	39.07064	96.93624		
7/30/18	1	gssb20180730-CEH-20	39.07064	96.93624		
7/30/18	1	gssb20180730-CEH-21	39.07064	96.93624		
7/30/18	2	gssb20180730-CEH-22	39.073422	96.908516		
7/30/18	2	gssb20180730-CEH-23	39.073422	96.908516		
7/30/18	2	gssb20180730-CEH-24	39.073422	96.908516		
7/30/18	2	gssb20180730-CEH-25	39.071909	96.908787		Snow Leopard Latrine
7/30/18	2	gssb20180730-CEH-26	39.071909	96.908787		Snow Leopard Latrine
7/30/18	2	gssb20180730-CEH-27	39.071909	96.908787		Snow Leopard Latrine
7/30/18	2	gssb20180730-CEH-28	39.071909	96.908787		Snow Leopard Latrine
7/30/18	2	gssb20180730-CEH-29	39.071909	96.908787		Snow Leopard Latrine
7/30/18	3	gssb20180730-MA-01				
7/30/18	3	gssb20180730-MA-02				
7/30/18	3	gssb20180730-MA-03				
7/30/18	3	gssb20180730-MA-04				
7/30/18	3	gssb20180730-MA-05				



Date	Transect	Sample ID	Latitude	Longitude	Altitude	Comments
7/30/18	3	gssb20180730-MA-06				
7/30/18	3	gssb20180730-MA-07				
7/30/18	2	QLS1	39.65	96.7244444	3795	
7/30/18	2	QLS2	39.2275	96.98055556	3240	
7/30/18	2	QLS3	39.2008333	97.19055556	3245	
7/30/18	2	QLS4	39.2008333	97.19055556	3245	
7/30/18	2	QLS5	39.2008333	97.19055556	3245	
7/30/18	2	QLS6	39.2097222	97.181111	3222	
7/30/18	2	QLS7	39.2097222	97.181111	3222	
7/30/18	2	QLS8	39.184444	97.03	3278	
7/30/18	2	QLS9	39.184444	97.03	3278	
7/30/18	2	QLS10	39.184444	97.03	3278	
7/30/18	2	QLS11	39.2958333	97.07305556	3281	
7/30/18	2	QLS12	39.2958333	97.07305556	3281	
7/30/18	2	QLS13	39.2958333	97.07305556	3281	
7/30/18	2	QLS14	39.2958333	97.07305556		
7/31/18	1	gssb20180731-CEH-01	39.069973	96.892471	3554	
7/31/18	1	gssb20180731-CEH-02				Was initially missing but was later found
7/31/18	2	gssb20180731-CEH-03	39.069973	96.892471	3554	
7/31/18	2	gssb20180731-CEH-04	39.0080663	96.90220607	3689	
7/31/18	2	gssb20180731-CEH-05	39.0670526	96.90217175	3695	
7/31/18	2	gssb20180731-CEH-06	39.0670526	96.90217175	3695	
7/31/18	2	gssb20180731-CEH-07	39.0057965	96.90215486	3204	
7/31/18	2	gssb20180731-CEH-08	39.0041099	96.90209386	3718	
7/31/18	2	gssb20180731-CEH-09	39.0037797	96.90206707	3714	
7/31/18	2	gssb20180731-CEH-10	39.0037797	96.90206707	3714	
7/31/18	2	gssb20180731-CEH-11	39.0037797	96.90206707	3914	
7/31/18	2	gssb20180731-CEH-12	39.9922558	96.89833202	3781	
7/31/18	2	gssb20180731-CEH-13	39.9922558	96.89833202	3781	
7/31/18	2	gssb20180731-CEH-14	39.9922558	96.89833202	3781	
7/31/18	2	gssb20180731-CEH-15	39.896446	96.896445	3793	
7/31/18	2	gssb20180731-CEH-16	38.9925897	96.89633077	3795	
7/31/18	2	gssb20180731-CEH-17	38.9911819	96.89350345	3817	
7/31/18	2	gssb20180731-CEH-18		N/A		
7/31/18	3	gssb20180731-CEH-19	39.001534	96.9053072	3749	
7/31/18	3	gssb20180731-CEH-20	39.0013191	96.90798511	3766	
7/31/18	3	gssb20180731-CEH-21		N/A		NOT SCAT - A ROCK

Date	Transect	Sample ID	Latitude	Longitude	Altitude	Comments
7/31/18	3	gssb20180731-CEH-22		N/A		
7/31/18	4	gssb20180731-CEH-23		N/A		
7/31/18	4	gssb20180731-CEH-24		N/A		
7/31/18	5	gssb20180731-CEH-25	39.042204	96.9261965	3556	Found randomly during camera trapping
7/31/18	5	gssb20180731-CEH-26	39.0423877	96.9257793	3551	Found randomly during camera trapping
7/31/18	6	gssb20180731-zy-01	39.0147713	96.90307687		
7/31/18	6	gssb20180731-zy-02	39.0147713	96.8919215		
7/31/18	6	gssb20180731-zy-03	39.0045755	96.8919215		
7/31/18	6	gssb20180731-zy-4	39.0002168	96.87870317		
7/31/18	6	gssb20180731-zy-04	39.0002168	96.87870317		
7/31/18	6	gssb20180731-zy-05	39.0002168	96.87870317		
7/31/18	6	gssb20180731-zy-06	39.0002168	96.87870317		
7/31/18	6	gssb20180731-zy-07	39.0002168	96.87870317		
7/31/18	6	gssb20180731-zy-08	39.000022	96.8795		
7/31/18	6	gssb20180731-zy-09	39.000022	96.8795		Almost all hair, no casing left
7/31/18	6	gssb20180731-zy-11	39.001057	96.885591		
7/31/18	6	gssb20180731-zy-12	39.001057	96.885591		
7/31/18	6	gssb20180731-zy-13	39.001057	96.885591		
7/31/18	6	gssb20180731-zy-14	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-15	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-16	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-17	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-18	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-19	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-20	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-21	39.023518	96.899672		
7/31/18	6	gssb20180731-zy-22	39.023518	96.899672		
7/31/18	7	QLS40	39.1391667	97.1175	3653	
7/31/18	7	QLS41	39.1391667	97.1175	3653	
7/31/18	7	QLS42	39.1391667	97.1175	3653	
7/31/18	7	QLS43	39.1391667	97.1175	3653	
7/31/18	7	QLS44	39.1391667	97.1175	3653	
7/31/18	7	QLS45	39.1391667	97.1175	3653	
7/31/18	7	QLS46	39.2447222	97.01194444	3503	
7/31/18	7	QLS47	39.2552778	97.012	3481	
7/31/18	7	QLS48	39.2552778	97.012	3481	
7/31/18	7	QLS49	39.2552778	97.012	3481	

Date	Transect	Sample ID	Latitude	Longitude	Altitude	Comments
7/31/18	7	QLS50	39.2552778	97.012	3481	
7/31/18	7	QLS51	39.2552778	97.012	3481	
7/31/18	7	QLS52	39.3108333	97.0361111	3463	
7/31/18	7	QLS53	39.3108333	97.0361111	3463	
7/31/18	7	QLS54	39.3108333	97.0361111	3463	
7/31/18	7	QLS55	39.3108333	97.0361111	3463	
7/31/18	7	QLS56	39.3108333	97.0361111	3463	
7/31/18	7	QLS57	39.3108333	97.0361111	3463	
7/31/18	7	QLS15	39.2308333	96.9366667	3672	
7/31/18	7	QLS16	39.2308333	96.9366667	3672	
7/31/18	7	QLS17	39.2105556	96.9188889	3727	
7/31/18	7	QLS18	39.2105556	96.9188889	3727	
7/31/18	7	QLS19	39.2105556	96.9188889	3727	
7/31/18	7	QLS20	39.2105556	96.9188889	3727	
7/31/18	7	QLS21	39.2105556	96.9188889	3727	
7/31/18	7	QLS22	39.2105556	96.9188889	3727	
7/31/18	7	QLS23	39.2105556	96.9188889	3727	
7/31/18	7	QLS24	39.2105556	96.9188889	3727	
7/31/18	7	QLS25	39.2530556	96.9847222	3645	
7/31/18	7	QLS26	39.2530556	96.9847222	3645	
7/31/18	7	QLS27	39.2530556	96.9847222	3645	
7/31/18	7	QLS28	39.2530556	96.9847222	3645	
7/31/18	7	QLS30	39.2530556	96.9847222	3645	
7/31/18	7	QLS31	39.1402778	97.1144444	3645	
7/31/18	7	QLS32	39.1391667	97.1175	3649	
7/31/18	7	QLS33	39.1391667	97.1175	3653	
7/31/18	7	QLS34	39.1391667	97.1175	3653	
7/31/18	7	QLS35	39.1391667	97.1175	3653	
7/31/18	7	QLS36	39.1391667	97.1175	3653	
7/31/18	7	QLS37	39.1391667	97.1175	3653	
7/31/18	7	QLS38	39.1391667	97.1175	3653	
7/31/18	7	QLS39	39.1391667	97.1175	3653	

Appendix I: Supplemental Material 5. Information for samples collected in East Burhanbuda Mountain, Dulan County, China.

Date	Sample ID	Latitude	Longitude	Altitude	Comments
7/25/18	qhdul-20180725-CEH-01	35.649375	98.468429		
7/25/18	qhdul-20180725-CEH-02	35.649414	98.468423		
7/25/18	qhdul-20180725-CEH-03	35.648749	98.468031		Found next to CEH04
7/25/18	qhdul-20180725-CEH-04	35.648749	98.468031		Found next to CEH03
7/25/18	qhdul-20180725-CEH-05	35.649977	98.467329		
7/25/18	qhdul-20180725-CEH-06	35.650071	98.466905		
7/25/18	qhdul-20180725-CEH-07	35.650505	98.466094		
7/25/18	qhdul-20180725-CEH-08	35.649971	98.464286		
7/25/18	qhdul-20180725-CEH-09	35.648475	98.464423	4128	
7/25/18	DL4	35.7975	98.54305556	4190	
7/25/18	DL5	35.7975	98.54305556	4190	
7/25/18	DL6	35.7580556	98.57027778	4322	
7/25/18	DL7	35.7427778	98.58	4389	
7/25/18	DL8	35.7283333	98.58583333	4430	
7/25/18	DL9	35.7152778	98.58861111	4472	
7/25/18	DL10	35.7152778	98.58861111	4472	
7/25/18	DL11	35.7152778	98.58861111	4472	
7/25/18	DL12	35.7152778	98.58861111	4472	
7/25/18	DL13	35.7152778	98.58861111	4472	Almost all hair, not casing left
7/25/18	DL14	35.7142222	98.5880556	4476	
7/25/18	DL15	35.7142222	98.5880556	4476	
7/25/18	DL16	35.7142222	98.5880556	4476	
7/25/18	DL17	35.7	98.59361111	4541	
7/25/18	DL18	35.7	98.59361111	4541	
7/25/18	DL19	35.7	98.59361111	4541	
7/25/18	DL20	35.6997222	98.593333	4541	
7/25/18	DL21	35.6997222	98.593333	4541	
7/25/18	DL22	35.6997222	98.593333	4541	
7/25/18	DL23	35.6916667	98.5994444	4573	
7/25/18	DL24	35.6911111	98.61372222	4550	
7/25/18	DL25	35.6911111	98.61372222	4550	
7/25/18	DL26	35.6911111	98.61372222	4550	
7/25/18	DL27	35.6911111	98.61372222	4550	
7/25/18	DL28	35.6925	98.62583333	4543	
7/25/18	DL29	35.6986111	98.64277778	4519	
7/25/18	DL30	35.7005556	98.65305556	4516	

Date	Sample ID	Latitude	Longitude	Altitude	Comments
7/25/18	DL31	35.7005556	98.65305556	4516	
7/25/18	DL32	35.7005556	98.65305556	4516	
7/25/18	DL33	35.7005556	98.65305556	4516	
7/25/18	DL34	35.7005556	98.65305556	4516	
7/25/18	DL35	35.6861111	98.59303335	4576	
7/25/18	DL36	35.6861111	98.59303335	4576	
7/25/18	DL37	35.6861111	98.59303335	4576	
7/25/18	DL38	35.6861111	98.59303335	4576	
7/25/18	DL39	35.6683333	98.5836111	4602	
7/25/18	DL40	35.6547222	98.54083333	4606	
7/25/18	DL41	35.6547222	98.54083333	4606	
7/25/18	DL42	35.9061111	98.75805556	4612	
7/25/18	DL43	35.9061111	98.75805556	4612	
7/25/18	DL43				
7/25/18	DL44	35.9061111	98.75805556	4612	
7/25/18	DL45	35.9061111	98.75805556	4612	
7/25/18	DL46	35.6583333	98.7361111	4559	
7/25/18	DL47	35.6583333	98.7361111	4559	
7/25/18	DL48	35.6738889	98.7225	4498	
7/25/18	DL49	35.6738889	98.7225	4498	
7/25/18	DL50	35.6738889	98.7225	4498	
7/25/18	DL51	35.6738889	98.7225	4498	
7/25/18	DL52	35.6738889	98.7225	4498	
7/26/18	qhdul-20180726-CEH-01	35.53319	98.33194		
7/26/18	qhdul-20180726-CEH-02	35.530873	98.333451		
7/26/18	qhdul-20180726-CEH-03	35.530384	98.334096		Found next to CEH04
7/26/18	qhdul-20180726-CEH-04	35.530384	98.334096		All hair, casing washed away
7/26/18	qhdul-20180726-CEH-05	35.530336	98.334322		
7/26/18	qhdul-20180726-CEH-06	35.526189	98.33631		
7/26/18	qhdul-20180726-CEH-07	35.526077	98.336375		Found next to CEH08 and CEH09
7/26/18	qhdul-20180726-CEH-08	35.526077	98.336375		Found next to CEH07 and CEH09
7/26/18	qhdul-20180726-CEH-09	35.526077	98.336375		Found next to CEH07 and CEH08
7/26/18	qhdul-20180726-CEH-10	35.524529	98.336275		
7/26/18	qhdul-20180726-CEH-11	35.5244	98.336469		
7/26/18	DL53	35.7702778	98.58916667	4513	
7/26/18	DL54	35.7725	98.33777778	4523	
7/26/18	DL55	35.7236111	98.4294444	4543	

Date	Sample ID	Latitude	Longitude	Altitude	Comments
7/26/18	DL56	35.7236111	98.4294444	4543	
7/26/18	DL57	35.7236111	98.4294444	4543	
7/26/18	DL58	35.7038889	98.46638889	4568	
7/26/18	DL59	35.7038889	98.46638889	4568	
7/26/18	DL60	35.7038889	98.46638889	4568	
7/26/18	DL61	35.7038889	98.46638889	4568	
7/26/18	DL62	35.7036111	98.4705556	4429	Bone with some fecal material around it.
7/26/18	DL63	35.7036111	98.4705556	4429	
7/26/18	qhdul-20180726-CEH-12	35.62393	98.50206		
7/26/18	qhdul-20180726-CEH-13	35.624138	98.501112		
7/26/18	qhdul-20180726-CEH-14	35.621776	98.504351		
7/26/18	qhdul-20180726-CEH-15	35.622859	98.500227		
7/26/18	DL64	35.7036111	98.56055556	4009	
7/26/18	DL65	35.7008333	98.5433333	4024	
7/26/18	DL66	35.71	98.5380556	4011	
7/26/18	DL67	35.71	98.5380556	4011	Almost all hair, not casing left
7/26/18	DL68	35.71	98.5380556	4011	
7/26/18	DL69	35.71	98.5380556	4011	
7/27/18	qhdul-20180727-CEH-01	35.983757	97.218387		
7/27/18	qhdul-20180727-CEH-02	35.915355	97.182176		
7/27/18	qhdul-20180727-CEH-03	35.913939	97.17653		
7/27/18	qhdul-20180727-CEH-04	35.913872	97.176372		
7/27/18	qhdul-20180727-CEH-05	35.91313	97.171644		
7/27/18	qhdul-20180727-CEH-06	35.913274	97.170487		Found next to CEH07
7/25/18	qhdul-20180725-CEH-10	35.719299	98.17720912		
7/25/18	qhdul-20180725-CEH-11	35.7197341	98.17145137		
7/25/18	qhdul-20180725-CEH-12	35.7201691	98.16569362		Found next to CEH02
7/25/18	qhdul-20180725-CEH-13	35.7206042	98.15993587		Found next to CEH01
7/25/18	qhdul-20180725-CEH-14	35.7210392	98.15417812		
7/25/18	qhdul-20180725-CEH-15	35.7214743	98.14842037		
7/25/18	qhdul-20180725-CEH-16	35.7219093	98.14266262		
7/25/18	qhdul-20180725-CEH-17	35.7223443	98.13690487		
7/25/18	qhdul-20180725-CEH-18	35.7227794	98.13114712	4378.870287	
7/25/18	DL4	35.7232144	98.12538937	4377.020081	
7/25/18	DL5	35.7236495	98.11963162	4375.169875	
7/25/18	DL6	35.7240845	98.11387387	4373.31967	
7/25/18	DL7	35.7245196	98.10811612	4371.469464	

Date	Sample ID	Latitude	Longitude	Altitude	Comments
7/25/18	DL8	35.7249546	98.10235837	4369.619258	
7/25/18	DL9	35.7253896	98.09660061	4367.769052	
7/25/18	DL10	35.7258247	98.09084286	4365.918846	
7/25/18	DL11	35.7262597	98.08508511	4364.06864	
7/25/18	DL12	35.7266948	98.07932736	4362.218435	
7/25/18	DL13	35.7271298	98.07356961	4360.368229	Almost all hair, not casing left
7/25/18	DL14	35.7275649	98.06781186	4358.518023	
7/25/18	DL15	35.7279999	98.06205411	4356.667817	
7/25/18	DL16	35.728435	98.05629636	4354.817611	
7/25/18	DL17	35.72887	98.05053861	4352.967405	
7/25/18	DL18	35.729305	98.04478086	4351.1172	
7/25/18	DL19	35.7297401	98.03902311	4349.266994	
7/25/18	DL20	35.7301751	98.03326536	4347.416788	
7/25/18	DL21	35.7306102	98.02750761	4345.566582	
7/25/18	DL22	35.7310452	98.02174986	4343.716376	
7/25/18	DL23	35.7314803	98.01599211	4341.86617	
7/25/18	DL24	35.7319153	98.01023436	4340.015965	
7/25/18	DL25	35.7323503	98.00447661	4338.165759	
7/25/18	DL26	35.7327854	97.99871886	4336.315553	
7/25/18	DL27	35.7332204	97.99296111	4334.465347	
7/25/18	DL28	35.7336555	97.98720336	4332.615141	
7/25/18	DL29	35.7340905	97.98144561	4330.764935	
7/25/18	DL30	35.7345256	97.97568786	4328.91473	
7/25/18	DL31	35.7349606	97.96993011	4327.064524	
7/25/18	DL32	35.7353956	97.96417236	4325.214318	
7/25/18	DL33	35.7358307	97.9584146	4323.364112	

Appendix I: Supplemental Material 6. Information for samples collected in Suojia, Yushu Prefecture, Qinghai Province, China.

Lab Name	Collection Date	Longitude	Latitude	Suspected Scat Source
SUJ04	6/25/16	93.83305556	34.30444444	Brown Bear
SUJ02	5/21/16			Brown Bear
SUJ09	6/25/16	93.83166667	34.30444444	Brown Bear
SUJ16	7/15/16	93.86	34.26944444	Brown Bear
SUJ06				
SUJ10	7/14/16	93.84333333	34.31333333	
SUJ11				
SUJ20	5/25/16	92	34	Snow Leopard
SUJ22	7/14/16	94.24666667	34.32944444	Snow Leopard
SUJ14	4/29/16			Snow Leopard
SUJ18	5/6/16	93.97722222	34.99777778	Snow Leopard
SUJ19				Snow Leopard
SUJ21	4/20/16			Snow Leopard
SUJ28	4/27/16	93.95472222	35.00916667	Snow Leopard
SUJ12	4/20/16	94.24527778	34.32833333	Snow Leopard
SUJ13	4/23/18	93.89194444	34.25972222	Snow Leopard
SUJ15	4/30/16			Snow Leopard
SUJ17	4/30/16			Snow Leopard
SUJ23	4/30/16	93.89472222	34.26333333	Snow Leopard
SUJ24	4/23/16	93.92	34.26	Snow Leopard
SUJ25	4/23/16	93.89416667	34.26388889	Snow Leopard
SUJ26		N/A	N/A	Snow Leopard
SUJ27	4/27/16	93.95277778	34.99638889	Snow Leopard
SUJ01	7/15/16	93.8325	34.6747222	Wolf
SUJ03	7/15/16	93.82722222	34.29138889	Wolf
SUJ05	7/15/16	93.8325	34.29111111	Wolf
SUJ07	7/14/16	93.82305556	34.18222222	Wolf
SUJ08	5/14/16	93.83277778	34.12638889	Wolf



Appendix I: Supplemental Material 7. Information for samples collected in Duocai, Yushu Prefecture, Qinghai Province, China.

Date	Township	Sample ID	Latitude	Longitude	Altitude	Comments
7/22/18	Diangjongrong	qhyus-20180722-CEH-01	33.507226	95.5278		Road nearby
7/22/18	Diangjongrong	qhyus-20180722-CEH-02	33.507226	95.5278		Road nearby
7/22/18	Diangjongrong	qhyus-20180722-CEH-03	33.507749	95.527903		Road nearby
7/22/18	Diangjongrong	qhyus-20180722-CEH-04	33.50775	95.527563		Suspected to be bear origin
7/22/18	Diangjongrong	qhyus-20180722-CEH-05	33.508858	95.526798		
7/22/18	Diangjongrong	qhyus-20180722-zhangyu-01	33.586037	95.495846		Collected next to zhangyu 02
7/22/18	Diangjongrong	qhyus-20180722-zhangyu-02	33.586037	95.495846		Collected next to zhangyu 01
7/22/18	Diangjongrong	qhyus-20180722-zhangyu-03	33.586175	95.496142		
7/22/18	Diangjongrong	DAI001	33.499	95.522	4699	
7/22/18	Diangjongrong	DAI002	33.699	95.251	4625	
7/22/18	Diangjongrong	DAI003	33.499	95.52	4663	
7/22/18	Diangjongrong	DAI004	33.4428	95.5204	4647	
7/22/18	Diangjongrong	DAI005	33.499	95.521	4713	
7/22/18	Diangjongrong	DAI006	33.499	95.521	4643	
7/22/18	Diangjongrong	DAI007	33.499	95.521	4643	
7/22/18	Diangjongrong	DAI008	33.499	95.521	4643	
7/22/18	Diangjongrong	DAI009	33.499	95.521	4643	
7/22/18	Diangjongrong	DAI010	33.49908	95.52288	4602	
7/22/18	Diangjongrong	ZD1	33.5191667	95.76361111	4592	
7/22/18	Diangjongrong	ZD2	33.5191667	95.76361111	4592	
7/22/18	Diangjongrong	ZD3	33.5191667	95.76361111	4592	
7/22/18	Diangjongrong	BB01DAI				Suspected to be bear origin
7/22/18	Diangjongrong	ZD4	33.5191667	95.76361111	4592	
7/22/18	Diangjongrong	ZD5	33.5191667	95.76361111	4592	
7/22/18	Diangjongrong	ZD6	33.5191667	95.76361111	4592	
7/22/18	Diangjongrong	ZD7	33.6188889	95.7058333	4404	Full foot of a vole with white hair
7/22/18	Diangjongrong	ZD8	33.6188889	95.7058333	4404	
7/22/18	Diangjongrong	ZD9	33.6188889	95.7058333	4404	
7/22/18	Diangjongrong	ZD10	33.6188889	95.7058333	4404	
7/23/18	Senlongrong	DAI011	33.7234	95.3687	4550	
7/23/18	Senlongrong	DAI012	33.728	96.37046	4556	
7/23/18	Senlongrong	qhyus-20180723-CEH-01				
7/23/18	Senlongrong	qhyus-20180723-CEH-02				
7/23/18	Senlongrong	qhyus-20180723-CEH-03				
7/23/18	Senlongrong	qhyus-20180723-CEH-04	33.723536	95.371109		Collected next to CEH-05
7/23/18	Senlongrong	qhyus-20180723-CEH-05	33.723536	95.371109		Collected next to CEH-04

Date	Township	Sample ID	Latitude	Longitude	Altitude	Comments
7/23/18	Senlongrong	qhyus-20180723-zhangyu-01	33.723753	95.370934		Outside of blue sheep carcass
7/23/18	Senlongrong	qhyus-20180723-zhangyu-02	33.723753	95.370934		Inside blue sheep carcass
7/23/18	Senlongrong	qhyus-20180723-zhangyu-03				
7/23/18	Senlongrong	qhyus-20180723-zhangyu-04				
7/23/18	Senlongrong	ZD11	33.8388889	95.36694444	4536	
7/23/18	Senlongrong	ZD12	33.8388889	95.36694444	4535	
7/23/18	Senlongrong	ZD13	33.8388889	95.36694444	4535	
7/23/18	Senlongrong	ZD14	33.8388889	95.6238889	4552	
7/23/18	Senlongrong	ZD15	33.8388889	95.6238889	4552	
7/23/18	Senlongrong	ZD16	33.8388889	95.6238889	4552	
7/23/18	Senlongrong	ZD17	33.8388889	95.6238889	4552	
7/23/18	Senlongrong	ZD18	33.8516667	95.6186111	4575	
7/23/18	Senlongrong	ZD19	33.8572222	95.61361111	4594	
7/23/18	Senlongrong	ZD20	33.8572222	95.61361111	4594	
7/23/18	Senlongrong	ZD21	33.8572222	95.61361111	4594	
7/23/18	Senlongrong	ZD22	33.8572222	95.61361111	4594	
7/23/18	Senlongrong	ZD23	33.8572222	95.61361111	4594	
7/23/18	Senlongrong	ZD24	33.8641667	95.60694444	4608	
7/23/18	Senlongrong	ZD25	33.8641667	95.60694444	4608	
7/23/18	Senlongrong	ZD26	33.8641667	95.60694444	4608	
7/23/18	Senlongrong	ZD27	33.8641667	95.6033333	4628	
7/23/18	Senlongrong	ZD28	33.8641667	95.6033333	4628	
7/23/18	Senlongrong	ZD29	33.8641667	95.6033333	4628	
7/23/18	Senlongrong	ZD30	33.8641667	95.6033333	4628	
7/23/18	Senlongrong	ZD31	33.8641667	95.6033333	4628	
7/23/18	Senlongrong	ZD32	33.8886111	95.5811111	4667	
7/23/18	Senlongrong	ZD33	33.8886111	95.5811111	4667	
7/23/18	Senlongrong	ZD34	33.8886111	95.5811111	4667	
7/23/18	Senlongrong	ZD35	33.8886111	95.5811111	4667	All casing washed away
7/23/18	Senlongrong	ZD36	33.8886111	95.5811111	4667	
7/23/18	Senlongrong	ZD37	33.8213889	95.51888889	4558	
7/23/18	Senlongrong	ZD38	33.8213889	95.51888889	4558	
7/23/18	Senlongrong	ZD39	33.8213889	95.51888889	4558	
7/23/18	Senlongrong	ZD40	33.8213889	95.51888889	4558	
7/23/18	Senlongrong	ZD41	33.8213889	95.51888889	4558	
7/23/18	Senlongrong	ZD42	33.8213889	95.51888889	4558	May not be scat
7/23/18	Senlongrong	ZD43	33.8213889	95.51888889	4558	

Date	Township	Sample ID	Latitude	Longitude	Altitude	Comments
7/23/18	Senlongrong	qhyus-20180723-CEH-06	33.665353	95.439427		
7/23/18	Senlongrong	qhyus-20180723-CEH-07				Inside of yak skull
7/23/18	Senlongrong	qhyus-20180723-CEH-08	33.663222	95.438731		Found next to CEH-09 and 10
7/23/18	Senlongrong	qhyus-20180723-CEH-09	33.663222	95.438731		Found next to CEH-08 and 10
7/23/18	Senlongrong	qhyus-20180723-CEH-10	33.663222	95.438731		Found next to CEH-08 and 09

Appendix I: Supplemental Material 8. Information for samples collected in Southwestern Mongolia.

Sample ID	Region	Study Site	Latitude	Longitude
MnMj001	Govi-Altay	eastern Govi-Altay province	45.1732	97.1437
MnMj002	Govi-Altay	eastern Govi-Altay province	45.1778	97.139
MnMj003	Govi-Altay	eastern Govi-Altay province	45.1778	97.139
MnMj004	Govi-Altay	eastern Govi-Altay province	45.1778	97.139
MnMj005	Govi-Altay	eastern Govi-Altay province	45.178	97.1381
MnMj006	Govi-Altay	eastern Govi-Altay province	45.1426	97.1342
MnMj008	Govi-Altay	eastern Govi-Altay province	45.1595	97.1347
MnMj009	Govi-Altay	eastern Govi-Altay province	45.2484	97.2424
MnMj010	Govi-Altay	eastern Govi-Altay province	45.2484	97.2424
MnMj011	Govi-Altay	eastern Govi-Altay province	45.2484	97.2423
MnMj012	Govi-Altay	eastern Govi-Altay province	45.2529	97.2577
MnMj013	Govi-Altay	eastern Govi-Altay province	45.2534	97.2574
MnMj014	Govi-Altay	eastern Govi-Altay province	45.2534	97.2574
MnMj015	Govi-Altay	eastern Govi-Altay province	45.2534	97.2574
MnMj016	Govi-Altay	eastern Govi-Altay province	45.2534	97.2574
MnMj017	Govi-Altay	eastern Govi-Altay province	45.2533	97.2573
MnMj018	Govi-Altay	eastern Govi-Altay province	45.2739	97.2349
MnMj019	Govi-Altay	eastern Govi-Altay province	45.2737	97.2351
MnMj020	Govi-Altay	eastern Govi-Altay province	45.2737	97.2351
MnMj021	Govi-Altay	eastern Govi-Altay province	45.2697	97.2401
MnMj022	Govi-Altay	eastern Govi-Altay province	45.2697	97.2401
MnMj023	Govi-Altay	eastern Govi-Altay province	45.2697	97.2401
MnMj024	Govi-Altay	eastern Govi-Altay province	45.2732	97.2378
MnMj025	Govi-Altay	eastern Govi-Altay province	45.2732	97.2378
MnMj026	Govi-Altay	eastern Govi-Altay province	45.2735	97.2369
MnMj027	Govi-Altay	eastern Govi-Altay province	45.2735	97.2369
MnMj028	Govi-Altay	eastern Govi-Altay province	45.2735	97.2369
MnMj029	Govi-Altay	eastern Govi-Altay province	45.2758	97.2344
MnMj030	Govi-Altay	eastern Govi-Altay province	45.2866	97.2319
MnMj031	Govi-Altay	eastern Govi-Altay province	45.2866	97.2319
MnMj032	Govi-Altay	eastern Govi-Altay province	45.2865	97.2318
MnMj033	Govi-Altay	eastern Govi-Altay province	45.2865	97.2318
MnMj034	Govi-Altay	eastern Govi-Altay province	45.2987	97.2353
MnMj035	Govi-Altay	eastern Govi-Altay province	45.2987	97.2353
MnMj036	Govi-Altay	eastern Govi-Altay province	45.2987	97.2353
MnMj037	Govi-Altay	eastern Govi-Altay province	45.2987	97.2353

Sample ID	Region	Study Site	Latitude	Longitude
MnMj038	Govi-Altay	eastern Govi-Altay province	45.2908	97.2295
MnMj039	Govi-Altay	eastern Govi-Altay province	45.2908	97.2295
MnMj040	Govi-Altay	eastern Govi-Altay province	45.2419	97.2526
MnMj041	Govi-Altay	eastern Govi-Altay province	45.2419	97.2526
MnMj042	Govi-Altay	eastern Govi-Altay province	45.242	97.2528
MnMj043	Govi-Altay	eastern Govi-Altay province	45.242	97.2528
MnMj044	Govi-Altay	eastern Govi-Altay province	45.1836	97.2608
MnMj045	Govi-Altay	eastern Govi-Altay province	45.3093	97.1389
MnMj046	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3093	97.1389
MnMj047	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3093	97.1389
MnMj048	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3093	97.1389
MnMj050	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3094	97.1386
MnMj051	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3094	97.1386
MnMj052	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3088	97.1383
MnMj053	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3087	97.14
MnMj054	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3103	97.1375
MnMj055	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3103	97.1375
MnMj056	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3163	97.1346
MnMj057	Govi-Altay	10km northeast of eastern Govi-Altay province	45.319	97.1363
MnMj058	Govi-Altay	10km northeast of eastern Govi-Altay province	45.319	97.1363
MnMj059	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3191	97.1365
MnMj060	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3191	97.1365
MnMj061	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3191	97.1388
MnMj062	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3191	97.1388
MnMj063	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3193	97.139
MnMj064	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3193	97.139
MnMj065	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3344	97.1314
MnMj066	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3344	97.1314
MnMj067	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj068	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj069	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj070	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj071	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj072	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj073	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3346	97.1315
MnMj075	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3395	97.1317
MnMj076	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3395	97.1317

Sample ID	Region	Study Site	Latitude	Longitude
MnMj077	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3395	97.1317
MnMj078	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3395	97.1317
MnMj079	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3395	97.1317
MnMj097	Govi-Altay	10km northeast of eastern Govi-Altay province	45.316	97.1444
MnMj098	Govi-Altay	10km northeast of eastern Govi-Altay province	45.316	97.1444
MnMj100	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3266	97.1493
MnMj101	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3266	97.1493
MnMj102	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3246	97.1493
MnMj103	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3246	97.1493
MnMj104	Govi-Altay	10km northeast of eastern Govi-Altay province	45.3246	97.1493
MnMj115	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.1928
MnMj116	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.1928
MnMj118	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.1928
MnMj119	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.1928
MnMj120	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.1928
MnMj121	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9785	100.1959
MnMj122	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9785	100.1959
MnMj123	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9785	100.1959
MnMj124	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.203
MnMj125	Govi-Altay	10km northeast of eastern Govi-Altay province	44.975	100.203
MnMj126	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9753	100.2041
MnMj127	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9753	100.2041
MnMj128	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9753	100.2041
MnMj129	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9753	100.2041
MnMj130	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9757	100.206
MnMj131	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9757	100.206
MnMj132	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9758	100.206
MnMj133	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9758	100.206
MnMj134	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9758	100.206
MnMj135	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9756	100.2048
MnMj141	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9462	100.2577
MnMj142	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9462	100.2577
MnMj143	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9462	100.2577
MnMj144	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9462	100.2577
MnMj145	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9462	100.2577
MnMj146	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9462	100.2575
MnMj149	Govi-Altay	10km northeast of eastern Govi-Altay province	44.9449	100.2442

Sample ID	Region	Study Site	Latitude	Longitude
MnMj150	Govi-Altay	10km northeast of eastern Govi-Altay province	44.945	100.2439
MnMj151	Govi-Altay	10km northeast of eastern Govi-Altay province	44.945	100.2439
MnMj152	Govi-Altay	10km northeast of eastern Govi-Altay province	44.945	100.2439
MnMj153	Govi-Altay	10km northeast of eastern Govi-Altay province	44.945	100.2439
MnMj154	Govi-Altay	central Bayanhongor Province	44.945	100.2439
MnMj155	Govi-Altay	central Bayanhongor Province	44.9451	100.2441
MnMj156	Govi-Altay	central Bayanhongor Province	44.9451	100.2441
MnMj157	Govi-Altay	central Bayanhongor Province	44.9451	100.2441
MnMj158	Govi-Altay	central Bayanhongor Province	44.9454	100.243
MnMj159	Govi-Altay	central Bayanhongor Province	44.9459	100.2417
MnMj160	Govi-Altay	central Bayanhongor Province	44.9459	100.2417
MnMj169	Ovorhangay	central Bayanhongor Province	44.7846	101.6328
MnMj177	Ovorhangay	central Bayanhongor Province	44.8308	101.5945
MnMj178	Ovorhangay	central Bayanhongor Province	44.8308	101.5945
MnMj179	Ovorhangay	central Bayanhongor Province	44.823	101.5851
MnMj180	Ovorhangay	central Bayanhongor Province	44.823	101.5851
MnMj181	Ovorhangay	central Bayanhongor Province	44.823	101.5851
MnMj183	Ovorhangay	central Bayanhongor Province	44.823	101.5846
MnMj184	Ovorhangay	central Bayanhongor Province	44.8231	101.5846
MnMj185	Ovorhangay	central Bayanhongor Province	44.8231	101.5846
MnMj186	Ovorhangay	central Bayanhongor Province	44.8231	101.5846
MnMj187	Ovorhangay	central Bayanhongor Province	44.8231	101.584
MnMj188	Ovorhangay	central Bayanhongor Province	44.8231	101.5835
MnMj189	Ovorhangay	central Bayanhongor Province	44.8231	101.5835
MnMj190	Ovorhangay	central Bayanhongor Province	47.3742	103.8445

Appendix I: Supplemental Material 9. Information pertaining to samples collected in China and Mongolia for landscape genetics analyses.

Sample Name	Population Name	Latitude	Longitude
TSH045	Tsaagan Turgen	50.27610	91.23392
TSH34	Tsaagan Turgen	50.25053	91.12779
TSH36	Tsaagan Turgen	50.25053	91.12779
TSH59	Tsaagan Turgen	50.25949	91.11188
TSH77	Tsaagan Turgen	50.28355	91.11361
TS13B	Tsaagan Turgen	-	-
TS19B	Tsaagan Turgen	49.39722	91.16444
TU003B	Tsaagan Turgen	49.41222	91.16583
TU003	Tsaagan Turgen	49.34111	91.180556
TU007	Tsaagan Turgen	49.47638	91.16583
TU019B	Tsaagan Turgen	49.39722	91.16444
TU024B	Tsaagan Turgen	49.78277	91.17444
SLJAR16	Jargalant Bumbat	47.61948	92.52560
SLJAR19	Jargalant Bumbat	47.62271	92.52757
SLJAR2	Jargalant Bumbat	47.57097	92.56608
SLJAR22	Jargalant Bumbat	47.63631	92.53319
SLJAR3	Jargalant Bumbat	-	-
SLJAR35	Jargalant Bumbat	47.55006	92.58719
MH004M	Jargalant Bumbat	-	-
MH006M	Jargalant Bumbat	-	-
M38	Jargalant Bumbat	47.57443	92.57291
SLBUM02	Jargalant Bumbat	47.37081	92.92378
SLBUM16	Jargalant Bumbat	47.38892	92.04081
SLBUM18	Jargalant Bumbat	47.38408	93.03635
SLBUM20	Jargalant Bumbat	47.38486	93.06050
MnMJ013	Burkhan Buudai	45.2534	97.2574
MnMJ014	Burkhan Buudai	45.2534	97.2574
MnMJ022	Burkhan Buudai	45.2697	97.2401
MnMJ095	Burkhan Buudai	-	-
MnMJ096	Burkhan Buudai	-	-
MnMJ113	Burkhan Buudai	-	-
MnMJ157	Tergun Bogd	44.9451	100.2441
MnMJ158	Tergun Bogd	44.9454	100.243
MnMJ163	Tergun Bogd	-	-
MnMJ137	Tergun Bogd	-	-
MnMJ140	Tergun Bogd	-	-
MnMJ182	Mayangan Yamaat (Baga Bogd)	-	-
MnMJ186	Mayangan Yamaat (Baga Bogd)	44.8231	101.5846
MnMJ187	Mayangan Yamaat (Baga Bogd)	44.8231	101.584
BABM31	Mayangan Yamaat (Baga Bogd)	-	-
BABM59	Mayangan Yamaat (Baga Bogd)	-	-
CO35	Mayangan Yamaat (Baga Bogd)	44.9409	101.52176
CO37	Mayangan Yamaat (Baga Bogd)	44.93765	101.62374
SCT001rA	Mayangan Yamaat (Baga Bogd)	44.9051	101.46031
ABM19	Mayangan Yamaat (Baga Bogd)	44.43577	102.04642
ABM46	Mayangan Yamaat (Baga Bogd)	44.52221	102.58220
SCTB40	Mayangan Yamaat (Baga Bogd)	-	-
BABM50	Mayangan Yamaat (Baga Bogd)	-	-
BBJ014	Gurvan Saikhan	43.87522	102.51924
BBJ17	Gurvan Saikhan	43.87693	102.51628



Sample Name	Population Name	Latitude	Longitude
EBJ19	Gurvan Saikhan	-	-
EBJ22	Gurvan Saikhan	43.46601	104.18143
WBM025	Gurvan Saikhan	43.46601	104.18143
WBM007	Gurvan Saikhan	43.84595	103.2389
WBM41	Gurvan Saikhan	-	-
NB03	Tost Noyon	43.082400	101.992700
NB05	Tost Noyon	43.082400	101.992650
NB12	Tost Noyon	43.151767	102.027233
NB27	Tost Noyon	43.215217	101.939000
TJ10mn	Tost Noyon	43.175917	100.578200
TA01mn	Tost Noyon	43.172278	100.570056
TNB01	Tost Noyon	43.240100	100.343450
TNB06	Tost Noyon	43.239100	100.339950
SBC54	Yanchiwan	39.069973	96.892471
SBC74	Yanchiwan	-	-
SBC30	Yanchiwan	39.071909	96.908787
SBC75	Yanchiwan	-	-
SBC83	Yanchiwan	39.00021683	96.87870317
SBC66	Yanchiwan	39.896446	96.896445
SBC55	Yanchiwan	39.00806626	96.90220607
SBC29	Yanchiwan	39.071909	96.908787
SBC63	Yanchiwan	39.069973	96.892471
SBC08	Yanchiwan	39.091812	96.956743
SBC90	Yanchiwan	39.001057	96.885591
SBC68	Yanchiwan	38.99118193	96.89350345
SBC11	Yanchiwan	39.090209	96.957599
SBC07	Yanchiwan	39.091812	96.956743
SBC64	Yanchiwan	39.9922558	96.89833202
QLS34	Qilian Shan	38.21628	100.52988
QLS36	Qilian Shan	38.7764	97.769261
QLS73	Qilian Shan	38.35599	99.15693
QLS75	Qilian Shan	38.18485	99.67938
QS37	Qilian Shan	-	-
QS31	Qilian Shan	38.64463	98.84902
QS16	Qilian Shan	-	-
QS17	Qilian Shan	-	-
AKS34	Akesai	38.52015	94.99235
AKS53	Akesai	38.391267	95.254967
AKS64	Akesai	38.391267	95.254967
H4	East Burhanbuda Mountain	-	-
H6	East Burhanbuda Mountain	-	-
H8	East Burhanbuda Mountain	-	-
DUL50	East Burhanbuda Mountain	-	-
DUL53	East Burhanbuda Mountain	35.65833333	98.7361111
DUL123	East Burhanbuda Mountain	36.096544	96.703657
DUL09	East Burhanbuda Mountain	35.648475	98.464423
DUL47	East Burhanbuda Mountain	35.65472222	98.54083333
DUL100	East Burhanbuda Mountain	35.912015	97.174806

## Appendix II: Supplementary Tables

Appendix II: Supplemental Material 1. Results of population assignment tests performed in GenAlEx when samples were divided into the three proposed subspecies as delineated by Janecka et al. (2017). *Italicized* samples are those which were added to the original dataset.

Sample	Population	Central	Western	Northern	Assigned Population	
TJ04cn	Central	-23.152	-35.829	-34.064	1	Central
TJ01cn	Central	-27.320	-36.581	-37.045	1	Central
AKS34	Central	-31.142	-38.430	-40.020	1	Central
<i>DUL09</i>	<i>Central</i>	<i>-29.331</i>	<i>-42.818</i>	<i>-48.167</i>	<i>1</i>	<i>Central</i>
<i>DUL123</i>	<i>Central</i>	<i>-22.910</i>	<i>-39.007</i>	<i>-46.464</i>	<i>1</i>	<i>Central</i>
<i>DUL47</i>	<i>Central</i>	<i>-35.700</i>	<i>-49.535</i>	<i>-49.619</i>	<i>1</i>	<i>Central</i>
H4	Central	-20.112	-29.632	-30.862	1	Central
H6	Central	-23.487	-35.735	-39.185	1	Central
H7	Central	-17.417	-24.630	-26.842	1	Central
H8	Central	-29.784	-41.055	-44.691	1	Central
<i>SBC29</i>	<i>Central</i>	<i>-28.551</i>	<i>-38.379</i>	<i>-49.948</i>	<i>1</i>	<i>Central</i>
<i>SBC30</i>	<i>Central</i>	<i>-35.580</i>	<i>-48.204</i>	<i>-58.343</i>	<i>1</i>	<i>Central</i>
<i>SBC54</i>	<i>Central</i>	<i>-30.168</i>	<i>-42.271</i>	<i>-48.265</i>	<i>1</i>	<i>Central</i>
<i>SBC55</i>	<i>Central</i>	<i>-32.519</i>	<i>-45.956</i>	<i>-52.644</i>	<i>1</i>	<i>Central</i>
NQ104	Central	-21.436	-34.500	-37.433	1	Central
NQ197	Central	-26.822	-38.824	-39.819	1	Central
NG202	Central	-31.151	-48.029	-49.041	1	Central
ZH301	Central	-25.357	-43.083	-42.671	1	Central
ZD13	Central	-20.748	-29.169	-37.716	1	Central
<i>SUJ11</i>	<i>Central</i>	<i>-27.982</i>	<i>-37.805</i>	<i>-43.535</i>	<i>1</i>	<i>Central</i>
<i>SUJ15</i>	<i>Central</i>	<i>-26.360</i>	<i>-38.227</i>	<i>-45.660</i>	<i>1</i>	<i>Central</i>
<i>SUJ17</i>	<i>Central</i>	<i>-29.216</i>	<i>-47.541</i>	<i>-51.303</i>	<i>1</i>	<i>Central</i>
<i>SUJ25</i>	<i>Central</i>	<i>-27.715</i>	<i>-42.711</i>	<i>-53.704</i>	<i>1</i>	<i>Central</i>
<i>SUJ26</i>	<i>Central</i>	<i>-29.219</i>	<i>-41.702</i>	<i>-51.165</i>	<i>1</i>	<i>Central</i>
<i>LXC040</i>	<i>Central</i>	<i>-17.267</i>	<i>-24.545</i>	<i>-29.919</i>	<i>1</i>	<i>Central</i>
<i>CT10</i>	<i>Central</i>	<i>-21.978</i>	<i>-31.023</i>	<i>-38.935</i>	<i>1</i>	<i>Central</i>
<i>CT11</i>	<i>Central</i>	<i>-34.283</i>	<i>-36.130</i>	<i>-42.142</i>	<i>1</i>	<i>Central</i>
<i>CT12</i>	<i>Central</i>	<i>-27.710</i>	<i>-31.337</i>	<i>-42.609</i>	<i>1</i>	<i>Central</i>
<i>CT2</i>	<i>Central</i>	<i>-20.546</i>	<i>-31.458</i>	<i>-39.022</i>	<i>1</i>	<i>Central</i>
<i>CT3</i>	<i>Central</i>	<i>-25.166</i>	<i>-30.120</i>	<i>-40.423</i>	<i>1</i>	<i>Central</i>
<i>CT7</i>	<i>Central</i>	<i>-21.736</i>	<i>-23.953</i>	<i>-33.224</i>	<i>1</i>	<i>Central</i>
SZ10	Central	-21.869	-27.662	-32.669	1	Central
SZ23	Central	-25.628	-30.033	-38.889	1	Central
SL64	Central	-24.418	-31.879	-34.267	1	Central

Sample	Population	Central	Western	Northern	Assigned Population	
SL99	Central	-27.391	-37.111	-43.325	1	Central
SL121	Central	-25.541	-35.673	-43.234	1	Central
SL242	Central	-25.400	-34.465	-37.180	1	Central
NP28	Central	-17.150	-21.884	-28.670	1	Central
NP38	Central	-20.183	-31.896	-33.595	1	Central
NP40	Central	-24.295	-34.872	-39.613	1	Central
NP44	Central	-23.960	-30.434	-33.560	1	Central
NJK105	Central	-15.956	-23.944	-24.982	1	Central
NJK113	Central	-25.049	-33.651	-39.095	1	Central
LD03	Western	-37.850	-28.899	-46.131	2	Western
LD04	Western	-28.518	-19.705	-28.527	2	Western
LD09	Western	-32.553	-21.374	-29.924	2	Western
LD18	Western	-33.285	-24.858	-37.502	2	Western
SCT52	Western	-35.927	-30.376	-39.342	2	Western
SCT77	Western	-40.161	-30.489	-43.994	2	Western
SCT83	Western	-30.387	-23.949	-34.915	2	Western
SCT90	Western	-37.094	-23.644	-32.980	2	Western
RTJ12	Western	-45.617	-31.176	-40.968	2	Western
RTJ14	Western	-35.540	-27.988	-31.395	2	Western
RTJ16	Western	-38.692	-33.356	-42.397	2	Western
RTJ18	Western	-39.758	-28.671	-39.263	2	Western
<i>KSZH10A</i>	<i>Western</i>	<i>-42.493</i>	<i>-23.525</i>	<i>-29.546</i>	2	<i>Western</i>
<i>KZSH21A</i>	<i>Western</i>	<i>-38.816</i>	<i>-26.688</i>	<i>-36.221</i>	2	<i>Western</i>
<i>KZSH2A</i>	<i>Western</i>	<i>-36.387</i>	<i>-20.165</i>	<i>-23.615</i>	2	<i>Western</i>
<i>KZSH74A</i>	<i>Western</i>	<i>-38.034</i>	<i>-25.109</i>	<i>-36.668</i>	2	<i>Western</i>
<i>KZSH78A</i>	<i>Western</i>	<i>-36.067</i>	<i>-26.632</i>	<i>-33.756</i>	2	<i>Western</i>
S3ky21	Western	-35.361	-22.749	-31.610	2	Western
S4ky	Western	-33.414	-21.363	-26.609	2	Western
S7kyG1	Western	-35.211	-23.001	-27.655	2	Western
SO2ky	Western	-34.743	-26.755	-33.590	2	Western
TSH34	Northern	-38.118	-34.376	-19.794	3	Northern
TSH36	Northern	-40.011	-32.770	-26.062	3	Northern
TSH59	Northern	-37.460	-29.633	-25.310	3	Northern
TSH77	Northern	-35.430	-30.707	-21.314	3	Northern
TS13B	Northern	-37.229	-30.714	-18.619	3	Northern
TS19B	Northern	-35.407	-29.803	-20.434	3	Northern
TU003	Northern	-40.055	-34.550	-20.575	3	Northern
TU007	Northern	-30.025	-23.885	-18.375	3	Northern
TU003B	Northern	-33.723	-26.528	-19.126	3	Northern
TU019B	Northern	-42.143	-32.455	-19.586	3	Northern

Sample	Population	Central	Western	Northern	Assigned Population	
TU024B	Northern	-41.189	-33.070	-24.673	3	Northern
MH004M	Northern	-28.008	-28.968	-22.057	3	Northern
M38	Northern	-44.425	-34.427	-26.504	3	Northern
RJaltM2	Northern	-43.906	-36.679	-23.456	3	Northern
RJaltM4	Northern	-31.421	-25.571	-18.804	3	Northern
BABM31	Northern	-37.059	-32.992	-26.877	3	Northern
BABM59	Northern	-37.359	-37.266	-23.812	3	Northern
ABM19	Northern	-41.459	-38.752	-22.823	3	Northern
ABM46	Northern	-46.259	-41.479	-31.744	3	Northern
WBM41	Northern	-36.715	-29.396	-17.424	3	Northern
BBJ014	Northern	-33.771	-30.150	-15.194	3	Northern
EBJ19	Northern	-26.650	-20.158	-14.332	3	Northern
TA01mn	Northern	-31.982	-28.479	-20.802	3	Northern
TJ10mn	Northern	-32.920	-27.372	-21.400	3	Northern
TNB01	Northern	-44.132	-37.193	-24.362	3	Northern
TNB06	Northern	-43.548	-34.782	-18.282	3	Northern
NB03	Northern	-35.408	-29.456	-16.376	3	Northern
NB05	Northern	-26.941	-23.609	-20.580	3	Northern
NB12	Northern	-52.691	-43.515	-27.675	3	Northern
NB27	Northern	-48.221	-45.830	-28.621	3	Northern

Appendix II: Supplemental Material 2. Results of population assignment test performed in GenAlEx when samples were divided into the seven sampling regions as delineated by Janecka et al. (2017). *Italicized* samples are those which were added to the original dataset.

Sample	Population	NQ	SQ	HIM	IP	TK	WM	SM	Assigned Pop	
TJ04cn	NQ	-21.873	-26.713	-28.656	-41.308	-37.173	-38.783	-34.901	1	NQ
TJ01cn	NQ	-26.621	-34.497	-29.912	-37.286	-42.786	-41.972	-37.968	1	NQ
AKS34	NQ	-31.680	-35.447	-37.718	-41.197	-42.298	-42.791	-42.362	1	NQ
<i>DUL09</i>	<i>NQ</i>	<i>-28.926</i>	<i>-30.796</i>	<i>-37.077</i>	<i>-49.000</i>	<i>-44.131</i>	<i>-51.797</i>	<i>-51.713</i>	<i>1</i>	<i>NQ</i>
<i>DUL123</i>	<i>NQ</i>	<i>-25.986</i>	<i>-24.841</i>	<i>-29.829</i>	<i>-42.025</i>	<i>-42.977</i>	<i>-47.847</i>	<i>-49.795</i>	<i>2</i>	<i>SQ</i>
<i>DUL47</i>	<i>NQ</i>	<i>-34.470</i>	<i>-37.223</i>	<i>-45.475</i>	<i>-53.981</i>	<i>-52.125</i>	<i>-52.429</i>	<i>-52.067</i>	<i>1</i>	<i>NQ</i>
H4	NQ	-20.180	-27.599	-25.021	-33.534	-31.614	-32.555	-34.336	1	NQ
H6	NQ	-24.693	-30.538	-28.750	-41.190	-37.412	-40.786	-44.208	1	NQ
H7	NQ	-18.390	-15.254	-19.637	-24.150	-26.040	-28.166	-27.481	2	SQ
H8	NQ	-30.060	-34.796	-37.246	-41.713	-43.845	-47.045	-46.888	1	NQ
<i>SBC29</i>	<i>NQ</i>	<i>-28.203</i>	<i>-32.805</i>	<i>-33.440</i>	<i>-44.050</i>	<i>-41.169</i>	<i>-51.544</i>	<i>-52.760</i>	<i>1</i>	<i>NQ</i>
<i>SBC30</i>	<i>NQ</i>	<i>-31.106</i>	<i>-44.461</i>	<i>-47.950</i>	<i>-54.008</i>	<i>-49.965</i>	<i>-59.261</i>	<i>-61.969</i>	<i>1</i>	<i>NQ</i>
<i>SBC54</i>	<i>NQ</i>	<i>-30.326</i>	<i>-30.119</i>	<i>-41.226</i>	<i>-48.522</i>	<i>-45.284</i>	<i>-49.321</i>	<i>-51.723</i>	<i>2</i>	<i>SQ</i>
<i>SBC55</i>	<i>NQ</i>	<i>-30.265</i>	<i>-36.873</i>	<i>-42.609</i>	<i>-48.247</i>	<i>-50.232</i>	<i>-55.511</i>	<i>-56.056</i>	<i>1</i>	<i>NQ</i>
NQ104	SQ	-21.049	-23.182	-28.457	-38.005	-36.792	-37.665	-40.798	1	NQ
NQ197	SQ	-28.891	-25.020	-32.350	-38.851	-44.502	-40.935	-44.164	2	SQ
NG202	SQ	-32.848	-30.707	-40.803	-47.919	-52.886	-48.856	-56.004	2	SQ
ZH301	SQ	-26.919	-22.725	-34.937	-45.949	-49.087	-41.498	-51.992	2	SQ
ZD13	SQ	-25.571	-19.492	-22.951	-28.458	-36.384	-39.843	-41.994	2	SQ
<i>SUJ11</i>	<i>SQ</i>	<i>-30.800</i>	<i>-25.752</i>	<i>-34.520</i>	<i>-38.431</i>	<i>-47.032</i>	<i>-44.989</i>	<i>-48.538</i>	<i>2</i>	<i>SQ</i>
<i>SUJ15</i>	<i>SQ</i>	<i>-28.882</i>	<i>-24.931</i>	<i>-30.786</i>	<i>-40.901</i>	<i>-42.942</i>	<i>-49.150</i>	<i>-47.891</i>	<i>2</i>	<i>SQ</i>
<i>SUJ17</i>	<i>SQ</i>	<i>-32.470</i>	<i>-31.368</i>	<i>-37.193</i>	<i>-50.584</i>	<i>-51.450</i>	<i>-50.571</i>	<i>-59.162</i>	<i>2</i>	<i>SQ</i>
<i>SUJ25</i>	<i>SQ</i>	<i>-33.163</i>	<i>-26.139</i>	<i>-32.458</i>	<i>-48.352</i>	<i>-44.574</i>	<i>-51.910</i>	<i>-62.777</i>	<i>2</i>	<i>SQ</i>
<i>SUJ26</i>	<i>SQ</i>	<i>-30.947</i>	<i>-26.052</i>	<i>-36.376</i>	<i>-45.767</i>	<i>-44.249</i>	<i>-52.329</i>	<i>-56.903</i>	<i>2</i>	<i>SQ</i>

Sample	Population	NQ	SQ	HIM	IP	TK	WM	SM	Assigned Pop	
LXC040	HIM	-20.046	-20.993	-18.734	-24.012	-26.392	-29.444	-34.140	3	HIM
CT10	HIM	-27.549	-29.336	-20.203	-32.804	-34.280	-40.892	-42.666	3	HIM
CT11	HIM	-40.193	-43.678	-30.105	-37.575	-40.164	-46.859	-46.054	3	HIM
CT12	HIM	-33.629	-37.125	-25.740	-36.751	-34.095	-44.458	-47.823	3	HIM
CT2	HIM	-24.891	-27.560	-21.219	-35.704	-32.238	-41.273	-40.634	3	HIM
CT3	HIM	-31.257	-32.372	-23.567	-28.570	-38.617	-42.560	-44.325	3	HIM
CT7	HIM	-27.335	-27.972	-19.542	-22.788	-28.936	-35.722	-39.192	3	HIM
SZ10	HIM	-27.052	-31.264	-19.365	-27.765	-30.609	-33.364	-37.163	3	HIM
SZ23	HIM	-31.478	-33.052	-24.856	-35.488	-29.556	-42.609	-41.432	3	HIM
SL64	HIM	-28.997	-31.759	-26.561	-33.778	-34.588	-37.851	-35.608	3	HIM
SL99	HIM	-35.763	-37.788	-24.265	-39.758	-43.138	-44.275	-49.393	3	HIM
SL121	HIM	-29.240	-33.731	-24.728	-41.009	-38.278	-45.727	-46.216	3	HIM
SL242	HIM	-32.582	-35.242	-21.453	-37.388	-37.843	-39.153	-40.183	3	HIM
NP28	HIM	-22.081	-22.674	-14.925	-19.921	-26.977	-32.474	-30.151	3	HIM
NP38	HIM	-27.406	-29.276	-18.132	-32.377	-39.229	-36.566	-37.198	3	HIM
NP40	HIM	-33.715	-29.849	-22.300	-38.216	-39.442	-41.626	-45.707	3	HIM
NP44	HIM	-30.825	-33.215	-20.555	-30.656	-38.036	-38.815	-35.197	3	HIM
NJK105	HIM	-20.554	-21.803	-14.958	-25.012	-26.120	-22.675	-30.978	3	HIM
NJK113	HIM	-33.857	-32.370	-21.413	-34.112	-39.367	-42.620	-43.079	3	HIM
LD03	IP	-41.674	-38.928	-39.887	-25.282	-36.399	-45.107	-51.263	4	IP
LD04	IP	-30.858	-32.130	-28.538	-16.371	-26.970	-28.310	-31.623	4	IP
LD09	IP	-34.767	-39.463	-33.017	-18.050	-27.761	-32.097	-32.146	4	IP
LD18	IP	-37.066	-41.275	-33.339	-24.374	-30.588	-38.791	-42.603	4	IP
SCT52	IP	-42.286	-43.647	-35.376	-29.659	-36.609	-42.055	-43.118	4	IP
SCT77	IP	-44.520	-47.061	-40.124	-36.106	-35.852	-47.168	-45.859	5	TK
SCT83	IP	-36.237	-38.519	-28.978	-22.419	-31.829	-37.045	-39.364	4	IP

Sample	Population	NQ	SQ	HIM	IP	TK	WM	SM	Assigned Pop	
SCT90	IP	-45.048	-42.794	-36.647	-23.543	-31.534	-33.248	-37.845	4	IP
RTJ12	TK	-51.121	-57.564	-44.810	-39.939	-32.731	-41.908	-46.584	5	TK
RTJ14	TK	-40.336	-45.413	-35.022	-30.541	-31.566	-31.055	-35.509	4	IP
RTJ16	TK	-43.737	-47.193	-40.170	-41.494	-35.429	-41.728	-50.106	5	TK
RTJ18	TK	-42.651	-45.815	-41.077	-29.886	-32.243	-40.123	-42.524	4	IP
<i>KSZH10A</i>	<i>TK</i>	<i>-51.807</i>	<i>-50.448</i>	<i>-41.519</i>	<i>-40.429</i>	<i>-19.698</i>	<i>-32.337</i>	<i>-34.199</i>	5	<i>TK</i>
<i>KZSH21A</i>	<i>TK</i>	<i>-43.436</i>	<i>-44.609</i>	<i>-40.247</i>	<i>-37.528</i>	<i>-27.545</i>	<i>-37.888</i>	<i>-42.730</i>	5	<i>TK</i>
<i>KZSH2A</i>	<i>TK</i>	<i>-43.772</i>	<i>-43.246</i>	<i>-37.038</i>	<i>-32.119</i>	<i>-17.290</i>	<i>-24.378</i>	<i>-26.120</i>	5	<i>TK</i>
<i>KZSH74A</i>	<i>TK</i>	<i>-46.707</i>	<i>-45.570</i>	<i>-35.574</i>	<i>-38.691</i>	<i>-22.201</i>	<i>-39.790</i>	<i>-40.853</i>	5	<i>TK</i>
<i>KZSH78A</i>	<i>TK</i>	<i>-41.907</i>	<i>-41.908</i>	<i>-36.836</i>	<i>-39.865</i>	<i>-23.466</i>	<i>-33.362</i>	<i>-40.384</i>	5	<i>TK</i>
S3ky21	TK	-46.773	-43.326	-32.944	-35.515	-20.474	-30.887	-39.357	5	TK
S4ky	TK	-40.350	-37.836	-31.687	-30.946	-19.064	-27.051	-28.739	5	TK
S7kyG1	TK	-45.993	-46.114	-33.075	-36.393	-20.395	-25.037	-36.283	5	TK
SO2ky	TK	-42.682	-42.616	-34.660	-34.727	-25.762	-36.145	-36.138	5	TK
TSH34	WM	-42.767	-47.325	-41.613	-39.916	-35.892	-19.690	-25.382	6	WM
TSH36	WM	-47.649	-47.767	-42.058	-43.591	-32.899	-22.785	-35.043	6	WM
TSH59	WM	-45.005	-52.202	-35.646	-40.716	-29.277	-24.051	-31.032	6	WM
TSH77	WM	-39.890	-43.364	-39.014	-38.133	-31.905	-17.632	-30.279	6	WM
TS13B	WM	-42.741	-46.627	-39.630	-36.439	-32.160	-18.086	-24.656	6	WM
TS19B	WM	-40.316	-44.444	-37.484	-38.501	-30.359	-16.938	-28.119	6	WM
TU003	WM	-43.859	-51.255	-44.497	-44.488	-34.179	-18.120	-28.573	6	WM
TU007	WM	-36.922	-37.609	-29.295	-31.257	-23.175	-15.479	-25.343	6	WM
TU003B	WM	-42.689	-42.808	-32.889	-36.589	-27.097	-22.805	-19.823	7	SM
TU019B	WM	-47.342	-49.990	-46.142	-37.275	-32.798	-20.695	-21.806	6	WM
TU024B	WM	-45.157	-53.603	-42.608	-40.953	-34.628	-25.186	-28.245	6	WM
MH004M	WM	-34.636	-36.053	-31.385	-35.546	-30.405	-20.254	-28.776	6	WM

Sample	Population	NQ	SQ	HIM	IP	TK	WM	SM	Assigned Pop	
M38	WM	-46.492	-57.608	-46.485	-40.057	-33.661	-27.633	-28.809	6	WM
RJaltM2	WM	-52.504	-57.068	-43.811	-45.505	-34.981	-20.509	-30.174	6	WM
RJaltM4	WM	-36.843	-40.938	-33.830	-30.843	-25.632	-16.128	-23.996	6	WM
BABM31	SM	-39.754	-45.198	-40.111	-45.671	-30.302	-36.096	-24.767	7	SM
BABM59	SM	-41.014	-43.187	-41.142	-47.103	-37.348	-29.609	-24.127	7	SM
ABM19	SM	-45.656	-41.754	-46.903	-41.948	-44.751	-28.582	-20.926	7	SM
ABM46	SM	-46.571	-46.820	-52.905	-46.968	-43.299	-35.766	-33.059	7	SM
WBM41	SM	-42.237	-48.568	-37.174	-41.156	-28.260	-21.994	-18.615	7	SM
BBJ014	SM	-40.316	-40.524	-36.341	-38.202	-31.616	-19.807	-13.471	7	SM
EBJ19	SM	-31.339	-37.547	-25.286	-26.436	-20.264	-14.700	-16.363	6	WM
TA01mn	SM	-36.001	-40.603	-33.375	-34.772	-30.330	-27.041	-18.363	7	SM
TJ10mn	SM	-36.356	-46.172	-33.630	-31.941	-29.790	-25.461	-22.322	7	SM
TNB01	SM	-48.271	-52.438	-45.098	-47.760	-36.122	-30.456	-21.928	7	SM
TNB06	SM	-49.143	-52.206	-46.117	-43.342	-36.147	-23.504	-16.205	7	SM
NB03	SM	-39.056	-43.649	-36.712	-38.015	-29.510	-21.127	-15.340	7	SM
NB05	SM	-30.084	-39.256	-26.296	-24.526	-26.925	-23.676	-20.038	7	SM
NB12	SM	-58.325	-59.302	-55.781	-50.575	-43.579	-33.487	-24.672	7	SM
NB27	SM	-51.196	-56.206	-49.833	-51.897	-47.090	-33.914	-26.047	7	SM



Appendix II: Supplemental Material 3. Species compiled into the reference file used for MT-CO1 and MT-RNR1 read mapping.

Genetic Marker Examined	Common Name	Scientific Name
MT-CO1	domestic sheep	<i>Ovis aries</i>
	argali	<i>Ovis ammon</i>
	blue sheep	<i>Pseudois nayaur</i>
	domestic goat	<i>Capra aegagrus hircus</i>
	Siberian ibex	<i>Capra sibirica</i>
	markhor	<i>Capra falconeri</i>
	alpine ibex	<i>Capra ibex</i>
	Himalayan tahr	<i>Hemitragus jemlahicus</i>
Tibetan gazelle	<i>Procapra picticaudata</i>	
MT-RNR1	domestic sheep	<i>Ovis aries</i>
	argali	<i>Ovis ammon</i>
	blue sheep	<i>Pseudois nayaur</i>
	domestic goat	<i>Capra aegagrus hircus</i>
	Siberian ibex	<i>Capra sibirica</i>
	markhor	<i>Capra falconeri</i>
	Himalayan tahr	<i>Hemitragus jemlahicus</i>
	musk deer	<i>Moschus moschiferus</i>
	marmot	<i>Marmota himalayana</i>
	mountain hare	<i>Lepus timidus</i>
	northern pika	<i>Ochotona hyperborea</i>
	plateau pika	<i>Ochotona curzoniae</i>
	house mouse	<i>Mus musculus</i>
	snowcock	<i>Tetraogallus</i>
	rhesus macaque	<i>Macaca mulatta</i>
	domestic yak	<i>Bos grunniens</i>
	dromedary camel	<i>Camelus dromedarius</i>
	snow leopard	<i>Panthera uncia</i>
	Pallas's cat	<i>Otocolobus manul</i>
	Eurasian lynx	<i>Lynx lynx</i>
wolf	<i>Canis lupus</i>	
domestic dog	<i>Canis familiaris</i>	
red fox	<i>Vulpes vulpes</i>	
brown bear	<i>Ursus arctos</i>	
Eurasian badger	<i>Meles meles</i>	
human	<i>Homo sapiens</i>	

Appendix II: Supplemental Material 4. The accession numbers and respective taxa designation used to design MT-CO1 primers.

Species	Accession Numbers				
<i>Capra falconeri</i>	NC_020622.1				
<i>Hemitragus jemlahicus</i>	KF317915	KF317904			
<i>Ovis ammon</i>	HM236188.1	KT781689.1	JX101654.1		
<i>Ovis ammon darwini</i>	KX609626.1				
<i>Capra sibirica</i>	AB743816	NC_020626.1	AB743816.1		
	KP231536	KM360063	KC679018	KC679017	KM998968
	JQ735456	KC679016	KF317905	KP195269	JQ735457
	KP273589	KP677510	KP677511	KF317903	KP662716
	KJ920215	KJ920216	KJ920217	KJ920218	KJ920219
<i>Capra aegagrus hircus</i>	AB735755	AB735758	AB735759	AB735766	AB735767
	AB735769	AB735775	AB735776	AB735780	AB736098
	JN245994	HQ269428	AB736109	HQ269434	HQ603176
	AB736122	AF533441	HQ269452	HQ269437	HQ269451
	GU229279	GU229280	GU229281	FJ958337	
	KF938358	KF938355	KF938350	KF938349	KF317902
	KC669588	KC669582	KF938348	KF938342	KF938341
	KF938340	KF938339	KF938335	KF938333	KF938332
<i>Ovis aries</i>	KF938331	KF938329	KF938327	KF938326	KF938325
	KP998472	KP998471	JX567087	DQ320096	HE577847
	HE577850	JN245995	EF092411	KP998470	HQ603178
	DQ320097	JN850772	FJ958344	EU834864	EF092405
	EF092406	EF490451	EF092408	EF490456	AY858379
<i>Pseudois nayaur</i>	HQ269457	HQ269459	KP998469		
<i>Procapra picticaudata</i>	KC679009	KC679008	KC679005	KC679004	KC678998
	KJ954145				

Appendix II: Supplemental Material 5. Comparison between two methods used for molecular dietary analysis after an initial run returned a substantial number of occurrences with domestic mouse (*Mus musculus*). The first method was use of the Illumina NextSeq 500 accompanied by gel excision. The second was use of the Illumina MiSeq with the use of bead purification.

Sample	NextSeq # of Sequences	MiSeq # of Sequences	NextSeq # of Mapped Reads	MiSeq # of Mapped Reads	NextSeq Species ID	MiSeq Prey Species ID
KZSH8	124,382	102,835	83,466	45,447	mouse	mouse
KZSH10	142,944	132,139	87,848	82,607	mouse	none
SLBUM10	93,453	118,982	75,439	49,908	mouse	marmot
SLBUM18	88,492	128,923	77,037	43,752	mouse	mouse
SLBUM20	92,972	136,506	68,262	59,596	mouse	domestic sheep
SLBUM21	112,704	63,629	88,861	11,324	mouse	Siberian ibex
SLJAR25	142,677	98,293	101,703	56,427	mouse	none
SLJAR36	160,430	112,058	131,597	61,800	mouse	Siberian ibex
WBM042	121,088	199,474	166,917	24,813	mouse	pika
WBM025	49,845	159,775	40,658	60,435	mouse	argali
SCT12	265,282	142,586	166,465	61,201	mouse	domestic sheep
SCT23	294,653	144,420	177,787	47,095	mouse	none
SCT31	54,065	81,815	31,611	12,376	mouse	blue sheep
SCT34	36,007	160,094	30,969	84,351	mouse	markhor
SCT52	125,918	182,487	92,478	30,661	mouse	mouse
SCT74	87,499	214,576	72,353	39,501	mouse	markhor
SCT80	79,560	118,074	58,501	42,222	mouse	markhor
CT12	160,652	316,383	134,433	77,725	mouse	argali
Average	124,035	145,169	93,688	49,513	-	-

## Appendix III: Interview Sheets

Appendix III: Supplemental Material 1. The interview sheet used in the study, Hacker *et al.* (2020) *Determinants of herder attitudes towards the threatened snow leopard (Panthera uncia) in Yushu Prefecture, China.* Oryx. DOI:10.1017/S0030605319001315.

Please mark how much you agree with each sentence. 请标记你对每个问题的同意程度	Strongly Disagree 强烈不同意				Strongly Agree 强烈同意			
I am concerned that snow leopards will kill my animals 我担心雪豹会杀死我的动物	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
We need to keep snow leopards safe. 我们需要保护雪豹.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Snow leopards are important to my religion. 雪豹在我的宗教信仰里很重要	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I am afraid of snow leopards. 我害怕雪豹	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
We need snow leopards in the wild. 我们需要野外有雪豹	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I enjoy seeing snow leopards. 我喜欢看雪豹	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Please mark how much you agree with each sentence. 请标记你对每个问题的同意程度	None 零				Many 多			
How many snow leopards are in the wild. 野外有多少雪豹	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
How many blue sheep are in the wild. 野外有多少岩羊	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
How many argali are in the wild. 野外有多少盘羊	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Are your animals your main source of money? 你的动物是你收入的主要来源? Yes 是的    No 不是的

If no, what is your main source of money? 如果不是, 那你的主要收入来源是: \_\_\_\_\_

How many of each animal do you own?    Yak 牦牛    Goat 山羊    Sheep 绵羊    Horse 马    Other 其他

你饲养有多少动物?                      \_\_\_\_\_    \_\_\_\_\_    \_\_\_\_\_    \_\_\_\_\_    \_\_\_\_\_

<b>Please circle all that apply to how you care for your animals: 请在你采用的动物管理方式上画圈</b>	My animals are guarded during the day. 动物整天被看守	My animals are kept in predator-proof corrals. 动物关在防捕食的畜栏
	I have animal insurance. 有动物保险	My animals wander unattended during the day. 整天无人看管

Have you lost any of your animals to snow leopards in the last 5 years? 过去 5 年, 你的家畜被雪豹危害过?  
Yes 有    No 没有

If so, please write year, time of year, type of animal, how many, and where:  
如果有, 请写出发生的年份、具体时间、危害家畜的种类、数量和地点:

Do you know if any snow leopards were killed by humans in the last 5 years? 过去 5 年, 是否有雪豹被猎杀?  
Yes 有    No 没有                      If so, how many? 如果有, 被猎杀的数量是多少: \_\_\_\_\_

Was this because the snow leopard killed animals? 这种猎杀是因为雪豹危害了家畜吗?    Yes 是    No 不是

What are you most worried about losing your animals to from most (1) to least (4). 关于危害家畜, 你最担心的是什么? (按重要性从 1-4 排序)	Sickness 疾病	Killed by Predator 被捕杀	Drought 干旱	Grassland Degradation 草场退化
	_____	_____	_____	_____

Age 年龄: \_\_\_\_\_ Gender 性别: \_\_\_\_\_ Last Grade Completed in School 学历: \_\_\_\_\_

Number of Children 孩子数量: \_\_\_\_\_

Appendix III: Supplemental Material 2. The English interview sheet used to record herder responses prior to the start of the Foxlight study

Pre-Foxlight Questionnaire

Date:                      Village:                      Longitude:                      Latitude:  
 Name:                      Herder Code:  
 Family Size:                      Years of Education:                      Elevation:

Is livestock rearing your primary means of income?	Yes	No			
If no, what is your primary means of income?					
What livestock do you own?	Yak	Goat	Sheep	Horse	Other
	---	---	---	---	---
How many adult males of each?	---	---	---	---	---
How many young males of each?	---	---	---	---	---
How many adult females of each?	---	---	---	---	---
How many young females of each?	---	---	---	---	---
Do you have insurance for your livestock?	Y/N	Y/N	Y/N	Y/N	Y/N
Are you satisfied with your insurance?	Yes	No	No opinion		
Please circle what do you like about your insurance:	Payment is enough	Payment timely	Evidence easy to collect	Insurance coverage is inexpensive	
Please circle what you do not like about your insurance:	Payment is not enough	Payment is delayed	Evidence is hard to collect	Insurance coverage is expensive	

Do you use dogs to protect livestock?    Yes    No

How effective do you think they dogs are at deterring predators?    1 (not effective)                        7 (very effective)       

Did you train your dog to guard livestock?    Yes    No

Pre-Foxlight Questionnaire

How often do you move your livestock per year?

When do you move your livestock (months)?

Reasons for moving?

Please circle the predators currently living in your area:					
Wolf	Snow Leopard	Lynx	Bear	Fox	Other

Which of these do you find most threatening to livestock?

Have you lost any livestock to predators in previous 2 years?      Yes      No

Date	Predator Responsible	Distance to House	Deterrents in Place (corrals, dogs, etc.)	Livestock Type	# Adult Female	# of Subadult Female	# Baby Female	# Adult Male	# of Subadult Male	# Baby Male

How did you decide which predators are responsible for kills?

Pre-Foxlight Questionnaire

Do you know of any incidents where predators were trapped or killed by humans? Yes No

Was this in retaliation for livestock losses? Yes No

If so, provide year, season, number, and predator:

Please circle the deterrents you are currently using to protect your livestock from predators:

Guard Dogs	Shepherd / Human Guarding	Housing Newborns Inside	Predator Proof Corrals	Noise Maker	Electric fencing	Electric Shockers	Scarecrow	Light	Other (Specify)
------------	---------------------------	-------------------------	------------------------	-------------	------------------	-------------------	-----------	-------	-----------------

Out of the above, which deterrents would you consider most effective at stopping animal loss?

Out of the above, which deterrents would you consider least effective at stopping animal loss?

If using human guarding do you guard your livestock:

All day during the day	All night during the night	Only occasionally during the day	Only occasionally at night
------------------------	----------------------------	----------------------------------	----------------------------

Other Notes / Comments:

Appendix III: Supplemental Material 3. The Mandarin Chinese interview sheet used to record herder responses prior to the start of the Foxlight study. The content of this interview sheet is the same as the content presented in the English interview sheet.

Pre-Foxlight Questionnaire

日期: 村: 经度: 纬度: 海拔:  
 姓名: 牧民代码:  
 家庭人口: 受教育几年:

你的主要收入来源是饲养牲畜嘛?	是	否			
如果否, 那么你的主要收入来源是什么?					
你有哪些牲畜?	牦牛	山羊	绵羊	马	其他
	—	—	—	—	—
雄性多少头?	—	—	—	—	—
雄性幼体多少头?	—	—	—	—	—
雌性多少头?	—	—	—	—	—
雌性幼体多少头?	—	—	—	—	—
牲畜是否入保?	是/否	是/否	是/否	是/否	是/否
对当前保险是否满意?	是	否	无意见		
你喜欢保险的哪些特点:	赔偿金额充足	赔偿及时	容易取证	保险便宜	
你不喜欢保险的哪些特点:	赔偿金额较低	赔偿不及时	不容易取证	保险较贵	

你是否用狗来保护牲畜?	是	否				
你觉得用狗来震慑捕食者效果如何?	1 (无效)					7 (非常有效)
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
你家的牧羊犬是否受过训练	是	否				



Pre-Foxlight Questionnaire

你每年转移牲畜几次? \_\_\_\_\_

几月份对其转移? \_\_\_\_\_

转移原因?

请圈出当前生活在附近的捕食者					
狼	雪豹	豺狼	棕熊	狐狸	其他

你觉得哪种对牲畜的威胁最大?

在过去两年里, 有没有捕食者捕食你家的牲畜?                    是                    否

日期	捕食者	到房屋距离	防护措施 (围栏, 狗, 其他请 列出)	牲畜类 型	成体雌 性数量	亚成体 雌性数 量	雌性 幼仔	成体雄 性数量	亚成体 雄性数 量	雄性 幼仔

Pre-Foxlight Questionnaire

你怎么知道是哪种动物杀死了牲畜?

你知道有野生动物被捕杀的事件嘛? 是 否

是由于牲畜损失带来的报复性猎杀吗? 是 否

如果是, 提供年份、季节、数量和捕食者

请圈出你当前使用保护牲畜的措施

狗 牧人看守 把牲畜幼仔放在房屋里 防护围栏 噪音 电围栏 电击 稻草人 灯光 其他(具体列出)

以上措施你认为哪种最有效?

以上措施你认为哪种最无效?

如果用人来保护牲畜, 你会选择:

整天守着 整晚守着 白天偶尔守着 晚上偶尔守着

其他备注:

Appendix III: Supplemental Material 4. The English interview sheet used to record the responses of herders who were not provided a Foxlight deterrent.

Post-No Foxlight Questionnaire

Date:                      Village:                      Longitude:                      Latitude:

Name:                      Herder Code:

Elevation:

What livestock do you own?	Yak	Cow	Goat	Sheep	Horse	Other
	—	—	—	—	—	—
How many males of each?	—	—	—	—	—	—
How many females of each?	—	—	—	—	—	—

Have you moved your livestock since the first interview?    Yes                      No  
If yes, where and when?

Have you lost any livestock to predators since using the first interview?    Yes                      No

Date	Predator Responsible	Distance to House	Deterrents in Place (corrals, dogs, etc.)	Livestock Type	# Adult Female	# of Subadult Female	# Young Female	# Adult Male	# of Subadult Male	# Young Male

Post-No Foxlight Questionnaire

How does livestock loss compare to this time of the year the last two years?	More loss	Less loss	Same loss
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If less loss, why do you think that is?

If more loss, why do you think this is?

Please circle other causes of livestock mortality since first interview (December 2019):				
	Accident	Old Age	Disease	Other (specify)
Number of Livestock Lost:	_____	_____	_____	_____
Details:				

Please circle the predators currently living in your area:					
Wolf	Snow Leopard	Lynx	Bear	Fox	Other

Please list the top three reasons why you lose livestock to predators:	
1.	
2.	
3.	

Please circle the deterrents you are currently using to protect your livestock from predators:								
Guards Dogs	Shepherd / Human Guarding	Housing Newborns Inside	Predator Proof Corrals	Noise Maker	Electric Shockers	Scarecrow	Light	Other (Specify)

Other Notes / Comments:

Appendix III: Supplemental Material 5. The Mandarin Chinese interview sheet used to record the responses of herders who were not provided a Foxlight deterrent.

Post-No Foxlight Questionnaire

日期                      村名                      经度                      纬度

牧民姓名                  牧民编号

海拔

你家中有哪些牲畜?	牦牛	奶牛	山羊	绵羊	马	其他
	—	—	—	—	—	—
雄性多少个?	—	—	—	—	—	—
雌性多少个?	—	—	—	—	—	—

第一次访谈后，是否转移牲畜?    是                      否

如果转移，何时转移?  
转移到哪?

第一次访谈后，你的牲畜有被野生动物捕食过吗?    有                      无

日期	肇事动物	距离房子距离	有无防护 (如围栏, 狗等)	损失牲畜类型	成体雌性数量	亚成体雌性数量	幼体雌性数量	成体雄性数量	亚成体雄性数量	幼体雄性数量

Post-No Foxlight Questionnaire


与两年前的这个时间相比，牲畜损失变化	增加	减少	不变
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如果牲畜损失减少，你觉得是什么原因造成的？

如果牲畜损失增加，你觉得是什么原因造成的？

请圈出第一次访谈以来牲畜损失的其他原因(2019年12月):

	意外	老死	疾死	其他 (请列出)
牲畜损失数量	_____	_____	_____	_____
请详细说明				

请圈出当前生活在附近的捕食者

狼	雪豹	豺狼	熊	狐狸	其他
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请列出三个牲畜被捕食的主要原因

1.
2.
3.

请圈出目前使用的防护措施

狗	找人看守	把牲畜幼仔放在房屋里	围栏	制造噪音	电围栏	稻草人	灯	其他请列出
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其他意见:

Appendix III: Supplemental Material 6. The English interview sheet used to record the responses of herders who were provided a Foxlight deterrent.

Post-Foxlight Questionnaire

Date: \_\_\_\_\_ Village: \_\_\_\_\_ Longitude: \_\_\_\_\_ Latitude: \_\_\_\_\_  
 Name: \_\_\_\_\_ Herder Code: \_\_\_\_\_  
 Elevation: \_\_\_\_\_ Start Date of Foxlight Use: \_\_\_\_\_

What livestock do you own?	Yak	Cow	Goat	Sheep	Horse	Other
	_____	_____	_____	_____	_____	_____
How many males of each?	_____	_____	_____	_____	_____	_____
How many females of each?	_____	_____	_____	_____	_____	_____

Have you moved your livestock since the first interview? Yes No

If yes, where and when?

How often did you use the foxlight?	Nightly	~3 nights a week	~1 night a week	~1 night a month	Didn't use
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If you did not use the foxlight, why?

Did you have any issues with the foxlight?	Not bright enough	Battery died	Would not charge / solar panel did not work	Stolen	Lost	Other (specify)
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Did your use of the foxlight increase or decrease based on time of year? Yes No  
 If yes, which months and why?

Did you move the foxlight or keep it in the same location? Moved Same Location

If moved, how often? \_\_\_\_\_

Of the locations you put the foxlight, which worked best?

How many foxlights would you need to be effective?	1	2	more than 3	more than 5
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Post-Foxlight Questionnaire

Have you lost any livestock to predators since using the first interview?      Yes      No

Date	Predator Responsible	Distance to House	Deterrents in Place (corrals, dogs, etc.)	Livestock Type	# Adult Female	# of Subadult Female	# Young Female	# Adult Male	# of Subadult Male	# Young Male	Was Foxlight in Use?	Distance of Foxlight from Attack	Direction Predator Approached From



Post-Foxlight Questionnaire

How does livestock loss compare to this time of the year the last two years?	More loss	Less loss	Same loss
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If less loss, do you think this is due to the foxlight?    Yes        No

If yes, why do you think the foxlight worked at keep away predators?

If no, what do you think is causing less loss?

If more loss, why do you think this is?

Please circle other causes of livestock mortality since first interview (December 2019):				
	Accident	Old Age	Disease	Other (specify)
Number of Livestock Lost:	_____	_____	_____	_____
Details:				

Please circle the predators currently living in your area:					
Wolf	Snow Leopard	Lynx	Bear	Fox	Other

Please list the top three reasons why you lose livestock to predators:
1.
2.
3.

On a scale of 1 (not effective) to 7 (very effective) how effective do you think the foxlight was at deterring the following predators?					
Wolf	Snow Leopard	Lynx	Bear	Fox	Other
_____	_____	_____	_____	_____	_____

If not effective for any of the above predators, why?

Post-Foxlight Questionnaire

On a scale of 1 (not confident at all) to 7 (very confident) how confident are you in your ability to continue implementing the foxlight? (Ohrens et al. 2019)						
1	2	3	4	5	6	7

If not confident, why?

On a scale of 1 (no pressure at all) to 7 (a lot of pressure) how much social pressure do you feel in implementing foxlights? (Ohrens et al. 2019)						
1	2	3	4	5	6	7

Please circle the deterrents you are currently using to protect your livestock from predators:								
Guards Dogs	Shepherd / Human Guarding	Housing Newborns Inside	Predator Proof Corrals	Noise Maker	Electric Shockers	Scarecrow	Light	Other (Specify)

Will you continue using the foxlight?      Yes                      No

What suggestions do you have to improve the foxlight?

Other Notes / Comments:

Appendix III: Supplemental Material 2. The Mandarin Chinese interview sheet used to record the responses of herders who were provided a Foxlight deterrent. The content of this interview sheet is the same as the content presented in the English interview sheet.

Post-Foxlight Questionnaire

日期                      村名                      经度                      纬度

牧民姓名                  牧民编号

海拔                      狐灯开始使用日期

你有哪些牲畜?	牦牛	奶牛	山羊	绵羊	马	其他
	—	—	—	—	—	—
雄性多少个?	—	—	—	—	—	—
雌性多少个?	—	—	—	—	—	—

第一次访谈后，是否转移牲畜?      是                      否

如果转移，何时转移?  
转移到哪?

使用狐灯频率	每晚	每周三晚	每周一晚	每月一晚	不使用
--------	----	------	------	------	-----

如果不使用狐灯，为什么?

使用狐灯时出现什么问题没	不够亮	电池没电	不能充电/太阳能天池无法使用	被偷	丢失	其他(请列出)
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你是否在一年中某个时间段停止使用狐灯，然后又重新使用狐灯?      是                      否

如果是，请问在一个月? 为什么?

是否移动狐灯位置?    移动                      未移动

如果移动，多久一次? \_\_\_\_\_

你把狐灯放在哪个位置效果最好?

你认为你应该持有多少个狐灯才能发挥保护牲畜的效果?	1	2	>3	>5
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Post-Foxlight Questionnaire

第一次访谈后，你的牲畜有被野生动物捕食过吗？      有                      无

日期	肇事动物	距离房子距离	有无防护（如围栏，狗等）	损失牲畜类型	成体雌性数量	亚成体雌性数量	幼体雌性数量	成体雄性数量	亚成体雄性数量	幼体雄性数量	是否使用狐灯	狐灯距离肇事点距离	捕食者从哪个方向靠近





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