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Regional comparison of snow leopard diet using DNA metabarcoding

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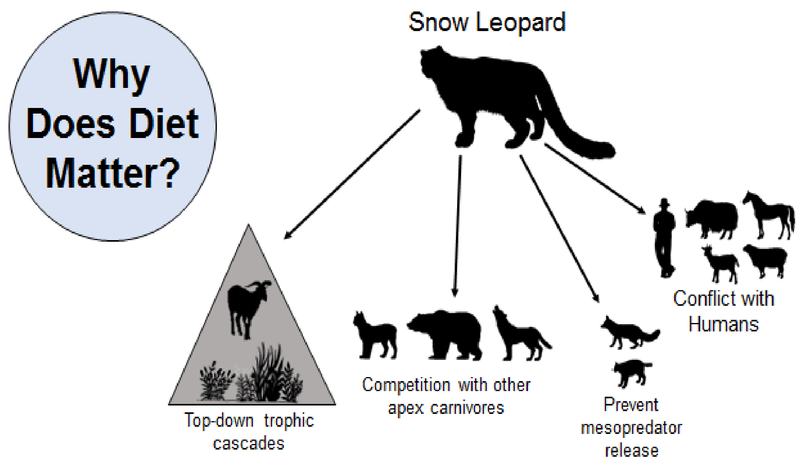
Snow leopards are endangered and distributed only in Central Asia.



Author	Year	Title	Method
Oli	1993	Diet of the snow leopard (Panthera uncia) in the	Microhistological
Chundawat and Rawat	1994	Food habits of snow leopard in Ladakh, India	Microhistological
Jackson	1996	Home range, movements and habitat use of snow leopard	Microhistological
Lhagvasuren and Munkhtsog	2002	The yak population in Mongolia and its relation with snow leopards as a prey	Microhistological
Bagchi and Mishra			Microhistological
Lovari et al.			Microhistological
Anwar et al.			Microhistological
Wegge et al.		predation on livestock and wild prey in a mountain valley	Microhistological
Suryawanshi et al.	2017	Impact of wild prey availability on lifestock predation by snow	Microhistological
Khatoon et al.	2017	Diet selection of snow leopard (Panthera uncia) in Chitral,	Microhistological
Chetri et al.	2017	Snow leopard and Himalayan wolf: food habits and prey	Microhistological

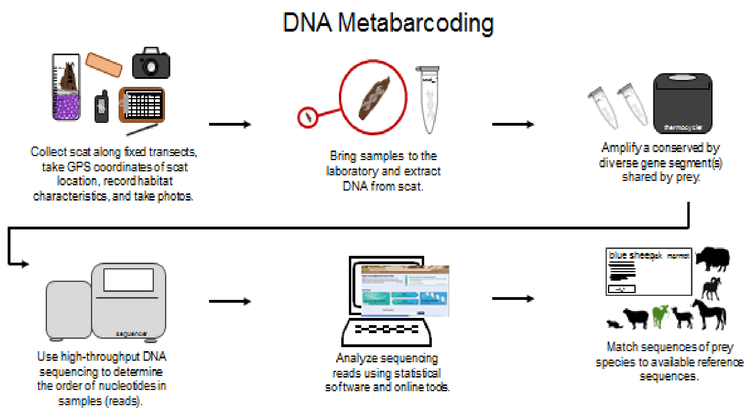
Predator misidentification
Laborious
Phenotypic variation
Interobserver bias

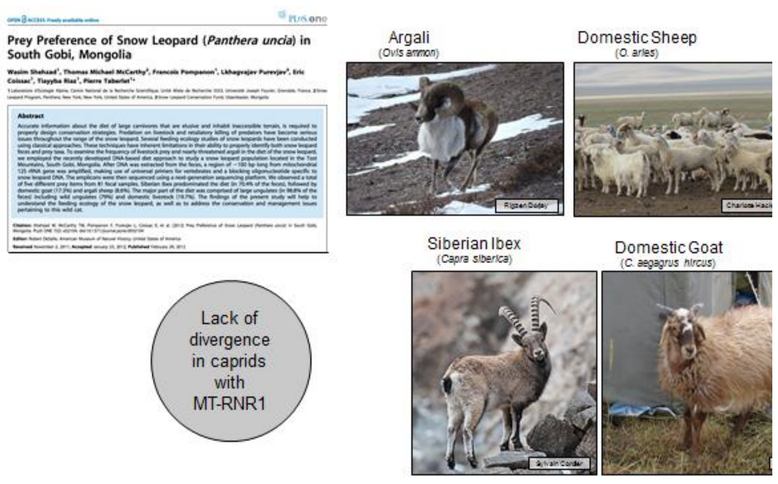
Author	Year	Title	Method
Devkota et al.	2013	Prey density and diet of snow leopard (Uncia uncia) in Shey Phoksundo National Park,	Interviews and Microhistological
Oli et al.	1994	Snow leopard *Panthera uncia* predation of livestock: an	Interviews
Aryal et al.			Interviews
Devkota et al.	2017	Abundance of snow leopard (Panthera uncia) and its wild	Interviews
Lyngdoh et al.			Data Synthesis
Johansson et al.	2015		GPS coordinates
Shehzad et al.	2012	Prey preference of snow leopard (Panthera uncia) in	Genetics

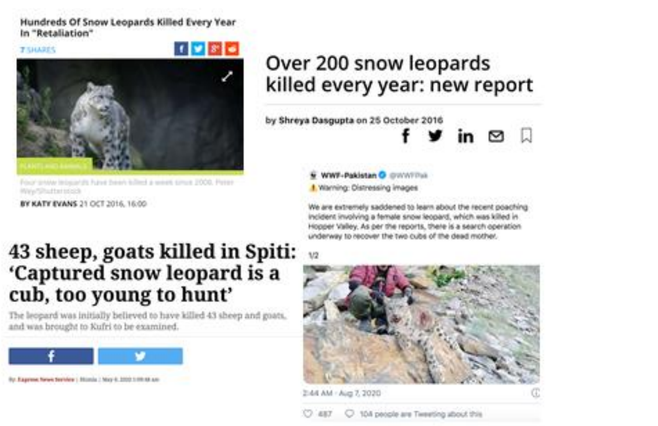
Public opinion
Inaccurate recollections
Differing methodologies
regional diet specificity holds
Site specific

Noninvasive
Sampling can be done over a wide area
More accurate results
Less time consuming from start to finish

A number of previous studies have examined snow leopard diet using different methods. All of these methods have their pros and cons. However, we were interested in using a next-generation sequencing approach called DNA metabarcoding.



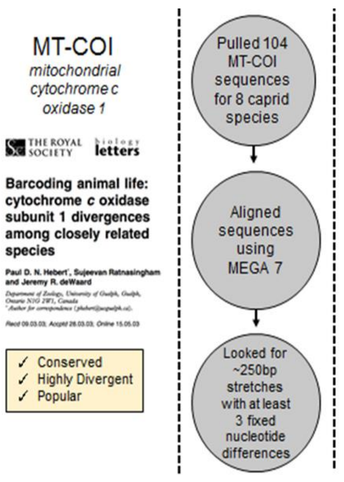


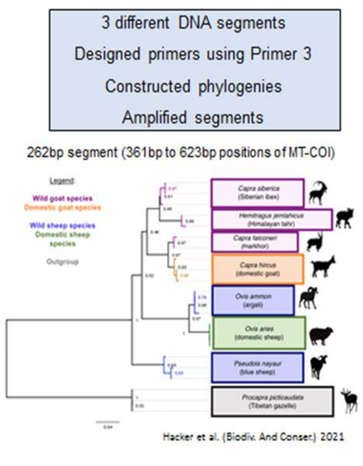


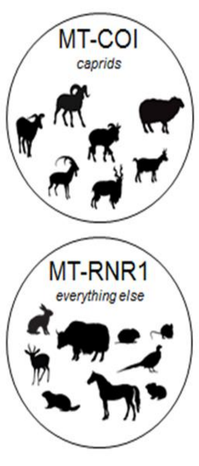
For DNA metabarcoding, we collect snow leopard positive scat samples in the field, then extract the DNA from the scat. We next amplify gene segments that all of our possible snow leopard prey items have and sequenced those segments to determine the order of nucleotides to create "reads". We then take those "reads" and match them to a reference database of prey sequences that we have compiled from online databases to genetically determine the prey items in each snow leopard scat collected.

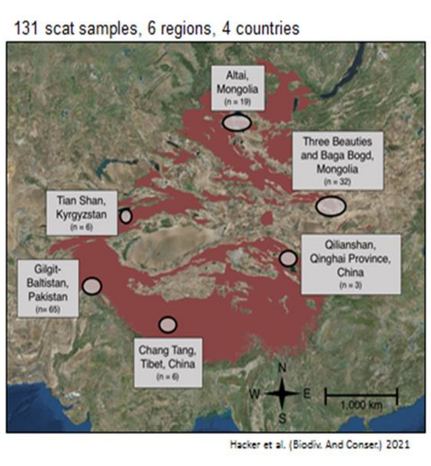
Previous work using DNA metabarcoding has been successful. However, the genetic marker used, MT-RNR1, was not able to genetically differentiate wild versus domestic goat and sheep species.

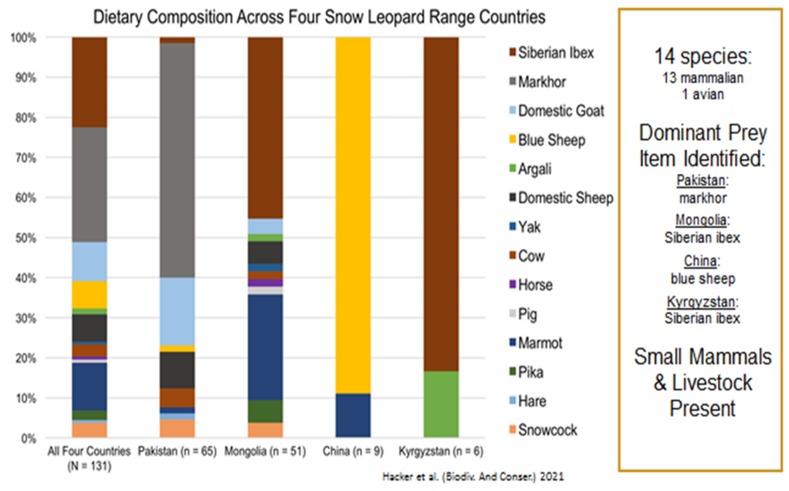
Being able to determine domestic wild goat and sheep species is important because livestock loss in snow leopard habitat is a large issue. Livestock loss can lead to large financial losses for herders, negative attitudes towards snow leopards and retaliatory killings.











14 species:
13 mammalian
1 avian

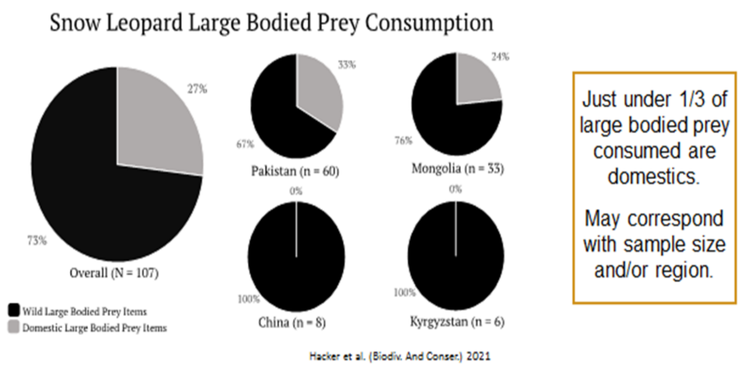
Dominant Prey Item Identified:
Pakistan: markhor
Mongolia: Siberian ibex
China: blue sheep
Kyrgyzstan: Siberian ibex

Small Mammals & Livestock Present

Our goal was to find a genetic marker that could work in tandem with MT-RNR1 to discern wild versus domestic goat and sheep species. We ended up selecting MT-COI, which has been used in past dietary studies. We designed three different primer pairs to amplify three different segments of MT-CO1 specific to goats and sheep and found out which one worked the best. But we also wanted to make sure that this would work across snow leopard habitat, so we wanted to use scat samples that covered different regions of snow leopard range.

So, we moved forward with using MT-CO1 to determine all of goat and sheep prey, and MT-RNR1 to determine all other prey items. We had samples from four different countries representative of six different regions of snow leopard range, and were able to determine the prey items in 131 snow leopard scats.

We found that our markers worked successfully in determine prey items for snow leopards across the four areas. We found 14 unique prey species, with each country having a dominate prey item present that varied regionally based on prey availability and range. We also found birds and small mammals in snow leopard diet in addition to larger hoof stock, and we also found livestock in snow leopard diet. These included domestic goat, domestic sheep, yak, cattle, horse, and pig. This indicated that snow leopards are capable of taking a wide variety of livestock and may be more opportunistic in their hunting strategies than previous studies have suggested.



Just under 1/3 of large bodied prey consumed are domestics.

May correspond with sample size and/or region.

Conclusions & Conservation Outcomes

- **MT-COI and MT-RNR1** together provide an **accurate and holistic** view of snow leopard diet.
- Important **prey species vary by country** and should be protected.
- The **role of small mammals and birds** in snow leopard diet **needs more research**.
- Snow leopard **conflict with humans** should be addressed and mitigated.
- DNA metabarcoding can **capture unexpected species** in diet.
- **More reference sequences are needed** for small mammals and other closely related species.

Future Directions

- Use **DNA metabarcoding** with **other carnivores** in snow leopard habitat.
- Investigate **fine scale spatial differences** in diet.
- **Examine** mechanisms of **niche-partitioning** among carnivore species.
- Study how carnivore **diet changes** across **seasons** and **years**.
- Work with local communities to **test non-lethal predator deterrents** to prevent livestock loss.

Larger bodied hoofstock species were examined in more detail to compare prey items that were wide versus domestic.
We found that nearly a third of larger hoofstock species in snow leopard diet were domestic species. In Pakistan, one third were livestock and in Mongolia, nearly one quarter were livestock. These are relatively high numbers, indicating that conflicts between herders and snow leopards in these sampling areas may be high or that wild prey may not be very abundant. It appears that livestock play an important role in contributing to snow leopard diet, which may also impact local ecosystem functions if snow leopards are not targeting and controlling wild prey populations.
There was no livestock found in the diets of snow leopards in China and Kyrgyzstan, but this is not unsurprising due to low sample sizes of both areas.
We also did not expect to find livestock in snow leopard samples from Kyrgyzstan since livestock are not kept in the sampling area.

In conclusion, we were able to show that the two genetic markers used for DNA metabarcoding were able to determine snow leopard diet across different areas of their range.
We also found that important prey species vary by country, and that these species require protection in snow leopard conservation efforts. Small mammals and birds were found in snow leopard diet. It is not clear what role these species play, but they likely help sustain snow leopards between larger kills.
Were also found that DNA metabarcoding can reveal unique species in snow leopard diet. For example, domestic pig is not a commonly known livestock animal lost.
Lastly, reference sequences for many species are still needed to reveal species level dietary items. For example, this study was unable to discern pika down to species level due to a lack of available online reference sequences.

We have many future initiatives using this technique.
First, we will be using DNA metabarcoding with other carnivore species living in snow leopard habitat, such as wolves, lynx, and bears.
We also hope to do more site specific diet studies and to look at how different carnivores share resources by examining dietary niche overlap.
We also aim to assess how diet changes seasonally, with a focus on livestock loss based upon season.
Over the long term, we hope that dietary monitoring will help in determining how adaptable snow leopards and other carnivore species are to large scale landscape shifts in an effort to better predict species outcomes due to climate change.
Lastly, were also currently putting our livestock loss data into practice by testing predator deterrents to see if they reduce livestock presence in snow leopard diet.