



## Monitoring of snow leopards in the Sarychat-Ertash State Reserve (Kyrgyzstan), between 2011 and 2019, through scat genotyping

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### Abstract

Snow leopards (*Panthera uncia*) are a keystone species of Asia's high mountain ecosystem. The species is assessed as Vulnerable by the IUCN Red List of Threatened Species and is elusive, limiting accurate population assessments that could inform conservation actions. Non-invasive genetic monitoring conducted by citizen scientists offers avenues to provide key data on this species. From 2011 to 2019, OSI-Panthera citizen science expeditions tracked signs of presence of snow leopards and collected scat samples along transects in the main valleys and crests of the Sarychat-Ertash State Reserve (Kyrgyzstan). Scat samples were genotyped at

twenty autosomal microsatellite loci and at a X/Y locus (sex identification), allowing an estimation of a minimum of 17 individuals. The genetic recapture of 12 of them provided indications of individuals' habitat use throughout the reserve. We found putative family relationships between several individuals; however, further research is needed to validate these findings. Our results demonstrate the potential of a citizen science program to collect meaningful data that can inform the conservation management of snow leopards.

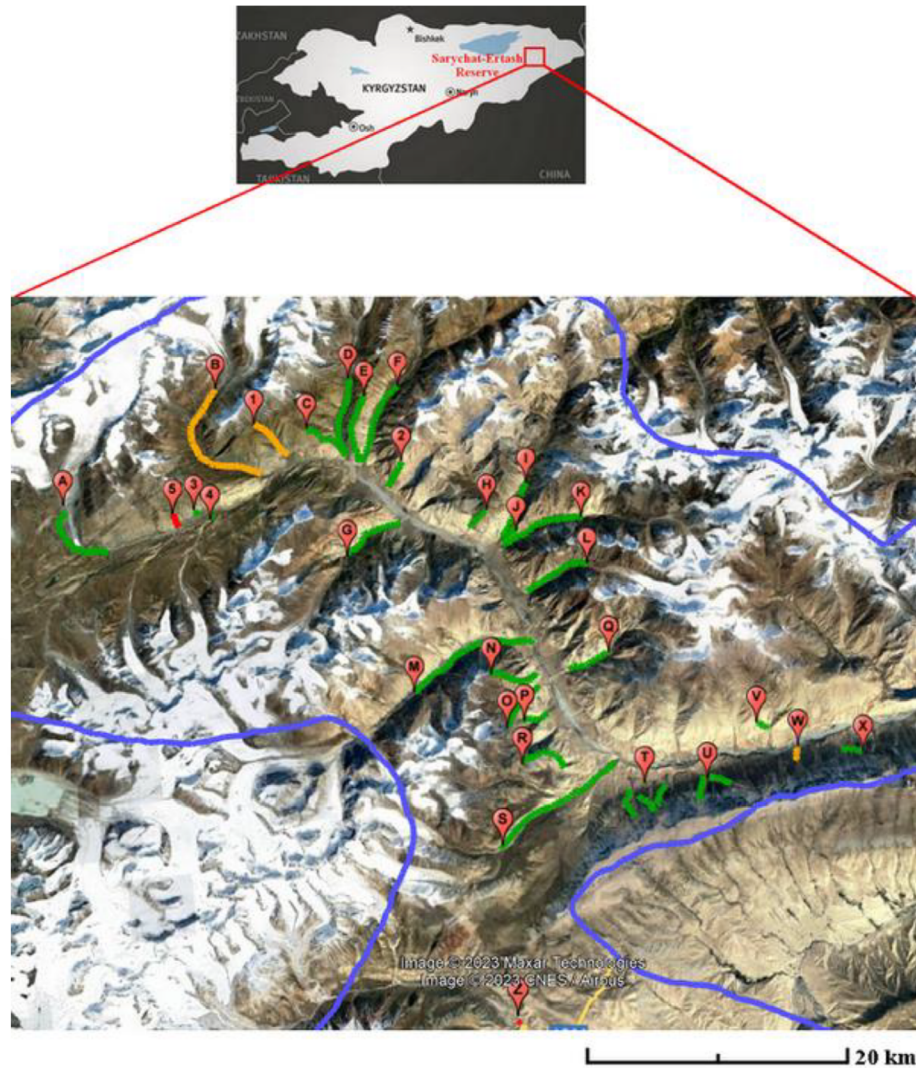
## Introduction

Threats facing snow leopard (*Panthera uncia*) populations include habitat loss, loss of prey base, human-wildlife conflicts, and illegal killing (Jackson and Hunter 1996; McCarthy and Chapron 2003; McCarthy and Mallon 2016; Nowell, et al. 2016). Because of these, snow leopards were assessed as Endangered by the IUCN from 1986 to 2016. The species was down listed to Vulnerable (C1) in 2017. Estimates of the total number of mature individuals indicate between 3,500 and 7,000 snow leopards across the species range (McCarthy, et al. 2017; Snow Leopard Trust, 2021). However, many range countries lack up-to-date information on snow leopard population sizes and demographic trends. The elusive snow leopard inhabit remote mountainous regions, which makes ecological, behavioural and population studies particularly challenging (Macdonald and Loveridge 2010; McCarthy and Mallon 2016). Research that seeks to provide current and accurate demographic trends is a main priority highlighted in the Snow Leopard Survival Strategy (McCarthy and Chapron 2003; Snow Leopard Network 2014).

In Kyrgyzstan, snow leopard numbers

have decreased at an alarming rate over the last few decades, with 650 to 800 individuals estimated in the 1990s, against 150 to 200 in 2000 (Koshkarev and Vyrypaev 2000). Latest estimates are around 350 to 400 individuals (National Academy of Sciences of Kyrgyzstan, unpublished data; McCarthy and Mallon 2016). Protection efforts in Kyrgyzstan have mainly focused on preventing illegal killing, one of the most important threats to wildlife since the country's transition in 1991 (Koshkarev and Vyrypaev 2000; McCarthy and Mallon 2016). The largest protected area, the Sarychat-Ertash State Reserve (SESR), established in 1995, is located in the Tien-Shan mountain range of Kyrgyzstan (Fig. 1). Besides illegal killing, major threats to biodiversity in the SESR include climate change, mining, overgrazing, and over-hunting (SER 2007). The SESR highlands are also surrounded by long-established ungulate hunting concessions, which increase pressure on snow leopards and their prey. The SESR is divided into fourteen districts, each monitored by a ranger. Several studies based on genetic analyses and camera trapping estimated the snow leopard population size in the SESR to be approximately 20 individuals (McCarthy, et al. 2008; Jumabay-Uulu, et al. 2014; McCarthy and Mallon 2016). However, a long term, accurate understanding of population status and changes is still lacking.

Non-invasive capture-mark-recapture methods, along with subsequent genetic analyses, are effective tools for estimating the demographic parameters needed to develop conservation plans (Bhagavatula and Singh 2006; Mondol, et al. 2009; Sugimoto, et al. 2012; Aziz, et al. 2017). The main objectives of the present study were to assess the suitability of



**Figure 1:** Map of Sarychat-Ertash State Reserve (Kyrgyzstan) highlighting transects performed from 2011 to 2019, red indicates where no traces of snow leopards were found, orange indicates where some traces were found except scat and green were snow leopard traces and scat were collected. Letters and numbers corresponding to the location of transects are provided in (*Supplementary Table S1*) along with the dates of inspections.

non-invasive genetic techniques for studying the snow leopard population in the SESR and to provide preliminary insights into genetic relatedness among individuals. All samples obtained in the field were collected through seasonal citizen science expeditions, where participants were trained by local rangers and

scientific experts on the methodology of scat sampling.

## Materials and methods

### Study Area

Before 1995, the SESR served as a grazing area for herders from the USSR (Union of Soviet

Socialist Republics), who lived there year-round with livestock herds numbering in the thousands. The SESR boundary encompasses 1,340 km<sup>2</sup>, with a 720 km<sup>2</sup> core zone and a 620 km<sup>2</sup> buffer zone (SER 2007) (Fig. 1). The relief is characterized by large flat valleys about one kilometer wide surrounded by high mountains of altitudes ranging from 2,000 to 5,500 m (SER 2007). The climate is continental, with low average temperatures even during the summer months (-21.5°C in January; +4.5°C in June). Vegetation types include arid grasslands and alpine meadows, with a majority of bushy and blanket cover type plants that are able to sustain the harsh and windy climate (SER 2007). Beside the snow leopard, several carnivores are found in the reserve, including wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), Tian Shan brown bears (*Ursus arctos isabellinus*), Pallas's cat (*Otocolobus manul*), Eurasian lynx (*Lynx lynx isabellinus*), as well as several mustelids (*Martes foina*, *Mustela erminea*). Large and medium herbivores, which are snow leopard prey species, are also found, including Siberian ibex (*Capra sibirica*), argali (*Ovis ammon*), grey marmots (*Marmota baibacina*) and Tolai hares (*Lepus tolai*). Several species of birds are present, a few of which represent prey species for snow leopards, such as snowcocks (*Tetraogallus himalayensis*) and chukar partridges (*Alectoris chukar*) (SER 2007; McCarthy and Mallon 2016).

### **Monitoring of snow leopard presence**

In this study, snow leopards within the SESR were monitored during citizen science expeditions led by the OSI-Panthera research program (osi-panthera.org). These two to four week expeditions were conducted by local rangers and guides, OSI scientific educators,

and volunteers. Volunteers are eco-tourists, with around a half of them being ecology and wildlife students or professionals with diverse specialties such as botany or ornithology. Monitoring effort increased over time, with two expeditions conducted in 2011 (July and August), three both in 2012 and 2013 (June, July, and August each year), four in 2014 and 2015 (June, early July, straddling July and August and late August each year), three in 2016 (June, July, and August), three in 2017 (June, July, and August), one in 2018 (July and August) and two in 2019 (July and August).

Snow leopard presence was recorded based on specific signs (presence of scats, hairs, scratch marks, tracks, urine sprays on rocks, and carcasses of prey species), and based on pictures from camera traps set at known locations. Incidental species were also recorded to obtain information on prey presence and biodiversity level.

The protocol consisted of searching for snow leopard signs along transects (Fig. 1). As snow leopards are more likely found in steep and rocky environments and travel along topographic edges (McCarthy and Mallon 2016), transects were designed along waterbodies, ridgelines and cliffs, as well as in narrow valleys and canyons (McCarthy and Mallon 2016). Most transects were set within a sampling area of about 500 km<sup>2</sup> within the SESR core zone, around the main valley in which the Ertash River flows, and at the entry of secondary valleys (Fig. 1). For each expedition 10 to 15 transects were surveyed, with transect length ranging from several hundred meters in valley bottoms to more than three kilometers along crest lines. The same transects were surveyed on a regular basis over the years

**(Supplementary Table S1).**

Glaciers, which are not considered high quality habitat for snow leopards (McCarthy and Mallon 2016) were only searched once due to low accessibility and time constraints. As snow leopards are territorial (McCarthy and Mallon 2016), our large sampling area which was covering most of the SESR and included snow leopard's preferential habitats enabled us to estimate a minimum number of individuals at the reserve scale and gain insights on their habitat use within it. The list of ridgelines covered and information on the presence of putative snow leopards' signs and number of scat samples collected can be found in **Supplementary Table S1**.

***Collection of scat samples***

Putative snow leopard scats were identified based on size, shape, vegetation content, as well as proximity to tracks, scratch marks and carcasses. Because scats are used by snow leopards for territory marking, only a small portion of each was collected. Due to exposure to harsh weather conditions in high mountain environments (such as UV rays, rainfall, wind, and trampling), the scats degraded within several months and could thus not be retrieved from one year to the next. This was supported through GPS recordings and reports from science coordinators across the years.

Each sample was collected with disposable latex gloves, and stored in a tube, with silicagel at room temperature for several weeks before being frozen and later sent to the lab for genotyping. Timeline to process samples following collection and freezing ranged from several months to several years. To avoid cross-contamination, individual sampling kits were

prepared before each expedition. Different volunteers collected samples on a given transect, to prevent the manipulation of different samples by the same person and to minimize potential cross contamination. Samples that had signs of humidity were dried in open air to prevent molding and degradation. In 2015, the storage and DNA preservation protocol was improved by including systematic drying, and use of a coffee filter to protect the samples from the silicagel. These improvements were combined with a shortened processing time between sample collection and genotyping, which led to an increase in the number of samples successfully genotyped. A total of 151 putative snow leopard scat samples were collected and processed (6 in 2011, 11 in 2012, 18 in 2013, 21 in 2014, 8 in 2015, 18 in 2016, 25 in 2017, 16 in 2018 and 28 in 2019).

***DNA extraction***

DNA extraction of each sample was conducted under sterile conditions in a designated area of the lab. The mucosal layer of each sample was swabbed to collect animal cells that were placed in a numbered microtube to proceed to DNA extraction. Sample tubes were surrounded by both negative extraction controls (blanks) and positive extraction controls consisting of snow leopard samples previously analyzed and validated in terms of DNA quality and genotyping success on microsatellite markers. Samples, as well as positive and negative extraction controls, were lysed overnight at 56°C, DNA was isolated and purified using purification columns and vacuum filtration according to the manufacturer's instructions (Nucleospin 96 Tissue Kit, Macherey-Nagel).

### ***Microsatellite markers genotyping and sex identification***

For each DNA sample, 20 microsatellite markers (Menotti-Raymond, et al. 1999) and one marker for sex identification (ZFGY) (Pilgrim, et al. 2005) were amplified in three multiplex PCRs (Polymerase Chain Reaction) referred to as A (6 loci), B (9 loci), and C (8 loci) (**Supplementary Table S2**) and genotyped with an automated sequencer. Each DNA sample was genotyped twice (multiple-tube approach) (Taberlet, et al. 1996). Previous research conducted in our lab on non-invasive samples from various species compared the results obtained from using two versus three replicates, and only little improvement was gained when adding a third replicate. Therefore, using two replicates effectively minimized costs while preserving the maximum number of samples for further analysis.

PCR reactions were prepared step-by-step following a unidirectional workflow. Three negative controls (blanks) and three positive controls (DNA previously analysed and validated in terms of genotyping success and quality) were included per PCR reaction plate. PCR amplifications were then performed in a 8 or 10 µl final volume containing 4 or 5 µl of mastermix Taq Polymerase (Type-It Microsatellite PCR Kit, Qiagen), 0.91 µL of pool A, 0.80 µL of pool B, or 1.66 µL of pool C, with primers pair concentrations ranging from 0.12 µM to 1.00 µM, and a mean of 30 ng of genomic DNA. For each pair of primers, one was coupled to a fluorescent dye. Our PCR thermal protocol consisted of 95°C for five minutes, followed by 35 cycles of 95°C for 30 seconds, 57°C (A) or 59°C (B) or 56.8°C (C) for 90 seconds, and 72°C for 30 (A&B) or 45 (C) seconds,

ending with an extension of 60°C for 30 minutes.

PCR products were resolved on a calibrated ABI PRISM 3130 XL capillary sequencer (ThermoFisher Scientific) under denaturing conditions (HiDi Formamide, ThermoFisher Scientific) with an internal size marker prepared once and dispatched equally in all sample wells of each marker run. This internal size marker guarantees the same calibration for all samples. As all the samples were distributed on multiple plates and each plate contained the same positive reference controls (previously genotyped once), all positive controls were run multiple times on each marker to guarantee both amplification and capillary resolution repeatability.

The electropherograms were analysed using GENEMAPPER 4.1 (ThermoFisher Scientific) and independently assessed by two analysts to determine the allele sizes for each marker for each sample. Analysts are trained to differentiate between peaks due to true alleles and those due to artefacts. They only kept annotations corresponding to true alleles and erased those due to artefacts. Thus, any remaining allele after analysts scoring was considered a true allele and retained in the consensus genotype of the sample. When the genotypes identified by each analyst did not match, the electropherograms were read again, and reading errors were resolved to create a consensus genotype for each sample. In addition, markers with more than two true alleles retained, or with alleles for which a persistent disagreement occurred on the call, were subsequently considered missing data. The genotype of each positive control was compared to its known reference to ensure the repeatability of the analyses. A quality

index (QI) was calculated for each sample by comparing each replicate genotype at each marker to the consensus genotype (Miquel, et al. 2006). The QI were averaged over all repeats for each locus, and then over all loci for each sample to obtain a QI per sample. Only samples presenting a QI superior to 0.5, or a minimum of ten markers successfully amplified were included in the subsequent analyses.

### *Genetic recaptures and genotyping error rates*

Genotypes were pairwise compared among all samples. Samples with identical or very close genotypes were associated to a single individual, specifically, when all markers were identical, or when a difference from the consensus on one or two markers occurred and could be attributed to allelic dropout or to a false allele.

The different genotypes assigned to an individual were compared to the individual consensus to calculate residual allelic dropout and false allele by direct counting over all loci and all samples.

### *Species confirmation*

To confirm the species of the identified individuals, the mitochondrial cytochrome b sequence was amplified and sequenced using a cocktail of designed primers, including three forward and three reverse primers:

PCa-Cytb-F1  
(5'-ATGACCAACATYCGAAAATCRYACC-3'),  
PCa-Cytb-F2  
(5'-ATGACCAACATYCGAAAAYCYCACC-3'),  
PCa-Cytb-F3  
(5'-ATGACCAACATTCGYAAAACYCACC-3'),  
PCa-Cytb-R1

(5'-AGGATRAARTGGAARGCGAAGAATCG-3'),  
PCa-Cytb-R2  
(5'-AGGATGAARTGGAATGCRAARAATCG-3'),  
and PCa-Cytb-R3  
(5'-AGGATRAAGTGGGAARGCRAAGAATCG-3').

This standard set of primers was designed to amplify cytochrome b sequences from all mammal species. PCR reactions were performed in a final volume of 10 µl, containing 5 µl of mastermix Taq Polymerase (Type-It PCR Kit, Qiagen), 0.21 µM of each primer pair and 2 µl of DNA extract of the highest quality sample assigned to each individual. The PCR thermal protocol consisted of 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 55°C for 90 seconds, and 72°C for 45 seconds, ending with an extension step of 60°C for 30 minutes. The PCR products were sequenced bidirectionally following the Sanger method with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and the same primers. Following purification, the sequences were analyzed using an ABI PRISM 3130 XL capillary sequencer (Applied Biosystems), with electropherograms interpreted using SeqMan Pro software (DNASTAR). For each sample, the resulting consensus sequence was compared to public databases with the BLAST online software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). We then analyzed identity scores and E-values to determine the species of each sample (cf. **Supplementary Table S8**).

### *Test of Hardy-Weinberg Proportions*

Hardy-Weinberg proportions were tested for each locus using ML Relate (Kalinowski, et al. 2006) with a 5% threshold. To consider multiple comparisons, the threshold was modified using the Bonferroni correction (Bonferroni, 1936).

Because such correction is often too stringent to allow for the detection of true positives, we also used a less stringent approach, the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) to control for false discovery.

### ***Distribution of pairwise genetic distances according to kinship***

Monitoring individuals requires the ability to identify them based on their respective genotypes. To do so, the probability that two unrelated individuals would have the same genotype ( $P_{uni (unrelated)}$ ) and the probability that two individuals with the same genotype are full siblings ( $P_{uni (sib)}$ ) were estimated (Evetts and Weir, 1998).

We also computed the expected distribution of the genetic distance (the number of different alleles between individuals) for each type of relationship, including parent-offspring, full siblings, half siblings, and unrelated. As there is no simple formula to calculate genetic distance greater than zero, an R script was used to compute these distributions using simulations (Pairwise\_distance\_genotypes.r in GitHub “jmorode/Genetics”) based on genotypes pairs generated for each type of relationship.

The simulated genotypes were generated as follows:

- for two unrelated individuals, at each locus, the allele frequencies in the SESR population were used to generate the genotype of each individual by randomly sampling two alleles;
- for parent-offspring, the parent genotype was generated as above. The genotype of the offspring was generated by sampling one allele randomly from its parent and the other randomly following the allele frequencies in the population;
- for full siblings, both unrelated parents were

generated as unrelated individuals. Each offspring was then generated by randomly sampling one allele from each parent;

- for half siblings, two unrelated fathers and one mother were first generated as unrelated individuals. One offspring was then generated by randomly sampling one allele from the mother and the other from one father. The other offspring was generated by randomly sampling one allele from the same mother and the other from the other father.

For each kind of relationship, one thousand simulations were performed to estimate the empirical distribution of pairwise genetic distances for a given relationship. Data analyses were carried out in the R statistical environment (version 3.4.3) (R Core Team 2021).

### ***Estimation of genealogical relationships***

Relatedness between individual snow leopards was tested using ML Relate software (Kalinowski, et al. 2006). To assess the accuracy of the genealogical relationships inferred by the software, one thousand families of known genotypes were simulated as describe above, using the R script GenerateIndividual.r available on GitHub “jmorode/Genetics”. A mother, two unrelated fathers, two full siblings and two pairs of half siblings were generated in each family to test the four ML Relate relationships: parent-offspring, full siblings, half siblings and unrelated. For each family, for the six unrelated individuals, the pair of full siblings, both pairs of half siblings and the six pairs of parent-offspring, the ML Relate inferred relationship was compared to the known one. The accuracy of the software was then estimated based on the percentage of correct relationships found by ML Relate over the 1,000 families.



## Results

### Scat genotypes

Among the samples gathered during the 2011 to 2019 sampling sessions in the SESR, DNA was extracted from 151 putative snow leopard scat samples, which were genotyped at 20 microsatellite loci. Of these, 65% (n = 98) had a QI equal to or above 0.50 or at least ten markers were successfully genotyped (mean QI of the 98 samples = 0.81) (**Supplementary Table S3**). For comparison, the 53 unusable samples had a mean QI of 0.19. On average, out of the 98 usable samples, 18 loci were successfully genotyped per sample, with 19 to 20 loci genotyped for 57 samples, and 10 to 18 loci genotyped for the other 41 samples.

Direct counting estimated 0.0008 of residual false alleles and 0.011 of residual allelic drop-outs over all samples.

Twenty-one unique genotypes were identified from the 98 samples successfully genotyped.

### Species confirmation

Mitochondrial cytochrome b sequences of 503 bp were obtained for 16 of these individuals (excluding SL17, for which the best sample did not yield a sufficiently high quality sequence for analysis). All 16 individuals presented the same haplotype. Using the NCBI BLAST tool, this sequence matched with 100% percent identity over its entire length with one haplotype (GenBank accession number KP202269.1) which was identified in several sequences obtained from snow leopard *Panthera uncia* samples (GenBank accession numbers MT423701.1 to MT423723.1 for example). This confirms that the sampled individuals were snow leopards and highlights the low poly-

morphism of this mitochondrial region at the geographical scale studied.

### Test of Hardy-Weinberg Proportions

At the 5% threshold, Hardy-Weinberg proportions were rejected for two loci. However, when multiple testing was considered, either with the Bonferoni correction or with the Benjamini-Hochberg procedure, no locus showed a significant deviation from Hardy-Weinberg proportions (**Supplementary Table S6**). As no significant heterozygote deficiency could be found, there was no evidence of inbreeding and/or population structuring and/or null alleles.

### Observed distributions of genetic distances

Genetic distances, defined as the number of different alleles between two individuals, were computed between the 21 unique genotypes. Most distances were between 14 and 24. Very few distances were smaller or equal to three (Fig. 2, Table 1).

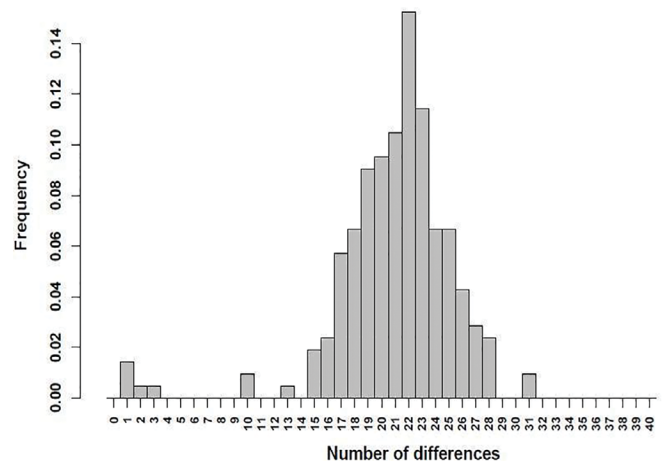


Figure 2: Distribution of pairwise allelic difference counts between individuals.

### Expected distribution of genetic distances according to genealogical relationships

The calculated  $P_{uni (unrelated)}$  and  $P_{uni (sib)}$  were low ( $P_{uni (unrelated)} = 1.13e-13$  and  $P_{uni (sib)} = 2.18e-6$ ). Using simulations, we also obtained the expected distribution of genetic distances according to kinship level, considering allele frequencies at each locus in the population (Supplementary Fig. S3). Considering only distances with a probability > 5%, we found that genetic distances should be in the range of 15 to 22 differences for unrelated individuals, 9 to 14 for parent-offspring, 7 to 14 for full siblings, and 12 to 18 for half siblings.

This indicates that a genetic distance smaller or equal to three would be very unlikely even between full siblings (1 on 1000, Figure S4), and would more likely result from genotyping errors between samples from a same individual. For genotypes with such small distances between them, a consensus genotype was built by retaining the most frequent allele at the locus with probable errors. With this method, the 21 unique genotypes originally identified were assumed to correspond to 17 snow leopards (11 males and 6 females; Supplementary Table S5).

Allele frequencies and expected and observed heterozygosity were computed for these 17 individuals (Supplementary Table S6).

### Relationships between snow leopards

We computed the relationships between snow leopards by using ML Relate on the 17 unique individuals identified (Supplementary Table S7) and identified seven parent-offspring, two full siblings, and 16 half-siblings relationships. Each individual was related to at least one and up to six other individuals (Fig. 3).

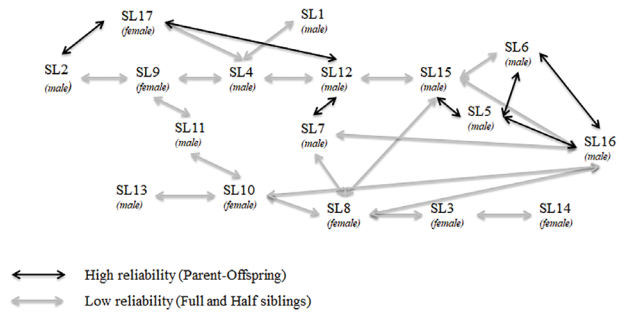


Figure 3: Relationships between the different individuals and their reliability inferred using the ML-Relate software.

To determine the reliability of the results from MLRelate, we assessed whether the program was able to accurately identify the relationships between the simulated genotypes of individuals of known kinship. The program has an overall identification accuracy rate of 74%, as it properly identified 93% of parent-offspring relationships, 67% of full-sibling relationships, 58% of half-sibling relationships, and 81% of unrelated individuals.

### Monitoring of individuals

Over the study period, 12 individuals were sampled several times (Fig. 4, Table 1). The other five (SL8 (female), SL10 (female), SL11 (male), SL16 (female), and SL17 (male)) were sampled only once, respectively in Uch Baital in 2012, Jaman-suu glacier in 2015, Uch Baital in 2015, Tchong saryetchki in 2019, and Chomoi in 2019. Among the 12 individuals sampled several times, two were sampled only during one year, with SL1 (male) sampled twice in Solomo and once at a location in front of Bashkul (2011), and SL7 (male) sampled three times at Djili suu (2014).

On the contrary, SL2 (male) was sampled multiple times during the study, in 2012, 2013, 2014, 2016, and 2017, in several locations all

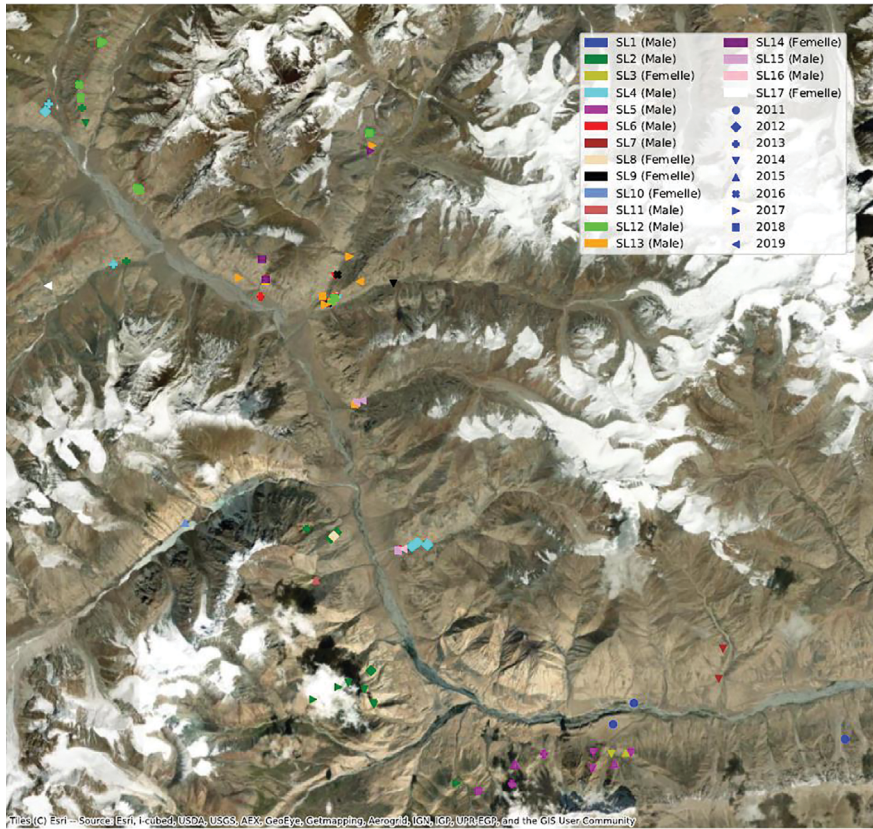


Figure 4: Map of sampled and genotyped snow leopards inside the SESR from 2011 to 2019. Individual snow leopards are highlighted in different colors and years are indicated with different symbols.

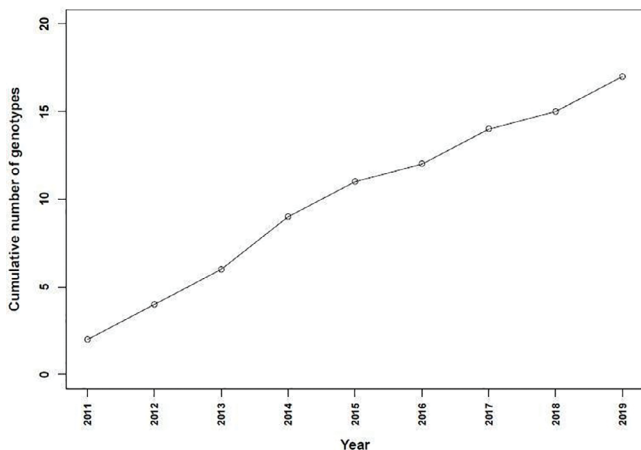
	Recapture	Sex	SL1	SL1_bis	SL1_ter	SL2	SL2_bis	SL3	SL4	SL5	SL5_bis	SL6	SL7	SL8	SL9	SL10	SL11	SL12	SL13	SL14	SL15	SL16	SL17	
SL1	1	Male																						
SL1_bis	1	Male	2																					
SL1_ter	1	Male	1	3																				
SL2	10	Male	21	23	22																			
SL2_bis	7	Male	21	23	22	1																		
SL3	2	Female	20	19	19	21	21																	
SL4	10	Male	17	15	18	15	16	19																
SL5	9	Male	19	19	20	22	23	26	21															
SL5_bis	1	Male	19	19	20	23	23	26	22	1														
SL6	12	Male	17	16	18	23	23	26	19	10	10													
SL7	3	Male	24	23	23	22	22	19	25	20	20	21												
SL8	1	Female	22	21	21	23	23	19	21	20	20	18	15											
SL9	4	Female	23	21	22	20	19	22	15	24	24	21	24	22										
SL10	1	Female	23	22	22	21	22	18	23	19	20	20	16	13	25									
SL11	1	Male	23	21	22	17	18	20	17	24	25	23	25	20	18	17								
SL12	12	Male	22	21	23	18	17	23	17	22	22	20	19	17	22	21	24							
SL13	13	Male	26	26	27	22	23	27	21	26	27	24	25	25	26	18	23	25						
SL14	3	Female	20	19	21	18	18	18	16	22	22	20	23	25	22	22	22	24	22					
SL15	4	Male	22	21	23	20	20	19	19	18	18	19	20	19	23	22	22	16	24	21				
SL16	1	Male	27	25	28	24	24	27	26	17	17	18	20	21	23	22	28	24	31	25	21			
SL17	1	Female	22	24	22	17	17	26	21	28	28	25	28	24	20	27	25	22	31	25	25	19		

Table 1: Pairwise allelic difference counts between individuals. Red background: distance = 1; blue background: distance = 2; green background: distance = 3

along and on both banks of the Ertash River with a total of 17 scats retrieved. SL5 (male) was also sampled 4 years in a row, from 2013 to 2016, in the Solomo and Sirdibai (ten scats in total).

Other individuals were resampled in different areas of the reserve for two or three years. On the male side: SL6 (12 scats) in 2013, 2014, 2016 and SL12 (12 scats) from 2016 to 2019, both on the upper part of the Ertash River; SL4 (ten scats) in 2012 and 2013 along the Ertash River; SL13 (13 scats) from 2016 to 2019, centered on and around the Koilou valley; and SL15 (four scats) sampled three times in Kitchi Sary Etchki (2018 and 2019), and once in Tchong Sary Etchki (2018). For the three remaining females, SL3 (two scats) was sampled in Solomo in 2014 and 2015; SL9 (four scats) in Kitchi Koilou in 2014 and Chong Koilou and Ortho Koilou in 2016; finally SL14 (three scats) was sampled in Chong Koilou and Kirk choro in 2017-2018.

We detected new individuals every year, and thus far this number has not plateaued (Fig. 5).



**Figure 5:** Cumulative number of identified individual snow leopards by year based on the minimum number of individuals retrieved by grouping samples with genotyping errors (cf. text).

## Discussion

A total of 98 of the 151 collected samples (65%) were genotyped with at least ten markers and a QI equal to or above 0.5. Samples were presumably difficult to genotype because of their degradation due to high mountain weather conditions and UV light, which led to 53 unusable samples (mean QI = 0.19). For the remaining 98 usable samples, genotyping was rather robust based on mean QI (0.81) as well as resulting dropout (0.011) and false allele (0.0008) rates. From 2015, the improvement of the storing protocol – systematic drying, use of silicagel and a coffee filter to protect from the silicagel – appeared to help slow DNA degradation. Reduced time between sample collection and DNA extraction was also linked to an increase in the number of samples successfully genotyped.

From the genotyped samples, 17 individuals were identified in the SESR, with a sex ratio of 11:6 male to female. This number of snow leopards is similar to previous estimates from genetic sampling (18 in 2009) and camera trapping (15 in 2014) performed within the SESR (McCarthy and Mallon 2016). At present, there are more males than females, but snow leopard sex ratio is known to be highly variable (Sharma, et al. 2014), and hence it should be re-examined in the coming years to note any changes.

New individuals were sampled every year, which could be explained by

- i) a partial sampling of the whole population each year;
- ii) the presence of new offspring in the area; or
- iii) individuals dispersing or moving in or through the SESR from outside areas.

### ***Genealogical relationships***

With our data set, some putative relationships were identified: seven parent-offspring relationships, two full siblings and 16 half-siblings. However, these results should be interpreted with caution until further verification is conducted. The seven identified parent-offspring relationships included SL6 and SL5 (both sampled between 2013 and 2016); SL5 and SL15 (sampled in 2018 and 2019); SL16 (sampled in 2019) with both SL5 and SL6; SL12 and SL7, respectively sampled from 2016 to 2018 and in 2014; and finally SL17 (sampled in 2019) with both SL2 (sampled between 2011 and 2017) and SL12 (sampled from 2016 to 2018). Using simulated data, ML Relate identified parent-offspring relationships effectively, achieving 93% accuracy rate. This suggests that the observed relationships may have some degree of validity. However, SL16 and SL17 have incomplete genotypes, making their genealogical relationships less reliable. In the future, more data and more evidence to support parent-offspring relationships could be gathered by camera trapping as it is possible to observe cubs with their mother over two years before dispersal (Jackson, et al. 2006; Alexander, et al. 2016; Rode, et al. 2021). Other full-siblings and half-sibling relationships are less certain, as we found that ML Relate was unable to effectively identify these relationships using simulated genotypes, achieving identification rates of 67% and 58%, respectively.

### ***Monitoring of individuals over time***

Among the 17 identified individuals, 12 were detected multiple times. Males have been shown to have larger home ranges than females

(Johansson, et al. 2015). SL2 was detected across the largest area within the reserve, with samples collected from seven different transects along both banks of the Ertash River. The transects were located approximately 25 kilometers apart at their furthest points. Other individuals were detected around a couple of valleys at various locations along the Ertash River and nearby. In addition, some individuals were detected on both banks of the Ertash River, for which there is no documented evidence that they cross yet.

The close proximity of samples collected from different individuals indicates that various snow leopards may visit marking areas; however, collar data from other studies suggests that snow leopards are generally territorial (Ahlborn and Jackson 1988; McCarthy, et al. 2005; Johansson et al. 2016; Rode, et al. 2021).

It is also worth noting that feces from juveniles (under one year old) were likely missed because they are difficult to recognize and may degrade faster than adult's scats, as observed for other species such as the brown bear (Sentilles, Delrieu & Quenette, 2016). Furthermore, in May 2018, the WWF carried out a genetic study in the SESR and collected all samples identified as snow leopard scats within a week. Consequently, during our subsequent missions that year, we missed part of the scats deposited earlier in the year and were only able to collect those deposited after May.

While this microsatellite-based genetic study represents a costly monitoring effort, it offers valuable insights that complement camera-trap analyses by providing a deeper understanding of the genetic status of the SESR snow leopard population, including potential insights into inbreeding and kinship.

Cross-referencing the results of this study with camera-trap data from the same area would yield additional information on behavior, precise sighting dates, and litter composition.

### ***Citizen sciences sample collection***

The citizen science program of OSI Panthera has allowed the sampling of snow leopard feces over nine summer seasons inside the SESR. In addition, the program fulfils an educational mission by raising awareness among volunteers about the importance of protecting wildlife and ecosystems. It trains participants in non-invasive wildlife observation techniques, specifically focusing on detecting signs of snow leopard presence. Another of the program's educational aims is to enhance the training of park rangers in data collection methodologies, empowering them to effectively apply their naturalist expertise for wildlife and ecosystem research. The program is financially supported by paid registrants, with volunteers going in the field to support scientists while they perform the collection of samples and other environmental data, and set up/control camera traps. As a result, data retrieval is not solely dependent on research grants and could, in some circumstances, be more sustainable in the long term (Couvet, et al. 2008).

### **Conclusion**

This study allowed us to gather information on the snow leopard population of the SESR between 2011 and 2019, during which we identified a minimum of 17 individuals. The recapture of 12 individuals over the years provided insights into the areas they were utilizing within the SESR. Additionally, we

attempted to assess genetic relationships between individuals, although this requires further validation. We will continue the long-term monitoring of this population through non-invasive sampling and by incorporating data obtained from camera traps. This approach will further refine our understanding of the population status, which is crucial for informing future conservation actions.

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### **Ethics approval**

An authorization from the Kirgiz Ministry of Natural Resources, Ecology and Technical Oversight was given to OSI-Panthera program

allowing scat collection, in accordance to the Nagoya protocol.

## Competing Interests

No known conflicts of interests.

## Data Availability

All data are available in supplementary materials.

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**Table S1: List of prospected transects from 2011 to 2019**

<b>Index</b>	<b>Place</b>	<b>Date</b>	<b>Snow leopard evidence (0: no, 1: yes)</b>	<b>Number of scats collected</b>	<b>Number of scats genotyped</b>
W	After Kocheteuk	July 2014	0	0	0
A	Atcha	July 2019	1	0	0
		August 2018	1	1	1
		August 2017	1	0	0
		August 2016	0	0	0
		June 2016	1	0	0
		June 2014	1	0	0
		June 2013	1	0	0
P	Bir Baital	August 2019	1	4	4
		July 2017	1	3	3
		August 2015	1	1	0
		August 2014	1	1	1
		June 2014	1	3	3
		June 2013	1	3	0
		August 2012	1	2	2
		July 2012	1	1	1
C	Bordu	August 2017	1	1	1
		August 2016	1	2	2
		June 2013	1	5	1
		June 2012	1	3	1
B	Boroko	July 2019	0	0	0
		August 2018	1	0	0
		August 2017	0	0	0
		June 2013	1	0	0
G	Chomoi	July 2019	1	3	3
		June 2013	1	6	3
O	Eki Baital	August 2015	1	0	0
		July 2015	1	1	0
		August 2014	1	1	0
X	Near Bashkul	June 2013	1	1	0

		August 2011	1	2	1
F	Chong Bordu	July 2019	1	1	1
		August 2018	1	0	0
		June 2016	0	0	0
		June 2014	1	1	1
I	Chong Koilou (waterfall and beyond)	July 2019	1	1	1
		August 2018	1	3	3
		August 2017	1	2	2
		June 2016	1	2	2
		June 2014	1	1	1
M	Jaman-suu	June 2017	1	0	0
		August 2016	0	0	0
		July 2016	1	1	1
		July 2015	1	0	0
		June 2015	1	2	1
		August 2014	1	1	0
		August 2013	1	1	0
R	Kichi Kashka tor	August 2011	1	1	0
		September 2018	1	0	0
		June 2017	1	0	0
		August 2016	1	1	0
S	Chong Kashka tor	August 2015	0	0	0
		June 2017	1	0	0
		July 2015	0	0	0
H	Kirk-choro	June 2014	1	1	0
		July 2019	1	1	1
		August 2018	1	3	3
		June 2014	1	6	2
		June 2013	1	5	3
		July 2012	0	0	0
W	Kocheuteuk	June 2012	1	0	0
		August 2012	0	0	0
		July 2012	0	0	0
		August 2011	0	0	0
Z	Koyendou	July 2011	1	0	0
		July 2015	0	0	0

		June 2015	0	0	0
E	Orto-bordu	July 2019	1	3	3
		August 2018	1	2	2
		August 2017	1	8	8
		June 2016	1	3	3
		June 2013	1	3	1
		July 2012	1	1	0
		June 2012	1	0	0
		June 2014	1	1	1
J	Orto Koilou	July 2019	1	2	2
		August 2018	1	2	2
		August 2017	1	4	4
		August 2016	1	0	0
		June 2016	1	3	3
		June 2014	1	4	4
		June 2013	1	7	4
D	Kichi Bordu	June 2016	1	0	0
		June 2014	1	1	0
K	Kichi Koilou	June 2014	1	1	1
		June 2013	0	0	0
		July 2012	0	0	0
Q	Tchong Sary Etchki	July 2019	1	3	3
		August 2018	1	2	2
		July 2015	0	0	0
		August 2014	0	0	0
		June 2013	1	5	2
		July 2012	1	1	1
		June 2012	1	3	3
		August 2011	1	0	0
		July 2011	1	5	0
L	Kitchi Sary Etchki	July 2019	1	5	5
		August 2018	1	2	2
T	Sirdibai	July 2017	0	0	0
		June 2017	1	2	2
		August 2016	1	1	1
		July 2016	1	1	1

		August 2015	1	2	1
		July 2015	1	0	0
		June 2015	1	3	2
		July 2014	1	1	0
		June 2014	1	2	0
		August 2013	1	4	1
		July 2013	1	3	2
		August 2012	1	1	0
		July 2012	1	2	0
		June 2012	0	0	0
		August 2011	1	0	0
		July 2011	1	0	0
U	Solomo	July 2017	0	0	0
		June 2017	1	0	0
		August 2016	0	0	0
		July 2016	0	0	0
		August 2015	0	0	0
		July 2015	1	1	1
		June 2015	1	2	1
		August 2014	1	2	1
		July 2014	1	1	1
		June 2014	1	3	2
		August 2013	1	1	0
		July 2013	1	4	0
		August 2012	0	0	0
		June 2012	1	3	0
		August 2011	1	2	2
		July 2011	1	3	2
N	Uch Baital	July 2016	1	2	2
		August 2015	1	1	0
		July 2015	1	2	2
		August 2013	1	1	0

		July 2013	1	1	1
		August 2012	1	4	1
		July 2012	0	0	0
		June 2012	1	3	2
		August 2011	1	0	0
		July 2011	1	1	1
V	Jili Boulak	June 2014	1	3	3
1	Kizil djar	July 2019	0	0	0
		August 2018	1	0	0
Crossing of IJK	Koilou – valley entrance	July 2019	1	2	2
		August 2018	1	0	0
		August 2017	1	3	3
		June 2016	1	1	0
		June 2014	1	7	0
		June 2013	1	0	0
		July 2012	1	1	0
2	Kyzyl keregue	July 2019	1	0	0
		August 2018	1	1	1
		August 2017	1	2	2
		June 2016	1	3	3
3	Face to Oroï suu	July 2019	1	0	0
4	Oroï suu	July 2019	1	2	2
5	Vallon entre Atcha et Boroko	July 2019	1	1	1

**Table S2 – Microsatellite markers**

<b>Mix</b>	<b>Locus name</b>	<b>Dye</b>	<b>Publication</b>
A	F37	PET <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA008	6FAM	Menotti-Raymond <i>et al.</i> 1999
B	FCA024	PET <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B & C	FCA026	PET <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA043	NED <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA045	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA058	6FAM	Menotti-Raymond <i>et al.</i> 1999
C	FCA069	6FAM	Menotti-Raymond <i>et al.</i> 1999
C	FCA075	NED <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
A	FCA077	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
A	FCA085	NED <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA096	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA124	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
A	FCA126	PET <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
C	FCA220	NED <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
C	FCA229	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
C	FCA310	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
C	FCA453	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
A	FCA547	VIC <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
B	FCA668	NED <sup>TM</sup>	Menotti-Raymond <i>et al.</i> 1999
A & C	ZFX Y	NED <sup>TM</sup>	Pilgrim <i>et al.</i> 2005

**Table S3 – Genotypes**

Session	Lab Code	N° Animal	F37	FCA008	FCA024	FCA043	FCA045	FCA058	FCA077	FCA085	FCA096	FCA124	FCA126	FCA547	FCA668	FCA026	FCA069	FCA075	FCA220	FCA229	FCA310	FCA453	ZF XY	Number of replicates	QI	Marker OK	Sex	Year	
S02_2020	SL-SE-5-28/07/2017	SL2	000000	00000	00000	120120	141147	203203	144144	131135	000000	090096	000000	000000	000000	148150	097101	118120	000000	000000	130130	000000	XY	2	0,46	10	Male	2017	
S02_2020	SL-SE-6-16	SL6	000000	132142	000000	114114	145147	000000	140144	127135	000000	090090	000000	000000	000000	148148	097097	118120	000000	000000	130130	000000	XY	2	0,46	11	Male	2016	
S02_2020	SL-SE-13-8/08/18	SL12	000000	136142	000000	118118	141145	201203	144144	127135	000000	096100	000000	000000	000000	095000	118120	000000	000000	130130	000000	XY	2	0,46	10	Male	2018		
S02_2020	SL-SE-3-28/07/2017	SL2	242242	000000	000000	118120	141147	201203	144144	131135	000000	000000	151151	000000	000000	148150	097101	118120	000000	000000	130130	000000	XY	2	0,50	11	Male	2017	
S01_2020	39_2014	SL3	000000	142142	000000	118118	145147	201201	000000	129131	000000	090100	000000	000000	000000	146148	093101	118118	000000	110110	130132	189193	XX	2	0,50	12	Female	2014	
S01_2020	4_2012	SL4	256272	132136	220222	118118	141147	201203	140144	135135	206206	090090	000000	247247	158158	148150	095101	120120	208208	110110	130130	189193	XY	2	0,54	19	Male	2012	
S02_2020	SL-SE-22-12/08/2017	SL12	242242	136142	000000	118118	141145	201203	144144	127135	000000	096100	000000	247247	000000	000000	095095	118120	000000	000000	130130	000000	XY	2	0,54	12	Male	2017	
S02_2020	SL-SE-5-2016	SL12	000000	136136	000000	118118	141145	201203	144144	127135	000000	096100	000000	000000	000000	148150	095095	118120	000000	000000	130130	000000	XY	2	0,54	12	Male	2016	
S02_2020	SL-SE-8-08/08/2017	SL13	000000	136136	000000	118124	141141	000000	140142	135135	000000	100102	139147	000000	000000	148148	097101	120120	000000	000000	130130	197197	XY	2	0,54	13	Male	2017	
S02_2020	SL-SE-15-10/08/2017	SL13	000000	136136	000000	118124	141141	201201	140142	131135	000000	100102	000000	000000	000000	148148	097101	120120	000000	106108	130130	000000	XY	2	0,58	12	Male	2017	
S02_2020	SL-SE-14-10/08/2017	SL14	000000	132132	000000	118118	141147	201203	140144	131135	200206	000000	000000	251251	000000	148148	093097	120120	000000	000000	130132	000000	XX	2	0,58	12	Female	2017	
S01_2020	10_2011	SL1	000000	136142	000000	118120	141147	201203	140146	129131	000000	090096	000000	247247	158158	146148	095095	118126	000000	110110	130130	000000	XY	2	0,59	14	Male	2011	
S01_2020	40_2014	SL2	000000	140142	000000	118120	141147	201203	144144	131135	000000	090096	000000	000000	158158	148150	097101	118120	000000	106106	130130	185193	XY	2	0,63	14	Male	2014	
S01_2020	10_2015	SL3	250256	142142	000000	118118	145147	201201	142144	129131	000000	000000	000051	000000	247251	000048	093101	118118	208208	110110	130132	189193	XX	2	0,63	15	Female	2015	
S02_2020	SL-SE-10-16	SL9	000000	132132	000000	118118	000000	000000	140144	129135	000000	090090	141149	247247	000000	146150	101101	118120	208208	000000	130130	193193	XX	2	0,63	14	Female	2016	
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S02_2020	SL-SE-6-08/08/2017	SL12	242242	136142	000000	118118	141145	201203	144144	127135	000000	096100	147153	000000	158158	148150	095095	118120	000000	000000	130130	000000	XY	2	0,67	14	Male	2017	
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S02_2020	SI-SE-04-6/08/18	SL13	256272	136136	000000	118124	141141	201201	140142	131135	000000	000000	000047	000000	000048	148101	197120	120100	000008	106130	130197	197197	XY	2	0,67	14	Male	2018
S01_2020	37_2013	SL2	000000	140142	000000	118120	141147	201203	144144	131135	000000	090096	141151	000000	158158	148101	197120	118100	000006	106130	130193	185193	XY	2	0,68	15	Male	2013
S01_2020	13_2012	SL2	242242	140142	000000	118120	141147	201203	144144	131135	000000	090096	141151	000000	158158	148101	197120	118108	208206	106130	130193	185193	XY	2	0,69	18	Male	2012
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S01_2020	23_2012	SL2	242242	140142	220220	118120	141147	201203	144144	131135	200206	090096	141151	247251	158158	148101	197120	118108	208206	106130	130193	185193	XY	2	0,75	20	Male	2012
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S02_2020	SL-SE-7-16	SL9	242272	132132	000000	118118	147147	201203	140144	129135	000000	090090	141149	247200	000050	146101	101120	118108	208210	106130	130193	193193	XX	2	0,79	18	Female	2016
S02_2020	SI-SE-01-5/08/18	SL12	242242	136142	000000	118118	141145	201203	144144	127135	204206	096100	000000	000058	158150	148195	197120	118108	204210	106130	130189	189189	XY	2	0,79	17	Male	2018
S02_2020	SL-SE-8-16	SL13	000000	136136	000000	118124	141141	201201	140142	131135	000000	100102	139147	247247	158160	148148	197101	120120	000000	000030	130197	197197	XY	2	0,79	16	Male	2016
S02_2020	SL SE-11-7/08/18	SL15	250256	132136	220222	114118	141147	201203	144144	127129	204206	096100	147157	247247	158160	148148	197097	118118	000000	000030	130100	000000	XY	2	0,79	17	Male	2018
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S02_2020	SL-SE-20-12/08/2017	SL12	242242	136142	000000	118118	141145	201203	144144	127135	204206	000000	147153	247247	158158	148150	197195	118120	204208	106110	130130	189189	XY	2	0,83	18	Male	2017
S02_2020	SL-SE-21-12/08/2017	SL12	242242	136142	000000	118118	141145	201203	144144	127135	204206	096100	147153	247247	158158	148195	197120	118100	000010	106130	130189	189189	XY	2	0,83	18	Male	2017
S01_2020	6_2011	SL1	256256	136142	222222	118120	141147	201203	140146	129131	206208	090096	153157	247247	158158	148148	197195	118126	208210	110130	130193	189193	XY	2	0,84	20	Male	2011
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S01_2020	8_2015	SL11	250272	132136	220220	118118	141147	199201	144144	129131	200200	090090	141151	247247	158158	146148	197101	118120	206206	108110	130130	193193	XY	2	0,88	20	Male	2015



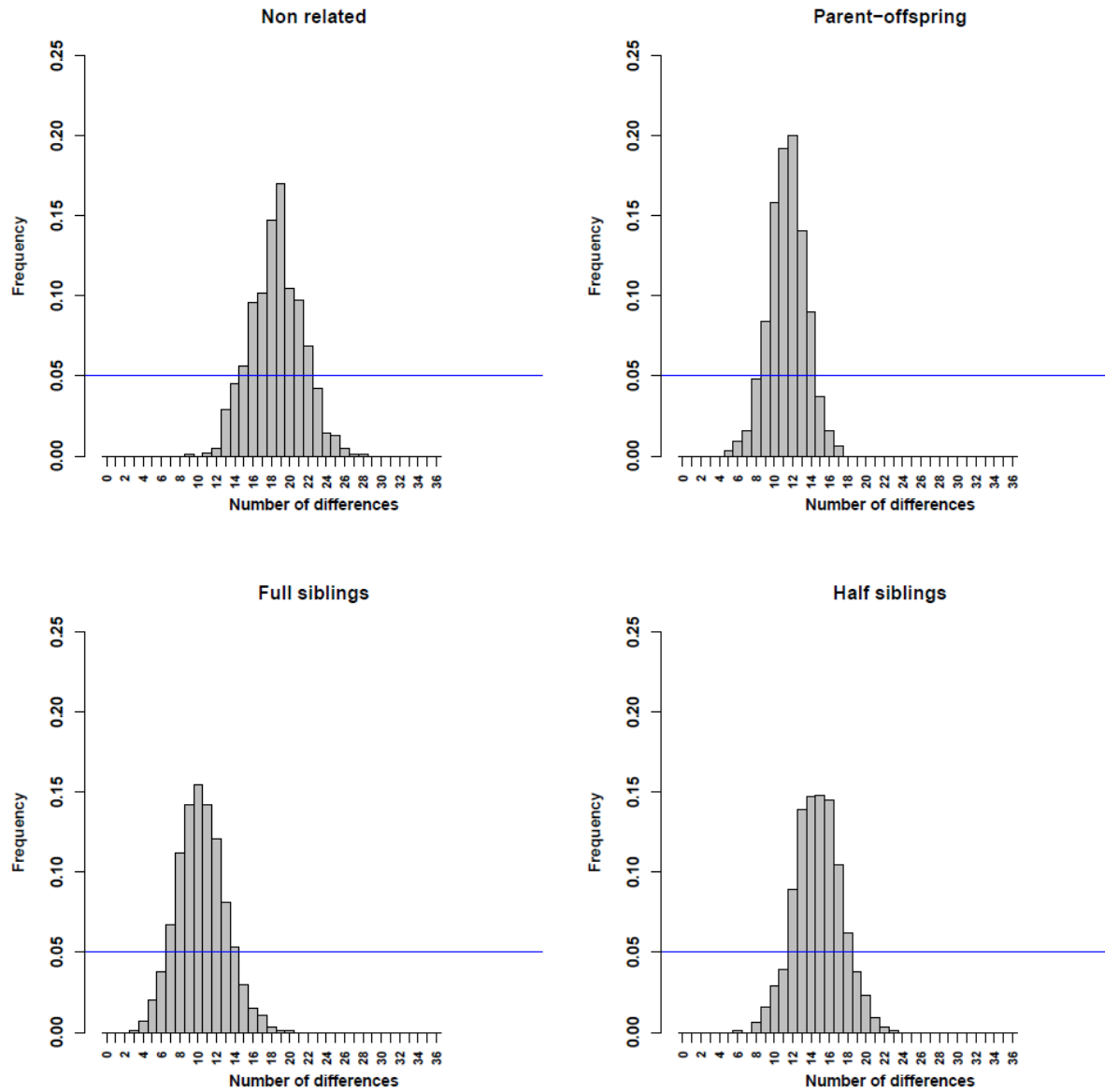
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S01_2020	6_2015	SL5	250272	132140	222222	114118	145147	203203	140144	127135	204208	090096	000000	247247	158158	148148	097097	118118	208210	106110	130130	185189	XY	2	0,90	19	Male	2015
S01_2020	7_2011	SL1	256256	136142	222222	118118	141147	201203	140146	129131	206208	090090	153157	247247	158158	148148	095095	118126	208210	110110	130130	189193	XY	2	0,91	20	Male	2011
S01_2020	12_2013	SL2	242272	140142	220220	118120	141147	201203	144144	131135	200206	090096	141151	247251	158158	148150	097101	118120	208208	106106	130130	185193	XY	2	0,92	20	Male	2013
S01_2020	17_2012	SL4	256272	132136	220222	118118	141147	201203	140144	135135	206206	090090	000000	247247	158158	148150	095101	120120	208208	110110	130130	189193	XY	2	0,92	19	Male	2012
S01_2020	6_2013	SL4	256272	132136	220222	118118	141147	201203	140144	135135	206206	090090	000000	247247	158158	148150	095101	120120	208208	110110	130130	189193	XY	2	0,92	19	Male	2013
S01_2020	13_2013	SL4	256272	132136	000000	118118	141147	201203	140144	135135	206206	090090	141153	247247	158158	148150	095101	120120	208208	110110	130130	189193	XY	2	0,92	19	Male	2013
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S01_2020	42_2013	SL5	250272	132140	222222	114118	145147	203203	140144	127135	204208	090096	000000	247247	158158	148148	097097	118118	208210	106110	130130	185189	XY	2	0,92	19	Male	2013
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S01_2020	2_2015	SL5	250272	132140	222222	114118	145147	203203	140144	127135	204208	090096	000000	247247	158158	148148	097097	118118	208210	106110	130130	185189	XY	2	0,92	19	Male	2015
S01_2020	20_2014	SL6	250254	132142	222222	114114	145147	201203	140144	127135	206208	090090	000000	247247	158158	148148	097097	118120	206210	106110	130130	189189	XY	2	0,92	19	Male	2014
S01_2020	6_2014	SL7	242256	140142	220220	118118	145147	201203	140144	127131	202204	100147	139151	247251	158160	146148	095097	118118	204208	106110	130130	185189	XY	2	0,92	20	Male	2014
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S01_2020	8_2014	SL7	242256	140142	220220	118118	145147	201203	140144	127131	202204	100147	139151	247251	158160	146148	095097	118118	204208	106110	130130	185189	XY	2	0,93	20	Male	2014
S01_2020	19_2014	SL6	250254	132142	222222	114114	145147	201203	140144	127135	206208	090090	157157	247247	158158	148148	097097	118120	206210	106110	130130	189189	XY	2	0,94	20	Male	2014
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S01_2020	10_2014	SL2	242272	140142	220220	118120	141147	201203	144144	131135	200206	090096	141151	247251	158158	148150	097101	118120	208208	106106	130130	185193	XY	2	0,96	20	Male	2014
S01_2020	11_2014	SL2	242272	140142	220220	118120	141147	201203	144144	131135	200206	090096	141151	247251	158158	148150	097101	118120	208208	106106	130130	185193	XY	2	0,96	20	Male	2014
S01_2020	9_2014	SL2	242272	140142	220220	118120	141147	201203	144144	131135	200206	090096	141151	247251	158158	148150	097101	118120	208208	106106	130130	185193	XY	2	0,96	20	Male	2014
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S03_2022	KG-SE-15-	SL13	000000	13613 6	0000 00	1181 24	1411 41	2012 01	1401 42	1311 35	0000 00	1001 02	0000 00	0000 00	1581 60	1481 48	0971 01	1201 20	0000 00	0000 00	1301 30	1971 97	XY	2	0,6 7	13	Male	2020

S03_2022	15/07/2019	17-KG-SE-	<b>SL12</b>	000000	136142	000000	118118	141145	201203	144144	127135	000000	096100	000000	158158	148150	095095	118120	000000	000000	130130	189189	XY	2	0,60	13	Male	2020
S03_2022	16/07/2019	21-KG-SE-	<b>SL17</b>	000000	142142	000000	118120	141147	201203	144144	127135	000000	090096	000000	158158	150150	095101	118120	000000	104106	130130	189193	XX	2	0,60	14	Female	2020

Mean QI : 0.81

Figure S4 - Distribution of pairwise distances between genotypes for different kinships.



**Table S5 – Consensus individual genotypes for the 17 snow leopards identified in this study.**

<b>Name</b>	<b>Recaptures</b>	<b>F37</b>	<b>FCA008</b>	<b>FCA024</b>	<b>FCA043</b>	<b>FCA045</b>	<b>FCA058</b>	<b>FCA077</b>	<b>FCA085</b>	<b>FCA096</b>	<b>FCA124</b>	<b>FCA126</b>
SL1	3	256256	136142	222222	118120	141147	201203	140146	129131	206208	090096	153157
SL2	17	242272	140142	220220	118120	141147	201203	144144	131135	200206	090096	141151
SL3	2	250256	142142	220222	118118	145147	201201	142144	129131	200206	090100	147153
SL4	10	256272	132136	220222	118118	141147	201203	140144	135135	206206	090090	141153
SL5	10	250272	132140	222222	114118	145147	203203	140144	127135	204208	090096	149157
SL6	12	250254	132142	222222	114114	145147	201203	140144	127135	206208	090090	157157
SL7	3	242256	140142	220220	118118	145147	201203	140144	127131	202204	100102	139147
SL8	1	256256	136142	220220	114118	147147	201201	140144	127129	0	090100	141147
SL9	4	242242	132132	0	118118	147147	201203	140144	129135	206206	090090	141149
SL10	1	250272	136142	220222	118118	141147	201201	140144	127131	200204	090100	139147
SL11	1	250272	132136	220220	118118	141147	199201	144144	129131	200200	090090	141151
SL12	12	242242	136142	220220	118118	141145	201203	144144	127135	204206	096100	147153
SL13	13	256272	136136	220222	118124	141141	201201	140142	131135	200206	100102	139147
SL14	3	256256	132142	222222	118118	141147	201203	140144	131135	200206	090102	149159
SL15	4	250256	132136	220222	114118	141147	201203	144144	127129	204206	096100	147157
SL16	1	0	140142	0	114118	145147	201203	140144	127129	204206	090090	0
SL17	1	0	142142	0	118120	141147	201203	144144	127135	0	090096	0

<b>FCA547</b>	<b>FCA668</b>	<b>FCA026</b>	<b>FCA069</b>	<b>FCA075</b>	<b>FCA220</b>	<b>FCA229</b>	<b>FCA310</b>	<b>FCA453</b>	<b>ZFXY</b>	<b>Marker OK</b>	<b>Sex</b>
247247	158158	148148	095095	118126	208210	110110	130130	189193	XY	20	Male
247251	158158	148150	097101	118120	208208	106106	130130	185193	XY	20	Male
247251	158160	146148	093101	118118	208208	110110	130132	189193	XX	20	Female
247247	158158	148150	095101	120120	208208	110110	130130	189193	XY	20	Male
247247	158158	148148	097097	118118	208210	106110	130130	185189	XY	20	Male
247247	158158	148148	097097	118120	206210	106110	130130	189189	XY	20	Male
247251	158160	146148	095097	118118	204208	106110	130130	185189	XY	20	Male
247247	158158	146148	095101	118118	206208	106110	130130	189189	XX	19	Female
247247	158160	146150	101101	118120	0	106110	130130	193193	XX	18	Female
247247	158158	146148	097101	118118	206206	106108	130132	185189	XX	20	Female
247247	158158	146148	097101	118120	206206	108110	130130	193193	XY	20	Male
247247	158158	148150	095095	118120	204208	106110	130130	189189	XY	20	Male
247247	158160	148148	097101	120120	206210	106108	130130	197197	XY	20	Male

247251	158158	148148	093097	120120	206208	110112	130132	193193	XX	20	Female
247247	158160	148148	097097	118118	204208	106106	130130	189193	XY	20	Male
0	158158	148148	097097	118118	0	0	130130	185189	XY	14	Male
0	158158	150150	095101	118120	0	104106	130130	189193	XX	14	Female

**Table S6 – Allele frequencies, allele richness, expected and observed heterozygosity, Hardy-Weinberg (H-W) test p-values and their Benjamini-Hofberg (B-H) associated thresholds.**

	<b>F37</b>	<b>FCA008</b>	<b>FCA024</b>	<b>FCA043</b>	<b>FCA045</b>	<b>FCA058</b>	<b>FCA077</b>	<b>FCA085</b>	<b>FCA096</b>	<b>FCA310</b>										
<b>Allele frequency</b>	242	0,2	132	0,2353	220	0,5357	114	0,1765	141	0,3235	199	0,0294	140	0,3235	127	0,2647	200	0,2333	130	0,9118
	250	0,2	136	0,2647	222	0,4643	118	0,7059	145	0,1765	201	0,5882	142	0,0588	129	0,2059	202	0,0333	132	0,0882
	254	0,0333	140	0,1176			120	0,0882	147	0,5	203	0,3824	144	0,5882	131	0,2353	204	0,2		
	256	0,3667	142	0,3824			124	0,0294					146	0,0294	135	0,2941	206	0,4333		
	272	0,2000															208	0,1		
<b>He</b>	0,74	0,71	0,50	0,46	0,61	0,51	0,54	0,75	0,71	0,16										
<b>Ho</b>	0,67	0,76	0,36	0,47	0,82	0,71	0,71	0,94	0,80	0,18										
<b>Number of alleles</b>	5	4	2	4	3	3	4	4	5	2										
<b>H-W Test p-value</b>	0,1049	0,6153	0,0149	0,3226	0,9409	0,9018	0,9219	0,9639	0,5975	0,0905										
<b>B-H threshold</b>	0,0100	0,0375	0,0025	0,0250	0,0475	0,0425	0,0450	0,0500	0,0325	0,0075										
	<b>FCA124</b>	<b>FCA126</b>	<b>FCA547</b>	<b>FCA668</b>	<b>FCA026</b>	<b>FCA069</b>	<b>FCA075</b>	<b>FCA220</b>	<b>FCA229</b>	<b>FCA453</b>										
<b>Allele frequency</b>	90	0,5294	139	0,1	247	0,8667	158	0,8529	146	0,1765	93	0,0588	118	0,6176	204	0,1071	104	0,0313	185	0,1471
	96	0,1765	141	0,1667	251	0,1333	160	0,1471	148	0,6471	95	0,2353	120	0,3529	206	0,2857	106	0,4063	189	0,4412
	100	0,2059	147	0,2333					150	0,1765	97	0,4118	126	0,0294	208	0,4643	108	0,0938	193	0,3529
	102	0,0882	149	0,1							101	0,2941			210	0,1429	110	0,4375	197	0,0588
			151	0,0667													112	0,0313		
			153	0,1333																
			157	0,1667																
			159	0,0333																
<b>He</b>	0,64	0,85	0,23	0,25	0,52	0,69	0,49	0,67	0,63	0,66										
<b>Ho</b>	0,71	0,93	0,27	0,29	0,53	0,59	0,41	0,64	0,69	0,59										
<b>Number of alleles</b>	4	8	2	2	3	4	3	4	5	4										

<b>H-W Test p-value</b>	0,7636	0,5675	0,1839	0,2889	0,3304	0,1546	0,1821	0,2227	0,6071	0,0166
<b>B-H threshold</b>	0,0400	0,0300	0,0175	0,0225	0,0275	0,0125	0,0150	0,0200	0,0350	0,0050

Mean He: 0.629

Mean Ho: 0,670

F = (Mean He – Mean Ho) / Mean He = -0.007

*(B-H threshold = p-value rank /20\*0.05)*



**Table S7 – ML Relate Output**

**a. Maximum likelihood estimates of relatedness**

	SL1	SL2	SL3	SL4	SL5	SL6	SL7	SL8	SL9	SL10	SL11	SL12	SL13	SL14	SL15	SL16	SL17
SL1	1																
SL2	0,02	1															
SL3	0	0	1														
SL4	0,17	0,11	0	1													
SL5	0,04	0	0	0	1												
SL6	0,05	0	0	0	0,51	1											
SL7	0	0,01	0,12	0	0,04	0	1										
SL8	0,01	0	0,26	0	0	0	0,25	1									
SL9	0	0,18	0	0,38	0	0	0	0	1								
SL10	0	0	0,1	0	0,07	0	0,18	0,25	0	1							
SL11	0	0,15	0	0,01	0	0	0	0,02	0,16	0,28	1						
SL12	0,05	0	0	0,21	0	0,04	0,45	0,03	0,1	0	0	1					
SL13	0	0	0	0	0	0	0,09	0	0	0,23	0,11	0	1				
SL14	0	0,04	0,16	0,12	0	0	0	0	0,02	0	0	0	0,04	1			
SL15	0	0	0	0,04	0,5	0,15	0,09	0,14	0	0	0	0,13	0	0	1		
SL16	0	0,02	0	0	0,64	0,54	0,3	0,17	0	0,13	0	0	0	0	0,38	1	
SL17	0,24	0,56	0	0,21	0,01	0	0	0	0,07	0	0	0,5	0	0	0	0	1

**b. Estimates of relationships**

	SL1	SL2	SL3	SL4	SL5	SL6	SL7	SL8	SL9	SL10	SL11	SL12	SL13	SL14	SL15	SL16	SL17
SL1	-																
SL2	U	-															
SL3	U	U	-														
SL4	HS	U	U	-													
SL5	U	U	U	U	-												
SL6	U	U	U	U	PO	-											
SL7	U	U	U	U	U	U	-										
SL8	U	U	HS	U	U	U	HS	-									
SL9	U	HS	U	HS	U	U	U	U	-								
SL10	U	U	U	U	U	U	U	HS	U	-							

SL11	U	U	U	U	U	U	U	U	HS	HS	-								
SL12	U	U	U	HS	U	U	PO	U	U	U	U	-							
SL13	U	U	U	U	U	U	U	U	U	HS	U	U	-						
SL14	U	U	HS	U	U	U	U	U	U	U	U	U	U	-					
SL15	U	U	U	U	PO	HS	U	HS	U	U	U	U	U	U	-				
SL16	U	U	U	U	PO	PO	FS	HS	U	HS	U	U	U	U	U	FS	-		
SL17	U	PO	U	HS	U	U	U	U	U	U	U	PO	U	U	U	U	U	-	

**c. Log-likelihood for all pairs of individuals**

Columns refer to the following quantities (Kalinowski et al., 2006):

- R: Relationship with the highest likelihood

- LnL(R): Log-likelihood of R

- Delta Ln(L): Delta log-likelihoods for each relationship. 9999 indicates that the relationship is impossible.

Individual 1	Individual 2	R	LnL(R)	Delta Ln(L)			
				U	HS	FS	PO
SL2	SL1	U	-66,77	-	3,69	8,49	9999
SL3	SL1	U	-69,54	-	1,53	6	9999
SL3	SL2	U	-65,76	-	2,83	8,29	9999
SL4	SL1	HS	-62,97	0,28	-	2,64	9999
SL4	SL2	U	-59,47	-	0,16	3,32	9999
SL4	SL3	U	-62,24	-	2,31	6,62	9999
SL5	SL1	U	-67,69	-	1,24	4,72	9999
SL5	SL2	U	-63,92	-	1,36	6,73	9999
SL5	SL3	U	-66,68	-	5,16	12,6	9999
SL5	SL4	U	-60,39	-	2,6	8,41	9999
SL6	SL1	U	-67,96	-	1,1	4,68	9999
SL6	SL2	U	-64,18	-	4,72	11,32	9999
SL6	SL3	U	-66,95	-	4,37	11,81	9999
SL6	SL4	U	-60,66	-	2,49	7,36	9999
SL6	SL5	PO	-57,25	7,86	3,22	2,23	-
SL7	SL1	U	-70,19	-	3,65	10,49	9999
SL7	SL2	U	-66,41	-	1,46	5,51	9999
SL7	SL3	U	-69,18	-	0,07	2,88	9999

SL7	SL4	U	-62,89	-	5,23	12,05	9999
SL7	SL5	U	-67,33	-	1,37	4,69	9999
SL7	SL6	U	-67,6	-	4,73	10,44	9999
SL8	SL1	U	-57,59	-	0,78	4,41	9999
SL8	SL2	U	-54,66	-	3,12	9,14	9999
SL8	SL3	HS	-57,02	0,41	-	3,12	0,38
SL8	SL4	U	-51,06	-	0,92	4,64	9999
SL8	SL5	U	-53,96	-	3,62	8	9999
SL8	SL6	U	-55	-	1,67	5,61	9999
SL8	SL7	HS	-55,07	0,29	-	2,34	0,29
SL9	SL1	U	-61,2	-	3,81	10,01	9999
SL9	SL2	HS	-57,9	0,23	-	4,02	9999
SL9	SL3	U	-60,9	-	1,65	7,03	9999
SL9	SL4	HS	-52,36	2,24	-	1,39	9999
SL9	SL5	U	-58,35	-	2,61	7,81	9999
SL9	SL6	U	-58,13	-	2,94	6,64	9999
SL9	SL7	U	-60,78	-	3,58	8,25	9999
SL9	SL8	U	-49,94	-	0,91	4,18	9999
SL10	SL1	U	-66,81	-	4,95	11,4	9999
SL10	SL2	U	-63,04	-	2,21	7,51	9999
SL10	SL3	U	-65,81	-	0,21	2,63	9999
SL10	SL4	U	-59,51	-	4,44	9,89	9999
SL10	SL5	U	-63,96	-	2,35	6,02	9999
SL10	SL6	U	-64,23	-	2,58	8,55	9999
SL10	SL7	U	-66,45	-	0,32	1,57	9999
SL10	SL8	HS	-53,33	0,6	-	1,18	9999
SL10	SL9	U	-57,21	-	4,22	10,6	9999
SL11	SL1	U	-68,9	-	4,59	10,42	9999
SL11	SL2	U	-65,13	-	0,23	1,78	9999
SL11	SL3	U	-67,89	-	1,72	6,62	9999
SL11	SL4	U	-61,6	-	0,97	4,17	9999
SL11	SL5	U	-66,05	-	4,71	11,12	9999
SL11	SL6	U	-66,31	-	3,53	9,3	9999
SL11	SL7	U	-68,54	-	5,7	12,81	9999

SL11	SL8	U	-55,48	-	0,46	4,23	9999
SL11	SL9	HS	-59,01	0,28	-	2,75	9999
SL11	SL10	HS	-63,71	1,46	-	0,76	9999
SL12	SL1	U	-65,65	-	1,66	5,43	9999
SL12	SL2	U	-61,88	-	1,06	4,44	9999
SL12	SL3	U	-64,65	-	2,3	7,92	9999
SL12	SL4	HS	-57,76	0,59	-	3,4	9999
SL12	SL5	U	-62,8	-	2,64	7,62	9999
SL12	SL6	U	-63,07	-	3,89	7,98	9999
SL12	SL7	PO	-63,39	1,9	0,35	2,57	-
SL12	SL8	U	-53,39	-	0,62	3,3	9999
SL12	SL9	U	-56,24	-	2,45	5,63	9999
SL12	SL10	U	-61,92	-	2,5	7,83	9999
SL12	SL11	U	-64,01	-	5,36	11,34	9999
SL13	SL1	U	-76,98	-	3,73	10,81	9999
SL13	SL2	U	-73,2	-	3,71	9,18	9999
SL13	SL3	U	-75,97	-	2,97	8,54	9999
SL13	SL4	U	-69,68	-	2,02	6,56	9999
SL13	SL5	U	-74,12	-	5,68	13,72	9999
SL13	SL6	U	-74,39	-	4,03	10,1	9999
SL13	SL7	U	-76,62	-	3,31	7,94	9999
SL13	SL8	U	-64,87	-	2,42	8,19	9999
SL13	SL9	U	-67,37	-	3,79	10,12	9999
SL13	SL10	HS	-72,04	1,2	-	1,56	9999
SL13	SL11	U	-75,33	-	0,15	5,31	9999
SL13	SL12	U	-72,09	-	3,05	9,97	9999
SL14	SL1	U	-71,21	-	2,14	6,05	9999
SL14	SL2	U	-67,43	-	2,11	5,54	9999
SL14	SL3	HS	-69,75	0,45	-	3,94	9999
SL14	SL4	U	-63,91	-	0,03	3	9999
SL14	SL5	U	-68,35	-	2,99	8,71	9999
SL14	SL6	U	-68,62	-	2,55	6,6	9999
SL14	SL7	U	-70,85	-	3,39	9,13	9999
SL14	SL8	U	-59,1	-	4,25	9,75	9999

SL14	SL9	U	-62,55	-	1	5	9999
SL14	SL10	U	-67,48	-	3,02	8,25	9999
SL14	SL11	U	-69,56	-	1,36	6,07	9999
SL14	SL12	U	-66,32	-	4,98	12,02	9999
SL14	SL13	U	-77,64	-	0,92	4,85	9999
SL15	SL1	U	-64,73	-	1,95	6,06	9999
SL15	SL2	U	-60,96	-	2,88	7,79	9999
SL15	SL3	U	-63,72	-	1,94	5,78	9999
SL15	SL4	U	-57,43	-	3,85	7,93	9999
SL15	SL5	PO	-60,12	1,76	0,45	3,03	-
SL15	SL6	HS	-61,94	0,21	-	3,95	9999
SL15	SL7	U	-64,37	-	0,18	3,52	9999
SL15	SL8	HS	-52,42	0,05	-	2,97	9999
SL15	SL9	U	-55,32	-	2,37	7,52	9999
SL15	SL10	U	-61	-	0,74	4,95	9999
SL15	SL11	U	-63,08	-	3,06	8,08	9999
SL15	SL12	U	-59,84	-	0,01	1,74	9999
SL15	SL13	U	-71,16	-	2,53	7,83	9999
SL15	SL14	U	-65,39	-	4,09	10,57	9999
SL16	SL1	U	-43,66	-	1,76	5,01	9999
SL16	SL2	U	-38,06	-	0,73	3,16	9999
SL16	SL3	U	-42,61	-	1,47	5,06	9999
SL16	SL4	U	-37,15	-	2,86	6,28	9999
SL16	SL5	PO	-35,2	5,42	2,29	0,31	-
SL16	SL6	PO	-35,47	2,95	1,05	0,4	-
SL16	SL7	FS	-41,35	1,46	0,51	-	9999
SL16	SL8	HS	-33,31	0,19	-	0,59	9999
SL16	SL9	U	-40,03	-	2,54	6,18	9999
SL16	SL10	HS	-38,25	0,02	-	1,85	0,55
SL16	SL11	U	-39,69	-	2,46	6,71	9999
SL16	SL12	U	-38,45	-	1,34	4,21	9999
SL16	SL13	U	-49,04	-	4,83	10,82	9999
SL16	SL14	U	-39,99	-	2,68	6,3	9999
SL16	SL15	FS	-37,85	0,43	0,37	-	9999

SL17	SL1	U	-46,41	-	0,96	2,21	9999
SL17	SL2	PO	-37,87	3,93	1,55	0,64	-
SL17	SL3	U	-46,2	-	2,89	7,13	9999
SL17	SL4	HS	-40,35	0,33	-	0,98	9999
SL17	SL5	U	-41,97	-	2,4	5,6	9999
SL17	SL6	U	-40,55	-	1,97	4,63	9999
SL17	SL7	U	-43,07	-	2,45	6,86	9999
SL17	SL8	U	-39,82	-	1,22	4,37	9999
SL17	SL9	U	-42,94	-	0,25	3,27	9999
SL17	SL10	U	-42,02	-	1,86	5,8	9999
SL17	SL11	U	-42,82	-	2,62	5,96	9999
SL17	SL12	PO	-38,36	2,91	1,1	1,58	-
SL17	SL13	U	-53,56	-	4,06	10,08	9999
SL17	SL14	U	-45,53	-	2,24	6,1	9999
SL17	SL15	U	-41,87	-	1,85	4,44	9999
SL17	SL16	U	-35,61	-	2,21	5,93	9999

#### d. Confidence Set (95%)

List of relationships consistent with the genetic data at 95% confidence level:

<u>Ind1</u>	<u>Ind2</u>	<u>Relationships</u>	<u>Ind1</u>	<u>Ind2</u>	<u>Relationships</u>	<u>Ind1</u>	<u>Ind2</u>	<u>Relationships</u>
SL1	SL2	U	SL2	SL17	HS, FS, PO	SL5	SL8	U
SL1	SL3	U, HS	SL3	SL4	U, HS	SL5	SL9	U, HS
SL1	SL4	U, HS, FS	SL3	SL5	U	SL5	SL10	U, HS
SL1	SL5	U, HS	SL3	SL6	U	SL5	SL11	U
SL1	SL6	U, HS	SL3	SL7	U, HS	SL5	SL12	U
SL1	SL7	U	SL3	SL8	U, HS, PO	SL5	SL13	U
SL1	SL8	U, HS	SL3	SL9	U, HS	SL5	SL14	U
SL1	SL9	U	SL3	SL10	U, HS	SL5	SL15	U, HS, PO
SL1	SL10	U	SL3	SL11	U, HS	SL5	SL16	FS, PO
SL1	SL11	U	SL3	SL12	U, HS	SL5	SL17	U, HS
SL1	SL12	U, HS	SL3	SL13	U	SL6	SL7	U
SL1	SL13	U	SL3	SL14	U, HS	SL6	SL8	U, HS

SL1	SL14	U, HS	SL3	SL15	U, HS	SL6	SL9	U
SL1	SL15	U, HS	SL3	SL16	U, HS	SL6	SL10	U, HS
SL1	SL16	U, HS	SL3	SL17	U	SL6	SL11	U
SL1	SL17	U, HS	SL4	SL5	U, HS	SL6	SL12	U
SL2	SL3	U	SL4	SL6	U, HS	SL6	SL13	U
SL2	SL4	U, HS	SL4	SL7	U	SL6	SL14	U, HS
SL2	SL5	U, HS	SL4	SL8	U, HS	SL6	SL15	U, HS
SL2	SL6	U	SL4	SL9	HS, FS	SL6	SL16	HS, FS, PO
SL2	SL7	U, HS	SL4	SL10	U	SL6	SL17	U, HS
SL2	SL8	U	SL4	SL11	U, HS	SL7	SL8	U, HS, FS, PO
SL2	SL9	U, HS	SL4	SL12	U, HS	SL7	SL9	U
SL2	SL10	U, HS	SL4	SL13	U, HS	SL7	SL10	U, HS
SL2	SL11	U, HS	SL4	SL14	U, HS	SL7	SL11	U
SL2	SL12	U, HS	SL4	SL15	U	SL7	SL12	HS, FS, PO
SL2	SL13	U	SL4	SL16	U	SL7	SL13	U
SL2	SL14	U, HS	SL4	SL17	U, HS, FS	SL7	SL14	U
SL2	SL15	U	SL5	SL6	FS, PO	SL7	SL15	U, HS
SL2	SL16	U, HS	SL5	SL7	U, HS	SL7	SL16	HS, FS

SL7	SL17	U, HS	SL11	SL17	U, HS
SL8	SL9	U, HS	SL12	SL13	U
SL8	SL10	U, HS, FS	SL12	SL14	U
SL8	SL11	U, HS	SL12	SL15	U, HS, FS
SL8	SL12	U, HS	SL12	SL16	U, HS
SL8	SL13	U, HS	SL12	SL17	HS, FS, PO
SL8	SL14	U	SL13	SL14	U, HS
SL8	SL15	U, HS	SL13	SL15	U, HS
SL8	SL16	U, HS, FS	SL13	SL16	U
SL8	SL17	U, HS	SL13	SL17	U
SL9	SL10	U	SL14	SL15	U
SL9	SL11	U, HS	SL14	SL16	U
SL9	SL12	U	SL14	SL17	U, HS
SL9	SL13	U	SL15	SL16	FS
SL9	SL14	U, HS	SL15	SL17	U, HS

SL9	SL15	U, HS	SL16	SL17	U, HS
SL9	SL16	U, HS			
SL9	SL17	U, HS			
SL10	SL11	U, HS, FS			
SL10	SL12	U, HS			
SL10	SL13	U, HS, FS			
SL10	SL14	U			
SL10	SL15	U, HS			
SL10	SL16	U, HS, FS, PO			
SL10	SL17	U, HS			
SL11	SL12	U			
SL11	SL13	U, HS			
SL11	SL14	U, HS			
SL11	SL15	U			
SL11	SL16	U, HS			



**Table S8 – Species Identification**

The Quality Index (QI) value corresponds to the one of the best quality samples assigned to the individual and is based on the microsatellite genotype of the sample. The mitochondrial cytochrome b haplotype obtained for each sample is indicated as well as the GenBank Accession number of the best match, with the scientific and common names of the published haplotype, as well as the percentage of identity and sequence coverage.

Microsatellite QI best sample assigned	N_Animal	Cytochrome b sequence (503bp)	GenBank Accession number	Scientific name	Common name	Percent identity (%)	Query cover (%)
1,00	SL12	CCCTTATCAAAATTATCAATCACTCATTGATCTTCCCACTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTACAAATCTCACCGGCTCTTTCTAGCCATACACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTA AATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTA CTATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	<i>Panthera uncia</i>	Snow leopard	100	100
0,96	SL14	CCCTTATCAAAATTATCAATCACTCATTGATCTTCCCACTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTACAAATCTCACCGGCTCTTTCTAGCCATACACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTA AATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTA CTATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	<i>Panthera uncia</i>	Snow leopard	100	100
0,96	SL2	CCCTTATCAAAATTATCAATCACTCATTGATCTTCCCACTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTACAAATCTCACCGGCTCTTTCTAGCCATACACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTA AATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTA CTATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	<i>Panthera uncia</i>	Snow leopard	100	100
1,00	SL13	CCCTTATCAAAATTATCAATCACTCATTGATCTTCCCACTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTACAAATCTCACCGGCTCTTTCTAGCCATACACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTA AATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTA CTATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	<i>Panthera uncia</i>	Snow leopard	100	100
1,00	SL15	CCCTTATCAAAATTATCAATCACTCATTGATCTTCCCACTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTACAAATCTCACCGGCTCTTTCTAGCCATACACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTA AATTATGGCTGA	KP202269.1	<i>Panthera uncia</i>	Snow leopard	100	100

ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATACATACACGTAGGACGA  
GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGACTATTGCTCGCAGT  
CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA  
TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC  
TCAGTAGATAAAGCCACCTTGACA

0,96

SL5

CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA  
ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTCCTACAAATTTCTCACC GGCTCTTTCTAGCCATACACT  
ATACATCAGACACAATAAACCCTTTCTCGTCAGTACCCACATCTGCCGCGACGTAAATTTATGGCTGA  
ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATACATACACGTAGGACGA  
GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGACTATTGCTCGCAGT  
CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA  
TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC  
TCAGTAGATAAAGCCACCTTGACA

KP202269.1

Panthera uncia

Snow leopard

100

100

CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA  
ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTCCTACAAATTTCTCACC GGCTCTTTCTAGCCATACACT  
ATACATCAGACACAATAAACCCTTTCTCGTCAGTACCCACATCTGCCGCGACGTAAATTTATGGCTGA  
ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATACATACACGTAGGACGA  
GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGACTATTGCTCGCAGT  
CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA  
TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC  
TCAGTAGATAAAGCCACCTTGACA

0,79

SL9

KP202269.1

Panthera uncia

Snow leopard

100

100

CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA  
ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTCCTACAAATTTCTCACC GGCTCTTTCTAGCCATACACT  
ATACATCAGACACAATAAACCCTTTCTCGTCAGTACCCACATCTGCCGCGACGTAAATTTATGGCTGA  
ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATACATACACGTAGGACGA  
GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGACTATTGCTCGCAGT  
CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA  
TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC  
TCAGTAGATAAAGCCACCTTGACA

0,91

SL1

KP202269.1

Panthera uncia

Snow leopard

100

100

CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA  
ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTCCTACAAATTTCTCACC GGCTCTTTCTAGCCATACACT  
ATACATCAGACACAATAAACCCTTTCTCGTCAGTACCCACATCTGCCGCGACGTAAATTTATGGCTGA  
ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATACATACACGTAGGACGA  
GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGACTATTGCTCGCAGT  
CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA  
TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC  
TCAGTAGATAAAGCCACCTTGACA

0,85

SL8

KP202269.1

Panthera uncia

Snow leopard

100

100

CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA  
ACTTTGGCTCCCTGTTAGGAGTATGTTTAACTCCTACAAATTTCTCACC GGCTCTTTCTAGCCATACACT  
ATACATCAGACACAATAAACCCTTTCTCGTCAGTACCCACATCTGCCGCGACGTAAATTTATGGCTGA  
ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATACATACACGTAGGACGA  
GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGACTATTGCTCGCAGT  
CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA  
TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC  
TCAGTAGATAAAGCCACCTTGACA

0,96

SL6

KP202269.1

Panthera uncia

Snow leopard

100

100

0,96	SL4	CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAATCCTACAAAATTCTCACCGGCTCTTTCTAGCCATACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTAATAATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTAATAATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACTATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	Panthera uncia	Snow leopard	100	100
0,96	SL7	CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAATCCTACAAAATTCTCACCGGCTCTTTCTAGCCATACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTAATAATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTAATAATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACTATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	Panthera uncia	Snow leopard	100	100
0,63	SL3	CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAATCCTACAAAATTCTCACCGGCTCTTTCTAGCCATACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTAATAATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTAATAATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACTATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	Panthera uncia	Snow leopard	100	100
0,92	SL10	CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAATCCTACAAAATTCTCACCGGCTCTTTCTAGCCATACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTAATAATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTAATAATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACTATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	Panthera uncia	Snow leopard	100	100
0,88	SL11	CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAATCCTACAAAATTCTCACCGGCTCTTTCTAGCCATACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTAATAATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTAATAATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA TCACCAATCTCCTATCAGCAATCCCATACTATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTTC TCAGTAGATAAAGCCACCTTGACA	KP202269.1	Panthera uncia	Snow leopard	100	100
0,69	SL16	CCCTTATCAAAATTATCAATCACTCATTCAATTGATCTTCCCCTCCATCCAACATCTCCGCATGATGAA ACTTTGGCTCCCTGTTAGGAGTATGTTTAATCCTACAAAATTCTCACCGGCTCTTTCTAGCCATACT ATACATCAGACACAATAACCGCTTTCTCGTCAGTCACCCACATCTGCCGCGACGTAATAATTATGGCTGA ATTATCCGATACCTACACGCCAACGGAGCCTCCATATTCTTTATCTGCCTATACATACACGTAGGACGA GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAACATTGGAGCCGTAATAATTGCTCGCAGT CATGGCTACAGCCTTCATGGGATATGTCTTACCCTGAGGCCAAATATCCTTCTGAGGAGCAACCGTGA	KP202269.1	Panthera uncia	Snow leopard	100	100

TCACCAATCTCCTATCAGCAATCCCATACATTGGGAGCAACCTAGTAGAATGGATCTGAGGGGGCTT  
TCAGTAGATAAAGCCACCTTGACA

0,60

SL17

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**References:**

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