## Original Article

# Range-Wide Snow Leopard Phylogeography Supports Three Subspecies 

Jan E. Janecka, Yuguang Zhang, Diqiang Li, Bariushaa Munkhtsog, Munkhtsog Bayaraa, Naranbaatar Galsandorj, Tshewang R. Wangchuk, Dibesh Karmacharya, Juan Li, Zhi Lu, Kubanychbek Zhumabai Uulu, Ajay Gaur, Satish Kumar, Kesav Kumar, Shafqat Hussain, Ghulam Muhammad, Matthew Jevit, Charlotte Hacker, Pamela Burger, Claudia Wultsch, Mary J. Janecka, Kristofer Helgen, William J. Murphy, and Rodney Jackson


#### Abstract

From the Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282 (J. E. Janecka and Hacker); Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, China (Yu-Guang and Di-Qiang); The Key Laboratory of Forest Ecology and Environment of State Forestry Administration, Beijing, China (Yu-Guang and Di-Qiang); Institute of General and Experimental Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia (Munkhtsog and Galsandorj), Irbis Mongolia, Ulaanbaatar, Mongolia (Munkhtsog, Bayaraa, and Galsandorj); Bhutan Foundation, Washington, DC (Wangchuk); Wildlife Biology Program, University of Montana, Missoula, MT (Wangchuk); Center for Molecular Dynamics, Kathmandu, Nepal (Karmacharya); Panthera, New York, NY (McCarthy and Li); Center for Nature and Society, College of Life Sciences, Peking University, Beijing, China (Li and Zhi); Department of Environmental Science, Policy and Management, University of California, Berkeley, CA (Li); Shan Shui Conservation Center, Beijing, China (Zhi); Snow Leopard Trust, Seattle, WA (Uulu); Center for Cellular and Molecular Biology, Hyderabad, India (Gaur, S. Kumar, and K. Kumar); Trinity College, Hartford, CT (Hussain); Baltistan Wildlife Conservation and Development Organization, Skardu, Pakistan (Hussain and Muhammad); Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A\&M University, College Station, TX (Jevit and Murphy); Research Institute of Wildlife Ecology, Vienna, Austria (Burger); American Museum of Natural History, New York, NY (Wultsch); Department of Biology, Texas A\&M University, College Station, TX (M. J. Janecka); University of Adelaide, Adelaide, Australia (Helgen); and Snow Leopard Conservancy, Sonoma, CA 95476 (Jackson).


Address correspondence to J. E. Janecka at the address above, or e-mail: janeckaj@duq.edu.
Received April 8, 2017; First decision April 10, 2017; Accepted April 29, 2017.
Corresponding Editor: Alfred Roca


#### Abstract

The snow leopard, Panthera uncia, is an elusive high-altitude specialist that inhabits vast, inaccessible habitat across Asia. We conducted the first range-wide genetic assessment of snow leopards based on noninvasive scat surveys. Thirty-three microsatellites were genotyped and a total of 683 bp of mitochondrial DNA sequenced in 70 individuals. Snow leopards exhibited low genetic diversity at microsatellites ( $A_{N}=5.8, H_{o}=0.433, H_{E}=0.568$ ), virtually no mtDNA variation, and underwent a bottleneck in the Holocene ( $\sim 8000$ years ago) coinciding with increased temperatures, precipitation, and upward treeline shift in the Tibetan Plateau. Multiple analyses supported 3 primary genetic clusters: (1) Northern (the Altai region), (2) Central (core Himalaya


and Tibetan Plateau), and (3) Western (Tian Shan, Pamir, trans-Himalaya regions). Accordingly, we recognize 3 subspecies, Panthera uncia irbis (Northern group), Panthera uncia uncia (Western group), and Panthera uncia uncioides (Central group) based upon genetic distinctness, low levels of admixture, unambiguous population assignment, and geographic separation. The patterns of variation were consistent with desert-basin "barrier effects" of the Gobi isolating the northern subspecies (Mongolia), and the trans-Himalaya dividing the central (Qinghai, Tibet, Bhutan, and Nepal) and western subspecies (India, Pakistan, Tajikistan, and Kyrgyzstan). Hierarchical Bayesian clustering analysis revealed additional subdivision into a minimum of 6 proposed management units: western Mongolia, southern Mongolia, Tian Shan, Pamir-Himalaya, Tibet-Himalaya, and Qinghai, with spatial autocorrelation suggesting potential connectivity by dispersing individuals up to $\sim 400 \mathrm{~km}$. We provide a foundation for global conservation of snow leopard subspecies, and set the stage for in-depth landscape genetics and genomic studies.

Subject areas: Population structure and phylogeography; Conservation genetics and biodiversity
Keywords: Asia, genetics, microsatellites, Panthera uncia, phylogeography, snow leopard, subspecies

The snow leopard (Panthera uncia), considered the world's most elusive large felid, inhabits a vast area ( $\sim 1.6$ million $\mathrm{km}^{2}$ ) across 12 countries in Asia (Jackson et al. 2008; McCarthy et al. 2016) (Figure 1). It is a high-altitude specialist that primarily occupies mountains above 3000 m in elevation (Hemmer 1972). This region is characterized by low oxygen levels, temperature extremes, aridity, low productivity, and harsh climatic condition, yet harbors many distinctive taxa, including the Tibetan fox (Vulpes ferrilata, Harris 2014), Chinese desert cat (Felis bieti, Riordan et al. 2015), argali (Ovis ammon, Harris and Reading 2008), markhor (Capra falconeri, Michel and Rosen 2015), urial (Ovis orientalis, Valdez 2008), and Tibetan antelope (Pantholops hodgsonii, IUCN SSC Antelope Specialist Group 2016). The endangered snow leopard is a flagship species for Asia, the largest carnivore in its high-altitude communities, and yet is under substantial threat throughout its range (Jackson et al. 2010; Rosen and Zeller 2016). Research on distribution (Jackson et al. 2006, Janecka et al. 2008, 2011a; McCarthy et al. 2008; Lovari et al. 2009; Karmacharya et al. 2011; Alexander et al. 2016), ecology (Jackson and Ahlborn 1989; McCarthy et al. 2005; Anwar et al. 2011; Lovari et al. 2013; Johansson et al. 2016; Chetri et al. 2017), adaptation to high-altitude (Cho et al. 2013; Janecka et al. 2015), and conservation (Hussain 2000; Mishra et al. 2003; Jackson and Wangchuk 2004; Rosen et al. 2012; Li et al. 2014; Kachel et al. 2016) has provided many insights into snow leopard abundance, habitat use, behavior, movement patterns, and feeding ecology that are important for guiding conservation and management actions needed to ensure its persistence.

Although snow leopards prefer high-altitude mountainous habitat (e.g., Himalaya, Pamir, Alay, Kunlun, Tian Shan) (Hemmer 1972), they also occur in lower, isolated massifs (e.g., Tost and Noyon Uul in the southern Mongolia, Janecka et al. 2011a; Johansson et al. 2016) and have been observed moving through flat or rolling terrain (Schaller 1998; McCarthy et al. 2005; Johansson et al. 2016). However, limited information is available on the level of connectivity among snow leopard populations. Two recent studies modeled snow leopard habitat and connectivity primarily based on topography and climate (Riordan et al. 2015; Li et al. 2016b). Genetic analyses are needed to provide more direct information on permeability of the landscape, dispersal, and demographic fluctuations, and to identify barriers to movement (Avise 1994, 2000).

Taxonomic classification, phylogeography, and population structure serve as the basis for conservation, management, and research


Figure 1. Locations of 21 sampling localities for snow leopards ( $n=70$ ) included in this study. Snow leopard DNA was obtained noninvasively across 7 regions including western Mongolia (1. Tsagaan Shuvuut, $n=6$; 2. Turgen, $n=5$; 3. Jargalant, $n=4$ ), southern Mongolia (4. Baga Bogd, $n=3$; 5. Arts Bogd, $n=3$; 6. Tost and Noyon, $n=8$; 7. Western Beauties, $n=2$; 8 . Eastern Beauties, $n=1$ ), northern Qinghai (9. Tianjun, $n=2$; 10. Akesai, $n=1$; 11 . Dulan, $n=3$ ), southern Qinghai (12. Zhiduo, $n=2$; 13. Nangqian, $n=2$ ), Tibet-Nepal-Bhutan (14. Jiduo, $n=1$; 15. Shenza, $n=2$ ), (16. Gasa, $n=4 ; 17$. Kangchenjunga, $n=6$ ), India-Pakistan (18. Ladakh, $n=4$; 19. Baltistan, $n=4$ ), Tajikistan-Kyrgyzstan (20. Murghab, $n=4 ; 21$. Tian Shan, $n=4$ ). Snow leopard range map was compiled by the International Snow Leopard Trust and the World Conservation Society in 2008 (version 2016-3).
(Wilson and Brown 1953; Avise 1990; Schwartz et al. 2007). Both ecological and molecular data are needed to understand the species, prioritize populations for conservation, and develop recovery or management plans (O’Brien 1991; Avise 1994; Moritz 1994; Haig et al. 2006; Rodgers and Janecka 2013). The snow leopard remains the last large felid to be the subject of a comprehensive subspecies assessment, phylogeographic analysis, and population structure study. Previous range-wide phylogeography studies of felids have primarily relied on samples from captive animals, telemetry studies, hunter-harvested individuals, or museum specimens (Culver et al. 2000; Eizirik et al. 2001; Uphyrkina et al. 2001; Luo et al. 2004).

The gap in knowledge for snow leopards is a direct result of the following challenges: 1) they inhabit remote, inaccessible regions that are often politically unstable, 2) opportunities for radio/GPS telemetry are limited because they are difficult to observe and trap in the wild, and 3) most founders of the captive population have an unknown origin.

Noninvasive genetic sampling via collection of scat along wildlife trails and marking sites are an effective and efficient way to survey snow leopard populations and have become an important approach for studying snow leopards and numerous other felids (e.g., Janecka et al. 2008; Gilad et al. 2011; Rodgers and Janecka 2013; Rodgers et al. 2015; Wultsch et al. 2016a, 2016b). Our collaborative efforts to conduct noninvasive surveys of this species have yielded snow leopard DNA samples from all major parts of the range. Here, we present the results of the first, to our knowledge, range-wide snow leopard genetic study to establish a framework for understanding its taxonomy, population history, and phylogeography using noninvasively collected scat samples.

## Materials and Methods

## Sample Collection, DNA Extraction, and Genotyping

We collected snow leopard scat in 21 localities distributed throughout the range (Figure 1; Supplementary Table S1) using noninvasive genetic surveys following Janecka et al. (2008, 2011a). DNA was extracted with the Qiagen DNA Stool Kit (Qiagen, Inc., Valencia, CA). Snow leopard scats were identified by amplifying and aligning a 96 bp fragment of the mitochondrial cytochrome $b$ gene with reference sequences following Janecka et al. (2008), or via a species-specific PCR assay from Janecka et al. (2014). We initially identified individuals by genotyping 8 microsatellite loci in triplicate using fluorescently labeled primers (Janecka et al. 2011a; 2014). Sex was determined by amplification of the Y-linked AMELY marker (Murphy et al. 1999) following methods in Janecka et al. (2008). Only those scat samples with no detectable errors in the initial microsatellite panel were used in the phylogeographic study and genotyped at 25 additional microsatellite loci (Supplementary Table S2).

## Genetic Analysis

The majority of felid phylogeographic studies have relied on microsatellites developed for the domestic cat (Felis catus) linkage and radiation hybrid maps (Menotti-Raymond et al. 1999, 2003). To improve PCR-based genotyping success in snow leopard scat we sequenced 49 microsatellites in 2 captive-bred snow leopard samples using Sanger sequencing, following the methods in Janecka et al. (2008). We designed new snow leopard-specific primers from repeatmasked microsatellite motif flanking sequences (i.e., no SINES, LINES, or LTRs), which were positioned closer to the repeats so the amplicon size was between 100 and 150 bp . These new primers were designated by the prefix "PUN" and the respective locus number used by Menotti-Raymond et al. $(1999,2003)$. We selected a final set of 33 microsatellites based on amplification intensity, unambiguous allele peaks, and chromosomal location, and genotyped these loci in 70 individuals (primers provided in Supplementary Table S2 and PCR conditions in Supplemental Material). Three individuals were genotyped in replicate at all 33 loci, and the genotypes for 2 microsatellite loci were replicated in all 70 individuals to ensure consistency. In addition, we sequenced 3 mtDNA segments in 70 individuals including 96 bp of cytochrome $b, 244 \mathrm{bp}$ of the hyper variable region II, and 323 bp of the central conserved region and aligned the sequences with SEQUENCHER 5.5.5 (Gene Codes

Corporation, Ann Arbor, MI). The only variable site was found in the central conserved region in one individual, therefore the mtDNA was not informative for population structure.

## Genetic Diversity, Population Structure, and Coalescent Simulations

Standard estimates of genetic diversity were derived in GENALEX 6.502 (Peakall and Smouse 2006) including the number of alleles $\left(A_{\mathrm{N}}\right)$, number of private alleles $\left(A_{P}\right)$, effective number of alleles $\left(A_{E}\right)$, observed heterozygosity $\left(H_{0}\right)$, expected heterozygosity $\left(H_{E}\right)$, and fixation index $\left(F_{l}, 1-\left(H_{O} / H_{E}\right)\right.$. Loci were tested for linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE) with significant $P$-values adjusted for multiple comparisons.

Recent changes in the effective population $\left(N_{E}\right)$ size in snow leopards were investigated using coalescent simulations implemented in MSVAR 1.3 (Beaumont 1999; Storz 2002; Girod et al. 2011) using 32 polymorphic microsatellite loci (i.e., one monomorphic locus was removed). The model assumes a single stable ancestral population $N_{E 1}$ in the past that experienced a demographic alteration (bottleneck or expansion) starting at time $t$ and subsequently changed exponentially in size to the current population $N_{E O}$. We simulated 2 different demographic scenarios: 1) larger prior distribution values for the contemporary population size $N_{E 0}$ than the ancestral $N_{E 1}$ (expansion) and 2) larger priors for $N_{E 1}$ than $N_{E 0}$ (bottleneck). We tested various prior distributions for each scenario to assess the independency of the posterior estimates for the parameters $N_{E O}$, $N_{E 1}$, and $t$. In lieu of a snow leopard-specific microsatellite mutation rate we choose an average mammalian mutation rate (Brinkmann 1998; Rooney 1999) of $1 \times 10^{-4}$ sub/site/year allowing rate variation between $10^{-3}$ and $10^{-5}$. We ran four coalescent simulations for each population with $2.5 \times 10^{9}$ iterations of the Markov chain Monte Carlo (MCMC) algorithm, discarding the first $20 \%$ as burnin. Convergence of the chains from each population simulated with 4 different priors, respectively, were assessed with Gelman-Rubin's diagnostic (Brooks 1998) implemented in the R package boa (Smith 2007). Gelman-Rubin's convergence tests of the MCMC algorithm for the independent runs and each variable resulted in values below the threshold of 1.1 (Gelman et al. 2004).

We assessed population structure using several methods. First, we estimated the pairwise fixation index ( $F_{S T}$, Weir and Cockerham 1984) to examine gene flow among predefined population groups (NQ, northern Qinghai, this included Aksei in northern Gansu; SQ, southern Qinghai; HIM, Tibet-Nepal-Bhutan; IP, India-Pakistan; TK, Tajikistan-Kyrgyzstan; WM, western Mongolia; SM, southern Mongolia) (Figure 2a). $F_{S T}$ values and their significance were estimated using the analysis of molecular variance (AMOVA) framework in GENALEX. $F_{S T}$ values were also calculated using ARLEQUIN 3.5 (Excoffier et al. 2007), but no substantial difference was found so we present the results from GENALEX. We also performed population assignment tests with frequency methods to evaluate the level of differentiation in GENALEX. These tests yield probabilities that an individual came from each population, based on its genotype and allele frequencies. If assignment probability was highest for a population in which that individual was not observed, it was considered genetically misassigned. There is a direct correlation between the misassignment rate and dispersal (Rannala and Mountain 1997; Paetkau et al. 2004; Janecka et al. 2011b). In addition, to assess genetic similarity among individual snow leopards without making assumptions regarding HWE and LD, we conducted a principal component analysis (PCA) in adegenet 1.4.2 (Jombart 2008) using R 3.2.4.


Figure 2. Range-wide genetic structure analysis of snow leopards ( $n=70$ ) using genotype data from 33 microsatellites. (a) Three subspecies (Panthera uncia uncia, Panthera uncia uncioides, and Panthera uncia irbis) identified across different regions of the snow leopard range (NQ, northern Qinghai; SQ, southern Qinghai; HIM, Himalayas-Bhutan, Nepal, Tibet; IP, India and Pakistan; TK, Tajikistan and Kyrgyzstan; WM, western Mongolia; SM, southern Mongolia). (b) PCA of snow leopards. Scatterplot of principal components 1 and 2 with points representing individual genotypes sampled across different geographic regions and $95 \%$ inertia ellipses. (c-i). Bayesian clustering analysis in STRUCTURE 2.3.4 (Pritchard et al. 2000) was conducted for snow leopards range-wide (c and d, $n=70$ ) and at first (e. $n=40$; f. $n=46$ ) and second (g. $n=24$; h. $n=16$; i. $n=30$ ) hierarchical levels. The second level analysis of Central, Western, and Northern groups, respectively, was used to delineate 6 MUs .

We also used individual-based Bayesian clustering approaches that explored the number of genetic clusters ( $K$ ) within our samples (Pritchard et al. 2000; Guillot et al. 2005). The first approach used STRUCTURE 2.2.4 to identify genetic clusters and estimate ancestry for each individual (Pritchard et al. 2000). This method estimates the likelihoods of different numbers of genetic clusters as well as cluster membership $(Q)$ for each individual. The analysis was done using the following model: admixture, alpha inferred from the data (initial value 1.0), correlated allele frequencies, sampling locations as prior (LOCPRIOR), and $2 \times 10^{6} \mathrm{MCMC}$ iterations after a burn-in of $2 \times 10^{5}$ replicates. We varied the number of potential genetic clusters from 1 to 10 . The most likely value of $K$ was determined using 10 independent runs for each value of $K$. We analyzed our results by applying the posterior probability (Pritchard et al. 2000) and the $\Delta K$ method (Evanno et al. 2005), as implemented by pophelper (Francis 2017) in R 3.2.4. The Q for each individual was averaged across all ten STRUCTURE runs. To examine hierarchical genetic structure, we conducted additional Bayesian analysis within identified genetic clusters until no further genetic subdivision was detected, or
inference was impossible due to low sample sizes (Balkenhol et al. 2014; Wultsch et al. 2016a). We also used a second Bayesian clustering approach that incorporates a spatially explicit model to generate priors as implemented in GENELAND 4.0.6 (Guillot et al. 2005). We applied the spatial model with uncorrelated allele frequencies and simulated the number of $K$ from 1 to 10 using 10 independent runs. Each run consisted of $1 \times 10^{6} \mathrm{MCMC}$ iterations with a thinning of 100. The level of spatial uncertainty was set to 50 m .

We also explored whether a smaller number of microsatellites typically used in noninvasive genetic surveys of snow leopards (i.e., 6-8 loci) would be sufficient to assign individuals to the major genetic groups identified in this study. We therefore created a reduced matrix with only six loci (PUN82, PUN100, PUN124, PUN225, PUN229, and PUN327) and included 26 additional samples from Ladakh, India. Probability of identity for unrelated ( $P_{\mathrm{ID}-\mathrm{urr}}$ ) and related ( $P_{\text {ID-sib }}$ ) individuals was estimated in GENALEX. We used the STRUCTURE test for migrants with $K=3$ to assign these 26 samples to 1 of the 3 clusters. The following parameters were used: admixture, alpha inferred from the data (initial value 1.0), correlated allele
frequencies, sampling locations for reference samples as spatial prior (LOCPRIOR), population information used to test for migrants, and $8 \times 10^{5} \mathrm{MCMC}$ iterations after a burn-in of $4 \times 10^{5}$ replicates.

We examined isolation-by-distance (IBD) patterns by correlating genetic and geographic distances via the Mantel test using ecodist 1.2.9 (Goslee and Urban 2007) in R 3.2.4. We also conducted spatial autocorrelation analysis in GENALEX to assess the spatial extent of genetic structure by examining genetic similarity between pairs of snow leopard individuals at several spatial distance classes ( 25,50 , $100,250,500,750,1000,1500,2000,2500$, and 3000 km ). The spatial autocorrelation coefficient $(r)$ was evaluated at each distance class against the null hypothesis of no genetic structure $(r=0)$ via permutation (10000 simulations) and bootstrapping (1000 repeats).

## Results

We obtained scat samples from 21 localities in 7 geographic regions (northern Qinghai [this region included Aksei in northern Gansu], southern Qinghai, Tibet-Nepal-Bhutan, India-Pakistan, TajikistanKyrgyzstan, western Mongolia, and southern Mongolia) (Figures 1 and 2a; Supplementary Table S1). A total of 70 representative individuals were genotyped at 33 microsatellite loci. Measures of genetic diversity were consistently low across the entire snow leopard range ( $A_{\mathrm{N}}$ of 2.6-3.3; $H_{\mathrm{O}}$ of 0.399-0.508; $H_{E}$ of 0.434-0.485; Table 1; Supplementary Table S3). Southern Mongolia and southern Qinghai had the lowest measures of diversity and the highest frequency of private alleles. When all samples were pooled, 16 loci were out of HWE, in contrast to only 3 loci within each of the 7 locations, indicative of the Wahlund effect. The coalescent simulations supported a demographic contraction scenario with the time estimate for the last bottleneck of $t=7782$ years ago (ya) (range of 4574-11 893; highest probability density, HPD, of 1084-107484; Supplementary Table S4 and Supplementary Figure S1) with an ancestral effective population size $N_{E 1}=8235$ (range of 7287-9852; HPD of 1951-47374) and current effective population size $N_{E 0}=1279$ (range of 1091-1504; HPD of 249-4902). We performed the analysis for all samples pooled and for the 3 main genetic clusters individually (Supplementary Figure S2). There was only a single variable site in the 683 bp concatenated mtDNA alignment, with 69 individuals having one haplotype, and the second haplotype observed in only a single individual.

Among these 7 geographic regions, the greatest genetic similarity based on the pairwise $F_{S T}$ was between northern Qinghai and southern Qinghai ( $F_{S T}=0.039, P=0.174$ ), between India-Pakistan
and Tajikistan-Kyrgyzstan ( $F_{S T}=0.007, P=0.412$ ), and between western Mongolia and southern Mongolia ( $F_{S T}=0.057, P<0.000$; Table 2). The most divergent $F_{S T}$ values ( $>0.25$ ) indicated high levels of differentiation for southern Mongolia versus southern Qinghai ( $F_{S T}=0.308, P<0.000$ ), western Mongolia versus southern Qinghai ( $F_{S T}=0.287, P<0.000$ ), and Tibet-Bhutan-Nepal versus southern Mongolia ( $F_{S T}=0.258, P<0.000$ ). The 2 Mongolian regions were the most differentiated with respect to the other regions.

The PCA revealed 3 major groups consistent with the geographic distribution of sampled localities (Figure 2b). Specifically, the snow leopards from the Tibetan Plateau (northern Qinghai, southern Qinghai, and Tibet) and the principal portion of the Himalaya (Bhutan and Nepal) clustered together into a "Central group," the snow leopards from Western Himalaya (India), Karakorum, Pamir, Alay, and Tian Shan (Tajikistan, Kyrgyzstan) formed a "Western group," and those from Altai (western Mongolia) and Southern Gobi (southern Mongolia) formed a "Northern group" (Figure 2b). Population assignment tests also supported strong genetic differentiation of the 3 groups. There was an $8.5 \%$ misassignment rate when samples were divided into 7 populations. When samples were divided into the Northern, Central, and Western populations there was no misassignment (Supplementary Tables S5 and S6).

Bayesian clustering in STRUCTURE tested for the presence of $K$ of $1-10$ genetic clusters using the admixture model with correlated allele frequencies and sampling locations as priors (Supplementary Figures S3 and S4). The Evanno et al. (2005) ad hoc method supported division into 2 groups ( $K=2$ ) with the first cluster comprised exclusively of samples from Mongolia (i.e., the Northern group) and the second cluster composed of the individuals from all remaining sites (Figure 2c). The majority of individuals were assigned ( $Q>90 \%$ ) to 1 of these 2 clusters. Snow leopards in Tajikistan and Kyrgyzstan showed evidence of genetic admixture with Mongolia. At $K=3$, the Central, Western, and Northern groups supported by PCA analysis and the assignment tests were recovered (Figure 2d). The spatially explicit Bayesian model implemented in GENELAND detected the same 3 clusters, although with more admixture (Figure 3). When a reduced matrix was created with only 6 microsatellite loci ( $P_{\mathrm{ID} \text {-unr }}=0.000033, P_{\mathrm{ID} \text {-sib }}=0.0097$ ), a number commonly used for individual identification in noninvasive surveys, the STRUCTURE test to detect migrants assigned $96 \%$ of 26 additional samples from Ladakh (India) to the appropriate genetic cluster (i.e., the Western group) in which they were sampled (Supplementary Table S7).

Table 1. Genetic diversity estimated in 70 snow leopards genotyped at 33 microsatellites

| Geographic region | $n$ | A | $A_{P}$ | $A_{\text {E }}$ | $H_{0}$ | $H_{E}$ | $F_{I}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Snow leopard | 70 | 5.8 | n. a. | 2.8 | 0.433 | 0.568 | 0.246 |
| Central group | 24 | 4.2 | 28 | 2.5 | 0.446 | 0.521 | 0.138 |
| Northern Qinghai | 6 | 3.1 | 10 | 2.3 | 0.457 | 0.484 | 0.053 |
| Southern Qinghai | 5 | 2.6 | 1 | 2.1 | 0.450 | 0.434 | -0.019 |
| Tibet/Nepal/Bhutan | 13 | 3.3 | 12 | 2.2 | 0.441 | 0.452 | 0.012 |
| Western group | 16 | 4.0 | 19 | 2.6 | 0.461 | 0.522 | 0.126 |
| India/Pakistan | 8 | 3.2 | 10 | 2.3 | 0.508 | 0.473 | -0.082 |
| Tajikistan/Kyrgyzstan | 8 | 3.3 | 6 | 2.4 | 0.415 | 0.485 | 0.174 |
| Northern group | 30 | 3.9 | 22 | 2.3 | 0.408 | 0.481 | 0.152 |
| Western Mongolia | 15 | 3.1 | 5 | 2.2 | 0.416 | 0.442 | 0.058 |
| Southern Mongolia | 15 | 3.3 | 14 | 2.1 | 0.399 | 0.450 | 0.110 |

$n$, samples size; $A_{N}$, number of alleles; $A_{P}$, private alleles; $A_{E}$, effective number of alleles ( $1 /\left(\sum p^{2}\right) ; H_{O}$, observed heterozygosity; $H_{E}$, expected heterozygosity; $F_{l}$, fixation index ( $1-H_{O} / H_{E}$ ).

Table 2. Pairwise $A M O V A F_{S T}$ estimates between 7 geographic regions for snow leopards

|  | Northern <br> Qinghai | Southern <br> Qinghai | Tibet/Nepal/Bhutan | India/Pakistan | Tajikistan/ Kyrgyzstan | Western <br> Mongolia | Southern <br> Mongolia |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Northern Qinghai | - | 0.174 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 |
| Southern Qinghai | 0.039 | - | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Tibet/Nepal/Bhutan | 0.121 | 0.177 | - | 0.000 | 0.000 | 0.000 | 0.000 |
| India/Pakistan | 0.138 | 0.211 | 0.166 | - | 0.412 | 0.000 | 0.000 |
| Tajikistan/Kyrgyzstan | 0.133 | 0.204 | 0.143 | 0.007 | - | 0.000 | 0.000 |
| Western Mongolia | 0.218 | 0.287 | 0.227 | 0.142 | 0.092 | - | 0.000 |
| Southern Mongolia | 0.220 | 0.308 | 0.258 | 0.161 | 0.122 | 0.057 | - |

$F_{S T}$ (below diagonal) and associated $P$-values (above diagonal) were based on 33 microsatellites.


Figure 3. Range-wide genetic structure in snow leopards ( $n=70$ ) using Bayesian clustering analysis in GENELAND 4.0 .3 (Guillot et al. 2005) of 33 microsatellites. Snow leopard scat samples were collected across localities within northern Qinghai (NQ), southern Qinghai (SQ), Himalaya (HIM; Bhutan, Nepal, Tibet), India and Pakistan (IP), Tajikistan and Kyrgyzstan (TK), western Mongolia (WM), and southern Mongolia (SM). Black dots represent sample locations based on latitude and longitude coordinates. Maps show posterior probabilities of genetic cluster memberships for the 3 significant clusters (a. cluster 1-central group, Panthera uncia uncioides; b. cluster 2-northern group, Panthera uncia irbis; c. Cluster 3-western group, Panthera uncia uncia).

Bayesian clustering analysis in STRUCTURE was performed at 2 additional hierarchical levels to further explore differentiation within the Central, Western, and Northern groups. The first level analyzed the Central and Western groups together (samples from northern Qinghai, southern Qinghai, Tibet, Bhutan, Nepal, India, Pakistan, Tajikistan, and Kyrgyzstan; Figure 2e) and in a separate analysis the Western and Northern groups were analyzed together (samples from India, Pakistan, Tajikistan, Kyrgyzstan, western Mongolia, and southern Mongolia; Figure 2f). Both analyses resulted in $K=2$, with the clusters corresponding to those identified with the full data set for $K=3$, and the PCA and population assignment analyses. There was no evidence for genetic admixture between Central and Western groups at this spatial scale (Figure 2e). In contrast, when Western and Northern groups were analyzed together, although the snow leopards sampled from Tajikistan and Kyrgyzstan clustered with India and Pakistan, they did exhibit low levels of admixture with Mongolia (Figure 2f). The second level of hierarchy included separate analyses of the Central (northern Qinghai, southern Qinghai, Tibet, and Himalaya; Figure 2g), Western (India, Pakistan, Tajikistan, and Kyrgyzstan; Figure 2h), and Northern (western Mongolia and southern Mongolia; Figure 2i) groups. Together, these analyses revealed additional structure within these regions. In the Central group, northern and southern Qinghai clustered separately from Tibet, Bhutan, and Nepal with admixture in the central part of the Tibetan Plateau (Figure 2g). The Western group had 2 clusters, one composed exclusively of Kyrgyzstan and the other of India, Pakistan, and Tajikistan (Figure 2h). Finally, in the Northern group, western and
southern Mongolia formed 2 distinct clusters, with a few individuals showing mixed ancestry (Figure 2i). The genetic clusters observed in the hierarchical analysis largely corresponded to those recovered with the full dataset for $K=6$ (Supplementary Figure S3), which had the highest posterior probability in the STRUCTURE analysis with 70 samples (Supplementary Figure S4a).

The simple Mantel test showed weak IBD $(r=0.183, P=0.020$; Supplementary Figure S5). Spatial autocorrelation analysis detected IBD in the first four distance classes $(25,50,100$, and 250 km ; Figure 4). The $x$-intercept of $r$ was between 250 and 500 km , indicating that snow leopard populations located within these distances are potentially connected by dispersing individuals.

## Discussion

The snow leopard, first described by Schreber (1775), is a highaltitude specialist that occupies mountains primarily between 1500 and 4500 m , with confirmed sightings to 6000 m in the Himalaya (Hemmer 1972). Historically, several subspecies have been proposed including the nominate Panthera uncia uncia (Stroganov 1962), Panthera uncia uncioides in Nepal (Horsfield 1855), Panthera uncia schneideri in Sikkim (India, Zukowsky 1950), and Panthera uncia baikalensis-romanii in the Transbaikal (Russia, Medvedev 2000) based on coat color differences. The lack of collection information for many museum specimens and the difficulty of observing and trapping snow leopards in the wild have to date prevented comprehensive taxonomic assessments and therefore this species is


Figure 4. Spatial autocorrelation in snow leopards ( $n=70$ ). Correlogram visualizing the autocorrelation coefficient ( $r$ ) at various distance classes (km). Dashed lines represent upper ( U ) and lower ( L ) bounds under the null hypothesis of no spatial structure ( $r=0 ; 10000$ permutations). 95\% confidence intervals about $r$ indicated by error bars (1000 bootstraps).
considered monotypic. Here, we address the taxonomic question regarding subspecies designation of the snow leopard using genetic data from noninvasive scat samples. Subspecies are generally considered distinct populations that correspond to geographic boundaries and meet discreteness and significance criteria (Wilson and Brown 1953; Haig et al. 2006). Based on the differentiation of nuclear loci that separated samples into 3 discrete and significant genetic clusters (Western, Central, and Northern groups) occurring in nonoverlapping geographic regions we propose the snow leopard be classified into 3 subspecies; P. u. uncia (type locality restricted to Central Asia including Tian Shan, Alay, Pamir, Karakoram, and trans-Himalaya), P. u. uncioides with schneideri as a junior synonym (core Himalaya and the Tibetan Plateau), and P. u. irbis with baikalensis-romanii as a junior synonym (the Altai and Southern Gobi of Mongolia) (Figures 1a and 2b).

Although historically recommendations for subspecies delineations have also included mitochondrial divergence and monophyly (Moritz 1994), phylogeographic studies over the last decade indicate that this strict criterion may be unreasonable because of mitonuclear discordance (Toews and Brelsford 2012). First, mitogenomes are frequently paraphyletic, notably in Felidae, due to introgression from past hybridization events (Roca et al. 2005; Li et al. 2016a). Second, the mtDNA represents a single linked locus (i.e., no recombination) with a smaller $N_{E}$ (i.e., haploid and only passed through females) therefore it is more sensitive to incomplete lineage sorting than nuclear loci (Avise 2000). Although our mtDNA data revealed no haplotype differences across the snow leopard range, the consistent recovery using nuclear markers of 3 discrete allopatric genetic clusters with significant differentiation, each occurring in unique geographic regions, warrants subspecies delineation.

There are several possible reasons why we did not observe different mtDNA haplotypes in the 3 subspecies. First, more extensive mitogenome sequencing may be required to detect polymorphism. Second, the snow leopard mitogenome may have undergone a selective sweep. Mitochondria are responsible for oxidative respiration and therefore may be under selective pressures in hypoxic environments (da Fonseca 2008; Hassanin et al. 2009). In addition, previous studies have shown that the snow leopard lineage underwent mitochondrial replacement after hybridization with the African lion lineage (Li et al. 2016a), and therefore may have been subject to adaptive introgression that resulted in low mtDNA variation (Toews and Brelsford 2012). Finally, mtDNA has a 4 -fold smaller $N_{E}$ compared to nuclear DNA (Hudson and Turelli 2003), and therefore more ancestral polymorphism would have been lost during the bottleneck
~8000 ya detected with microsatellites. The lack of distinct mtDNA lineages is consistent with previous studies in the Tibetan region showing weaker Pleistocene refugia effects (Qu and Lei 2009; Yang et al. 2009; Zhan et al. 2011) compared to those observed in European and North American taxa (Taberlet et al. 1998; Petit et al. 2003; Shafer et al. 2010). Sequencing of mitochondrial and nuclear genomes in the 3 subspecies would shed light on the mechanisms that contributed to the observed mitonuclear discordance.

Snow leopards face threats including low prey densities, retaliatory killing by farmers and herdsmen in response to livestock depredation, illegal wildlife trade, climate change, and development of roads, rails, mining, and hydropower facilities (Jackson et al. 2010; McCarthy et al. 2016). Traditional pastoralism and agro-pastoralism dominate local economies within the snow leopard range often leading to human-wildlife conflict (Mishra et al. 2003, 2016). Successful community-based conservation initiatives have been implemented in several areas, including Mongolia, Nepal, and Pakistan (Jackson et al. 2010). Recently, there has been an effort to coordinate conservation range-wide with a comprehensive Global Snow Leopard © Ecosystem Protection Program that seeks to secure populations in 20 different landscapes by 2020 (Snow Leopard Secretariat 2013). Our phylogeographic assessment strongly highlights the importance of large-scale international efforts (Rosen and Zahler 2016). Snow leopard populations exhibit cross-boundary connectivity in several important parts of their range, such as between Pakistan and India on the western portion of the Himalaya, and between Nepal, Bhutan, and southern Tibet. It is critical that international corridors between these populations are maintained, and that synchronized conservation actions are realized so that no single area becomes isolated, or a population sink, contributing to decline in neighboring countries.

Presently, the International Union for Conservation of Nature (IUCN) considers the snow leopard a monotypic species and applies criteria for "Endangered (EN) Category 1 (C1)" status range-wide. These include <2500 mature individuals and an estimated $20 \%$ decline in 2 generations, corresponding to $\sim 16$ years in snow leopards (IUCN Standards and Petitions Subcommittee 2016). Applying the results from our phylogeographic analysis, we generated a preliminary population size estimate for each subspecies using population size estimates in McCarthy et al (2016b) by summing those within the approximate range of each respective subspecies (Supplementary Table S8 and Supplementary Figure S6). The estimate for P. u. uncia was 2124-3356 individuals, for P. u. uncioides it was 1402-3083, and for $P$. u. irbis it was 741-1646. This suggests the latter 2 subspecies may meet IUCN EN C1 criteria. These population estimates are
preliminary and additional research is needed to both definitively assign populations to subspecies, and obtain abundance information in areas where quantitative data is not available, particularly on the Tibetan Plateau.

In order to determine if assignment of individuals to subspecies could be made with fewer microsatellites we created a reduced matrix of 6 loci and tested 26 additional samples from Ladakh, India. Even with this reduced matrix, we were able to correctly assign $96 \%$ of the individuals to $P$. $u$ uncia, thus illustrating the utility of this reference data set and the substantial level of differentiation between the subspecies. We have performed whole genome amplification of a subset of the samples yielding synthetically derived amplicons not subject to CITES restrictions (Janecka et al. 2006, 2007). We will make these available to other laboratories to ensure uniform allele designations, thus facilitating direct comparisons with our data set in future studies.

The low microsatellite diversity and lack of mtDNA variation within snow leopards is typical of felids or subspecies that have either been historically isolated, or have undergone recent population bottlenecks, such as the Far Eastern leopard (Panthera pardus orientalis, Uphyrkina et al. 2001), Sumatran tiger (Panthera tigris sumatrae, Luo et al. 2004), North American puma (Puma concolor cougar, Culver et al. 2000), North American ocelot (Leopardus pardalis albescens, Janecka et al. 2011b), and the Asiatic cheetah (Acinonyx jubatus venaticus, Charruau et al. 2011). Although we sequenced a limited amount of mtDNA, Luo et al. (2004) detected 4-6 haplotypes for the same segments in tigers despite analyzing fewer individuals. The microsatellite and mtDNA diversity in snow leopards is consistent with the low genome-wide polymorphism previously reported for a single snow leopard from Mongolia (Cho et al. 2013). We estimated the most recent bottleneck occurred during the middle Holocene, potentially as a consequence of changing climatic and habitat conditions (Zhang et al. 2006; Yang et al. 2009). This finding is reinforced by the uniformly reduced variation across the snow leopard range. Alternatively, if anthropogenic factors were the primary cause of lower variation in extant snow leopards, the diversity would likely vary across populations.

The estimated time of the bottleneck in snow leopards coincides with the start of the Holocene Thermal Maximum (approximately 6000-8000 years ago), a period of warming and increased precipitation in the Tibetan Plateau and a synchronous treeline shift to higher elevations (Zhao et al. 2011). The correspondence of these events with the snow leopard bottleneck has important implications for understanding the potential impact of global climate change. Similar climatological trends are occurring throughout the world, with particularly elevated warming trends in the Tibetan Plateau and the Himalaya (Liu and Chen 2000; Walther et al. 2002; Farrington and Li 2016). Recent studies examining the potential impact of climate change have predicted a substantial reduction in snow leopard habitat and increased fragmentation (Forrest et al. 2012; Li et al. 2016b). Our inferences on the demographic contraction in the Holocene lend support to models that indicate snow leopards are susceptible to global warming.

Major landscape features in Asia correlate with the observed phylogeographic patterns. In the north, the $P$. u. irbis populations that occupy low-altitude mountains of the Gobi in southern Mongolia are separated from the Qilian Shan in northern Qinghai by the Alashan Plateau, with $>400 \mathrm{~km}$ of unsuitable habitat. This potential movement barrier corresponds to the greatest observed genetic differentiation within the species, and is consistent with the recent habitat and connectivity models (Riordan et al. 2015; Li et al. 2016b). The admixture observed in Kyrgyzstan, on the other side of China, indicates the presence of more recent introgression between the subspecies
or unsampled populations with intermediate allele frequencies. The Dzungarian Basin ( $\sim 500 \mathrm{~km}$ wide) is likely an impenetrable barrier for snow leopards. However, there are isolated mountains to the west along the boundary of Kazakhstan and China, and to the east in Xinjiang between Tian Shan and the Gobi in southern Mongolia that may act as stepping stones between P. u. uncia and P. u. irbis (Li et al. 2016b). However, both of these possible routes appear too far north and west to connect Qinghai/Gansu (P. u. uncioides) with the northern subspecies. The barrier separating the Central P. u. uncioides and the Western P. u. uncia, between Nepal and India (Ladakh), is not as obvious because the trans-Himalaya form a nearly continuous chain. One possibility is that the combination of their height (i.e., many peaks $>6000 \mathrm{~m}$ above sea level), linear distance ( $\sim 1000 \mathrm{~km}$ ), and the presence of several major rivers in the region may limit connectivity. Another possibility is that in the past this area may have been covered by extensive uninhabitable glacial fields. These explanations are mutually compatible and both may contribute to the observed differentiation in snow leopards between the Central and Western groups, which is consistent with the Li et al. (2016b) model of snow leopard habitat that indicates more fragmentation in this area than predicted by the Riordan et al. (2015) model.

The observed population genetic structure also provides a coherent and objective basis with which to define management units (MUs). In the north, western Mongolia (Altai MU) and southern Mongolia (Gobi $\mathrm{MU})$ formed separate genetic clusters in the hierarchical analysis. In this area, snow leopards primarily occupy the Altai mountain range, which runs east into the Gobi Altai, with the mountains becoming lower and more isolated, often separated by $>100 \mathrm{~km}$ of flat desert. Nonetheless, there was evidence for transient dispersal between each of these areas suggesting that the smaller massifs act as stepping stones for migration between larger habitat patches. Long distance movements have been observed among radio and GPS-collared snow leopards in Mongolia (McCarthy et al. 2005; Johansson et al. 2016). In the southern portion of Tibetan Plateau there was connectivity with the Himalaya, consistent with snow leopard habitat models (Riordan et al. 2015; Li et al. 2016b). Northern Qinghai/Gansu and southern Qinghai (Qinghai MU) were genetically divergent from Tibet, Bhutan, and Nepal (TibetHimalaya MU) similar to phylogeographic patterns observed in other species (Qu and Lei 2009; Yang et al. 2009; Qu et al. 2010; Zhan et al. 2011). Within Central Asia, the Tajikistan, Pakistan, and India (Pamir-Himalaya MU) region also appeared connected. The Pamirs in Tajikistan are separated from the Tian Shan in Kyrgyzstan (Tian Shan MU) by $\sim 600 \mathrm{~km}$, some of which includes river valleys (e.g., Vakhsh, Kyzyl-Suu, and Naryn Rivers) which may explain the differentiation in this area. Our subspecies designations, and the delineation of the Qinghai MU and Tibet-Himalaya MU, also correspond with 4 physiography and prey types zones described from a recent meta-analysis of feeding ecology studies (Lyngdoh et al. 2014). Snow leopard conservation efforts need to focus on maintaining natural connectivity within these MUs and to develop context-specific conservation programs. These efforts should take priority over attempts to establish corridors that would cross natural phylogeographic boundaries. Additional sampling is urgently needed to better understand population structure within the MUs and landscape factors that affect connectivity.

## Conclusions

We conducted the first range-wide genetic analysis of wild snow leopard populations and delineate 3 subspecies. The criteria for IUCN's Red List should be applied to each of these individually. The snow leopard underwent a bottleneck $\sim 8000$ ya in the Holocene
coinciding with global warming that occurred during the epoch. Population structure within the subspecies indicates a minimum of 6 MUs, 3 of which span multiple countries. Each of these may require different conservation initiatives. Our results serve as a foundation for understanding landscape connectivity of snow leopards and set the stage for more in-depth genomic studies.

## Supplementary Material

Supplementary data are available at Journal of Heredity online.

## Funding

The work was supported by the National Geographic Society (grant 8369-07 to J.E.J., W.J.M., L.Q., and Z.Y.), the Snow Leopard Conservancy (grant G1400042 to J.E.J., R.J., B.M., and W.J.M.), Britten Foundation (grants 12121 and 11104 to R.J.), Larry Bowman Foundation (grants 16092 and 14031 to R.J.), The College of Veterinary Medicine of Texas A\&M University to J.E.J. and W.J.M., The Snow Leopard Conservation Grants Program (grants 120808 and G1500042 to J.E.J.), B.M., N.G., and M.J.), Duquesne University to J.E.J., NSF (grant EF0629849 to W.J.M.), the University of Montana to T.R.W., Council of Scientific and Industrial Research, India (grant BSC0207 to A.G. and S.K.), and Department of Biotechnology, India (grant GAP0374 to A.G. and S.K.)

## Acknowledgments

We would like to thank the rangers, biologists, herders, and local communities that helped in collection of samples. All samples were obtained in accordance with the appropriate agencies and all international shipments were in compliance with CITES requirements.

## Data Availability

Microsatellite data was archived in DRYAD (doi:10.5061/dryad.sb20c) and sequences for microsatellite flanking regions and mtDNA segments were deposited in GenBank under accessions KY967522-KY967572.

## References

Alexander JS, Zhang C, Shia K, Riordan P. 2016. A granular view of a snow leopard population using camera traps in Central China. Biol Conserv. 197:27-31.
Anwar MB, Jackson R, Nadeem MS, Janecka JE, Hussain S, Beg MA, Ghulam M, Qayyum M. 2011. Food habits of snow leopard Panthera uncia (Schreber, 1775) in Baltistan, Northern Pakistan. Eur J Wildl Res. 57:1077-1083.
Avise JC. 1994. Molecular markers, natural history, and evolution. New York: Chapman \& Hall.
Avise JC. 2000. Phylogeography: the history and formation of species. Cambridge (MA): Harvard University Press.
Avise JC, Ball RM. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys Evol Biol. 7:45-67.
Balkenhol N, Holbrook JD, Onorato D, Zager P, White C, Waits LP. 2014. A multi-method approach for analyzing hierarchical genetic structures: a case study with cougars Puma concolor. Ecography. 37:552-563.
Beaumont MA. 1999. Detecting population expansion and decline using microsatellites. Genetics. 153:2013-2029.
Brinkmann B, Klintschar M, Neuhuber F, Hühne J, Rolf B. 1998. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. Am J Hum Genet. 62:1408-1415.

Brooks SP, Gelman A. 1998. General methods for monitoring convergence of iterative simulations. J Comp Graph Stat. 7:434-455.
Charruau P, Fernandes C, Orozco-Terwengel P, Peters J, Hunter L, Ziaie H, Jourabchian A, Jowkar H, Schaller G, Ostrowski S, et al. 2011. Phylogeography, genetic structure and population divergence time of cheetahs in Africa and Asia: evidence for long-term geographic isolates. Mol Ecol. 20:706-724.
Chetri M, Odden M, Wegge P. 2017. Snow leopard and Himalayan wolf: food habits and prey selection in the central Himalayas, Nepal. PLoS One. 12: 00170549.
Cho YS, Hu L, Hou H, Lee H, Xu J, Kwon S, Oh S, Kim HM, Jho S, Kim S, et al. 2013. The tiger genome and comparative analysis with lion and snow leopard genomes. Nat Commun. 4:2433.
Culver M, Johnson WE, Pecon-Slattery J, O'Brien SJ. 2000. Genomic ancestry of the American puma (Puma concolor). J Hered. 91:186-197.
da Fonseca RR, Johnson WE, O’Brien SJ, Ramos MJ, Antunes A. 2008. The adaptive evolution of the mammalian mitochondrial genome. BMC Genomics. 9:119.
Eizirik E, Kim JH, Menotti-Raymond M, Crawshaw PG Jr, O’Brien SJ, Johnson WE. 2001. Phylogeography, population history and conservation genetics of jaguars (Panthera onca, Mammalia, Felidae). Mol Ecol. 10:65-79.
Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 14:2611-2620.
Excoffier L, Laval G, Schneider S. 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 1:47-50.
Farrington JD, Li J. 2016. Climate change impacts on snow leopard range. In: Nyhus PJ, McCarthy T, Mallon D, editors. Snow leopards. Biodiversity of the world: conservation from genes to landscapes. London: Elsevier. p. 85-96.

Forrest JL, Wikramanayake E, Shrestha R, Areendran G, Gyeltshen K, Maheshwari A, Mazumdar S, Naidoo R, Thapa GJ, Thapa K. 2012. Biological conservation. 150:129-135.
Francis RM. 2017. pophelper: an R package and web app to analyse and visualize population structure. Mol Ecol Resour. 17:27-32.
Gelman A, Carlin JB, Stern HS, Rubin DB. 2004. Bayesian data analysis. Boca Raton (FL): Chapman and Hall/CRC Press.
Guillot, G, Mortier F, Estoup A. 2005. GENELAND: a computer package for landscape genetics. Mol Ecol Resour. 5:712-715.
Gilad O, Janecka JE, Armstrong F, Tewes ME, Honeycutt RL. 2011. Mountain lions in Guadalupe Mountains National Park: estimates of occurrence and distribution through DNA analysis. Southwest Nat. 56:297-304.
Girod C, Vitalis R, Leblois R, Fréville H. 2011. Inferring population decline and expansion from microsatellite data: a simulation-based evaluation of the Msvar method. Genetics. 188:165-179.
Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. J Stat Softw. 22:1-19.
Haig SM, Beever EA, Chambers SM, Draheim HM, Dugger BD, Dunham S, Elliott-Smith E, Fontaine JB, Kesler DC, Knaus BJ, et al. 2006. Taxonomic considerations in listing subspecies under the U.S. Endangered Species Act. Conserv Biol. 20:1584-1594.
Harris RB. 2014. Vulpes ferrilata. The IUCN Red List of Threatened Species. e.T23061A46179412. [cited 2017 Mar 21]. http://dx.doi.org/10.2305/ IUCN.UK.2014-3.RLTS.T23061A46179412.en.
Harris RB, Reading R. 2008. Ovis ammon. The IUCN Red List of Threatened Species. e.T15733A5074694. http://dx.doi.org/10.2305
Hassanin A, Ropiquet A, Couloux A, Cruaud C. 2009. Evolution of the mitochondrial genome in mammals living at high altitude: new insights from a study of the tribe Caprini (Bovidae, Antilopinae). J Mol Evol. 68:293-310.
Hemmer H. 1972. Uncia uncia. Mamm Species. 20:1-5.
Horsfield T. 1855. Brief notices of several new or little-known species of Mammalia, lately discovered or collected in Nepal by Brian Houghton Hodgson, Esq. Ann Mag Nat Hist. 16:101-114.
Hudson RR, Turelli M. 2003. Stochasticity overrules the "three-times rule": genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution. 57:182-190.

Hussain S. 2000. Protecting the snow and enhancing farmer's livelihoods: a pilot insurance scheme in Baltistan. Mountain Res Dev. 20:226-231.
IUCN SSC Antelope Specialist Group. 2016. Pantholops hodgsonii. The IUCN Red List of Threatened Species. e.T15967A50192544; [cited 2017 Mar 21]. http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS. T15967A50192544.en. Accessed on 21 March 2017.
IUCN Standards and Petitions Subcommittee. 2016. Guidelines for Using the IUCN Red List Categories and Criteria. Version 12; [cited 2017 Mar 21]. http://www.iucnredlist.org/documents/ RedListGuidelines.pdf.
Kachel SM, McCarthy KP, McCarthy T, Oshurmamadov N. 2016. Investigating the potential impact of trophy hunting of wild ungulates on snow leopard Panthera uncia conservation in Tajikistan. Oryx. doi:10.1017/ S0030605316000193
Karmacharya DB, Thapa K, Shrestha R, Dhakal M, Janecka JE. 2011 Noninvasive genetic population survey of snow leopards (Panthera uncia) in Kangchenjunga conservation area, Shey Phoksundo National Park and surrounding buffer zones of Nepal. BMC Res Notes. 4:516.
Jackson R, Ahlborn G. 1989. Snow leopards (Panthera uncia) in Nepal: home range and movements. Natl Geogr Res. 5:161-175.
Jackson R, Mallon D, McCarthy T, Chundaway RA, Habib B. 2008. Panthera uncia. The IUCN Red List of Threatened Species. e.T22732A9381126; [cited 2017 Mar 21]. http://dx.doi.org/10.2305/ IUCN.UK.2008.RLTS. T22732A9381126.en.
Jackson RM, Mishra C, McCarthy TM, Ale SB. 2010. Snow leopards: conflict and conservation. In: MacDonald DW, Loveridge AJ, editors. Biology and conservation of wild felids. London: Oxford University Press. p. 417-430.

Jackson R, Roe JD, Wangchuk R, Hunter DO. 2006. Estimating snow leopard population abundance using photography and capture-recapture techniques. Wildl Soc Bull. 34:772-781.
Jackson R, Wangchuck R. 2004. A community-based approach to mitigating livestock depredation by snow leopards. Hum Dimens Wildl. 9:307-315.
Janecka JE, Grassman LI Jr, Derr JN, Honeycutt RL, Eiadthong W, Tewes ME. 2006. Rapid whole genome amplification of DNA from felids: applications for conservation genetics. Wildl Soc Bull. 34:1134-1141.
Janecka JE, Grassman LI Jr, Honeycutt RL, Tewes ME. 2007. Whole genome amplification for sequencing and applications in conservation genetics. $J$ Wildl Manag. 71:1357-1360.
Janecka JE, Jackson R, Munkhtsog B, Murphy WJ. 2014. Characterization of 9 microsatellites and primers in snow leopards and a species-specific PCR assay for identifying noninvasive samples. Conserv Genet Resour. 6:369-373.
Janecka JE, Jackson R, Yuquang Z, Diqiang L, Munkhtsog B, Buckley-Beason V, Murphy WJ. 2008. Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. Anim Conserv. 11:401411.

Janecka JE, Munkhtsog B, Jackson RM, Naranbaatar G, Mallon DP, Murphy WJ. 2011a. Comparison of noninvasive genetic and camera-trapping techniques surveying snow leopards. J Mammal. 92:771-783.
Janecka JE, Nielsen SS, Andersen SD, Hoffmann FG, Weber RE, Anderson T, Storz JF, Fago A. 2015. Genetically based low oxygen affinities of felid hemoglobins: lack of biochemical adaptation to high-altitude hypoxia in the snow leopard. J Exp Biol. 218:2402-2409.
Janecka JE, Tewes ME, Laack LL, Caso A, Grassman LI Jr, Haines AM, Shindle D, Davis B, Murphy WJ, Honeycutt RL. 2011b. Reduced genetic diversity and isolation of remnant ocelot populations occupying a severely fragmented landscape in southern Texas. Anim Conserv. 14:608-619.
Johansson O, Rauset GR, Samelius G, McCarthy T, Andren H, Tumursukh L, Mishra C. 2016. Land sharing is essential for snow leopard conservation. Biol Conserv. 203:1-7
Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 24:1403-1405.
Li G, Davis BW, Eizirik E, Murphy WJ. 2016a. Phylogenomic evidence for ancient hybridization in the genomes of living cats (Felidae). Genome Res. 26:1-11.

Li J, McCarthy TM, Wang H, Weckworth B, Schaller GB, Mishra C, Lua Z, Beissinger SR. 2016b. Climate refugia of snow leopards in High Asia. Biol Conserv. 203:188-196.
Li J, Wang D, Yin H, Zhaxi D, Jiagong Z, Schaller GB, Mishra C, McCarthy TM, Wang H, Wu L, et al. 2014. Role of Tibetan Buddhist monasteries in snow leopard conservation. Conserv Biol. 28:87-94.
Liu XD, Chen BD. 2000. Climatic warming in the Tibetan Plateau during recent decades. Int J Climatol. 20:1729-1742.
Lovari S, Boesi R, Minder I, Mucci N, Randi E, Dematteis A, Ale S. 2009. Restoring a keystone predator may endanger a prey species in a humanaltered ecosystem: the return of the snow leopard to Sagarmatha National Park. Anim Conserv. 12:559-570.
Lovari S, Minder I, Ferretti F, Mucci N, Randi E, Pellizzi B. 2013. Common and snow leopards share prey, but not habitats: competition avoidance by large predators? J Zool. 291:127-135.
Luo SJ, Kim JH, Johnson WE, van der Walt J, Martenson J, Yuhki N, Miquelle DG, Uphyrkina O, Goodrich JM, Quigley HB, et al. 2004. Phylogeography and genetic ancestry of tigers (Panthera tigris). PLoS Biol. 2:e442.
Lyngdoh S, Shrotriya S, Goyal SP, Clements H, Hayward MW, Habib B. 2014. Prey preferences of the snow leopard (Panthera uncia): regional diet specificity holds global significance for conservation. PLoS One. 9:e88349.
McCarthy K, Fuller T, Ming M, McCarthy T, Waits L, Jumabaev K. 2008. Assessing estimators of snow leopard abundance. J Wildl Manag. 72:1826-1833.
McCarthy TM, Fuller TK, Munkhtsog B. 2005. Movements and activities of snow leopards in southwestern Mongolia. Biol Conserv. 124:527-537.
McCarthy T, Mallon D, Sanderson EW, Zahler P, Fisher K. 2016b. Biogeography and status overview. In: Nyhus PJ, McCarthy T, Mallon D, editors. Snow leopards. Biodiversity of the world: conservation from genes to landscapes. London: Elsevier. p. 23-42.
Michel S, Rosen T. 2015. Capra falconeri. The IUCN Red List of Threatened Species. e.T3787A97218336; [cited 2017 Mar 21]. http://www.iucnredlist.org/details/3787/0.
Medvedev DG. 2000. Morfologicheskie otlichiya irbisa iz Yuzhnogo Zabaikalia. [Morphological differences of the snow leopard from Southern Transbaikalia]. Vestnik Irkutskoi Gosudarstvennoi sel'skokhozyaistvennoi akademyi. Proc Irkutsk State Agric Acad. 20:20-30.
Menotti-Raymond M, David VA, Lyons LA, Schäffer AA, Tomlin JF, Hutton MK, O’Brien SJ. 1999. A genetic linkage map of microsatellites in the domestic cat (Felis catus). Genomics. 57:9-23.
Menotti-Raymond M, David VA, Roelke ME, Chen ZQ, Menotti KA, Sun S, Schäffer AA, Tomlin JF, Agarwala R, O’Brien SJ, et al. 2003. Second-generation integrated genetic linkage/radiation hybrid maps of the domestic cat (Felis catus). J Hered. 94:95-106.
Murphy WJ, Sun S, Chen ZQ, Pecon-Slattery J, O’Brien SJ. 1999. Extensive conservation of sex chromosome organization between cat and human revealed by parallel radiation hybrid mapping. Genome Res. 9:12231230.

Mishra C, Allen P, McCarthy T, Madhusudan MD, Bayarjargal A, Prins HH. 2003. The role of incentive programs in conserving the snow leopard. Conserv Biol. 17:1512-1520.
Mishra C, Redpath SR, Suryawanshi KR. 2016. Livestock predation by snow leopards: conflicts and the search for solutions. In: In: Nyhus PJ, McCarthy T, Mallon D, editors. Snow leopards. Biodiversity of the world: conservation from genes to landscapes. London: Elsevier. p. 59-68.
Moritz C. 1994. Defining 'evolutionarily significant units' for conservation. Trends Ecol Evol. 9:373-375.
O’Brien SJ, Mayr E. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. Science. 251:1187-1188.
Paetkau D, Slade R, Burden M, Estoup A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol. 13:55-65.
Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 6:288-295.

Petit R, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M, et al. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. Science. 300:1563-1565.
Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155:945-959.
Qu Y, Lei F. 2009. Comparative phylogeography of two endemic birds of the Tibetan plateau, the white-rumped snow finch (Onychostruthus taczanowskii) and the Hume's ground tit (Pseudopodoces humilis). Mol Phylogenet Evol. 51:312-326.
Qu Y, Lei F, Zhang R, Lu X. 2010. Comparative phylogeography of five avian species: implications for Pleistocene evolutionary history in the QinghaiTibetan plateau. Mol Ecol. 19:338-351.
Rannala B, Mountain JL. 1997. Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA. 94:9197-9201.
Riordan P, Cushman S, Mallon D, Shi E, Hughes J. 2015. Predicting global population connectivity and targeting conservation action for snow leopard across its range. Ecography. 38:1-8.
Riordan P, Sanderson J, Bao W, Abdukadir A, Shi K. 2015. Felis bieti. The IUCN Red List of Threatened Species. e.T8539A50651398. [cited 2017 Mar 21]. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T8539A50651398.en.
Roca AL, Georgiadis N, O'Brien SJ. 2005. Cytonuclear genomic dissociation in African elephant species. Nat Genet. 37:96-100.
Rodgers TW, Giacalone J, Heske EJ, Janecka JE, Jansen PA, Phillips CA, Schooley RL. 2015. Socio-spatial organization and kin structure in ocelots from integration of camera trapping and noninvasive genetics. J Mammal. 96:120-128.
Rodgers TW, Janecka JE. 2013. Applications and techniques for non-invasive faecal genetics research in felid conservation. Eur J Wildl Res. 59:1-16.
Rooney AP, Honeycutt RL, Davis SK, Derr JN. 1999. Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. J Mol Evol. 49:682-690.
Rosen T, Hussain S, Mohammad G, Jackson R, Janecka JE, Michel S. 2012. Reconciling sustainable development of mountain communities with large carnivore conservation. Mountain Res Dev. 32:286-293.
Rosen T, Zahler P. 2016. Transboundary initiatives and snow leopard conservation. In: In: Nyhus PJ, McCarthy T, Mallon D, editors. Snow Leopards. Biodiversity of the world: conservation from genes to landscapes. London: Academic Press. p. 267-276.
Schaller GS. 1998. Wildlife of the Tibetan steppe. Chicago (IL): University of Chicago Press.
Schreber JCD. 1775. Felis uncia. Die Säugthiere in Abbildungen nach der Natur. Tafeln. 100:1774-1855.
Schwartz MK, Luikart G, Waples RS. 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol Evol. 22:25-33.
Shafer AB, Cullingham CI, Côté SD, Coltman DW. 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. Mol Ecol. 19:4589-4621.
Smith BJ. 2007. boa: an R package for MCMC output convergence assessment and posterior inference. J Stat Softw. 21:1-37.

Snow Leopard Secretariat. 2013. Global Snow Leopard \& Ecosystem Protection Program. Bishkek city, Kyrgyz Republic; [cited 2017 Mar 22]. http:// www.globalsnowleopard.org/.
Storz JF, Beaumont MA. 2002. Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. Evolution. 56:154-166.
Stroganov SU. 1962. Carnivorous mammals of Siberia. Jerusalem (Israel): Israel Program Science.
Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol Ecol. 7:453-464.
Toews DP, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol. 21:3907-3930.
Uphyrkina O, Johnson WE, Quigley H, Miquelle D, Marker L, Bush M, O'Brien SJ. 2001. Phylogenetics, genome diversity and origin of modern leopard, Panthera pardus. Mol Ecol. 10:2617-2633.
Valdez R. 2008. Ovis orientalis. The IUCN Red List of Threatened Species. e.T15739A5076068; [cited 2017 Mar 22]. http://dx.doi.org/10.2305/ IUCN.UK.2008.RLTS.T15739A507 6068.en.
Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJ, Fromentin JM, Hoegh-Guldberg O, Bairlein F. 2002. Ecological responses to recent climate change. Nature. 416:389-395.
Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. Evolution. 38:1358-1370.
Wilson EO, Brown WL. 1953. The subspecies concept and its taxonomic application. Syst Zool. 2:97-111.
Wultsch C, Caragiulo A, Dias-Freedman I, Quigley H, Rabinowitz S, Amato G. 2016a. Genetic diversity and population structure of Mesoamerican jaguars (Panthera onca): implications for conservation and management. PLoS One. 11:e0162377.
Wultsch C, Waits LP, Kelly MJ. 2016b. A comparative analysis of genetic diversity and structure in jaguars (Panthera onca), pumas (Puma concolor), and ocelots (Leopardus pardalis) in fragmented landscapes of a critical Mesoamerican linkage zone. PLoS One. 11:1-30.
Yang S, Dong H, Lei F. 2009. Phylogeography of regional fauna on the Tibetan Plateau: a review. Prog Nat Sci. 19:789-799.
Zhao Y, Yu Z, Zhao W. 2011. Holocene vegetation and climate histories in the eastern Tibetan Plateau: controls by insolation-driven temperature or monsoon-derived precipitation changes? Quat Sci Rev. 30: 1173-1184.
Zhan X, Zheng Y, Wei F, Bruford MW, Jia C. 2011. Molecular evidence for Pleistocene refugia at the eastern edge of the Tibetan Plateau. Mol Ecol. 20:3014-3026.
Zhang F, Jiang Z. 2006. Mitochondrial phylogeography and genetic diversity of Tibetan gazelle (Procapra picticaudata): implications for conservation. Mol Phylogenet Evol. 41:313-321.
Zukowsky L. 1950. Grossäuger, die Hagenbeck entdeckte. Der Zoologischer Garten. 17:211-221.

