



Conservation importance of the strategic, centrally located snow leopard population in the western Himalayas, India: a genetic perspective

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Abstract

The snow leopard population in Union Territory of Ladakh (UTL), India is at the centre of five out of eight mountain ranges within the species' habitat in the high-mountain Asia. Its strategic location is of immense conservation significance to maintain genetic connectivity and metapopulation dynamics of snow leopards (*Panthera uncia*). Therefore, we provide the first estimates of the snow leopard's individual-based spatial genetic characteristics from UTL. Multi-locus genotyping ($n = 14$ loci) of individuals ($n = 19$) revealed moderate genetic diversity in the population (mean number of alleles = 5.86 ± 0.55 , observed heterozygosity = 0.48 ± 0.05 , expected heterozygosity = 0.65 ± 0.03 , allelic richness = 2.65 ± 0.15). We did not observe any evidence of population structuring (using STRUCTURE and Factorial Correspondence Analysis) or isolation by distance. However, the clustering approach based on genetic distance (Nei's standard distance and Cavalli-Sforza and Edwards distance) and subsequent discriminant analysis of principal components (DAPC) revealed three sub-clusters of related individuals within the study population without any spatial correlates. We observed 1.2% first-order relatives, suggesting sufficient dispersal and panmixia in the UTL population. We observed high fixation index ($F_{IS} = 0.26 \pm 0.05$; 0.17 ± 0.03 upon removing loci with null alleles) and presence of individuals from genetically divergent populations in UTL. Hence, the high positive F_{IS} value could be attributed to both Wahlund effect and inbreeding. Prioritization and effective conservation planning of the UTL population as a source would benefit the global snow leopard population by (i) maintaining connectivity between the Himalayas and the central Asian mountain ranges, and (ii) providing refuge during future climate change-related range contraction.

Keywords *Panthera uncia* · Western Himalayas · Microsatellite · Genetic diversity · Functional connectivity

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Introduction

The snow leopard (*Panthera uncia*) is the apex predator of the high-altitude ecosystem in the south and central Asian mountain systems, also known as high-mountain Asia. Conservation of this species is imperative in maintaining the stability of prey communities and ecosystem services (Ripple et al. 2014). The current snow leopard range is distributed across 12 countries spanning the high-mountain Asia (McCarthy and Chapron 2003). Though snow leopards are known to use several habitat types to some extent, the fragile ecosystem of high alpine meadows is the most utilized habitat. They prefer cold and arid tracts between 3200 and 5200 m altitudes though there are recent reports of snow leopard presence in < 3200 m (Sathyakumar unpublished; Valentová 2017).

The snow leopard is categorized as 'Vulnerable' in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as their population (between 7446 and 7996 individuals) is small with a decreasing trend compared to the distribution range (between 1,77,6000 and 3,30,0000 km²) (McCarthy et al. 2017; Li et al. 2020). Due to the remote, rugged, and largely inhospitable habitat, the snow leopard is one of the least studied species among the big cats (McCarthy et al. 2017; Watts et al. 2019). The key threats to the conservation of the species are fragmentation and loss of habitat, depletion of prey biomass, poaching and retaliatory killings (McCarthy and Chapron 2003; Crooks et al. 2011; Riordan et al. 2016; McCarthy et al. 2017; Maheshwari and Niraj 2018; Maheshwari and Sathyakumar 2020). Implications of global climate change further amplify fragmentation of remaining habitats supporting large mammal populations in harsh conditions (Cardillo et al. 2005; Morrison et al. 2007). Climate change projections suggest that the warmer and wetter conditions in the snow leopard range could result in shifting of the treeline, with forests ascending to the alpine regions (Forrest et al. 2012). Further fragmentation of the snow leopard habitat would ultimately lead to the possibility of genetic isolation between populations, thereby disrupting the ecological and evolutionary processes in the high-mountain Asia (Riordan et al. 2016). Hence, ensuring habitat connectivity has been suggested to minimize genetic stochasticity due to climate change and other factors associated with Anthropocene (Sgrò et al. 2011; Padrón et al. 2018). Therefore, delineation of seven snow leopard landscape conservation units (LCU) and ten linkages between these have been carried out to achieve species conservation goals (Li et al. 2020). Additionally, genetic data of nuclear microsatellites identified three subspecies of the snow leopard with distinct geographic ranges (Janečka et al. 2017).

Conservation of the snow leopard, occupying a vast geographic range, requires insight into the species' ecology besides assessing suitable habitat to obtain optimal conservation benefits with limited resources (Li et al. 2020). Despite vast literature on its ecology (Forrest et al. 2012; Li et al. 2016, 2020; Riordan et al. 2016; Mahmood et al. 2019; Watts et al. 2019; Maheshwari and Sathyakumar 2020; Singh et al. 2020), assessments of genetic diversity and functional connectivity is rare (Karmacharya et al. 2011; Janečka et al. 2017; Poyarkov et al. 2020; Rode et al. 2020).

Union Territory of Ladakh (UTL), India, is one of the strongholds of the snow leopard, while being the westernmost limit of the species in the Himalayas. UTL accounts only for 0.56% of the total species range while contributing to 7.4% of the total occurrences across its range, even after considering only one record per 1 km² (Li et al. 2020). About 60% of the Indian snow leopard population is estimated to be residing in UTL, whereas ~30% of UTL's total

geographical area (~30,000 km²) was high to medium suitability habitat (Watts et al. 2019). The region is the centre of the snow leopard's geographic distribution aiding connectivity (<300 km) with the surrounding mountain ranges (Fig. 1a). The area has been identified as one of the 'climate refugia' for the snow leopard (Li et al. 2016). Hence, the UTL region is critical as a conservation priority area for the snow leopard.

Because of the strategic central location, UTL is a core source population of the snow leopard within the landscape comprised of Tibetan plateau, Pamir-Alay, Kunlun Shan, Hindukush, Karakoram, and Himalayan mountain ranges. The need for including genetic diversity parameters for devising management policies of wild populations in the Anthropocene has recently been emphasized (DeWoody et al. 2021). Hence, this study aims to characterize the levels of genetic diversity, relatedness, inbreeding, and gene flow in the snow leopard population of UTL using non-invasive genetic sampling. We also provide individual-based genetic relationships, in addition to population genetic characteristics, to obtain further insight to develop effective conservation strategies for the species.

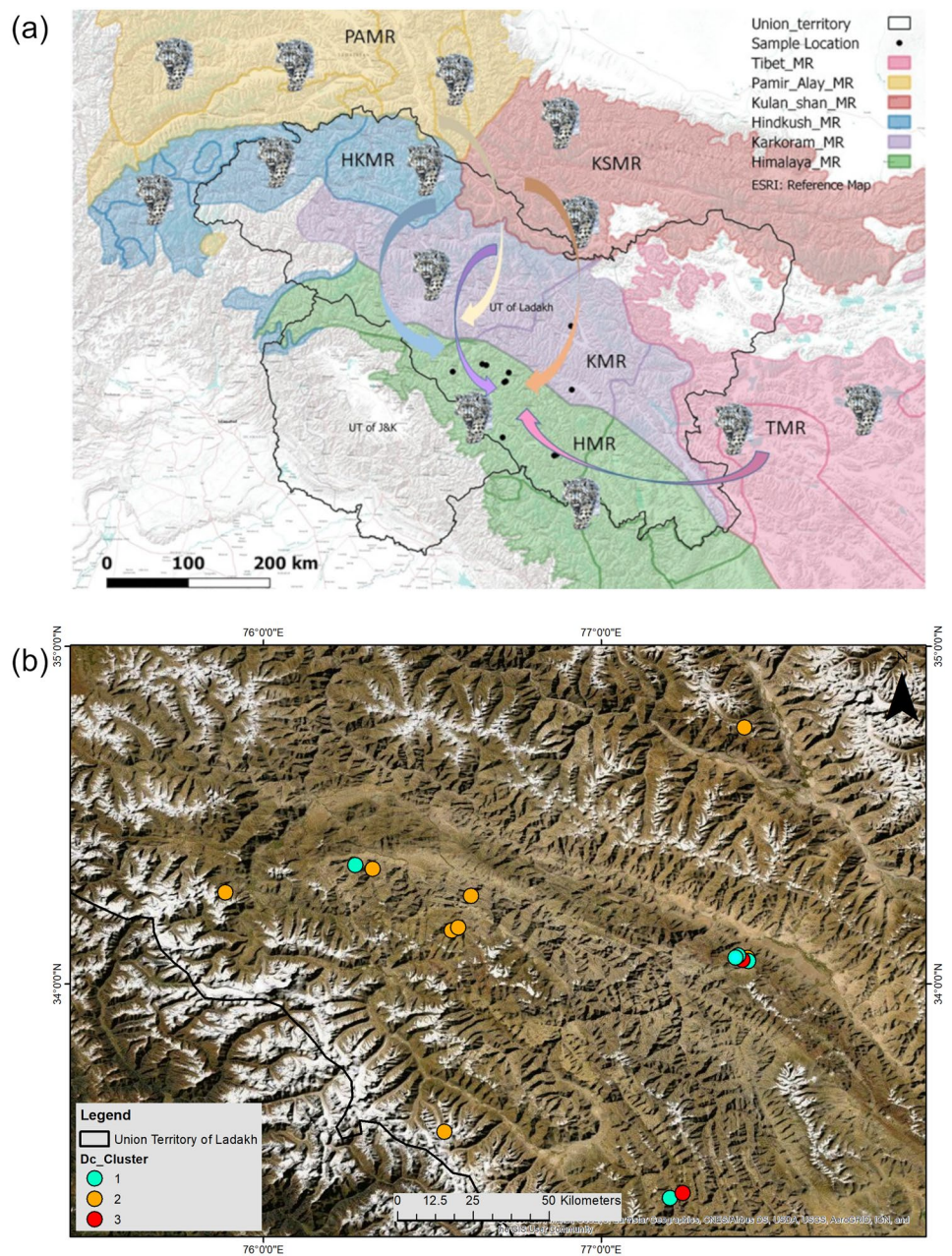
Material and methods

Sample collection, DNA extraction and species identification

We used a total of 58 carnivore scat samples, putatively of snow leopard origin, which were collected from the UTL region from 2009 to 2012 (Fig. 1a) as a part of a research project involving few of our co-authors, for which DNA-based species confirmation was undertaken. Additionally, we used tissue samples from two individuals, which died due to natural causes, from the study area provided to the repository of Wildlife Institute of India, Dehradun, India, by the erstwhile Department of Wildlife Protection, Government of Jammu and Kashmir. We extracted genomic DNA from the scraped outer layer of the scat samples using Qiagen QIAmp DNA stool kit following the protocols provided by the manufacturer with minor modifications, while using Qiagen DNeasy blood and tissue kit for DNA isolation from tissue samples. Separate dedicated facilities for tissue and faecal DNA extraction were used to avoid contamination, and negative controls during each batch of extraction were also used for the same purpose.

We amplified a 146 bp segment of the mitochondrial Cytochrome *b* (Cytb) from the scat samples using a carnivore-specific primer pair designed by Farrell et al. (2000) following amplification protocols suggested by the authors to ascertain the species identity. After a reaction clean-up step using Exonuclease I and Shrimp Alkaline

Fig. 1 a Snow leopard population of Union Territory of Ladakh, western Himalaya, India in relation to other populations distributed across the Asian mountains range. (Arrows indicate the possibility of snow leopard immigration to Union Territory of Ladakh, India); **b** locations of individuals belonging to three different clusters as observed from genetic distance (D_C) dendrogram



Phosphatase, the amplicons were Sanger sequenced from both directions using BigDye Terminator v3.1 Cycle Sequencing kit (Invitrogen), followed by capillary electrophoresis in an ABI 3130 Genetic Analyzer. Sequence quality and base calls were manually verified using BioEdit v7.2 (Hall 1999). Species of origin were confirmed using the BLAST algorithm (Altschul et al. 1990) on NCBI GenBank and comparing with reference sequence repository of Wildlife Forensic Cell of Wildlife Institute of India, Dehradun. We used a similarity threshold of 95% for species delimitation between the snow leopard and other sympatric carnivores using Cytb as suggested in the literature (Tobe et al. 2010).

Microsatellite amplification and fragment analysis

We selected 14 highly polymorphic microsatellite markers—PUN82, PUN100, PUN124, PUN132, PUN225, PUN229, PUN327 (Janečka et al. 2008), FCA272, FCA126, FCA077, FCA742, FCA441 (Menotti-Raymond et al. 1999), PLE23 and PLE57 (Singh et al. 2002). We used a multi-tube genotyping approach (Taberlet et al. 1996) as we typed each sample three times with independent PCR runs to minimize genotyping errors, i.e., allelic drop-out and false allele amplification. Each reaction contained 1 μ L of the DNA extract, 5 μ L of the 2X Qiagen Multiplex PCR kit, 1 μ g BSA and 0.4 μ M of each primer. The thermal profile

for the reactions was as follows—initial denaturation at 94 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 35 s, annealing at 53 °C for primers described by Janečka et al. (2008), 51 °C for Menotti-Raymond et al. (1999) and Singh et al. (2002) for 60 s and extension at 72 °C for 90 s followed by a final extension for 30 min at 72 °C. We performed all amplifications on ABI 9600 Fast (Applied Biosystem) thermocycler. The amplicons were subjected to fragment analysis with a size standard on the ABI 3130 Genetic Analyzer (Applied Biosystems). The raw data were manually verified and scored using GeneMapper 3.7 (Applied Biosystems), and consensus genotypes for the samples were constructed.

Data analysis

We used the software MICRO-CHECKER (van Oosterhout et al. 2004) to check for genotyping errors, e.g., miscalling of alleles due to stuttering and large allele drop-outs besides detecting the presence of null alleles. We performed Fisher exact tests between all pairs of loci to detect any evidence of linkage disequilibrium using GENEPOP (Rousset 2008) with 1000 dememorization steps, 100 batches and 1,000 iterations per batch. We calculated the mean number of alleles per locus (MNA), observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (AR), and inbreeding coefficient (F_{IS}) while also estimating the 95% confidence interval and statistical significance of F_{IS} by bootstrap resamplings ($n=9999$) using the R package diveRsity (Keenan et al. 2013; R Core Team 2019) as well performing exact tests to detect departure from Hardy–Weinberg equilibrium (HWE).

We employed a Bayesian individual-based clustering algorithm implemented by the software STRUCTURE (Pritchard et al. 2000) to understand population sub-structuring within UTL. For STRUCTURE, we used a total of 500,000 Markov-chain Monte Carlo iterations per assumed number of clusters (K), with the first 50,000 discarded as burn-in. We determined the number of clusters using the $L(K)$ (Pritchard et al. 2000) and ΔK method (Evanno et al. 2005) implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012) while we averaged the results from ten independent runs using CLUMPAK (Kopelman et al. 2015).

Nei's standard genetic distance (D_S) and Cavalli-Sforza and Edwards distance (D_C) were computed using the software POPULATIONS v1.2.32 (Langella 2002) and constructed a neighbour-joining dendrogram with 100 bootstraps. D_C is known to perform better than D_S in the presence of null alleles (Chapuis and Estoup 2007; Séré et al. 2017). FIGTREE v1.4.2 (Rambaut 2014) was used for visualization and annotating the dendrogram. Additionally, we used a multivariate test, discriminant analysis of principal

components (DAPC) using R package ADEGENET (Jombart 2008), to visualize the degree of differentiation between the genetic clusters identified by the genetic distance dendrograms. We performed three-dimensional factorial correspondence analysis (FCA) using GENETIX 4.05.2 (Belkhir et al. 2004). A mantel test for congruence of the matrices of pairwise genetic and geographic distances was performed to examine the presence of isolation by distance in the study population. We computed the pairwise relatedness between the individual dyads using the software COANCESTRY v1.0.1.9 (Wang 2011). The mean relatedness for each individual with every other individual was calculated, and the values were interpolated across UTL, India, employing an inverse-distance-weighted (IDW) algorithm using ArcGIS 10.2 (ESRI). We used the software BOTTLENECK (Piry et al. 1999) to test for departures from mutation drift equilibrium using three microsatellite mutation models—infinite allele model (IAM), two-phase model (TPM) and stepwise mutation model (SMM). We assumed a two-phase mutation model with 70% stepwise mutations, 30% multiple-step mutations and significance of heterozygosity excess over all loci (P) was determined with a one-tailed Wilcoxon sign rank test.

Results

Genetic diversity

Alongside the 2 tissue samples, we identified 17 snow leopard individuals from 58 Cytb sequences followed by multi-locus genotyping ($n=17$; for confirmed snow leopard samples). Cytb sequences (GenBank accession numbers: ON805826–ON805828) did not indicate any polymorphism within the amplified segments across samples. Nineteen samples were used for the final data analyses, including two tissue samples, with a mean success rate of 91.4% across 14 felid specific microsatellite markers. None of the microsatellite loci displayed any indication of large allele drop-out. Of the 91 Fisher exact probabilities between loci to test for linkage disequilibrium, only 1 pair (PUN82 and PUN327) was significant ($p < 0.01$). The markers PLE57 and PUN132 showed the possibility of scoring errors due to stuttering, whereas we observed the presence of null alleles in PUN132 (0.33), PUN124 (0.15), FCA441 (0.24), and PLE57 (0.21). The cumulative P_{ID} and $P_{ID_{sib}}$ for the 14 loci were 7.41×10^{-12} and 2.28×10^{-5} , respectively. A relatively high proportion (24.4%) of the alleles had frequency < 0.05 though there were no alleles below the frequency of 0.01 (Fig. S1).

The level of genetic diversity in the studied snow leopard population was moderate. Out of the 14 loci, 10 deviated from Hardy–Weinberg equilibrium (HWE) based on Fisher

exact tests. Overall mean observed heterozygosity (H_O) and expected heterozygosity (H_E) were 0.48 (0.21–0.84) and 0.65 (0.40–0.82), respectively (Table 1). The overall allelic richness in the UTL population was 2.65. We found the presence of 82 alleles across the 14 loci with frequencies ranging from 0.03 to 0.76. Due to heterozygote deficiency, we observed a positive inbreeding coefficient ($F_{IS}=0.26$) which was statistically significant ($\alpha=0.05$) based on 9999 permutations. To test the impact of null alleles on the F_{IS} estimate, we removed the four loci showing its presence, and the reanalysis still yielded a positive estimate of $F_{IS}=0.17 \pm 0.03$.

Population genetic structure

Though the highest value of ΔK obtained from STRUCTURE was at $K=2$ (Fig. S2a), we did not observe any assignment of individuals, all admixtures with $Q < 0.8$, to either of the clusters within the UTL population (Fig. S2c). In support of the ΔK method, inspection of the $L(K)$ plot (Fig. S2b) revealed that there was no structuring within UTL as the variance of Ln probability increased significantly at $K=2$ as compared to $K=1$.

Nei's standard genetic distance (D_S) between the individuals ranged from 0.3 (5437 and 5438) to 2.4 (5426 and 9949). Genetic distances (D_S) of 0.8 to 1.1 were most frequent amongst all pairs of individuals. The neighbour-joining dendrogram (Fig. 2a), constructed from D_S calculated from the microsatellite data, revealed the presence of multilineage clusters. The clusters ($n=3$) comprised of nine,

seven and three individuals, respectively, while the first cluster was subdivided into three distinct sub-clusters. The dendrogram from Cavalli-Sforza and Edwards distance (D_C) showed a similar grouping of individuals into three clusters and further subgroupings (Fig. 2b). These clusters were separated in the discriminant space with no ellipses overlapping using DAPC (Fig. 3). However, based on geo-coordinates, the clustering within UTL samples did not show any spatial patterns (Fig. 1b).

Factorial correspondence analysis (FCA; Fig. 4) did not show any close grouping of individuals with few outliers, indicating a lack of strong population structuring. Mantel test revealed that the congruence between the matrices of genetic distance and geographic distance between the sampled individuals was not statistically significant ($p > 0.05$), thereby ruling out the possibility of isolation by distance (Fig. S3).

Relatedness estimates

The Queller–Goodnight (QG) relatedness estimates indicated that 85.4% of the individual dyads were unrelated to each other ($QG < 0.125$), whereas the proportions of third ($0.25 > QG \geq 0.125$) and second-order ($0.5 > QG \geq 0.25$) relatives were 8.2% and 6.4% in the UTL snow leopard population, respectively (Figs. S4, S5). The QG values showed a decreasing pattern with increasing geographic distance (GD) between the individuals (Fig. S5), but GD could only explain 1.9% of the variations in dyads

Table 1 Genetic diversity parameters of the snow leopard in the Union Territory of Ladakh, India

Loci	AR	H_O	H_E	F_{IS}	P_{ID}	P_{IDsib}
FCA742	2.46	0.57	0.65	0.12	0.18	0.46
FCA441	2.36	0.26 ⁺	0.57	0.54*	0.19	0.48
FCA272	2.54	0.56	0.61	0.09	0.17	0.46
FCA126	4.13	0.84	0.82	-0.03	0.06	0.36
FCA077	2.31	0.53 ⁺	0.62	0.14	0.12	0.42
PLE57	1.97	0.21 ⁺	0.40	0.47	0.20	0.49
PLE23	2.14	0.38 ⁺	0.49	0.24	0.05	0.35
PUN82	2.74	0.53 ⁺	0.66	0.21	0.21	0.52
PUN327	2.71	0.47 ⁺	0.66	0.29	0.29	0.58
PUN229	3.09	0.65	0.74	0.13	0.11	0.41
PUN225	2.28	0.47 ⁺	0.64	0.27	0.18	0.47
PUN132	2.59	0.22 ⁺	0.73	0.69*	0.20	0.49
PUN124	3.28	0.59 ⁺	0.82	0.28	0.38	0.64
PUN100	2.46	0.47 ⁺	0.64	0.27	0.20	0.48
Overall	2.65	0.48 ⁺	0.65	0.26*	7.41×10^{-12} **	2.28×10^{-5} **

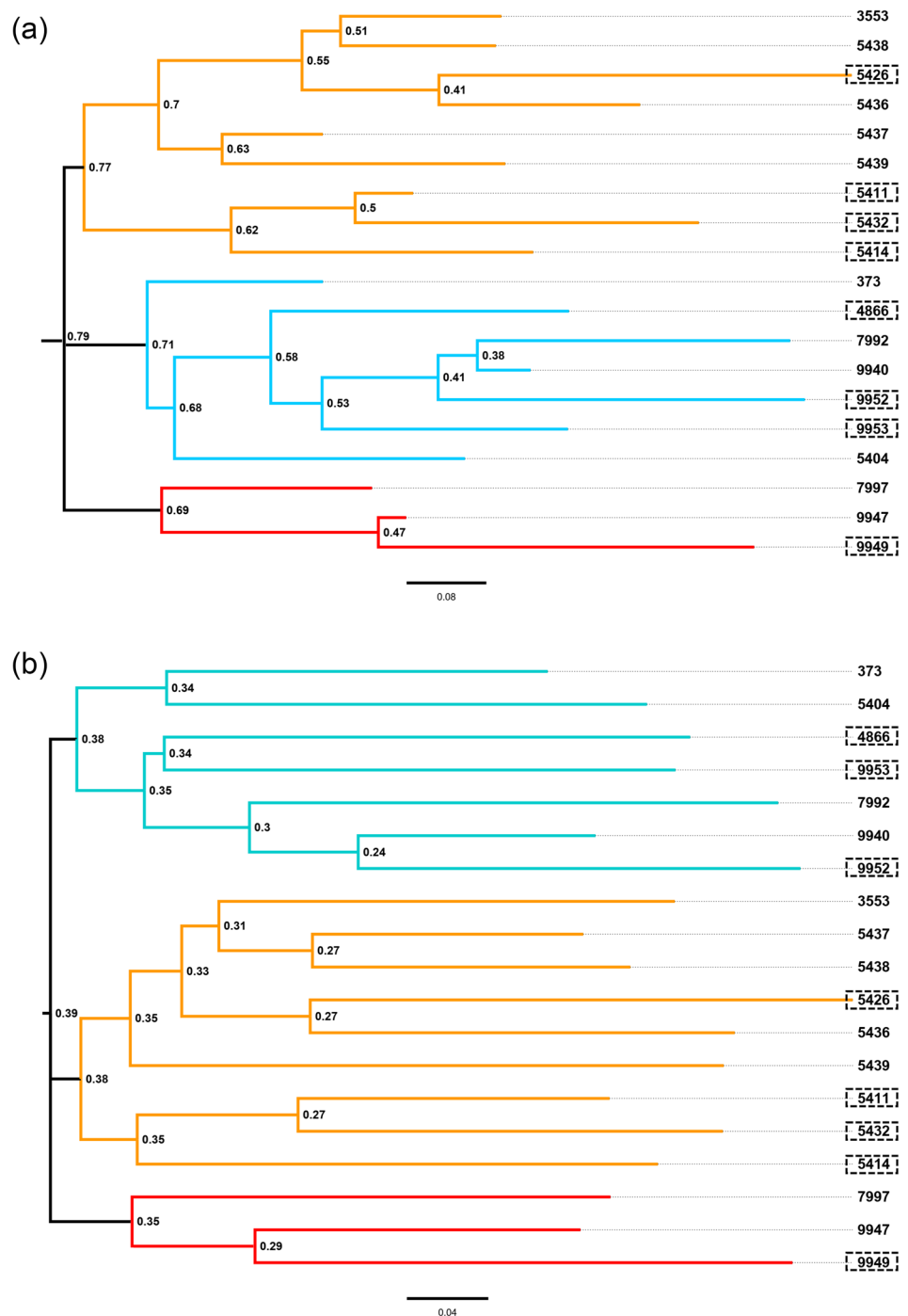
AR Allelic richness, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient (fixation index), P_{ID} probability of identity, P_{IDsib} sibling probability of identity

*Denotes statistical significance based on 9999 permutations

⁺Denotes significant deviation from Hardy–Weinberg equilibrium based on exact tests

**Cumulative values

Fig. 2 Dendrogram of **a** Nei's standard genetic distance (D_S) and **b** Cavalli-Sforza and Edwards distance (D_C) for the snow leopard individuals in Union Territory of Ladakh, India. Tip labels indicate unique sample ID. Three different clusters with reference to the D_C dendrogram—Clusters 1, 2 and 3 are denoted by cyan, gold and red coloured bars, respectively. Dashed boxes indicate outlier individuals with reference to Factorial Correspondence Analysis



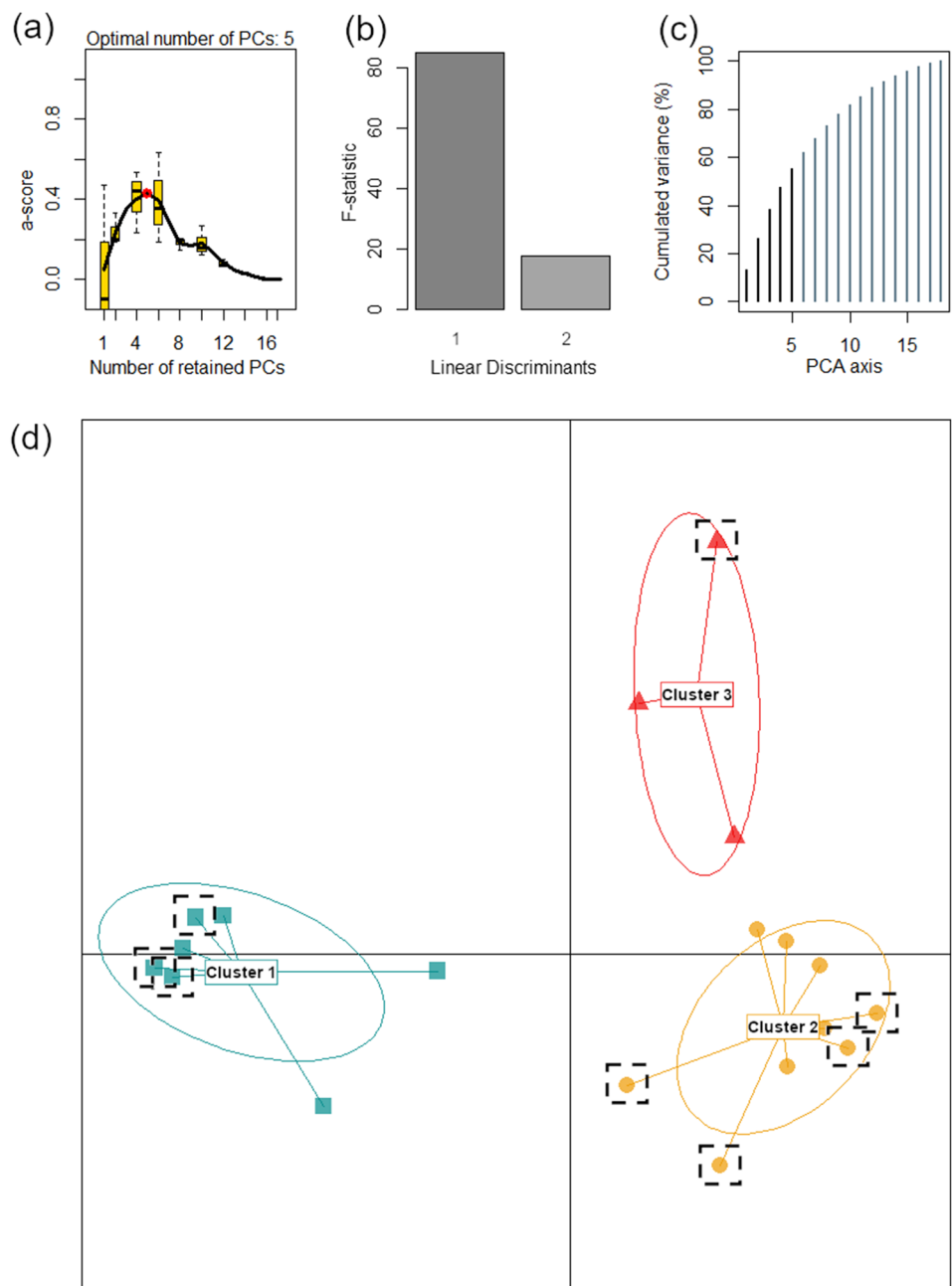
with $QG \geq 0.125$ and 3.4% with $QG \geq 0.25$. Out of the 171 possible dyads of individuals, 156 (91.2%) were not related to each other, 13 (7.6%) were half-siblings, and two (1.2%) were determined as full siblings at $\alpha = 0.01$. We also observed that the geographic distance between pairs of samples was uniformly distributed across the landscape (Fig. S6), while $> 80\%$ of the pairs had relatedness values < 0.10 . Mean QG estimates within clusters ($n = 3$) identified by the D_C dendrogram (Fig. 2b) were higher

(between 0.04 ± 0.03 and 0.18 ± 0.11) than mean QG values across different clusters (-0.11 ± 0.01).

Departures from mutation drift equilibrium

The results of the BOTTLENECK analysis showed that there was no consistent or strong signal for a departure from mutation drift equilibrium. To assess if the results were influenced by having included four loci with null alleles,

Fig. 3 Discriminant analysis of principal components (DAPC) between the genetic clusters ($n=3$) observed using the dendrogram of pairwise genetic distances (D_C)—**a** optimum alpha score, **b** discriminant function eigenvalues, **c** principal component eigenvalues and **d** scatter plot of the snow leopard individuals of Union Territory of Ladakh, India. Dashed boxes indicate outlier individuals with reference to Factorial Correspondence Analysis



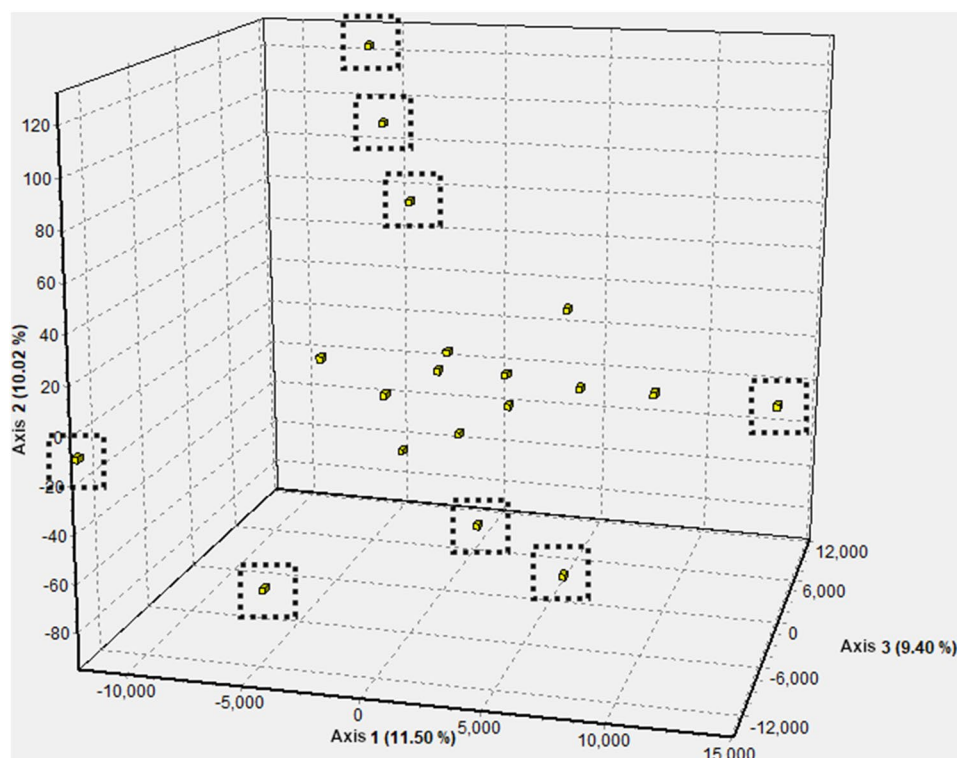
the analyses were repeated just including ten microsatellites. The population showed a significant excess of heterozygotes ($p=0.00684$) only under the IAM model based on the Wilcoxon sign rank test. However, the test was not significant when 14 loci were used (IAM; $p=0.12061$).

Discussion

The UTL population of snow leopards, because of its strategic location, provides biological connectivity within high-mountain Asia. Hence, this population is of immense

conservation importance because this area may serve as climate refugia (Li et al. 2016). Maintaining connectivity between these mountain ranges is thus important to allow natural dispersal and improve snow leopard population persistence in this landscape (Li et al. 2016, 2020; Janečka et al. 2017). This study provides the first attempt to document the genetic diversity of the source population in UTL based on 19 confirmed snow leopard individuals.

Fig. 4 Factorial correspondence analysis (FCA) of the snow leopard individuals sampled from Union Territory of Ladakh, India. Dashed boxes indicate outlier individuals



Genetic diversity in UTL population

We observed a moderate level of genetic variability in the UTL snow leopard population. The MNA (5.9) in UTL was higher compared to other studies in terms of MNA (3.2–3.3), while in terms of H_O and H_E , UTL ($H_O = 0.48$; $H_E = 0.65$) was similar to other adjacent populations ($H_O = 0.41–0.66$; $H_E = 0.30–0.72$) (between 8 and 107 samples/site) (Janečka et al. 2017; Rode et al. 2020) (Table S1). Similar trends were observed based on the shared loci used in a recent range-wide study (Janečka et al. 2017) (Table S2).

The majority of the felid species are solitary with male-mediated dispersal (Packer 1986). Such aspects of species ecology are known to affect genetic characteristics. Published heterozygosity values of different felid species are not directly comparable across species because of ascertainment bias due to the use of different markers across studies (Garner et al. 2005; Li and Kimmel 2013). However, the observed heterozygosity in the UTL snow leopards (0.48 ± 0.05) were comparable to recent studies on other solitary felids, e.g., mountain lions (0.34–0.53), tiger (0.55–0.77), leopard (0.36–0.83) and jaguar (0.66) (Gustafson et al. 2019; Kolipakam et al. 2019; Bhatt et al. 2020; Lorenzana et al. 2020).

Lack of genetic sub-structure and inbreeding within UTL

Small, isolated populations and the presence of cryptic barriers in spite of landscape contiguity are often at the centre of conservation concern due to the possibility of genetic drift, inbreeding and resultant inbreeding depression, characterized by morphological aberrations and reduced population vigour (Keller and Waller 2002; O'Brien 2005). Therefore, understanding genetic characteristics of threatened species is a key factor for conservation planning. Our study indicates high levels of gene flow in the UTL snow leopards and is supported by multiple approaches (STRUCTURE, FCA and IBD analyses). The absence of genetic differentiation found in this study illustrates the mobility and dispersal ability of snow leopards. Snow leopards are capable of long-distance movement in search of prey or a mate (Jackson 1996; Oli 1997; McCarthy et al. 2005; Johansson et al. 2016; Poyarkov et al. 2020). Similarly, snow leopard populations in other landscapes (e.g., Mongolia) exhibiting dispersal over long distances also show limited phylogeographic structuring (Janečka et al. 2017).

However, we also observed the presence of at least eight outlier individuals suggestive of individuals not originating in UTL, with a possibility of immigration from other

populations (Fig. 4). On the other hand, these outlier individuals were not conspicuous in terms of genetic distances (D_S , D_C) or DAPC (Figs. 2, 3). Additionally, we observed the presence of three clusters based on genetic distance dendrograms and subsequently verified the same using DAPC (Figs. 2, 3). There were no spatial segregation and, therefore, probably indicate towards three different ancestries immigrating and thereafter contributing to the UTL population. Our analyses revealed that the observed genetic clusters represent individuals who are more closely related to each other than to random members of the population. These related individuals may share alleles that differ in frequencies from those observed at other sites within the same metapopulation. Methods such as STRUCTURE and DAPC may not differentiate between family group and population structure (Patterson et al. 2006; Anderson and Dunham 2008) and therefore, are not suggestive of population structuring in the case of UTL snow leopards.

Several reasons could contribute to the presence of the highly positive F_{IS} value (indicating a deficit of heterozygotes) in the UTL population: the presence of null alleles, inbreeding, and Wahlund effect. Null alleles do not seem to explain the result since similar results were observed after excluding four loci having null allele frequency > 0.15 . With regard to inbreeding, we cannot exclude the possibility that this is an artifact, as such high F_{IS} values could be due to (i) low population density (Sharma et al. 2021) that resulted in the increased chance of mating by few dominant individuals, and (ii) high site fidelity or philopatry in the populations (Jackson 1996).

Within the sampled individuals, only 1.2% of pairwise relationships were categorized as first-order, thereby ruling out the possibility of imminent inbreeding in the study population. The network diagram (Fig. S4) also shows only a few individuals clustering with other individuals with high relatedness in the study area. Furthermore, the population of UTL is comprised of snow leopard individuals from genetically divergent populations, probably from surrounding mountain ranges due to the central location of the UTL habitat and thus, potentially producing Wahlund effect (Chesser 1991; Waples 2015). Unfortunately, our low sample size does not allow to distinguish the Wahlund effect from other causes of positive F_{IS} .

There were no indications of a recent demographic bottleneck in the UTL snow leopard population using the heterozygosity-excess test. Population structure as well as migration or admixture among subpopulations, can mask the effects of a bottleneck (Cornuet and Luikart 1996). We interpret that in metapopulations which are characterised by interconnected subpopulations with local extinction and recolonisation turnover dynamics (Hanski 1998), demographic bottlenecks may prove difficult to detect using genetic data, especially if the sample size is limited.

Genetic relatedness amongst the sampled individuals

Comparison of relatedness and geographic distances between dyads of individuals at two different cut-offs corresponding to third order (e.g., first-cousin) and higher ($QG \geq 0.125$) as well as second-order (e.g., half-sibling) and higher ($QG \geq 0.25$) did not reveal any pattern, indicating panmixia and connectivity with other snow leopard populations. IDW interpolation also suggests a uniform distribution of mean relatedness of the individuals in the study area except for few possible immigrants (Fig. 5). The distribution patterns of geographic distance (nearly even across classes) and genetic distance (D_S , D_C), as well as relatedness (unimodal) for dyads of individuals, also indicates the lack of geographic structuring of the UTL population (Fig. S6). FCA results lacked any clustering along any of the axes with few individuals as outliers suggesting the absence of genetic structure and immigration from other populations.

Significance of UTL landscape as a central snow leopard habitat

Mahmood et al. (2019) observed that snow leopards have been locally extinct across 408 PAs in Asia, along with an overall 69% decline. The snow leopard habitat is further projected to lose 23% of the current extent in the next 50 years while the Himalayan mountain ranges would be affected by the resulting fragmentation (Li et al. 2016). Therefore, securing core areas and maintaining landscape connectivity is of utmost importance for a conservation policy that integrates the climate projection component (Forrest et al. 2012). Li et al. (2016) suggested that providing legal protection in the form of establishing reserves in identified refugia is an effective conservation strategy as only 19% of the range is currently under protection. Riordan et al. (2016) suggested that legal protection alone was insufficient for snow leopard conservation though connectivity could be maintained through the unsuitable habitat.

All individuals sampled in this study were within ~150 km of each other, which is well within the dispersal capabilities of the snow leopard (250–500 km) (Janečka et al. 2017). However, there are examples in the literature where adjacent populations within the dispersal range developed strong genetic structuring in the presence of a barrier, as observed in the case of mountain lions in California, USA (Ernest et al. 2003). Our analyses did not reveal any such pattern for the snow leopard in the core source population of UTL in spite of adjacent mountain ranges such as Zaskar and Karakoram. This could probably be attributed to the plausible high connectivity of snow leopard populations with adjacent mountain ranges (Fig. 1). On a similar note, Riordan et al. (2016) predicted the western Himalayan

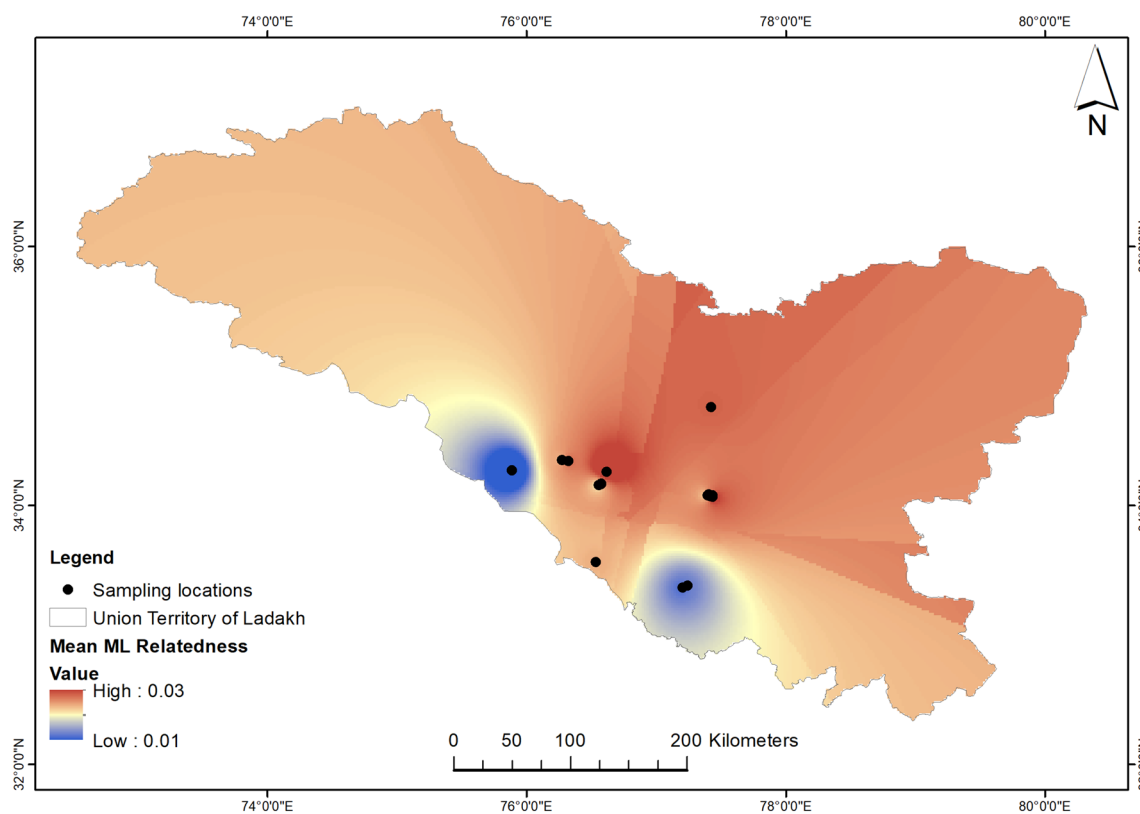


Fig. 5 Spatial interpolation of average maximum-likelihood relatedness for snow leopard individuals sampled from the Union Territory of Ladakh, India

region to maintain a moderate to high level of movement across a large area while maintaining connectivity between the QTP and Altai through Pamir and Alay mountain ranges. The uniform distribution of the branching in the dendrogram constructed from pairwise D_S as well as D_C indicate a long-term stable effective population size in UTL with high gene flow with adjoining populations (Spong et al. 2000).

Our findings substantiate the importance and contribution of the UTL population to the snow leopard gene pool of adjoining snow leopard habitats. UTL provides refuge (Li et al. 2016) and connectivity within high-mountain Asia for snow leopards. However, the effectiveness of the conservation efforts may be compromised with increasing change in land use patterns caused by anthropogenic activities and climate change-induced habitat loss. With most snow leopard populations across the range having no evidence of population structuring and a high dispersal capability, we believe there is no need for adaptive management, such as introduction of individuals to augment genetic diversity, for the UTL snow leopard population in the recent future with respect to inbreeding. Additionally, we suggest a trans-boundary, collaborative study for understanding the extent of the genetic linkages and significance of the UTL population

in the context of the Asian mountain ranges in developing conservation strategies using harmonized genetic markers.

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Author contributions Concept and supervision: SPG, BH, SSK, DS; fieldwork: AM; sample processing: SKS, BDJ; performed the experiments: SKS, BDJ; analyzed the data: RD, RS, SKS, BDJ; authored the original draft: RD; reviewed and commented on the draft manuscript: RS, SPG, SKS, BDJ, AM, SS, BH; approved the final draft: SKS, RD, RS, AM, BDJ, DS, SS, BH, SPG. RD and SKS contributed equally to this paper.

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Availability of data and material Raw genotypes used in this study will be made available upon reasonable request. All other data have been

included in the form of tables in the manuscript and the supplementary material.

Code availability Not applicable as we did not use any custom code for this study.

Declarations

Conflict of interest The authors declare no conflict of interests.

Ethics approval This study did not involve handling of live animals. We used tissue samples from the repository of Wildlife Institute of India, Dehradun, India. Non-invasive faecal samples used in this study were collected under an earlier study by one of the authors and the permission was accorded by the erstwhile Department of Wildlife Protection, Government of Jammu and Kashmir.

Consent to participate Not applicable as the study did not include any human participants.

Consent for publication Not applicable as this study does not contain data from any individual person. All authors approved and consented to the submission of the final draft manuscript.

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