Janecka *et al.* Supplementary Material

Methods

Study areas

GPS locations and elevation of survey sites:

(1) 1 site in Ladakh, India (N34º20'01.13", E77º08'08.17", elevation range of 3,950–4420 m)

(2) 2 sites in Dulan County, Qinghai, China (N36º04'07.95", E96º57'38.02", elevation range of 3543–3930 m and N3604'12.01, E98 32'48.10", elevation range of 4135-4278 m)

(3) and 3 sites in South Gobi, Mongolia (N43º05'07.56", E101º59'52.86"; N43º09'06.77", E102º01'30.86"; N43º13'22.87", E101º55'42.95", elevation range of 2054–2250 m; Fig. 1

Species identification with mitochondrial cytochrome *b* primers

A 148-bp region of the mitochondrial cytochrome *b* gene was amplified by PCR using carnivore-specific primers (Farrell, Roman & Sunquist, 2000). Amplifications were conducted in 10 µl volumes, containing 0.2 mM of each dNTP, 1x PCR buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM *KCl; Eppendorf, Germany)*, 0.25 units of HotMaster™ *Taq* (Eppendorf), 1 µg of Bovine Serum Albumin (New England BioLabs, USA), 0.24 mM forward and reverse primers, and 1 µl of DNA extract. The PCR reaction conditions included an initial denaturing step of 94ºC for 1 min, followed by 50 cycles of 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 60 sec, and a final extension step of 72ºC for 2 min. PCR products were fractionated on a 1.2% agarose minigel, stained with ethidium bromide (0.5 µg/ml), and visualized under ultraviolet light. PCR products were

purified using PrepEase® PCR Purification 96-well Plates (USB Corporation, USA), sequenced using ABI BigDye Terminator chemistry (Applied Biosystems, USA), and resolved on an ABI 3730 automated sequencer. Sequences were obtained from both strands and contigs were assembled using SEQUENCHER v4.7 (Gene Codes Corporation, USA).

Sequences were examined in SEQUENCHER, trimmed of primers and ambiguous bases, and aligned with reference sequences in CLUSTAL (Thompson *et al.*, 1997). Reference sequences of sympatric carnivores (Wilson & Reeder, 2005) were either downloaded from GenBank or sequenced in the lab from tissue extractions (GenBank XXXXX-XXXXX). Several closely related carnivores (tiger [*Panthera tigris*] and Asian leopard cat [*Prionailurus bengalensis*]) were included for the purpose of reconstructing an informative phylogeny for species identification. We included several divergent haplotypes for 3 species (e.g., tiger, wolf, and domestic cat) to determine the amount of intraspecific divergence expected in this short region of cytochrome *b*. When it was not possible to obtain reference sequences for sympatric taxa (i.e., Chinese desert cat [*Felis bieti*] and Pallas cat [*Otocolobus manul*]) we included a species from their same evolutionary lineage; the wild cat was a surrogate for the Chinese desert cat and the Asian leopard cat was a surrogate for the Pallas cat (Johnson *et al.*, 2006).

A 100-bp segment of cytochrome *b* was used to reconstruct a neighbor-joining phylogeny using the Kimura (1980) two-parameter model of evolution in PAUP* (Swofford, 1998) using reference carnivores and the haplotypes observed in scats. The significance of nodes was evaluated by bootstrapping with 100 replicates and a pair-wise distance matrix was generated to examine levels of divergence. Haplotypes obtained

from scats that clustered with high bootstrap support (>90%) in a monophyletic group with a reference sequence, and showed intraspecific levels of sequence divergence (<0.03, see Results) were considered to originate from that species. Nucleotide diversity (π) among the haplotypes observed in scats was estimated in DNASP (Rozas & Rozas, 1999).

Redesigning microsatellite primers

We evaluated 14 microsatellite loci for individual identification from scat that were previously found to be variable in the snow leopard (Waits *et al.*, 2007). These loci were originally isolated from the domestic cat (*Felis catus-FCA*) genome (Menotti-Raymond *et al.*, 1999). There is approximately 20 million years (my) of divergence between *Panthera* and *Felis* (Johnson *et al.*, 2006), making accumulation of mutations in the primer sites likely. Primer mismatches can increase the incidence of null alleles and cause weak amplifications (Callen *et al.*, 1993; Menotti-Raymond *et al.*, 2003; Menotti-Raymond *et al.*, 1999). Because of the degradation of DNA in many noninvasive samples, most studies recommend amplicons be shorter than 200 bp (Taberlet, Waits & Luikart, 1999). In addition, microsatellites frequently occur in repetitive elements of vertebrate genomes, often found within or adjacent to SINEs, LINEs, and LTRs (Buchanan *et al.*, 1993; Lopez-Giraldez *et al.*, 2006; Nadir *et al.*, 1996). Primers designed within a repetitive element adjacent to a microsatellite can cause weak amplification, background peaks, and locus specific dropout (Lopez-Giraldez *et al.*, 2006).

To avoid the pitfalls described above, we redesigned primers to (1) precisely match the snow leopard sequence, (2) amplify segments < 150 bp, and (3) avoid

designing primers in flanking sequences that contain SINEs, LINEs, and LTRs. The original domestic cat sequences for each of the 14 FCA microsatellite loci (Menotti-Raymond *et al.*, 1999) were downloaded from GenBank and primers were designed using PRIMER3 (Rozen & Skaletsky, 2000) to amplify a fragment including approximately 100 bp flanking each side of the particular microsatellite short tandem repeat (STR). When the original GenBank accession did not contain sufficient flanking sequence, we identified additional flank from the feline genome assembly at the UCSC database. These new primers were then used to amplify and sequence each of the 14 microsatellite loci in high quality snow leopard DNA (primers available from authors upon request). Microsatellite primers that amplified multiple fragments were cloned before sequencing using the TOPO TA Cloning® Kit (*Invitrogen*) following manufacturer recommendations.

Snow leopard microsatellites and their flanking sequences were screened against the domestic cat 2X genome assembly using BLAT (Kent, 2002), REPEATMASKER (Smit, Hubley & Green, 2004), and BLAST of domestic cat trace archive sequences to identify and mask flanking sequences that contain interspersed repeats. New primers were designed immediately flanking the STR repeat in PRIMER3 to amplify smaller fragments (<150 bp) and include only single-copy sequence. These new primers were designated as "PUN" with the same number as the respective FCA locus described by Menotti-Raymond *et al.* (1999).

Microsatellite loci were PCR amplified from scat samples in 10 µl volumes containing 0.2 mM of each dNTP, 1x PCR buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM KCl; Eppendorf), 0.25 units of HotMaster™ *Taq* (Eppendorf), 1 µg of Bovine Serum Albumin (New England BioLabs), 0.24 mM fluorescent dye-labeled

forward primer (6FAM, PET, NED, or VIC; Applied Biosystems), 0.24 mM reverse primer, and 1 µl of extracted DNA. The PCR conditions included an initial denaturing step of 94ºC for 1 min, followed by 50 cycles of 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 60 sec, and a final extension step of 72ºC for 30 min. All amplifications of DNA extracted from scats were set up in triplicate in a pre-PCR dedicated lab area. The PCR products were sized using an ABI 3730 automated sequencer and the alleles scored using GENEMAPPER v4.0 (Applied Biosystems). We compared amplifications with the PUN primers and the original FCA primers for 6 loci using DNA extracted from 17 scat samples that were verified to be of snow leopard origin by cytochrome *b* sequencing.

MtDNA panel for genetic structure

108-bp segment of Hyper Variable Segment 1:

PUN-HVS1-F, TAAAAACAACTCCATAGCTTCCAT

PUN-HVS1-R, GGGGCAGTTG ATCGGTTT

185-bp segment of Hyper Variable Segment 2:

PUN-HVS2-F, TTAGTA AATAATTAGCTTAAACAAACC

PUN-HVS2-R, GGATTGTTGGGCGTGTAAAT

185- and 153-bp segments of the Central Conserved Region:

PUN-CCR-F, CACCTGG CCTCGAGAAAC

PUN-CCR-R254, CCAAATGCATGACACCACAG

PUN-CCR-F173, CAACTTTCTCAAATAGGACATCTCG

PUN-CCR-R, TATATGTCCTGCGACCATTGA

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Table S1. Comparison of the number of alleles and the probability of identity (P_{ID}) observed for snow leopards by Waits et al. (2007) with estimates from snow leopard scat collected in Ladakh and South Gobi.

	Alleles			P_{ID}		
Locus	Waits *et al.*	Ladakh	South Gobi	Waits *et al.*	Ladakh	South Gobi
82	4	2	1	0.026	0.375	0.386
100	5	3	3	0.089	0.283	0.254
124	6	4	4	0.089	0.169	0.132
132	5	3	2	0.094	0.248	0.689
225	4	3	2	0.111	0.283	0.386
229	6	3	3	0.094	0.248	0.341
327	5	4	2	0.021	0.169	0.689

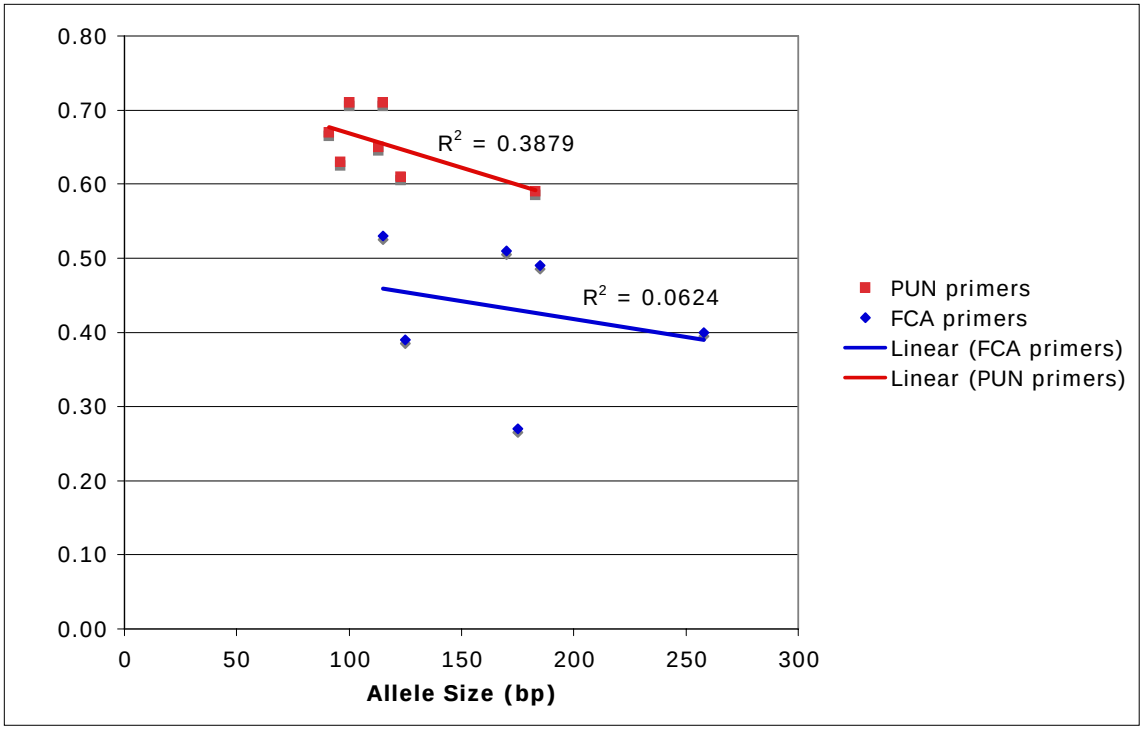

Figure S1. A plot of the maximum allele size versus quality index (Q) for loci amplified with PUN and FCA primers from 17 snow leopard scats.