Forest without prey: livestock sustain a leopard *Panthera pardus* population in Pakistan

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SUPPLEMENTARY MATERIAL1 Primer design and DNA sequencing

A primer pair *PantF/PantR* specific to the common leopard was designed on the 12S mitochondrial rRNA gene (Table S1). The specificity of this primer pair, amplifying a 79 bp fragment, was validated in silico, using *ecoPCR* (Bellemain et al., 2010; Ficetola et al., 2010). The PCRs were carried out in a total volume of 20 µl, with 8 mM Tris–HCl (pH 8.3), 40 mM KCl, 2 mM $MgCl_2$, 0.2 µM of each primer, 5 µg bovine serum albumin (BSA; Roche Diagnostic, Basel, Switzerland) and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, USA), using 2 µl as a DNA template. The PCR conditions chosen were an initial 10-minute denaturation at 95°C followed by 45 cycles of denaturation at 95°C for 30 seconds and annealing at 53°C for 30 seconds. Thus, the primary identification of the samples was based on the presence of a PCR product of the suitable length revealed by electrophoresis on a 2% agarose gel. Successfully amplified samples were selected for further analysis.

To amplify the DNA extracted from the faeces we used the universal primer pair for vertebrates *12SV5F*/*12SV5R* (Riaz et al., 2011), amplifying a c. 100 bp fragment of the 12S ribosomal gene used for studying the diet of carnivores (Shehzad et al., 2012b). To prevent the amplification of predator DNA, the *PantB* (Table S1) blocking oligonucleotide specific to leopards was designed as suggested by Vestheim & Jarman (2008). It overlaps the amplification primer *12SV5R* by six nucleotides (Shehzad et al., 2012b).

All DNA amplifications were carried out in a final volume of 25 µl, using 2 µl of DNA extract as a template. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase, 10 mM Tris–HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 0.1 µM of each universal primer for vertebrates (*12SV5F/12SV5R*), with 2 µM of *PantB* and 5 µg of bovine serum albumin. The PCR mixture was denatured at 95°C for 10 minutes followed by 45 cycles of 30 seconds at 95°C and 30 seconds at 60°C; as the target sequences are c. 100 bp long, the elongation step was removed to reduce the +A artifact (Brownstein et al., 1996; Magnuson et al., 1996), which may decrease the efficiency of the first step of the sequencing process (blunt-end ligation).

Because all samples were pooled in a single run for sequencing, each PCR was performed with primers bearing a sample-specific tag of seven nucleotides. This tag was used to sort out the sequence reads obtained according to the relevant sample (Valentini et al., 2009). All PCR products were purified using the MinElute PCR purification kit (QIAGEN GmbH, Hilden, Germany) and then titrated using capillary electrophoresis (QIAxcel, QIAGEN GmbH, Hilden, Germany) and multiplexed in equimolar concentration before sequencing.

The sequencing was carried out on the Illumina/Solexa Genome Analyzer IIx (Illumina Inc., San Diego, USA), using the Paired-End Cluster Generation Kit V4 and the Sequencing Kit V4 and following the manufacturer's instructions. A total of 108 nucleotides were sequenced on each extremity of the DNA fragments.

TABLE S1 Amplification primers, with their oligonucleotide sequences, used to study the prey profile of the common leopard *Panthera pardus* in Ayubia National Park, Pakistan (Fig. 1). The length of amplified fragments (excluding length of primers) with *Pant* and *12SV5* primer pairs was 79 bp and ≥101 bp, respectively.

Primer	Oligonucleotide sequence (5′–3′)	Reference
PantF	GTCATACGATTAACCCGG	Ficetola et al., 2010
PantR	TGCCATATTTTTATATTAACTGC	Ficetola et al., 2010
12SV5F	TTAGATACCCCACTATGC	Riaz et al., 2011
12SV5R	TAGAACAGGCTCCTCTAG	Riaz et al., 2011
PantB[*]	CTATGCTTAGCCCTAAACCTAGATAGTTAGCCCAAACAAAACTAT-C3	This study

[*] *PantB* is a blocking oligonucleotide.

TABLE S2 Overview of sequence counts at various stages of the analysis.

	Number of reads (% of properly assembled sequences[1])	Number of different sequences[2]
Number of properly assembled sequences	652,090	36,929
Filtering sequence length ≥ 60 bp & count≥ 100	283,757 (43.51)	150
Filtering for most of the PCR/sequencing errors	257,190 (39.44)	56
Perfectly assigned taxa	228,983 (35.11)	12

[1]Direct and reverse sequence reads corresponding to a single DNA molecule were aligned and merged, producing what we called a properly assembled sequence.

[2] Non-strictly identical sequences correspond to different sequences.