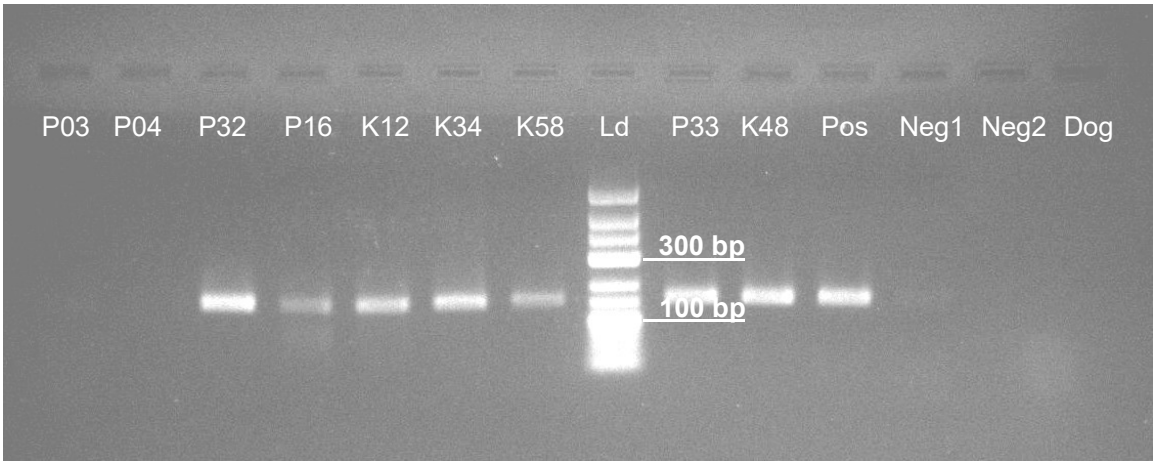
Figure A5. Illustrative example of predator identification with a 150 bp region of cytochrome-b mitochondrial DNA, which is known to be specific to snow leopards. Gel electrophoresis patterns after polymerase chain reaction (PCR) of five scat samples from Pin Valley (P03, P04, P32, P16, and P33), and four from Kibber (K12, K34, K58, and K48) are shown, along with PCR-control (Neg1) and extraction-control (Neg2), positive-control (Pos), extract from dog (Dog), and ladder (L). The bands in the ladder that correspond to 100 bp and 300 bp, respectively are labeled. Seven scats show band at ca 150 bp, and two scats do not show band.



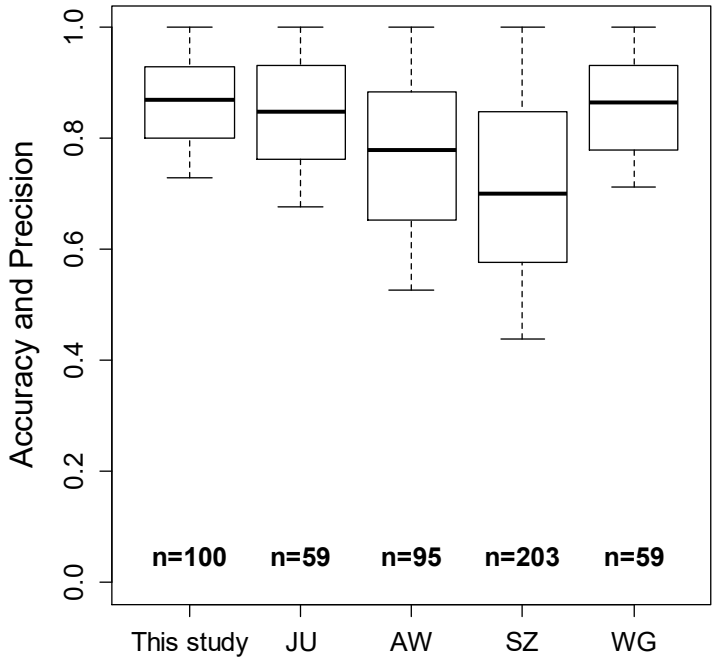
Appendix 5

Comparison of precision and accuracy of molecular identification with other studies

The DNA-based method of screening scats for predator identification is susceptible to both type-I and type-II error. Type-I error arises from a possibility of contamination of scats before they are collected by the researcher, and this contributes to false positives. A snow leopard scat can be deposited near/over a wolf scat, and subsequently both may test positive for snow leopard DNA. Even if one attempts overcome this by screening for multiple DNA matches in each scat, we still encounter problems with type-II error. Type-II errors can arise from fragmented DNA, chiefly from UV-photodegradation, and primers fail to amplify the target sequences during PCR-cycles. This contributes to false negatives, as significant UV-photodegradation of DNA can occur with 1-2 weeks (Woodruff et al. 2015). Admittedly, the likelihood of false negatives may be higher than false positives.

We explore these inherent shortcomings through randomized re-sampling iterations to evaluate accuracy and precision in our samples a posteriori, and compared them against other reports. Let p represent the number of scats testing positive for snow leopard DNA, and n represent the number of scats testing negative. Now, **accuracy** of identification is $a = \frac{p}{p+n}$. Considering false negatives (e.g. degraded scats), we examined accuracy for $72 \leq p \leq 100$ and $0 \leq n \leq 28$ in our samples. This accounts for potential false negatives, and assumes that false positives are absent. The variability among random re-sampling iterations would represent **precision**. For comparison we used the reported values of p and n in recent studies and assessed their accuracy and precision (Anwar et al. 2011, Shehzad et al. 2012, Wegge et al. 2012, Jumbay-Uulu et al. 2014). Results after 1000 random re-sampling iterations showed that accuracy and precision of our study compares favorably against other reports (Fig. A6).

Figure A6. Box-and-whisker plot (median and inter-quartile ranges) representing accuracy and precision of predator identification through DNA-screening of scats in our study, compared against other reports - (AW: Anwar et al. 2011; JU: Jumbay-Uulu et al. 2014; SZ: Shehzad et al. 2012; WG: Wegge et al. 2012). Number of scats screened in each case is also shown. Calculations are based iterative 1000 random re-samples to account for potential false negatives. The accuracy and precision of our samples compares favorably against the other reports.



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