Description of Supplemental Information appendixes for:

# Multiple species-specific molecular markers using nanofluidic array as a tool to detect prey DNA from carnivore scats

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**Appendix S1.** Reference sequences from NCBI, with accession number for the 18 target species and the list of literature citations for the published sequences.

**Appendix S2.** Target position, allele of the target species, and allele of the non-target species for 207 developed genetic markers for 18 target species.

Footnote for Appendix S2:
† The target position refers to the moose reference sequence AJ000026 (1140 bp). For the two forest bird species, Western capercaillie and black grouse, the position refers to the black grouse reference sequence EF571183 (1143 bp), followed by the corresponding homological position in the moose reference sequence AJ000026 within brackets.

**Appendix S3.** Allele, sequence with target position, STA sequence, LSP sequences (locus specific sequence), and ASP sequence (allele specific sequence) of 207 developed genetic markers for 18 target species. The sequences contain IUPAC ambiguity codes, indicating where there is variation within species. One column indicates if the assay is included in the final selection of 80 markers and another column indicates the four best markers per species (based on t test, frequency of overlap, and the distance between the minimum specific sample and the maximum non-specific sample).

Footnote for Appendix S3:
† The specific target amplification (STA) was excluded during the method optimization and not used in the final presented method.
‡ We used two sets of identical ASP primer sequences but with different fluorescence.

**Appendix S4.** Results from two-sample t-test for the 80 selected genetic markers. Assay name, t value, p value, p value adjusted with BY correction method for multiple testing (Benjamini & Yekutieli, 2001), average and standard deviation of non-specific and specific reference tissue samples, and the frequency of overlap, which is the proportion of non-specific reference tissue samples that overlap with the minimum of the specific reference tissue samples. For the four fallow deer markers we conducted instead a one-sample t-test since we had only one specific sample available, following the ROX outlier filtering.

Footnote for Appendix S4:
† When i) the adjusted p value was non-significant (n = 10), or ii) the test gave a positive t value(which means higher intensity of the non-specific reference tissues compared to the specific reference tissues therefore no separation) (n = 1), or iii) we could only run a one-sample t-test because only one specific reference tissue was available (n = 4), we additionally ran a two-sample t-test and estimated the frequency of overlap from a different run (results in Appendix S5).

**Appendix S5.** Results from additional two-sample t-test and estimate of frequency of overlap on a different run for 15 genetic markers.

**Appendix S6.** Location of wolf scats (n = 80) collected during winters (October - March) from 2009 to 2018 in Sweden.

**Appendix S7.** Flow chart of the threshold setting procedure to get a binary detection for prey species in each scat sample, for each scenario of minimum amplifying markers required to determine the presence of DNA from a target species