



Supplementary Materials: DNA Footprints: Using Parasites to Detect Elusive Animals, Proof of Principle in Hedgehogs

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A.vasorum A.abstrusus C.striatum C.vulpes	TGTTTGTCGAACGGC	AACAATGGTTGT AGTNTTAGTCGT AGTCGAAAACTCGG	30 240 CETCGTC-GTCGTCGT TATCGACTGACGATGT CTTCAAG-AAAGTCGT GCTTCACG-AAAGTC	TTGATGCGATCGA CGATGCGA <mark>T</mark>	TGATTCCCGT	FTCAGTGAGGA FCTAGTTGAGA	CTGAG <mark>ATGATT</mark>	GCAACGTGTAA	300 CAA
	310		30 340	350	360	370	380	390 • • • • • • • •	400
A.vasorum	TAATTATATATATAT	ATTATTACACAAAA	CGTAATATGTGATC	ATGTGGTTGTAT	GCTAGTAATG	TCATTGCTAC	CGTCATCGAT	TTGCTGATTT	CAA
A.abstrusus	CAACGATATTGGTAC	TATGTTACATTGAG	CGTAATGTGTATGT	ATGTGCATTTAT	GCTAGTGATA	TCATTACTAT	CATCGTTGAT	STTGGTGGTTT!	CAA
C.striatum	AACATATATTAT	CGTTTTGCATCGAA	TGTGATGTGTATTC	GTGTGAATGTGT	GTATTTGTTG	TGAACG-TAT	GCTAGATAGT	TAAATCAT	TGC
C.vulpes	AACACATTAGT	GTTTTACACTGAAT	GTGATGTGTATTCG1	FGTGAATGTGTG	TATTTGT	GTACG-TATG	CTAGATAGTGT	AGATCATCAT	'GC
	410	420 4	30 440	450	460	470	480	490	500
A.vasorum	TGAGTGTCGTTGAGA	ATCGTGAAA	TAAGAAGTAATATT	ATCGATCGA	TGGATGAATG	GATGAAA-TG	 TATTTCATATO	GTAATGACAA	AAA
A. abstrusus	TGGGTATCGTTGAGA		TGGAGAACATCGTT					ATGATGANGA	
C.striatum	TGA-TGTCGTCGAGG	TGGTTTTCAAGCGT	TGTGATTGGAGATC	ATATATAAA	TATTCGACGA	TTGACAA-TA	TGTGATGATCA	GCGATGATA-	GACA
C.vulpes	TAC-TATCGTCGAGG	GGTTTTCAAACGT	IGTGATTGAAGATCA	TATAT <mark>G ATGAI</mark>	ATT GA GAT	TGATAAA AA	TTGATGATCAG	CAA <mark>T</mark> GAGAAG	ACA

Figure S1. Sequence alignment of ITS-2 sequences showing positions of discriminatory primers resulting in specific PCR products differing in length by 50bp.

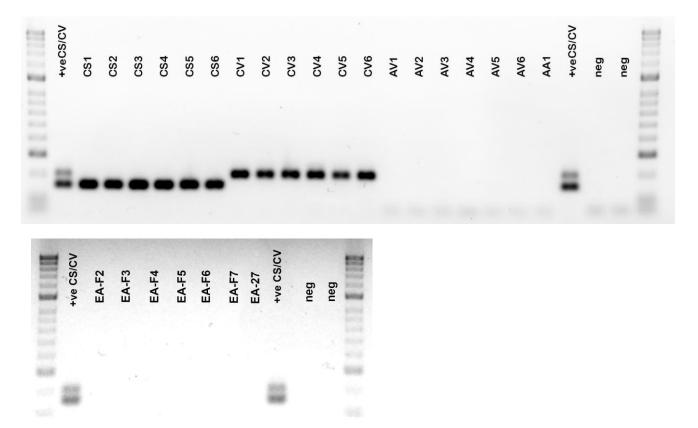


Figure S2. PCR of extracted nematode DNA with CS/CV multiplexed primer set illustrating specific amplification.

PCRs produced expected bands: 157 bp for *C. striatum* (CS), 207 bp for *C. vulpis* (*CV*) and no product amplified for other nematode species tested. **Gel 1** shows PCR of DNA extracts from *Crenosoma striatum* (CS) (hedgehog) *Crenosoma vulpis* (CV) (fox), *Angiostrongylus vasorum* (AV) (fox), and *Aelurostrongylus abstrusus* (AA) (cat) (kindly donated by S. Morelli). **Gel 2** shows PCR of DNA extracts from *Eucoleus aerophilus* (*Capillaria aerophila*) (EA) (fox). PCRs included a positive control mixture of DNA from *Crenosoma striatum* and *Crenosoma vulpis* and negative H₂0 controls. All DNA was isolated from morphologically identified adult lungworms and validated for PCR with universal primers before testing.