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*Wildlife Research*

### Supplementary Material

#### **Environmental DNA detection of spot-tailed quoll from soil is unlikely to be useful for routine monitoring**

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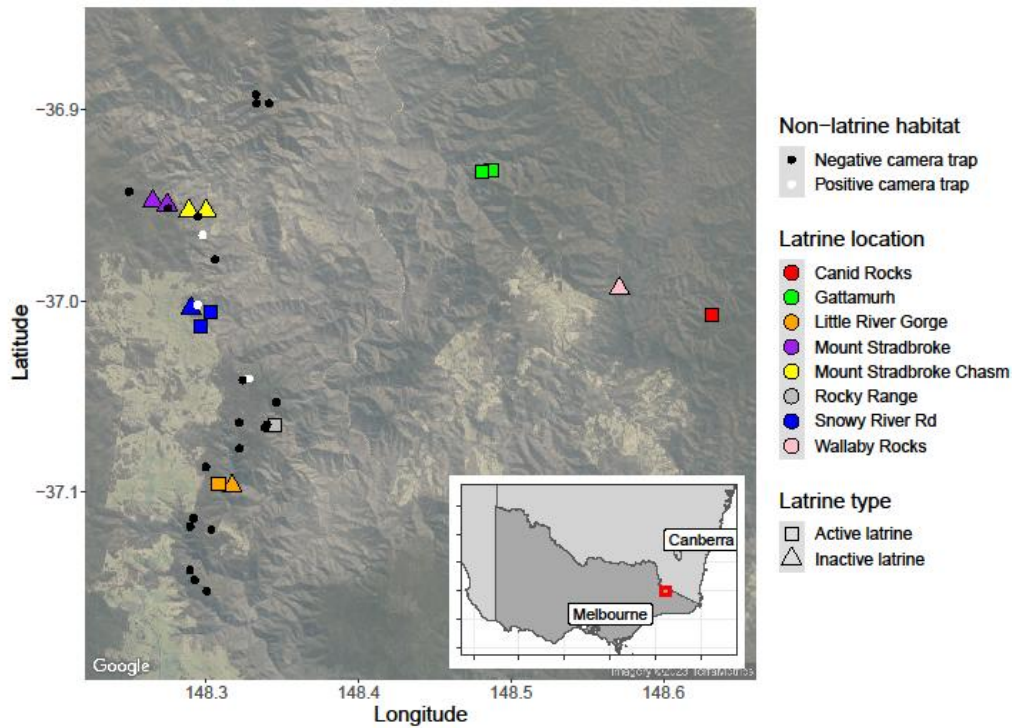
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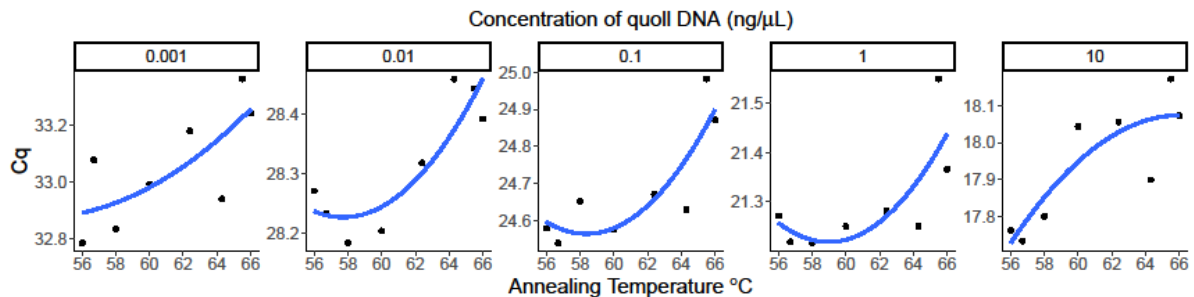
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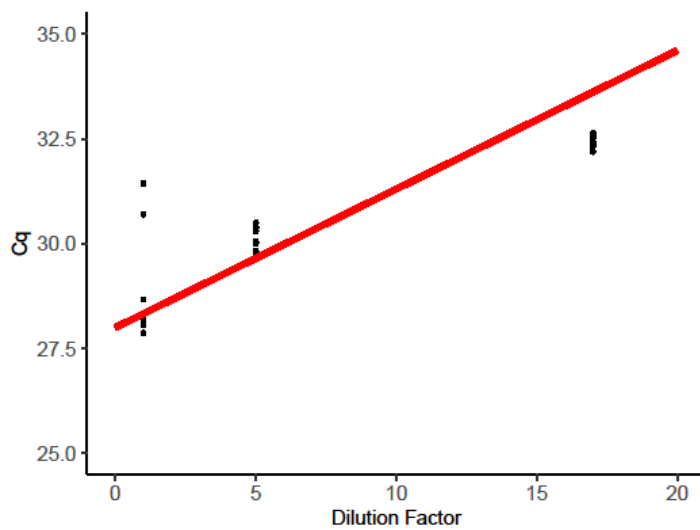
Supplementary Materials



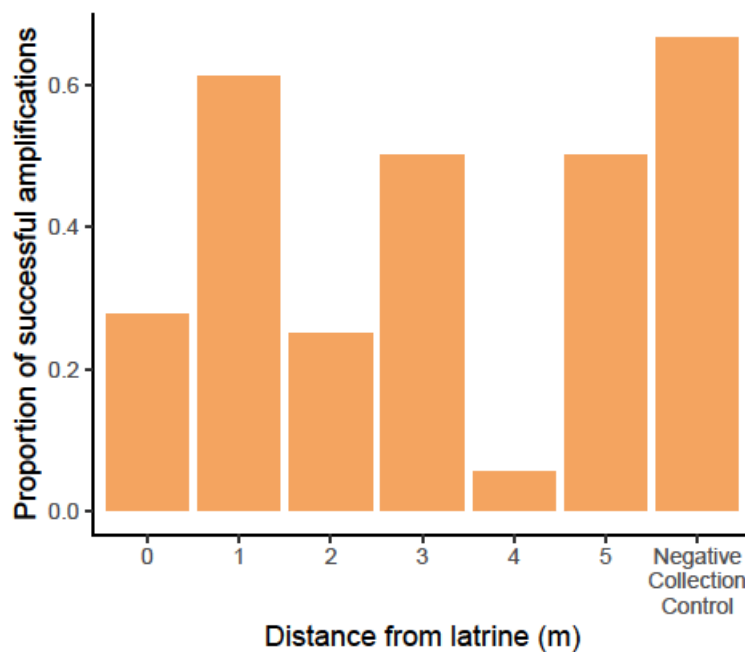
**Supplementary Figure S1.** Soil sampling sites in the Snowy River region, Victoria, Australia. The red square in the map inset shows the sampling location in Victoria, Australia. Colours of points represent broad localities in which one or more latrine site are found.



**Supplementary Figure S2.** Evaluation of annealing temperature from a dilution of STQ tissue sample extract. Each point represents a qPCR replicate. Blue lines represent a quadratic linear regression computed using the R function *lm*.



**Supplementary Figure S3.** Spike in dilution test results. Each point represents a PCR replicate. The red line represents the expected relationship between dilution factor and Cq value when samples are un-inhibited.



**Supplementary Figure S4.** Proportion of positive amplifications from captive quoll enclosure soil sample extracts, taken at increasing distance from the quoll's latrine. All qPCR and extraction no-template control replicates failed to amplify. It is unclear whether the cross-contamination that led to positive amplification across 60% of the collection control qPCR replicates occurred during collection, transport or laboratory processing.

**Supplementary Table S1.** Amplification results for each sampling site and distance class.

Site Type	Site Subtype	Locality	Latrine	Sample Type	Distance From Latrine	N of Samples	N Extracts	N qPCR replicates	Successful Amplification	Proportion Success
Latrine	Active Latrine	Canid Rocks	CR	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Canid Rocks	CR	Soil	0	5	15	30	1	1.0
Latrine	Active Latrine	Canid Rocks	CR	Soil	1	5	15	30	25	0.2
Latrine	Active Latrine	Gattamurh	GA	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Gattamurh	GA	Soil	0	2	6	12	7	0.4
Latrine	Active Latrine	Gattamurh	GA	Soil	1	2	6	12	12	0.0
Latrine	Active Latrine	Gattamurh	GAL02	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Gattamurh	GAL02	Soil	0	5	15	30	22	0.3
Latrine	Active Latrine	Gattamurh	GAL02	Soil	1	5	15	30	30	0.0
Latrine	Active Latrine	Little River Gorge	LDL01	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Little River Gorge	LDL01	Soil	0	5	15	30	30	0.0
Latrine	Active Latrine	Little River Gorge	LDL01	Soil	1	5	15	30	30	0.0
Latrine	Active Latrine	Snowy River Rd	MHS2	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Snowy River Rd	MHS2	Soil	0	5	15	30	10	0.7
Latrine	Active Latrine	Snowy River Rd	MHS2	Soil	1	5	15	30	21	0.3
Latrine	Active Latrine	Rocky Range	RRL02	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Rocky Range	RRL02	Soil	0	5	15	30	0	1.0
Latrine	Active Latrine	Rocky Range	RRL02	Soil	1	5	15	30	29	0.0
Latrine	Active Latrine	Snowy River Rd	SRL02	Collection blank	NA	1	3	6	6	0.0
Latrine	Active Latrine	Snowy River Rd	SRL02	Soil	0	5	15	30	13	0.6
Latrine	Active Latrine	Snowy River Rd	SRL02	Soil	1	5	15	30	28	0.1
Latrine	Inactive Latrine	Little River Gorge	LDL02	Collection blank	NA	1	3	6	3	0.5
Latrine	Inactive Latrine	Little River Gorge	LDL02	Soil	0	5	15	30	29	0.0
Latrine	Inactive Latrine	Little River Gorge	LDL02	Soil	1	5	15	30	29	0.0
Latrine	Inactive Latrine	Mount Stradbroke Chasm	MSC	Collection blank	NA	1	3	6	6	0.0
Latrine	Inactive Latrine	Mount Stradbroke Chasm	MSC	Soil	0	5	15	30	13	0.6
Latrine	Inactive Latrine	Mount Stradbroke Chasm	MSC	Soil	1	5	15	30	21	0.3

Latrine	Inactive Latrine	Mount Stradbroke Chasm	MSC_21	Collection blank	NA	1	3	6	6	0.0
Latrine	Inactive Latrine	Mount Stradbroke Chasm	MSC_21	Soil	0	1	3	6	6	0.0
Latrine	Inactive Latrine	Mount Stradbroke Chasm	MSC_21	Soil	1	1	3	6	0	1.0
Latrine	Inactive Latrine	Mount Stradbroke	MSL01	Collection blank	NA	1	3	6	6	0.0
Latrine	Inactive Latrine	Mount Stradbroke	MSL01	Soil	0	5	15	30	30	0.0
Latrine	Inactive Latrine	Mount Stradbroke	MSL01	Soil	1	5	15	30	30	0.0
Latrine	Inactive Latrine	Mount Stradbroke	MSL02	Collection blank	NA	1	3	6	6	0.0
Latrine	Inactive Latrine	Mount Stradbroke	MSL02	Soil	0	5	15	30	26	0.1
Latrine	Inactive Latrine	Mount Stradbroke	MSL02	Soil	1	5	15	30	30	0.0
Latrine	Inactive Latrine	Snowy River Rd	SRL01	Collection blank	NA	1	3	6	6	0.0
Latrine	Inactive Latrine	Snowy River Rd	SRL01	Soil	0	5	15	30	30	0.0
Latrine	Inactive Latrine	Snowy River Rd	SRL01	Soil	1	5	15	30	30	0.0
Latrine	Inactive Latrine	Wallaby Rocks	WR	Collection blank	NA	1	3	6	6	0.0
Latrine	Inactive Latrine	Wallaby Rocks	WR	Soil	0	1	3	6	5	0.2
Latrine	Inactive Latrine	Wallaby Rocks	WR	Soil	1	1	3	6	0	1.0
Non-latrine habitat	Negative camera trap		RRS1	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		RRS1	Soil	NA	3	9	18	18	0.0
Non-latrine habitat	Negative camera trap		RRS4	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		RRS4	Soil	NA	3	9	18	18	0.0
Non-latrine habitat	Negative camera trap		STQ134	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		STQ134	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap		STQ210	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		STQ210	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap		STQ214	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		STQ214	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap		STQ223	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		STQ223	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap		STQ224	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		STQ224	Soil	NA	4	12	24	23	0.0
Non-latrine habitat	Negative camera trap		STQ230	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap		STQ230	Soil	NA	4	12	24	24	0.0

Non-latrine habitat	Negative camera trap	STQ236	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ236	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ237	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ237	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ239	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ239	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ259	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ259	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ261	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ261	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ263	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ263	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ266	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ266	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ280	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ280	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ289	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ289	Soil	NA	4	12	24	22	0.1
Non-latrine habitat	Negative camera trap	STQ297	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ297	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ299	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ299	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Negative camera trap	STQ300	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Negative camera trap	STQ300	Soil	NA	4	12	24	24	0.0
Non-latrine habitat	Positive camera trap	STQ120	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Positive camera trap	STQ120	Soil	NA	5	15	30	28	0.1
Non-latrine habitat	Positive camera trap	STQ272	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Positive camera trap	STQ272	Soil	NA	5	15	30	29	0.0
Non-latrine habitat	Positive camera trap	STQ191	Collection blank	NA	1	3	6	6	0.0
Non-latrine habitat	Positive camera trap	STQ191	Soil	NA	5	15	30	30	0.0

**Supplementary Materials Table S2.** Estimated concentration of STQ DNA in soil at each latrine site.

Site Type	Locality	Latrine	Distance from latrine	Estimated Concentration (molecules/mg)		
				Mean	lower 95% CI	upper 95% CI
Active Latrine	Canid Rocks	CR	0	81.48	8.48	794.34
Active Latrine	Canid Rocks	CR	1	1.44	0.03	60.32
Active Latrine	Gattamurh	GA	0	1.55	0.04	47.21
Active Latrine	Gattamurh	GA	1	0.03	0.00	3.59
Active Latrine	Gattamurh	GAL02	0	0.41	0.03	5.05
Active Latrine	Gattamurh	GAL02	1	0.01	0.00	0.38
Active Latrine	Little River Gorge	LDL01	0	0.00	0.00	0.05
Active Latrine	Little River Gorge	LDL01	1	0.00	0.00	0.00
Active Latrine	Snowy River Rd	MHS2	0	48.65	5.56	483.24
Active Latrine	Snowy River Rd	MHS2	1	0.86	0.02	36.70
Active Latrine	Rocky Range	RRL02	0	37.68	3.74	347.64
Active Latrine	Rocky Range	RRL02	1	0.67	0.01	26.40
Active Latrine	Snowy River Rd	SRL02	0	6.55	0.74	56.96
Active Latrine	Snowy River Rd	SRL02	1	0.12	0.00	4.33
Inactive Latrine	Mount Stradbroke Chasm	MSC	0	24.12	2.77	226.15
Inactive Latrine	Mount Stradbroke Chasm	MSC	1	0.43	0.01	17.17
Inactive Latrine	Mount Stradbroke Chasm	MSC_21	0	27.90	0.37	2130.81
Inactive Latrine	Mount Stradbroke Chasm	MSC_21	1	0.49	0.00	161.82
Inactive Latrine	Mount Stradbroke	MSL01	0	0.00	0.00	0.05
Inactive Latrine	Mount Stradbroke	MSL01	1	0.00	0.00	0.00
Inactive Latrine	Mount Stradbroke	MSL02	0	0.06	0.00	1.18
Inactive Latrine	Mount Stradbroke	MSL02	1	0.00	0.00	0.09
Inactive Latrine	Snowy River Rd	SRL01	0	0.00	0.00	0.05
Inactive Latrine	Snowy River Rd	SRL01	1	0.00	0.00	0.00
Inactive Latrine	Wallaby Rocks	WR	0	75.69	1.27	5164.44
Inactive Latrine	Wallaby Rocks	WR	1	1.34	0.00	392.20