

Supplementary Information

Tables S1 and S2

**APPLYING MALDI-TOF MS TO RESOLVE MORPHOLOGIC AND GENETIC SIMILARITIES BETWEEN TWO
DERMACENTOR TICK SPECIES OF PUBLIC HEALTH IMPORTANCE**

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Table S1. Oligonucleotide used in the pathogen screening and tick gene sequencing analyzes.

TARGET ORGANISM	GENE TARGET	OLIGONUCLEOTIDE NAME	SEQUENCE 5'-3'	PCR TYPE	AMPLICON SIZE (bp)	PRIMER DESIGN REFERENCES
Tick	12S rDNA	T1B	AAACTAGGATTAGATACCT	PCR	335	55
		T2A	AATGAGAGCGACGGGCGATGT			
Tick	ITS2*	DITS2_F1	GTTTGGCAGAGAGTCGGAAG	PCR	365	72
		DITS2_R1	CTGGAGTGGCCCAGTCTTT			
Genus <i>Rickettsia</i>	23S rRNA (rrl)	PanR8_F	AGCTTGCTTTTGGATCATTGG	real-time PCR	111	68
		PanR8_R	TTCTTGCCTTTTCATACATCTAGT			
		PanR8_P	CCTGCTTCTATTTGTCTTGCAGTAACACGCCA			
<i>Rickettsia akari</i> , <i>R. amblyommatis</i> , <i>R. australis</i> , <i>R. canadensis</i> , <i>R. conorii</i> , <i>R. felis</i> , <i>R. honei</i> , <i>R. massiliae</i> , <i>R. montanensis</i> , <i>R. parkeri</i> , <i>R. rhipicephali</i> , <i>R. sibirica</i> , <i>R. slovaca</i> , <i>R. rickettsii</i> , <i>R. prowazekii</i> , and <i>R. typhi</i>	<i>ompA</i> *	RR190-70	ATGGCGAATATTTCTCCAAAA	Primary stage Nested PCR	632	63,64
		RR190-701	GTTCCGTTAATFGCAGCATCT			
		RR190-70	ATGGCGAATATTTCTCCAAAA	Semi-nested stage PCR	532	
		RR190-602	AGTGCAGATTGCTCCCCCT			
Genera <i>Anaplasma</i> , <i>Ehrlichia</i> , <i>Neoehrlichia</i> , <i>Neorickettsia</i> , and <i>Wolbachia</i>	16S	ECHSYBR-F	AACACATGCAAGTCGAACGG	real-time PCR	146 to 154	67
		ECHSYBR-R	CCCCCGCAGGGATTATACA			
<i>Rickettsia bellii</i>	<i>gltA</i> *	RBELLIF	ATCCTGATTTGCTGAATTTTT	real-time PCR	338	69
		RBELLIR	TGCAATACCAGTACTGACG			
		RBELLII_P3_CR610	ATGATGTTTGCCACACCTTGTGAAAA			
	rrs	Out2F	GATAGCGGAATTCCTAGTGTAGAGGTG	PCR	599	6,71
		317Pan	AAAGGAGGTAATCCAGC			
	<i>gltA</i> *	Abov_gltA2F	CGGAAATTACTTTTATAGATGG	PCR	826	6
		Abov_gltA2R	CATACCAYTGAGAAACCCAAC			
<i>Anaplasma bovis</i> -like	groEL	HS1-f	CGTCAGTGGGCTGGTAATGAA	Primary stage Nested PCR	1360	65, 70
		HS6-r	CCWCCWGGTCWACACCTTC			
		HS3-f	ATAGTYATGAAGGAGAGTGAT	Semi-nested stage PCR	1320	70
		HSV-r	TCAACAGCAGCTCTAGTWG			

*ITS2: internal transcribed spacer 2 region; *ompA*: outer membrane protein A; *gltA*: citrate synthase.

Table S2. Accession numbers of the nuclear ITS2 and mitochondrial 12S ribosomal sequences deposited in NCBI.

Target	Organism	Accession number	Sample	Geographical origin (USA state)
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757077	<i>Dermacentor variabilis</i> TN 114	TN
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757078	<i>Dermacentor variabilis</i> OH 731	OH
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757079	<i>Dermacentor variabilis</i> DE 2383	DE
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757080	<i>Dermacentor variabilis</i> GA 2606	GA
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757081	<i>Dermacentor variabilis</i> NJ 2292	NJ
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757082	<i>Dermacentor variabilis</i> IA 3267	IA
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757083	<i>Dermacentor variabilis</i> SC 940	SC
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757084	<i>Dermacentor variabilis</i> WI 1532	WI
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757085	<i>Dermacentor variabilis</i> MI 2368	MI
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757086	<i>Dermacentor variabilis</i> ND 1353	ND
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757087	<i>Dermacentor variabilis</i> DE 2399	DE
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757088	<i>Dermacentor variabilis</i> NE 3145	NE
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757089	<i>Dermacentor variabilis</i> GA 2601	GA
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757090	<i>Dermacentor variabilis</i> NY 1721	NY
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757091	<i>Dermacentor variabilis</i> NJ 2291	NJ
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757092	<i>Dermacentor variabilis</i> TN 115	TN
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757093	<i>Dermacentor variabilis</i> NY 1719	NY
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757094	<i>Dermacentor variabilis</i> CT 2063	CT
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757095	<i>Dermacentor variabilis</i> OH 729	OH
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757096	<i>Dermacentor variabilis</i> WI 1531	WI
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757097	<i>Dermacentor variabilis</i> IA 3280	IA
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757098	<i>Dermacentor variabilis</i> OH 741	OH
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757099	<i>Dermacentor variabilis</i> NE 3136	NE
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757100	<i>Dermacentor variabilis</i> GA 2604	GA
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757101	<i>Dermacentor variabilis</i> MI 2369	MI
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757102	<i>Dermacentor variabilis</i> OH 727	OH
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757103	<i>Dermacentor variabilis</i> OH 724	OH
internal transcribed spacer 2	<i>Dermacentor variabilis</i>	OR757104	<i>Dermacentor variabilis</i> SC 924	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840660	<i>Dermacentor variabilis</i> OH 727	OH
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840661	<i>Dermacentor variabilis</i> ND 1353	ND
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840662	<i>Dermacentor variabilis</i> WI 1530	WI
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840663	<i>Dermacentor variabilis</i> MI 2371	MI
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840664	<i>Dermacentor variabilis</i> GA 2606	GA
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840665	<i>Dermacentor variabilis</i> TN 114	TN
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840666	<i>Dermacentor variabilis</i> NY 1719	NY
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840667	<i>Dermacentor variabilis</i> NE 3145	NE
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840668	<i>Dermacentor variabilis</i> SC 928	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840669	<i>Dermacentor variabilis</i> SC 924	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840670	<i>Dermacentor variabilis</i> ND 1356	ND
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840671	<i>Dermacentor variabilis</i> OH 736	OH

Table S2. continuation.

Target	Organism	Accession number	Sample	Geographical origin (USA state)
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840672	<i>Dermacentor variabilis</i> WI 1529	WI
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840673	<i>Dermacentor variabilis</i> NY 1721	NY
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840674	<i>Dermacentor variabilis</i> NJ 2293	NJ
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840675	<i>Dermacentor variabilis</i> GA 2601	GA
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840676	<i>Dermacentor variabilis</i> SC 943	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840677	<i>Dermacentor variabilis</i> SC 938	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840678	<i>Dermacentor variabilis</i> SC 930	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840679	<i>Dermacentor variabilis</i> NY 1797	NY
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840680	<i>Dermacentor variabilis</i> NJ 2291	NJ
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840681	<i>Dermacentor variabilis</i> DE 2399	DE
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840682	<i>Dermacentor variabilis</i> SC 940	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840683	<i>Dermacentor variabilis</i> CT 2063	CT
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840684	<i>Dermacentor variabilis</i> CT 2065	CT
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840685	<i>Dermacentor variabilis</i> NY 1723	NY
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840686	<i>Dermacentor variabilis</i> CT 2130	CT
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840687	<i>Dermacentor variabilis</i> SC 927	SC
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840688	<i>Dermacentor variabilis</i> NE 3235	NE
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840689	<i>Dermacentor variabilis</i> WI 1531	WI
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840690	<i>Dermacentor variabilis</i> WI 1532	WI
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840691	<i>Dermacentor variabilis</i> GA 2600	GA
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840692	<i>Dermacentor variabilis</i> GA 2605	GA
small subunit ribosomal RNA gene	<i>Dermacentor variabilis</i>	OR840693	<i>Dermacentor variabilis</i> DE 2384	DE
internal transcribed spacer 2	<i>Dermacentor similis</i>	OR734979	<i>Dermacentor similis</i> CA 2666	CA
internal transcribed spacer 2	<i>Dermacentor similis</i>	OR734980	<i>Dermacentor similis</i> WA 3059	WA
internal transcribed spacer 2	<i>Dermacentor similis</i>	OR734981	<i>Dermacentor similis</i> CA 2680	CA
internal transcribed spacer 2	<i>Dermacentor similis</i>	OR734982	<i>Dermacentor similis</i> CA 2761	CA
internal transcribed spacer 2	<i>Dermacentor similis</i>	OR734983	<i>Dermacentor similis</i> CA 2738	CA
internal transcribed spacer 2	<i>Dermacentor similis</i>	OR734984	<i>Dermacentor similis</i> CA 2686	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085218	<i>Dermacentor similis</i> CA 2664	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085219	<i>Dermacentor similis</i> WA 3065	WA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085220	<i>Dermacentor similis</i> CA 2683	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085221	<i>Dermacentor similis</i> WA 3092	WA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085222	<i>Dermacentor similis</i> CA 2686	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085223	<i>Dermacentor similis</i> CA 2658	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085224	<i>Dermacentor similis</i> CA 2680	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085225	<i>Dermacentor similis</i> CA 2663	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085226	<i>Dermacentor similis</i> CA 2736	CA
small subunit ribosomal RNA gene	<i>Dermacentor similis</i>	PP085227	<i>Dermacentor similis</i> CA 2738	CA