Ixodes scapularis does not harbor a stable midgut microbiome

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Supplementary Figures

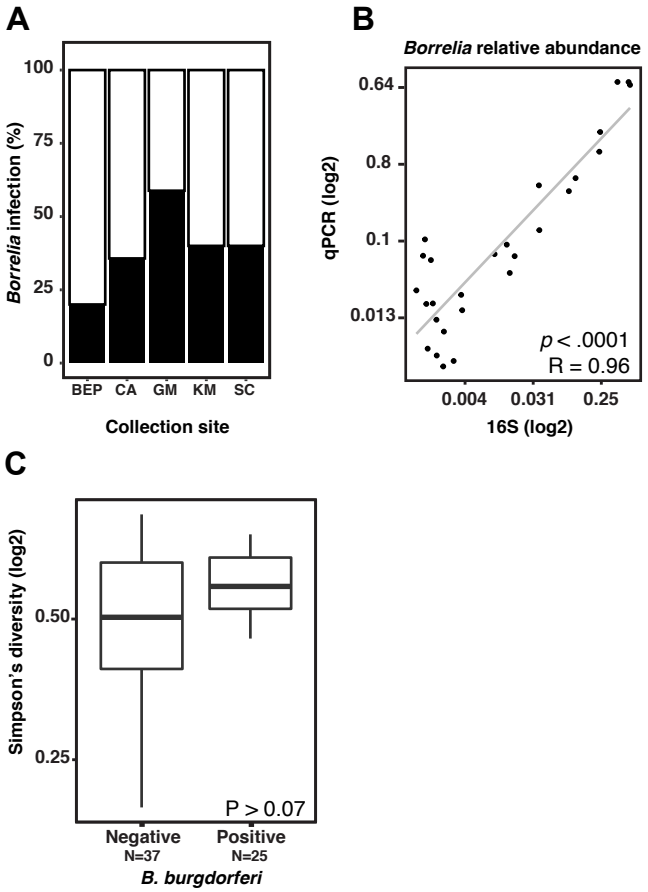

Figure S1. Characterization of *Borrelia burgdorferi* infection in wild *I. scapularis*.

(A) *Borrelia* infection frequency as determined by qPCR for *B. burgdorferi flaB* gene, plotted by collection site**. (B)** The relative abundance of *B. burgdorferi* as measured by qPCR with *flaB* primers calculated as a percent of total 16S rRNA gene counts exhibits a strong positive correlation between the relative abundance of *Borrelia* by 16S rRNA gene sequencing (Pearson $p < 0.0001$, $r = 0.96$). **(C)** Alpha diversity as quantified by the Simpson's diversity metric, plotted for *B. burgdorferi* infected and uninfected ticks. No significant difference is observed (t-test, $p > 0.07$).

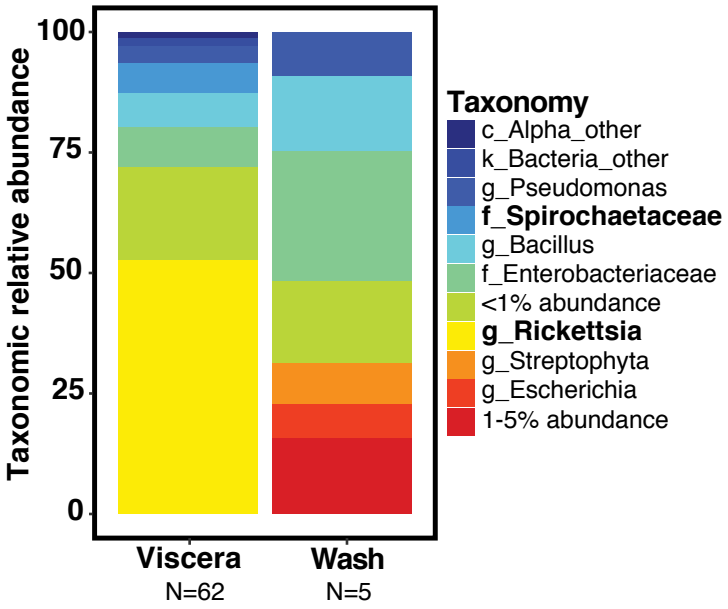

Figure S2. Taxonomic characterization of the *I. scapularis* microbiota.

Relative taxonomic abundance for bacteria detected by 16S rRNA gene sequencing averaged across all adult *I. scapularis* internal viscera samples and external washes. Taxa previously known to be associated with *I. scapularis* are bolded in legend.

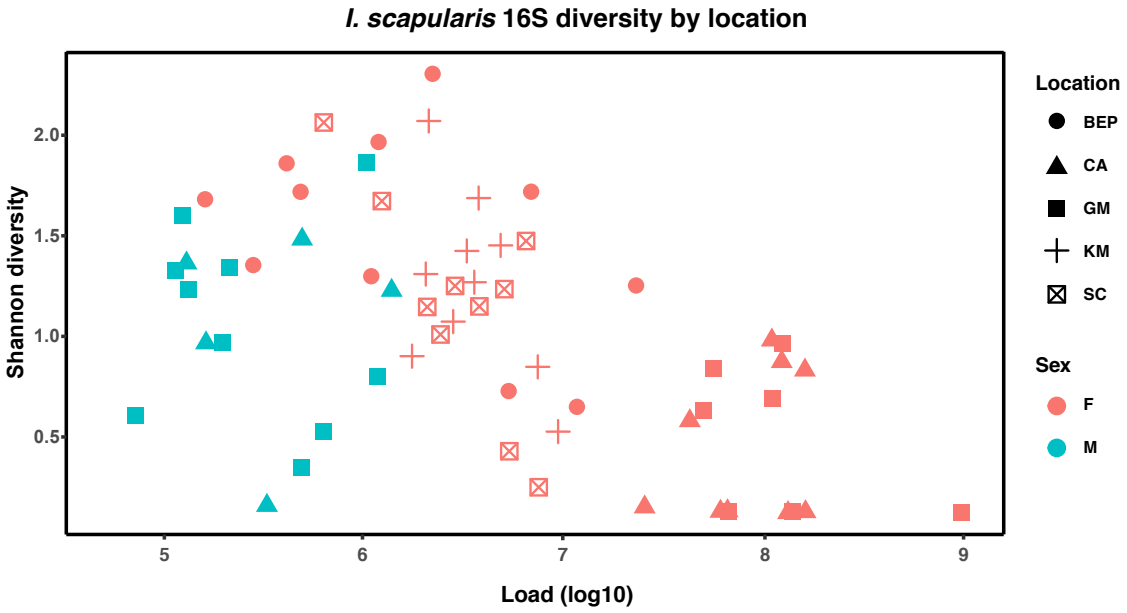

Figure S3. Bacterial load and diversity in adult female *I. scapularis* ticks across sample collection sites.

Shannon diversity (y-axis) and bacterial load as measured by qPCR (x-axis) of adult female *I. scapularis* ticks. Samples cluster by sex, but not by geographic collection site. Big Eau Pleine (BEP), Carlos Avery (CA), Gordie Mikkelson (GM), Kettle Moraine (KM), Sandberg Conservancy (SC).

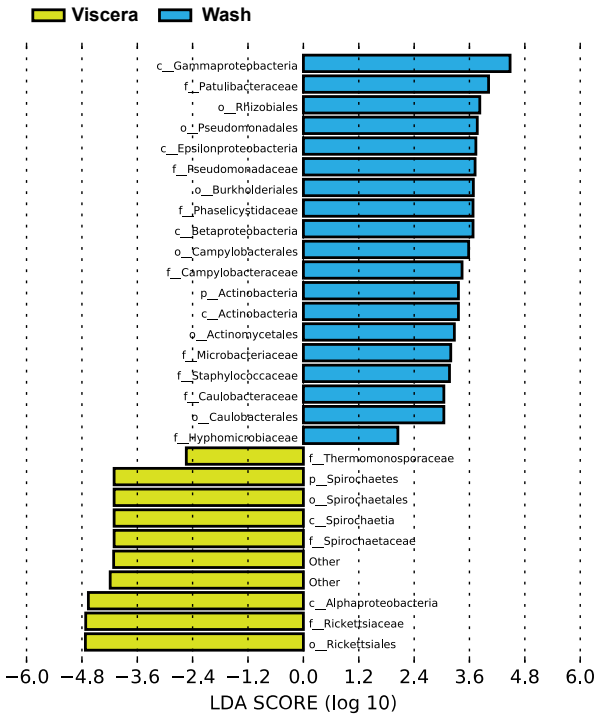

Figure S4. LEfSe plot of *I. scapularis* samples.

Taxa enriched in either the viscera (yellow) or the wash samples (blue) as measured by the LDA score are indicated.

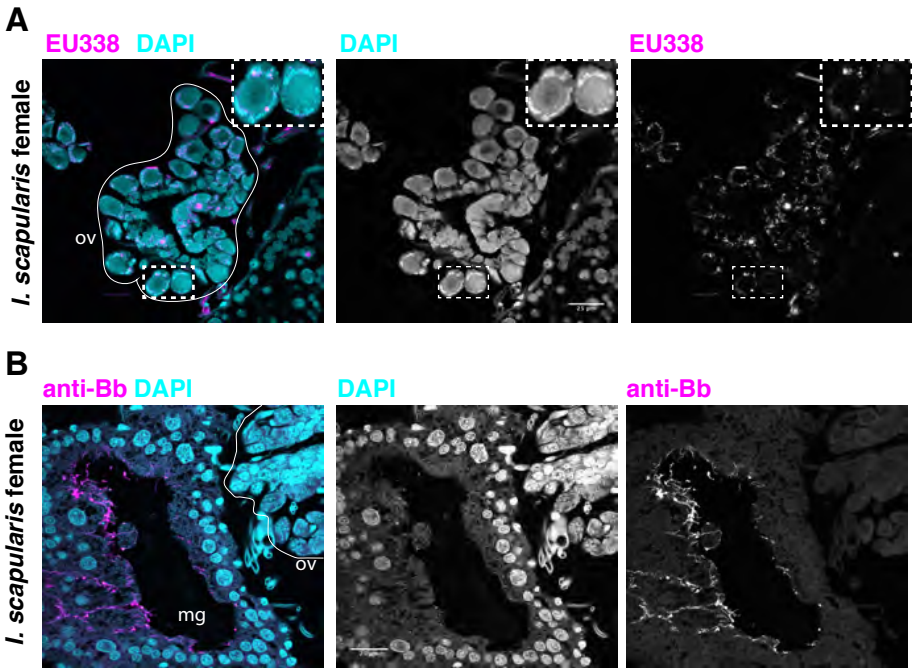

Figure S5. Detection of internal bacteria within *I. scapularis* by confocal microscopy. **A)** DAPI (blue) and EU338-TSA-ISH (magenta) staining of the central section of the ovary of a female adult *I. scapularis* tick. The ovary is outlined in white and region of inset zoom is indicated by a dashed white rectangle. **(B)** Anti-*B. burgdorferi* (magenta) and DAPI (blue) staining of a midgut cross-section and ovary of an infected *I. scapularis* adult female tick. The midgut lumen is indicated by "mg", and ovary is outlined in white. Scale bars are 25 microns.

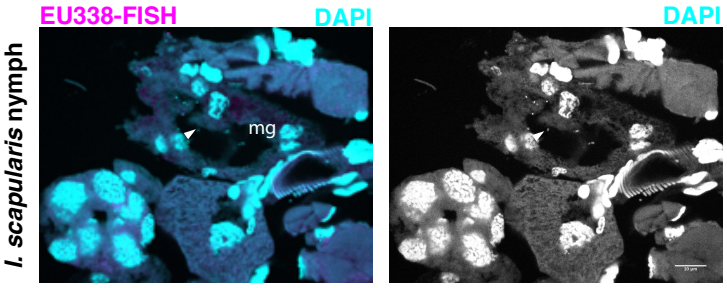

Figure S6. Confocal microscopy of *I. scapularis* nymph.

DAPI (blue) and EU338-TSA-ISH (magenta) staining of an *I. scapularis* nymph. Midgut is indicated by an arrowhead. Scale bar is 10 microns.

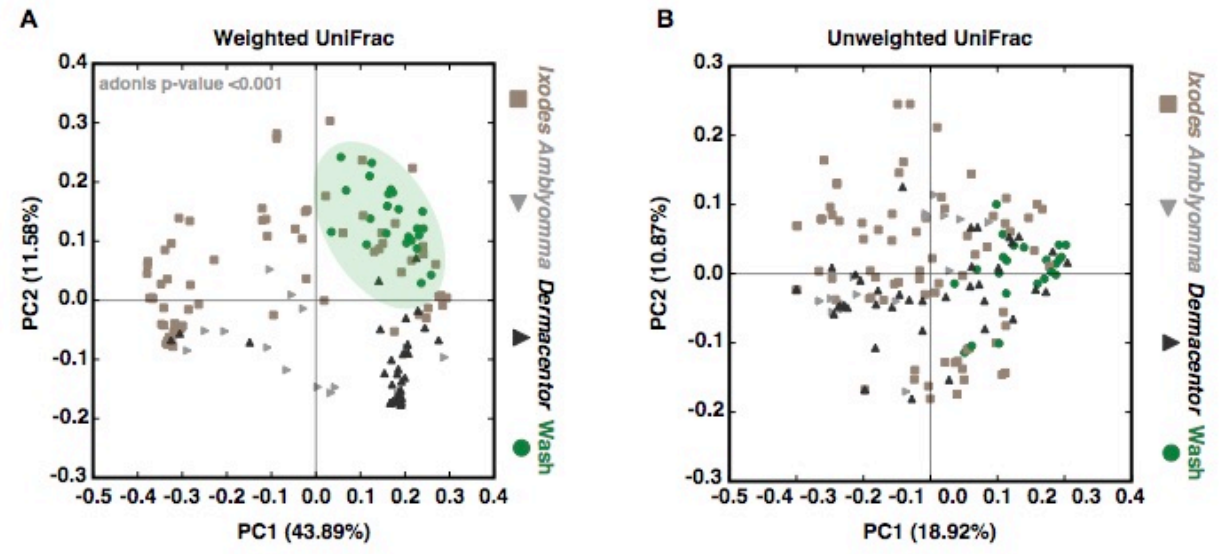

Figure S7. Beta diversity analysis of adult hard tick samples.

A) Beta diversity analysis using weighted UniFrac (which accounts for taxon relative abundance within samples) reveals clustering by external wash samples and by tick species. **B)** Beta diversity analysis using the unweighted UniFrac metric (in which the relative abundance of taxa within samples is not taken into account) shows a lack of species-specific clustering.

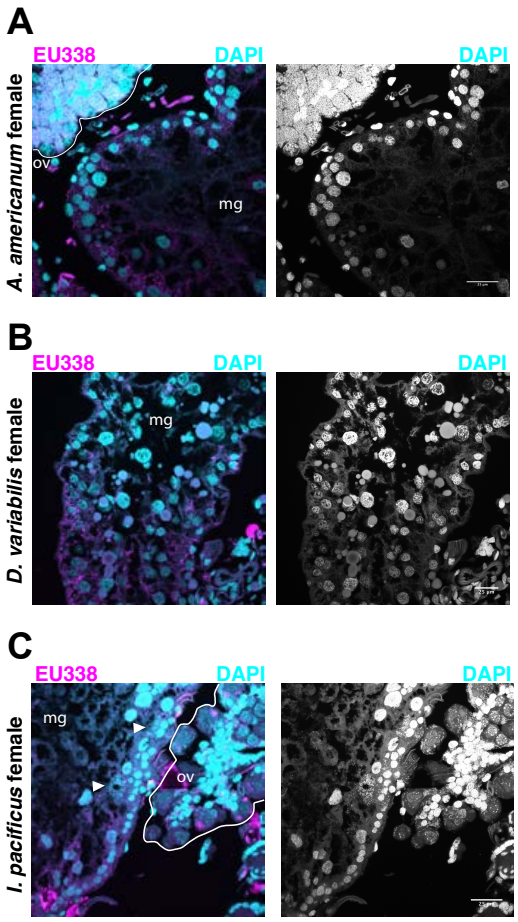

Figure S8. Confocal microscopy of non-*I. scapularis* hard ticks.

(A) *Amblyomma maculatum* adult female, **(B)** *Dermacentor variabilis* adult female, **(C)** and *Ixodes pacificus* adult female. All ticks stained with DAPI (blue) and EU338-TSA-ISH (magenta), with the midgut lumen indicated with "mg" and ovary with "ov". Arrowheads indicate bacterial micro-colonies within midgut. Ovaries are outlined in white in A and C. Scale bars are 25 microns.

Table S1. 84 Spirochaetes, Deferribacteres, tick endosymbiont, and pathogen genomes analyzed in this study.

Genome	Strain	Assembly
Alkalispirochaeta alkalica	DSM 8900	GCF_000373545.1
Anaplasma phagocytophilum	HZ2	GCF_000439755.1
Anaplasma phagocytophilum	HZ	GCF_000013125.1
Anaplasma phagocytophilum	JM	GCF_000439775.1
Anaplasma phagocytophilum	Norway_variant2	GCF_000689635.2
Borrelia afzelii	HLJ01	GCF_000304735.1
Borrelia afzelii	K78	GCF_000962775.1
Borrelia afzelii	PKo	GCF_000222835.1
Borrelia afzelii	Tom3107	GCF_000741005.1
Borrelia burgdorferi	B31	GCF_000008685.2
Borrelia burgdorferi	CA-11.2A	GCF_000172315.2
Borrelia duttonii	Ly	GCF_000019685.1
Borrelia garinii	BgVir	GCF_000239475.1
Borrelia garinii	NMJW1	GCF_000300045.1
Borrelia garinii	SZ	GCF_000691545.1
Borrelia hermsii	CC1	GCF_000956315.1
Borrelia hermsii	HS1	GCF_001660005.1
Borrelia miyamotoi	CT14D4	GCF_000807295.1
Borrelia miyamotoi	LB-2001	GCF_000445425.4
Borrelia persica	No12	GCF_000500045.1
Borrelia parkeri	HR1	GCF_000512145.1
Borrelia turicatae	91E135	GCF_000012085.1
Borrelia recurrentis	A1	GCF_000019705.1
Brachyspira hyodysenteriae	ATCC_27164	GCF_001676785.2
Brachyspira hyodysenteriae	WA1	GCF_000022105.1
Brachyspira intermedia	PWS_A	GCF_000223215.1
Brachyspira murdochii	DSM_12563	GCF_000092845.1
Brachyspira pilosicoli	95_1000	GCF_000143725.1
Brachyspira pilosicoli	B2904	GCF_000296575.1
Brachyspira pilosicoli	P43_6_78	GCF_000325665.1
Caldithrix abyssi	DSM_13497	GCF_000241815.1
Coxiella-like endosymb. of Amblyomma	C904	GCF_000815025.1
Coxiella burnettii	RSA 493	GCF_000007765.1
Ehrlichia chaffeensis	Arkansas	GCF_000013145.1
Ehrlichia chaffeensis	Heartland	GCF_000632815.1
Ehrlichia chaffeensis	Jax	GCF_000632865.1
Ehrlichia chaffeensis	Liberty	GCF_000632885.1
Ehrlichia chaffeensis	Osceola	GCF_000632905.1

Ehrlichia chaffeensis	Saint Vincent	GCF_000632925.1
Ehrlichia chaffeensis	Wakulla	GCF_000632945.1
Ehrlichia chaffeensis	West Paces	GCF_000632965.1
Geovibrio sp. L21	Ace-BES	GCF_000421105.1
Leptospira alstonii	GWTS #1	GCF_001729245.1
Leptospira biflexa	serovar Patoc 1 (Ames)	GCF_000017605.1
Leptospira biflexa	serovar Patoc 1 (Paris)	GCF_000017685.1
Leptospira borgpetersenii	serovar Ballum	GCF_001444465.1
Leptospira borgpetersenii	serovar Hardjo-bovis str. JB197	GCF_000013965.1
Leptospira borgpetersenii	serovar Hardjo-bovis str. L550	GCF_000013945.1
Leptospira interrogans	serovar Bratislava	GCF_001010765.1
Leptospira interrogans	serovar Copenhageni str. Fiocruz_L1-130	GCF_000007685.1
Leptospira interrogans	serovar Hardjo str. Norma	GCF_001293065.1
Leptospira interrogans	serovar Lai str. 56601	GCF_000092565.1
Leptospira interrogans	serovar Lai str. IPAV	GCF_000231175.1
Leptospira interrogans	serovar Linhai str. 56609	GCF_000941035.1
Leptospira interrogans	serovar Manilae	GCF_001047655.1
Leptospira interrogans	Grippotyphosa UI 12764	GCF_000244315.2
Leptospira kirschneri	H1	GCF_000243915.1
Leptospira santarosai	serovar Arenal str. MAVJ 401	GCF_000243835.1
Leptospira santarosai	serovar Shermani str. LT 821	GCF_000313175.2
Rickettsia endosymb. of I. scapularis		GCF_000160735.1
Rickettsia peacockii	Rustic	GCF_000021525.1
Rickettsia belli	RML369-C	GCF_000012385.1
Salinispira pacificus	L21-RPul-D2	GCF_000507245.1
Sediminispirochaeta bajacaliforniensis	DSM 16054	GCF_000378205.1
Sediminispirochaeta smaragdinae	DSM 11293	GCF_000143985.1
Sphaerochaeta globosa	Buddy	GCF_000190435.1
Sphaerochaeta coccoides	DSM 17374	GCF_000208385.1
Sphaerochaeta pleomorpha	Grapes	GCF_000236685.1
Spirochaeta lutea	JC230	GCF_000758165.1
Spirochaeta africana	DSM_8902	GCF_000242595.2
Spirochaeta aurantia	M1	IMG: 2579779150
Spirochaeta thermophila	DSM_6192	GCF_000147075.1
Spirochaeta thermophila	DSM_6578	GCF_000184345.1
Treponema brennaborense	DSM_12168	GCF_000212415.1
Treponema caldarium	DSM_7334	GCF_000219725.1
Treponema denticola	ATCC 35405	GCF_000008185.1
Treponema pallidum subsp. pallidum	Nichols	GCF_000008605.1
Treponema pallidum subsp. pallidum	Sea 81-4	GCF_000604125.1
Treponema pedis	T A4	GCF_000447675.1
Treponema phagedenis	4A	GCF_000513775.1

Treponema primitia	ZAS-2	GCF_000214375.1
Treponema saccharophilum	DSM 2985	GCF_000255555.1
Treponema succinifaciens	DSM 2489	GCF_000195275.1
Turneriella parva	DSM 21527	GCF_000266885.1

Table S2. Wild-collected hard ticks analyzed in this study.

Genus	Species	Life Stage	Sex	Collected	Sequenced	Analyzed by Microscopy
Amblyomma	*maculatum*	Adult	Female	20	10	10
Amblyomma	*maculatum*	Adult	Male	20	10	10
Amblyomma	*maculatum*	Nymph	N/A	17	N/A	10
Dermacentor	*andersoni*	Adult	Female	10	10	N/A
Dermacentor	*andersoni*	Adult	Male	10	10	N/A
Dermacentor	*variabilis*	Adult	Female	22	10	10
Dermacentor	*variabilis*	Adult	Male	19	10	9
Dermacentor	*variabilis*	Nymph	N/A	14	N/A	N/A
Ixodes	*pacificus*	Adult	Female	37	10	10
Ixodes	*pacificus*	Adult	Male	25	10	10
Ixodes	*scapularis*	Adult	Female	14	9	2
Ixodes	*scapularis*	Adult	Male	16	5	2
Ixodes	*scapularis*	Nymph	N/A	1	N/A	N/A
Ixodes	*scapularis*	Adult	Female	13	7	2
Ixodes	*scapularis*	Adult	Male	22	10	2
Ixodes	*scapularis*	Nymph	N/A	15	N/A	10
Ixodes	*scapularis*	Adult	Female	20	10	10
Ixodes	*scapularis*	Adult	Female	20	10	10
Ixodes	*scapularis*	Adult	Female	20	10	10
Ixodes	*scapularis*	Adult	Female	5	N/A	N/A
Ixodes	*scapularis*	Adult	Male	9	N/A	3

Table S2. Wild-collected hard ticks analyzed in this study.

Genus	Species	Site of Collection	Latitude/Longitude
Amblyomma	*maculatum*	USA: McPherson Preserve, Stillwater, OK	36.13 N 97.20 W
Amblyomma	*maculatum*	USA: McPherson Preserve, Stillwater, OK	36.13 N 97.20 W
Amblyomma	*maculatum*	USA: McPherson Preserve, Stillwater, OK	36.13 N 97.20 W
Dermacentor	*andersoni*	USA: Klickitat River Canyon, WA	45.78 N 121.20 W
Dermacentor	*andersoni*	USA: Klickitat River Canyon, WA	45.78 N 121.20 W
Dermacentor	*variabilis*	USA: Carlos Avery WMA, Columbus, MN	45.29 N 93.13 W
Dermacentor	*variabilis*	USA: Carlos Avery WMA, Columbus, MN	45.29 N 93.13 W
Dermacentor	*variabilis*	USA: Carlos Avery WMA, Columbus, MN	45.29 N 93.13 W
Ixodes	*pacificus*	USA: Klickitat River Canyon, WA	45.78 N 121.20 W
Ixodes	*pacificus*	USA: Klickitat River Canyon, WA	45.78 N 121.20 W
Ixodes	*scapularis*	USA: Carlos Avery WMA, Columbus, MN	45.29 N 93.13 W
Ixodes	*scapularis*	USA: Carlos Avery WMA, Columbus, MN	45.29 N 93.13 W
Ixodes	*scapularis*	USA: Carlos Avery WMA, Columbus, MN	45.29 N 93.13 W
Ixodes	*scapularis*	USA: Gordie Mikkelson WMA, East Bethel, MN	45.37 N 93.13 W
Ixodes	*scapularis*	USA: Gordie Mikkelson WMA, East Bethel, MN	45.37 N 93.13 W
Ixodes	*scapularis*	USA: Gordie Mikkelson WMA, East Bethel, MN	45.37 N 93.13 W
Ixodes	*scapularis*	USA: Sandburg Woods Conservancy, Madison, WI	43.13 N 89.31 W
Ixodes	*scapularis*	USA: Kettle Moraine State Forest Southern Unit, Kewaskum, WI	43.49 N 88.18 W
Ixodes	*scapularis*	USA: Big Eau Pleine County Park, Mosinee, WI	44.76 N 89.87 W
Ixodes	*scapularis*	USA: Wolf Creek State Park, Windsor, IL	39.48 N 88.69 W
Ixodes	*scapularis*	USA: Wolf Creek State Park, Windsor, IL	39.48 N 88.69 W