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Table S1. Characteristics of *I. ricinus* tick collection localities in north-eastern Poland.

Region	Province	Locality	Geographical coordinates	Habitats
West	Iława	Szałkowo	53°39'52.0"N 19°37'03.8"E	Ecotone (grassy path/forest)
		Sąpy	53°42'30.9"N 19°40'52.8"E	Ecotone (grassy path/forest)
		Wielowieś	53°46'39.5"N 19°44'58"E	Forest landscape
	Ostróda	Zawady Małe	53°41'39.2"N 20°05'25.1"E	Forest landscape
		Samborowo	53°40'22.3"N 19°47'47.7"E	Ecotone (grassy path/forest)
Central	Olsztyn	Kudypy	53°45'58.9"N 20°22'47.4"E	Ecotone (grassy path/forest)
		Zazdrość	53°41'45.6"N 20°31'21.3"E	Forest landscape
	Szczytno	Czarny Piec	53°32'35.6"N 20°37'38.8"E	Ecotone (grassy path/forest)
		Warchały	53°32'15.6"N 20°49'14.6"E	Forest landscape
		Leleszki	53°37′02.6"N 20°43′17.3"E	Ecotone (grassy path/forest
East	Mrągowo	Wierzbowo	53°48'24.0"N 21°19'25.6"E	Ecotone (grassy path/forest)
		Piecki	53°48'01.7"N 21°19'33.1"E	Forest landscape
		Sorkwity	53°50'19.0"N 21°06'22.6"E	Forest landscape
	Pisz	Krutyń	53°42'09.0"N 21°26'21.4"E	Forest landscape
		Wygryny	53°40'15.8"N 21°32'17.1"E	Ecotone (grassy path/forest)

Table S2. Statistical table of ANOVA (GLM) analysis of *I. ricinus* tick density in north-eastern Poland.

Population	General Linear Model (GLM)	Effects and effect interactions statistics
Total (females + males + nymphs)	**Year × Region × Habitat**	Main effect of Year: $F_{1, 89} = 3.6$, $p = 0.061$ Main effect of Region: $F_{2, 89} = 1.3$, $p = 0.281$ Main effect of Habitat: $F_{1, 89} = 0.5$, $p = 0.484$ Effect interaction of Year × Habitat: $F_{1, 89} = 0.0$, $p = 0.958$ Effect interaction of Year × Region: $F_{2, 89} = 3.5$, $p = 0.036$* Effect interaction of Habitat x Region : $F_{2, 89} = 0.1$, p=0.862 Effect interaction of Year × Region x Habitat: $F_{2, 89} = 0.4$, p=0.695
Nymphs	**Year × Region × Habitat**	Main effect of Year: $F_{1, 89} = 11.7$, $p = 0.001$* Main effect of Region: $F_{2, 89} = 5.7$, $p = 0.005$* Main effect of Habitat: $F_{1, 89} = 0.4$, $p = 0.529$ Effect interaction of Year × Habitat: $F_{1, 89} = 0.1$, $p = 0.762$ Effect interaction of Year × Region: $F_{2, 89} = 2.8$, $p = 0.068$

		Effect interaction of Habitat x Region : $F_{2, 89}$ = 0.3, p=0.752 Effect interaction of Year × Region x Habitat: $F_{2, 89}$ = 0.8, p=0.452
Females	**Year × Region × Habitat**	Main effect of Year: $F_{1, 89}$ = 2.0, p = 0.159 Main effect of Region: $F_{2, 89}$ = 3.8, p = 0.027* Main effect of Habitat: $F_{1, 89}$ = 0.0, p = 0.955 Effect interaction of Year × Habitat: $F_{1, 89}$ = 0.1, p = 0.707 Effect interaction of Year × Region: $F_{2, 89}$ = 0.4, p = 0.605 Effect interaction of Habitat x Region : $F_{2, 89}$ = 0.2, p=0.784 Effect interaction of Year × Region x Habitat: $F_{2, 89}$ = 0.0, p=0.981
Males	**Year × Region × Habitat**	Main effect of Year: $F_{1, 89}$ = 6.0, p = 0.016* Main effect of Region: $F_{2, 89}$ = 2.5, p = 0.092 Main effect of Habitat: $F_{1, 89}$ = 0.2, p = 0.647 Effect interaction of Year × Habitat: $F_{1, 89}$ = 0.1, p = 0.709 Effect interaction of Year × Region: $F_{2, 89}$ = 0.4, p = 0.656 Effect interaction of Habitat x Region : $F_{2, 89}$ = 2.8, p=0.066 Effect interaction of Year × Region x Habitat: $F_{2, 89}$ = 0.5, p=0.586

* statistically significant - $p<0.05$.

Table S3. Variants of *flaB* gene in spirochaetes species from *B. burgdorferi* s.l complex identified in questing *I. ricinus* in north-eastern Poland.

Species	**Variant**	**Isolate (n)**	**Accession number in GenBank**	**Similarity in GenBank**			
				Reference in GenBank	**Identity (%)**	**Host**	**Country**
B. bavariensis	BbavV1	1	MW963152	CP028872 (strain Pbi)	100	human	Germany
				MN958342		*I. ricinus*	Iran
B. burgdorferi	BbV1	5	MW963153	MF150052	100	*I. ricinus*	Poland
				CP001205 (strainZS7)		tick	Germany
				FJ231335		human	Czech Republic
B. valaisiana	BvV1	5	MW963154	MF150077	100	*I. ricinus*	Poland
				MT813126			Lithuania
				CP009117 (strain Tom4006)		*I. persulcatus*	Siberia
B. lusitaniae	BlV1	12	MW963155	MF150075	100	*I. ricinus*	central Poland

			KF422804 MW272741			western Poland Romania
	BlV2	5	MW963156	MF150075 KF422804 MW272741	99.8	*I. ricinus*	central Poland western Poland Romania
	BlV3	5	MW963157	KR782242 AB091809 KF894062	100	*I. ricinus* *Apodemus agrarius* (spleen)	Poland Turkey Poland
	BlV4	1	MW963158	MF150075 KF422804 MW272741	99.6	*I. ricinus*	central Poland western Poland Romania
B. garinii	BgV1	6	MW963159	MK604254 KF990320 JN828685	100	*I. ricinus*	Poland S-W Poland Czech Republic
	BgV2	5	MW963160	KF918606 MK604257 KF836510	100	*I. ricinus* fed on *Vulpes vulpes* *I. ricinus*	Poland S-W Poland
	BgV3	5	MW963161	KF422775 KF836512 HM345905	100	*I. ricinus* fed on *Nyctereutes procyonoides* *I. ricinus*	Poland S-W Poland western Poland
	BgV4	4	MW963162	MK604255 KF990320 JN828685	99.6	*I.ricinus*	Poland S-W Poland Czech Republic
	BgV5	3	MW963163	MK604261 KF894053 KF422816	100	*I. ricinus Apodemus flavicollis* (spleen) *I. ricinus* fed on *Dama dama*	Poland
	BgV6	3	MW963164	MK604253 AB091814	100	*I. ricinus*	Poland Turkey

			KJ577820		*I. ricinus* fed on *Myotis daubentonii*	Poland
	BgV7	3	MW963165	KF422783 KF894058 HM345902	100	I. ricinus *Apodemus flavicollis* (spleen) *I. ricinus* fed on *Turdus philomelos*	Poland
	BgV8	4	MW963166	KX646198 DQ650336 KF894055	100	*I. ricinus* *Apodemus agrarius* (spleen)	Poland
	BgV9	2	MW963167	MK604263 KF990322 MN958341	100	*I. ricinus*	Poland western Poland Iran
	BgV10	1	MW963168	MK604255 KF990320 JN828685	99.8	*I. ricinus*	Poland S-W Poland Czech Republic
	BgV11	1	MW963169	CP028861 (strain 200047) KR782222 KF894061	100	*I. ricinus* fed on bird *I. ricinus* *Apodemus agrarius* (spleen)	France Poland Poland
B. afzelii	BaV1	38	MW963171	CP018262 (strain BO23) CP009058 (strain K78) MK604271	100	human skin *I. ricinus*	Germany Austria Poland
	BaV2	2	MW963172	KR782215 KF422856 JN828691	100	*I. ricinus*	N-W Poland Poland Czech Republic