Ticks and Tick-borne Diseases 10 (2019) 101254

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Ixodes persulcatus/pavlovskyi natural hybrids in Siberia: Occurrence in sympatric areas and infection by a wide range of tick-transmitted agents

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ARTICLE INFO

Keywords:
Ixodes pavlovskyi
Ixodes persulcatus
Natural hybrids
Infectious agents
Genetic variability
Western Siberia

ABSTRACT

Ixodes persulcatus and *Ixodes pavlovskyi* ticks, two closely related species of the *I. ricinus* - *I. persulcatus* group, are widely distributed in the southern part of Western Siberia. Recently, the existence of natural hybrids of *I. persulcatus* and *I. pavlovskyi* ticks has been demonstrated. The aim of this study was to evaluate the abundance of *I. persulcatus/pavlovskyi* hybrids in several locations with different ratios of parental tick species and to investigate the prevalence and genetic variability of a wide range of infectious agents in these hybrids compared to the parental tick species. Natural hybrids of *I. persulcatus* and *I. pavlovskyi* ticks were identified in all examined locations in Altai and Novosibirsk, Western Siberia, Russia. The abundance of hybrids varied from 7% to 40% in different locations and was maximal in a location with similar proportions of *I. persulcatus* and *I. pavlovskyi* ticks. For the first time, it was shown that hybrids can be infected with the same agents as their parental tick species: tick-borne encephalitis and Kemerovo viruses, *Borrelia afzelii, Borrelia bavariensis, Borrelia garinii, Borrelia miyamotoi, Rickettsia helvetica, Rickettsia raoultii, Rickettsia sibirica,* "*Candidatus* Rickettsia tarasevichiae", *Anaplasma phagocytophilum, Ehrlichia muris,* "*Candidatus* Neoehrlichia mikurensis", and *Babesia microti.* The prevalence of most bacterial agents in hybrids was intermediate compared to their parental tick species. Most genetic variants of the identified agents have been previously found in the parental tick species. Wide distribution of *I. persulcatus/pavlovskyi* natural hybrids implies that *I. persulcatus*, *I. pavlovskyi* and their hybrids coexist in all *I. persulcatus* - *I. pavlovskyi* sympatric areas.

1. Introduction

The role of natural hybridization in the evolutionary history of many species is well known. This phenomenon has been recognized in organisms from different taxa, mainly in plants and more rarely in animals and invertebrates (Arnold, 2004; King et al., 2015). Hybridization leads to the introduction of new gene combinations into F_1 offspring; new genetic variation may be retained or broadened during hybrid backcrossing into parental species (Araya-Anchetta et al., 2013). Among ixodid ticks, heterospecific crossing has been demonstrated in laboratory experiments with different tick species: *Dermacentor andersoni* with *Dermacentor variabilis* (Oliver et al., 1972), *Dermacentor marginatus* with *Dermacentor reticulatus* (Zahler and Gothe, 1997), *Ixodes scapularis* with *Ixodes pacificus* (Oliver et al., 1993), and *Ixodes persulcatus* with *Ixodes ricinus* (Balashov et al., 1998; Bugmyrin et al., 2015); however, in some cases F_1 progeny were not fertile. Nevertheless, natural hybrids in tick populations have been found based on genetic and morphological features for *D. andersoni* with *D. variabilis* (Araya-Anchetta et al., 2013), *I. persulcatus* with *I. ricinus* (Bugmyrin et al., 2015; Kovalev et al., 2016), *Ixodes scapularis* with *Ixodes cookei* (Patterson et al., 2017), and *Hyalomma* spp. ticks (Rees et al., 2003).

Recently, possible existence of natural hybrids of *I. persulcatus* and *Ixodes pavlovskyi* ticks in Tomsk Province, Western Siberia has been demonstrated based on analysis of mitochondrial and genomic genetic loci (Kovalev et al., 2015). Both *I. persulcatus* and *I. pavlovskyi* ticks readily attack humans (Filippova, 1977; Romanenko and Leonovich, 2015; Ushakova et al., 1969). These tick species belong to the *I. ricinus* - *I. persulcatus* group and are closely related based on morphological and genetic similarity (Filippova, 1977; Livanova et al., 2015). In Russia, *I. persulcatus* ticks are widely distributed throughout the extensive wooded areas from the north-west to the Far East region of the country (Filippova, 1977). *Ixodes pavlovskyi* has a discontinuous distribution in

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https://doi.org/10.1016/j.ttbdis.2019.05.020
Received 9 November 2018; Received in revised form 6 May 2019; Accepted 28 May 2019

the Far Eastern and Western Siberian disjunctive regions (Bolotin et al., 1977; Filippova, 1977; Kolonin, 1981), and in both regions, *I. pavlovskyi* ticks coexist with *I. persulcatus* (Filippova, 1977; Livanova et al., 2015; Mal'kova et al., 2012; Romanenko and Leonovich, 2015). Previously, in Western Siberia, *I. pavlovskyi* ticks were found mainly in the Altai and Kuznetsk Alatau Mountains and the Salair Ridge, with a single observation in Novosibirsk Province (Belyantseva and Okulova, 1974; Kovalevskiy et al., 1975; Pomerantsev, 1948; Sapegina and Ravkin, 1969). However, in two last decades, *I. pavlovskyi* ticks have been repeatedly observed north of their usual habitat, and currently, the abundance of *I. pavlovskyi* is greater than that of *I. persulcatus* ticks in suburban areas of Novosibirsk and Tomsk (Livanova et al., 2011, 2015; Mal'kova et al., 2012; Romanenko and Leonovich, 2015). The reasons for this *I. pavlovskyi* expansion are not clear. Recently, it was demonstrated that *I. pavlovskyi* ticks can carry similar range of infectious agents as *I. persulcatus*, including tick-borne encephalitis and Kemerovo viruses (TBEV and KemV), as well as *Borrelia burgdorferi* sensu lato (s.l.), *Borrelia miyamotoi, Anaplasma phagocytophilum*, *Ehrlichia muris*, "*Candidatus* Neoehrlichia mikurensis", *Rickettsia* spp., and *Babesia microti* (Bakhvalova et al., 2016; Korenberg et al., 2010; Mukhacheva and Kovalev, 2014; Rar et al., 2017; Takano et al., 2014; Tkachev et al., 2014, 2017). However, the prevalence of some agents significantly differs between *I. persulcatus* and *I. pavlovskyi*: *I. pavlovskyi* ticks were significantly more often infected with *Borrelia garinii* and significantly less often with *Borrelia afzelii, Borrelia bavariensis,* "*Candidatus* R. tarasevichiae", and *E. muris* than *I. persulcatus* (Mukhacheva and Kovalev, 2014; Rar et al., 2017).

Recent discovery of natural hybrids of *I. persulcatus* and *I. pavlovskyi* (hereinafter referred to as *I. persulcatus/pavlovskyi* hybrids or hybrids) required further investigation of the occurrence of the hybrids in different habitats and the prevalence of tick-transmitted agents in these hybrids. To clarify these issues, the abundance of hybrids was investigated in several locations from the Republic of Altai and Novosibirsk Province, representing traditional and recently invaded habitats of *I. pavlovskyi* ticks, respectively. Notably, *I. pavlovskyi* predominated in sites from the Novosibirsk Province, while *I. persulcatus* was more abundant in the Republic of Altai. In addition, the prevalence and genetic diversity of a wide range of infectious agents in hybrids were compared with those found in the parental *I. persulcatus* and *I. pavlovskyi* species, which were simultaneously caught in the same locations and have been described previously (Rar et al., 2017).

2. Materials and methods

2.1. Field study

Adult questing *Ixodes* spp. ticks were collected by flagging in May–June of 2010–2015 in two locations in Western Siberia, Russia: in the northern part of the Altai Mountains (Altai), sites A1 and A2, and in parks and suburbs of the city of Novosibirsk, sites N1-N5 (Table 1, Fig. 1). The sampling sites were described in previous study (Rar et al., 2017). Briefly, sites A1 and A2 were located on mountain slopes near Artybash village in Turochaksky District (51° 47′ N, 87° 18′ E) and near the Altai Branch of the Central Siberian Botanic Garden in Shebalinsky District (51° 36′ N, 85° 48′ E), respectively. In Novosibirsk Province, site N1 was in the Central Siberian Botanic Garden (54° 48′ N, 83° 07′ E), site N2 in the floodplain of Zyryanka rivulet (54° 50′ N, 83° 05′ E), site N3 at the mouth of the Shadrikha River, (54° 46′ N, 83° 09′ E), site N4 in a ravine near Plotnikovo village (55° 00′ N, 83° 24′ E) and site N5 in the outskirts of Nizhnaya Yeltsovka (54° 53′ N, 83° 08′ E).

2.2. Tick species and hybrid identification

Tick species were identified using standard morphological criteria and genetic methods. Differentiation of *I. pavlovskyi* from *I. persulcatus* ticks was based on five morphological criteria: the form of the auriculae, conscutum color, scapular grooves profile, the form of basis capituli, and punctuations and form of scutum (Filippova, 1977).

Table 1
Distribution of *I. persulcatus* and *I. pavlovskyi* ticks and their hybrid variants in different sites.

Site	No. of genotyped ticks	No. (%) of ticks identified as			
		I. persulcatus[*]	*I. pavlovskyi*[*]	Hybrids[**]	Putative hybrids[#]
A1	53	33 (62.3)	11 (20.8)	6 (11.3)	3 (5.7)
A2	760	590 (77.6)	113 (14.9)	52 (6.8)	5 (0.7)
Total A1 and A2	**813**	**623 (76.6)**	**124 (15.3)**	**58 (7.1)**	**8 (1.0)**
N1	409	22 (5.4)	316 (77.3)	47 (11.5)	24 (5.9)
N2	27	0	22 (81.5)	5 (18.5)	0
N3	382	110 (28.8)	79 (20.7)	152 (39.8)	41 (10.7)
N4	63	14 (22.2)	22 (34.9)	24 (38.1)	3 (4.8)
N5	23	3 (13.0)	14 (60.9)	5 (21.7)	1 (4.3)
Total N1-N5	**904**	**149 (16.5)**	**453 (50. 1)**	**233 (25.8)**	**69 (7.6)**
All sites	**1717**	**772 (45.0)**	**577 (33.6)**	**291 (16.9)**	**77 (4.5)**

[*] - using both morphological and genetic characteristics.

[**] - ticks with hybrid genotype.

[#] - can be referred to different species based on morphology and genetic markers.

Extraction of total nucleic acids from ticks was performed as described previously (Rar et al., 2017). The species identity of each tick was defined based on two genetic loci: the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene and the nuclear internal transcribed spacer (ITS2), as previously described (Rar et al., 2017). For the *cox*1 gene, species-specific PCR was performed using common forward (Ixodes-F) and species-specific reverse (Ipers-R and Ipav-R) primers (Table S1). For the nuclear genome fragment ITS2, PCR fragments obtained for each tick with primers F-ITS2 and R1-ITS2 (Table S1) were sequenced. Ticks with ITS2 fragments, which were heterozygous at characteristic positions of both *I. persulcatus* and *I. pavlovskyi* ITS2 sequences (72, 73, 75–78 nucleotide positions of ITS2) were termed as ticks with hybrid ITS2 fragments (Fig. 2). Ticks with a hybrid nuclear locus and ticks with a mitochondrial locus belonging to one species and a nuclear ITS2 locus of the other species were designated ticks with hybrid genotype or hybrids.

2.3. Detection and genotyping of tick-transmitted agents

The detection and genotyping of examined pathogens were described in detail previously (Rar et al., 2017). To identify TBEV and KemV RNA and *Borrelia* spp., *Rickettsia* spp., Anaplasmataceae bacteria, and *Babesia* spp. DNA, nested PCR was carried out using primers indicated in Table S1. Amplified fragments corresponding to the E gene of TBEV, segment 1 of KemV, *p83/100* and *clpA* genes of *B. burgdorferi* (s.l.), *glpQ* gene of *B. miyamotoi*, *gltA* gene of *Rickettsia* spp., *groESL* operon of Anaplasmataceae, and 18S rRNA of *Babesia* spp. were sequenced.

2.4. Sequencing and phylogenetic analysis

The PCR products were purified using GFX Columns (Amersham Biosciences, USA). The Sanger sequencing reactions were conducted using "BigDye" Terminator v. 3.1 Cycle Sequencing kit" (Applied Biosystems Inc., Austin, TX, USA) in both directions with primers indicated in Table S1. The products were analyzed using an ABI 3500 Genetic Analyzer (Applied Biosystems Inc.). Obtained sequences were compared with those of reference strains available in the NCBI website using the BLASTN 2.2.31 + (https://www.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was performed using Maximum Likelihood (ML)

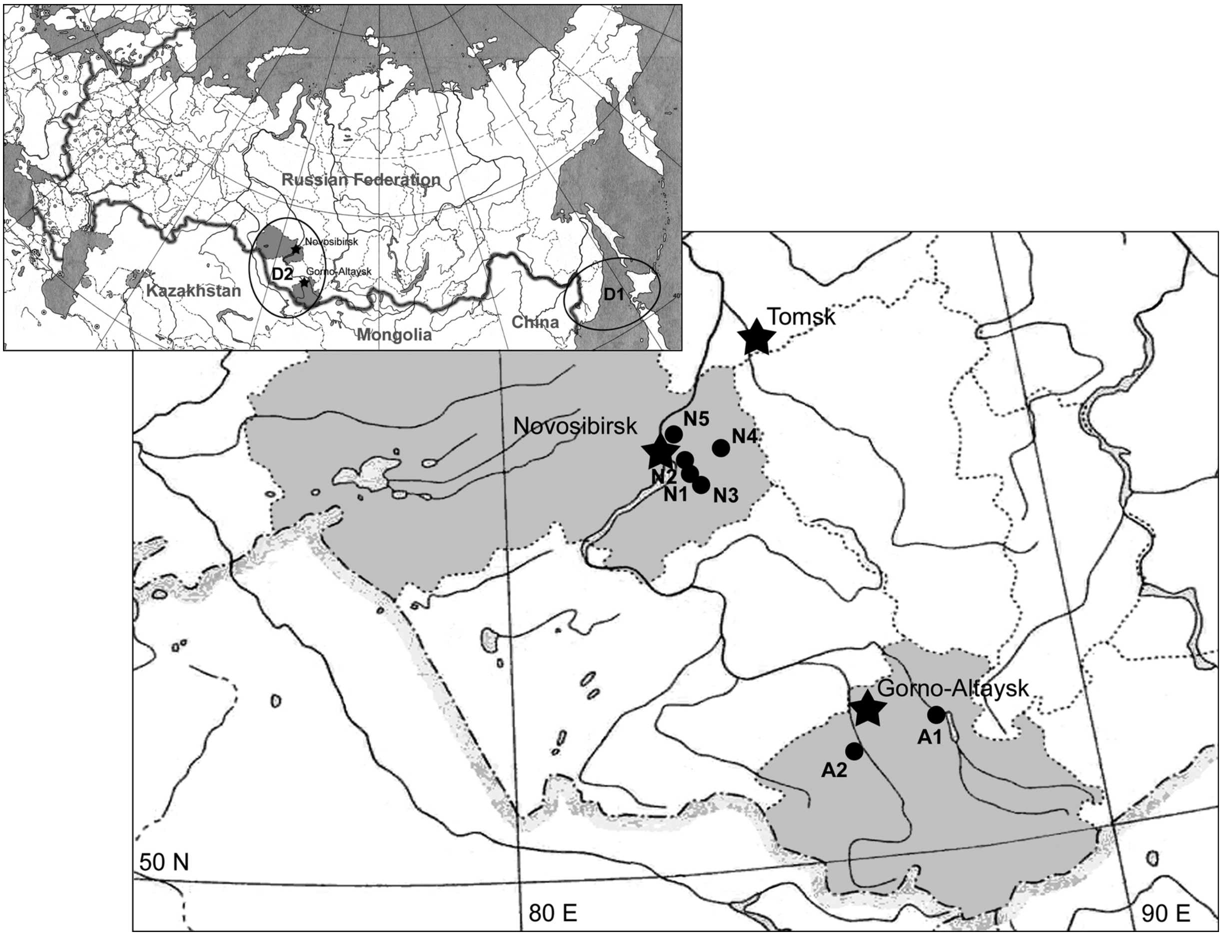

Fig. 1. Sites of tick collections in Western Siberia. *Legend*: D1 and D2, Far Eastern and Western Siberian disjunctive regions; A1-A2, sites located in the Republic of Altai; N1-N5, sites located in Novosibirsk Province.

method in MEGA 7.0 with 1000 bootstrap replicates (Kumar et al., 2016).

2.5. Statistical analysis

Statistical analysis was performed to compare the proportions of genotypes among the ticks collected in various locations and the prevalence of causative agents in ticks with different genotypes. The 95% confidence intervals (CI) for the prevalence of infectious agents in ticks were computed using an Excel spreadsheet (http://www.pedro.org.au/english/downloads/confidence-interval-calculator/). Differences in prevalence of infectious agents in ticks with different genotypes or ticks from different regions were computed with the Pearson $\chi 2$ goodness-of-fit test (http://www.socscistatistics.com/tests/chisquare/). $P < 0.05$ was regarded as significant.

2.6. Nucleotide sequence accession numbers

Nucleotide sequences determined in this study were deposited in GenBank under the following accession numbers: MG190874-MG190888, for TBEV; KX834333, KX834334 and KX834342, for KEMV; MG010818 (*clpA*) and MG010848-MG010851 (*p83/100*) for *B. afzelii*; MG010819-MG010828 (*clpA*) and MG010852-MG010866 (*p83/100*) for *B. bavariensis*; MG010829-MG010847 (*clpA*) and MG010867-MG010884 (*p83/100*) for *B. garinii*; MG010816-MG010817 for *B. miyamotoii*; MG022123-MG022125, for *R. helvetica*; MG022117-MG022122, for *R. raoultii*; MG022126 and MG022127, for *R. sibirica*; MG022128-MG022130, for "*Candidatus* R. tarasevichiae"; MG182152-MG182154; for *A. phagocytophilum*; MG182155 and MG182156, for *E. muris*; MG182157, for "*Candidatus* N. mikurensis"; MG182158 for *Bab. microti*.

3. Results

3.1. Tick genotype determination

A total of 2000 adult *Ixodes* spp. ticks were collected by flagging in seven sites of the Republic of Altai (n = 1035) and Novosibirsk Province (n = 965), Western Siberia (Fig. 1). After morphological determination, the species of 22 and 45 ticks from Altai and Novosibirsk regions, respectively, was not determined, because at least one morphological criterion was not congruent with other ones; these specimens were removed from the study. Among the remaining ticks (n = 1933), 200 specimens from Altai and 16 specimens from Novosibirsk were not successfully amplified using at least one genetic locus; these specimens also were excluded from further examination. Thus, only 813 ticks from Altai and 904 ticks from Novosibirsk were reliably characterized morphologically and genetically.

From the ticks collected in the Republic of Altai, 623 ticks were identified as *I. persulcatus* and 124 ticks, as *I. pavlovskyi* based on both morphological and genetic criteria; 58 ticks had hybrid genotypes and were designated as hybrids (Table 1). In addition, eight ticks can be referred to one species based on all the examined morphological criteria and to another species when genetic markers were used; these ticks were named "putative hybrids". In the Novosibirsk Province, 149 ticks were identified as *I. persulcatus*, 453 as *I. pavlovskyi*, 233 ticks had hybrid genotypes, and 69 ticks were designated as putative hybrids (Table 1). *I. persulcatus* and *I. pavlovskyi* ticks have been investigated

A

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                              6666666667 7777777778 8888888889
                              1234567890 1234567890 1234567890
I.pers (MG516557) EST         GCAGCACGCA TTTTTATGCT TTCTTGCGTT
I.pers (MG516558) EST         .......... .......... ..........
I.pers (MH784907) Omsk        .......... .......... ..........
I.pers (MH784904) Omsk        .......... .......... ..........
I.pers (MG516546) Sakh        .......... .......... ..........
I.pers (JF703109) Karel       .......... .......... ..........
I.pers (AB032837) China       .......... .......... ..........
I.pavl (D88860)   JPN         .......... .GC.ATAA.. ..........
I.pavl (D88859)   JPN         .......... .GC..TAA.. ..........
I.pavl (MG516563) Alt         .......... .GC.ATAA.. ..........
I.pavl (MG516564) Alt         .......... .AC.ATAA.. ..........
I.pavl (MG516565) Nov         .......... .GC.ATAA.. ..........
I.pavl (MG516566) Nov         .......... .AC.ATAA.. ..........
I.pavl (KP241978) Tomsk       .......... .GC.ATAA.. ..........
I.pavl (MG516568) KAZ         .......... .GC.ATAA.. ..........
```

B

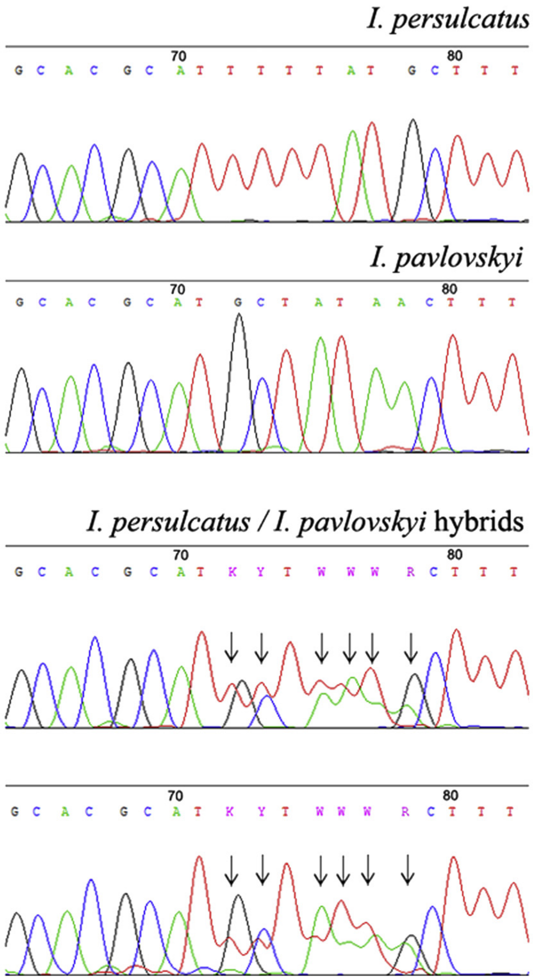

Fig. 2. ITS2 locus identification. (A) Aligment of ITS2 (61-90-bp fragments) of *I. persulcatus* and *I. pavlovskyi* ticks from different countries. All shown *I. persulcatus* sequences were from areas, which are not inhabited by *I. pavlovskyi* ticks. Omsk, Sakh, Karel, Alt, Nov, and Tomsk correspond to Russian regions Omsk, Sakhalin, Novosibirsk, Tomsk Provinces, Republic of Karelia, and Republic of Altai, respectively. (B) Traces of ITS2 fragments (64-82-bp) of *I. persulcatus*, *I. pavlovskyi* ticks and their hybrids. Heterozygous positions are indicated by arrows.

previously (Rar et al., 2017); ticks with hybrid genotypes and putative hybrids were examined in this study. As the “putative hybrids” could be recorded because of both error-prone morphological discrimination and inheritance peculiarities, hybrids and putative hybrids were examined separately.

Four variants of hybrid genotypes were revealed. The hybrid variant Iper/Hybr contained the mitochondrial *cox*1 gene of *I. persulcatus* and both *I. persulcatus* and *I. pavlovskyi* alleles of nuclear ITS2, that is, hybrid ITS2 (Fig. 2), while variant Iper/Ipav contained the *cox*1 gene of *I. persulcatus* and ITS2 of *I. pavlovskyi.* The variant designated Ipav/Hybr contained the *cox*1 gene of *I. pavlovskyi* and hybrid ITS2; the variant Ipav/Iper contained the *cox*1 gene of *I. pavlovskyi* and ITS2 of *I. persulcatus* (Fig. 3). The hybrid variants Iper/Hybr and Iper/Ipav originated from *I. persulcatus* females, while Ipav/Hybr and Ipav/Iper were from *I. pavlovskyi* females (Fig. 3). The genetic variants Iper/Hybr and Ipav/Hybr can be the result of the crossing of *I. persulcatus* and *I. pavlovskyi* ticks; thus, these variants correspond to the genotypes of F_1 progeny. The variants Iper/Ipav and Ipav/Iper with introgression of mitochondrial sequences can be the result of repeated crossing of hybrids with the parental species (Fig. 3). In putative hybrids, two genotypes were observed: pu:Iper/Iper and pu:Ipav/Ipav contained the *cox*1 gene and ITS2 belonging to one species, while the morphology of these ticks referred to another species.

Ticks with hybrid genotypes were identified at all studied sites with frequencies varying from 6.8% (site A2) to 39.8% (site N3) (Table S2). The distribution of different genotypes in Altai and Novosibirsk was variable. In Altai (sites A1 and A2), the frequencies of the four hybrid genotypes were not significantly different and varied from 1.6 to 2.1%. In Novosibirsk (sites N1-N5), the proportion of hybrids originated from *I. pavlovskyi* females (Ipav/Hybr and Ipav/Iper) was significantly higher ($\chi^2 = 118.4$, df = 1, $P < 0.001$) than that of hybrids originated from *I. persulcatus* females (Iper/Hybr and Iper/Ipav) (Table 2). Notably, the frequency of some hybrid genotypes depended on the sex of ticks: the genotype Ipav/Hybr was identified in females significantly more frequently ($\chi^2 = 66.6$, df = 1, $P < 0.001$) than in males, while the proportion of hybrid genotypes Iper/Ipav and Ipav/Iper in males was significantly higher than in females ($\chi^2 = 11.7$, df = 1, $P < 0.001$ and $\chi^2 = 21.9$, df = 1, $P < 0.001$, respectively) (Table 2).

Putative hybrids were recorded in Novosibirsk significantly more frequently ($\chi^2 = 44.2$, df = 1, $P < 0.001$) than in Altai (Table 1). The prevalence of both variants of putative hybrids, pu:Iper/Iper and pu:Ipav/Ipav, was similar (Table 2). The proportion of males and females with the genotype pu:Iper/Iper was comparable, while pu:Ipav/Ipav males were recorded significantly more often ($\chi^2 = 25.9$, df = 1, $P < 0.001$) than Ipav/Ipav females (Table 2).

The prevalence and genetic variability of tick-transmitted agents found in hybrids were compared with those detected in parental tick species, which were collected at the same time and locations and have been described previously (Rar et al., 2017).

3.2. Detection and genotyping of TBEV and KemV RNA

TBEV RNA was found in hybrids from both Altai and Novosibirsk with a prevalence of 5.2% in both case, and in 7.2% of putative hybrids from Novosibirsk (Table 3). TBEV was detected in ticks of all hybrid variants. Sequencing of positive samples revealed nine sequence variants of TBEV, all of which belonged to the Siberian subtype. Among them, four sequence variants (GenBank accession numbers MG190874, MG190875, MG190879, MG190884-MG190886) were new, while other variants were identical to those previously identified in *I. persulcatus* and *I. pavlovskyi* ticks in Siberia (Rar et al., 2017) (Fig. 4A).

KemV RNA was revealed in three ticks with a genotype Ipav/Iper from Altai and Novosibirsk (Table 3). Based on segment 1 fragment sequences, KemV isolates from hybrids were identical (KX834333, KX834342) or closely related (KX834334) to isolates from *I. persulcatus* and *I. pavlovskyi* ticks previously found in Western Siberia (Rar et al., 2017) (Fig. 4B).

For both TBEV and KemV, there was no significant difference in their prevalence between various hybrid variants and parental species or between Altai and Novosibirsk (Table S3).

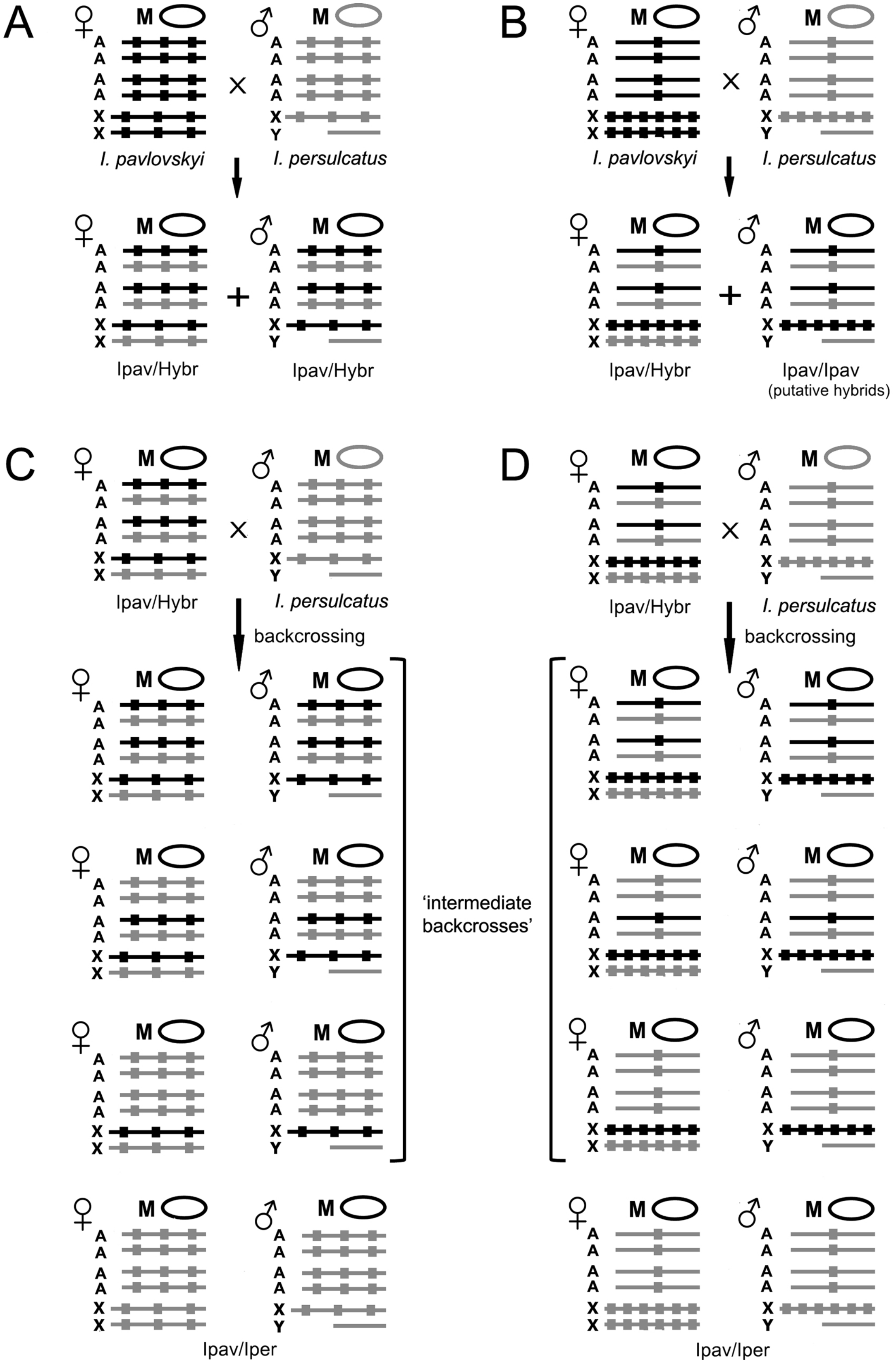

Fig. 3. The scheme of hybridization. Crossing of *I. pavlovskyi* female and *I. persulcatus* male (A, B) and multiple backcrossing of hybrids with *I. persulcatus* (C, D). The scheme includes two variants of ITS2 localization on chromosomes: A, C and B, D, respectively. *Legend*: Ipav/Hybr, Ipav/Iper, and pu:Ipav/Ipav – hybrid and putative hybrid genotypes; *I. pavlovskyi* is marked with black; *I. persulcatus* is marked with grey; A – autosomes, X and Y – sex chromosomes; ⬭ - mitochondrial genome; ■ – ITS2 locus on chromosomes.

Table 2

Prevalence of various hybrid and putative hybrid genotypes in Altai and Novosibirsk regions.

Site	Sex	No. of tested ticks	No. (%) of hybrids with genotype				No. (%) of putative hybrids with genotype	
			Iper/Hybr	Iper/Ipav	Ipav/Hybr	Ipav/Iper	pu:Iper/Iper	pu:Ipav/Ipav
Altai (A1 and A2)	female	497	7 (1.4)	3 (0.6)	11 (2.2)	2 (0.4)	4 (0.8)	2 (0.4)
	male	316	10 (3.2)	12 (3.8)	2 (0.6)	11 (3.5)	2 (0.6)	0
	total	813	17 (2.1)	15 (1.8)	13 (1.6)	13 (1.6)	6 (0.7)	2 (0.2)
Novosibirsk (N1- N5)	female	500	19 (3.8)	7 (1.4)	96 (19.2)	37 (7.4)	21 (4.2)	4 (0.8)
	male	404	1 (0.2)	12 (3.0)	4 (1.0)	57 (14.1)	14 (3.5)	30 (7.4)
	total	904	20 (2.2)	19 (2.1)	100 (11.1)	94 (10.4)	35 (3.9)	34 (3.8)
Both regions (all sites)	**female**	**997**	**26 (2.6)**	**10 (1.0)**	**107 (10.7)**	**39 (3.9)**	**25 (2.5)**	**6 (0.6)**
	male	**720**	**11 (1.5)**	**24 (3.3)**	**6 (0.8)**	**68 (9.4)**	**16 (2.2)**	**30 (4.2)**
	total	**1717**	**37 (2.2)**	**34 (1.9)**	**113 (6.6)**	**107 (6.2)**	**41 (2.4)**	**36 (2.1)**

3.3. Detection and genotyping of *B. burgdorferi (s.l.)*

Borrelia burgdorferi (s.l.) DNA was found in 37.8% of hybrid, in all genetic variants. The prevalence of *B. burgdorferi* (s.l.) in hybrids did not significantly differ between Altai and Novosibirsk and was 37.9% and 37.8%, respectively (Table 3). In both regions, three *Borrelia* species were identified in hybrids: *B. afzelii*, *B. bavariensis*, and *B. garinii* with overall prevalence of 5.2%, 13.7%, and 22.0%, respectively, including cases of mixed infection (Table 4). Thus, *B. afzelii* was detected in hybrids significantly less often than *B. bavariensis* (χ^2 = 12.6, df = 1, P < 0.001) and *B. garinii* (χ^2 = 35.2, df = 1, P < 0.001). The same three *Borrelia* species, *B. afzelii*, *B. bavariensis*, and *B. garinii*, were found in putative hybrids with the prevalence of 9.1% for each species (Table 4).

The comparison of *Borrelia* spp. prevalence in hybrids and parental species demonstrated that in total hybrids were significantly more often infected with *B. afzelii* (χ^2 = 10.6, df = 1, P = 0.001), *B. bavariensis* (χ^2 = 62.2, df = 1, P < 0.001), and less often with *B. garinii* (χ^2 = 29.6, df = 1, P < 0.001) compared to *I. pavlovskyi* ticks; and contrariwise, hybrids were significantly less often infected with *B. afzelii* (χ^2 = 8.4, df = 1, P = 0.004), *B. bavariensis* (χ^2 = 9.4, df = 1, P = 0.002), and more often with *B. garinii* (χ^2 = 37.6, df = 1, P < 0.001) compared to *I. persulcatus* (Table S3). The prevalence of *B. afzelii, B. bavariensis*, and *B. garinii* in most hybrid variants was

Table 3

Prevalence of tick-transmitted agents in *Ixodes* spp. specimens.

Region	*Ixodes* spp. genotype	No. of ticks	No. (%) of ticks infected by any of agent	No. (%) of ticks containing nucleic acids of tested agents[#]								
				TBEV	KEMV	B.burg	B.miyam	Rick.sp	A.phag	E.mur	Ca.N.m	Bab.m
Altai (A1, A2)	**All hybrids**	**58**	**42 (72.4)**	**3 (5.2)**	**1 (1.7)**	**22 (37.9)**	**5 (8.6)**	**29 (50.0)**	**1 (1.7)**	**4 (6.9)**	**0**	**0**
	Iper/Hybr	17	11 (64.7)	0	0	8 (47.1)	0	10 (58.8)	1 (5.9)	2 (11.8)	0	0
	Iper/Ipav	15	13 (86.7)	1 (6.7)	0	3 (20.0)	2 (13.3)	11 (73.3)	0	2 (13.3)	0	0
	Ipav/Hybr	13	7 (53.8)	2 (15.4)	0	5 (38.5)	2 (15.4)	3 (23.1)	0	0	0	0
	Ipav/Iper	13	11 (84.6)	0	1 (7.7)	6 (46.2)	1 (7.7)	5 (38.5)	0	0	0	0
	All putative hybrids	**8**	**3 (37.5)**	**0**	**0**	**1 (12.5)**	**0**	**4 (50.0)**	**2 (25.0)**	**1 (12.5)**	**0**	**0**
	pu:Iper/Iper[*]	6	3 (50)	0	0	1 (16.7)	0	2 (33.3)	0	1 (16.7)	0	0
	pu:Ipav/Ipav[**]	2	0	0	0	0	0	2 (100)	2 (100)	0	0	0
	I. pers	**185**	**173 (93.5)**	**7 (3.8)**	**2 (1.1)**	**70 (37.8)**	**11 (5.9)**	**162 (87.6)**	**15 (8.1)**	**27 (14.6)**	**0**	**0**
	I. pavl	**124**	**78 (62.9)**	**12 (9.7)**	**1 (0.8)**	**58 (46.8)**	**9 (7.3)**	**11 (8.9)**	**3 (2.4)**	**1 (0.8)**	**1 (0.8)**	**2 (1.6)**
Novosibirsk (N1- N5)	**All hybrids**	**233**	**147 (63.1)**	**12 (5.2)**	**2 (0.9)**	**88 (37.8)**	**9 (3.9)**	**61 (26.2)**	**14 (6.0)**	**17 (7.3)**	**3 (1.3)**	**8 (3.4)**
	Iper/Hybr	20	13 (65.0)	1 (5.0)	0	**4 (20.0)**	1 (5.0)	11 (55.0)	1 (5.0)	3 (15.0)	1 (5.0)	2 (10.0)
	Iper/Ipav	19	15 (78.9)	1 (5.3)	0	8 (42.1)	2 (10.5)	6 (31.6)	1 (5.3)	1 (5.3)	0	1 (5.3)
	Ipav/Hybr	100	63 (63.0)	7 (7.0)	0	46 (46.0)	3 (3.0)	18 (18.0)	4 (4.0)	5 (5.0)	1 (1.0)	1 (1.0)
	Ipav/Iper	94	56 (59.6)	3 (3.2)	2 (2.1)	30 (31.9)	3 (3.2)	26 (27.7)	8 (8.5)	8 (8.5)	1 (1.1)	4 (4.3)
	All putative hybrids	**69**	**44 (63.8)**	**5 (7.2)**	**0**	**18 (26.1)**	**3 (4.3)**	**22 (31.9)**	**5 (7.2)**	**6 (8.7)**	**0**	**1 (1.4)**
	pu:Iper/Iper[*]	35	20 (57.1)	1 (2.9)	0	7 (20.0)	1 (2.9)	11 (31.4)	1 (2.9)	1 (2.9)	0	1 (2.9)
	pu:Ipav/Ipav[**]	34	24 (70.6)	4 (11.8)	0	**11 (32.4)**	2 (5.9)	11 (32.4)	4 (11. 8)	5 (14.7)	0	0
	I. pers	**149**	**120 (80.5)**	**11 (7.4)**	**0**	**56 (37.6)**	**10 (6.7)**	**97 (65.1)**	**6 (4.0)**	**13 (8.7)**	**2 (1.3)**	**2 (1.3)**
	I. pavl	**453**	**258 (57.0)**	**22 (4.9)**	**1 (0.2)**	**187 (41.3)**	**28 (6.2)**	**36 (7.9)**	**16 (3.5)**	**1 (0.2)**	**8 (1.8)**	**0**
Both regions (all sites)	**All hybrids**	**291**	**189 (64.9)**	**15 (5.2)**	**3 (1.0)**	**110 (37.8)**	**14 (4.8)**	**90 (30.9)**	**15 (5.2)**	**21 (7.2)**	**3 (1.0)**	**8 (2.7)**
	Iper/Hybr	37	24 (64.9)	1 (2.7)	0	12 (32.4)	1 (2.7)	21 (56.8)	2 (5.4)	5 (13.5)	1 (2.7)	2 (5.4)
	Iper/Ipav	34	28 (82.4)	2 (5.9)	0	11 (32.4)	4 (11.8)	17 (50.0)	1 (2.9)	3 (8.8)	0	1 (2.9)
	Ipav/Hybr	113	70 (61.9)	9 (8.0)	0	51 (45.1)	5 (4.4)	21 (18.6)	4 (3.5)	5 (4.4)	1 (0.9)	1 (0.9)
	Ipav/Iper	107	67 (62.6)	3 (2.8)	3 (2.8)	36 (33.6)	4 (3.7)	31 (29.0)	8 (7.5)	8 (7.5)	1 (0.9)	4 (3.7)
	All putative hybrids	**77**	**47 (61.0)**	**5 (6.5)**	**0**	**19 (24.7)**	**3 (3.9)**	**26 (33.8)**	**7 (9.1)**	**7 (9.1)**	**0**	**1 (1.3)**
	pu:Iper/Iper[*]	41	23 (56.1)	1 (2.4)	0	8 (19.5)	1 (2.4)	13 (31.7)	1 (2.4)	**2 (4.9)**	0	1 (2.4)
	pu:Ipav/Ipav[**]	36	24 (66.7)	4 (11.1)	0	11 (30.6	2 (5.6)	13 (36.1)	6 (16.7)	5 (13.9)	0	0
	I. pers	**334**	**293 (87.7)**	**18 (5.4)**	**2 (0.6)**	**126 (37.7)**	**21 (6.3)**	**259 (77.5)**	**21 (6.3)**	**40 (12.0)**	**2 (0.6)**	**2 (0.6)**
	I. pavl	**577**	**336 (58.2)**	**34 (5.9)**	**2 (0.3)**	**245 (42.5)**	**37 (6.4)**	**47 (8.1)**	**19 (3.3)**	**2 (0.3)**	**9 (1.6)**	**2 (0.3)**

Abbreviations: I. pers - *I. persulcatus*; I. pavl - *I. pavlovskyi*; B.burg - *B. burgdorferi* (s.l.); B.miyam - *B. miyamotoi;* Rick.sp - *Rickettsia* spp.; A.phag. - *A. phagocytophilum*; E.mur - *E. muris*; Ca.N.m - "*Candidatus* N. mikurensis"; Bab.m - *Bab. microti*.

[#] Including cases of mixed sinfection.

[*] -putative hybrids with *I. pavlovskyi* morphology.

[**] - putative hybrids with *I. persulcatus* morphology.

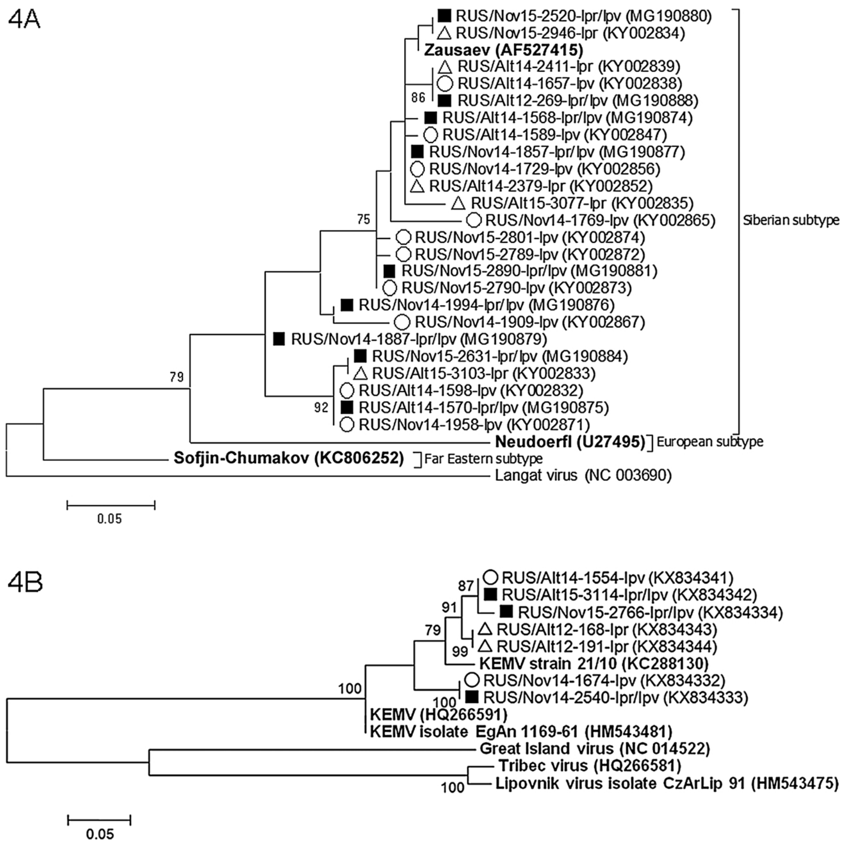

Fig. 4. Phylogenetic trees constructed by the ML method based on nucleotide sequences of 211-bp fragment of the E gene of TBEV (A), 328-bp fragment of KEMV genome segment 1 (B), 579-bp fragment of *clpA* gene of *Borrelia* spp. from *Borrelia burgdorferi* s.l. complex (C), 450-bp fragment of *gltA* gene of *Rickettsia* spp. (D), and 1189-1200-bp fragment of the *groEL* gene of Anaplasmataceae bacteria (E). Significant bootstrapping values (> 70%) are shown on the nodes. The sequences of prototype strains/isolates and from the *Borrelia* MLST website and GenBank database are in boldface. *Legend*: ○ – *I. pavlovskyi* ticks; △ – *I. persulcatus* ticks; ■– hybrids.

intermediate compared to parental tick species. Notably, hybrids originated from *I. persulcatus* females (Iper/Hybr and Iper/Ipav) were more often infected with *B. bavariensis* and less often with *B. garinii* compared to hybrids originated from *I. pavlovskyi* females (Ipav/Hybr and Ipav/Iper). However, the difference was not significant between all variants. The prevalence of *B. bavariensis* and *B. garinii* in putative hybrids also was intermediate compared to *I. pavlovskyi* and *I. persulcatus* ticks (Table 4, Fig. 5).

Analysis of *clpA* gene fragments of *B. burgdorferi* (s.l.) found in hybrids revealed two alleles of *B. afzelii*, nine alleles of *B. bavariensis*, and 16 alleles of *B. garinii*. One new *B. bavariensis* allele (MG010828) and two new *B. garinii* alleles (MG010846, MG010847), differing from known alleles by 2–3 nucleotides, were identified (Fig. 4C). All other determined alleles were previously found in parental tick species and deposited in the GenBank database and/or on the *Borrelia* MLST website (Margos et al., 2008).

To correct distinguish two closely related species, *B. bavariensis* and *B. garinii*, the second genetic marker, the *p83/100* gene, was included in this study. The use of this gene for adequate *Borrelia* species identification has been recently demonstrated (Sabitova et al., 2018). Phylogenetic analysis of determined *p83/100* gene sequences confirmed *Borrelia* species determination using *clpA* gene fragments. The *p83/100* gene sequences from hybrid ticks included three genetic variants of *B. afzelii*, eleven variants of *B. bavariensis*, and 14 variants of *B. garinii*. Of these genetic variants, two variants of *B. bavariensis* (MG010864-MG010866) and four variants of *B. garinii* (MG010881-MG010884) were new, while other variants were detected previously in *I. persulcatus* and/or *I. pavlovskyi* ticks from the studied region (Fig. S1). Notably, one new *B. garinii* variant (MG010883) detected in several hybrids from Novosibirsk was unusual and contained an 18-bp insertion in the *p83/100* gene (Fig. S1).

3.4. Detection and genotyping of *B. miyamotoi*

Borrelia miyamotoi DNA was found in 8.6% of hybrid ticks from Altai and 3.9% of hybrids from Novosibirsk as well as in 4.3% of putative hybrids from Novosibirsk (Table 3). *Borrelia miyamotoi* was identified in all genetic variants of hybrids and putative hybrids. The prevalence of *B. miyamotoi* in hybrids did not differ significantly from those in *I. persulcatus* and *I. pavlovskyi* ticks (Table S3). The sequences of all determined *glpQ* gene fragments of *B. miyamotoi* detected in hybrid ticks and putative hybrids were identical, and matched a sequence previously found in *I. persulcatus* and *I. pavlovskyi* ticks from Western Siberia (KY006159–KY006162), belonging to Asian-type *B. miyamotoi* (Fig. S2).

3.5. Detection and genotyping of *Rickettsia* spp

Rickettsia spp. DNA was identified in hybrid ticks from Altai significantly more often ($\chi^2 = 75.5$, df = 1, $P < 0.001$) than in Novosibirsk, with frequencies of 50.0% and 26.2%, respectively (Table 3). Five *Rickettsia* species were found in hybrids and putative hybrids: *Rickettsia heilongjiangensis*, *Rickettsia helvetica*, *Rickettsia raoultii*, *Rickettsia sibirica* and "*Candidatus* R. tarasevichiae" (Table 5). The prevalence of "*Candidatus* R. tarasevichiae" in hybrids and putative hybrids was significantly higher compared to other *Rickettsia* species ($P < 0.001$).

Comparison of *Rickettsia* spp. prevalence in hybrids and parental

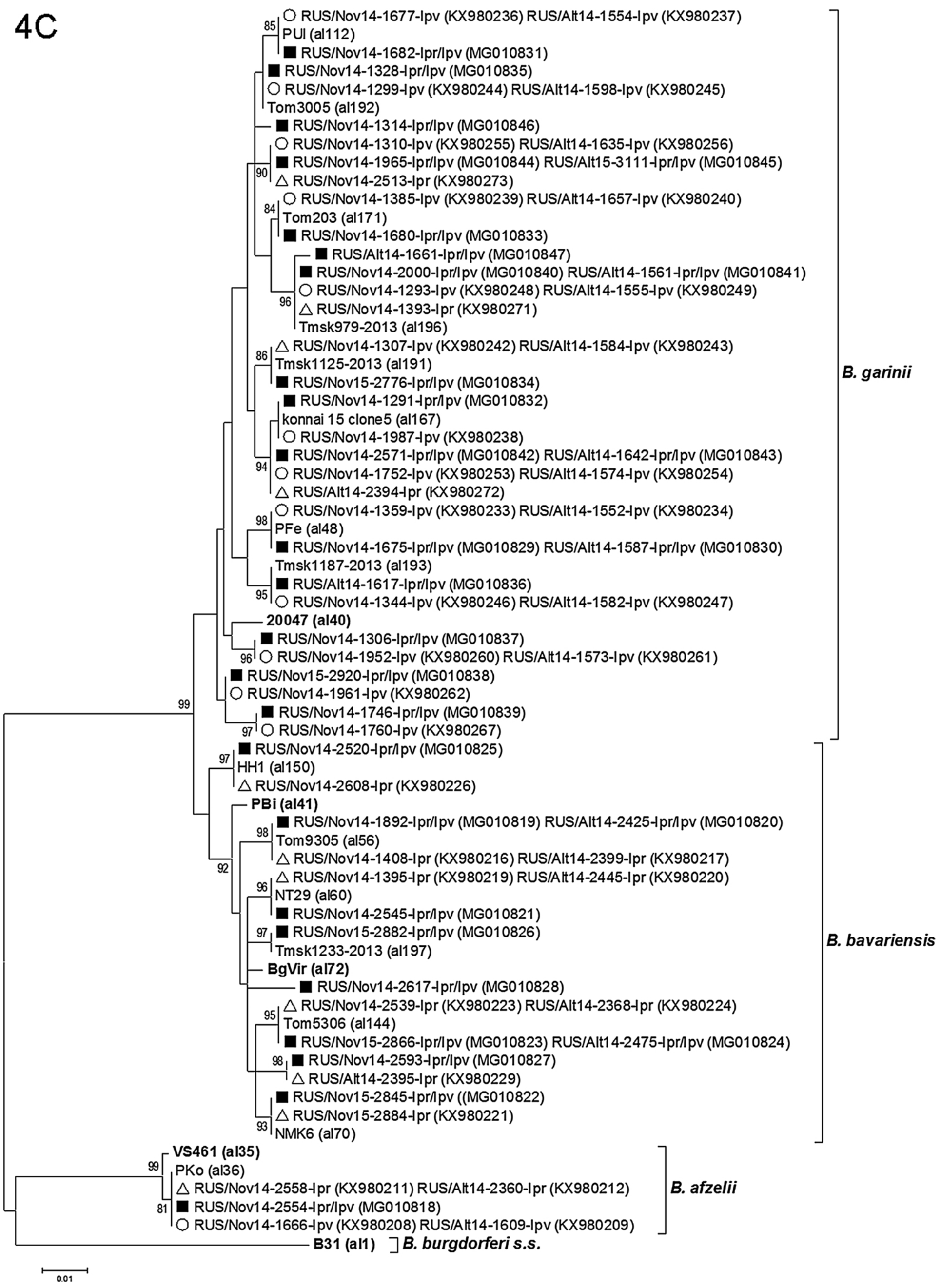

Fig. 4. (*continued*)

species demonstrated that all genetic variants of hybrids and putative hybrids were significantly more often ($P < 0.001$) infected with "*Candidatus* R. tarasevichiae" compared to *I. pavlovskyi* and significantly less often ($P < 0.01$) compared to *I. persulcatus* (Table 5, Fig. 5). For other *Rickettsia* species, differences were not significant. The comparison of various genetic variants of hybrids demonstrated that hybrids originated from *I. persulcatus* (Iper/Hybr and Iper//Ipav) were significantly more frequently ($P < 0.05$) infected with "*Candidatus* R. tarasevichiae" compared to hybrids originated from *I. pavlovskyi* (Ipav/Hybr and Ipav/Iper).

Rickettsia spp. isolates were characterized by the *gltA* gene. Most *gltA* gene sequences of *R. helvetica* in hybrids and putative hybrids were identical and corresponded to a variant previously identified in both *I. persulcatus* and *I. pavlovskyi* ticks from Western Siberia, however the *gltA* gene sequence of one isolate from Altai (MG022123) differed from them by one nucleotide substitution. *R. raoultii gltA* gene fragments from hybrid ticks belonged to genetic variants previously named RpA4, DnS14, and DnS14 or differed from them by single nucleotide substitutions. Two *gltA* gene fragments of *R. sibirica* from hybrid ticks (MG022126, MG022127) differed from each other by two nucleotide mismatches and from the known sequences of *R. sibirica* by one substitution. All *gltA* gene sequences of "*Candidatus* R. tarasevichiae" identified in hybrids and putative hybrids were identical to corresponding sequences previously found in parental tick species (Fig. 4D).

3.6. Detection and genotyping of Anaplasmataceae bacteria

Bacteria of three species belonging to the Anaplasmataceae family, *A. phagocytophilum, E. muris* and "*Candidatus* N. mikurensis", were found in hybrid ticks. *A. phagocytophilum* was found in 1.7% of hybrids from Altai and in 6.0%, from Novosibirsk; this difference was not significant (Table 3). The prevalence of *E. muris* in hybrid ticks from Altai

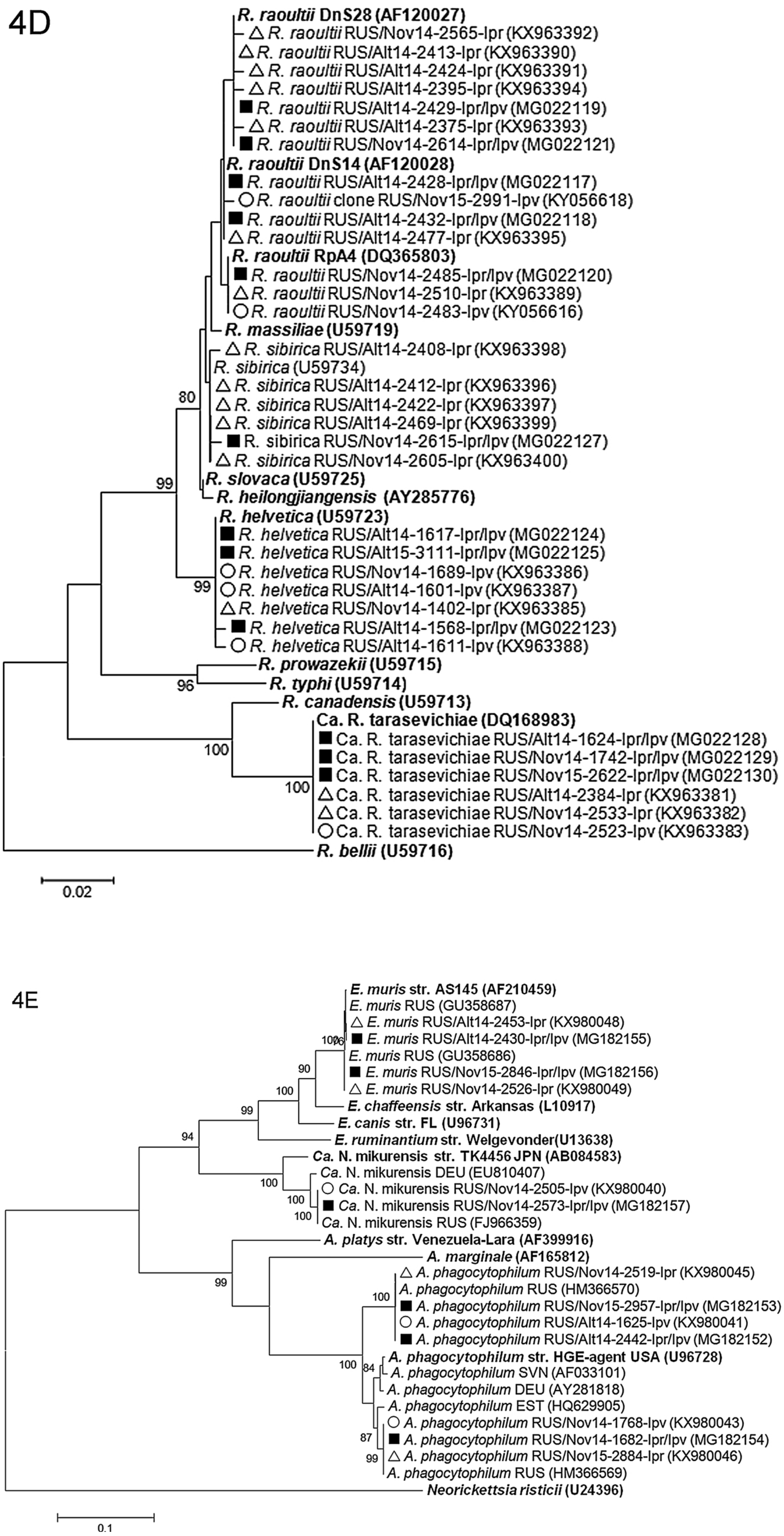

Fig. 4. (*continued*)

Table 4
Prevalence of *B. burgdorferi* (s.l.) in *Ixodes* spp. specimens.

Region	*Ixodes* spp. genotype	No. of ticks	No. (%) of ticks containing DNA of tested agents[#]				
			all *B. burgdorferi* (s.l.)	Ba	Bb	Bg	Bv
Altai (A1, A2)	**All hybrids**	**58**	**22 (37.9)**	**4 (6.9)**	**10 (17.2)**	**11 (19.0)**	**0**
	Iper/Hybr	17	8 (47.1)	2 (11.8)	6 (35.3)	1 (5.9)	0
	Iper/Ipav	15	3 (20.0)	1 (6.7)	3 (20.0)	0	0
	Ipav/Hybr	13	5 (38.5)	0	0	5 (38.5)	0
	Ipav/Iper	13	6 (46.2)	1 (7.7)	1 (7.7)	5 (38.5)	0
	All putative hybrids	**8**	**1 (12.5)**	**0**	**0**	**1 (12.5)**	**0**
	pu:Iper/Iper*	6	1 (16.7)	0	0	1 (16.7)	0
	pu:Ipav/Ipav**	2	0	0	0	0	0
	I. pers	**185**	**70 (37.8)**	**19 (10.3)**	**50 (27.0)**	**5 (2.7)**	**0**
	I. pavl	**124**	**58 (46.8)**	**3 (2.4)**	**0**	**56 (45.2)**	**0**
Novosibirsk (N1-N5)	**All hybrids**	**233**	**88 (37.8)**	**11 (4.7)**	**30 (12.9)**	**53 (22.7)**	**0**
	Iper/Hybr	20	4 (20.0)	0	4 (20.0)	0	0
	Iper/Ipav	19	8 (42.1)	0	4 (21.1)	4 (21.1)	0
	Ipav/Hybr	100	46 (46.0)	4 (4.0)	8 (8.0)	36 (36.0)	0
	Ipav/Iper	94	30 (31.9)	7 (7.4)	14 (14.9)	13 (13.8)	0
	All putative hybrids	**69**	**18 (26.1)**	**7 (10.1)**	**7 (10.1)**	**6 (8.7)**	**0**
	pu:Iper/Iper*	35	7 (20.0)	3 (8.6)	3 (8.6)	2 (5.7)	0
	pu:Ipav/Ipav**	34	11 (32.4)	4 (11.8)	4 (11.8)	4 (11.8)	0
	I. pers	**149**	**56 (37.6)**	**20 (13.4)**	**28 (18.8)**	**13 (8.7)**	**1 (0.7)**
	I. pavl	**453**	**187 (41.3)**	**5 (1.1)**	**6 (1.3)**	**178 (39.3)**	**0**
Both regions (all sites)	**All hybrids**	**291**	**110 (37.8)**	**15 (5.2)**	**40 (13.7)**	**64 (22.0)**	**0**
	Iper/Hybr	37	12 (32.4)	2 (5.4)	10 (27.0)	1 (2.7)	0
	Iper/Ipav	34	11 (32.4)	1 (2.9)	7 (20.6)	4 (11.8)	0
	Ipav/Hybr	113	51 (45.1)	4 (3.5)	8 (7.1)	41 (36.3)	0
	Ipav/Iper	107	36 (33.6)	8 (7.5)	15 (14.0)	18 (16.8)	0
	All putative hybrids	**77**	**19 (24.7)**	**7 (9.1)**	**7 (9.1)**	**7 (9.1)**	**0**
	pu:Iper/Iper*	41	8 (19.5)	3 (7.3)	1 (2.4)	5 (12.2)	0
	pu:Ipav/Ipav**	36	11 (30.6)	4 (11.1)	4 (11.1)	5 (13.9)	0
	I. pers	**334**	**126 (37.7)**	**39 (11.7)**	**78 (23.4)**	**18 (5.4)**	**1 (0.3)**
	I. pavl	**577**	**245 (42.5)**	**8 (1.4)**	**6 (1.0)**	**234 (40.6)**	**0**

Abbreviations: I. pers - *I. persulcatus*; I. pavl - *I. pavlovskyi*; Ba - *B. afzelii*; Bb - *B. bavariensis*; Bg - *B. garinii*; Bv - *B. valaisiana.*

[#] Including cases of mixed infection.

* -putative hybrids with *I. pavlovskyi* morphology.

** - putative hybrids with *I. persulcatus* morphology.

and Novosibirsk was similar, 6.9% and 7.3%, respectively, while "*Candidatus* N. mikurensis" was found only in Novosibirsk in 1.3% of hybrids (Table 3). *A. phagocytophilum* and *E. muris* were also detected in both genetic variants of putative hybrids.

All genetic variants of hybrids and putative hybrids were significantly more often infected with *E. muris* compared to *I. pavlovskyi* ticks ($P < 0.001$); the difference between hybrids and *I. persulcatus* was not significant (Table 3, Fig. 5). As for *A. phagocytophilum*, the difference in the prevalence between hybrids and *I. pavlovskyi* was significant only for hybrids with a genotype Ipav/Iper ($\chi^2 = 4.2$, df = 1, $P < 0.05$) and putative hybrids with a genotype pu:Ipav/Ipav ($\chi^2 = 15.5$, df = 1, $P < 0.001$).

Two sequence variants of the *A. phagocytophilum groESL* operon were identified in hybrids and putative hybrids; these sequences corresponded to two distinct genetic groups of *A. phagocytophilum* previously found in *I. persulcatus* and *I. pavlovskyi* ticks from the same regions (Fig. 4E). The determined *groESL* operon sequences of *E. muris* and "*Candidatus* N. mikurensis" were highly conserved and identical to corresponding sequences (Fig. 4E), which have been previously identified in both *I. persulcatus* and *I. pavlovskyi* ticks (Rar et al., 2017).

3.7. Detection and genotyping of Babesia spp

Babesia sp. DNA was found in 3.4% and 1.4% of hybrids and putative hybrids, respectively, from Novosibirsk but was not detected in Altai (Table 3). Sequences of the 18S rRNA gene fragment of *Babesia* sp. were identical to corresponding sequences of US-type *Bab. microti* previously identified in *I. persulcatus* and *I. pavlovskyi* ticks from the studied region in Western Siberia (Fig. S3).

4. Discussion

This investigation was a continuation of a previously published study that included the examination of the abundance of *I. persulcatus* and *I. pavlovskyi* ticks in the same sites of the Republic of Altai and Novosibirsk Province and the prevalence of different tick-transmitted agents and their genetic variability (Rar et al., 2017). In this study, *I. persulcatus/pavlovskyi* hybrids were found in all sites examined within the studied regions that – together with previous findings of such hybrids in the Tomsk Province, Western Siberia (Kovalev et al., 2015) and Khabarovsk Province, the Far East (MG210488-MG210491 and our unpublished data) – demonstrated probable ubiquity of hybrids in *I. persulcatus - I. pavlovskyi* sympatric areas. Importantly, the proportion of hybrids in the total *Ixodes* spp. population depended on the ratio of parental species. The proportion of hybrids was relatively low (7–12%) in sites where one tick species dominated over the other (sites A2 and N1) and reached 38–40% in sites with similar abundance of both parental tick species (sites N3 and N4) (Table 1).

Two genetic markers were used for the genetic characterization of ticks: the mitochondrial *cox*1 gene and the nuclear ITS2 fragment. The direction of hybrid crosses was determined by the mitochondrial *cox*1 gene, while the presence of hybridization was defined using ITS2. These markers have been frequently used for tick species determination (Burger et al., 2014; Kanduma et al., 2016; Kovalev et al., 2015, 2016; Livanova et al., 2015; Lv et al., 2014). Despite the large number of studies concerning the phylogeny of ticks, their genome organization is poorly studied. In *I. scapularis,* tandem copies of ribosomal DNA regions, including ITS2, are located on two pairs of autosomes and the sex X chromosome (Meyer et al., 2010); however, the number of ITS2 copies on different chromosomes remains unknown. In contrast to *I.*

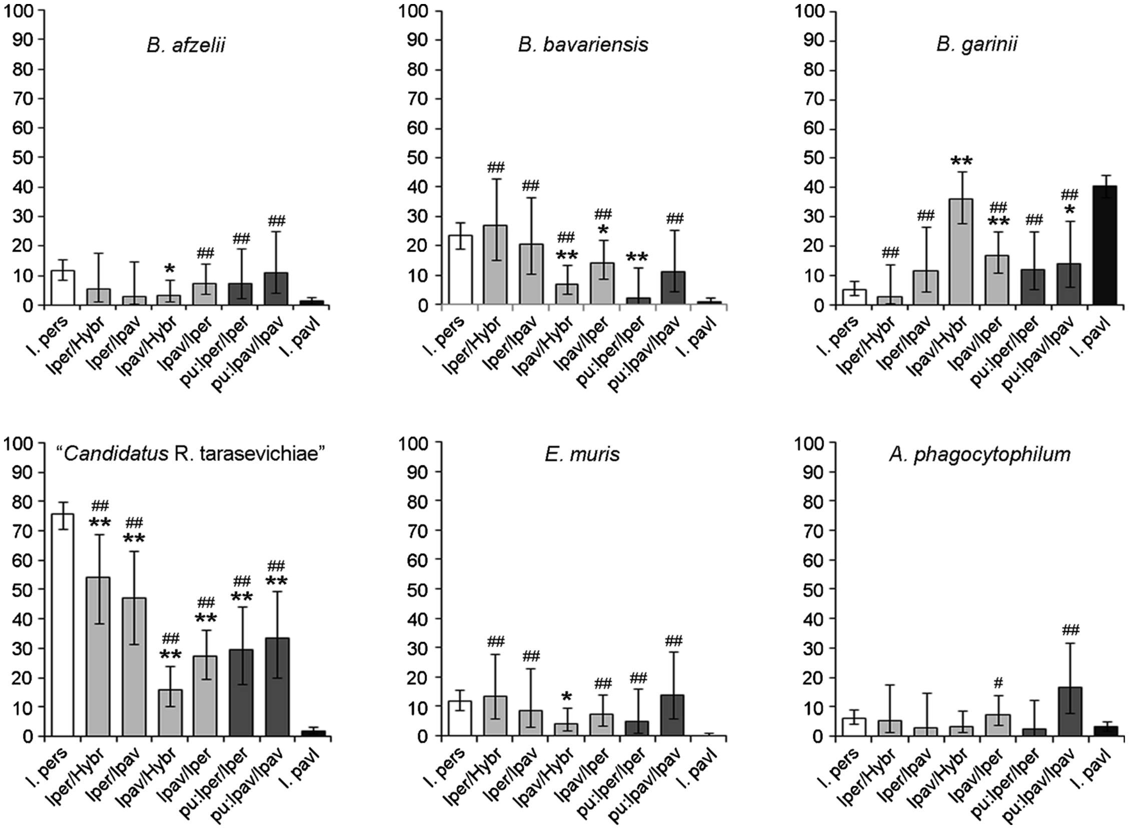

Fig. 5. The prevalence of infectious agents in different hybrid and putative hybrid variants and parental tick species. The significant difference between hybrid variants and *I. persulcatus* was marked by * ($P < 0.05$) and ** ($P < 0.01$). The significant difference between hybrid variants and *I. pavlovskyi* was marked by # ($P < 0.05$) and ## ($P < 0.01$).

scapularis, ribosomal DNA regions are located only on one autosome in *Rhipicephalus microplus* (Hill et al., 2009). So, three hypotheses should be considered here: i) the number of ITS2 copies on autosomes prevails over those on the sex X chromosome; ii) their number is similar; iii) the number of ITS2 copies on the sex X chromosome is substantially greater than those on autosomes. In all cases, non-Mendelian inheritance would be observed.

If the first or second hypothesis is correct, the autosomes play the main role in the inheritance of the ITS2 (Fig. 3A, 3C); however, while considering the third hypothesis, the autosomes might be ignored in the inheritance of the ITS2 locus because the presence of a low number of ITS2 copies could not be identified by sequencing (Fig. 3B, 3D). Regardless of if the autosomes or the X chromosome play the main role in the inheritance of the ITS2, the crossing of *I. persulcatus* and *I. pavlovskyi* should lead to the appearance of ticks with only two hybrid variants (Iper/Hybr and Ipav/Hybr) (Fig. 3A, 3B).

The backcrossing of F_1 hybrids (Iper/Hybr or Ipav/Hybr) with parental tick species because of random distribution of chromosomes with ITS2 locus can lead to the appearance of 'intermediate backcrosses' with different ratio of *I. persulcatus* and *I. pavlovskyi* ITS2 (Fig. 3C, 3D). Notably, some of these 'intermediate backcrosses' cannot be identified by sequencing because of the small proportion of ITS2 of one tick species. The subsequent multiple backcrossing of 'intermediate backcrosses' with a parental tick species could result to appearance of ticks with genotypes Iper/Ipav and Ipav/Iper (Fig. 3). Hybrid variants (Iper/Ipav and Ipav/Iper) could not appear if *I. persulcatus/pavlovskyi* hybrids were not fertile. The observed high abundance of backcrosses in some locations (10–20%) also strongly supports this suggestion about the fertility of *I. persulcatus/pavlovskyi* hybrids (Table S2).

Notably, in the first or second variant of the ITS2 inheritance, the number of revealed females and males with hybrid F_1 genotypes should be similar (Fig. 3A); in the third variant, hybrid males can not be detected by ITS2 fragment analysis (Fig. 3B). The comparison of hybrids with different genotypes collected in nature showed that among the hybrids resulted from *I. persulcatus* females and *I. pavlovskyi* males crossing (Iper/Hybr), the ratio of females and males was similar (Tables 2, S2). On the contrary, females significantly prevailed among hybrids originated from *I. pavlovskyi* females and *I. persulcatus* males (Ipav/Hybr). In addition, most of putative hybrids with the genotype of *I. pavlovskyi* and phenotype of *I. persulcatus* (pu:Ipav/Ipav) were males (Tables 2, S2, Fig. 3B). The observed discrepancy in sex-related inheritance for two directions of crossing could be explained by the different ratios of ITS2 copies on the X chromosome and autosomes in *I. persulcatus* and *I. pavlovskyi* ticks. In addition, different hybrid variants of males and females might have different viability in nature. Further experiments including laboratory crossing of *I. persulcatus* with *I. pavlovskyi* are required for direct confirmation of hybrid fertility and for clarifying the mode of inherence of ribosomal DNA regions.

Since *I. persulcatus* and *I. pavlovskyi* ticks carry a similar spectrum of tick-transmitted agents, one might expect that their hybrids can also be infected with the same agents. Indeed, a wide spectrum of infectious agents, namely, TBEV, KemV, *B. afzelii*, *B. bavariensis*, *B. garinii*, *B. miyamotoi*, *R. heilongjiangensis*, *R. helvetica*, *R. raoultii*, *R. sibirica*, "*Candidatus* R. tarasevichiae", *A. phagocytophilum*, *E. muris*, "*Candidatus* N. mikurensis", and *Bab. microti* were found in ticks with hybrid genotypes and/or putative hybrids (Tables 3–5). Notably, this is the first time the above tick-transmitted agents have been found in *I. persulcatus/pavlovskyi* hybrids. Previously, it has been shown that the prevalence of some agents differs significantly between *I. persulcatus* and *I. pavlovskyi* ticks (Rar et al., 2017). For these agents, their prevalence in hybrids and putative hybrids was intermediate compared to the parental tick species. Interestingly, the hybrids originated from *I.*

Table 5
Prevalence of *Rickettsia* spp. in *Ixodes* spp. specimens.

Region	*Ixodes* spp. genotype	No. of ticks	No. (%) of ticks containing DNA of tested agents[#]						
			all *Rickettsia* spp.	Rh	Rr	Rs	Rt	Rhlg	Rsp
Altai (A1, A2)	**All hybrids**	**58**	**29 (50.0)**	**4 (6.9)**	**3 (5.2)**	**1 (1.7)**	**26 (44.8)**	**0**	**0**
	Iper/Hybr	17	10 (58.8)	1 (5.9)	0	1 (5.9)	9 (52.9)	0	0
	Iper/Ipav	15	11 (73.3)	0	2 (13.3)	0	11 (73.3)	0	0
	Ipav/Hybr	13	3 (23.1)	2 (15.4)	0	0	1 (7.7)	0	0
	Ipav/Iper	13	5 (38.5)	1 (7.7)	1 (7.7)	0	5 (38.5)	0	0
	All putative hybrids	**8**	**4 (50.0)**	**0**	**0**	**0**	**4 (50.0)**	**0**	**0**
	pu:Iper/Iper[*]	6	2 (33.3)	0	0	0	2 (33.3)	0	0
	pu:Ipav/Ipav[**]	2	2 (100)	0	0	0	2 (100)	0	0
	I. pers	**185**	**162 (87.6)**	**0**	**13 (7.0)**	**6 (3.2)**	**161 (87.0)**	**1 (0.5)**	**1 (0.5)**
	I. pavl	**124**	**11 (8.9)**	**10 (8.1)**	**0**	**0**	**1 (0.8)**	**1 (0.8)**	**0**
Novosibirsk (N1-N5)	**All hybrids**	**233**	**61 (26.2)**	**0**	**3 (1.3)**	**1 (0.4)**	**57 (24.5)**	**0**	**0**
	Iper/Hybr	20	11 (55.0)	0	0	0	11 (55.0)	0	0
	Iper/Ipav	19	6 (31.6)	0	1 (5.3)	0	5 (26.3)	0	0
	Ipav/Hybr	100	18 (18.0)	0	1 (1.0)	0	17 (17.0)	0	0
	Ipav/Iper	94	26 (27.7)	0	1 (1.1)	1 (1.1)	24 (25.5)	0	0
	All putative hybrids	**69**	**22 (31.9)**	**1 (1.4)**	**0**	**0**	**20 (29.0)**	**1(1.4)**	**0**
	pu:Iper/Iper[*]	35	11 (31.4)	0	0	0	10 (28.6)	1 (2.9)	0
	pu:Ipav/Ipav[**]	34	11 (32.4)	1 (2.9)	0	0	10 (29.4)	0	0
	I. pers	**149**	**97 (65.1)**	**1 (0.7)**	**7 (4.7)**	**2 (1.3)**	**92 (61.7)**	**0**	**1 (0.7)**
	I. pavl	**453**	**36 (7.9)**	**10 (2.2)**	**15 (3.3)**	**0**	**9 (2.0)**	**4 (0.9)**	**0**
Both regions (all sites)	**All hybrids**	**291**	**90 (30.9)**	**4 (1.4)**	**6 (2.1)**	**2 (0.7)**	**83 (28.5)**	**0**	**0**
	Iper/Hybr	37	21 (56.8)	1 (2.7)	0	1 (2.7)	20 (54.1)	0	0
	Iper/Ipav	34	17 (50.0)	0	3 (8.8)	0	16 (47.1)	0	0
	Ipav/Hybr	113	21 (18.6)	2 (1.8)	1 (0.9)	0	18 (15.9)	0	0
	Ipav/Iper	107	31 (29.0)	1 (0.9)	2 (2.0)	1 (0.9)	29 (27.1)	0	0
	All putative hybrids	**77**	**26 (33.8)**	**1 (1.3)**	**0**	**0**	**24 (31.2)**	**1 (1.3)**	**0**
	pu:Iper/Iper[*]	41	13 (31.7)	0	0	0	12 (29.3)	1 (2.4)	0
	pu:Ipav/Ipav[**]	36	13 (36.1)	1 (2.8)	0	0	12 (33.3)	0	0
	I. pers	**334**	**259 (77.5)**	**1 (0.3)**	**20 (6.0)**	**8 (2.4)**	**253 (75.7)**	**1 (0.3)**	**2 (0.6)**
	I. pavl	**577**	**47 (8.1)**	**20 (3.5)**	**15 (2.6)**	**0**	**10 (1.7)**	**5 (0.9)**	**0**

Abbreviations: I. pers - *I. persulcatus*; I. pavl - *I. pavlovskyi*; Rhlg – *R. heilongjiangensis*; Rh – *R. helvetica*; Rr – *R. raoultii*; Rs – *R. sibirica*; Rt – "*Candidatus* R. tarasevichiae"; Rsp – new *Rickettsia* genovariants.

[#] Including cases of mixed infection.

[*] -putative hybrids with *I. pavlovskyi* morphology.

[**] - putative hybrids with *I. persulcatus* morphology.

persulcatus females (Iper/Hybr and Iper/Ipav) were similar to *I. persulcatus* ticks regarding their high infectious rates with *B. bavariensis*, "*Candidatus* R. tarasevichiae", and *E. muris*. In contrast, the hybrids originated from *I. pavlovskyi* females as parental *I. pavlovskyi* were more often infected with *B. garinii* compared to hybrids originated from *I. persulcatus* females (Tables 3–5, Fig. 5).

As to the genetic variability of tick-transmitted agents detected in hybrids, most genetic variants of these agents have been previously found in the parental tick species (Igolkina et al., 2016; Fomenko et al., 2007; Mukhacheva and Kovalev, 2013; Rar et al., 2010, 2011, 2017; Sabitova et al., 2018; Tkachev et al., 2017). However, several new genetic variants of *B. bavariensis, B. garinii*, *R. helvetica*, *R. raoultii*, and *R. sibirica* were detected in hybrids. Nevertheless, given the high genetic variability of these infectious agents previously recorded in Western Siberia, we expect that these new genetic variants will be found in the parental tick species in the future.

Importantly, hybrid ticks and their backcrosses have a wider spectrum of gene combinations than their parental species and may therefore be better adapted to different infectious agents. Thus, we hypothesize that the existence of natural hybrids may facilitate adaptation of tick-transmitted agents, which first adapt to interspecies hybrids, and then adapt to the parental species which had not previously been a usual host for that agent. For example, *B. afzelii*, *B. bavariensis*, "*Candidatus* R. tarasevichiae", and *E. muris*, which are typical for *I. persulcatus*, have been found in single *I. pavlovskyi* ticks, but a number of hybrids from sympatric areas.

Since only one nuclear locus for genetic characterization of ticks was used, only general trends could be observed and some detailed relationships might be missed. So, the use of other nuclear loci, preferably coding sequences, should be used. However, such loci that might distinguish *I. persulcatus* and *I. pavlovskyi* have not yet been identified.

Recently, a new tick species, *Ixodes inopinatus*, which is morphologically similar to *I. ricinus* was discovered (Estrada-Peña et al., 2014). This tick species co-exists with *I. ricinus* in different European regions (Chitimia-Dobler et al., 2018) that require correct genetic characterization of *Ixodes* ticks collected from the sympatric areas. In addition, possible existence of natural hybrids of morphologically close tick species necessitates genetic analysis of both chromosomal and mitochondrial loci. Although vector competence of both *I. pavlovskyi* and *I. inopinatus* for tick-transmitted agents remains to be proven, epidemiology of tick-transmitted diseases in *I. ricinus* – *I. inopinatus* and *I. persulcatus* - *I. pavlovskyi* sympatric areas should be studied more accurately.

5. Conclusion

In summary, we observed that natural hybrids of *I. persulcatus* and *I. pavlovskyi* ticks were ubiquitous, and the abundance of hybrids was maximal in locations with similar proportions of the parental tick species. For the first time, TBEV, KemV, *B. afzelii*, *B. bavariensis, B. garinii*, *B. miyamotoi*, *R. helvetica*, *R. raoultii*, *R. sibirica*, "*Candidatus* R. tarasevichiae", *A. phagocytophilum, E. muris*, "*Candidatus* N. mikurensis", and *Bab. microti* have been found in hybrids, and the prevalence of most bacterial agents in hybrids was intermediate compared to their parental tick species. We assume that "*I. persulcatus - I. pavlovskyi"* sympatric areas are in fact "*I. persulcatus* - *I. pavlovskyi* - hybrids" sympatric areas.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Funding

The work was supported by Russian State funded budget project of ICBFM SB RAS # AAAA-A17-117020210027-9.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.05.020.

References

Araya-Anchetta, A., Scoles, G.A., Giles, J., Busch, J.D., Wagner, D.M., 2013. Hybridization in natural sympatric populations of *Dermacentor* ticks in northwestern North America. Ecol. Evol. 3, 714–724. https://doi.org/10.1002/ece3.496.

Arnold, M.L., 2004. Natural hybridization and the evolution of domesticated, pest and disease organisms. Mol. Ecol. 13, 997–1007. https://doi.org/10.1111/j.1365-294X.2004.02145.x.

Bakhvalova, V.N., Chicherina, G.S., Potapova, O.F., Panov, V.V., Glupov, V.V., Potapov, M.A., Seligman, S.J., Morozova, O.V., 2016. Tick-borne encephalitis virus diversity in ixodid ticks and small mammals in South-Western Siberia. Russia. Vector Borne Zoonotic Dis. 16, 541–549. https://doi.org/10.1089/vbz.2015.1834.

Balashov, Y.S., Grigor'eva, L.A., Oliver, J., 1998. Reproductive isolation and interspecific hybridization in ixodid ticks of the *Ixodes ricinus – I. persulcatus* group (Acarina Ixodidae). Entomol. Rev. 78, 500–508.

Belyantseva, G.I., Okulova, N.M., 1974. Seasonal changes in the activity of ixodid ticks in the natural focus of tick-borne encephalitis in the south of the Kemerovo region. Med. Parasit. Parasit. Bolezni. 43 (6), 710–715 (In Russian).

Bolotin, E., Kolonin, G., Kiselev, A., Matjushina, O., 1977. The distribution and ecology of *Ixodes pavlovskyi* (Ixodidae) on Sykhote-Alin. Parazitologiya. 3, 225–229 (In Russian).

Bugmyrin, S.V., Belova, O.A., Ieshko, E.P., Bespyatova, L.A., Karganova, G.G., 2015. Morphological differentiation of *Ixodes persulcatus* and *I. ricinus* hybrid larvae in experiment and under natural conditions. Ticks Tick. Dis. 6, 129–133. https://doi.org/10.1016/j.ttbdis.2014.11.001.

Burger, T.D., Shao, R., Barker, S.C., 2014. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, contains a cryptic species. Mol. Phylogenet. Evol. 76, 241–253. https://doi.org/10.1016/j.ympev.2014.03.017.

Chitimia-Dobler, L., Rieß, R., Kahl, O., Wölfel, S., Dobler, G., Nava, S., Estrada-Peña, A., 2018. *Ixodes inopinatus* − Occurring also outside the Mediterranean region. Ticks Tick. Dis. 9, 196–200. https://doi.org/10.1016/j.ttbdis.2017.09.004.

Estrada-Peña, A., Nava, S., Petney, T., 2014. Description of all the stages of *Ixodes inopinatus* n. sp. (Acari: Ixodidae). Ticks Tick Borne Dis. 5, 734–743. https://doi.org/10.1016/j.ttbdis.2014.05.003.

Filippova, N.A., 1977. Ixodid ticks of the subfamily ixodinae. The Fauna of the USSR. Arachnida. New Ser., vol. 4 Nauka Publishing House, Leningrad (In Russian).

Fomenko, N.V., IuB, Sabitova, Khasnatinov, M.A., Gol'tsova, N.A., Danchinova, G.A., Zh, Bataa, Ambed, D., Stronin, O.V., 2007. Heterogeneity of the gene P83/100 of *Borrelia burgdorferi* sensu lato complex. Mol. Gen. Mikrobiol. Virusol. 4, 31–37 (In Russian).

Hill, C.A., Guerrero, F.D., Van Zee, J.P., Geraci, N.S., Walling, J.G., Stuart, J.J., 2009. The position of repetitive DNA sequence in the southern cattle tick genome permits chromosome identification. Chromosome Res. 17, 77–89. https://doi.org/10.1007/s10577-008-9003-0.

Igolkina, Y., Bondarenko, E., Rar, V., Epikhina, T., Vysochina, N., Pukhovskaya, N., Tikunov, A., Ivanov, L., Golovljova, I., Ivanov, M., Tikunova, N., 2016. Genetic variability of *Rickettsia* spp. in *Ixodes persulcatus* ticks from continental and island areas of the Russian Far East. Ticks Tick. Dis. 7, 1284–1289. https://doi.org/10.1016/j.ttbdis.2016.06.005.

Kanduma, E.G., Mwacharo, J.M., Githaka, N.W., Kinyanjui, P.W., Njuguna, J.N., Kamau, L.M., Kariuki, E., Mwaura, S., Skilton, R.A., Bishop, R.P., 2016. Analyses of mitochondrial genes reveal two sympatric but genetically divergent lineages of *Rhipicephalus appendiculatus* in Kenya. Parasit. Vectors 9, 353. https://doi.org/10.1186/s13071-016-1631-1.

King, K.C., Stelkens, R.B., Webster, J.P., Smith, D.F., Brockhurst, M.A., 2015. Hybridization in parasites: consequences for adaptive evolution, pathogenesis, and public health in a changing world. PLoS Pathog. 11, e1005098. https://doi.org/10.1371/journal.ppat.1005098.

Kolonin, G.V., 1981. World Distribution of Ixodid Ticks (genus *Ixodes*). Nauka Publishing House, Moscow (In Russian).

Korenberg, E.I., Nefedova, V.V., Romanenko, V.N., Gorelova, N.B., 2010. The tick *Ixodes pavlovskyi* as a host of spirochetes pathogenic for humans and its possible role in the epizootiology and epidemiology of borrelioses. Vector Borne Zoonotic Dis. 10, 453–458. https://doi.org/10.1089/vbz.2009.0033.

Kovalev, S.Y., Mikhaylishcheva, M.S., Mukhacheva, T.A., 2015. Natural hybridization of the ticks *Ixodes persulcatus* and *Ixodes pavlovskyi* in their sympatric populations in Western Siberia. Infect. Genet. Evol. 32, 388–395. https://doi.org/10.1016/j.meegid.2015.04.003.

Kovalev, S.Y., Golovljova, I.V., Mukhacheva, T.A., 2016. Natural hybridization between *Ixodes ricinus* and *Ixodes persulcatus* ticks evidenced by molecular genetics methods. Ticks Tick. Dis. 7, 113–118. https://doi.org/10.1016/j.ttbdis.2015.09.005.

Kovalevskiy, Yu.V., Kuksgauzen, N.A., Zhmaeva, Z.M., 1975. Materials on *Ixodes pavlovskyi* Pom. distribution in Altai. Parazitologiia. 9, 518–921 (In Russian).

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 836 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Livanova, N.N., Livanov, S.G., Panov, V.V., 2011. Characteristics of the distribution of ticks *Ixodes persulcatus* and *Ixodes pavlovskyi* at the border between the forest and forest-steppe zones in the territory near Ob River. Parazitologiia. 45, 94–103 (In Russian).

Livanova, N.N., Tikunov, A.Y., Kurilshikov, A.M., Livanov, S.G., Fomenko, N.V., Taranenko, D.E., Kvashnina, A.E., Tikunova, N.V., 2015. Genetic diversity of *Ixodes pavlovskyi* and *I. persulcatus* (Acari: Ixodidae) from the sympatric zone in the south of Western Siberia and Kazakhstan. Exp. Appl. Acarol. 67, 441–456. https://doi.org/10.1007/s10493-015-9947-7.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., Lin, X., 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). Parasit. Vectors 7, 93. https://doi.org/10.1186/1756-3305-7-93.

Mal'kova, M.G., Iakimenko, V.V., Tantsev, A.K., 2012. Changes in the ranges of pasture ixodid ticks of the genus *Ixodes* Latr., 1795 (Parasitiformes, Ixodinae) in Western Siberia. Parazitologiia 46, 369–383 (In Russian).

Margos, G., Gatewood, A.G., Aanensen, D.M., Hanincová, K., Terekhova, D., Vollmer, S.A., Cornet, M., Piesman, J., Donaghy, M., Bormane, A., Hurn, M.A., Feil, E.J., Fish, D., Casjens, S., Wormser, G.P., Schwartz, I., Kurtenbach, K., 2008. MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. Proc. Natl. Acad. Sci. U.S.A. 105, 8730–8735. https://doi.org/10.1073/pnas.0800323105.

Meyer, J.M., Kurtti, T.J., Van Zee, J.P., Hill, C.A., 2010. Genome organization of major tandem repeats in the hard tick, *Ixodes scapularis*. Chromosome Res. 18, 357–370. https://doi.org/10.1007/s10577-010-9120-4.

Mukhacheva, T.A., Kovalev, S.Y., 2013. Multilocus sequence analysis of *Borrelia burgdorferi* s.l. In Russia. Ticks Tick. Dis. 4, 275–279. https://doi.org/10.1016/j.ttbdis.2013.02.004.

Mukhacheva, T.A., Kovalev, S.Y., 2014. *Borrelia* spirochetes in Russia: genospecies differentiation by real-time PCR. Ticks Tick. Dis. 5, 722–726. https://doi.org/10.1016/j.ttbdis.2014.05.016.

Oliver, J.H.Jr., Wilkinson, P.R., Kohls, G.M., 1972. Observations on hybridization of three species of North American *Dermacentor* ticks. J. Parasitol. 58, 380–384.

Oliver, J.H.Jr., Owsley, M.R., Hutcheson, H.J., James, A.M., Chen, C., Irby, W.S., Dotson, E.M., McLain, D.K., 1993. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). J. Med. Entomol. 30, 54–63.

Patterson, J.W., Duncan, A.M., McIntyre, K.C., Lloyd, V.K., 2017. Evidence for genetic hybridization between *Ixodes scapularis* and *Ixodes cookei*. Can. J. Zool. 95, 527–537.

Pomerantsev, B.I., 1948. New ticks of Ixodes family (Ixodidae). Parasit. Sbornik ZIN AS USSR. 9, 36–46 (In Russian).

Rar, V.A., Livanova, N.N., Panov, V.V., Doroschenko, E.K., Pukhovskaia, N.M., Vysochina, N.P., Ivanov, L.I., 2010. Genetic diversity of *Anaplasma* and *Ehrlichia* in asian part of Russia. Ticks Tick. Dis. 1, 57–65. https://doi.org/10.1016/j.ttbdis.2010.01.002.

Rar, V.A., Epikhina, T.I., Livanova, N.N., Panov, V.V., 2011. Genetic diversity of *Babesia* in *Ixodes persulcatus* and small mammals from North Ural and West Siberia, Russia. Parasitology 138, 175–182. https://doi.org/10.1017/S0031182010001162.

Rar, V., Livanova, N., Tkachev, S., Kaverina, G., Tikunov, A., Sabitova, Y., Igolkina, Y., Panov, V., Livanov, S., Fomenko, N., Babkin, I., Tikunova, N., 2017. Detection and genetic characterization of a wide range of infectious agents in *Ixodes pavlovskyi* ticks in Western Siberia. Russia. Parasit. Vectors. 10, 258. https://doi.org/10.1186/s13071-017-2186-5.

Rees, D.J., Dioli, M., Kirkendall, L.R., 2003. Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae). Mol. Phylogenet. Evol. 27, 131–142.

Romanenko, V., Leonovich, S., 2015. Long-term monitoring and population dynamics of ixodid ticks in Tomsk city (Western Siberia). Exp. Appl. Acarol. 66, 103–118. https://doi.org/10.1007/s10493-015-9879-2.

Sabitova, Y., Fomenko, N., Tikunov, A., Stronin, O., Khasnatinov, M., Abmed, D., Danchinova, G., Golovljova, I., Tikunova, N., 2018. Multilocus sequence analysis of *Borrelia burgdorferi* sensu lato isolates from Western Siberia, Russia and Northern Mongolia. Infect. Genet. Evol. 62, 160–169. https://doi.org/10.1016/j.meegid.2018.04.015.

Sapegina, V.F., Ravkin, Yu.S., 1969. The find of *ixodes pavlovskyi* pom. in North-East Altai. Parazitologiia. 3 (1), 22–23 (In Russian).

Takano, A., Toyomane, K., Konnai, S., Ohashi, K., Nakao, M., Ito, T., Andoh, M., Maeda, K., Watarai, M., Sato, K., Kawabata, H., 2014. Tick surveillance for relapsing fever spirochete *Borrelia miyamotoi* in Hokkaido, Japan. PLoS One 9, e104532. https://doi.org/10.1371/journal.pone.0104532.

Tkachev, S., Panov, V., Dobler, G., Tikunova, N., 2014. First detection of Kemerovo virus in *Ixodes pavlovskyi* and *Ixodes persulcatus* ticks collected in Novosibirsk region, Russia. Ticks Tick. Dis. 5, 494–596. https://doi.org/10.1016/j.ttbdis.2014.03.003.

Tkachev, S.E., Tikunov, A.Y., Babkin, I.V., Livanova, N.N., Livanov, S.G., Panov, V.V., Yakimenko, V.V., Tantsev, A.K., Taranenko, D.E., Tikunova, N.V., 2017. Occurrence and genetic variability of Kemerovo virus in *Ixodes* ticks from different regions of Western Siberia, Russia and Kazakhstan. Infect. Genet. Evol. 47, 56–63. https://doi.org/10.1016/j.meegid.2016.11.007.

Ushakova, G.V., Filippova, N.A., Panova, I.V., 1969. About the species of *Ixodes persulcatus* group (Parasitiformes, Ixodidae) IV. New data in the ecology of *I. pavlovskyi* Pom. from East Kazakhstan. Parazitologiya. 3, 436–439 (In Russian).

Zahler, M., Gothe, R., 1997. Evidence for the reproductive isolation of *Dermacentor marginatus* and *Dermacentor reticulatus* (Acari: Ixodidae) ticks based on cross-breeding, morphology and molecular studies. Exp. Appl. Acarol. 21, 685–696.