Prevalence of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in ixodid ticks in the Far East of Russia

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1. Introduction

Lyme borreliosis is one of the most common vector-borne zoonotic bacterial diseases in the world. Tens of thousands of cases are reported annually in temperate regions of North America, Eurasia and Australia (Rosa et al., 2005). Lyme disease has both acute (with a transient inflammatory skin rash known as erythema migrans, arthritis, carditis, neuropathies) and persistent clinical manifestations with chronic arthritis, neuroborreliosis, small-point rash, erythema migrans and other skin problems. The prevalence of particular Lyme disease symptoms varies between North America and Europe, with arthritis more common in the United States and neurological and skin disorders more common in Europe. These distinct clinical manifestations of Lyme disease might reflect the geographical distribution of *Borrelia* genospecies. The bacteria that cause human Lyme disease belong to a clade of 16 named species called *Borrelia burgdorferi sensu lato*. Among these species, *Borrelia burgdorferi sensu stricto* (s.s.) *Borrelia garinii*, *Borrelia afzelii* and "*Borrelia bavariensis*" sp. nov. are well-known causes of Lyme disease in North America, Europe (Casjens et al., 2011) and Russia (Rar et al., 2017). *Borrelia finlandensis* isolated from an *Ixodes ricinus* tick in Finland is closely related to *B. burgdorferi* s.s. and is suggested as candidate for new-species status (Casjens et al., 2011). Infection by *B. burgdorferi s.s.* is frequently associated with arthritis, *B. garinii* with neurological disease and *B. afzelii* with chronic skin disorders, although the correlation...
is not absolute.

Besides *B. burgdorferi* s.l. another genetically distant *Borrelia* spp. are associated with hard ticks including *Borrelia miyamotoi* and *Borrelia* sp. HM, *Borrelia* sp. HF, *Borrelia* sp. HK, *Borrelia* sp. HL in Haemaphysalis spp. ticks (Furuno et al., 2017; GenBank accession numbers LC170019-LC170035). *B. miyamotoi* phylogenetically clusters with the relapsing fever *Borrelia* species (Krause et al., 2015). The distinctive feature of *B. miyamotoi* and other relapsing fever group species includes expression of a glycrophosphodiester phosphodiesterase (GlpQ) gene (Krause et al., 2015). Transovarial transmission is another biological feature that distinguishes relapsing fever *Borrelia* species including *B. miyamotoi* from *B. burgdorferi* (Krause et al., 2015). Human cases of *B. miyamotoi* infection were first reported in 2011 in Russia (Platonov et al., 2011) and subsequently in the United States, Europe, and Japan. The most common clinical manifestations of *B. miyamotoi* infection are fever, fatigue, headache, chills, myalgia, arthralgia, and nausea with possible severe sequellae, including meningoencephalitis (Krause et al., 2015). Spirochetes represent a phylum of bacteria phylogenetically distinct from other main bacterial groups (Rosa et al., 2005). *B. burgdorferi* (B31 strain) was the third microbial genome ever sequenced (Fraser et al., 1997). Overall, *B. burgdorferi*’s genome consists of one megabase linear chromosome and a variety of circular and linear plasmids ranging in size from 9 to 62 kb. The chromosome, unlike many other eubacteria, has no relation to either the bacteria’s virulence or to the host-parasite interaction (Fraser et al., 1997). Some of the plasmids are necessary for the *B. burgdorferi* life cycle in wild nature but not for propagation of the bacteria in culture. The genetic variations of *Borrelia burgdorferi* contribute to varying degrees of infection and dissemination (Theisen et al., 1995; Kurtenbach et al., 2006).

Mechanisms controlling the *Borrelia* selection are multiple-niche polymorphism and negative frequency-dependent selection (Kurtenbach et al., 2006; Samuels and Radolf, 2010). Multiple-niche polymorphism is maintained within a population due to the varying amount possible ecological niches such as various biotopes, reservoir hosts and vectors. Both genetic diversity, various gene expression patterns and protein polymorphism are required for *B. burgdorferi* adaptation to phylogenetically divergent arthropods and vertebrate hosts. In negative frequency-dependent selection, *B. burgdorferi* rare low-frequency variants may have a selective advantage because of lack of an immunological response of arthropod hosts.

Despite high prevalence of Lyme borreliosis in the Far East of Russia (3.3–5.45 confirmed cases per 100,000 population) little is currently known about *Borrelia* biodiversity (Sato et al., 1996; Mediannikov et al., 2005; Liu et al., 2012). High prevalence of *B. burgdorferi* s.l. (Korenberg et al., 1993), predominance of *B. garinii* over *B. afzelii* (Li et al., 1998; Mediannikov et al., 2005) and absence of *Borrelia burgdorferi* sensu stricto or *Borrelia japonica* isolates (Li et al., 1998; Morozov and Morozova, 2012 and references therein) were thoroughly examined. Meanwhile, genetic diversity, geographic distribution, natural hosts and vectors of *B. miyamotoi* are at the beginning of study (Mukhacheva et al., 2015). Our aim was the comparative analysis of *Borrelia* species in ixodid ticks collected from various biotopes in the Far East of Russia.

### 2. Materials and methods

#### 2.1. Ticks

Were collected from vegetation by using 60 × 100 cm cotton cloths (flag), ticks were removed from the “flag” and placed in cotton wet bandage and cotton bag. Average number of ixodid ticks was calculated as “ticks per one flag - hour” is the score of relative abundance of ticks that is calculated as the number of unfed adult ticks averaged per 1 h, 

\[
\frac{\sum \text{all collected ticks}}{\sum \text{all collected flags}} \times \frac{1}{\text{hours}}
\]

Ticks species were determined on the base of their morphological traits according to (Filippova; 1985). Adult questing ticks were flagged from vegetation during May and June (when ticks are the most active in the region) 1999–2014 in the Far East (Amur region, Jewish Autonomous region, Khabarovsk territory and Sakhalin region) of Russia in sampling sites 1–19 differing in their geographic location, climatic conditions, vegetation and anthropogenic pressure (Fig. 1 and Table 2). Sites 1–6 are located in the Amur region; sites 7–9 - in the Jewish Autonomous region, 10–17 on the Khabarovsk territory; and sites 18 and 19 - in the Sakhalin region. A few premature ticks can also be collected from vegetation but we focused on adult ixodid ticks due to their epidemiological importance.

#### 2.2. Borrelia DNA detection

**Arthropod suspensions** were prepared from individual ticks in disposable tubes with pestles in liquid nitrogen. Since 2013 «TissueLyser II» (Qiagen, Germany) kit was used.

**Total nucleic acids** were isolated from individual tick suspensions using phenol-chloroform deproteinization with subsequent alcohol
precipitation ("Vector Best", Russia) during 1999–2009 and later in 2010–2014 it was replaced with adsorption of nucleic acids on silica by using "Ribosorb" ("InterLabService", Russia).

**PCR** with primers SL (Demeraeschlack et al., 1995) and mastermix ("Syntol", Russia) with subsequent electrophoresis was performed in 1999–2006; test-system for *Borrelia burgdorferi* s.l. detection ("IzoGen", Russia) was used during 2007–2009. Real time PCR detection and quantitation were began to use in 2010 initially with the kit "AmpLiSens B. burgdorferi sensu lato - FL" and later in 2011–2014 with "AmpLy-Sens TBE, B. burgdorferi, A. phagocytophila, E. muris/E. chaffensis - FL" ("InterLabService", Russia). *Borrelia miyamotoi* DNA was detected by PCR with subsequent electrophoresis using primers specific to p66 gene (Fomenko et al., 2010) in 2011–2013 and by real time PCR in 2014 with the kit "Vector Borrelia miyamotoi - FL" (Vector Best, Russia) according to the manufacturer's instructions.

### 2.3. Bacterial loads

Bacterial loads were estimated using quantitative real time PCR as described above with calibration curve of dependence between quantities of *Borrelia burgdorferi* s.s. strain B31 DNA with the known concentrations and threshold cycles (Ct) of fluorescence as previously published (Morozova et al., 2011).

### 2.4. Nucleotide sequences

Nucleotide sequences of PCR products were determined using primers (Fomenko et al., 2008, 2010), BigDye 3.1 Terminator Cycle Sequencing Kit and DNA analyzer ABI 3500 (Applied Biosystems, USA). GenBank (http://www.ncbi.nlm.nih.gov) accession numbers of the *Borrelia* spp. determined in our study are shown in *Table 1*. Phylogenetic analysis was performed using MEGA 6.06 (5 alternative algorithms, 1,000 replications) (Tamura et al., 2013). Reference nucleotide sequences determined in our study and examined by means of PCR for *Borrelia burgdorferi* s.l. detection. In 2011–2014 part of them (519 *I. persulcatus*) was also assayed to detect *B. miyamotoi* DNA. The *B. miyamotoi* DNA detection rate in the Far East of Russia was 36.4 ± 1.2%, whereas it was 68.8 ± 6.8 in larch-birch forest (with domination) in the Amur region (Table 2). Structure of tick populations of the Sakhalin Island differed from mainland, *I. persulcatus* was the only detected tick species (Table 2). Humidity of soil seems to be a limited factor for ixodid ticks development and is especially crucial parameter for *I. persulcatus* (Filippova; 1985). Therefore, the species remains abundant and predominant in coniferous–broadleaved forests far away from towns and villages. Other tick species including *Haemaphysalis* spp. can survive in less humid conditions and gradually replace the former prevailing *I. persulcatus*.

### 2.5. Statistical comparisons

We were carried out using percentages with the Standard Error of the Percentage (SEP) (Sheskin, 2011). Continuous variables were compared using Student's t-test. *P* values < 0.05 were assumed to be significant.

### 3. Results

#### 3.1. Distribution of ixodidae ticks in the Far East of Russia

Ixodid ticks were collected in the Far East of Russia both in the mainland part (the Amur, the Jewish Autonomous regions and the Khabarovsk territory) and on Sakhalin Island during 1999–2014 (Fig. 1, *Table 2*). Totally, 2,158 individual ixodid ticks were collected, identified and examined by means of PCR for *Borrelia burgdorferi* s.l. detection. In 2011–2014 part of them (519 *I. persulcatus*) was also assayed to detect *B. miyamotoi* DNA. The *B. miyamotoi* DNA detection rate in the Far East of Russia was 36.4 ± 1.2%, whereas it was 68.8 ± 6.8 in larch-birch forest (with domination) in the Amur region (site 17) and 6.9 ± 4.8% and 14.3 ± 5.1% in larch-white birch forest (with *H. concinna* domination) on the Khabarovsk territory (site 18) and in coniferous–broadleaved forests (sites 4–6, 10, 12, 15) was essentially less than in larch-birch and pine-birch forests (site 1, 3) or in the coniferous forest landscapes with *I. persulcatus* prevalence on the Khabkhtin mountains (Bolshokhekkhtizsky wildlife reserve, site 11) and in Solnechniy district (site 14). In the Jewish Autonomous region in coniferous–broadleaved forest (site 9) predominant *I. persulcatus* ticks reached the maximum population density (Table 2). Structure of tick populations of the Sakhalin Island differed from mainland, *I. persulcatus* was the only detected tick species (Table 2). Humidity of soil seems to be a limited factor for ixodid ticks development and is especially crucial parameter for *I. persulcatus* (Filippova; 1985). Therefore, the species remains abundant and predominant in coniferous–broadleaved forests far away from towns and villages. Other tick species including *Haemaphysalis* spp. can survive in less humid conditions and gradually replace the former prevailing *I. persulcatus*.

### 3.2. PCR detection of Borrelia DNA

**PCR detection of Borrelia burgdorferi** s.l. in *I. persulcatus* ticks was successful in all explored biotops and areas. *Borrelia* DNA detection rate reached 69.0 ± 4.6% in larch-small-leaved forest (with *I. persulcatus* domination) on the Khabarovsk territory (site 17) and 68.8 ± 6.8 in larch-birch forest (with *H. concinna* domination) in the Amur region (site 1) with low anthropogenic pressure and relatively high tick population density. *Borrelia* DNA detection rate 0–28.6% for other tick species was significantly below the corresponding values for *I. persulcatus* collected from the same regions (*P* < 0.01) (Table 3). For comparison, totally in the Far East of Russia the average infection rate of *I. persulcatus* during 1999–2014 was 36.4 ± 1.2%, whereas *H. japonica* - 14.3 ± 5.1%, *D. silvarum* - 6.9 ± 4.8% and *H. concinna* -

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**Table 1**

| Borrelia species | 16S RNA sequence | 5S–23S IGS sequence | p66 sequence | gplQ sequence |
|------------------|------------------|---------------------|--------------|--------------|
| *B. garinii*     | KY312010-KY312015, KY312118, KY346892, KY346970, KY346973, KY348890, KY924779, KY932076, KY932077, KY963154, KY963161 | - | - | - |
| *B. afzelii*     | KX622580-KX622581, KX622582, KX668604 | - | - | - |
| *B. miyamotoi*   | KX769484-KX769851 | - | - | - |
Table 2
Geographic locations of collection places and ratio of the ixodid tick species.

| Site № | District                  | Collection place (or exact location) | Biotope                          | Geographic coordinates of the site | Year | Average number (ticks per 1 flag-hour) | Tick species ratio (%) |
|--------|---------------------------|--------------------------------------|----------------------------------|-----------------------------------|------|----------------------------------------|------------------------|
|        |                           |                                      |                                  |                                   |      |                                        |                        |
| Amur region                                   |                                        |                                      |                                  |                                   |      |                                        |                        |
| 1      | Magdagachinsky            | Ductui                               | larch-birch forest               | 53°22′                            | 2011 | 85.3                                   | 33.6 65.6 * 0          | 0.8                   |
| 2      | Zeisky                    | Zeya                                 | pine–birch forest                | 53°44′                            | 2010 | N/A**                                  |                        | 0                     |
| 3      | Shimanovsky               | Belkovach                            | larch-birch forest               | 52°19′                            | 2011 | 122.0                                  | 4.9 95.1 0            | 0                     |
| 4      | Blagoveshchensky          | Raduga camp                          | deciduous forest                 | 50°40′                            | 2007 | 41.2                                   | 15.5 84.5 0           | 0                     |
| 5      | Blagoveshchensky          | Raduga camp                          | deciduous forest                 | 50°40′                            | 2008 | 13.8                                   | 41.4 58.6 0           | 0                     |
| 6      | Svoobodensky              | Kosmodrom                            | broadleaved forest               | 51°53′                            | 2011 | 30.0                                   | 8.9 88.9 0            | 2.2                   |
| 7      | Amur region               |                                      |                                  |                                   |      |                                        |                        | 0                     |
| 8      | Jewish Autonomous region  |                                      |                                  |                                   |      |                                        |                        | 0                     |
| 9      | Oktyabrsky                | Stolbovoye                           | broadleaved forest               | 47°55′                            | 2013 | 19.3                                   | 6.9 51.7 24.1        | 17.3                  |
| 10     | Leninsky                  | Churki                               | coniferous–broadleaved forest    | 48°04′                            | 2013 | 14.3                                   | 38.0 44.0 18.0        | 0                     |
| 11     | Biobidziansky             | Brhosse, 17th km                    | coniferous–broadleaved forest    | 48°41′                            | 2013 | 71.4                                   | 54.0 35.2 10.8        | 0                     |
| Khabarovsk territory                          |                                        |                                      |                                  |                                   |      |                                        |                        | 0                     |
| 10     | Iazo                      | Kiimik                               | deciduous forest                 | 47°59′                            | 2014 | 38.3                                   | 2.6 93.0 4.4         | 0                     |
| 11     | Khabarovsk                | Chekhtizir                           | coniferous–broadleaved forest    | 48°15′                            | 2014 | 182.9                                  | 88.9 2.5 5.4         | 3.2                   |
| 12     | Nanaisky                  | Troitzkoe                            | mixed broadleaved forest         | 49°22′                            | 2014 | 13.8                                   | 40.6 52.2 7.2        | 0                     |
| 13     | Solnechny                 | Gorny                                | coniferous–broadleaved forest    | 50°45′                            | 2009 | 62.0                                   | 100 0 0              | 0                     |
| 14     | Solnechny                 | Solnechny                           | mixed coniferous–small-leaved    | 50°26′                            | 2014 | 103.5                                  | 99.0 1.0 0           | 0                     |
| 15     | Komsomolsky               | Khurba                               | secondary fine-leaved forest     | 50°24′                            | 2009 | 25.0                                   | 18.0 72.0 0          | 0                     |
| 16     | Komsomolsky               | Taethny                             | coniferous–broadleaved forest    | 50°35′                            | 2014 | 4.0                                    | 90.0 10.0 0          | 0                     |
| 17     | Vaninsky                  | Toki                                 | larch–small-leaved forest        | 49°07′                            | 2011 | 57.0                                   | 100 0 0              | 0                     |
| Sakhalin region                               |                                        |                                      |                                  |                                   |      |                                        |                        | 0                     |
| 18     | Kholmsky                  | Pionery                              | mixed coniferous–small-leaved    | 47°16′                            | 2011 | 39.0                                   | 100 0 0              | 0                     |
| 19     | Yuzhno-Sakhalinsky        | Ivestkoy                             | mixed coniferous–small-leaved    | 46°50′                            | 2011 | 60.9                                   | 100 0 0              | 0                     |

Notes: *-the dominant ixodid species percent s are marked in bold; **- average numbers of ixodid ticks were not calculated.
Table 3
PCR detection of the *Borrelia burgdorferi* sensu lato DNA in the individual ixodid ticks collected from vegetation.

| Site No | Year | District               | *Ixodes persulcatus* | *Haemaphysalis concinna* | *Haemaphysalis japonica douglasi* | *Dermacentor silvarum* |
|---------|------|------------------------|----------------------|---------------------------|-----------------------------------|------------------------|
|         |      |                        | Number of studied ticks | Number of ticks with the Borrelia DNA | Rate (%) | Number of studied ticks | Number of ticks with the Borrelia DNA | Rate (%) | Number of studied ticks | Number of ticks with the Borrelia DNA | Rate (%) |
| Amur region | |                         |                       |                           |          |                       |                           |          |                       |                           |          |
| 1       | 2011 | Magdagachinsky         | 48                    | 33                        | 68.8 ± 6.8 | 51                    | 2                        | 3.9 ± 2.7 | 0                       |                           | 1        |
| 2       | 2011 | Shimanovsky            | 9                     | 2                         | 22.2 ± 14.7 | 89                    | 4                        | 4.5 ± 2.2 | 0                       |                           | 2        |
| 3       | 2011 | Svobodnensky           | 12                    | 2                         | 16.7 ± 11.2 | 85                    | 3                        | 3.5 ± 2.0 | 0                       |                           | 3        |
| 4       | 2010 | Selendzhinsky          | 0                     | 0                         | 0          | 56                    | 0                        | 0          | 0                       |                           | 0        |
| 5       | 2010 | Zeisky                 | 82                    | 34                        | 41.5 ± 5.5 | 0                     |                           | 0          | 17                      | 2                         | 11.8 ± 8.1 |
| 6       | 2007 | Blagoveschenskoy       | 30                    | 8                         | 26.7 ± 8.2 | 70                    | 0                        | 0          | 0                       |                           | 0        |
| 6       | 2008 | Blagoveschenskoy       | 27                    | 12                        | 44.4 ± 9.7 | 73                    | 0                        | 0          | 0                       |                           | 0        |
| 1-6     | 2007-2011 |                  | 208                   | 91                        | 43.8 ± 3.4 | 424                   | 9                        | 2.1 ± 0.7 | 0                       |                           | 23        |
| Jewish Autonomous region | |                         |                       |                           |          |                       |                           |          |                         |                           | 8.7 ± 6.0 |
| 7       | 2013 | Oktyabrsky             | 20                    | 2                         | 10.0 ± 6.9 | 2                     | 0                        | 0          | 0                       |                           | 5        |
| 8       | 2013 | Leninsky               | 23                    | 6                         | 26.1 ± 9.4 | 0                     |                           | 0          | 0                       |                           | 0        |
| 9       | 2013 | Birolzhinsky           | 50                    | 27                        | 54.0 ± 7.1 | 13                    | 0                        | 0          | 0                       |                           | 0        |
| 7-9     | 2013 |                        | 93                    | 35                        | 37.6 ± 5.1 | 15                    | 0                        | 12         | 0                       |                           | 5        |
| Khabarovsk territory | |                         |                       |                           |          |                       |                           |          |                         |                           | 0        |
| 10      | 2014 | Lazo                   | 15                    | 3                         | 20.0 ± 10.7 | 21                    | 1                        | 4.8 ± 4.8 | 13                      |                           | 0        |
| 11      | 1999-2009 |                  | 510                   | 152                       | 287 ± 2.0 | 0                     |                           | 0          | 0                       |                           | 0        |
| 11      | 2010-2014 |                  | 250                   | 136                       | 544 ± 3.2 | 0                     |                           | 0          | 0                       |                           | 0        |
| 12      | 2014 | Nansiky               | 26                    | 9                         | 346 ± 9.5 | 21                    | 4                        | 19.0 ± 8.8 | 3                       | 1                         | 33.3 ± 33.3 |
| 13      | 2009 | Solnechny             | 40                    | 10                        | 250 ± 6.9 | 0                     |                           | 0          | 0                       |                           | 0        |
| 14      | 2014 | Solnechny             | 66                    | 17                        | 258 ± 5.4 | 0                     |                           | 0          | 0                       |                           | 0        |
| 15      | 2009 | Komsomolsky           | 50                    | 10                        | 200 ± 5.7 | 0                     |                           | 0          | 0                       |                           | 0        |
| 16      | 2014 | Komsomolsky           | 21                    | 7                         | 333 ± 10.5 | 0                     |                           | 21         | 6                        | 28.6 ± 10.1 | 0        |
| 17      | 2011 | Vaninsky              | 100                   | 69                        | 690 ± 4.6 | 0                     |                           | 0          | 0                       |                           | 0        |
| 10-17   | 1999-2014 |                 | 1098                  | 413                       | 37.6 ± 1.5 | 42                    | 5                        | 11.9 ± 5.1 | 37                      | 7                         | 18.9 ± 6.5 |
| Sakhalin region | |                         |                       |                           |          |                       |                           |          |                         |                           | 0        |
| 18      | 2011 | Kholsky               | 100                   | 19                        | 190 ± 3.9 | 0                     |                           | 0          | 0                       |                           | 0        |
| 19      | 2011 | Yurhno-Sakhalinsky    | 100                   | 24                        | 240 ± 4.3 | 0                     |                           | 0          | 0                       |                           | 0        |
| 18, 19  | 2011 |                        | 200                   | 43                        | 215 ± 2.9 | 0                     |                           | 0          | 0                       |                           | 0        |
| Far East | |                         |                       |                           |          |                       |                           |          |                         |                           | 6.9 ± 4.8 |
| 1-19    | 1999-2014 |                | 1599                  | 582                       | 36.4 ± 1.2 | 481                   | 14                        | 2.9 ± 0.8 | 49                      | 7                         | 14.3 ± 5.1 |

Notes: 1 – results of real time PCR, 2 – data of PCR with subsequent electrophoresis.
was detected in 8.9 ± 1.3% of the studied ticks. The Borrelia miyamotoi DNA detection in I. persulcatus ticks from various regions of the Russian Far East is presented in Table 4. DNA was detected in 2.0–27.8% of the ticks studied.

### Table 4

| Region          | No of tick collection site | Ixodidae tick species | Number of samples with the Borrelia DNA | Ct range (min-max) | Average Ct | Average quantity of genome-equivalents per a tick | B. miyamotoi monoinfection | B. miyamotoi + B. burgdorferi s.l. | B. miyamotoi in total |
|-----------------|---------------------------|-----------------------|----------------------------------------|--------------------|------------|------------------------------------------------|---------------------------|-------------------------------|--------------------------|
| Amur region     |                           | I. persulcatus        | 33                                     | 11.47–31.28        | 22.84 ± 7.05 | 1.46 × 10(5)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 2                                      | 15.05–17.35        | 16.20 ± 1.63 | 1.46 × 10(7)                                   |                           |                               |                          |
| Amur region     |                           | I. persulcatus        | 2                                      | 19.43–19.73        | 19.58 ± 0.21 | 1.40 × 10(6)                                   |                           |                               |                          |
| Khabarovsk region |                           | I. persulcatus        | 34                                     | 18.93–30.32        | 24.73 ± 2.70 | 5.93 × 10(5)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 2                                      | 12.44–14.74        | 13.59 ± 1.63 | 5.35 × 10(9)                                   |                           |                               |                          |
| Amur region     |                           | I. persulcatus        | 27                                     | 10.68–17.44        | 13.41 ± 1.85 | 6.06 × 10(9)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 10                                     | 14.65–30.04        | 19.90 ± 8.79 | 6.72 × 10(7)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 136                                    | 10.62–31.84        | 20.03 ± 6.42 | 6.16 × 10(7)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 9                                      | 10.42–23.73        | 18.48 ± 6.33 | 1.81 × 10(8)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 17                                     | 10.79–25.97        | 17.97 ± 5.59 | 2.57 × 10(8)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 7                                      | 11.01–22.53        | 14.58 ± 4.01 | 2.69 × 10(9)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 69                                     | 9.33–33.91         | 22.56 ± 7.03 | 1.07 × 10(7)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 19                                     | 11.71–31.64        | 19.28 ± 7.57 | 1.04 × 10(8)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | I. persulcatus        | 24                                     | 11.27–31.05        | 20.07 ± 7.20 | 5.99 × 10(7)                                   |                           |                               |                          |
| Total           |                           | I. persulcatus        | 390                                    | 9.33–33.91         | 20.16 ± 0.18 | 5.63 × 10(7)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. concinna           | 2                                      | 29.49–34.20        | 31.85 ± 3.33 | 2.84 × 10(2)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. concinna           | 4                                      | 29.98–32.21        | 32.02 ± 1.41 | 2.52 × 10(2)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. concinna           | 3                                      | 30.30–33.01        | 31.29 ± 1.49 | 4.18 × 10(2)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. concinna           | 1                                      | 30.69              | 30.69      | 3.81 × 10(4)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. concinna           | 4                                      | 24.42–26.48        | 24.95 ± 0.94 | 2.88 × 10(6)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. concinna           | 14                                     | 24.42–34.20        | 26.53 ± 3.60 | 1.13 × 10(4)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. japonica           | 1                                      | 13.96              | 13.96      | 4.14 × 10(9)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. japonica           | 6                                      | 20.47–21.86        | 21.22 ± 0.48 | 4.50 × 10(5)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | H. japonica           | 7                                      | 13.96–21.86        | 20.18 ± 2.78 | 5.55 × 10(7)                                   |                           |                               |                          |
| Jewish Autonomous region |                   | D. silverum           | 2                                      | 28.35–28.94        | 28.65 ± 0.42 | 2.61 × 10(3)                                   |                           |                               |                          |

Notes: 1 – data of PCR with electrophoresis detection; 2 – results of real-time PCR.

2.9 ± 0.8% of the studied ticks were positive for Borrelia miyamotoi DNA. DNA was detected in 2.0–33.3% of the I. persulcatus ticks collected from various biotopes of the Jewish Autonomous region, the Sakhalin region, and the Khabarovsk territory (Table 4). B. miyamotoi was not detected in the Amur region (Table 4) despite availability of I. persulcatus in the examined region. On an average, B. miyamotoi was detected in 8.9 ± 1.3% I. persulcatus ticks (as mono infection in 4.0 ± 0.9% or as mixed infection with B. burgdorferi s.l. in 4.8 ± 0.9%) with reduced rate in comparison with B. burgdorferi s.l. (Tables 3 and 4).

### 3.3. Quantitative estimations of Borrelia DNA in ixodid ticks

Average threshold cycles (Ct) of real time PCR with the analyzed B. burgdorferi s.l. DNA significantly differed among four examined tick species with the maximal deduced bacterial loads for I. persulcatus and...
Multiple locus sequence analysis (MLSA) of *B. miyamotoi* nucleotide sequences of three fragments of 16S rRNA, glpQ and p66 genes showed similar patterns (Fig. 3). Isolates of *B. miyamotoi* from the Far East of Russia belong to the Asian group previously found in Japan, China, Siberia and Ural (Fig. 3). All our nucleotide sequences of 16S rRNA and 5S rRNA were identical to each other and to the strain FR64b (CP004217) from Switzerland (Fig. 2) with the single nucleotide polymorphism (SNP) in 5S–23S rRNA ITS of *Borrelia* isolate Vanino (KX685729) similar to *Borrelia* sp. isolate Tokachi-J-IP21f (EF160140) from Japan. One should note that the only *B. afzelii* isolates from ixodid ticks was identical to *B. afzelii* HLJ01 strain (CP003882) from China and clustered with strain VS461 (GenBank accession number NR164748) from Switzerland (Fig. 2) with the single nucleotide polymorphism (SNP) in 5S–23S rRNA ITS of *Borrelia* isolate Vanino 2011-2 (KX685729) similar to *Borrelia* sp. isolate Tokachi-J-IP21f (EF160140) from Japan. One should note that the only *B. garinii* (9 isolates) were found on the Sakhalin Island despite the simultaneous circulation of both *B. garinii* (23 isolates) and *B. afzelii* (12 isolates) in the continental part of the Far East of Russia. Observed fluctuations of ratios between *B. garinii* and *B. afzelii* in two areas of the Khabarovsk territory with prevalence of *B. garinii* over *B. afzelii* (18:8, respectively) and in two collection places of the Amur region with the corresponding ratio species between *B. garinii* and *B. afzelii* near 1 (5:4) were not significant due to small sampling sizes (Fig. 2). Phylogenetic analysis of nucleotide sequences of both 16S rRNA gene and 5S–23S rRNA ITS does not permit to distinguish between *B. garinii* and *Borrelia bavariensis* (Fig. 2). Both species belong to the same clade with good bootstrap indexes (Fig. 2).

### 3.5. MLSA analysis of *B. miyamotoi*

Multiple locus sequence analysis (MLSA) of *B. miyamotoi* nucleotide sequences of three fragments of 16S rRNA, glpQ and p66 genes showed similar patterns (Fig. 3). Isolates of *B. miyamotoi* from the Far East of Russia belong to the Asian group previously found in Japan, China, Siberia and Ural (Fig. 3). All our nucleotide sequences of 16S rRNA and p66 genes were identical to each other and to the strain FR64b (CP004217) from Japan, the only glpQ gene sequence includes the SNP identical to Siberian strain 57Nsk (FJ940729).
Fig. 2. Phylogenetic analysis of the nucleotide sequences of *Borrelia* 16S rRNA gene fragment of 733 bp long (A) and SS–23S ITS of 211–218 bp long (B) using Mega 6.06 software, UPGMA algorithm and 1,000 replications. Phylogenetic trees constructed by means of 5 alternative algorithms (Maximum likelihood, Neighbor-Joining, Minimum-Evolution, UPGMA and Maximum Parsimony) show similar topologies and reasonable bootstrap support. Nucleotide sequences of *Borrelia* isolates determined in our study are shown in bold. The number of identical sequences of *Borrelia* isolates determined in these ixodid ticks is shown in parentheses. Branches corresponding to the reference strains are underlined.
(caption on next page)
persulcatus P. Schulze, 1930; Haemaphysalis phasiana Saito and Wassef, 1974; Haemaphysalis concinna Koch, 1844; Haemaphysalis japonica Nuttall et Warburton, 1915 and Dermacentor silvarum Olenev, 1932 were found on the Khabarovsk territory. The four tick species including I. persulcatus, H. concinna, H. japonica, D. silvarum predominated and have epidemiological importance (Volkov, 2005). During our period of observation 1999–2014 the four prevailing tick species - I. persulcatus, H. concinna, H. japonica, D. silvarum were found in all examined regions. Minor tick species were not observed, therefore, they were not analyzed. Monodominant populations of ixodids with the only species I. persulcatus remain on the Sakhalin Island and on Kamchatka Peninsula (Pukhovskaya et al., 1991, 2010). Tick numbers and dominance structure depend on anthropogenic influence. In afforestation biotopes both total number of ixodid ticks and proportion of taiga ticks dissipate (Volkov, 2005). Wide distribution of Borrelia DNA in the ixodid ticks of different species collected from all the studied areas of the Far East of Russia including Kamchatka Peninsula (Pukhovskaya et al., 2010) was shown (Tables 2–4). Nevertheless, maximal values of Borrelia DNA detection rate and bacterial loads were observed for I. persulcatus that together with its wide distribution throughout forest zone of Eurasia suggest its leading role as vector of B. burgdorferi s.l. Our comparative analysis confirmed the previous observation of principal role of I. persulcatus to cause Lyme disease in China and at the border with Russia (Liu et al., 2012).

Phylogenetic analysis of 16S rRNA gene and 5S–23S rRNA intergenic spacer nucleotide sequences revealed two species of B. burgdorferi s.l. complex, Borrelia garinii of prevailing Asian type NT29 with a few isolates of European type 20047 and Borrelia afzelii close toVS461 reference strain in ixodid ticks collected from all the examined regions besides the Sakhalin Island with the B. garinii only. One should note that currently according to BLAST homology search among GenBank deposited nucleotide sequences Borrelia DNA genetic variability in I. persulcatus ticks remains low: 1) for 16S RNA gene - 0% for B. afzelii, 0–1% for B. garinii, 0–2% for B. miyamotoi; 2) for 5S –23S ITS - 0–2% for B. garinii and 1–2% for B. afzelii; 3) for glpQ –0–4% for B. miyamotoi; 4) for p66 –0–5% for B. miyamotoi. One should note that variability of B. miyamotoi glpQ and p66 gene fragments slightly exceeded nucleotide change levels of 16S RNA gene and 5S–23S ITS. The remarkable genetic stability of Borrelia might suggest epigenetic regulation for adaptation to evolutionary divergent invertebrate vectors and vertebrate.

Molecular typing of Borrelia on the base of a single fragment of coding region or intergenic spacer as well as numerous attempts to use random set of several genetic loci were not always successful (Baranton and Postic, 2006). It may be caused by orthologous and paralogous genes localized on numerous plasmids with varying copy numbers in wild populations and possible loss during laboratory passaging. Therefore, description of natural diversity of Borrelia by analysis of laboratory strains or single locus (Sato et al., 1996) and especially fragment of the plasmid gene encoding outer surface protein A (Mediannikov et al., 2005) can lead to misrepresentations and wrong interpretations. MLSA of wild isolates (without a laboratory passage) with similar topologies of phylogenetic trees with reasonable bootstrap support (Figs. 2 and 3) provided the convincing evidences of spatial homogeneity and temporal stability of species. Differences exist between Borrelia isolates according to tick vector and geographic region, but little genetic difference has been found between isolates within a given geographic area or with the same tick vector association (Figs. 2 and 3) (Morozov and Morozova, 2012 and references therein). Despite detection of Borrelia valaisiana in China (Hao et al., 2011) they were not detected in the Far East of Russia (Fig. 2). Low-frequency Borrelia variants were not found in ixodid ticks surviving without immune system at ambient temperatures (Fig. 2, 3). Genetic similarity was observed for Borrelia isolates from invertebrate vectors and vertebrate reservoir hosts as well as from patients (Figs. 2 and 3). The available data support concept of multiple ecological niches (such as various vectors, reservoir hosts and biotops) with minimal genetic polymorphism. But further study of Borrelia adaptation to divergent arthropods and vertebrate hosts is required. Rich ixodofauna of the Far East of Russia along with multiple vertebrate reservoir hosts (Volkov, 2005) provide multiple ecological niches not only for tick-borne borrelia with high spacial and temporal genetic stability but even for relative stable wild populations of RNA-containing tick-borne encephalitis virus (Pukhovskaya et al., 2018).

However, recent study revealed that in Siberia main part of borrelioses was caused by B. miyamotoi but not B. burgdorferi s.l. as previously believed (Titkov et al., 2018). The recently described infection with B. miyamotoi that is transmitted by the same vector - I. persulcatus ticks and proceeds as fever with unspecific symptoms without erythema and relatively rare serious complications (Krause et al., 2015). Despite lower bacterial loads and decreased infection rate of ticks with B. miyamotoi compared to B. burgdorferi s.l. the epidemiological significance is emerging. High abundance of ixodid ticks in the Far East of Russia, high infection rate of different tick species with Borrelia spp. with enormous bacterial loads and absence of vaccines may be reasons of stable and high Lyme disease rate.

5. Conclusion

Comparative analysis of both infection rate and bacterial loads of four prevailing species of ixodid ticks with Borrelia burgdorferi s.l. revealed the leading role of Ixodes persulcatus in the Far East of Russia. Genetic homogeneity and temporal stability of B. garinii, B. afzelii and B. miyamotoi suggest multiple ecological niches as mechanism providing their stability.

Conflicts of interest

There are no conflicts to declare.

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