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ORIGINAL PAPER

The diversity and prevalence of hard ticks attacking human hosts in Eastern Siberia (Russian Federation) with first description of invasion of non-endemic tick species

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Abstract Hard ticks are the vectors of many pathogens including tick-borne encephalitis virus and the Lyme disease agent *Borrelia burgdorferi* sensu lato. In Eastern Siberia, *Ixodes persulcatus*, *Dermacentor nuttalli*, *Dermacentor silvarum* and *Haemaphysalis concinna* are regarded as aggressive to humans. Recently, significant changes in world tick fauna have been reported and this affects the spread of tick-borne pathogens. We studied the current species diversity, population structure and prevalence of tick-borne pathogens of hard ticks (Acari: Ixodidae) that attacked humans in Eastern Siberia (Irkutsk region, Russia). In total, 31,892 individual ticks were identified and analysed during the years 2007–2014. The majority (85.4 %) of victims was bitten by *I. persulcatus*, 14.55 % of attacks on humans were caused by *D. nuttalli* and *D. silvarum*, whereas *H. concinna* was documented only in 15 cases (0.05 %). The seasonal activity and the age/gender structure of the tick population were studied as well. Among all the studied ticks, three unconventional species, i.e. *Rhipicephalus sanguineus*, *Dermacentor reticulatus* and *Amblyomma americanum*, were identified. Analysis of tick bite histories indicates at least three events of invasion of non-endemic ticks into the ecosystems of northern Eurasia with harsh continental climates. Invading ticks are able to reach the adult life stage and are aggressive to the local human population. Phylogenetic analysis of mt 16S rRNA gene fragments suggests multiple independent routes of tick migration to Eastern Siberia. Possible implications to human health and epidemiology of tick-borne infections are discussed.

Keywords *Ixodes* · *Dermacentor* · *Haemaphysalis* · *Rhipicephalus* · *Amblyomma* · Human host · Invasion · Russia

Background

Ixodid ticks are vectors of several dangerous human pathogens. The most important of them are tick-borne encephalitis virus (TBEV, *Flaviviridae*) and *Borrelia burgdorferi* sensu lato (*Spirochaetaceae*). TBEV annually causes about 3000 cases of tick-borne encephalitis (TBE) in Europe and up to 10,000 cases in the Russian Federation (Korenberg and Likhacheva 2006; Donoso Mantke et al. 2008). The agent of Lyme disease (LD) *B. burgdorferi* sensu lato is spread worldwide and causes approximately 85,500 cases of disease annually, including up to 9000 cases per year in the Russian Federation (Hubalek 2009; Korenberg and Likhacheva 2006). Several "emerging" tick-borne diseases have been described in recent decades including human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME) caused by

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Anaplasma phagocytophilum and *Ehrlichia chaffeensis*, respectively (reviewed in Ismail et al. 2010).

In the Irkutsk region (Eastern Siberia, Russia), tick-borne diseases pose serious threats to the health of local residents and visitors. To reduce the risk of human infection, the "Centre for Diagnosis and Prevention of Tick-Borne Diseases" (hereinafter, "the Centre") was established at the Federal Budgetary Scientific Center for Family Health and Human Reproduction Problems (FBSC FHHRP) in Irkutsk. Each tick is routinely tested for infection with TBEV and *B. burgdorferi* sensu lato, and if the pathogen is detected in the tick, the patient receives the treatment with anti-TBEV immunoglobulins and/or antibiotics to prevent the disease according to modern Russian healthcare regulations (Sanitary rules SP3.1.3.2352-08 2008). Approximately 7000 bite victims attend the Centre after tick bites annually, and more than half of them deliver the causative tick specimen (Khasnatinov et al. 2012). Six species of hard ticks are common in Eastern Siberia—*Ixodes persulcatus* Schulze, 1930; *Ixodes lividus* Koch, 1844; *Ixodes trianguliceps* Birula, 1895; *Dermacentor nuttalli* Olenev, 1929; *Dermacentor silvarum* Olenev, 1932; and *Haemaphysalis concinna* Koch, 1844. Besides these, several findings of *Ixodes subterraneus* Filippova, 1961; *Ixodes crenulatus* Koch, 1844; *Ixodes berlesei* Birula, 1895; and *Ixodes stromi* Filippova, 1957 have been reported from the natural landscapes of Eastern Siberia and neighbouring territories. However, only four of them, *I. persulcatus*, *D. nuttalli*, *D. silvarum* and *H. concinna* have been reported as vectors of tick-borne diseases to humans (Korenberg and Lebedeva 1969; Filippova 1977; Kolonin 1981; Emelyanova et al. 1985; Danchinova et al. 2006).

In recent decades, significant changes in tick fauna caused by the increased distribution range of *Ixodes ricinus* Linnaeus, 1758; *Ixodes pavlovskyi* Pomerantsev, 1946; and *Rhipicephalus sanguineus* Latreille, 1806 have been described in many countries (Mal'kova et al. 2012; Jaenson et al. 2012; Gray et al. 2009; Dantas-Torres 2010). The key drivers of this spread are presumed to be changes in climate, anthropogenic modifications of habitat conditions and local ecological/geographical factors (Medlock et al. 2013). As each of these key drivers is present in Eastern Siberia, in this study, we evaluated the current species diversity and prevalence of Ixodid ticks attacking humans in Irkutsk region and neighbouring territories during eight consecutive seasons of tick activity between 2007 and 2014. The study revealed that, besides the well-known endemic ticks, local residents are regularly attacked by uncommon tick species, whose natural distribution ranges are localised thousands of kilometres from Irkutsk City and whose optimal habitat conditions are far different from the climate, vegetation and vertebrate host diversity of Eastern Siberia.

Materials

The study was performed in Irkutsk City and neighbouring territories within a range of about 100 km (Fig. 1). This area is situated in Eastern Siberia, Russia, with landscapes formed by typical taiga forests and harsh continental (borderline subarctic) climate.

Ticks were delivered to the Centre by bitten people between the 25th of March, 2007 and the 28th of October, 2014. Each patient was interviewed to collect the following information: the time, geographic location and circumstances of tick attack; the age, gender and occupation of the patient; the history of vaccination against TBE; the history of tick bites and tick-borne diseases in the past; and, finally, the current state of the patient's health. Written informed consent for participation in the research was also obtained from each patient. The design of the research was approved by the Committee for Biomedical Ethics of FSPSI "SC FHHR".

Methods

Tick species identification

Each tick was washed twice in 70 % ethanol and once in distilled water and dried on filter paper. Its life stage and species were identified, and then it was immediately dissected for pathogen detection. The developmental stage and identification of tick species was done using key guides to tick fauna of Russia and adjacent countries (Filippova 1977; Serdjukova 1956; Filippova 1997), USA (Goddard and Norment 2006) and the world (Walker et al. 2000). Due to the time restrictions, we did not differentiate the *D. nuttalli* and *D. silvarum* species; however, any uncommon *Dermacentor* sp. ticks were identified to species level.

Previously, it has been demonstrated that mitochondrial 16S ribosomal RNA (rRNA) gene is useful both to identify the tick species (Lv et al. 2014) and to analyse the phylogeny, population genetics and phylogeography of ticks (Black and Piesman 1994; Mangold et al. 1998; Nava et al. 2012). Therefore, we amplified by polymerase chain reaction (PCR), sequenced and analysed a fragment of mt 16S rRNA gene from each available uncommon tick specimen and from several reference specimens of endemic species.

Extraction of nucleic acids from ticks and polymerase chain reaction

The DNA was extracted using a RiboPrep kit (AmpliSens, Moscow) from the remnants of individual ticks after pathogen detection tests. Approximately 100 ng of total DNA was used as template in 25 μl of PCR reaction mix with 10 pmol of each primer and HS Taq DNA-polymerase kit (Evrogen, Moscow) according to the manual of the supplier. To amplify the 440-bp

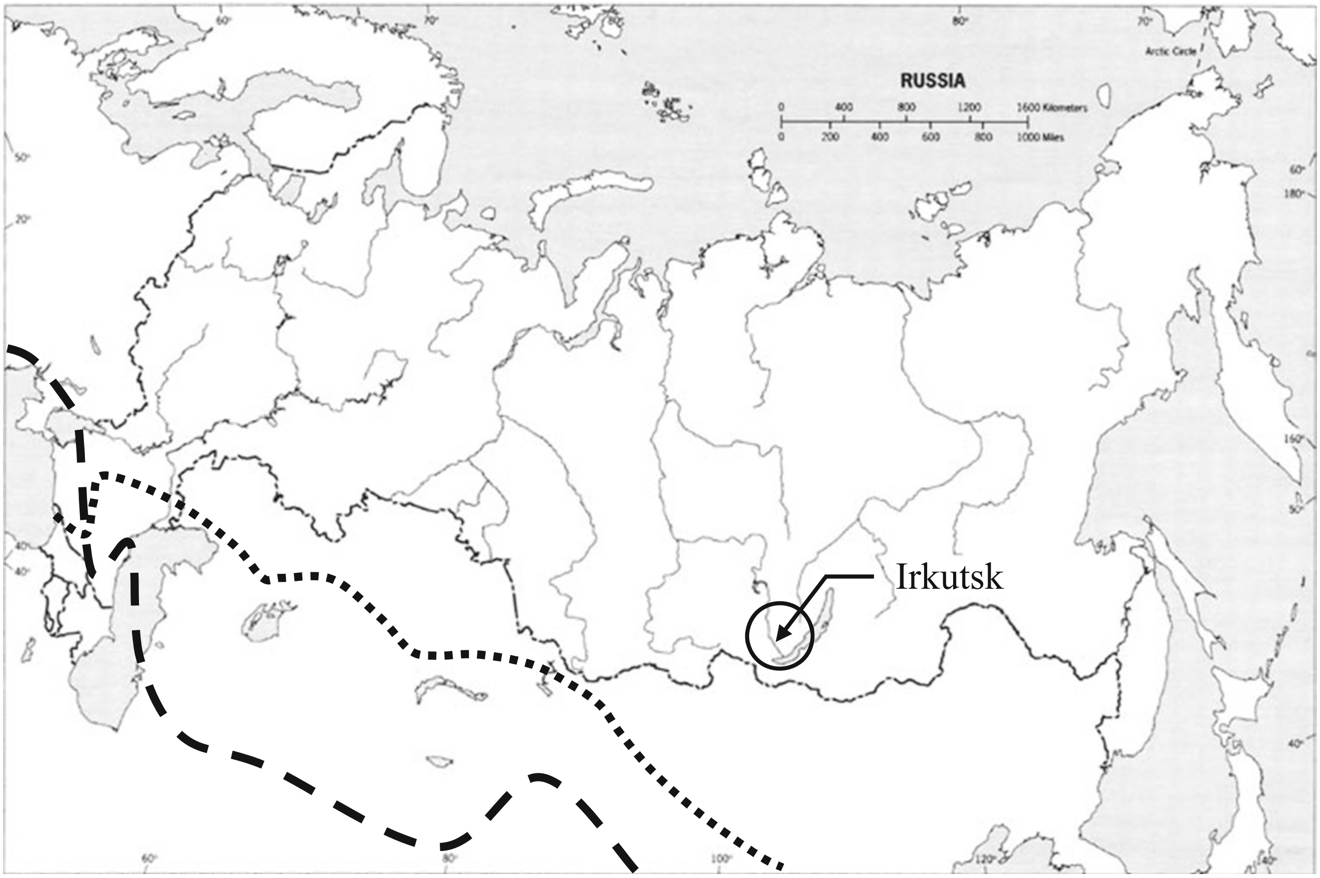

Fig. 1 The map of the research area. The approximate area of surveillance of tick bites to human hosts is designated by an *open circle*. The *arrowhead* corresponds to Irkutsk City. The *thick dotted line* designates the northern border of the distribution range of *R. turanicus*. The *thick dashed line* designates the northern border of the Eurasian distribution range of *R. sanguineus* (according to Kolonin 2009)

fragment of the mt 16S rRNA gene, we used the modified primers according to Black and Piesman (1994): 16Sf (5′-TTGCTGTGGTATTTTGACTA-3′) and 16Sr (5′-CCGGTCTGAACTCAGATC-3′). The amplification was performed with the following conditions: initial denaturation at 95 °C for 3 min followed by 30 cycles of 94 °C for 15 s, 60 °C for 15 s, 72 °C for 40 s and final extension at 72 °C for 5 min. Fragments of the expected size were detected and purified by electrophoresis in a 0.8 % agarose gel with ethidium bromide staining. From 1 to 2 μl of DNA solution containing the PCR fragment was used as a template for sequencing reactions with the ABI Prism Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems, Switzerland) according to the manufacturer's instructions. Both positive and negative strands of each PCR product were sequenced using 3 pmol of PCR primers. The products of the sequencing reactions were run on an ABI 3500xL Genetic Analyzer (Applied Biosystems).

Statistical and phylogenetic analysis

All data were presented as mean values with 95 % confidence intervals (95 % CI) estimated for each dataset. Calculations were performed using the MS Office Excel software package. The nucleotide sequences were edited manually and aligned using the ClustalW program implemented in BioEdit software (Hall 1999). The phylogenetic analysis was performed by three different methods, i.e. maximum likelihood (ML), neighbour joining (NJ) and maximum parsimony (MP). The reliability of the phylogenetic reconstructions was evaluated by bootstrap analysis with 1000 replications. Evolutionary analyses were conducted in MEGA6 software (Tamura et al. 2013).

Results

Species diversity and population structure of ticks attacking human hosts

In total, 31,892 individual ticks were identified and studied for the presence of tick-borne pathogens between the years 2007 and 2014. The diversity of ticks that attacked humans comprised six species. The most abundant species was *I. persulcatus* with a mean incidence of 3405±220 bites per year. Two closely related species *D. nuttalli* and *D. silvarum* provided a mean incidence of 579±130 bites per year, whereas another endemic species, *H. concinna*, appeared to be less aggressive to humans with only 1.9±1.1 attacks per year. In total, during 8 years of observations, *I. persulcatus* caused approximately 85.4 % of bites, *D. nuttalli*/*D. silvarum*—14.5 % and *H. concinna*—0.05 %. Surprisingly, three very uncommon tick species were identified among the ticks delivered for analysis, *R. sanguineus* (three cases), *Dermacentor reticulatus* (single case) and *Amblyomma americanum* (single case).

The earliest bite of *D. nuttalli/D.silvarum* was registered on the 13th of March 2009, whereas the earliest attack of *I. persulcatus* occurred on the 27th of March 2014. The latest attacks of these ticks on humans were documented on the 28th of October 2014 and 12th of October 2010, respectively. Bites of *H. concinna* were documented in 7 out of 8 years (with the exception for 2009) but exclusively during June and July. *I. persulcatus* that bit humans were most commonly females (91.6±2.3 % of all the attacks during each season), whereas males (6.7±0.6 %) and nymphs (1.7±2 %) attacked rarely. No larvae were delivered during 8 years of observations. The *Dermacentor* sp. had a more balanced gender structure with 60.4 % females, 39.2 % males and 0.4 % nymphs among all the ticks feeding on the human host. For *H. concinna*, the sample size was too small to elucidate the age-gender structure. *R. sanguineus* attacked residents of Irkutsk City in May, June and October, and single bites of *A. americanum* and *D. reticulatus* were documented on the 8th of June 2008 and the 9th of May 2009, respectively. The seasonal activity and population structure of ticks that attacked humans are summarised in Table 1.

Analysis of tick bite histories indicates invasion of non-endemic tick species into Eastern Siberian ecosystems

Analysing the circumstances of attacks of humans by ticks belonging to uncommon species, we revealed that ticks 1408-09 (*D. reticulatus*) and 3358-14 (*R. sanguineus*) were from people who were bitten while visiting remote regions in the European part of Russia where these ticks are common—Tula region and Rostov-on-Don City, respectively. However, three other ticks, i.e. 3465-08, 2550-13 and 6138-14, were delivered by people who were bitten in the suburbs of Irkutsk City (Table 2). According to morphological features, we attributed the ticks to species *A. americanum* (3465-08, Fig. 2a, b) and *R. sanguineus* (2550-13 and 6138-14, Fig. 2c, d). All three cases occurred in different localities near Irkutsk City in the years 2008, 2013 and 2014. Each case was caused by a questing adult tick belonging to the non-endemic species that are not inhabiting Eastern Siberian ecosystems. According to interview data, all three bitten residents neither travelled outside Irkutsk region nor contacted with vertebrate animals imported from other regions of Russia or from other countries. Thus, we hypothesised that these cases represent three independent events of the invasion of non-endemic ticks *A. americanum* and *R. sanguineus* into Eastern Siberia.

Genetic identification of tick species and evolutionary relationships of invading ticks in Eastern Siberia

To confirm the species identity of uncommon ticks, we analysed the mt 16S rRNA gene fragments of seven tick specimens from Irkutsk region including three specimens of *R. sanguineus* and four specimens of the endemic Eastern Siberian ticks *I. persulcatus* (both female and male), *D. nuttalli* and *H. concinna* that were delivered to the Centre by bitten humans during the season of 2014 (Table 2). Unfortunately, the samples 3465-08 for *A. americanum* and 1408-09 for *D. reticulatus* were not available for DNA extraction and PCR amplification. Phylogenetic analysis included reference sequences of 19 species of ticks from the genera *Ixodes*, *Dermacentor*, *Haemaphysalis* and *Rhipicephalus*. The sequence of the soft tick *Ornitodoros porcinus* was taken as an

Table 1 Seasonal activity and population structure of ticks that attacked humans

Tick species	Annual structure of the seasonal activity, date±95 % CI (days)			Duration of tick activity, days	Annual number of documented tick bites, mean value±95 % CI			
	First tick bite	Maximal number of bites	Last tick bite		Total	Female	Male	Nymph
I. persulcatus	April, 9±10	June, 4±6	September, 21±13	165±13	3405±220	3079±264 (91.6 %)[a]	225±35 (6.7 %)[a]	55±62 (1.7 %)[a]
Dermacentor sp.	April, 1±12	May, 11±13	October, 23±4	205±15	579.25±130	348±58 (61.8 %)[a]	226±81 (38 %)[a]	2±2 (0.3 %)[a]
H. concinna	June, 13±7	NA	July, 30±61	47±55	1.9±1.1	1.1±0.8	<1	<1
R. sanguineus	May 27	NA	October, 21	137	<1	<1	0	0
D. reticulatus	May, 9	NA	NA	NA	<1	0	<1	0
A.americanum	June, 8	NA	NA	NA	<1	0	<1	0

NA not applicable due to small sample volume

[a] Average percentage is shown in parenthesis

Table 2 Specimens of ticks characterised in this study

Case ID	Tick species, gender	Date of bite	Geographic location of tick bite	mt 16S rRNA gene, GenBank ID
Uncommon tick specimens				
3465-08	*Amblyomma americanum*, ♂	08 June 2008	Suburban area of Irkutsk City	NA
1408-09	*Dermacentor reticulatus*, ♂	09 May 2009	Tula region, Russia	NA
2550-13	*Rhipicephalus sanguineus*, ♀	27 May 2013	Suburban area of Irkutsk City	KP866203
3358-14	*Rhipicephalus sanguineus*, ♀	07 June 2014	Suburban area of Rostov-on-Don City Rostov region, Russia	KP866202
6138-14	*Rhipicephalus sanguineus*, ♀	21 October 2014	Suburban area of Irkutsk City	KP866201
Eastern Siberian endemic tick specimens				
3874-14	*Haemaphysalis concinna*, ♀	13 June 2014	Mishelevka town, ~100 km NW of Irkutsk City	KP866207
4882-14	*Ixodes persulcatus*, ♀	26 June 2014	Suburban area of Irkutsk City	KP866204
5332-14	*Ixodes persulcatus*, ♂	08 July 2014	Listvyanka town, ~70 km SE of Irkutsk City	KP866205
6136-14	*Dermacentor nuttalli*, ♂	18 October 2014	Suburban area of Irkutsk City	KP866206

NA specimen was not available for PCR and sequencing

outgroup (Fig. 3). The overall topology of the tree of hard ticks was congruent with the phylogeny of *Ixodida* that has been resolved previously (Black and Piesman 1994). The evolutionary relationships reconstructed by ML, NJ and MP methods were identical, and the nodes that discriminate different tick species had high statistical support (bootstrap values >70 %). Phylogenetic analysis confirmed the species identity established by morphological signatures. The samples 2550-13, 3358-14 and 6138-14 belonged to the *R. sanguineus* sensu lato cluster. The tick 2550-13 grouped with *Rhipicephalus turanicus* L34303 as described elsewhere (Black and Piesman 1994), whereas the latter two samples significantly differed both from *R. sanguineus* and from *R. turanicus* reference sequences. Samples 4882-14 and

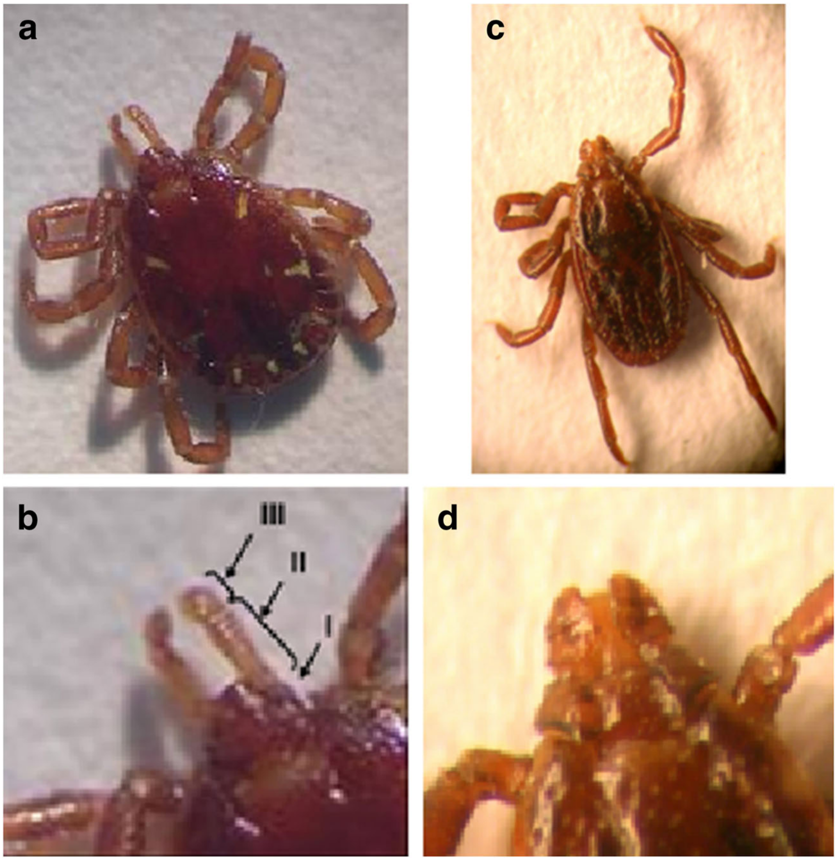

Fig. 2 Morphology of identified tick specimens: **a** male *Amblyomma americanum* 3465-08; **b** the gnathosoma morphology with palp articles designated I, II and III; **c** female *Rhipicephalus sanguineus* 2550-13; and **d** the hexagonal shape of basis capituli

5332-14 grouped with the *I. persulcatus* reference sequence; sample 6136-14 grouped with a *D. nuttalli* specimen from China and 3874-14 with *H. concinna*. The evolutionarily closely related and geographically close species *D. silvarum*, *I. ricinus*, *I. pavlovskyi* and *H. japonica* formed clearly separate branches (Fig. 3).

To better understand the phylogeographic relationships of invading *R. sanguineus*, we analysed the extended set of 119 *Rhipicephalus* sp. sequences published in GenBank with *I. persulcatus* as an outgroup (Fig. 4). It appeared that every tick from Eastern Siberia belongs to a separate cluster inside the *R. sanguineus* sensu lato complex. The sample 6138-14 falls into "*R. sanguineus sensu* Walker (2000)" cluster (Dantas-Torres et al. 2013) that included ticks from tropical ecosystems of Asia and South America. The most closely related sequences were derived from *R. sanguineus* inhabiting Taiwan and Thailand as well as isolates belonging to tropical lineages A–C from Colombia and Brazil (Moraes-Filho et al. 2011). The South African isolate of *R. turanicus* "SAfr2" branched in this cluster as well (Fig. 4). The cluster that united sample 2550-13 and *R. turanicus* L34303 in this analysis was composed of three sequences of *R. sanguineus* from China and one additional sequence of *R. turanicus* (Figs. 3 and 4). However, the sequence of the collection voucher specimen of *R. turanicus* "haplotype 13" (GenBank: KF145150) grouped in a clearly separate cluster along with the majority of conventional *R. turanicus* isolates (Dantas-Torres and Otranto 2015). The last studied specimen of *R. sanguineus* 3358-14 had attacked a human in the European part of Russia (Rostov-on-Don City). According to the sequence of its 16S rRNA gene, it clustered with a large group of sequences of *R. sanguineus* from the Europe Union (Spain, France and Germany) and South America (Nava et al. 2012) including temperate climate species of *R. sanguineus* sensu lato (Moraes-Filho et al. 2011). This group also included two specimens of *R. turanicus* (GenBank: GU553082 and Z97885), the latter of which had been previously described as a laboratory strain originating in Spain (Mangold et al. 1998).

Thus, the phylogenetic analysis confirmed the species identification and indicated that there were at least two independent events of introduction of *R. sanguineus* from two genetically divergent populations into the Eastern Siberian ecosystem.

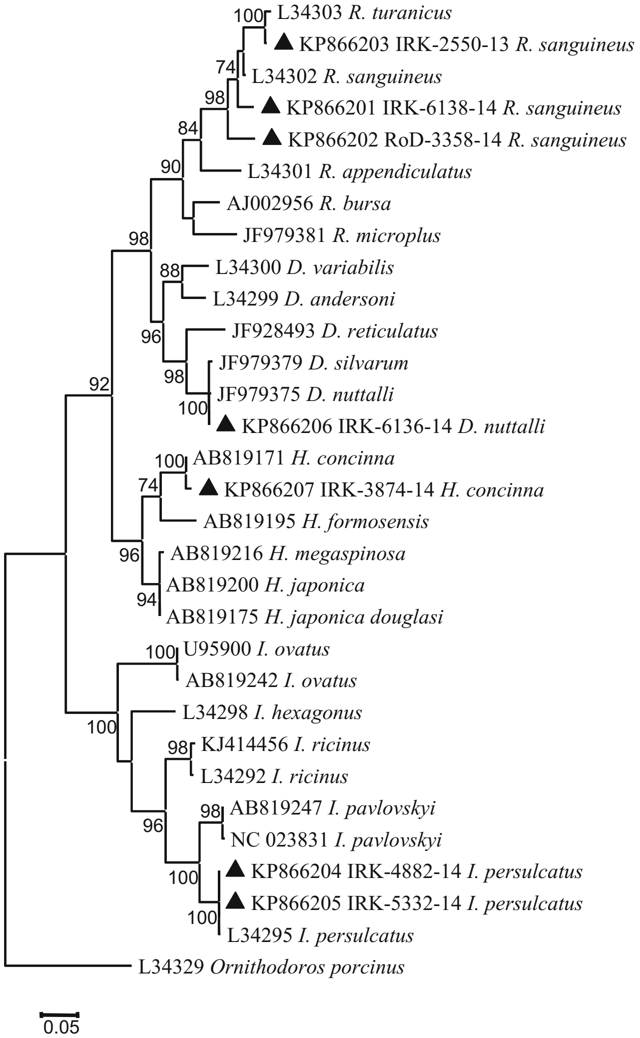

Fig. 3 Phylogenetic relationships of *Ixodidae* ticks reconstructed from a ~390-bp fragment of the mitochondrial 16S rRNA gene. The samples from Irkutsk are labelled by *black triangles*. The tree topology was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). Bootstrap analysis was performed on the basis of 100 replications to evaluate the reliability of the analysis. The bootstrap support values are shown in percentages next to the branches; values less than 70 % are hidden. The scale, representing the number of substitutions per site, is drawn below the tree. Evolutionary analyses were conducted in MEGA6 software (Tamura et al. 2013)

Discussion

At the present time, *I. persulcatus* remains the most important vector of tick-borne diseases to humans in Eastern Siberia, *D. nuttalli* and *D. silvarum* are much less aggressive to humans, whereas *H. concinna* does not play any significant role as a disease vector in Eastern Siberia, though its abundance in natural landscapes may reach 14 ticks per 1 km (Vershinin et al. 2014). This is probably caused by the very mosaic distribution of *H. concinna*. Indeed, the natural conditions optimal for these ticks, such as light humid deciduous forests and shrub meadows (Nosek 1971), are relatively rare in Eastern Siberia. Besides the common tick species, we revealed five specimens of uncommon ticks that bit residents of Irkutsk City. Two specimens belonging to *R. sanguineus* and *D. reticulatus* species occurred in regions where those ticks are endemic. But three other findings including one specimen of *A. americanum* and two specimens of *R. sanguineus* were of local origin.

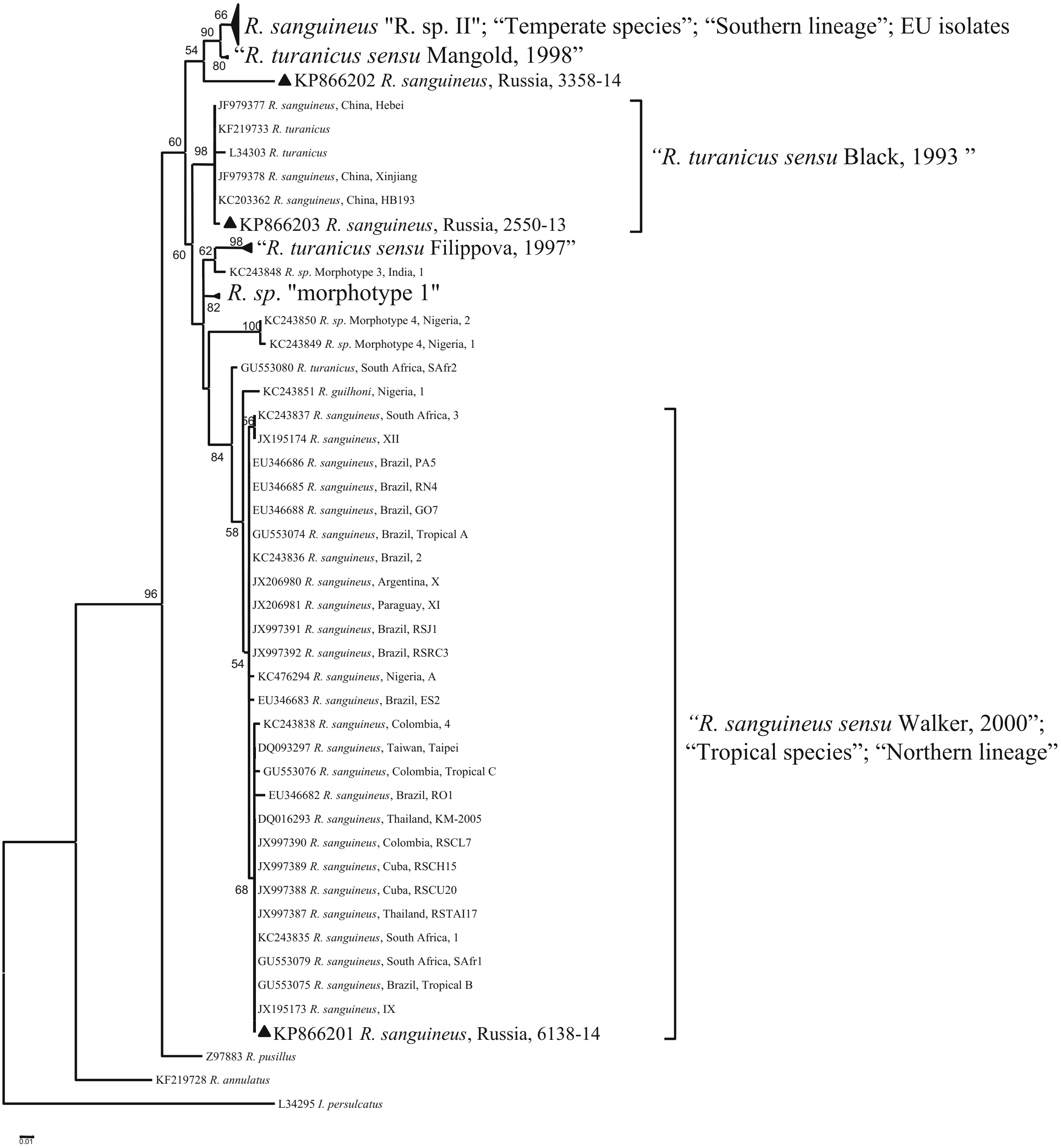

Fig. 4 Phylogenetic relationships of *Rhipicephalus* sp. reconstructed from a ~245-bp fragment of the mitochondrial 16S rRNA gene. The samples from Irkutsk are labelled by *black triangles*. The numbers in square brackets in the descriptions of tree clusters designate the corresponding reference in the bibliography. The tree topology was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). Bootstrap analysis was performed on the basis of 100 replications to evaluate the reliability of the analysis. The bootstrap support values are shown in percentages next to the branches; values less than 50 % are hidden. The scale, representing the number of substitutions per site, is drawn below the tree. Evolutionary analyses were conducted in MEGA6 software (Tamura et al. 2013)

The most surprising invader, *A. americanum*, is a three-host non-nidicolous tick distributed in the south-eastern USA. The normal distribution range of *A. americanum* is restricted to forests and suburbs of Mexico (Coahuila, Nuevo Léon, Tamaulipas) and the USA as far north as New York and Iowa and as far west as Kansas, Oklahoma and Texas (Kolonin 2009). Interestingly, it may form transient foci or even sustainable populations well outside its usual range (Merten and Durden 2000). In our case, the unfed male of *A. americanum* was indeed questing and attacked a man in a suburban forest in Irkutsk.

The most likely scenario of this tick's migration would be a patient being bitten while visiting the USA and detecting the tick on return to Eastern Siberia (as it was proven in cases 1408-09 and 3358-14). However, this route was excluded because the patient had lived in Irkutsk City for his entire life and reported the absence of contact with any kind of exported animals or travelling people. Thus, at least one nymph of *A. americanum* must have travelled about 10,000 km from its usual habitation, invaded the local ecosystem near Irkutsk (probably finding a vertebrate host), survived over the winter and successfully moulted into the imago. Another possible route might include zoonotic transfer with migrating birds or animals. The white-tailed deer is the most important vertebrate host for *A. americanum* because it can feed all three life stages of the tick and would usually deposit the fed ticks in habitats that maximise tick survival. However, larvae and nymphs also infest various ground-feeding birds, medium- and large-sized mammals and, on occasion, small mammals (reviewed in Childs and Paddock 2003). It has been previously shown that nymphs of *A. americanum* are definitely able to travel for more than 1900 km from the southern USA to Canada with migrating thrushes and flycatchers, whereas other *Amblyomma* sp. ticks have been documented crossing the 5000-km distances via direct seasonal migrations of birds (Scott et al. 2001). Recently, it was also discovered that migrating birds are able to cross the Pacific Ocean, covering more than 7000 km just in 5 days (Gill et al. 2009). At the same time, the feeding time of larvae and nymphs of *A. americanum* may range between 3 and 9 days, whereas females may take blood for up to 15 days (Troughton and Levin 2007). Thus, hypothetically, any stage of *A. americanum* could survive trans-pacific migration. Finally, there are several bird species that perform annual migrations between Asia and North America, including the bar-tailed godwit (*Limosa lapponica*), the sandhill crane (*Grus canadensis*), the yellow wagtail (*Motacilla flava*), the arctic warbler (*Phylloscopus borealis*) and, most interestingly, a grey-cheeked thrush (*Catharus minimus*) that has been found to be responsible for the spread of *A. americanum* in Canada (Scott et al. 2001; Gill et al. 2009; Krechmer et al. 1978; Ehrlich et al. 1988; Alström et al. 2011). The high possibility of regular tick migration between North America and Asia and associated pathogen transfer is also supported by recent findings of North American Powassan virus in ecosystems of Russian Far East (Leonova et al. 2009; Deardorff et al. 2013).

R. sanguineus is a cosmopolitan species inhabiting both Old and New Worlds; however, the northern edge of its distribution range in Eurasia does not reach the northern coast of the Caspian Sea (Fig. 1). The closely related *R. turanicus* is an abundant tick species inhabiting Mediterranean countries, the south of Russia, the Arabian peninsula and other Middle Eastern countries. The northern bound of distribution of *R. turanicus* lies much further north than that of *R. sanguineus* and runs along the northern coast of the Caspian Sea in the Russian Federation to the head of the Irtysh River in South Kazakhstan and is restricted by western China on the eastern side (Kolonin 2009). In fact, the distance to the nearest established natural population would be around 3500 km for *R. sanguineus* and 2500 km for *R. turanicus*. Abundant migrations of birds and humans between the European part of Russia, Central Asian countries and the Asian part of Russia make the random drift of *Rhipicephalus* sp. to non-endemic ecosystems highly probable. The most interesting finding is that, according to phylogenetic analysis, *Rhipicephalus* sp. ticks from Irkutsk belong to different evolutionary lineages suggesting the existence of several independent migration routes. Indeed, *R. sanguineus* 6138-14 was introduced not from the nearest East European or Central Asian populations, but rather from tropical ecosystems (Fig. 4) with a minimum travelled distance to Eastern Siberia of ~3600 km (Taiwan, GenBank accession DQ093297). Another specimen, *R. sanguineus* 2550-13, clustered with a number of *R. sanguineus* and *R. turanicus* isolates that differed both from conventional *R. sanguineus* and from the *R. turanicus* type strain. The closest relatives to the *R. sanguineus* specimens had been collected in the Chinese provinces Xinjiang and Hebei that are situated in approximately 1200 and 1500 km from Irkutsk, respectively (Lv et al. 2014). In summary, phylogeographic analysis suggests that *R. sanguineus* ticks invading Eastern Siberia are significantly different from those living in European parts of Russia or in EU countries. With high probability, these ticks were independently transferred from geographically distant populations of *R. sanguineus* sensu lato inhabiting subtropical and tropical ecosystems of Asia via different migration routes.

Recently, the concept of "climate niche" was suggested as a way to map the potential range of tick invasion. According to this approach, it is mainly the climate trends that underpin the expansion or retraction of the historical range of some tick species. Several reports are beginning to confirm this for such tick species as *I. ricinus*, *Boophylus annulatus*, *Dermacentor marginatus*, *Hyalomma marginatum*, *Hyalomma excavatum*, *Rhipicephalus bursa* and *R. turanicus* (reviewed in Estrada-Peña 2008). Mathematical analysis of climate conditions and *R. sanguineus* distribution in Europe over the last 40 years also indicated that since the 1960s the European ecosystems have evolved towards more suitable biological conditions for maintaining or increasing *R. sanguineus* populations (Beugnet et al. 2011). Thus, the detection of *R. sanguineus* and *A. americanum* attacks on human host in Eastern Siberia may be the first sign of changes at climate conditions that results in increased survival rates of non-endemic ticks. Further development of this process could be advantageous to the establishment of sustainable populations of novel tick species in northern Eurasia; however, comprehensive ecological research and modelling is necessary to check this hypothesis.

In spite being an aggressive biter of humans, *A. americanum* was regarded as a tick of minor importance as a vector of zoonotic pathogens to humans. However, in recent decades, this tick has been recognised as a significant vector of the HME agents *E. chaffeensis*, *Ehrlichia ewingii*, *Coxiella burnetii*, *Francisella tularensis*, *Rickettsia amblyommii* and *Borrelia lonestari* (Childs and Paddock 2003). *R. sanguineus* s.l. is a recognised parasite biting humans both at imago and at nymphal stages and is able to infest densely populated urban areas (Vatansever et al. 2008). This tick species also have been regarded as a vector for a number of pathogenic microorganisms, including significant human pathogens such as Crimean-Congo hemorrhagic fever virus, Thogotovirus Thogoto (reviewed in Hubálek and Rudolf 2012), *C. burnetii* (agent of Q-fever), *Ehrlichia canis*, *E. chaffeensis* (HME), *Rickettsia conorii* (Mediterranean spotted fever), *Rickettsia massiliae* (spotted fever) and *Rickettsia rickettsii* (Rocky Mountain spotted fever) (Dantas-Torres and Otranto 2015). Assuming the ability of the abovementioned ticks to establish sustainable populations in novel territories, these infections should be considered as a possible emerging threat to the health of local residents in Eastern Siberia.

Conclusion

For the first time, we describe the events of invasion of *A. americanum* and *R. sanguineus* into ecosystems of northern and central Asia with a harsh continental climate. Those ticks have multiple independent routes of migration to non-specific regions, are able to survive at least during the adult life stage and can be aggressive to local human populations. Therefore, changing climate conditions may trigger rapid changes in tick fauna composition and the diversity of microorganisms associated with invading ticks. This may further lead to the emergence of novel tick-borne infections in north Asian ecosystems. Further comprehensive analysis of the evolution of local ecosystems is necessary to evaluate the risk of spread of *A. americanum* and *R. sanguineus* to novel territories in northern Eurasia.

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Compliance with ethical standards The study was approved by the Committee for biomedical ethics of FSPSI “SC FHHR” and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. All applicable international, national, and institutional guidelines for the care and use of animals were followed. Informed consent was obtained from all individual participants included in the study.

Conflicts of interests The authors declare that they have no competing interests.

Authors’ contributions MAK, AVL and GAD designed the study. GAD and IVP collected the specimens and interviewed the patients. MAK, AVL, ELM and NVK performed the experiments. MAK and AVL analysed the results. MAK, AVL, GAD and NVK drafted the manuscript. All authors read and approved the final manuscript.

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