

# Genetic diversity of *Babesia* in *Ixodes persulcatus* and small mammals from North Ural and West Siberia, Russia

V. A. RAR<sup>1\*</sup>, T. I. EPIKHINA<sup>1</sup>, N. N. LIVANOVA<sup>1,2</sup> and V. V. PANOV<sup>1,2</sup>

<sup>1</sup>Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, pr. Lavrent'eva 8, Novosibirsk, 630090 Russian Federation

<sup>2</sup>Institute of Systematics and Ecology of Animals, Siberian Branch, Russian Academy of Sciences, ul. Frunze 11, Novosibirsk, 630091 Russian Federation

(Received 8 May 2010; revised 9 July 2010; accepted 10 July 2010; first published online 27 August 2010)

## SUMMARY

**Objective.** The aim of this work was to study the prevalence and genetic diversity of *Babesia* in *Ixodes persulcatus* ticks and small mammals from Ural and Siberia in Russia. **Methods.** In total, 481 small mammals and 922 questing adult *I. persulcatus* from North Ural (Sverdlovsk region) and West Siberia (Novosibirsk region) were examined for the presence of *Babesia* by nested PCR based on the 18S rRNA gene. **Results.** *Babesia microti* of the 'Munich'-type was found in 36.2% of blood samples of the small mammals from the Sverdlovsk region and *B. microti* of the 'US'-type in 5.3% of the animals from the Novosibirsk region. *Babesia* DNA was not detected in 133 analysed *I. persulcatus* from the Sverdlovsk region; however, it was found in 24 of 789 ticks from the Novosibirsk region. Three distinct *Babesia* species were detected in *I. persulcatus*. *B. microti* 'US'-type was identified in 10 ticks, *Babesia* closely related to *B. divergens*/*B. capreoli* in 2 ticks, and *Babesia* closely related to *B. venatorum* (EU1) in 12 ticks. **Conclusion.** To our knowledge, this is the first detection of *Babesia sensu stricto* in *I. persulcatus* ticks and of *B. microti* in *I. persulcatus* in the Asian part of Russia.

Key words: *Ixodes persulcatus*, *Babesia* spp., prevalence, genetic diversity, North Ural, West Siberia.

## INTRODUCTION

Babesiosis, which is caused by tick-borne intra-erythrocytic protozoan parasites of the genus *Babesia* (order *Piroplasmida*, phylum *Apicomplexa*), is a well-known disease affecting a wide range of wild and domestic animals, including cervids, cattle, sheep, horses, and dogs (Criado-Fornelio *et al.* 2003; Hunfeld *et al.* 2008). The interest in *Babesia* has recently increased with recognition of its role as a zoonotic agent of human babesiosis.

Several hundred cases of human babesiosis have been recorded in the USA and most of them were caused by *Babesia microti*, a natural parasite of microtine rodents and shrews. The clinical features in patients vary from asymptomatic to life threatening; the fatality rate is about 5% (Hunfeld *et al.* 2008). It is well established that *Ixodes scapularis* in the USA as well as *I. ricinus* and *I. trianguliceps* in Europe are vectors of *B. microti* (Foppa *et al.* 2002; Gray *et al.* 2002; Hunfeld *et al.* 2008). Phylogenetic analysis based on the comparison of the 18S rRNA, beta-tubulin and CCT-eta genes of *B. microti* has shown a high heterogeneity of this species (Goethert and Telford III, 2003; Nakajima *et al.* 2009). Thus, *B. microti* is now recognized as a genetically diverse

species complex that comprises several clusters. The most widely distributed cluster of *B. microti* (designated *B. microti* 'US'-type) contains the zoonotic *B. microti* strains G1, Gray, and Jena/Germany, as well as isolates from microtine rodents from different parts of Eurasia (Gray *et al.* 2010; Zamoto *et al.* 2004a,b). Some *B. microti* strains and isolates from rodents and ticks in Germany (Munich), Poland and the United Kingdom belong to a second cluster, *B. microti* 'Munich'-type (Pieniazek *et al.* 2006; Sinski *et al.* 2006; Nakajima *et al.* 2009). Two other clusters contain Japanese isolates of *B. microti* 'Kobe'-type and *B. microti* 'Hobetsu'-type (Tsuji *et al.* 2001).

In Europe, only 1 confirmed case of human babesiosis caused by *B. microti* infection has been reported (Hildebrandt *et al.* 2007), and about 40 cases have been caused by *Babesia divergens* and related parasites. Most cases have occurred in splenectomized individuals, and the case fatality rate is about 40% (Gray *et al.* 2010). In addition to *B. divergens*, *B. venatorum* (originally designated EU1) was shown to be the causative agent of 3 cases in Austria, Italy and Germany (Herwaldt *et al.* 2003; Häselbarth *et al.* 2007). The causative agents of 2 fatal cases of babesiosis in Portugal and the Canary Islands were initially identified as *B. divergens*; however, comparison of nucleotide sequences allowed them to be ascribed in latest reviews to *B. divergens*-like parasites (Hunfeld *et al.* 2008; Gray *et al.* 2010). In addition, *Babesia* closely related to *B. divergens* (*Babesia* sp.

\* Corresponding author: Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, pr. Lavrent'eva 8, Novosibirsk, 630090 Russian Federation. Tel: +7 383 3635177. Fax: +7 383 3635164. E-mail: rarv@niboch.nsc.ru

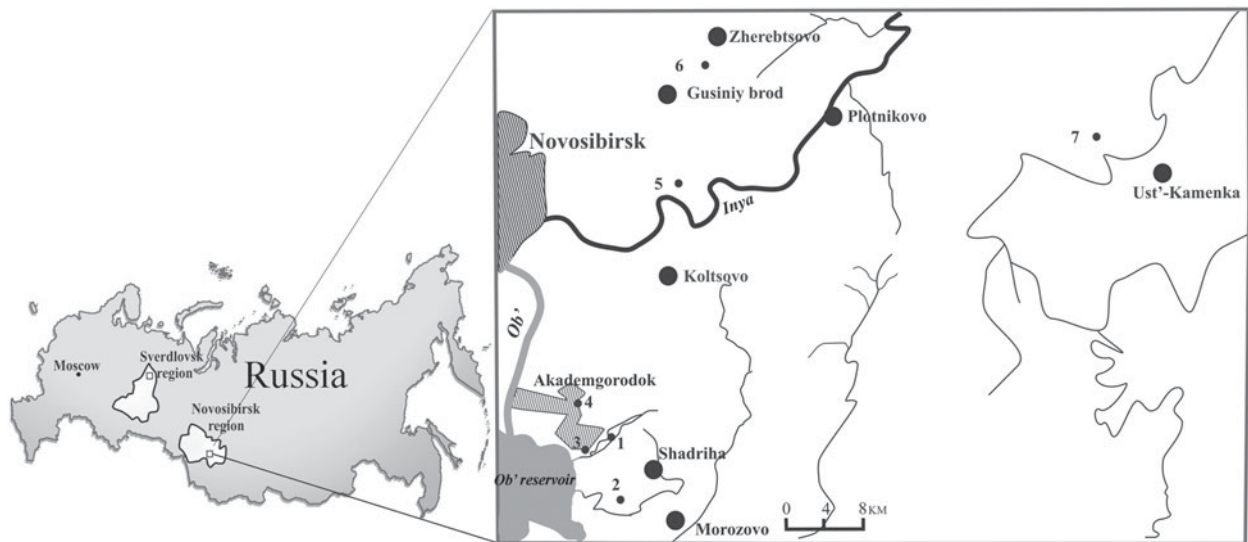


Fig. 1. Location of sampling sites in North Ural and West Siberia. The sites of tick collection in the Novosibirsk region are denoted as 1–7.

MO1) caused at least 3 cases of babesiosis in the USA (Herwaldt *et al.* 2004; Gray *et al.* 2010). Moreover, a lethal case of babesiosis in the Caucasian region of the former USSR was caused by *Babesia* sp. morphologically similar to *B. divergens* (Rabinovich *et al.* 1978).

The specific vector of both *B. divergens* and *B. venatorum* is *I. ricinus* (Bonnet *et al.* 2009; Becker *et al.* 2009); the natural hosts of *B. divergens* are cattle and of *B. venatorum* are roe deer (Zintl *et al.* 2003; Duh *et al.* 2005). One more *Babesia* species, *Babesia capreoli* has been found to infect cervids (roe deer, red deer, and sika deer) in different European countries. Despite a high phylogenetic similarity to *B. divergens*, *B. capreoli* differs from *B. divergens* in its host range and lack of zoonotic potential (Malandrin *et al.* 2010).

A few papers so far describe the prevalence of *Babesia* in nature in the distribution area of *Ixodes persulcatus* in Russia. Thus, the aim of this work was to study the prevalence and genetic diversity of *Babesia* in ticks and small mammals in North Ural and West Siberia.

## MATERIALS AND METHODS

### Sample collection

In total, 133 adult *I. persulcatus* ticks were collected by flagging the vegetation in the Sverdlovsk region, North Ural, (60°N, 59°E) in 2004 and 2009; in addition, 789 *I. persulcatus* were collected in 7 sampling sites of the Novosibirsk region, West Siberia, (55°N, 83°E) in 2008–2009 (Fig. 1). Ticks were stored at –20 °C until used for DNA extraction.

The sampling site in the Sverdlovsk region was located on the territory of Denezhkin Kamen' Reserve in mixed birch-aspen or pine–birch forests near the northern border of the *I. persulcatus* distribution area.

The sampling sites in the Novosibirsk region comprised: (1) mixed aspen–birch forest near Novosibirsk Scientific Centre (Akademgorodok), (2) pine forest about 8 km from Novosibirsk Scientific Centre, (3) pine forest near Novosibirsk Scientific Centre, (4) mixed aspen–birch forest near Novosibirsk Scientific Centre, (5) mixed birch–pine forest about 15 km from Novosibirsk, (6) mixed aspen–birch forest about 20 km from Novosibirsk and (7) mixed moist aspen–birch forest about 60 km from Novosibirsk.

Wild small mammals were trapped in the period of *I. persulcatus* activity. In total, 196 small mammals were caught using live traps in the Sverdlovsk region in June–July 2004–2005, and 285 small mammals were trapped in the Novosibirsk region in May–September 2003, 2006, and 2007. All experiments with animals were conducted in compliance with the Animal Welfare Act at the Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, according to the guidelines for experiments with laboratory animals (Supplement to the Order of the Russian Ministry of Health no. 755 of August 12, 1977). Trapped animals were anaesthetized with diethyl ether. Aliquots of blood were sampled into the tubes containing 50 mM EDTA.

### DNA extraction

Total DNA was extracted from crushed ticks using a Proba NK kit (DNA-Technology, Moscow, Russia). Total DNA was extracted from 200 µl of blood according to the method of Boom *et al.* (1990).

### PCR assay

All PCR reactions were performed in 20 µl of the reaction mixture containing 67 mM Tris-HCl

Table 1. Primers used for nested PCR

Target gene	Primer sequences (5'-3')	Annealing temperature
<i>Babesia</i> spp. 18S rRNA gene	BS1 (gacggtagggtattggcct)	58 °C
	BS2 (attcaccggatcactcgtac)	
	PiroA (attaccaatcctgacacaggg)	64 °C
	PiroC (ccaacaaatagaaccaargtcctac)	
<i>B. microti</i> 'US'-type 18S rRNA gene	PiroA (attaccaatcctgacacaggg)	55 °C
	Bm1 (ggaaaatagtagccgaaggcacc)	
<i>B. microti</i> 'Munich'-type 18S rRNA gene	PiroA (attaccaatcctgacacaggg)	50 °C
	Bm2 (agatagtagtaaccaattaaggatacc)	
<i>B. microti</i> 18S rRNA gene	BS3 (cgaggcagcaacgggtaacg)	60 °C
	BS4 (agggacgtagtcggcagcag)	
<i>Babesia sensu stricto</i> 18S rRNA gene	BS5 (cgaggcagcaacgggtaacg)	60 °C
	BS4 (agggacgtagtcggcagcag)	

(pH 8.9), 16.6 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, 0.01% Tween 20, 200 µM of each dNTP, 5% glycerol, 0.5 µM primers, 2 U of Taq DNA polymerase (Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia) and 2 µl of DNA for primary reactions or 2 µl of the primary PCR products for nested reactions. The PCR conditions comprised initial denaturation for 3 min at 94 °C followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at temperatures indicated in Table 1 and elongation for 1–1.5 min at 72 °C. DNA extracted from blood samples of the dogs with confirmed babesiosis (Rar *et al.* 2005) was used as a positive control.

For screening analysis, *Babesia* DNA was detected by nested PCR with the primers specific to the 18S rRNA gene of known *Babesia* species (Table 1.). The primers BS1 and BS2 were used for primary reactions, the primers PiroA (Armstrong *et al.* 1998, modified) and PiroC, for nested reactions, the final products had a length of 340–390 bp. Second rounds of PCR using the forward primer PiroA and the reverse primer Bm1 specific to *B. microti* 'US'-type or the reverse primer Bm2 specific to *B. microti* 'Munich'-type were performed to genotype the positive samples from small mammals.

For sequencing analysis, second rounds of PCR with the forward primer BS3 and reverse primer BS4 were performed to amplify the 1272–1277 bp fragments of *B. microti* 18S rRNA gene, and the reactions with the forward primer BS5 and reverse primer BS4 were conducted to amplify the 1238 bp fragments of *Babesia sensu stricto* 18S rRNA gene.

#### Sequencing of the PCR products

Nucleotide sequences of the PCR products purified with GFX Columns (Amersham Biosciences, Piscataway, NJ, USA) were determined at the DNA Sequencing Center of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk (<http://sequest.niboch.nsc.ru>) using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City,

CA, USA) and analysed by the BLASTN (<http://www.ncbi.nlm.nih.gov/BLAST>) and CLUSTALW (<http://www.ebi.ac.uk/clustalw/index.html>) programs. Phylogenetic analysis was performed using the MEGA 3.1 software (Kumar *et al.* 2004). A phylogenetic tree was constructed by the neighbor-joining (NJ) method and, in addition, by maximum parsimony (MP) and minimum evolution (ME) methods. The stability of the constructed trees was estimated by bootstrap analysis with 1000 replicates. The clustering of analysed organisms was identical regardless of which phylogenetic method was used.

#### Nucleotide sequence accession numbers

The partial 18S rRNA gene nucleotide sequences of *B. microti* were deposited in GenBank under accession numbers AY943957, AY943958 and GU057383. The partial 18S rRNA gene nucleotide sequences of *B. divergens* and *Babesia* sp. (EU1) were deposited under accession numbers GU057385 and GU734773, respectively.

#### RESULTS

##### Detection of Babesia DNA in small mammals

*Babesia* DNA was found using nested PCR in 71 of the 196 small mammals from the Sverdlovsk region and in 15 of 285 small mammals from the Novosibirsk region. Nucleotide sequences of the 18S rRNA gene were determined for 12 positive blood samples from the Sverdlovsk region and for 6 samples from the Novosibirsk region. All the determined *Babesia* 18S rRNA gene sequences from small mammals from the Sverdlovsk region were identical to each other (a typical sample was Ubl-104, GenBank accession no. AY943958) and to the sequence of *B. microti* strain Munich (GenBank accession no. AB071177). However, all the determined sequences from small mammals from the Novosibirsk region (a typical sample was Sbl-11, GenBank accession no. AY943957) were identical to the sequence of

Table 2. Detection of *Babesia microti* DNA in blood of small mammals by nested PCR

Region	Species	No. of studied animals	No. (%) of animals infected with	
			<i>B. microti</i> 'US'-type	<i>B. microti</i> 'Munich'-type
Sverdlovsk region	<i>Myodes rutilus</i>	99	—	41 (41,4)
	<i>M. rufocanus</i>	7	—	4 (57,1)
	<i>M. glareolus</i>	37	—	14 (37,8)
	<i>Sorex araneus</i>	47	—	8 (17,0)
	<i>Sorex</i> spp.	5	—	4 (80,0)
	<i>Sicista betulina</i>	1	—	1
	Total	196	—	71 (36,2)
Novosibirsk region	<i>M. rutilus</i>	50	5 (10,0)	—
	<i>M. rufocanus</i>	62	2 (3,2)	—
	<i>M. glareolus</i>	17	—	—
	<i>S. araneus</i>	60	2 (3,3)	—
	<i>Apodemus agrarius</i>	17	1 (5,9)	—
	<i>Microtus</i> spp.	44	4 (9,1)	—
	<i>Sicista betulina</i>	35	1 (2,9)	—
	Total	285	15 (5,3)	—

Table 3. Detection of *Babesia* spp. in *Ixodes persulcatus* in the Novosibirsk region

Sampling sites	Year	Total number of ticks	Number (%) of ticks containing DNA of		
			<i>B. microti</i>	<i>B. divergens</i> -like	<i>B. venatorum</i> -like
1	2008	43	1 (2·3)	2 (4·7)	3 (7·0)
	2009	58	0	0	0
2	2008	25	0	0	1 (4·0)
	2009	89	3 (3·4)	0	0
3	2008	80	0	0	0
4	2008	114	0	0	0
5	2009	80	1 (1·3)	0	0
6	2009	105	4 (3·8)	0	8 (7·6)
7	2009	195	1 (0·5)	0	0
Total	2008–2009	789	10 (1·3)	2 (0·3)	12 (1·5)

*B. microti* strain G1 (GenBank accession no. AF231348). Species-specific PCR was performed to genotype the other positive samples. All the positive samples from the Sverdlovsk region were successfully amplified with the primers specific to *B. microti* 'Munich'-type, and all positive samples from the Novosibirsk region, with primers specific to *B. microti* 'US'-type.

*B. microti* of both 'Munich'-type and 'US'-type were found in samples from different species of the examined small mammals: red-backed voles (*Myodes rutilus*), gray red-backed voles (*M. rufocanus*), common shrews (*Sorex araneus*), and northern birch mice (*Sicista betulina*). Moreover, *B. microti* 'US'-type was found in the blood samples from tundra voles (*Microtus oeconomus*), common vole (*Mi. arvalis*), East-European field vole (*Mi. rossiaemeridionalis*), and striped field mouse (*Apodemus agrarius*), and *B. microti* 'Munich'-type was detected in samples

from bank voles (*Myodes glareolus*) and shrews (*Sorex tundrensis*, *S. caecutiens*, and *S. isodon*) (Table 2).

#### Detection of *Babesia* DNA in *I. persulcatus*

*Babesia* DNA was undetectable in 133 analysed ticks from the Sverdlovsk region but was found in 24 of the 789 *I. persulcatus* from the Novosibirsk region. Positive ticks were collected in 5 of the 7 sampling sites located at a distance of 3–65 km from each other (Fig. 1, Table 3). Nucleotide sequences of *Babesia* 18S rRNA gene fragments (1174–1211 bp) were determined for all positive ticks. The sequences from 10 ticks collected in sampling sites (1), (2), (5), (6), and (7) were identical to each other (a typical sample was Nov-*Ip*307, GenBank accession no. GU057383) and to the sequence of *B. microti* strain G1. The sequences from Nov-*Ip*316 (GenBank accession no. GU057385) and Nov-*Ip*341 ticks from sampling

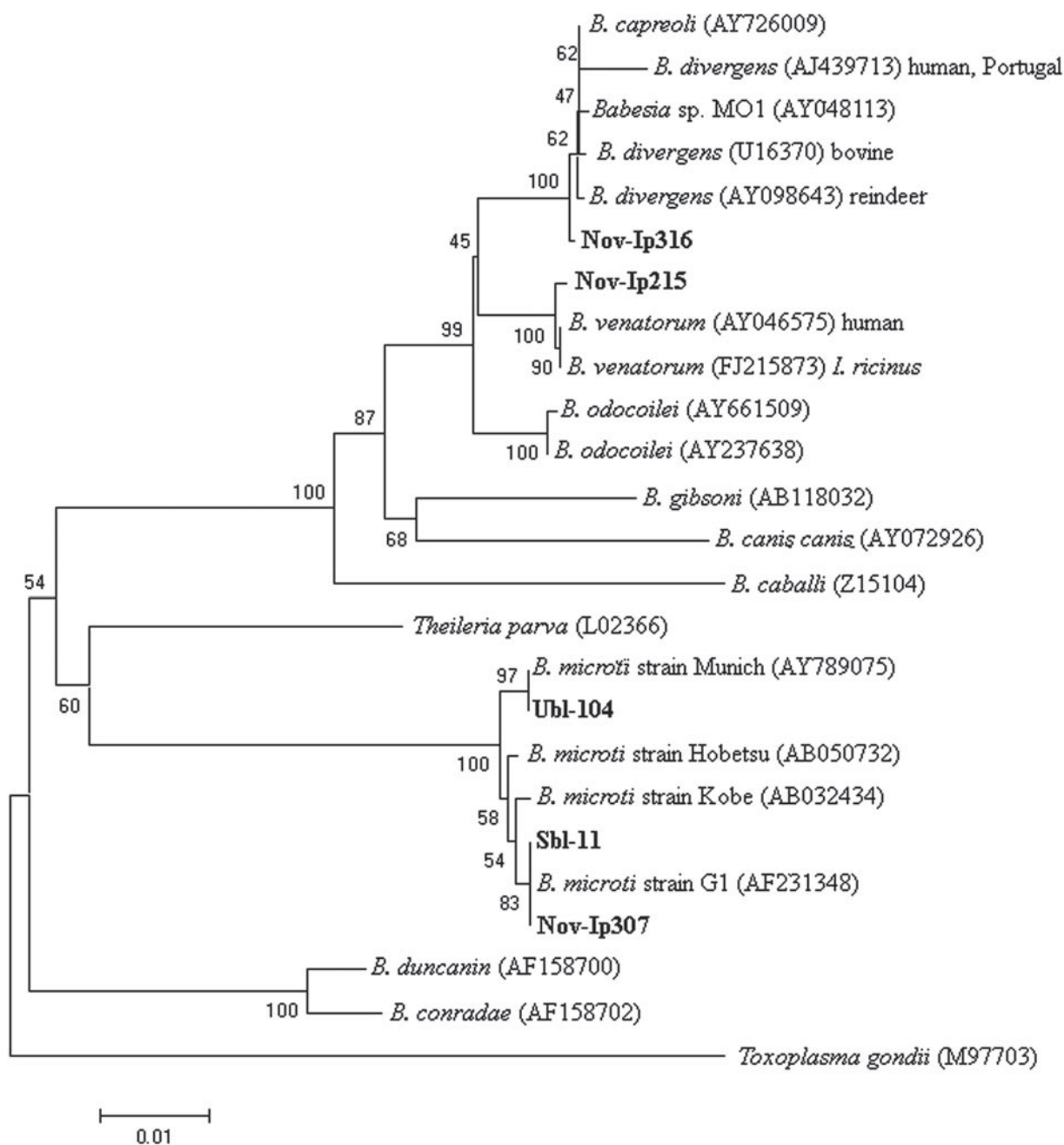


Fig. 2. The phylogenetic tree constructed by the NJ method using the 1200-bp sequences of *Babesia* spp. 18S rRNA gene. The scale bar indicates an evolutionary distance of 0.01 nucleotides per position in the sequence. *Toxoplasma gondii* was used as an outgroup. The specimens from *I. persulcatus* (Nov-Ip215, Nov-Ip307, Nov-Ip316) and *Mi. oeconomus* (Sbl-11) from the Novosibirsk region and *M. rutilus* from the Sverdlovsk region (Ubl-104) from this study are in boldface.

site (1) showed the highest level of identity (99.5%) to the sequences of *B. divergens*, *B. capreoli*, and *Babesia* sp. MO1 (GenBank accession no. U16370, FJ944827 and AY048113, respectively), and differed from all of them by 6 nucleotides. The determined babesial 18S rRNA gene sequences from 12 ticks from sampling sites (1), (2), and (6) were also identical to each other (a typical sample was Nov-Ip215, GenBank accession no. GU734773) and differed by only 2 of the overall 1174 nucleotides (99.8% similarity) from the 18S rRNA gene sequence of *B. venatorum* (GenBank accession no. AY046575).

Phylogenetic analysis showed that the sample Nov-Ip316 clustered together with known *B. divergens*, *B. divergens*-like, and *B. capreoli* isolates with a strong bootstrap support, and the sample Nov-Ip215 clustered together with *B. venatorum* isolates (Fig. 2).

#### DISCUSSION

The rodent parasite *B. microti* is spread worldwide in a wide range of small mammals, including *Myodes* spp., *Microtus* spp., *Apodemus* spp., and *Sorex* spp.

(Goethert and Telford III, 2003; Zamoto *et al.* 2004a). In Russia, *B. microti* were earlier found on the territory of West Ural in *M. glareolus*, *M. rufocanus*, *M. rutilus*, *Microtus oeconomus*, *Apodemus uralensis*, and *Sorex* spp. (Morozov *et al.* 2006), and in the Far East in *M. rufocanus* and *A. peninsulae* (Zamoto *et al.* 2004b). Confirming these data, we found *B. microti* in North Ural and West Siberia in most of the examined species of microtine rodents (*Myodes* spp., *Microtus* spp., *Apodemus agrarius*, *Sicista betulina*) and insectivores (*Sorex* spp).

The site of North Ural examined in this study is located near the northern border of the *I. persulcatus* distribution area; this site displayed a low abundance of *I. persulcatus* and small mammals and a mosaic distribution of ticks. In addition to *I. persulcatus*, *I. trianguliceps* is abundant in the examined location (Livanova and Livanov, 2010). Surprisingly, all the *B. microti*, found in small mammals from North Ural, were *B. microti* 'Munich'-type parasites, previously identified only in Europe. Note, that the *B. microti* detected in small mammals in the neighbouring regions in West Ural (Telford III *et al.* 2002; GenBank accession no. AY144693) and West Siberia were attributed to the widespread *B. microti* 'US'-type. We failed to find any *B. microti* 'Munich'-type in *I. persulcatus* despite a high prevalence of this *Babesia* in voles and shrews in North Ural. The reason of such discrepancy could be the low number of *I. persulcatus* analysed, or a smaller portion of infected adult ticks as compared with nymphs (Piesman *et al.* 1986). However, we analysed exclusively adult ticks, because only the adult stage of *I. persulcatus* could be readily collected by flagging. It was previously shown that *I. trianguliceps* in the United Kingdom is the most probable vector transmitting *B. microti* 'Munich'-type to voles (Bown *et al.* 2008). Thus, we cannot exclude involvement of *I. trianguliceps* in the transmission of *B. microti* 'Munich'-type to vertebrate hosts in the examined territory of North Ural.

To date, publications on the detection of *B. microti* in *I. persulcatus* ticks are sparse. *B. microti* DNA was, for the first time, found in 0.9% of *I. persulcatus* in the northwestern part of Russia (Alekseev *et al.* 2003). Then it was shown that 3.6–4.0% of *I. persulcatus* from China contained *B. microti* 'Hobetsu'-type, infective for rodents (Sun *et al.* 2008). In this study, *B. microti* DNA was, for the first time, found in *I. persulcatus* from Siberia. All *B. microti* parasites detected in the ticks were shown to be *B. microti* 'US'-type, also detected in small mammals in the same region.

In addition to *B. microti*, 2 different *Babesia* species closely related to but distinct from both *B. divergens*/*B. capreoli* and *B. venatorum* were found in *I. persulcatus* from the Novosibirsk region. To our knowledge, this is the first detection of *Babesia sensu*

*stricto* in *I. persulcatus* ticks. Note, that it is not always possible to reliably differentiate the species from *Babesia sensu stricto* cluster by phylogenetic analysis due to minor differences in nucleotide sequences. In particular, various bovine *B. divergens* isolates can differ from each other by 1–2 base pairs in the 18S rRNA gene (reviewed by Gray *et al.* 2010), whereas 2 apparently distinct species, *B. divergens* and *B. capreoli*, differ by only 3 bases in the same gene (Malandrin *et al.* 2010). So far, it remains disputable whether the *Babesia* isolates found in *I. persulcatus* are conspecific to any of the valid *Babesia* species.

Note, that the distribution of infected ticks was highly mosaic. In particular, *B. venatorum*-like parasites were found only in 3 of the 7 examined sampling sites and in 2 of them, about 7.0% of the ticks were positive. Furthermore, the portion of ticks infected with *Babesia* spp. in sampling site (1) varied in different years from 0 to 14%.

It is well known that different species of *Babesia sensu stricto* parasitize different vertebrate hosts. In Europe, parasites from the *B. divergens* group infect mainly cervids and cattle (Malandrin *et al.* 2010; Zintl *et al.* 2003), whereas in the USA *Babesia* sp. MO-1 naturally infect cottontail rabbits (Spencer *et al.* 2006). Further study is necessary to determine the possible hosts of *B. divergens*-like and *B. venatorum*-like parasites detected in *I. persulcatus* ticks in Siberia.

#### ACKNOWLEDGMENTS

The authors are grateful to S.G. Livanov (Institute of Systematics and Ecology of Animals) for his assistance in sampling and to N.V. Fomenko and S.E. Tkachev (Institute of Chemical Biology and Fundamental Medicine) for their assistance in DNA extraction, and to N.A. Titova for technical support.

#### FINANCIAL SUPPORT

This study was supported in part by the Siberian Branch of the Russian Academy of Sciences (Integration interdisciplinary project No. 63 and Integration project No. 6).

#### REFERENCES

- Alekseev, A. N., Semenov, A. V. and Dubinina, H. V. (2003). Evidence of *Babesia microti* infection in multi-infected *Ixodes persulcatus* ticks in Russia. *Experimental and Applied Acarology* **29**, 345–353. doi: 10.1023/A.1025841901909.
- Armstrong, P. M., Katavolos, P., Caporale, D. A., Smith, R. P., Spielman, A. and Telford, S. R. III (1998). Diversity of *Babesia* infecting deer ticks (*Ixodes dammini*). *American Journal of Tropical Medicine and Hygiene* **58**, 739–742.
- Becker, C. A., Bouju-Albert, A., Jouglin, M., Chauvin, A. and Malandrin, L. (2009). Natural transmission of zoonotic *Babesia* spp. by *Ixodes ricinus*

- ticks. *Emerging Infectious Diseases* **15**, 320–322. doi: 10.3201/eid1502.081247.
- Bonnet, S., Brisseau, N., Hermouet, A., Jouglin, M. and Chauvin, A.** (2009). Experimental in vitro transmission of *Babesia* sp. (EU1) by *Ixodes ricinus*. *Veterinary Research* **40**, 21. doi: 10.1051/vetres/2009004.
- Boom, R., Sol, C. J., Salimans, M. M., Jansen, C. L., Wertheim-van Dillen, P. M. and van der Noorda, J.** (1990). Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology* **28**, 495–503.
- Bown, K. J., Lambin, X., Telford, G. R., Ogden, N. H., Telfer, S., Woldehiwet, Z. and Birtles, R. J.** (2008). Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations. *Applied and Environmental Microbiology* **74**, 7118–7125. doi: 10.1128/AEM.00625-08.
- Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A. and Barba-Carretero, J. C.** (2003). Molecular studies on *Babesia*, *Theileria* and *Hepatozoon* in southern Europe. Part I. Epizootiological aspects. *Veterinary Parasitology* **113**, 189–201. doi:10.1016/S0304-4017(03)00078-5.
- Duh, D., Petrovec, M., Bidovec, A. and Avsic-Zupanc, T.** (2005). Cervids as Babesiae hosts, Slovenia. *Emerging Infectious Diseases* **11**, 1121–1123.
- Foppa, I. M., Krause, P. J., Spielman, A., Goethert, H., Gern, L., Brand, B. and Telford, S. R. III** (2002). Entomologic and serologic evidence of zoonotic transmission of *Babesia microti*, eastern Switzerland. *Emerging Infectious Diseases* **8**, 722–726.
- Goethert, H. K. and Telford, S. R. III** (2003). What is *Babesia microti*? *Parasitology* **127**, 301–309. doi: 10.1017/S0031182003003822.
- Gray, J., von Stedingk, L. V., Gürtelschmid, M. and Granström, M.** (2002). Transmission studies of *Babesia microti* in *Ixodes ricinus* ticks and gerbils. *Journal of Clinical Microbiology* **40**, 1259–1263. doi: 10.1128/JCM.40.4.1259-1263.2002.
- Gray, J., Zintl, A., Hildebrandt, A., Hunfeld, K. P. and Weiss, L.** (2010). Zoonotic babesiosis: Overview of the disease and novel aspects of pathogen identity. *Ticks and Tick-borne Diseases* **1**, 3–10. doi: 10.1016/j.ttbdis.2009.11.003.
- Häselbarth, K., Tenter, A. M., Brade, V., Krieger, G. and Hunfeld, K. P.** (2007). First case of human babesiosis in Germany – Clinical presentation and molecular characterisation of the pathogen. *International Journal of Medical Microbiology* **297**, 197–204. doi: 10.1016/j.ijmm.2007.01.002.
- Herwaldt, B. L., Cacciò, S., Gherlinzoni, F., Aspöck, H., Slemenda, S. B., Piccaluga, P., Martinelli, G., Edelhofer, R., Hollenstein, U., Poletti, G., Pampiglione, S., Löschenberger, K., Tura, S. and Pieniazek, N. J.** (2003). Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. *Emerging Infectious Diseases* **9**, 942–948.
- Herwaldt, B. L., de Bruyn, G., Pieniazek, N. J., Homer, M., Lofy, K. H., Slemenda, S. B., Fritsche, T. R., Persing, D. H. and Limaye, A. P.** (2004). *Babesia divergens*-like infection, Washington State. *Emerging Infectious Diseases* **10**, 622–629.
- Hildebrandt, A., Hunfeld, K. P., Baier, M., Krumbholz, A., Sachse, S., Lorenzen, T., Kiehntopf, M., Fricke, H. J. and Straube, E.** (2007). First confirmed autochthonous case of human *Babesia microti* infection in Europe. *European Journal of Clinical Microbiology and Infectious Diseases* **6**, 595–601. doi: 10.1007/s10096-007-0333-1.
- Hunfeld, K. P., Hildebrandt, A. and Gray, J. S.** (2008). Babesiosis: recent insights into an ancient disease. *International Journal for Parasitology* **38**, 1219–1237. doi: 10.1016/j.ijpara.2008.03.001.
- Kumar, S., Tamura, K. and Nei, M.** (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**, 150–163. doi: 10.1093/bib/5.2.150.
- Livanova, N. N. and Livanov, S. G.** (2010). Zoological presupposition of human tick-borne infections in the Northern Urals. *Zoological Journal* **89**, 1–8 (in Russian).
- Malandrin, L., Jouglin, M., Sun, Y., Brisseau, N. and Chauvin, A.** (2010). Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. *International Journal for Parasitology* **40**, 277–284.
- Morozov, A. V., Kovalevsky, U. V., Korenberg, E. I., Gorelova, N. V. and Podlesniy, L. A.** (2006). Preliminary results of *Babesia microti* DNA detection in small mammals inhabiting natural foci in Middle Ural. *Bulletin ESSC SB RAMS* **5**, 136–138 (in Russian).
- Nakajima, R., Tsuji, M., Oda, K., Zamoto-Niikura, A., Wei, Q., Kawabuchi-Kurata, T., Nishida, A. and Ishihara, C.** (2009). *Babesia microti*-group parasites compared phylogenetically by complete sequencing of the CC-Teta gene in 36 isolates. *Journal of Veterinary Medical Science* **71**, 55–68. doi: 10.1292/jvms.71.55.
- Pieniazek, N., Sawczuk, M. and Skotarczak, B.** (2006). Molecular identification of *Babesia* parasites isolated from *Ixodes ricinus* ticks collected in northwestern Poland. *Journal of Parasitology* **92**, 32–35.
- Piesman, J., Karakashian, S. J., Lewengrub, S., Rudzinska, M. A. and Spielman, A.** (1986). Development of *Babesia microti* sporozoites in adult *Ixodes dammini*. *International Journal for Parasitology* **16**, 381–385. doi: 10.1016/0020-7519(86)90118-9.
- Rabinovich, S. A., Voronina, Z. K., Stepanova, N. I., Maruashvili, G. M., Bakradze, T. L., Odisharija, M. S. and Gvasalija, N. I.** (1978). The first detection of human babesiosis in USSR and the short analysis of cases described in literature. *Medical Parasitology* **3**, 97–107 (in Russian).
- Rar, V. A., Maksimova, T. G., Zakharenko, L. P., Bolykhina, S. A., Dobrotvorsky, A. K. and Morozova, O. V.** (2005). *Babesia* DNA detection in canine blood and *Dermacentor reticulatus* ticks in southwestern Siberia, Russia. *Vector-Borne and Zoonotic Diseases* **5**, 285–287. doi: 10.1089/vbz.2005.5.285.
- Siński, E., Bajer, A., Welc, R., Pawełczyk, A., Ogrzewalska, M. and Behnke, J. M.** (2006). *Babesia microti*: prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes District of North-Eastern Poland. *International Journal of Medical Microbiology* **296** (Suppl. 40), 137–143. doi: 10.1016/j.ijmm.2006.01.015.

- Spencer, A. M., Goethert, H. K., Telford, S. R. III and Holman, P. J.** (2006). In vitro host erythrocyte specificity and differential morphology of *Babesia divergens* and a zoonotic *Babesia* sp. from eastern cottontail rabbits (*Sylvilagus floridanus*). *Journal of Parasitology* **92**, 333–340. doi: 10.1645/GE-662R.1.
- Sun, Y., Liu, G., Yang, L., Xu, R. and Cao, W.** (2008). *Babesia microti*-like rodent parasites isolated from *Ixodes persulcatus* (Acari: Ixodidae) in Heilongjiang Province, China. *Veterinary Parasitology* **156**, 333–339. doi: 10.1016/j.vetpar.2008.05.026.
- Telford, S. R. III, Korenberg, E. I., Goethert, H. K., Kovalevskii, U. V., Gorelova, N. B. and Spielman, A.** (2002). Detection of natural foci of babesiosis and granulocytic ehrlichiosis in Russia. *Zhurnal Mikrobiologii, Epidemiologii I Immunobiologii* **6**, 21–25 (in Russian).
- Tsuji, M., Wei, Q., Zamoto, A., Morita, C., Arai, S., Shiota, T., Fujimagari, M., Itagaki, A., Fujita, H. and Ishihara, C.** (2001). Human babesiosis in Japan: epizootiologic survey of rodent reservoir and isolation of new type of *Babesia microti*-like parasite. *Journal of Clinical Microbiology* **39**, 4316–4322. doi: 10.1128/JCM.39.12.4316-4322.2001.
- Zamoto, A., Tsuji, M., Kawabuchi, T., Wei, Q., Asakawa, M. and Ishihara, C.** (2004a). U.S.-type *Babesia microti* isolated from small wild mammals in Eastern Hokkaido, Japan. *Journal of Veterinary Medical Science* **66**, 919–926. doi: 10.1292/jvms.66.919.
- Zamoto, A., Tsuji, M., Wei, Q., Cho, S. H., Shin, E. H., Kim, T. S., Leonova, G. N., Hagiwara, K., Asakawa, M., Kariwa, H., Takashima, I. and Ishihara, C.** (2004b). Epizootiologic survey for *Babesia microti* among small wild mammals in northeastern Eurasia and a geographic diversity in the beta-tubulin gene sequences. *Journal of Veterinary Medical Science* **66**, 785–792. doi: 10.1292/jvms.66.785.
- Zintl, A., Mulcahy, G., Skerrett, H. E., Taylor, S. M. and Gray, J. S.** (2003). *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. *Clinical Microbiology Reviews* **16**, 622–636. doi: 10.1128/CMR.16.4.622-636.2003.