

New Records of *Bartonella* Spp. And *Rickettsia* Spp. In Lice Collected From Small Rodents

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Abstract

Background: Lice are blood-sucking insects that are of medical and veterinary significance as parasites and vectors for various infectious agents. More than half of described blood-sucking lice species are found on rodents. Rodents are important hosts of several *Bartonella* and *Rickettsia* species and some of these pathogens are characterised as human pathogens in Europe. Rodent ectoparasites, such as fleas and ticks, are important vectors of *Bartonella* spp. and *Rickettsia* spp., but knowledge about the presence of these bacteria in lice is limited. The aim of this study was to determine the prevalence of *Bartonella* and *Rickettsia* bacteria in lice collected from rodents in Slovakia.

Methods: The ectoparasites were collected from small rodents captured from 2010 to 2015 at four different sites in eastern Slovakia. The presence of *Bartonella* and *Rickettsia* pathogens in lice samples was screened by real-time PCR, targeting *ssrA* and *gltA* genes respectively. The molecular characterisation of the *Bartonella* strains was based on sequence analysis of partial *rpoB* and ITS genes, and of the *Rickettsia* species on sequence analysis of the *gltA* gene.

Results: A total of 1074 lice of seven species were collected from six rodent species in Slovakia from 2010 to 2015. *Bartonella* DNA was detected in three species of lice *Hoplopleura affinis* (collected from *Apodemus agrarius*, *A. flavicollis* and *Myodes glareolus*), *Polyplax serrata* (from *A. agrarius*) and *Hoplopleura* sp. (from *A. flavicollis*). Sequence analysis revealed that the *Bartonella* strains belonged to the *B. cooperplainsensis*, *B. tribocorum* and *B. taylorii* genogroups. *Rickettsia* DNR was detected in *H. affinis* and *P. serrata* lice collected from *A. agrarius*. Sequence analysis revealed the presence of two *Rickettsia* species: *R. helvetica* and *Rickettsia* sp.

Conclusions: To the best of the authors' knowledge, this is the first report on the occurrence and diversity of *Bartonella* spp. and *Rickettsia* spp. in lice collected from small rodents in Europe. This study is also the first to detect *B. cooperplainsensis* in Slovakia.

Background

Small rodents are important hosts of ectoparasites such as fleas, ticks, mites and lice and are reservoir hosts or carriers of medically important pathogens [1]. Rodent ectoparasites (fleas and ticks) are vectors of *Bartonella* spp. and *Rickettsia* spp. [1–3].

Bartonella spp. and *Rickettsia* spp. are gram-negative bacteria that can cause severe disease in humans and animals [4, 5]. Currently, 37 *Bartonella* species and three subspecies have been identified [6]. Small rodents represent an important group of potential reservoirs for many *Bartonella* infections. More than 20 *Bartonella* species have been detected in different rodent species. At least six *Bartonella* species found in rodents in Europe have been implicated in human illnesses [2]. *Bartonella* spp. have been reported in rodents and their ectoparasites (ticks, mites, and fleas) in Sweden [7], Denmark [8], Poland [9], Spain [10], Lithuania [11, 12], Slovakia [13, 14] and Germany [15].

Thirty-one *Rickettsia* species and two subspecies have been validated and published [16]. Members of the *Rickettsia* genus are divided into four groups: spotted fever group (SFG), typhus group (TG), ancestral group (AG) and transitional group (TRG) [17]. SFG rickettsia are widespread in Europe and are currently recognised as human and animal pathogens [5]. Several molecular studies conducted in Germany [18], Slovakia [3, 19, 20, 21], Hungary [22], Croatia [23], Poland [17] and Lithuania [24, 25] have demonstrated the presence of rickettsial DNA in small rodents and their ectoparasites (such as ticks, mites and fleas).

Sucking lice (Phthiraptera: Anoplura) are obligate blood-feeding insects and permanent ectoparasites of eutherian mammals. All their life cycle stages are closely related with their vertebrate hosts and they cannot survive without them. More than 540 species of blood-sucking lice have been described that parasitise over 840 mammal species belonging to 12 mammalian orders [26, 27]. Small rodents are the most common hosts of sucking lice: about 67% of the described sucking lice species are found on rodents [28]. The sucking lice are of medical and veterinary significance as vectors of louse-borne pathogens (viruses, bacteria, fungi and protozoa) to vertebrate hosts [29]. Blood-sucking lice are highly host-specific and each species of sucking louse parasitises a single host species or only a few closely related host species [26, 30]. In Europe, reports of pathogens in lice are scarce. Hornok et al. [29] were the first to report *Rickettsia* spp. in lice from livestock animals and proved that lice could be potential vectors of arthropod-borne pathogens. However, there is limited information available with regard to *Bartonella* spp. and *Rickettsia* in lice collected from rodents in Europe. The role of lice in the life cycles of *Rickettsia* and *Bartonella* is still not clear. The aim of this study was to determine the prevalence of *Bartonella* and *Rickettsia* species in lice collected from rodents in Slovakia.

Methods

Sample collection and identification

Small mammals were live-captured between 2010 and 2015 using Swedish bridge metal traps baited with sunflower seeds at four different sites in eastern Slovakia: two with mixed forest vegetation with a predominance of beech, hornbeam and spruce [Čermeľ (208–600 m a.s.l.; 48°45'46–67"N; 21°8'8–17"E) and Hyl'ov (500–750 m a.s.l.; 48°44'22–80" N; 21°4'18–90" E)], and two with deciduous forest vegetation [the Botanical garden in Košice (208 m a.s.l.; 48°44'6–84"N; 21°14'16–14" E) with a predominance of hornbeam, and the Rozhanovce game reserve (215 m a.s.l.; 48°45'36"N; 21°21'30"E), an ecotone of oak-hornbeam forest and pasture in a menagerie] (collection sites are more described in 3, 13, 31, 32).

At each site, 50 traps were placed 5 m apart in transects (approximately 250 m in length) for two consecutive nights. Captured animals were transported to the laboratory where they were determined to species level and euthanised under licenses from the Ministry of Environment of the Slovak Republic No.

4874/2011-2-2.

The ectoparasites (ticks, fleas, mites and lice) were collected and placed in 70 % ethanol until determination. Lice were then determined by species and sex using light microscopy according to Smetana [33] and Wegner [34].

Molecular analyses

Lice from each rodent host were grouped in pools by species, life stage and sex. A total of 275 sample pools (between one and ten lice per pool): 38 pools of larvae, 151 pools of females and 86 pools of males were analysed. DNA from lice was extracted using 2.5 % ammonium hydroxide solution [35]. *Bartonella* and *Rickettsia* DNA in samples was detected using a duplex TaqMan real-time PCR targeting 124 bp fragment of *ssrA* and 103 bp fragment of citrate synthase (*gltA*) genes respectively. The qPCR amplifications were carried out in a 15- μ l final volume consisting of 1 μ l of extracted DNA, (1x) SensiMix™ II Probe No-ROX Kit (Bioline Reagents Ltd, UK), 1 μ M of each primer and 0.5 μ M of each probe. The reaction was carried out in a real-time thermocycler Rotor-Gene Q 5plex model with software version 1.7 (Qiagen GmbH, Germany). The optimised thermal cycler programme was 95 °C for 10 minutes (1 cycle), followed by 50 cycles of denaturation at 95 °C for 20 seconds, annealing at 50 °C for 1 minute, and extension at 72 °C for 10 seconds. Results that satisfied the amplification cut-offs below 40 Ct (cycle threshold) when the threshold was 0.10101 indicated positive samples. *Bartonella*-positive samples were tested further in two PCRs using a set of genus-specific primers targeting the 795 bp fragment of the RNA polymerase β -subunit (*rpoB*) gene [36] and primers targeting the 16S-23S rRNA gene intergenic species region (ITS) (0.9–1.6 kb) [37, 38]. A nested PCR that targeted the partial *gltA* gene (338 bp fragment) [19] was used for amplification of *Rickettsia* spp. The primer sequences and target genes used in this study are presented in Table 1. Negative (dH₂O) and positive controls (DNA of *Bartonella*-infected rodents and the DNA of *Rickettsia*-infected ticks, confirmed by sequencing) were included in real-time PCR, conventional and nested PCRs runs. Products of amplification were identified in 1.5 % agarose gel after undergoing electrophoresis under standard conditions and staining with ethidium bromide solution (2 μ g/ml), and then visualised using the UV transilluminator (EASY Win32, Herolab, Germany).

Representative positive PCR products were extracted from the agarose gel and purified using the GeneJET Gel Extraction Kit (ThermoFisher Scientific, Lithuania) according to the manufacturer's instructions (Macrogen Europe, Netherlands). The obtained sequences were edited, aligned with one other and compared with the sequence data available from NCBI GenBank, using the Mega X program and the NCBI BLAST® blastn suite applet. The most appropriate model of nucleotide substitution for each alignment dataset was determined according to the Bayesian information criterion (BIC). Phylogenetic trees were constructed using the maximum-likelihood (ML) method with the Tamura-Nei model. Bootstrap support was calculated by means of 1,000 replicates. *Bartonella* and *Rickettsia* sequences obtained in this study were deposited in the GenBank database under the accession numbers MT840662 - MT840520 (*Bartonella* ITS region), MT876371 - MT876377, MT833866 (*Bartonellarpob* gene) and MT876378 - MT876382 (*RickettsiagltA* gene).

Statistical analysis

The prevalence of pathogens in lice was calculated as a minimum infection rate (MIR) with 95 % confidence intervals (CI). MIR was calculated as the ratio of the number of positive pools to the total number of lice tested. The underlying MIR assumption was that only one infected individual exists in a positive pool [39].

Results

A total of 1074 lice belonging to seven species (28 *Hoplopleura acanthopus*, 732 *Hoplopleura affinis*, 1 *Hoplopleura edentula*, 7 *Hoplopleura sp.*, 225 *Polyplax serrata*, 79 *Polyplax spinulosa*, and 2 *Polyplax sp.*) were collected from 216 small rodents representing six species (*Apodemus agrarius* n = 151, *Apodemus flavicollis* n = 35, *Microtus arvalis* n = 13, *Microtus subterraneus* n = 2, *Myodes glareolus* n = 11 and *Rattus norvegicus* n = 4). Both sexes of lice and larvae were found on the rodents (Table 2).

Bartonella infection in lice

Based on real-time PCR analysis, a total of 32 lice DNA pools (11.6%; 32/275 pools) were found to be positive for *Bartonella* spp. with an overall MIR of 3.0% (32/1074; 95% CI: 2.0–4.2%) and six lice pools were positive for *Rickettsia* spp. (2.2%; 6/275 pools) with an overall MIR of 0.6% (6/1074; 95% CI: 0.2–1.2%) (Table 2). Positive samples had Ct values of between 18 and 39.

Bartonella DNA was detected in three species of lice *H. affinis* (collected from *A. agrarius*, *A. flavicollis* and *M. glareolus*), *P. serrata* (collected from *A. agrarius*) and *Hoplopleura sp.* (collected from *A. flavicollis*). All three live stages of lice were found to be infected with *Bartonella* spp.: larvae (13.2% positive pools out of 38), males (8.1% out of 86) and females (13.3% out of 151). A higher prevalence of *Bartonella* spp. was detected in *H. affinis* (16.2% positive pools out of 148; MIR of 3.3% 24/732; 95% CI: 2.1–4.8%), followed by *P. serrata* (7.7% positive pools out of 91; MIR of 3.1% 7/225; 95% CI: 1.3–6.3%). One single specimen of seven tested for *Hoplopleura sp.* was positive.

Bartonella-positive PCR products of good quality were subjected to sequence analysis. A total of 14 good-quality sequences of *Bartonellarpob* (n=8) gene and ITS region (n=6) were obtained and analysed. The ITS region sequences of *Bartonella* derived from lice were 100 % identical to each other and 98–100 % identical to *Bartonella cooperplainsensis* and *Bartonella tribocorum* sequences deposited in GenBank (Fig. 1). Sequences (samples MT840662, MT840663, MT840664) derived from *H. affinis* (two pools of females and one pool of larvae collected from two *A. agrarius* rodents) were 100 % identical to each other, 100 % identical to *B. cooperplainsensis* sequences detected in *A. agrarius* from Lithuania (GenBank: MH547343) and 98 % identical to *B. cooperplainsensis* sequences detected in rats from Italy (GenBank: MK562489) and Australia (GenBank: EU111770) (Fig. 1). Sequences (samples MT840518, MT840519, MT840520) derived from *H. affinis* (two different pools of females collected from a single *A. flavicollis* and a single *M. glareolus*) and *P. serrata* (one pool of males collected from a single *A. agrarius*) were 100 % identical to each other and with *B. tribocorum* sequences detected in *A. agrarius* rodents from Lithuania (GenBank: MH687379) and South Korea (GenBank JN810856) (Fig. 1).

The *rpoB* gene sequences (samples MT876371, MT876372, MT876373, MT876374, MT876375, MT876376 and MT876377) derived from *H. affinis* (five pools of females and two pools of larvae from *A. agrarius* (n=3)) were 100 % identical to each other and to *B. coopersplainsensis* sequences detected in *A. agrarius* from Lithuania (GenBank: MH547343). These sequences showed 98 % similarity to *B. coopersplainsensis* sequences detected in rats from Australia (GenBank: EU111792) and Thailand (GenBank: MF105907) (Fig. 2). One *Bartonellarpob* sequence (sample MT833866) derived from *H. affinis* (one pool of females from a single *A. flavicollis*) was 100 % identical to the *B. taylorii* strain detected in *A. flavicollis* from Turkey (GenBank: MH932636) (Fig. 2).

Rickettsia infection in lice

Six lice pools of *H. affinis* (four pools) and *P. serrata* (two pools) collected from *A. agrarius* (n=6) were found to be positive for *Rickettsia* spp. *Rickettsia* pathogens were detected in males (2.3 % positive pools out of 86) and females (2.7 % out of 151). A total of five good-quality sequences of *Rickettsia gltA* gene were obtained and analysed. Sequence analysis of the partial *gltA* gene revealed the presence of two *Rickettsia* species: *Rickettsia helvetica* (n=4) and unrecognised *Rickettsia* sp. (n=1). *Rickettsia* sequences (samples MT876379, MT876380, MT876381 and MT876382) derived from *H. affinis* (three pools of females) and *P. serrata* (one pool of males) shared 99 % identity (with one nucleotide difference) and were 100 % identical to the *gltA* sequence of *R. helvetica* detected in *A. flavicollis* from Lithuania (GenBank: MF491764) and *R. helvetica* sequences detected in fleas from Slovakia (GenBank: MN276064, MK85717) and in *Ixodes ricinus* ticks from Slovakia (GenBank: MK85717), Poland (GenBank: EY779822) and Italy (GenBank: MN226407). The *Rickettsia* sequence (sample MT876378) isolated from *H. affinis* (one pool of males) was 100 % identical to the closely phylogenetically related sequences deposited in GenBank for *R. raoultii* (GenBank: MN550895; MH064450; MK875750; MK792599), *R. aeschlimann* (GenBank: JF803905) and *R. heilongjiangensis* (GenBank: JX945522).

Discussion

In Europe, lice collected from rodents have never been examined before for the presence of these bacteria. This study is the first report on the prevalence and diversity of *Bartonella* and *Rickettsia* species in lice collected from rodents in Slovakia. Phylogenetic analysis based on the *Bartonella rpoB* gene and *ITS* region and the *Rickettsia gltA* gene revealed the presence of *B. tribocorum*, *B. coopersplainsensis*, *B. taylorii*, *R. helvetica* and *Rickettsia* sp. in rodent lice.

In this study, *B. taylorii* was detected in *H. affinis* lice collected from *A. flavicollis*. In previous studies, *B. taylorii* has been confirmed in the small mammals *A. agrarius*, *A. flavicollis*, *M. glareolus*, *M. arvalis* and *Talpa europaea* in Slovakia [13, 14]. *B. taylorii* strains in small mammals and their ectoparasites have also been reported in several studies conducted in Europe, including in Germany [15], England [40], Lithuania [11, 12], Slovenia [41], Poland [9] and Spain [10]. *B. taylorii* can infect several sympatric woodland rodents at a given site. A high diversity of *B. taylorii* strains is frequently found in *Apodemus* mice and in *Myodes* and *Microtus* voles [2]. The pathogenic potential of *B. taylorii* is as yet unknown [11, 40].

In this study, the *B. tribocorum* infection was detected in *P. serrata* and *H. affinis* lice collected from *A. flavicollis*, *A. agrarius* and *M. glareolus*. This *Bartonella* species is pathogenic to humans [2]. Previous studies have strongly supported the association of *B. tribocorum* with rats of the genus *Rattus*. *B. tribocorum* has been detected in rats and their fleas in Thailand [42] and *Bartonella* strain closely related to *B. tribocorum* has been detected in louse (adult *P. spinulosa*) collected from rats in Egypt [43]. In the striped field mouse *A. agrarius*, *B. tribocorum* was detected for the first time in South Korea [44] and closely related strains were later confirmed in *A. agrarius* from Slovakia [13] and Lithuania [12].

The present study is the first to detect the *B. coopersplainsensis* infection in Slovakia in *H. affinis* lice collected from *A. agrarius*. Previously, *B. coopersplainsensis* has been isolated in rats from Australia [45] and New Zealand [46] and in one louse pool (*Hoplopleura* spp.) collected from rats in Thailand [42]. *B. coopersplainsensis* has also been reported in *A. agrarius* in Lithuania [12]. There is a lack of information on *B. coopersplainsensis*, therefore the public health impact of this bacteria is unknown [46].

The present study is also the first to demonstrate the presence of *R. helvetica* and *Rickettsia* sp. in lice collected from rodents in Slovakia. Two *R. helvetica* strains were detected in *H. affinis* and *P. serrata* lice collected from *A. agrarius*. *R. helvetica* are considered to be agents of human rickettsioses [17]. In recent studies conducted in Slovakia, *R. helvetica* has been identified in rodents and in fleas, mites and ticks collected from rodents [3, 19, 20, 21]. *R. helvetica* has also been reported in rodents and their ectoparasites in other European countries, such as the Netherlands [47], Hungary [22], Germany [18], Poland [17] and Lithuania [24, 25].

Based only on sequence analysis of the *gltA* gene, the *Rickettsia* sp. detected in this study in *H. affinis* lice pool collected from *A. agrarius* was not identified to species level. The obtained *gltA* sequence showed 100% identity with the corresponding sequences of *R. aeschlimann*, *R. heilongjiangensis* and *R. raoultii* in the GenBank database.

The presence of *Bartonella* spp. and *Rickettsia* spp. in lice may result from the acquisition of these bacteria via blood meals from infected rodents. *Bartonella* spp. are transmitted via horizontal transmission: arthropod vectors become infected with *Bartonella* bacteria while feeding on infected hosts, including rodents, and can then transfer the bacteria to another host [42]. Worldwide, the prevalence of *Bartonella* spp. in rodents ranges from 25 to 80%, which suggests a reciprocal adaptation between the bacteria and their reservoirs [1]. As a result of their blood-feeding habits, lice could transfer disease agents between closely-related host species [26, 29] and physical contact between individual rodents may promote the transmission of different *Bartonella* species [1].

Some SFG rickettsiae are thought to circulate in enzootic or epizootic cycles between wild vertebrates and arthropod vectors. The high prevalence of *R. helvetica* previously obtained in small rodents suggests that they may play an important role as potential natural reservoir hosts for this pathogen [18, 25].

The rodents from which the lice were collected have previously been tested for the presence of *Bartonella* and *Rickettsia* pathogens [3, 13, 14]. However, almost all the *Bartonella*-infected and *Rickettsia*-infected lice were derived from non-infected rodent hosts (except for two specimens of *A. agrarius*; data not

shown). In this case, lice could become infected by parasitising on other infected hosts. Examined small rodents infested with lice also harbour other ectoparasites species such as mites, fleas and *I. ricinus* ticks [3, 20]. The presence of *Bartonella* pathogens in lice may also result from acquisition pathogens by co-feeding with *Bartonella*-infected fleas. Fleas are the main vectors for the maintenance and transmission of *B. grahamii*, *B. taylorii* and *B. rochalimae* among populations of small mammals [2]. *B. tribocorum* has been detected in fleas and *B. coopersplainsensis* in ticks and lice [42]. Lice that infest rodents could acquire *Rickettsia* pathogens by co-feeding with infected *I. ricinus* ticks, fleas and mites. Horizontal transmission through a shared blood meal has been demonstrated for some rickettsial pathogens [48]. In a previous study conducted in Slovakia, *Rickettsia* spp. was detected in four species of mites, *I. ricinus* ticks and four flea species, with an overall prevalence of 9.3%, 17.2% and 3.5% respectively [3]. *R. helvetica* has been identified in fleas, ticks and mites [3, 21].

Although, the results of the present study confirm the circulation of *Bartonella* spp. and *Rickettsia* spp. in lice, the role of lice in the transmission of *Bartonella* and *Rickettsia* species remains unknown. Thus, future studies should be performed to determine the specific roles of different species of lice parasitising small rodents in the transmission of *Bartonella* spp. and *Rickettsia* spp. bacteria in order to estimate the potential risks for other mammals (e.g. cats) and humans.

Conclusions

To the best of the authors' knowledge, this is the first report on the occurrence and diversity of *Bartonella* spp. and *Rickettsia* spp. in lice collected from small rodents in Europe. The data presented in this paper add to knowledge about the distribution of *Bartonella* spp. and *Rickettsia* spp. in rodent ectoparasites, and demonstrate the presence of *Bartonella* pathogens in three species of lice – *H. affinis*, *P. serrata* and *Hoplopleura* sp. – and of *Rickettsia* pathogens in two lice species – *H. affinis* and *P. serrata*. This study is also the first to detect *B. coopersplainsensis* in Slovakia.

Abbreviations

SFG

Spotted fever group; TG:Typhus group; AG:ancestral group; TRG:transitional group; MIR:Minimum infection rate; CI:confidence interval; ML:maximum-likelihood method; BIC:Bayesian information criterion.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Small mammals were euthanized according to the laws of the Slovak Republic under the licences of the Ministry of Environment of the Slovak Republic No. 297/108/06–3.1 and No. 6743/2008–2.1.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request. Representative sequences were submitted to the GenBank database under the accession numbers MT840662 - MT840520, MT876371 - MT876377, MT833866, MT876378 - MT876382.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MS, AA, AP and JR designed the study data, participated in manuscript preparation, review & editing. MS and JF collected specimens for the study and performed the morphological description of specimens. AA and JR performed the experiments. AA, JR and AP conducted the data analysis. All authors read and approved the final manuscript.

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Tables

Table 1. Primers and probes used for real-time PCR, conventional and nested-PCRs.

Primer or probe	Sequence (5'-3')	Target in assay	Reference
ssrA-F1	AGTTGCAAATGACAACTATGCGG AAGGCTTCTGTTGCCAGGYG	<i>Bartonella</i> spp <i>ssrA</i> gene	[12, 49]
ssrA-R1	HEX-ACCCCGCTTAAACCTGCGACGGTT-BHQ1		
ssrA-P1 ^a			
RpoB-F	CGCATTGGYTTTRCTTCGTATG	<i>Bartonella</i> spp <i>rpoB</i> gene	[36]
RpoB-R	GTRGAYTGATTRGAACGYTG		
WITS-F ^b	ACCTCCTTTCTAAGGATGAT	<i>Bartonella</i> spp ITS region	[37, 38]
WITS-R ^b	CTCTTTCTTCAGATGATGATCC		
Bh311-332F ^c	CTCTTTCTTCAGATGATGATCC		
Bh473-452R ^c	AACCAACTGAGCTACAAGCCCT		
Rick F1	TGCMGAYCATGAGCACAATGCTTC	<i>Rickettsia</i> spp. <i>gltA</i> gene	This study
Rick R1	CCCAAAGTGAKGCAATACCCGT		
Rick P1 ^a	FAM-TGCCGGCTCATCYGGAGCTAACCC-BHQ1		
RpCS.877p ^b	GGGGACCTGCTCACGGCGG	<i>Rickettsia</i> spp. <i>gltA</i> gene	[19]
RpCS.1258n ^b	ATTGCAAAAAGTACAGTGAACC		
RpCS.896p ^c	GGCTAATGAAGCAGTGATAA		
RpCS.1233n ^c	GCGACGGTATACCCATAGC		

^aProbe, ^bExternal primers, ^cInternal primers.

Table 2 Presence of *Bartonella* spp. and *Rickettsia* spp. in lice collected from different species of small rodents.

Rodents species	<i>H. affinis</i>			<i>H. edentula</i>			<i>H. acanthopus</i>			<i>Hoplopleura sp</i>			<i>P. serrata</i>			<i>P. spinulosa</i>		
	No. positive pools/no. pools (no. lice in pools); MIR % [95 % CI]			No. positive pools/no. pools (no. lice in pools); MIR % [95 % CI]			No. positive pools/no. pools (no. lice in pools); MIR % [95 % CI]			No. positive pools/no. pools (no. lice in pools) %; MIR [95 % CI]			No. positive pools/no. pools (no. lice in pools) %; MIR [95 % CI]					
	L	0	0	L	0	0	L	0	0	L	0	0	L	0	0	L	0	0
<i>Bartonella</i> spp.																		
<i>A. agr</i>	3/6 (24)	2/41 (246)	12/78 (418)	-	-	-	-	0/1 (1)	-	0/4 (4)	-	-	0/16 (82)	3/18 (36)	3/38 (79)	-	0/1 (1)	-
<i>A. fla</i>	1/1(2)	1/6 (16)	3/7 (16)	-	-	-	-	-	-	1/1 (1)	-	-	0/3 (3)	0/3 (3)	1/10 (19)	-	0/1 (1)	0/2 (2)
<i>M. arv</i>	-	-	-	-	-	-	0/2 (4)	0/5 (9)	0/4 (13)	0/1 (1)	-	-	-	-	0/1 (1)	-	-	-
<i>M. sub</i>	-	-	0/1	-	-	-	-	-	-	0/1 (1)	-	-	-	-	-	-	-	-
<i>M. gla</i>	-	1/5(6)	1/3(3)	-	-	0/1 (1)	-	-	0/1 (1)	-	-	-	0/2 (2)	-	-	-	0/1 (2)	-
<i>R. nor</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1 (8)	0/2 (20)	0/5 (45)
Total	4/7 (26); 15.4[4.4-34.9]	4/52 (268); 1.5[0.4-3.8]	16/89 (438); 3.7[2.1-5.9]	-	-	0/1 (1)	0/2 (4)	0/6 (10)	0/5 (14)	1/7 (7); 14.3[0.4-57.9]	-	-	0/19 (85)	3/23 (41); 7.3 [1.5-19.9]	4/49 (99); 4.0[1.1-10.0]	0/1 (8)	0/5 (24)	0/7 (47)
<i>Rickettsia</i> spp.																		
	L	0	0	L	0	0	L	0	0	L	0	0	L	0	0	L	0	0
<i>A. agr</i>	0/6 (24)	1/42 (246)	3/78 (418)	-	-	-	-	0/1 (1)	-	0/4 (4)	-	-	0/16 (82)	1/15 (24)	1/40 (91)	-	0/1 (1)	-
<i>A. fla</i>	0/1 (2)	0/6 (16)	0/7 (16)	-	-	-	-	-	-	0/1 (1)	-	-	0/3 (3)	0/3 (3)	0/10 (19)	-	0/1 (1)	0/2 (2)
<i>M. arv</i>	-	-	-	-	-	-	0/2 (4)	0/5 (9)	0/4 (13)	0/1 (1)	-	-	-	-	0/1 (1)	-	-	-
<i>M. sub</i>	-	-	0/1	-	-	-	-	-	-	0/1 (1)	-	-	-	-	-	-	-	-
<i>M. gla</i>	-	0/5 (6)	0/3 (3)	-	-	0/1 (1)	-	-	0/1 (1)	-	-	-	0/2 (2)	-	-	-	0/1 (2)	-
<i>R. nor</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1 (8)	0/2 (20)	0/5 (45)
Total	0/7 (26)	1/53 (268); 0.4% [0.0-2.1]	3/89 (438); 0.7 [0.1-2.0]	-	-	0/1 (1)	0/2 (4)	0/6 (10)	0/5 (14)	0/7 (7)	-	-	0/19 (85)	1/20 (29); 3.4[0.0-17.8]	1/51 (111); 0.9[0.0-4.9]	0/1 (8)	0/5 (24)	0/7 (47)

Abbreviations: *A. agr* – *Apodemus agrarius*; *A. fla* – *Apodemus flavicollis*; *M. arv* – *Microtus arvalis*; *M. sub* – *Microtus subterraneus*; *M. gla* – *Myodes glareolus*; *R. nor.* – *Rattus norvegicus*

Figures

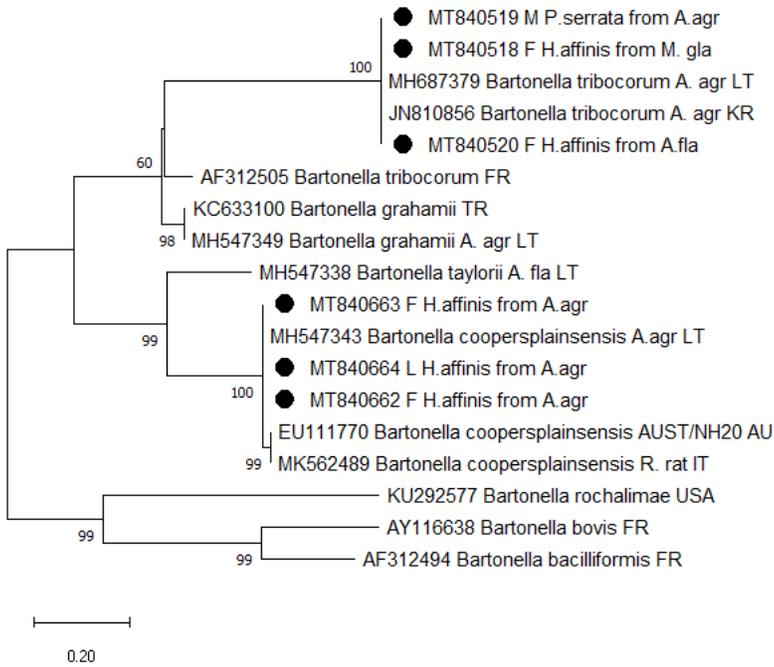


Figure 1

Maximum-likelihood phylogenetic tree for the partial ITS region of *Bartonella* spp. The phylogenetic tree was created using the Tamura-Nei model and bootstrap analysis of 1000 replicates. Samples sequenced in the present study are marked. Abbreviations: *A. agr* – *Apodemus agrarius*, *M. gla* – *Myodes glareolus*, *A. fla* – *Apodemus flavicollis*, *R. rat* – *Rattus norvegicus*, F – female, M – male, L – larva.

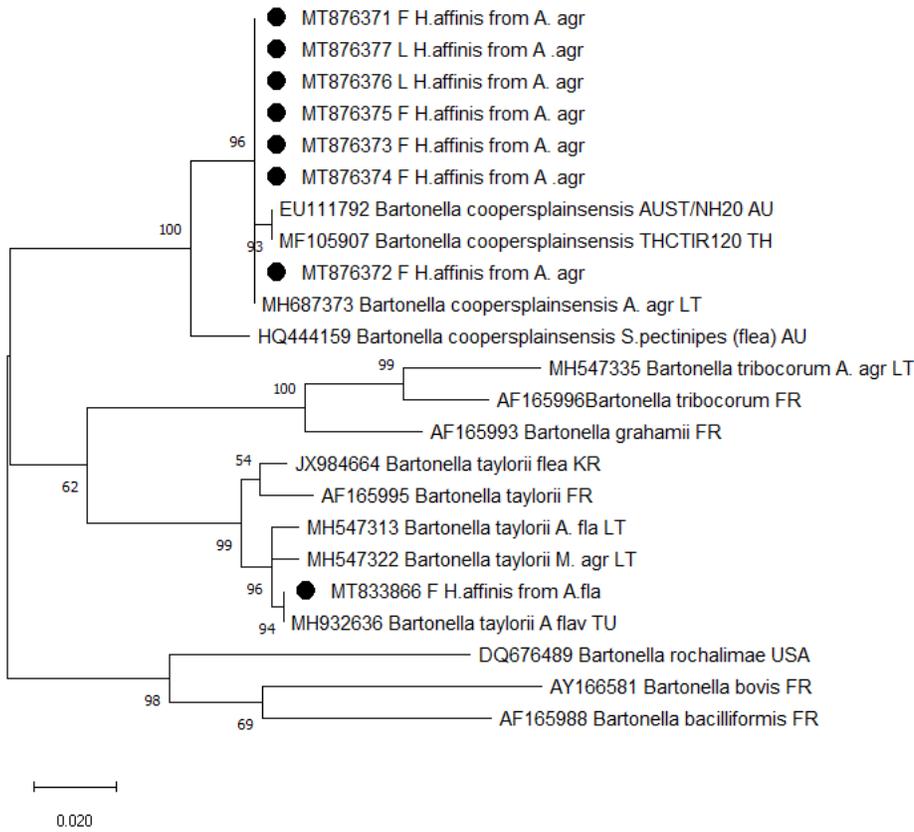


Figure 2

Maximum-likelihood phylogenetic tree for the partial rpoB gene of Bartonella spp. The phylogenetic tree was created using the Tamura-Nei model and bootstrap analysis of 1000 replicates. Samples sequenced in the present study are marked. Abbreviations: A. agr – Apodemus agrarius, M. agr – Microtus agrestis, A. fla - A. flavicollis, F – female, M – male, L – larva.

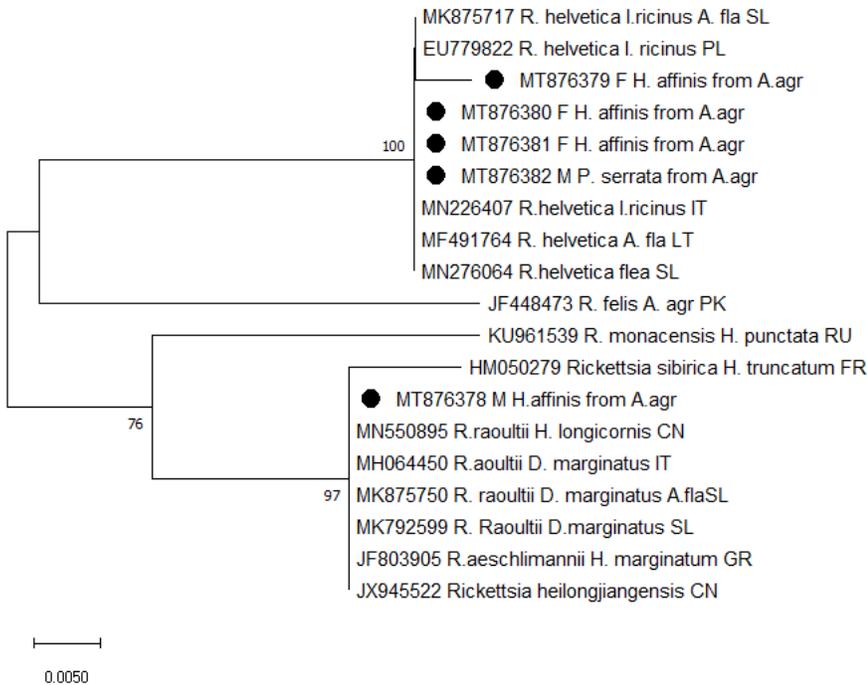


Figure 3

Maximum-likelihood phylogenetic tree for the partial *gltA* gene of *Rickettsia* spp. The phylogenetic tree was created using the Tamura-Nei model and bootstrap analysis of 1000 replicates. Samples sequenced in the present study are marked. Abbreviations: A. agr – *Apodemus agrarius*, A. fla – *Apodemus flavicollis*, F – female, M – male.