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Genetic diversity of *Bartonella taylorii* and *Bartonella grahamii* strains

Dalytė Mardosaitė-Busaitienė,

Paulina Amšiejūtė,

Jana Radzijevskaja*,

Algimantas Paulauskas

Faculty of Natural Sciences, Vytautas Magnus University, 8 Vileikos St, Kaunas 44404, Lithuania

Bacterial strains are a characteristic feature of many bacterial pathogens, including the species of the genus *Bartonella*. These bacteria are associated with different vertebrate hosts and vectors and have been detected in North America, Australia, Asia, and Europe. This study presents molecular characterization of *Bartonella* strains circulating in wild rodents. *B. taylorii* and *B. grahamii* are detected with high prevalence in mice, voles, and rats. However, there is a lack of knowledge on the geographical distribution and genetic diversity of *B. taylorii* and *B. grahamii* strains in wild rodents. The objectives of this study were to characterize the genetic diversity of *B. taylorii* and *B. grahamii* strains by sequence analysis of the housekeeping gene (*rpoB*) and the 16S-23S rRNA intergenic species region (ITS). Sequence analysis of *rpoB* gene revealed the presence of 15 *B. taylorii* genotypes and four *B. grahamii* genotypes in mice and voles captured in Lithuania. Sequence analysis of the ITS region revealed the presence of six *B. taylorii* genotypes and four *B. grahamii* genotypes in Lithuanian voles and mice. Analysis of genetic diversity demonstrated that *B. grahamii* strains derived from the same geographic region or from regions of close proximity more conservative, while *B. grahamii* strains from more distant areas are more variable. Genetic diversity of *B. taylorii* strains seems not to depend on geographic distance.

Keywords: genetic diversity, *Bartonella grahamii*, *Bartonella taylorii*, rodents, geographic distance

INTRODUCTION

Bacterial strains are a characteristic feature of many bacterial pathogens, including the species of the genus *Bartonella*. *Bartonella* are facultative intracellular, gram-negative bacteria that are transmitted to animals and humans by vectors such as fleas, mites, sand fleas and ticks (Birtles, Raoult, 1996; Kosoy et al., 2010). These bacteria have been detected in Asia, Australia, North America, and Europe (Vijay et al., 2012). Rodents play an important role as potential reservoirs of many infections caused by *Bartonella* pathogens such as Carrion's disease, endocarditis, fever, neuroretinitis, bacteremia, and others that have been reported worldwide (Okaro et al., 2017). *Bartonella* genus has one of the lowest recombination rates among intracellular bacteria. However, recombination events among *Bartonella* strains circulating within rodents occurred more frequently

* Corresponding author. Email: jana.radzijevskaja@vdu.lt

compared to human- and cat-adapted species, suggesting a broader host range for rodent-adapted species (Buffet et al., 2013). More than one of genetic variants of bacteria could circulate in the same rodent species (Gutierrez et al., 2015; Morick et al., 2011).

Bartonella taylorii and *Bartonella grahamii* are often found in wild rodents from Muridae and Cricetidae families (Okaro et al., 2017). *B. grahamii* infects many species of mice and voles and can be pathogenic for humans. The pathogenic potential of *B. taylorii* is yet unknown (Kevin et al., 2004). In Europe, *B. taylorii* and *B. grahamii* detected in the yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), striped field mouse (*Apodemus agrarius*), herb field mouse (*Apodemus uralensis*), steppe field mouse (*Apodemus witherbyi*), harvest mouse (*Micromys minutus*), common house mouse (*Mus musculus*), bank vole (*Myodes glareolus*), common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), root vole (*Microtus oeconomus*), and brown rat (*Rattus norvegicus*) (Špitalska et al., 2017; Gutierrez et al., 2015; Tołkacz et al., 2018; Obiegala et al., 2019). However, there is a lack of knowledge on the geographical distribution and genetic diversity of *B. taylorii* and *B. grahamii* strains in wild rodents.

The aim of this study was to investigate the genetic diversity of *B. taylorii* and *B. grahamii* strains in different species of rodents collected in Lithuania and compared with those submitted to GenBank based on the *rpoB* gene and the 16S-23S rRNA intergenic species region (ITS).

MATERIALS AND METHODS

In the present study, *Bartonella* DNA was collected form 30 *Bartonella*-infected rodents (trapped in different locations in Lithuania) representing seven species *Apodemus flavicollis*, *Apodemus agrarius*, *Micromys minutus*, *Myodes glareolus*, *Microtus oeconomus*, *Microtus agrestis,* and *Mus musculus*) (Table 1). A 795 bp fragment of *Bartonella* RNA polymerase β-subunit (*rpoB*) gene (Renestro et al., 2001) and 0.8–0.9 kb fragment of the 16S-23S rRNA gene intergenic species region (ITS) (Jensen et al., 2000; Kaewmongkol, 2012) were amplified by conventional and nested PCRs. The best quality PCR products were extracted from agarose gel and purified using GenJET PCR purification kit (Thermo Fisher Scientific, Lithuania) and were sent for sequencing (Macrogen Europe, Netherlands) after preparation.

Genetic polymorphism of *B. grahamii* and *B. taylorii* strains circulating in rodents from

Table 1. **Distribution of *B. grahamii* and *B. taylorii* in rodents in Lithuania**

Rodent species		*Bartonella grahamii*	*Bartonella taylorii*
Yellow-necked mouse (*Apodemus flavicollis*)		+	+
Striped field mouse (*Apodemus agrarius*)		+	+
Harvest mouse (*Micromys minutus*)		+	–
Common house mouse (*Mus musculus*)		+	–
Bank vole (*Myodes glareolus*)		+	+
Root vole (*Microtus oeconomus*)		+	+
Field vole (*Microtus agrestis*)		–	+
Location			
Rusnė	55°19'26.23"N, 21°20'24.15"E	+	+
Žalgiriai	55°18'40.0"N, 21°26'10.0"E	–	+
Beištrakiai	54°54'22.3"N, 24°20'28.6"E	+	+
Elektrėnai	54°45'37.22"N, 24°40'41.45"E	+	+
Dubingiai	55°03'38.1"N, 25°27'03.7"E	+	+
Lukštas	55°51'0.94"N, 26°12'6.11"E	+	+

+, *Bartonella*-infection present; –, *Bartonella* infection absent.

Lithuania and different parts of the world was analysed using the *rpoB* gene and ITS region sequences obtained in this study and previously submitted in GenBank. Sequences were analyzed using Mega X software package, v10.0.5 (Kumar et al., 2018) and the NCBI BLAST® software (http://blast.ncbi.nlm.nih.gov). Nucleotide diversity (π), haplotype diversity (Hd), and the average number of nucleotide differences (k) were calculated using DnaSP v5.10.01. Overall genetic distance (D), variable sites (V) and parsimony-informative sites (Pi) were calculated by using Mega X software, v10.0.5. (Librado, Rozas, 2009).

RESULTS

Genetic diversity of *B. grahamii* and *B. taylorii* strains

A total of 38 good-quality *B. grahamii* (n = 13) and *B. taylorii* (n = 25) *rpoB* gene and ITS region sequences obtained in this study were included in genetic analysis.

Sequence analysis of eight *B. grahamii rpoB* gene sequences revealed the presence of four *B. grahamii* genotypes (with difference at seven nucleotides positions and three parsimony-informative sites): first genotype detected in *A. agrarius*, second genotype detected in *A. agrarius*, *A. flavicollis*, *M. musculus* and *M. glareolus*, the third genotype in *M. glareolus*, and the fourth genotype in *A. agrarius* and *M. minutus*. Three different genotypes were detected in *A. agrarius* (differed at five nucleotide positions) and two in *M. glareolus* (differed at three nucleotide positions) rodent species (Table 2a; 4a). Four *B. grahamii* genotypes were identified based on five *B. grahamii* ITS region sequences (analysed sequences differed at 15 nucleotides positions and have one parsimony-informative site): the first genotype was detected in *A. agrarius*, the second in *A. agrarius* and

Table 2. **Comparison of the *rpoB* gene (a.) and ITS (b.) region sequences of *B. grahamii* obtained from rodents in Lithuania**

Genetic variant	Rodent species	Nucleotide position															
a.																	
							2		2		2		4		4		
				4		8	3		6		9		4		4		
				4		6	0		3		6		4		6		
1	*A. agrarius*			T		A	G		C		T		–		–		
2	*A. agrarius*			A		G	C		G		C		–		–		
	A. flavicollis			A		G	C		G		C		–		–		
	M. musculus			A		G	C		G		C		A		A		
	M. glareolus			A		G	C		G		C		A		A		
3	*M. glareolus*			.		G	C		G		C		G		G		
4	*A. agrarius*			A		G	C		G		C		G		G		
	M. minutus			A		G	C		G		C		G		G		
b.																	
							1	2	3	3	4	6	7	7	7	7	
			1	2	2	9	0	5	3	4	1	7	6	7	8	8	
		9	2	1	9	7	0	5	5	3	1	5	7	0	0	2	
1	*A. agrarius*	C	T	C	A	A	A	A	T	C	C	G	A	G	A	C	
2	*M. glareolus*	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.	
	A. agrarius	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.	
3	*M. oeconomus*	T	C	T	G	T	G	G	A	T	T	.	G	A	T	T	
4	*M. minutus*	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	

Table 3. **Comparison of the *rpoB* gene (a.) and ITS (b.) regions sequences of *B. taylorii* obtained from rodents in Lithuania**

Genetic variant	Rodent species	Nucleotide position																																									
a.																																												
														1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	4	4	4	4	4	5	5	6	6	6	6	6	6	6	7	7
				1	2	2	2	3	3	5	6	7	8	1	3	3	5	6	0	6	7	9	0	1	2	2	2	6	0	1	2	2	4	1	6	1	4	6	7	7	8	8	2	8
		8	9	1	3	4	6	2	8	0	2	7	3	3	4	7	5	7	9	3	5	3	2	4	3	8	9	6	7	7	3	8	3	5	3	7	1	6	1	7	0	7	8	0
1	*A. flavicollis*	C	G	C	T	C	G	T	A	G	G	G	G	G	C	T	A	T	G	T	G	T	T	G	T	G	G	C	C	C	G	A	-	-	-	-	-	-	-	-	-	-	-	-
2	*M. agrestis*	A	.	.	C	T	.	C	G	A	A	.	.	.	.	.	.	A	.	G	.	.	C	.	.	.	A	.	.	.	.	G	A	T	A	G	G	T	T	C	T	G	G	G
3	*M. agrestis*	A	.	.	C	T	.	C	G	A	A	.	.	.	.	.	.	A	.	G	.	.	C	.	.	.	A	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-
4*	*M.glareolus*	A	.	T	.	T	.	C	G	.	A	.	.	A	.	.	G	.	C	G	A	.	C	A	.	.	A	.	.	.	T	.	A	T	G	C	G	G	T	C	C	G	A	A
5	*M. glareolus*	A	.	T	.	T	.	C	G	.	A	.	.	A	.	.	G	.	C	G	A	.	C	A	.	.	A	.	.	.	T	.	G	C	G	C	G	T	T	C	C	G	A	-
6	*M. glareolus*	A	.	T	.	T	.	C	G	.	A	.	.	A	.	.	G	.	C	G	A	.	C	A	.	.	A	.	.	.	T	.	A	T	G	C	G	T	T	C	C	G	A	A
7	*M. glareolus*	A	.	.	.	.	A	C	.	T	A	.	.	.	T	.	.	A	.	G	.	C	C	.	C	.	A	.	.	.	.	.	A	T	G	G	G	T	T	C	C	G	A	A
8	*M.glareolus*	A	.	T	.	T	.	C	G	.	A	.	.	A	.	.	G	.	C	G	A	.	C	A	.	.	A	.	.	.	T	.	A	T	G	C	G	G	T	C	C	A	A	A
9	*M. oeconomus*	A	.	.	C	T	.	C	G	A	A	.	.	.	.	.	.	A	.	G	.	.	C	.	.	.	A	.	.	.	.	G	A	T	A	G	G	T	T	C	T	G	G	A
	M. agrestis	A	.	.	C	T	.	C	G	A	A	.	.	.	.	.	.	A	.	G	.	.	C	.	.	.	A	.	.	.	.	G	A	T	A	G	G	T	T	C	T	G	G	A
10	*M. oeconomus*	G	A	.	C	T	.	C	G	A	.	A	.	.	T	.	.	.	.	G	.	.	C	A	.	A	A	.	.	.	.	.	A	-	-	-	-	-	-	-	-	-	-	-
11	*M. oeconomus*	A	.	.	C	T	.	C	G	A	A	A	.	.	.	.	.	.	.	G	.	.	C	A	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	*M. agrestis*	A	.	.	.	T	.	C	G	.	A	A	.	A	.	C	G	.	C	G	A	.	C	A	.	.	A	.	.	T	T	.	G	C	G	C	G	T	T	C	C	G	A	A
13	*M. oeconomus*	A	.	.	C	T	.	C	G	.	A	.	.	A	.	.	G	.	C	G	A	.	C	A	.	.	A	T	T	.	T	.	A	T	G	C	A	T	A	C	C	G	A	A
14**	*M. glareolus*	A	.	.	C	T	.	C	G	A	A	.	A	.	.	.	.	A	.	G	.	.	C	.	.	.	A	.	.	.	.	G	A	T	A	G	G	T	T	A	T	G	G	A
15***	*M. agrestis*	A	.	T	.	T	.	C	G	.	A	A	.	A	.	C	G	.	C	G	A	.	C	A	.	.	A	.	.	T	T	.	G	C	G	C	G	T	T	C	C	G	A	A

Genetic variant	Rodent species	Nucleotide position																				
b.																						
			2	2	3	4	4	4	4	5	7	7	7	7	7	7	8	8	8	8	8	8
		6	3	7	1	0	3	3	6	3	0	7	7	7	8	9	0	0	5	6	7	8
		2	8	0	1	6	6	7	9	2	1	4	5	8	6	5	0	3	8	6	1	3
1	*A. agrarius*	G	T	C	G	G	A	A	C	G	T	A	G	C	A	A	G	T	A	A	G	A
2	*M. glareolus*	.	C	.	.	.	.	G	.	.	.	.	A	T	G	G	C	A	G	G	.	G
3	*M. glareolus*	.	C	T	A	A	.	.	.	.	.	G	A	.	.	.	C	A	.	.	.	.
4	*M. oeconomus*	A	C	T	A	.	.	.	.	A	.	.	A	.	G	G	C	A	G	.	A	.
5	*M. oeconomus*	A	.	.	A	.	.	.	A	A	.	.	A	.	G	G	C	A	G	.	A	.
6	*M. agrestis*	.	C	.	.	.	C	.	.	A	C	.	A	.	G	G	C	A	G	G	.	.

* – this same genetic variant was detected in one *Myodes glareolus* specimen; ** – the same genetic variant was detected in one *Myodes glareolus* specimen; *** – the same genetic variant was detected in one *Microtus agrestis* specimen.

M. glareolus, the third in *M. oeconomus*, and the fourth genotype in *M. minutus*. Two different genotypes were detected in *A. agrarius* (sequences differed at two nucleotide positions) (Table 2b; 4a).

Sequence analysis of 19 *B. taylorii rpoB* gene sequences revealed the presence of 15 *B. taylorii* genotypes (with difference at 43 nucleotide positions and 27 parsimony-informative sites): six different genotypes were detected in *M. glareolus* (differed at 27 nucleotide positions), five in *M. agrestis* (differed at 21 nucleotide positions), and four in *M. oeconomus* (differed at 23 nucleotide positions). One genetic variant was detected in *A. flavicollis* specimen. *M. agrestis* and *M. oeconomus* and shared the same genotype (Table 3a; 4a). Six *B. taylorii* genotypes were identified based on six ITS region sequences (analysed sequences differed at 21 nucleotides positions and have ten parsimony-informative sites): two different genotypes were detected in *M. glareolus* (differed at 11 nucleotide positions) and two in *M. oeconomus* (differed at three nucleotide positions). Different genotypes were detected in *M. agrestis* and *A. agrarius* (Table 3b; 4a).

B. taylorii strains isolated from rodents in Lithuania demonstrated a higher genetic diversity based on estimated parameters of haplotype diversity (Hd), nucleotide diversity (Π), the average number of nucleotide differences (k) compared with *B. grahamii* strains (Table 4a). *B. grahamii* strains demonstrated higher similarity to each other compared with *B. taylorii* strains (overall mean genetic distance (D) was higher for *B. taylorii*) (Table 4a).

In this study, we also used GenBank sequences of rodents from France, China, Russia, South Korea, Japan, USA, Canada, UK, Germany, and Slovakia to analyse genetic diversity of *B. grahamii* and *B. taylorii* strains (Table 4b). Among analysed sequences of both genetic regions, the higher number of genotypes was detected in *B. taylorii*, while haplotype diversity parameters were similar in both *Bartonella* species. The number of variable sites, parsimony-informative sites, nucleotide diversity, and the average number of nucleotide differences showed higher values for *B. grahamii* compared with those obtained for *B. taylorii* (Table 4b). The overall mean genetic distance among *B. grahamii* strains isolated from Lithuanian rodents was five times lower compared with the genetic distance estimated among *B. grahamii* strains from different geographical regions. Similar patterns were observed in values of nucleotide diversity (Table 4a, b).

Table 4. **Comparison of genetic diversity and nucleotide polymorphism of the *rpoB* gene and ITS region sequences of *B. taylorii* and *B. grahamii* between rodents from Lithuania (a) and rodents from Lithuania and other countries (b)**

Bartonella spp. (sq nr.)	Gene / region	Fragment size (bp)	Genotypes number	D (±SD)	V	Pi	Π (±SD)	Hd(±SD)	k
a.									
B. taylorii (19)	*rpoB*	797	15	0.0190 ± 0.0039	43	27	0.02241 ± 0.00262	0.895 ± 0.048	7.32749
B. grahamii (8)	*rpoB*	797	4	0.0047 ± 0.0019	7	3	0.00454 ± 0.00265	0.464 ± 0.020	1.42857
B. taylorii (6)	ITS	792	6	0.0117 ± 0.0026	21	10	0.01153 ± 0.00149	1.000 ± 0.096	9.13333
B. grahamii (5)	ITS	754	4	0.0083 ± 0.0028	15	1	0.00823 ± 0.00402	0.900 ± 0.161	6.20000
b.									
B. taylorii (31)	*rpoB*	797	23	0.019 ± 0.003	61	39	0.02334 ± 0.00189	0.929 ± 0.029	7.63226
B. grahamii (26)	*rpoB*	797	18	0.024 ± 0.004	76	41	0.02696 ± 0.00350	0.929 ± 0.035	8.49231
B. taylorii (19)	ITS	812	19	0.021 ± 0.003	67	26	0.02196 ± 0.00199	0.988 ± 0.021	4.65497
B. grahamii (16)	ITS	760	12	0.042 ± 0.009	59	40	0.03855 ± 0.00340	0.867 ± 0.079	15.1500

Abbreviations: D, genetic distance; V, variable sites; Pi, parsimony-informative sites; Π, nucleotide diversity; Hd, haplotype diversity; k, average number of nucleotide differences; SD, standard deviation; sq nr, sequences number.

DISCUSSION

In Lithuania, *B. taylorii* strains isolated from rodents demonstrated a high genetic diversity and were frequently found in *Myodes* and *Microtus* voles. *Bartonella* isolates belonging to the *B. grahamii* genetic group were detected more frequently in *Apodemus* mice compared with voles. *B. taylorii* showed higher prevalence among Lithuanian rodents than *B. grahamii*. Similar results were obtained in Slovakia, where *B. taylorii* was most common among captured wild rodents: 85% of sequences were ascribable to *B. taylorii* and detected in *A. flavicollis*, *M. glareolus*, *M. arvalis*, and *T. europaea*, only four sequences derived from *A. flavicollis* and *M. glareolus* were found to be similar to *B. grahamii* (Špitalska et al., 2017). In Austria, *B. taylorii* was the most frequently detected species without apparent host specificity, *B. grahamii* was found only in wood mice (Schmidt et al., 2014). In France, as in Lithuania, *B. taylorii* identified in bank voles, field voles, and wood mice with a much higher prevalence than *B. grahamii*, which was recovered only in *Myodes* voles (Buffet et al., 2013).

In this study, *B. grahamii* strains were detected in 13 out of 38 rodent specimens and showed lower sequence diversity compared with *B. taylorii*. Sequence analysis of the *rpoB* gene and ITS region revealed the presence of 15 and six *B. taylorii* genotypes, respectively. Only four *B. grahamii* genotypes were detected in voles and mice. In Europe, sequence analysis showed similar results. In France, where genetic analysis of *B. taylorii* and *B. grahamii* was performed, *B. taylorii* strains demonstrated a markedly higher genetic diversity than *B. grahamii* (Buffet et al., 2013). In contrast, research done in Heixiazi Island, China, showed different results. Genetic diversity estimated for *B. grahamii* was greater than the genetic diversity established for *B. taylorii* from the same area. It was suggested that all presently known isolates of *B. grahamii* from *Apodemus* spp. are split into two clades (one clade in Asia and another clade in Europe and North America). However, host species showed a more important role for the specificity of *Bartonella* genotypes than the geographic area distance (Li et al., 2015).

Similar results to ours were also observed in Sweden, where only three sequence types of *B. grahamii* were identified in four rodent species captured in three geographic locations separated by less than 30 km. The study showed that environmental barriers in the form of water can lead to isolation and loss of genomic variability in host-associated bacterial populations and suggested that genetically similar strains can infect a range of different hosts (Berglund et al., 2010). In another research carried out in Sweden in 2003, Ehrenborg and colleagues could not find any association between *B. grahamii* subtype and infected host species. *B. grahamii* variants were separated by short geographic distances and the evidence of restricted gene flow was most discernible among populations separated by natural boundaries but not by species boundaries between the captured small mammals (Ehrenborg et al., 2003).

Analysis of genetic diversity demonstrated that *B. grahamii* strains derived from the same geographic region or from regions of close proximity are more conservative, while *B. grahamii* strains from more distant areas are more variable. Genetic diversity of *B. taylorii* strains seems not to depend on geographic distance (Table 4a, b). The genotypic heterogeneity of these two bacteria species might be due to the several factors: the geographical distances, the environmental conditions, host animals, and specificity of vectors.

To better analyse the effects of geographical factors on *Bartonella* isolates, analysis of intra- and interspecies variations should be performed for more isolates of the same species of rodent hosts collected in different geographic locations. Since rodents live in a wide range of habitats that are frequented by humans and *Bartonella* species may be transmitted to humans by ticks, fleas, and lice (Jiypong et al., 2014), future surveys of the transmission and ecology of *Bartonella* are warranted in order to improve bartonellosis prevention.

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Dalytė Mardosaitė-Busaitienė, Paulina Amšiejūtė, Jana Radzijevskaja, Algimantas Paulauskas

***BARTONELLA TAYLORII* IR *BARTONELLA GRAHAMII* PADERMIŲ GENETINĖ ĮVAIROVĖ**

Santrauka

Skirtingų bakterijų padermės, įskaitant ir *Bartonella* rūšis, gali būti susijusios su skirtingais stuburiniais šeimininkais bei vektoriais ir aptinkamos skirtinguose geografiniuose regionuose – Šiaurės Amerikos, Australijos, Azijos ir Europos. *B. taylorii* ir *B. grahamii* rūšies bakterijos dažnai identifikuojamos pelėse, pelėnuose ir žiurkėse, tačiau vis dar trūksta žinių apie šių bakterijų padermių geografinį pasiskirstymą ir genetinę įvairovę. Šio tyrimo tikslas buvo nustatyti *B. taylorii* ir *B. grahamii* padermių genetinę įvairovę pagal *rpoB* geno ir 16S–23S rRNR vidinio transkribuojančio regiono (ITS) sekas. Atlikus Lietuvoje sugautų pelėnų ir pelių *rpoB* geno sekų analizę, buvo nustatyta penkiolika *B. taylorii* ir keturi *B. grahamii* genotipai; ITS regiono sekų analizės metu nustatyti šeši *B. taylorii* ir keturi *B. grahamii* genotipai. Genetinės įvairovės analizė rodo, kad *B. grahamii* padermės, kilusios iš to paties geografinio regiono ar nedideliu atstumu nutolusių vietovių, yra gana konservatyvios, o *B. grahamii* padermės, kilusios iš labai nutolusių vietovių, pasižymi didesne sekų įvairove. Geografinis atstumas *B. taylorii* padermių genetinei įvairovei įtakos neturi.

Raktažodžiai: genetinė įvairovė, *Bartonella grahamii*, *Bartonella taylorii*, graužikai, geografinis atstumas