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Isolated populations of ***Ixodes lividus*** ticks in the Czech Republic and Belgium host genetically homogeneous ***Rickettsia vini***

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1 **Isolated populations of Ixodes lividus ticks in the Czech Republic and**  
2 **Belgium host genetically homogeneous Rickettsia vini**

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## 35 **Abstract**

36 In the last two decades, advent of molecular methods revealed a remarkable diversity of  
37 rickettsiae (*Rickettsiales: Rickettsiaceae*) in invertebrates. Several species of these obligate  
38 intracellular bacteria are known to be associated with human infections, hence more attention  
39 has been directed towards human-biting ectoparasites. An uncharacterized spotted fever group  
40 rickettsia has recently been detected in *Ixodes lividus* ticks (Acari: Ixodidae). In order to  
41 determine whether this rickettsia varies among isolated tick populations, a total of 1758 and  
42 148 *Ixodes lividus* ticks (Acari: Ixodidae) were collected in the Czech Republic and Belgium,  
43 respectively, from nests of sand martins (*Hirundinidae: Riparia riparia*) or by mist-nets. All  
44 165 and 78 randomly selected ticks for screening and 3 females of *Ixodes arboricola* (Acari:  
45 Ixodidae), obtained previously in another study screened for the presence of rickettsial DNA  
46 yielded positive for *Rickettsia vini*, a spotted fever group rickettsia that has recently be found  
47 in the closely related *I. arboricola*. Maximum Likelihood analysis of the obtained rickettsial  
48 sequences showed that the most closely related genospecies to *Rickettsia vini* corresponds to  
49 an uncharacterized rickettsia detected in *Argas lagenoplastis* (Acari: Argasidae), a nidicolous  
50 soft tick of fairy martin (*Petrochelidon ariel*) (*Hirundinidae*) in Australia, followed by various  
51 rickettsial genospecies. Considering the apparently endosymbiotic nature of *R. vini*, the  
52 existence of *R. vini* genospecies infecting ticks of different genus and different geographical  
53 distributions, suggest the existence of an initial infection in a common Ixodidae ancestor.

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## 57 **Research Highlights**

- 58 • The tick *Ixodes lividus* contains *Rickettsia vini* with 100% prevalence.
- 59 • *Rickettsia vini* and its most closely related genospecies infect nidicolous ticks.
- 60 • A common ancestor of the genera *Ixodes* and *Argas* hosted a rickettsial endosymbiont.

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63 **Keywords:** *Ixodes*, *Argas*, *Rickettsia*, spotted fever group, *Riparia riparia*, coevolution

64

## 65 **Introduction**

66 Rickettsiae are obligate intracellular bacteria of invertebrates, some rickettsial species can  
67 propagate in vertebrates, to which they are transmitted via blood-sucking arthropods (Parola  
68 et al., 2013). Based on whole-genome analyses, a single topology of the genus *Rickettsia* has

69 been estimated (Murray 2016), while the members of the well-sampled spotted fever group  
70 (SFG) are mainly associated with ixodid ticks (Parola et al., 2013). As many ticks are  
71 common parasites of free-ranging vertebrates, some ancestral groups exhibit a nidicolous  
72 behaviour, parasitizing their hosts within caves, burrows, and nests, which is the case of  
73 representatives of the family Argasidae and many species of the genus *Ixodes* (Ixodidae)  
74 (Sonenshine and Roe, 2014).

75  
76 *Ixodes lividus* C.L. Koch, 1844, a nidicolous tick species, has been found to contain rickettsial  
77 DNA with the 100% prevalence (Elfving et al., 2010; Graham et al., 2010). The obtained  
78 sequences had been assigned to those available in GenBank, however, the rickettsial agent  
79 had remained uncharacterized.

80  
81 Recently, in *Ixodes arboricola* Schultze & Schlottke, 1930, a tick that strictly feeds on birds  
82 roosting and breeding inside tree cavities (Heylen et al., 2012) and that is closely related to *I.*  
83 *lividus* (ref Heylen 2014 => see phylogenetic tree) a new rickettsial organism *Rickettsia vini*  
84 has been detected with the prevalence of 94.4-100.00% (Palomar et al., 2012a; Palomar et al.,  
85 2015). Occasionally, this rickettsial organism has also been detected in immature stages of the  
86 field-dwelling *Ixodes ricinus* (Linnaeus, 1758) derived from passerine birds (Keskin et al.,  
87 2014; Palomar et al., 2012a; Palomar et al., 2012b; Špitalská et al., 2011; Novakova et al.,  
88 2015; Novakova et al., 2016).

89  
90 Both closely related *I. lividus* and *I. arboricola* tick species have Palearctic distribution  
91 (Guglielmone et al., 2014, Heylen et al., 2014). While a large proportion of *I. arboricola*'s  
92 hosts are birds showing a relatively low migratory potential, the main host of *I. lividus* ticks is  
93 sand martin, *Riparia riparia* (Hirundinidae), a passerine bird that breeds in Holarctis and  
94 migrates south in winter (Svensson et al., 2010).

95 During the breeding season, *R. riparia* colonizes river banks in high densities and nests in  
96 sand holes (burrows) that are up to 1 m long. It is also able to nest at human-made mining  
97 places (Heneberg et al., 2013).

98 The aim of this study is to provide molecular characterization of the rickettsial genospecies in  
99 *I. lividus* ticks (24 areas in the Czech Republic and 1 area in Belgium), see whether there are  
100 differences among rickettsial agents of geographically *I. lividus* tick populations and verify to  
101 what extent the *I. lividus*-associated rickettsial genospecies resembles *R. vini* found in *I.*  
102 *arboricola* .

103

## 104 **Materials and Methods**

105

106 Nest materials from 20 European bee-eater (*Merops apiaster*) burrows, 8 burrows made by *R.*  
107 *riparia* but inhabited by Eurasian tree sparrow (*Passer montanus*) and 325 *R. riparia* burrows,  
108 were collected in May and September 2013 at 24 sampling sites in the Czech Republic (Table  
109 1). Nest material was retrieved quantitatively from each burrow by a metal spoon attached to  
110 a 2 m pool. Material from each nest was placed into a separate zip-lock plastic bag, brought to  
111 laboratory and stored at fridge at 8 °C until analyzed. The nest material was spreaded on a  
112 white plastic plate and observed for the presence of ticks (each nest was examined for 15  
113 minutes).

114 Complementing the above specimens, bird-derived *I. lividus* ticks were collected from a  
115 random subset of 10 out of 49 infested individuals of a total of 135 *R. riparia* that were  
116 caught with mist-nets in August 2013 in Kieldrecht, Antwerp, Belgium (Table 2).

117 All ticks were stored in 70% ethanol and determined according to Nosek and Sixl (1972).

118 Species identification of larvae that were tested for the presence of rickettsial DNA was  
119 supported molecularly by amplification of partial sequence of the 16S rDNA mitochondrial  
120 gene (Table 3).

121 DNA was extracted according to Sangioni et al. (2005) from randomly selected *I. lividus*  
122 ticks, while a maximum of 10 immature ticks from each sampling site, each individual from a  
123 different nest hole (if applicable), and all adult ticks were chosen, and all *I. ricinus* ticks  
124 (Table 1-2). Additionally, rickettsial DNA from 3 females of *I. arboricola* collected in  
125 Břeclav, the Czech Republic, as a part of a previous study (Novakova et al., 2015) has been  
126 amplified.

127 Isolated DNA was subjected to polymerase chain reaction (PCR) under the following  
128 conditions: 25 µL of PCR reaction was composed of 12.5 µL Combi PPP Master Mix (Top  
129 Bio, Vestec, Czech Republic), 1.5 µL of each primer at 20 µM, 7.0 µL of PCR H<sub>2</sub>O, and 2.5  
130 µL DNA (10-24 ng/µl). Samples were tested by a battery of primers targeting fragments of  
131 six rickettsial genes (Table 3). When the amplification of the partial sequence of the *gltA* gene  
132 resulted negative, an additional PCR targeting the tick mitochondrial *16S rDNA* gene was  
133 performed in order to check whether the DNA extraction was successful.

134 For each reaction, negative (PCR H<sub>2</sub>O) and positive controls (DNA from *Rickettsia rickettsii*  
135 strain I12B-infected Vero cells, 15 ng/µl) were used. PCR products were visualized on 1.5 %

136 agarose gels (Roche Diagnostics, Basel, Switzerland) and chosen samples were purified by  
137 Gel/DNA Fragments Extraction Kit (Geneaid, Taipei City, Taiwan). Sanger dideoxy  
138 sequencing was performed by Macrogen (Amsterdam, Netherlands).  
139 Sequences were analyzed by BLAST (Basic Local Alignment Tool) to assign similarities to  
140 other *Rickettsia* sequences available in Nucleotide Database of National Center for  
141 Biotechnology Information (NCBI) (Altschul et al., 1990). Sequences were aligned via  
142 ClustalW version 2.1 (Larkin et al., 2007), using gap opening penalty 10, gap extension penalty  
143 1, DNA weight matrix IUB and transition weight 0.5. Bayesian inference in MrBayes version  
144 3.2.2 (Ronquist and Huelsenbeck, 2003) was performed in order to infer the phylogenetic tree  
145 topologies, using following parameters: mixed model of nucleotide substitution, gamma-  
146 distributed rates among sites, four Monte Carlo Markov chains for 2,000,000 cycles, chains  
147 were sampled every 1000<sup>th</sup> generation, first 25% of the samples was discarded as burn-in. The  
148 final tree topologies were consequently generated employing 50% majority-rule consensus. We  
149 obtained summary statistics of Bayesian analyses as follows: average standard deviation of split  
150 frequencies 0.0032-0.0079, maximum standard deviation of split frequencies 0.012-0.034,  
151 average potential scale reduction factor 1.000 for all the trees, and maximum potential scale  
152 reduction factor 1.003-1.010. In order to include as many rickettsial species as possible, only  
153 DNA sequences of the *gltA*, *ompA*, and *ompB* genes were aligned. The *gltA*, *ompA*, and *ompB*  
154 partial sequences were analyzed separately, and also concatenated.

155

## 156 **Results**

157

158 In total, 1,758 unfed *I. lividus* ticks (1,707 larvae, 15 nymphs, 10 males, 26 females) and 5  
159 unfed *I. ricinus* ticks (one larva, 4 nymphs) have been found in *R. riparia* nests. Ticks have  
160 been found at 17 out of 24 examined sampling sites in the Czech Republic (Table 1). 148  
161 ticks were derived from *R. riparia* individuals in Belgium (Table 2). The infestation  
162 prevalences for the adult birds (total captured: 61 individuals) and the fledgelings (total  
163 captured: 74 individuals) were 6.6% and 47.3%, respectively.

164 DNA was successfully isolated from 178 out of 243 *I. lividus* ticks (136/165 ticks from the  
165 Czech Republic, 42/78 ticks from Belgium), 5/5 *I. ricinus* ticks and 3/3 *I. arboricola* ticks.  
166 Rickettsial DNA was detected in all 178 *I. lividus* and 3 *I. arboricola* but none of *I. ricinus*  
167 ticks.

168 DNAs of 3 samples of *I. lividus* ticks from each locality from the Czech Republic (if  
169 applicable), three *I. arboricola* ticks and all samples from Belgium were sequenced.

170 The rickettsial agent in all the samples has been determined as *R. vini*. The identification has  
171 been supported by 100.00% sequence identity of all 5 loci previously sequenced from *R. vini*  
172 (Table 4).

173 The amplification of the partial sequence of the rickettsial 16S rRNA has not been successful  
174 for *I. arboricola* ticks and we were not able to repeat the experiment as no DNA of *I.*  
175 *arboricola* left. All sequences obtained in this study were identical to each other and to  
176 previously amplified sequences of *R. vini* isolated from *I. arboricola* ticks. The only  
177 difference (99.88% similarity, 808/809 nt) has been observed for the *sca4* sequence of all  
178 tested *I. lividus* ticks from Pouzďřany and Věmyslice compared to corresponding sequences  
179 obtained from *I. lividus* ticks from other Czech localities, Belgium and *I. arboricola* ticks  
180 from Břeclav.

181 We suggest that *Rickettsia* sp. IXL11 (Accession Numbers GU124744-5), isolated from *I.*  
182 *lividus* ticks in the United Kingdom (Graham et al., 2010) may belong to *R. vini*. Similarities  
183 are 100% (293/293 nt) for the *gltA* and 98.63% (289/293 nt) for the *ompA* partial sequences.  
184 The closest similarity to *R. vini* sequences showed uncharacterized *Rickettsia* sp. obtained  
185 from *Argas lagenoplastis* Froggatt, 1906 tick from Australia followed by several  
186 uncharacterized *Rickettsia* spp. from Australia and the Old World (Table 4). These rickettsiae  
187 were included in the phylogenetic analysis in case that *gltA*, *ompA*, and *ompB* sequences were  
188 available in NCBI. In concatenated analysis, *R. vini* clustered together with *Rickettsia* sp.  
189 from *A. lagenoplastis*, *Rickettsia* sp. from *Argas (Carios) dewae* Kaiser & Hoogstraal, 1974,  
190 *Rickettsia heilongjiangensis* and *Rickettsia japonica* (Figure 1). Analyses of single genes  
191 provided slightly different topologies within the cluster of the five above mentioned  
192 rickettsiae (S1-S3 in Supplemental Material).

193

## 194 **Discussion**

195

196 *R. vini* has been found to infect 100% of tested *I. lividus* ticks in the Czech Republic and  
197 Belgium, which is in compliance with previous studies when an uncharacterized *Rickettsia* sp.  
198 has been found infecting 100% of *I. lividus* ticks in Sweden and the Great Britain (Elfving et  
199 al., 2010; Graham et al., 2010). A surprising fact is that *Rickettsia* sp. with the highest  
200 similarity of DNA sequences to *R. vini* was detected in *A. lagenoplastis*, a nidicolous soft tick  
201 species associated with fairy martin (*Petrochelidon ariel*) (Hirundinidae) followed by  
202 *Rickettsia* sp. in *A. (C.) dewae*, a nidicolous bat tick from Australia, and various species of  
203 both free-living and nidicolous Asian hard tick species (Table 4).

204 The origin of the genus *Rickettsia* has been estimated to occur 150 million years ago (Weinert  
205 et al., 2009), while the origin of the order Passeriformes is dated about 40 millions years ago  
206 (Brusatte et al., 2015). An evolution of nest construction habits of Hirundinidae showed that  
207 nest burrowing is plausibly primitive and mud-nesting is more recent (Winkler and Sheldon,  
208 1993). This supports the fact that *R. riparia* (nest burrows) is not closely related with *P. ariel*  
209 (mud-nesting) (Sheldon et al., 2005). It indicates that coevolution of *R. vini*-like rickettsiae-  
210 tick associations developed independently on host bird species.

211 Most recent review of tick evolution by Mans et al. (2016) has presented that clades that  
212 possess both argasids and ixodids are estimated <150 millions years ago. It is believed that  
213 two sister clades of Ixodida, Argasidae and Ixodidae, have probably common ancestors  
214 (Klompen et al., 1996). Nidicolous way of life is typical for almost every species of  
215 Argasidae. In the sister clade Ixodidae, numerous species of the most ancient genus *Ixodes*  
216 show nidicolous behavior and is considered being ancestral in this genus (Sonenshine and  
217 Roe, 2014). Hence, evolution of rickettsial endosymbionts may correlate with evolution of  
218 ticks. Our study suggests that a common ancestor of the genera *Ixodes* and *Argas* hosted a  
219 rickettsial endosymbiont.

220 It has frequently been reported that there is no species-specific tick-host affinity of nidicolous  
221 ticks and the observed species-specificity is more probably due to an extremely unique habitat,  
222 such as nest burrows and tree holes (Sonenshine and Roe, 2014). The *Ixodes* spp. parasitizing  
223 swifts, and passerine birds including swallows appear to be able to feed on most host species  
224 they might contact (George, 1987; Elfving et al., 2010). One example, *P. montanus*, may  
225 occupy both these habitats, thus may be infested both by *I. lividus* and *I. arboricola* ticks  
226 (Novakova et al., 2015). These situations may cause shifts of rickettsiae among tick species  
227 by cofeeding, e.g., *R. vini* has already been detected in *I. ricinus* ticks collected from birds  
228 (Palomar et al., 2012a). However, no evidence of birds as reservoirs or amplifiers of  
229 rickettsiae has been shown (Ogrzewalska and Pinter, 2016) and transovarial transmission is  
230 still the main route of spotted fever group rickettsiae perpetuation in tick population  
231 (Socolovschi et al., 2009). We detected DNA of *R. vini* in all stages of *I. lividus* ticks. This  
232 supports successful transovarial and transstadial transmission of this bacteria.

233 *R. vini* has been found with high prevalence in two closely related species *I. lividus* and *I.*  
234 *arboricola*, which supports its endosymbiotic character. The genus *Ixodes* is the most ancient  
235 in the family Ixodidae (Sonenshine and Roe, 2014). The finding of the most closely related  
236 rickettsial genospecies to *R. vini* in *A. lagenoplastis* and *Argas dewae*, nidicolous tick species,  
237 endorses the theory that a nidicolous way of life is primitive in the family Ixodidae.



238 Further studies based on a wide range of argasid and ixodid ticks are needed to elucidate the  
239 rickettsia-tick coevolution.

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241

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246

247 **Conflict of interest statement:** The authors declare that the research was conducted in the  
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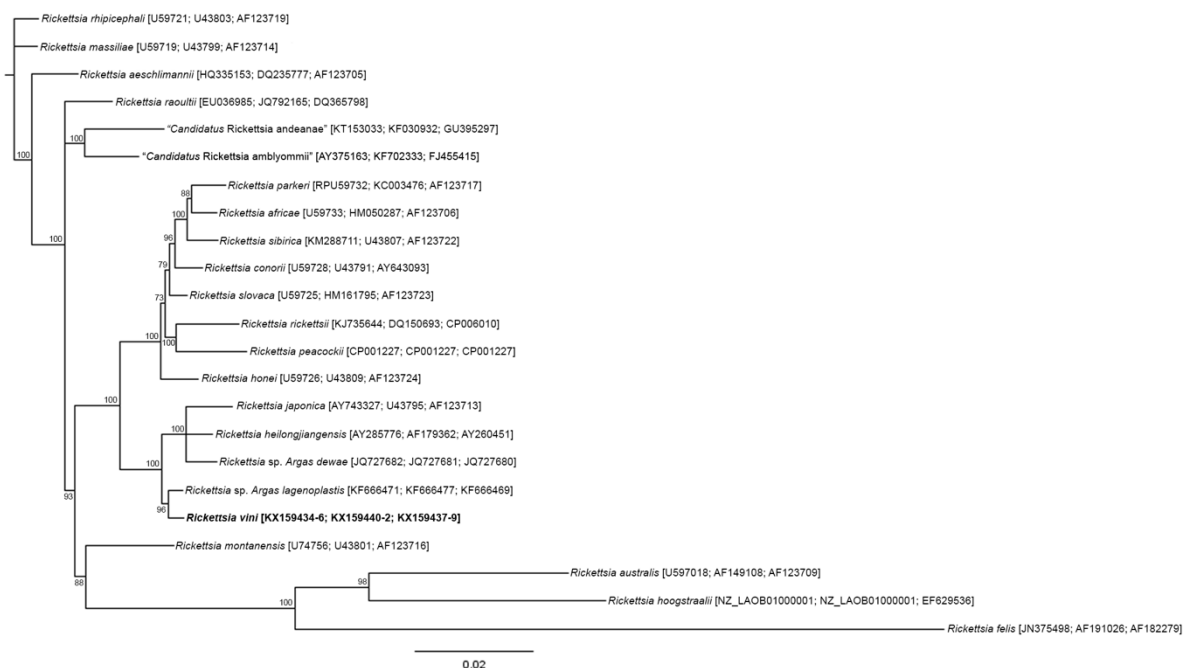
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## 417 **Figures:**

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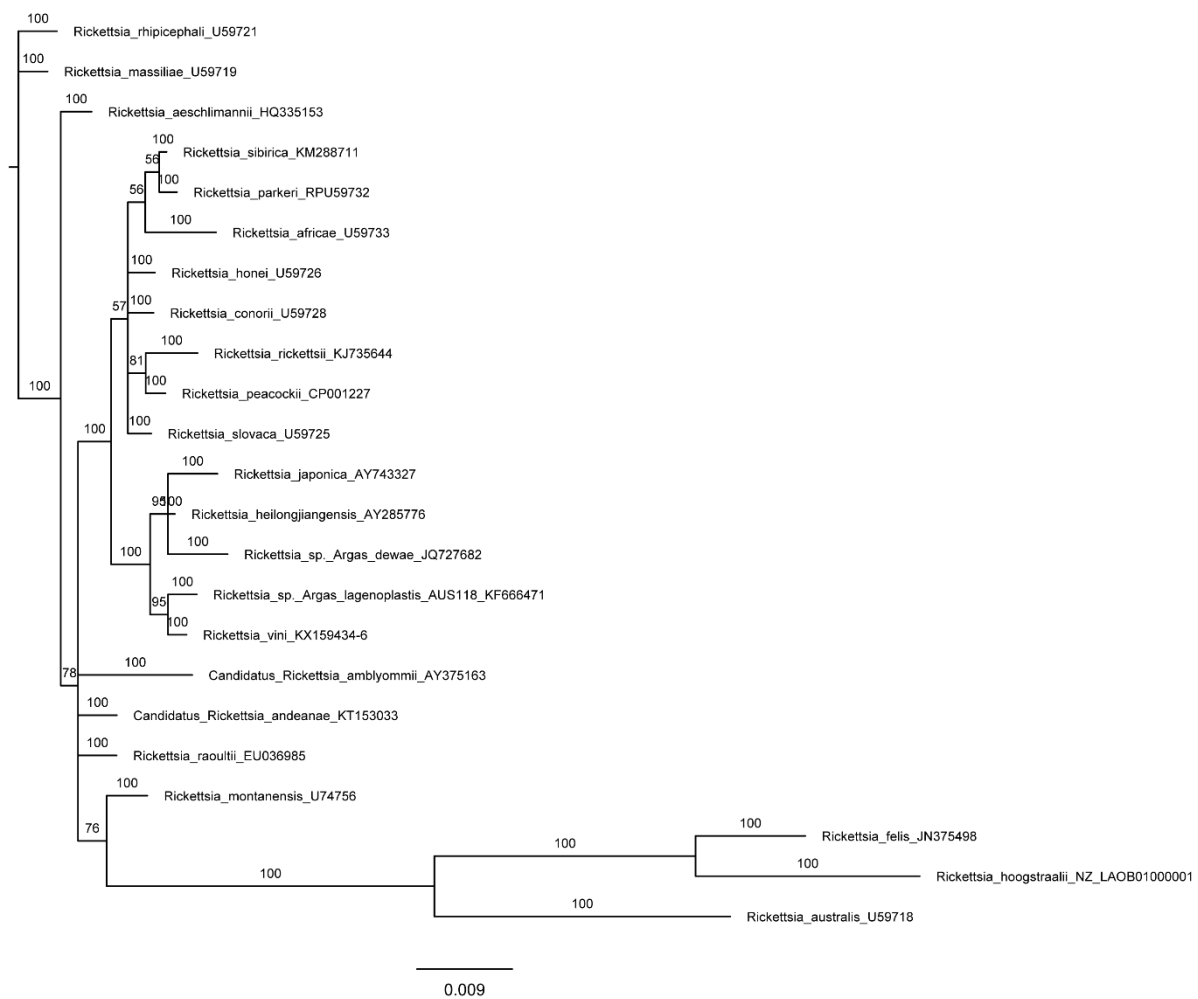


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421 **Fig. 1. Maximum-likelihood analysis of concatenated sequences of *gltA*, *ompA*, and *ompB***  
 422 **genes of *Rickettsia vini* and its closely related genospecies.**

423 A total of 2,092 unambiguously aligned nucleotide sites of the rickettsial genes *gltA*, *ompA*,  
 424 and *ompB* were concatenated and subjected to analysis by the maximum-likelihood method.  
 425 The tree is drawn to scale; bar, 0.02 substitutions per site. The GenBank accession numbers  
 426 are shown in brackets.

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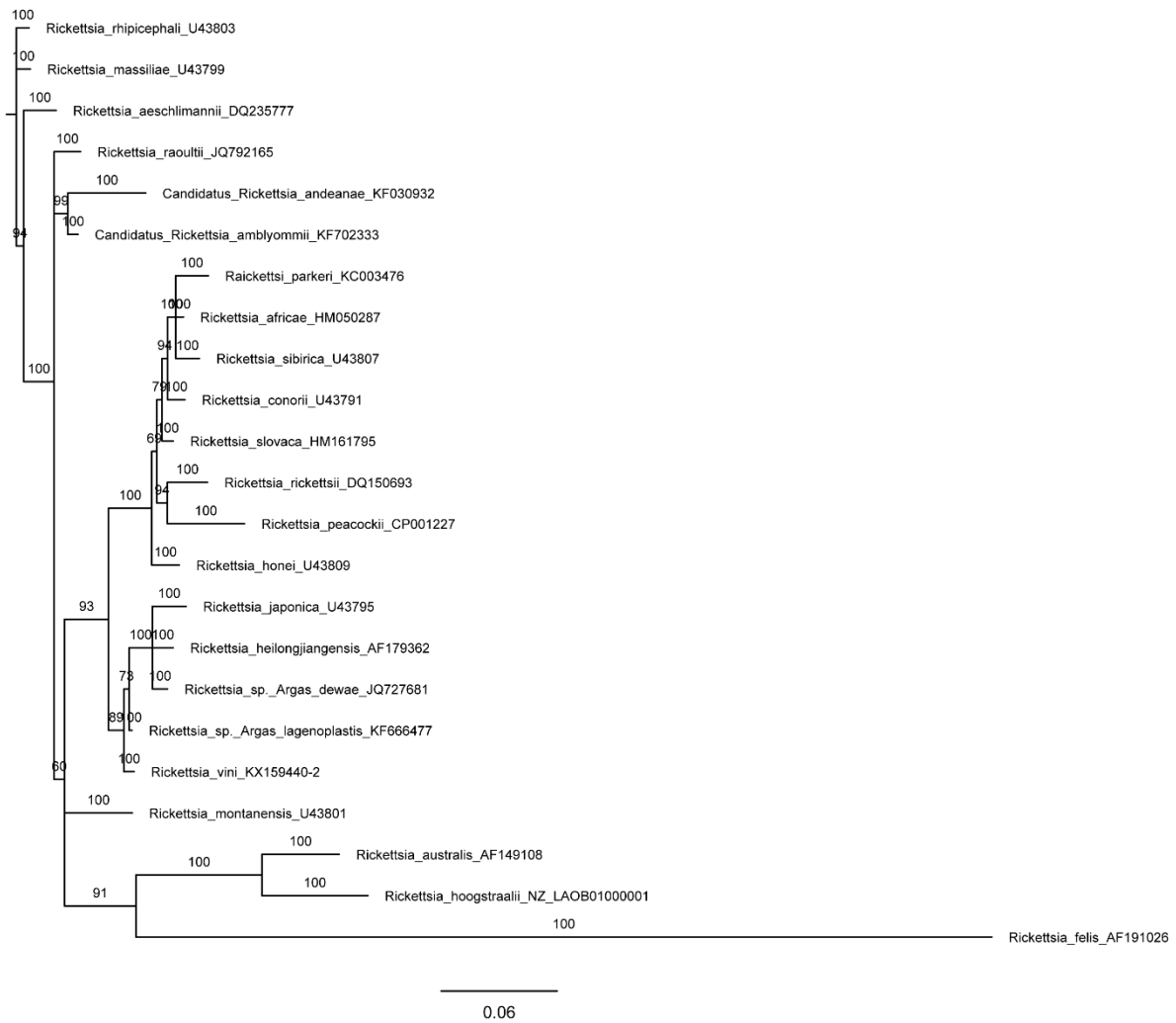


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429 **Fig. S1 Maximum-likelihood analysis of *gltA* gene of *Rickettsia vini* and its closely related**  
 430 **genospecies.**

431 A total of 1,075 unambiguously aligned nucleotide sites of the rickettsial gene *gltA* were  
 432 subjected to analysis. The tree is drawn to scale; bar, 0.009 substitutions per site. GenBank  
 433 accession numbers are shown in brackets.  
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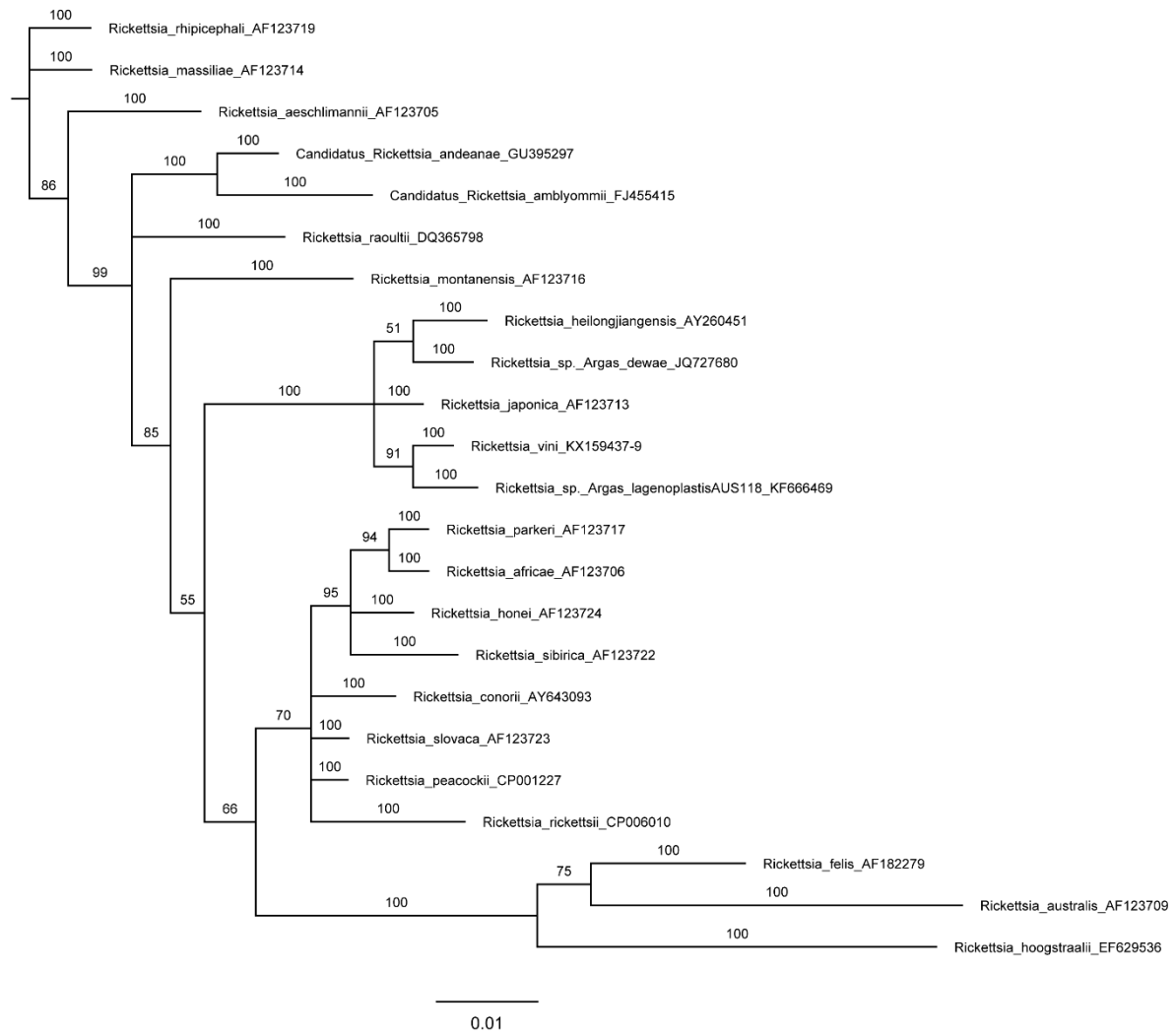


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**Fig. S2 Maximum-likelihood analysis of *ompA* gene of *Rickettsia vini* and its closely related genospecies.**

A total of 590 unambiguously aligned nucleotide sites of the rickettsial gene *ompA* were subjected to analysis. The tree is drawn to scale; bar, 0.06 substitutions per site. GenBank accession numbers are shown in brackets.





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447 **Fig. S3 Maximum-likelihood analysis of *ompB* gene of *Rickettsia vini* and its closely**  
 448 **related genospecies.**

449 A total of 455 unambiguously aligned nucleotide sites of the rickettsial gene *ompB* were  
 450 subjected to analysis. The tree is drawn to scale; bar, 0.01 substitutions per site. GenBank  
 451 accession numbers are shown in brackets.

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455 **Table 1**

456 Ticks collected from nest-holes of bird nest holes in the Czech Republic in 2013.

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<b>Location</b>		<b>Nest holes</b>			<b>Ticks in each nest hole</b>			
<b>Sampling site</b>	<b>District</b>	<b>Coordinates</b>	<b>Sampling date</b>	<b>No. of nest holes containing ticks/ Total No.</b>	<b>No. of nesting birds MA/PM/RR<sup>a</sup></b>	<b>Host bird species</b>	<b>Number, Stage<sup>b</sup>, (Species)<sup>c</sup></b>	<b>Ticks tested for the presence of rickettsial DNA</b>
ATC Merkur	Brno - venkov	48.90N, 16.57E	07-Sep	0/12	0/0/12			
Betlém	Brno - venkov	48.91N, 16.59E	07-Sep	0/2	2/0/0			
Běleč	Beroun	49.91N, 14.17E	04-May	1/13	0/0/13	RR	1L	1L
			08-Sep	1/3	3/0/0	MA	1N (IR)	1N
Cep I	Jindřichův Hradec	48.92N, 14.88E	06-Sep	3/8	0/0/12	RR	1F	1F
							65L	5L
							13L	5L
Černuc II	Kladno	50.32N, 14.21E	08-Sep	0/8	0/0/8			
Černuc - nová	Kladno	50.32N, 14.21E	08-Sep	7/12	0/0/12	RR	7L	1L
							2L	2L
							2L	2L
							30L	2L
							22L	2L
							1L	1L
2N (IR)	2N							

ČMŠ	Znojmo	48.82N, 16.26E	07-Sep	2/22	0/0/22	RR	1F 1F	1F 1F
Hýskov	Beroun	50.00N, 14.07E	04-May	4/19	0/0/19	RR	1L 3L 1F 3L	1L 3L 1F 3L
Liteň	Beroun	49.92N, 14.16E	04-May	2/5	0/1/4	RR	27L 29L	1L 2L
			08-Sep	8/20	0/0/20	RR	2F 7L, 1F 1F 18L, 1F 5L, 1F 1L 4L 66L	2F 1L, 1F 1F 1L, 1F 1L, 1F 1L 1L 2L
Lžín	Tábor	49.23N, 14.76E	06-Sep	1/11	0/0/11	RR	11L	10L
Martincova pískovna	Znojmo	48.82N, 16.25E	02-May	6/7	0/0/7	RR	36L 12L 9L 3L 2L 21L, 1N	2L 1L 1L 1L 1L 1L, 1N
			07-Sep	3/17	0/0/17	RR	33L 2L 1L (IR)	1L 1L 1L
Mladošovice	České Budějovice	48.95N, 14.70E	06-Sep	1/8	0/0/8	RR	1N (IR)	1N
Oleksovice	Znojmo	48.90N, 16.25E	02-May	14/14	0/0/14	RR	17L	1L

							14L	1L
							22L	1L
							24L	1L
							37L	1L
							17L	
							23L	1L
							17L	1L
							31L	1L
							4L	
							5L	
							11L	
							19L	1L
							11L	1L
Oslavany	Brno - venkov	49.11N, 16.34E	07-Sep 02-May	0/2 10/27	0/0/2 0/1/26	RR	7L	1L
							24L	1L
							1L	1L
							1L	1L
							3L	1L
							42L, 2F	1L, 2F
							1L	1L
							6L	1L
							4L	1L
Pňovice	Příbram	49.59N, 13.90E	04-May	8/16	0/0/16	RR	25L	1L
							4L	1L
							3L	1L
							7L, 1N	1L, 1N

								8L	1L
								1L	1L
								49L	1L
								2L	1L
								8L, 5N	1L, 1N
Pouzdrany	Břeclav	48.92N, 16.62E	02-May	18/26	0/3/23	PM		103L, 1N	1N
						RR		7L	
								5L	
								13L, 1N	1N
								36L	1L
								58L	1L
								4L	
								41L, 1N	1N
								45L	1L
								6L	
								34L	1L
								33L	1L
								44L	1L
								7L	
								4L	
								25L	
								36L, 1M	1M
			07-Sep	9/18	0/0/18	RR		16L	
								13L	
								12L	
								32L, 1N, 5F	5F
								3L	
								13L, 1M, 5F	1M, 5F

								35L	1L
								2M	2M
								62L, 3M	3M
								1L	
Roudnice nad Labem	Litoměřice	50.42N, 14.22E	04-May	2/6	0/1/5	RR	3L	3L	
							28L	7L	
Srbsko	Beroun	49.93N, 14.14E	04-May	1/1	0/0/1	RR	21L	10L	
Strachotín	Břeclav	48.91N, 16.64E	02-May	0/14	0/0/14				
Straškov	Litoměřice	50.35N, 14.23E	04-May	1/6	0/0/6	RR	1N	1N	
Únanov	Znojmo	48.89N, 16.07E	02-May	0/12	10/2/0				
			07-Sep	0/5	5/0/0				
V Holi	Strakonice	49.25N, 13.88E	08-Sep	0/8	0/0/8				
Vémyslice	Znojmo	49.03N, 16.23E	02-May	9/9	0/0/9	RR	5L	1L	
							12L, 1N, 1M	1N, 1M	
							5L	1L	
							14L	1L	
							9L	1L	
							5L	1L	
							12L, 1N, 1M	1N, 1M	
							19L, 2N, 1M, 4F	2N, 1M, 4F	
			07-Sep	1/4	0/0/4	RR	2L	1L	
Záblatí	Jindřichův Hradec	49.10N, 14.67E	06-Sep	0/14	0/0/14				
<b>TOTAL</b>				<b>112/349</b>	<b>20/8/325</b>		<b>1707L, 15N, 10M, 26F; 1L (IR), 4N (IR)</b>	<b>118L, 11N, 10M, 26F, 1L (IR), 4N (IR)</b>	

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<sup>a</sup> *Merops apiaster* (MA), *Passer montanus* (PM), *Riparia riparia* (RR)

<sup>b</sup> larva (L), nymph (N), male (M), female (F)

<sup>c</sup> *Ixodes lividus* if not mentioned elsehow, *Ixodes ricinus* (IR)

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492 **Table 2**493 Ticks collected from *Riparia riparia* in Czech Republic in August 2013.

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<b>Location</b>				<b><i>Riparia riparia</i> birds</b>		<b><i>Ixodes lividus</i> ticks</b>	
<b>Sampling site</b>	<b>Township</b>	<b>District</b>	<b>Coordinates</b>	<b>Age category<sup>a</sup></b>	<b>Number, Stage<sup>b</sup></b>	<b>Ticks subjected to DNA isolation</b>	
Kieldrecht	Kieldrecht	Antwerp	51.30N, 4.24E	J	3L, 20N	2L, 10N	
				J	2L, 12N	2L, 10N	
				J	2N	2N	
				J	24L, 7N	10L, 7N	
				J	6N	6N	
				J	28L, 26N	10L, 10N	
				A	1N	1N	
				A	9L, 2N	3L, 2N	
				A	4L	2L	
				A	2L	1L	
<b>TOTAL</b>			<b>10</b>	<b>72L, 76N</b>	<b>30L, 48N</b>		

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496 <sup>a</sup> juvenile (J), adult (A)497 <sup>b</sup> larva (L), nymph (N)

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**Table 3**  
Primer pairs used for amplification of tick and rickettsial genes

Target gene, primer pair no., and primers	Specificity	Nucleotide sequences (5' → 3')	Amplified fragment (bp)	Reference
<b>mitochondrial 16S rRNA</b>	ticks			
16S +1		CCG GTC TGA ACT CAG ATC AAG T	≈460	Mangold et al. (1998)
16S -1		GCT CAA TGA TTT TTT AAA TTG CTG T		Mangold et al. (1998)
<b>gltA</b>	<i>Rickettsia</i> spp.			
1 CS-78		GCA AGT ATC GGT GAG GAT GTA AT	401	Labruna et al. (2004a)
CS-323		GCT TCC TTA AAA TTC AAT AAA TCA GGA T		Labruna et al. (2004a)
2 CS-239		GCT CTT CTC ATC CTA TGG CTA TTA T	834	Labruna et al. (2004b)
CS-1069		CAG GGT CTT CGT GCA TTT CTT		Labruna et al. (2004b)
<b>ompA</b>	Spotted fever group			
Rr190.70p		ATG GCG AAT ATT TCT CCA AAA	632	Regnery et al. (1991)
190-701		GTT CCG TTA ATG GCA GCA TCT		Roux et al. (1996)
<b>ompB</b>	<i>Rickettsia</i> spp. <sup>a</sup>			
rompB OF		GTA ACC GGA AGT AAT CGT TTC GTA A	511	Choi et al. (2005)
rompB OR		GCT TTA TAA CCA GCT AAA CCA CC		Choi et al. (2005)
<b>htrA</b>	<i>Rickettsia</i> spp. <sup>a</sup>			
17kD1		GCT CTT GCA ACT TCT ATG TT	434	Webb et al. (1990)
17kD2		CAT TGT TCG TCA GGT TGG CG		Webb et al. (1990)
<b>sca4</b>	<i>Rickettsia</i> spp. <sup>a</sup>			
1 D1f		ATG AGT AAA GAC GGT AAC CT	929	Sekeyova et al. (2001)

	D928r	AAG CTA TTG CGT CAT CTC CG		Sekeyova et al. (2001)
	2 D767f	CGA TGG TAG CAT TAA AAG CT	624	Sekeyova et al. (2001)
	D1390r	CTT GCT TTT CAG CAA TAT CAC		Sekeyova et al. (2001)
<b>rickettsial 16S rRNA</b>		<i>Rickettsia</i> spp.		
	fD1	AGA GTT TGA TCC TGG CTC AG	426	Weisburg et al. (1991)
	Rc16S.452n	AAC GTC ATT ATC TTC CTT GC		Márquez et al. (1998)

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<sup>a</sup> Except some species of basal groups (i.e., *Rickettsia bellii*).

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**Table 4**

Similarities of partial sequences of rickettsial genes from this study to the corresponding sequences in NCBI, query cover 100.00%.

Sequences amplified in this study		Most similar sequences in NCBI			Accession Numbers
Gene	Host tick species/ Country <sup>a</sup>	Length [nt]	Similarity ≥ 99.00 [%]	Rickettsia species [Accession number(s)] <sup>b</sup>	
<i>gltA</i>		1092	100.00	<i>R. vini</i> [JF803266]	KX159434-6
			99.73	<i>R. sp. Argas lagenoplastis</i> [JF803266]	
				<i>Candidatus R. jingxinensis</i> [KT899089]	
				<i>R. sp. Haemaphysalis longicornis</i> [JQ697958; AB516964]	
				<i>R. heilongjiangensis</i> [CP002912; AB473812; AY285776; AY285776]	
			99.63	<i>R. heilongjiangensis</i> [AF178034]	
			99.54	<i>R. japonica</i> [AP011533]	
				" <i>R. hulinii</i> " [AF172943]	
99.45	<i>R. slovacae</i> [CP003375, CP002428; U59725]				
	<i>R. japonica</i> [U59724]				
		99.39	8 sqcs ( <i>R. sp. Amblyomma ovale</i> , <i>R. sibirica</i> ssp. <i>sibirica</i> , <i>R. parkeri</i> , <i>R. japonica</i> )		
<i>ompA</i>		590	100.00	<i>R. vini</i> [JF758828]	KX159440-2
			99.32	<i>R. sp. Argas lagenoplastis</i> [KF666477]	
<i>ompB</i>		455	100.00	<i>R. vini</i> [JF758826]	KX159437-9
			99.34	<i>R. sp. Argas lagenoplastis</i> [KF666469]	
			99.12	<i>R. japonica</i> [AP011533; AF123713; AB003681]	
				<i>Rickettsia</i> sp. strain Davousti [AY260452]	

			99.00	<i>R. sp. Argas dewae</i> [JQ727680] <i>R. heilongjiangensis</i> [CP002912; AY280712; AY260451]	
<i>htrA</i>		394	100.00	<i>R. vini</i> [KT187396; JF758827]	KX159431-3
			99.75	<i>Candidatus R. honei</i> [AY737683] <i>R. sp. Haemaphysalis longicornis</i> [AB516960] <i>R. sp. Haemaphysalis hystricis</i> [AB359457; AB114805]	
			99.50	<i>R. japonica</i> [LC101441; AP011533] <i>R. sp. Haemaphysalis japonica</i> [KR733074]	
<i>sca4</i>	IA CZ	1352	99.04	<i>R. sp. Argas lagenoplastis</i> [KF666473]	KX159443
	IL CZ	1330	99.00	<i>R. sp. Argas lagenoplastis</i> [KF666473]	KX159444
	BE	1321	99.01	<i>R. sp. Argas lagenoplastis</i> [KF666473]	KX159445
<i>16S rRNA</i> <sup>c</sup>		396	100.00	30 sqcs ( <i>R. rickettsii</i> , <i>R. peacockii</i> , <i>R. philipii</i> , <i>R. slovacca</i> , <i>R. vini</i> )	KX159448-9
			99.75	<i>R. sp. Argas lagenoplastis</i> [KF666475]	
			99.49	<i>R. sp. Argas dewae</i> [JQ727684]	

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<sup>a</sup> *Ixodes arboricola* (IA), *Ixodes lividus* (IL), Czech Republic (CZ), Belgium (BE). Except for the *sca4* partial gene sequence, sequences of other genes were identical to each other.

<sup>b</sup> *Rickettsia* (*R.*), sequences (sqcs)

<sup>c</sup> The amplification of the partial sequence of the *16S rRNA* gene from *Ixodes arboricola* ticks failed and no DNA left.