

High Prevalence and Low Diversity of *Rickettsia* in *Dermacentor reticulatus* Ticks, Central Europe

Appendix

Materials and methods

We isolated DNA from samples from the Czech Republic and Slovakia using alkaline hydrolysis in 1.25% NH₄OH (1) and samples from Hungary using a NucleoSpin tissue kit (Macherey-Nagel, <http://www.mn-net.com>) according to manufacturer instructions. For pathogen detection we ran real-time PCR using Probes Master mastermix on Light Cycler (both Roche Diagnostics, <https://www.roche.com>) and for sequencing we ran conventional PCR using PPP mastermix (Top-Bio, <http://www.top-bio.com>) and VWR Doppio gradient (VWR, <https://www.vwr.com>) (2–4) (Appendix Table).

Commercial provider Macrogen (<https://www.macrogen.com>) performed sequencing. We analyzed obtained sequences by BLAST algorithm (5) and aligned them using Geneious alignment with 21 relevant sequences downloaded from the GenBank database. We selected representative sequences of spotted fever group *Rickettsia* species based on BLAST analysis and phylogeny published elsewhere (6). We cut the final alignment to 600 bp and executed phylogenetic analysis in Geneious Prime software (7) with *Rickettsia typhi* (GenBank NC_006142) as an outgroup. We tested phylogenetic relationships by Bayesian inference analysis (8) and maximum likelihood analysis (9). We performed the Bayesian analysis by Geneious Prime plugin MrBayes version 3.2.6 (<https://www.geneious.com>) using the GTR (general time-reversible) substitution model for 10⁶ generations, with trees and parameters sampled every 200 generations. We summarized the trees after removing 10% burn-in. We carried out the maximum likelihood analysis by the Geneious Prime plugin PhyML 3.3.20180621 using the GTR substitution model. We calculated nodal supports with 1000 bootstrap replicates and visualized the tree using TreeGraph 2.12.0 (10).

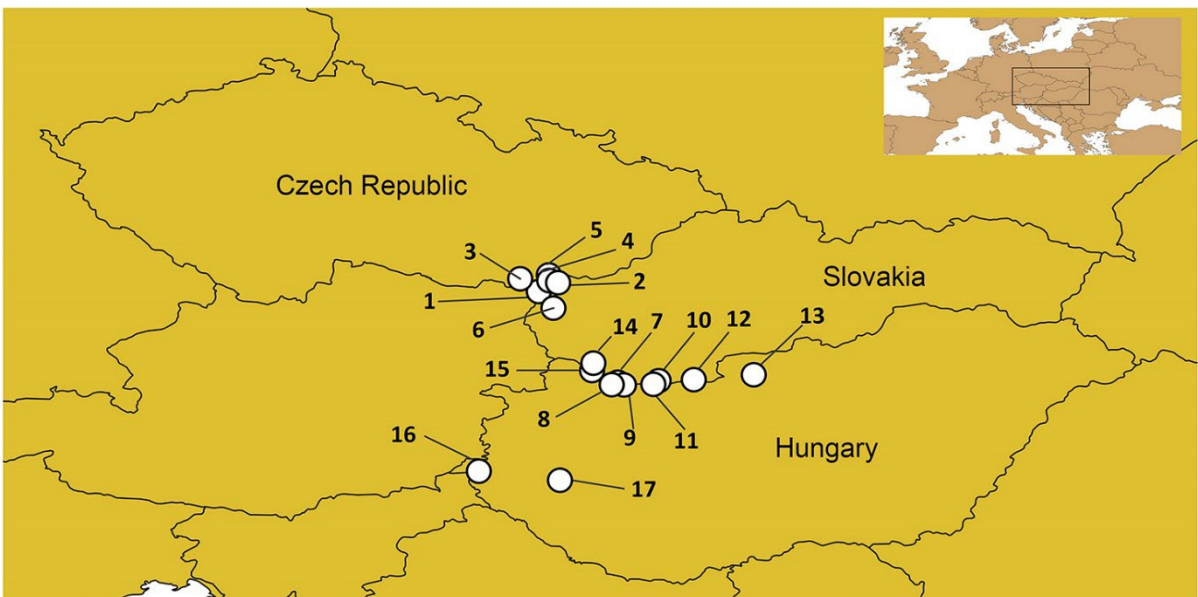
Appendix Table. Methods used for sample examination and preparation of gene fragments for sequencing*

Method	Target gene	Primers and probes, $\mu\text{mol/L}$	Sample volume/total volume, μL	Program				Ref
				Hotstart	Cycles	Final extension	Cooling	
Duplex quantitative PCR with probe	<i>gltA</i>	ApMSPf_upg 0.4 ApMSPr 0.4 gltA-CS-5 0.6 gltA-CS-6 0.6 5'Cy.5- ApMSPp-3'BHQ3 0.2 5'Hex- gltA-CS-3'BHQ2 0.4	2.0/20	95°C/10 min	45× [95°C for 10 s, 51°C for 30 s, 60°C for 30 s]	None	40°C for 10 min	(2)
PCR	<i>ompA</i>	Rr190.70p0.5 190.701 0.5	2.5/25	95°C/5 min	35× [95°C for 15 s, 54°C for 15 s, 72°C for 30 s]	72°C/3 min	10°C/ ∞	(3)
PCR	<i>ompB</i>	ompB.4362p 0.5 ompB.4836n 0.5	2.5/25	95°C/5 min	35× [95°C 15s, 54°C 15 s, 72°C 30 s]	72°C/3 min	10°C/ ∞	(4)

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| 1. Lanžhot | 4. Mikulčice | 7. Klučovec | 10. Dulov Dvor | 13. Szendehely | 16. Kondorfa |
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