Supplementary material

Table S1. PCR primers used in this study.

Primer	Sequence	Gene	Target organisms	Reference
27f	5'- AGAGTTTGATCCTGGCTCAG-3'	16S rRNA	Bacteria	(3)
1494r	5'- CTACGGCTACCTTGTTACGA -3'	16S rRNA	Universal	(3)
341f*	5'- CCTACGGGAGGCAGCAG-3'	16S rRNA	Bacteria	(4)
907r	5'- CCGTCAATTCMTTTGAGTTT-3'	16S rRNA	Bacteria	(3)
Rick653f	5'- GAGTGTAGTAGGGGATGATG -3'	16S rRNA	*Ricketssia*	This study
Rick982r	5'- CCACCATGTCAAGGGTTGGT -3'	16S rRNA	*Ricketssia*	This study
Cox sp434f	5'- CCTTTTGAGCGTTGACGTTA-3'	16S rRNA	*Coxiela sp.*	This study
Cox sp1004r	5'- CCAAAGGCACCAAGTCATTT -3'	16S rRNA	*Coxiela sp.*	This study
Acari412f	5'- CGGGACTCTTTTGAGGCC-3'	18S rRNA	Acari	This study
Acari990r	5'- ATCCTCCCAGTGTCCG-3'	18S rRNA	Acari	This study
T1BF	5'-AAACTAGGATTAGATACCCT-3'	12S rRNA	Arthropoda	(1)
T2AR	5'-AATGAGAGCGACGGGCGATGT-3'	12S rRNA	Arthropoda	(1)
SNR	5'-AATTGACATCCTATTTCAAA-3'	12S rRNA	*Rh. sanguineus*	This study
17kdf-1f	5'- ATGAGTAAAGACGGTAACCT-3'	Gene 'D' 17kd surface antigen	*Rickettsia*	(5)
17kd-1390r	5'- CTTGCTTTTCAGCAATATCAC-3'	Gene 'D' 17kd surface antigen	*Rickettsia*	(5)
OmpA-70f	5'- ATGGCGAATATTTCTCCAAAA-3'	Outer membrane protein A	*Rickettsia*	(2)
OmpA701r	5'- GTTCCGTTAATGGCAGCATCT-3'	Outer membrane protein A	*Rickettsia*	(2)

* For PCR-DGGE analyses a 5' GC rich tail was added to the primer: CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCC (4).

Table S2. PCR and qPCR conditions for the different primer pairs used in this study.

Primer pair	Use	Initial denaturing	No. of Cycles	Denaturing	Annealing	Extension	Final extension
27f-14942r	PCR	95°C, 5 min	35	95°C,30 sec	60°C,30 sec	72°C, 60 sec	72°C, 5 min
341f-907r	PCR	95°C, 5 min	35	95°C,30 sec	58°C,30 sec	72°C, 30 sec	72°C, 5 min
T1B-T2A	PCR	94°C, 5 min	35	94°C,30 sec	47°C,30 sec	72°C, 60 sec	72°C, 5 min
T1B-SNR	PCR	94°C, 5 min	35	94°C,30 sec	54°C,30 sec	72°C, 60 sec	72°C, 5 min
ompA-70f-701r	PCR	95°C, 5 min	35	95°C,30 sec	58°C,30 sec	72°C, 30 sec	72°C, 5 min
17kd-1f-1390r	PCR	95°C, 5 min	35	95°C,30 sec	60°C,30 sec	72°C, 60 sec	72°C, 5 min
341f (GC)-907r	PCR-DGGE	95°C, 5 min	35	95°C,30 sec	58°C,30 sec	72°C, 30 sec	72°C, 5 min
Rick653f-Rick982r	qPCR	95°C, 15 min	40	95°C,30 sec	60°C,30 sec	72°C, 30 sec	Melting protocol
Acari412f-Acari990r	qPCR	95°C, 15 min	40	95°C,30 sec	60°C,30 sec	72°C, 30 sec	Melting protocol
Cox sp-434f-1004r	qPCR	95°C, 15 min	40	95°C,30 sec	60°C,30 sec	72°C, 30 sec	Melting protocol

Table S3: The average standard curves slopes, correlation coefficients and amplification efficiencies of the various qPCR assays used in this study

Assay	Slope	R^2	Efficiency
Coxiella sp.	-3.33	0.99	99.74
Rickettsia	-3.37	0.98	99.11
Tick	-3.37	0.99	99.08

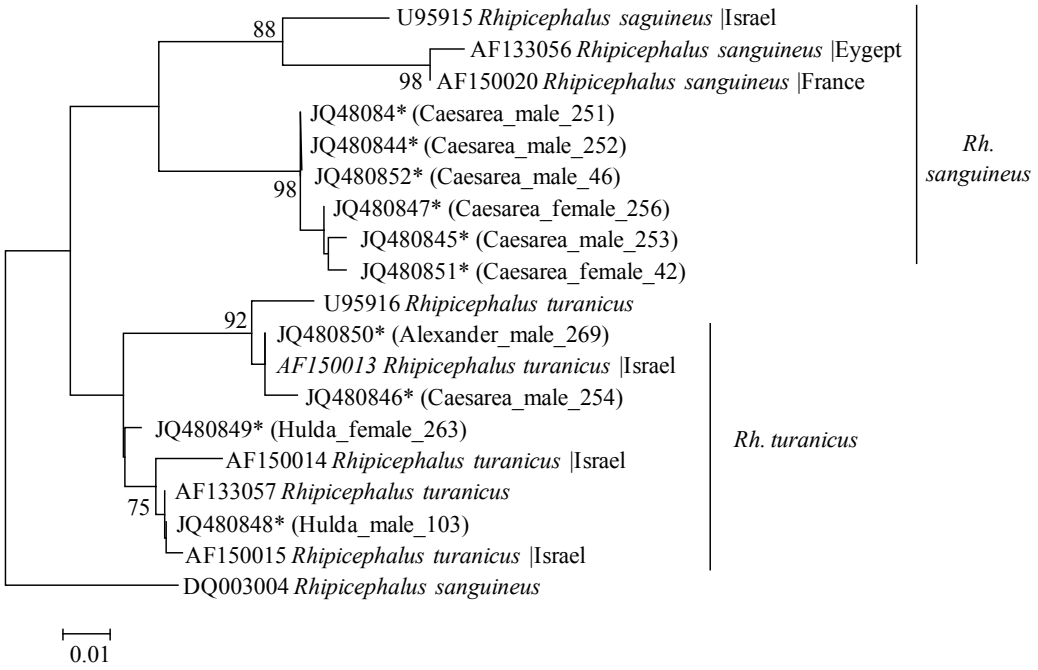

Figure S1. Phylogenetic tree based on Ticks 12S mitochondrial rRNA gene fragments. Maximum-likelihood tree based on Kimura 2-parameter model was constructed using MEGA software (version 5.05). Bootstrap analyses with 1,000 re-sampling were performed to test the robustness of the branching. Bootstrap values higher than 75% are presented. Sequences obtained in the present work are designated by asterisk. The place of collection the sex and the tick number are shown in parentheses. The bar indicates 0.01 substitutions per nucleotide per site.

References

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