SUPPLEMENTARY DATA

Ecological contacts and host specificity promote replacement nutritional endosymbionts in ticks

Microbial Ecology

Marie Buysse*, Florian Binetruy, Raz Leibson, Yuval Gottlieb* and Olivier Duron*

* Correspondence: olivier.duron@ird.fr; marie.buysse@ird.fr; gottlieb.yuval@mail.huji.ac.il

Table S1 List and origin of tick species used in this study for multi-locus typing of *Francisella*-LE.

Tick species	Origin	*n*	*Francisella*-LE type	Reference for primary description of *Francisella*-LE
Argasidae (soft ticks)				
Ornithodoros porcinus Walton, 1962	Laboratory colony derived from field specimens collected in Mahitsy, Madagascar, 2008-2010	3	Obligate endosymbiont	(1)
Ixodidae (hard ticks)				
Amblyomma (*Aponomma*) *latum* Koch, 1844	Collected on vegetation in Gabon, 2016	2	Obligate endosymbiont	Unpublished data
Dermacentor nitens Neumann, 1897	Collected on domestic horses (*Equus caballus*) in Macouria, French Guiana, 2016	2	Obligate endosymbiont	Unpublished data
Dermacentor occidentalis Marx, 1892	Collected on vegetation in Hopland, Mendocino Co, California, 1985	3	Obligate endosymbiont	(1)
Dermacentor reticulatus (Fabricius, 1794)	Collected on vegetation in Proveysieux, France, 2011	1	Obligate endosymbiont	(1)
Hyalomma aegyptium (Linnaeus, 1758)	Collected on an unidentified tortoise species in Yakir, Israel	5	Obligate endosymbiont	(2)
Hyalomma excavatum Koch, 1844	Collected on cattle (*Bos taurus*) in Mecitözü, Çorum, Turkey, 2011	3	Obligate endosymbiont	(1); (2)
	Collected on vegetation in the Crete island	1	Obligate endosymbiont	Unpublished data
	Collected on domestic horses (*Equus caballus*) in Israel	4	Obligate endosymbiont	Unpublished data
Hyalomma impeltatum Schulze and Schlottke, 1930	Collected on unidentified gerbil species in Sde Boker, Israel	5	Obligate endosymbiont	(1)
Hyalomma lusitanicum Koch, 1844	Collected on vegetation in Veneguera, Canaries, 2010	1	Obligate endosymbiont	(1)
Hyalomma marginatum Koch, 1844	Collected on vegetation in South of France	1	Obligate endosymbiont	(1); (2)
	Collected on domestic horses (*Equus caballus*) in Israel	3	Obligate endosymbiont	Unpublished data
Hyalomma rufipes Koch, 1844	Collected on various bird species at birding stations in Eilat and Jerusalem, Israel	7	Obligate endosymbiont	(2)
Ixodes ricinus (Linnaeus, 1758)	Collected on vegetation in diverse regions of France	7	Facultative endosymbiont	Unpublished data
Ixodes scapularis Say, 1821	Collected on vegetation at Queen's biology station, Ontario, Canada, 2013	2	Facultative endosymbiont	(1)
Rhipicephalus decoloratus Koch, 1844	Collected on zebu (*Bos indicus*) in Burkina-Faso, 2013	1	Facultative endosymbiont	(1)

Table S2 Genes and primers used in polymerase chain reaction (PCR) assays for multi-locus typing of *Francisella*-LE. Nested and semi-nested PCR assays were performed as follows: The first PCR run with the external primers was performed in a 10-µLvolume containing 20–50 ng of genomic DNA, 3 mM of each dNTP (Thermo Scientific), 8 mM of $MgCl_2$ (Roche Diagnostics), 3 µM of each primer, 1 µL of 10× PCR buffer (Roche Diagnostics), and 0.5 U of Taq DNA polymerase (Roche Diagnostics). A 1-µL aliquot of the PCR product from the first reaction was then used as a template for the second round of amplification. The second PCR was performed in a total volume of 25 µL and contained 8 mM of each dNTP (Thermo Scientific), 10 mM of $MgCl_2$ (ThermoScientific), 7.5 µM of each of the internal primers, 2.5 µL of 10×PCR buffer (Thermo Scientific), and 1.25 U of Taq DNA polymerase (Thermo Scientific). All PCR rounds were performed under the following conditions: initial denaturation at 93°C for 3 min, 35 cycles of denaturation (93°C, 30 s), annealing (Tm=50–56°C, depending on primers, 30 s), extension (72°C, 1–2 min), and a final extension at 72°C for 5 min.

Gene	Hypothetical product	Primers (5'-3')		Tm	Fragment size
16S rRNA	Small ribosomal subunit	FRCL_16S_F1	GCTATCTACTTCTGGAGCAACCC	52°C	1st round PCR: FRCL_16S_F1/FRCL_16S_R2: 1 295bp
		FRCL_16S_F2	CCAACAGCTAGTACTCATCGT		2nd round PCR (fragment 1): FRCL_16S_F1/FRCL_16S_R1: 773bp
		FRCL_16S_R1	AGAGTACGGTAGAGGAATGGG		2nd round PCR (fragment 2): FRCL_16S_F2/FRCL_16S_R2: 685bp
		FRCL_16S_R2	GGATACCAGTTGGAAACGAC		
rpoB	DNA-directed RNA polymerase beta chain	FRCL_rpoB_F2	GAGGCACACTTAGGTTTAGYTTC	56°C	1st round PCR: FRCL_rpoB_F2/FRCL_rpoB_R2: 420bp
		FRCL_rpoB_R1	GCATATACATATAACCAACTG		2nd round PCR: FRCL_rpoB_F2/FRCL_rpoB_R1: 379bp
		FRCL_rpoB_R2	CTAAACTATATGARCCAGTYGACC		
groEL	Chaperone protein GROEL	FRCL_groEL_F1	CAGATGAAGCTCGTACA	50°C	1st round PCR: FRCL_groEL_F1/FRCL_groEL_R2: 1 171bp
		FRCL_groEL_R1	TCACCAGCACCATCAATG		2nd round PCR: FRCL_groEL_F1/FRCL_groEL_R1: 981bp
		FRCL_groEL_R2	GCATCATCKACACGATC		
ftsZ	Cell division protein FtsZ	FRCL_ftsZ_F1	CAGCATATGTGTGAAGAGG	52°C	1st round PCR: FRCL_ftsZ_F1/FRCL_ftsZ_R2: 804bp
		FRCL_ftsZ_R1	GACATATCCATACCAGCTG		2nd round PCR: FRCL_ftsZ_F1/FRCL_ftsZ_R1: 713bp
		FRCL_ftsZ_R2	ACCAGCTATCACGATAGCC		
gyrb	DNA gyrase subunit B	FRCL_gyrB_F1	GGTAAGCTGGCAGACTGTC	56°C	1st round PCR: FRCL_gyrB_F1/FRCL_gyrB_R2: 1 145bp

	FRCL_gyrB_R1	CCAAAGCTGACCAGGGTTCA	2nd round PCR: FRCL_gyrB_F1/FRCL_gyrB_R1: 1 035bp
	FRCL_gyrB_R2	CGTGGCTCCACYTCATCACC	

16

Table S3 Key traits of the 29 tick species infected by *Francisella*-LE used in this study.

Tick species infected by *Francisella*-LE	Key traits			
	Tick family	Geographic distribution	Feeding preferences	*Francisella*-LE type
Ornithodoros porcinus	Argasidae (soft ticks)	Old World	Mammals	Obligate endosymbiont
Ornithodoros moubata	Argasidae (soft ticks)	Old World	Mammals	Obligate endosymbiont
Argas arboreus	Argasidae (soft ticks)	Old World	Birds	Unknown
Amblyomma latum	Ixodidae (hard ticks)	Old World	Reptiles	Obligate endosymbiont
Amblyomma dissimile	Ixodidae (hard ticks)	New World	Reptiles	Obligate endosymbiont
Amblyomma geayi	Ixodidae (hard ticks)	New World	Mammals	Facultative endosymbiont
Amblyomma goeldii	Ixodidae (hard ticks)	New World	Mammals	Obligate endosymbiont
Amblyomma humerale	Ixodidae (hard ticks)	New World	Reptiles	Obligate endosymbiont
Amblyomma latepunctatum	Ixodidae (hard ticks)	New World	Mammals	Facultative endosymbiont
Amblyomma longirostre	Ixodidae (hard ticks)	New World	Mammals and birds	Obligate endosymbiont
Amblyomma maculatum	Ixodidae (hard ticks)	New World	Mammals and birds	Obligate endosymbiont
Amblyomma oblongoguttatum	Ixodidae (hard ticks)	New World	Mammals	Obligate endosymbiont
Amblyomma pacae	Ixodidae (hard ticks)	New World	Mammals	Obligate endosymbiont
Amblyomma paulopunctatum	Ixodidae (hard ticks)	Old World	Mammals	Obligate endosymbiont
Amblyomma rotundatum	Ixodidae (hard ticks)	New World	Reptiles	Obligate endosymbiont
Amblyomma sculptum	Ixodidae (hard ticks)	New World	Mammals	Facultative endosymbiont
Amblyomma varium	Ixodidae (hard ticks)	New World	Mammals and birds	Obligate endosymbiont
Dermacentor nitens	Ixodidae (hard ticks)	New World	Mammals	Obligate endosymbiont
Dermacentor occidentalis	Ixodidae (hard ticks)	New World	Mammals	Obligate endosymbiont
Dermacentor reticulatus	Ixodidae (hard ticks)	Old World	Mammals	Obligate endosymbiont
Hyalomma aegyptium	Ixodidae (hard ticks)	Old World	Reptiles	Obligate endosymbiont
Hyalomma excavatum	Ixodidae (hard ticks)	Old World	Mammals and birds	Obligate endosymbiont
Hyalomma impeltatum	Ixodidae (hard ticks)	Old World	Mammals and birds	Obligate endosymbiont
Hyalomma lusitanicum	Ixodidae (hard ticks)	Old World	Mammals and birds	Obligate endosymbiont
Hyalomma marginatum	Ixodidae (hard ticks)	Old World	Mammals and birds	Obligate endosymbiont
Hyalomma rufipes	Ixodidae (hard ticks)	Old World	Mammals and birds	Obligate endosymbiont
Ixodes ricinus	Ixodidae (hard ticks)	Old World	Mammals, birds and reptiles	Facultative endosymbiont
Ixodes scapularis	Ixodidae (hard ticks)	New World	Mammals and birds	Facultative endosymbiont
Rhipicephalus decoloratus	Ixodidae (hard ticks)	Old World	Mammals	Facultative endosymbiont

Figure legends

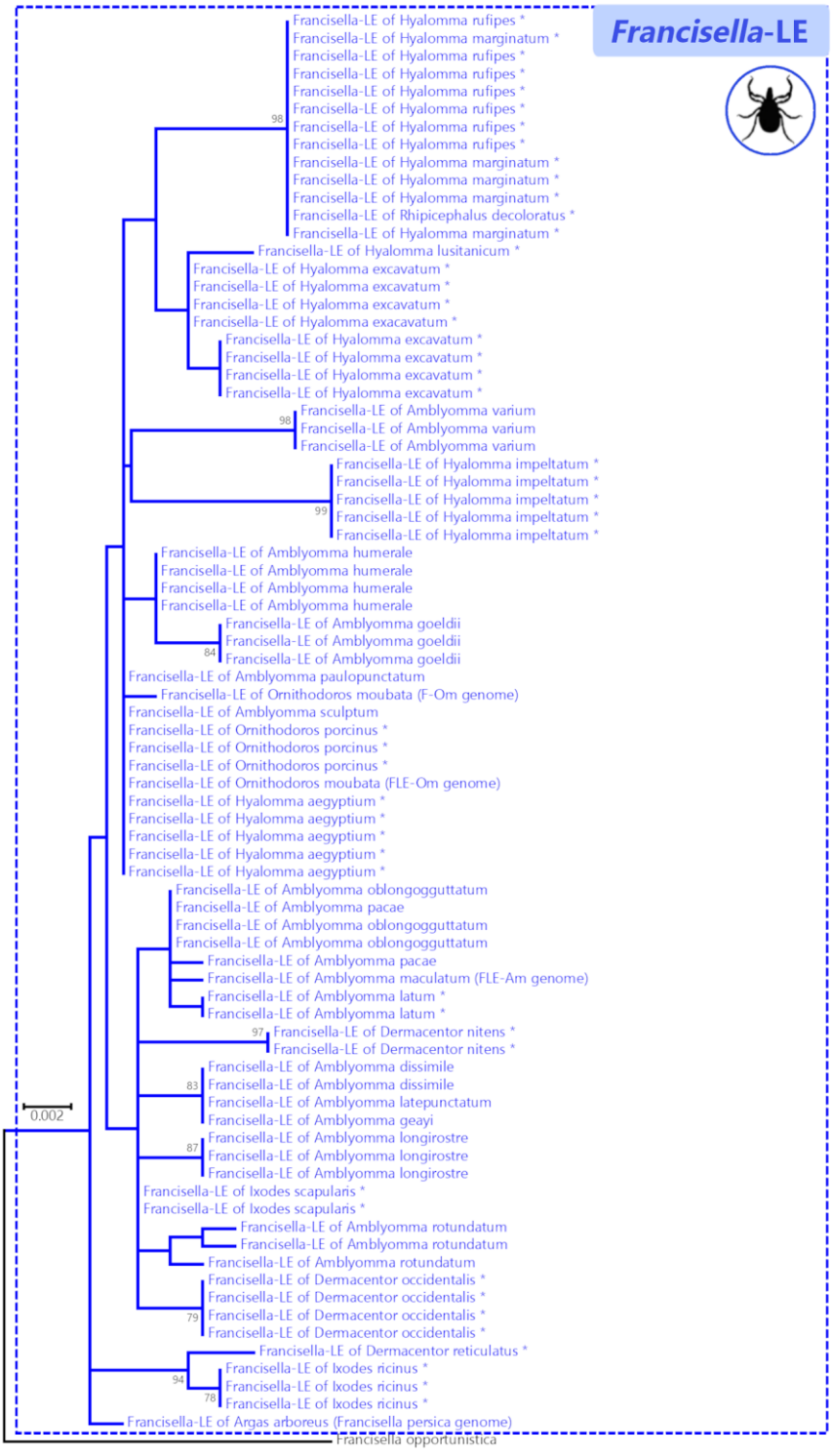
Fig. S1 *Francisella* phylogenetic tree constructed using maximum-likelihood (ML) estimations based on 16S rRNA nucleotidic sequences (695 unambiguously aligned bp; best-fit approximation for the evolutionary model: HKY+G) from *Francisella*-LE of 29 tick species and from other *Francisella* species. Branch numbers indicate percentage bootstrap support (1 000 replicates). Only bootstrap supports >70% are shown. The scale bar is in units of substitution/site. *, *Francisella*-LE sequences obtained in this study. *Francisella*-LE sequences are indicated by a blue font.

Fig. S2 *Francisella* phylogenetic tree constructed using maximum-likelihood (ML) estimations based on *rpoB* nucleotidic sequences (379 unambiguously aligned bp; best-fit approximation for the evolutionary model: TN93+I) from *Francisella*-LE of 29 tick species and from other *Francisella* species. Branch numbers indicate percentage bootstrap support (1 000 replicates). Only bootstrap supports >70% are shown. The scale bar is in units of substitution/site. *, *Francisella*-LE obtained in this study. *Francisella*-LE sequences are indicated by a blue font.

Fig. S3 *Francisella* phylogenetic tree constructed using maximum-likelihood (ML) estimations based on *groEL* nucleotidic sequences (981 unambiguously aligned bp; best-fit approximation for the evolutionary model: TN93+G) from *Francisella*-LE of 29 tick species and from other *Francisella* species. Branch numbers indicate percentage bootstrap support (1 000 replicates). Only bootstrap supports >70% are shown. The scale bar is in units of substitution/site. *, *Francisella*-LE obtained in this study. *Francisella*-LE sequences are indicated by a blue font.

Fig. S4 *Francisella* phylogenetic tree constructed using maximum-likelihood (ML) estimations based on *ftsZ* nucleotidic sequences (713 unambiguously aligned bp; best-fit approximation for the evolutionary model: GTR+G) from *Francisella*-LE of 29 tick species and from other *Francisella* species. Branch numbers indicate percentage bootstrap support (1 000 replicates). Only bootstrap supports >70% are shown. The scale bar is in units of substitution/site. *, *Francisella*-LE obtained in this study. *Francisella*-LE sequences are indicated by a blue font.

Fig. S5 *Francisella* phylogenetic tree constructed using maximum-likelihood (ML) estimations based on *gyrB* nucleotidic sequences (1,035 unambiguously aligned bp; best-fit approximation for the evolutionary model: GTR+G+I) from *Francisella*-LE of 29 tick species and from other *Francisella* species. Branch numbers indicate percentage bootstrap support (1 000 replicates). Only bootstrap supports >70% are shown. The scale bar is in units of substitution/site. *, *Francisella*-LE obtained in this study. *Francisella*-LE sequences are indicated by a blue font.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Francisella opportunistica

Fig. S1

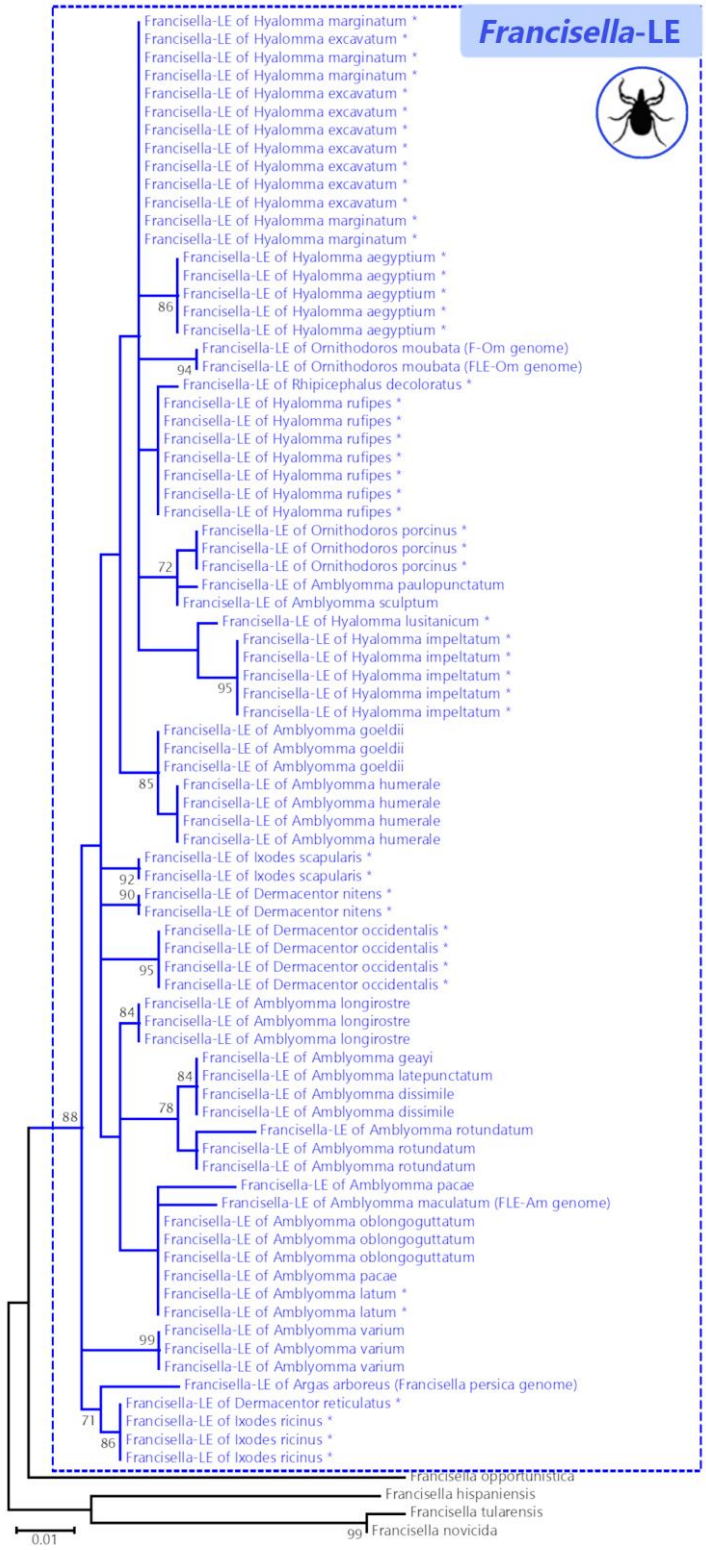
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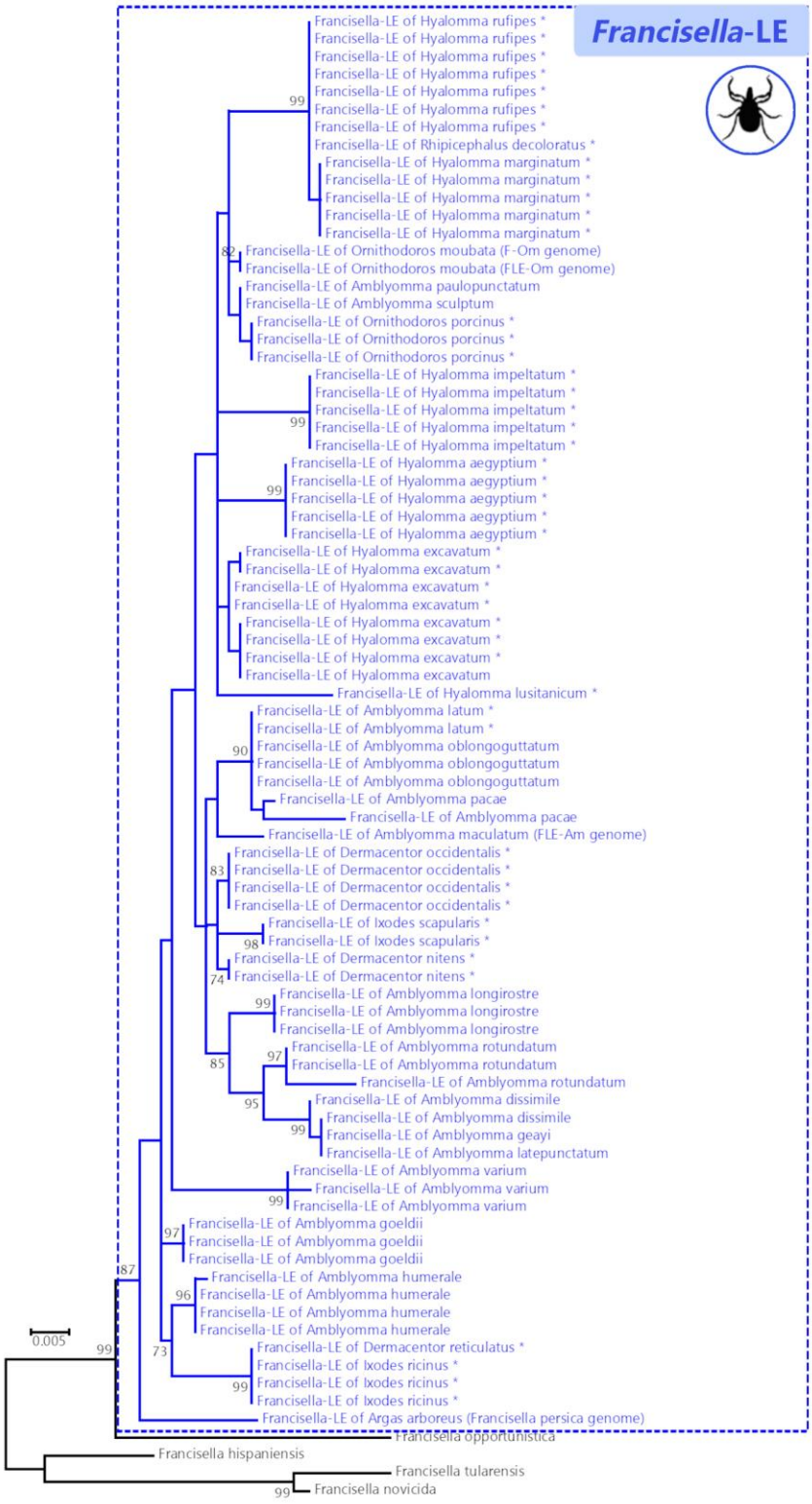
Fig. S2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Francisella tularensis
Francisella novicida
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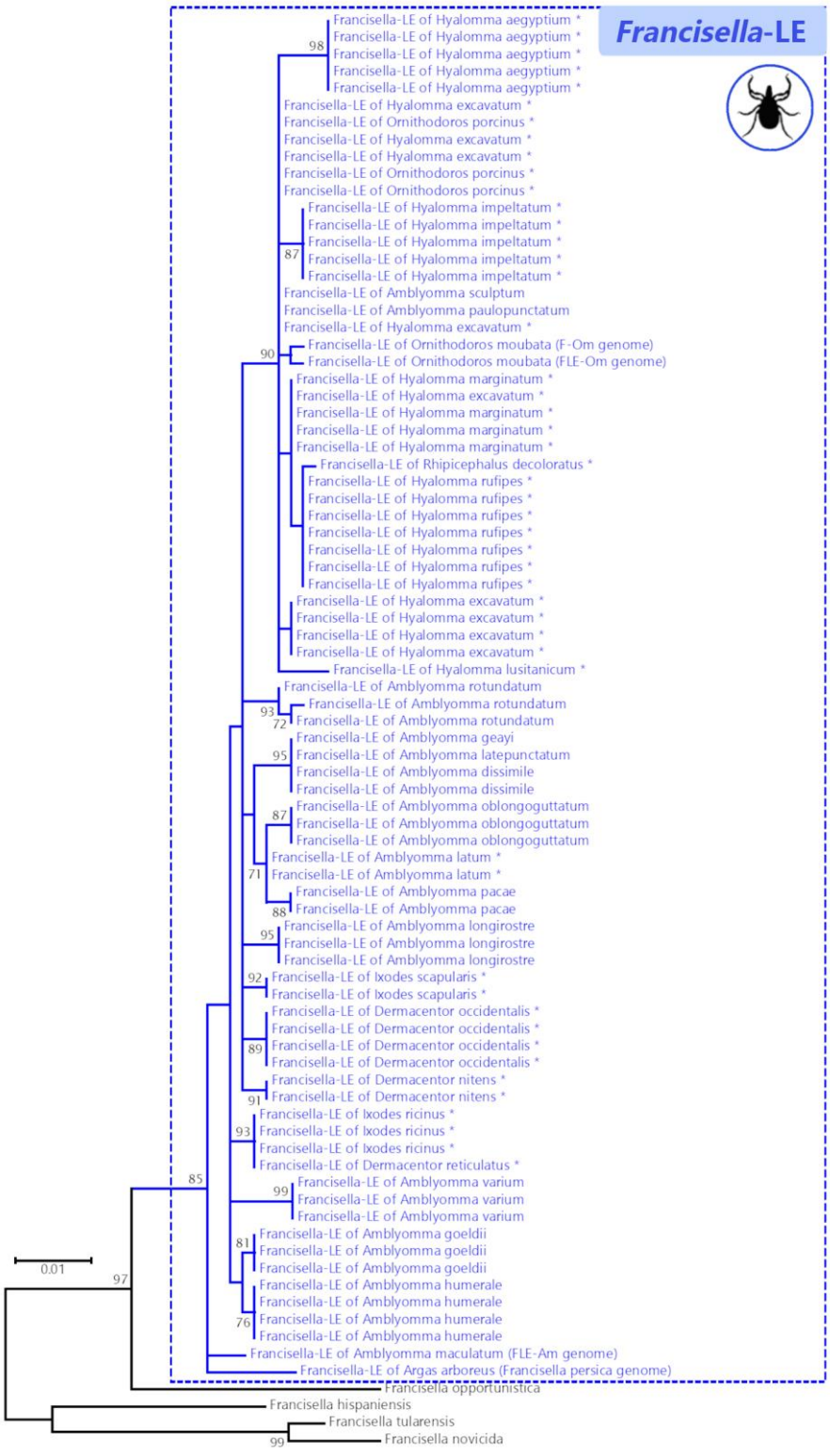
58

59 **Fig. S3**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Francisella-LE of Amblyomma sculptum
Francisella-LE of Amblyomma paulopunctatum
Francisella-LE of Hyalomma excavatum *
Francisella-LE of Ornithodoros moubata (F-Om genome)
Francisella-LE of Ornithodoros moubata (FLE-Om genome)
Francisella-LE of Hyalomma marginatum *
Francisella-LE of Hyalomma excavatum *
Francisella-LE of Hyalomma marginatum *
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Francisella-LE of Amblyomma geayi
Francisella-LE of Amblyomma latepunctatum
Francisella-LE of Amblyomma dissimile
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Francisella-LE of Ixodes scapularis *
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Francisella-LE of Dermacentor reticulatus *
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Francisella-LE of Amblyomma humerale
Francisella-LE of Amblyomma humerale
Francisella-LE of Amblyomma maculatum (FLE-Am genome)
Francisella-LE of Argas arboreus (Francisella persica genome)
Francisella opportunistica
Francisella hispaniensis
Francisella tularensis
Francisella novicida
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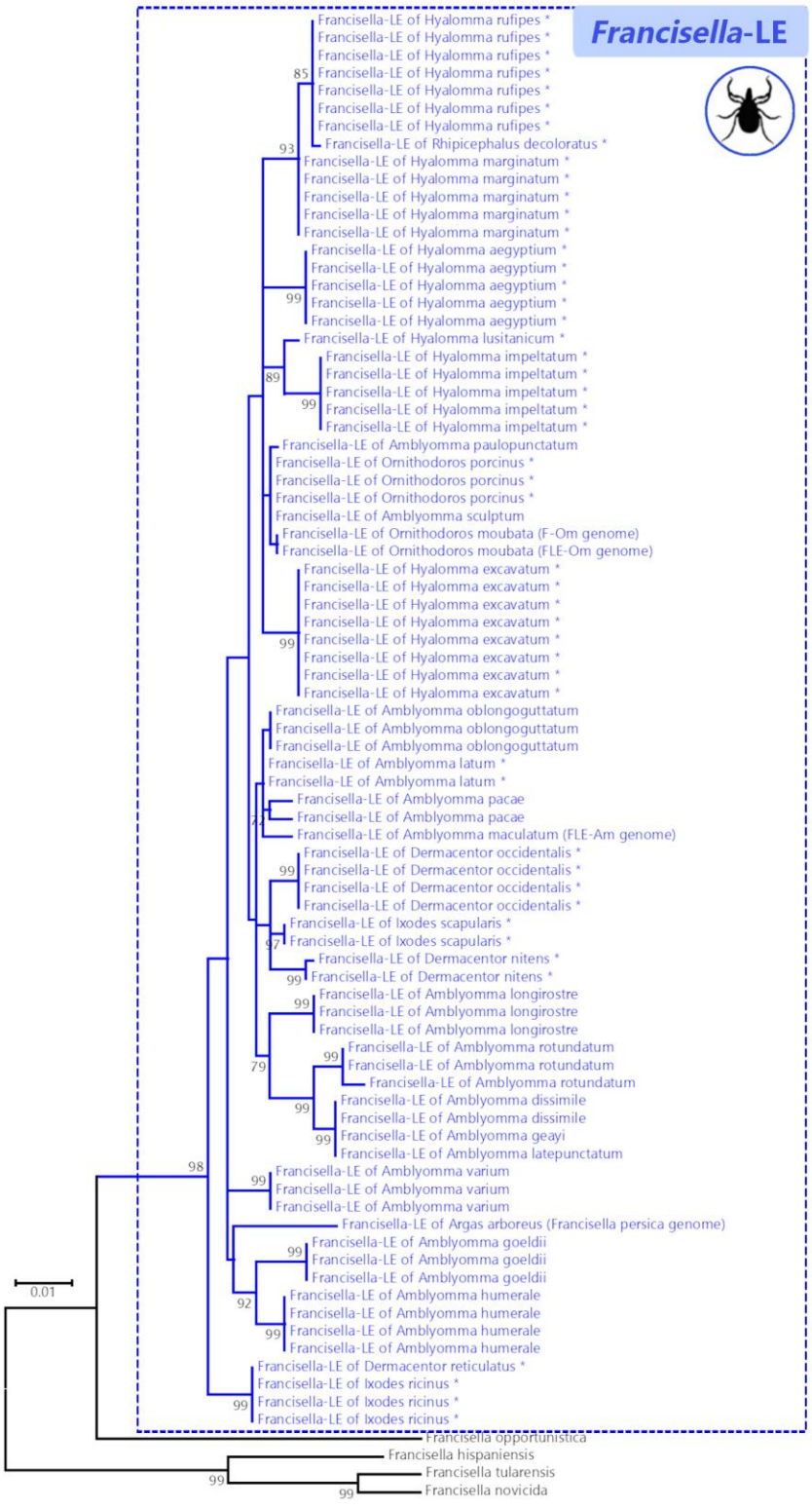
Fig. S4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Francisella hispaniensis
Francisella tularensis
Francisella novicida
0.01

Fig. S5

Supplementary references

1. Duron O, Binetruy F, Noel V, Cremaschi J, McCoy K, Arnathau C, Plantard O, Goolsby J, Perez De Leon AA, Heylen DJA, Raoul Van Oosten A, Gottlieb Y, Baneth G, Guglielmone AA, Estrada-Pena A, Opara MN, Zenner L, Vavre F, Chevillon C. 2017. Evolutionary changes in symbiont community structure in ticks. Mol Ecol 26:2905–2921.
2. Azagi T, Klement E, Perlman G, Lustig Y, Mumcuoglu KY, Apanaskevich DA, Gottlieb Y. 2017. *Francisella*-like endosymbionts and *Rickettsia* species in local and imported *Hyalomma* ticks. Appl Environ Microbiol 83:1–14.