Supplementary materials

Exploring the effect of the *Cardinium* endosymbiont on spiders

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Population	Country	Year of collection	Map	n	Prevalence (n)	95% c.i.
Cap Breton	France	2010	A	16	0.00 (0)	(0.00-0.21)
Toulouse	France	2010	B	19	0.16 (3)	(0.03-0.40)
Barcelona	Spain	2010	C	7	0.00 (0)	(0.00-0.41)
Castelnaudary	France	2009	D	21	0.86 (18)	(0.64-0.97)
Castres	France	2010	E	10	0.30 (3)	(0.07-0.65)
Perpignan	France	2010	F	17	0.18 (3)	(0.04-0.43)
Faveyrolles	France	2010	G	34	0.50 (17)	(0.32-0.68)
Béziers	France	2010	H	10	0.60 (6)	(0.26-0.88)
Montpellier	France	2009-2010	I	159	0.79 (126)	(0.72-0.85)
Saint Gilles	France	2010	J	28	0.07 (2)	(0.01-0.24)
Château-Renard	France	2010	K	18	0.72 (13)	(0.47-0.90)
Valence	France	2009	L	15	0.73 (11)	(0.45-0.92)
Pierrelatte	France	2010	M	11	0.27 (3)	(0.06-0.61)
Lyon Saint Exupéry	France	2010	N	10	0.10 (1)	(0.00-0.45)
Univeristé Lyon 1	France	2010	O	23	0.17 (4)	(0.05-0.39)
La Varenne	France	2009	P	10	0.10 (1)	(0.00-0.45)
Saint Romain en Gier	France	2009	Q	12	0.33 (4)	(0.10-0.65)
Plan de la Tour	France	2010	R	3	0.00 (0)	(0.00-0.71)
Sari d'Orcino	France (Corsica)	2010	S	14	0.00 (0)	(0.00-0.23)
Heraklion	Crete	2010	T	10	0.00 (0)	(0.00-0.31)
Petra	Jordan	2011	U	6	0.00 (0)	(0.00-0.46)
Sede Boqer Campus	Israel	2010	V	11	0.09 (1)	(0.00-0.41)
Merced	California	2010	W	12	0.00 (0)	(0.00-0.26)
Madera	California	2010	X	16	0.00 (0)	(0.00-0.21)
Mariposa	California	2010	Y	2	0.00 (0)	(0.00-0.84)
Yosemite	California	2010	Z	16	0.00 (0)	(0.00-0.21)

Table S1. Description of the *Holocnemus pluchei* samples: location, sample size and prevalence of *Cardinium*. Map letters indicate references in Figure 1. Confidence intervals (c.i.) were calculated from the binomial distribution.

Organism	Gene	Gene product	Primer (5' - 3')	Annealing temperature	Size	Positive control for infection screening	Reference (primers)
Holocnemus pluchei	*CO1*	Cytochrome oxidase subunit I	Ron – GGAGCYCCWGATATAGCTTTCCC * Nancy – CCTGGTAAAATTAAAATATAAACTTC *	52°C	488bp	_	(Simon et al. 1994)
Cardinium hertigii	*16S rRNA*	Small ribosomal subunit	ChF – TACTGTAAGAATAAGCACCGGC * ChR – GTGGATCACTTAACGCTTTCG *	54°C	ChF/ChR: 394bp	*H. pluchei*	(Zchori-Fein & Perlman 2004)
			CLOf1 – GAACCTTACCTGGGCTAGAATGTATT CLOr1 – GCCACTGTCTTCAAGCTCTACCAAC	54°C	CLOf1/CLOr1: 466bp ChF/CLOr1: 954bp	*H. pluchei*	(Gotoh et al. 2007)
	gyrb	DNA gyrase subunit B	gyrF – CCTATYCTTGTAGARGGRGAAA * gyrR – GCTCATCAAHACATTTTTGCGTTGG *	52°C	483bp	*H. pluchei*	This study
Wolbachia pipientis	*wsp*	Surface protein	81F – TGGTCCAATAAGTGATGAAGAAAC * 691R – AAAAATTAAACGCTACTCCA *	50°C	602bp	*Culex pipiens* (Diptera: Culicidae)	(Zhou et al. 1998)
Arsenophonus spp.	*ftsK*	Cell division protein (DNA translocase)	ftsKf – GTTGTYATGGTYGATGAATTTGC * ftsKr – GCTCTTCATCACYATCAWAACC *	52°C	445bp	*Hippobosca equina* (Diptera: Hippoboscidae)	(Duron et al. 2010)
Spiroplasma ixodetis	*16S rRNA*	Small ribosomal subunit	SpixoF – TTAGGGGCTCAACCCCTAACC * SpixoR – TCTGGCATTGCCAACTCTC *	52°C	810bp	*Cicadella viridis* (Hemiptera: Cicadellidae)	(Duron et al. 2008a)
Rickettsia spp.	*gltA*	Citrate synthase	RICS741F – CATCCGGAGCTAATGGTTTTGC * RCIT1197R – CATTTCTTTCCATTGTGCCATC *	50°C	470bp	*Meta sp.* (Aranea: Tetragnathidae)	(Goodacre et al. 2006) (Davis et al. 1998)

Table S2. Genes and primer features. The sizes of PCR products provided are those of positive controls in base pairs (bp). * Primers used in initial screen of the spider collection; the other primers were used to obtain additional sequences.

15 **References**

16 Davis, M. J., Ying, Z., Brunner, B. R., Pantoja, A. & Ferwerda, F. 1998 Rickettsial relative associated with Papaya bunchy top disease. *Current Microbiology*
17 26 , 80–84.

18 Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstadter, J. & Hurst, G. D. 2008a The diversity of reproductive parasites among arthropods:
19 *Wolbachia* do not walk alone. *BMC Biology* 6 , 27.

20 Duron, O., Wilkes, T. E. & Hurst, G. D. 2010 Interspecific transmission of a male-killing bacterium on an ecological timescale. *Ecol Lett* 13 , 1139-48.

21 Goodacre, S. L., Martin, O. Y., Thomas, C. F. & Hewitt, G. M. 2006 *Wolbachia* and other endosymbiont infections in spiders. *Mol Ecol* 15 , 517-27.

22 Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994 Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and
23 a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87 , 651–701.

24 Zchori-Fein, E. & Perlman, S. J. 2004 Distribution of the bacterial symbiont Cardinium in arthropods. *Mol Ecol* 13 , 2009-16.

25 Zhou, W., Rousset, F. & O'Neil, S. 1998 Phylogeny and PCR-based classification of Wolbachia strains using wsp gene sequences. *Proc Biol Sci* 265 , 509-15.

Figure legends

Figure S1. Numbers of eggs regressed against mother's tibia-patella length. Black squares, *Cardinium*-infected females (n = 50); with squares, uninfected females (n = 17).

Figure S2. Variation of *Cardinium* frequencies in the south of France and Spain. The figure encompasses 18 populations (from A to R on Figure 1). Left: distance measured from Castelnaudary (population D); right: distance measured from Béziers (population H).
Cardinium frequency significantly decreases from reference locations (Castelnaudary: $t_{16} = -2.056$; $P = 0.05$; Béziers: $t_{16} = -2.467$; $P = 0.02$). No *Cardinium*-infected specimen was found in populations more than 400km from reference locations.

Figure S3. *Cardinium* phylogeny constructed via maximum-likelihood using (a) *16S rRNA* and (b) *gyrb* sequences. The symbionts of arthropods have the prefix S followed by the proper name of their host, whereas other bacteria have proper binomial nomenclature. *Cardinium* hosts are encoded with shape symbols, with Araneae designated by diamonds, Acari by squares, Hemiptera by circles, Hymenoptera by black triangles and Diptera by white triangles. Associated GenBank accession numbers are shown in parentheses. Sequences from *Holocnemus pluchei* are underlined. Effect of infection is indicated in bold type if known (CI, cytoplasmic incompatibility; P, parthenogenesis; EF, enhancing of fecundity). The three *Cardinium* groups (a-c) are indicated. The closest known relative of *Cardinium*, the *Acanthamoeba* symbiont *Amoebophilus asiaticus*, was used as an outgroup. Numbers on branches indicate percentage bootstrap support for major branches (1000 replicates; only bootstrap values of 60% or more are shown). The scale bar is in units of substitutions/site.

Figure S4. Distribution of mtDNA haplotypes among populations, partitioned by *Cardinium* infection status. The sample letter above each box corresponds to the populations listed in Table S1. Within each population, the colours within bars indicate the relative proportion of haplotypes within each infection status (U = uninfected, I = infected). Numbers within bars indicate the number of specimens.

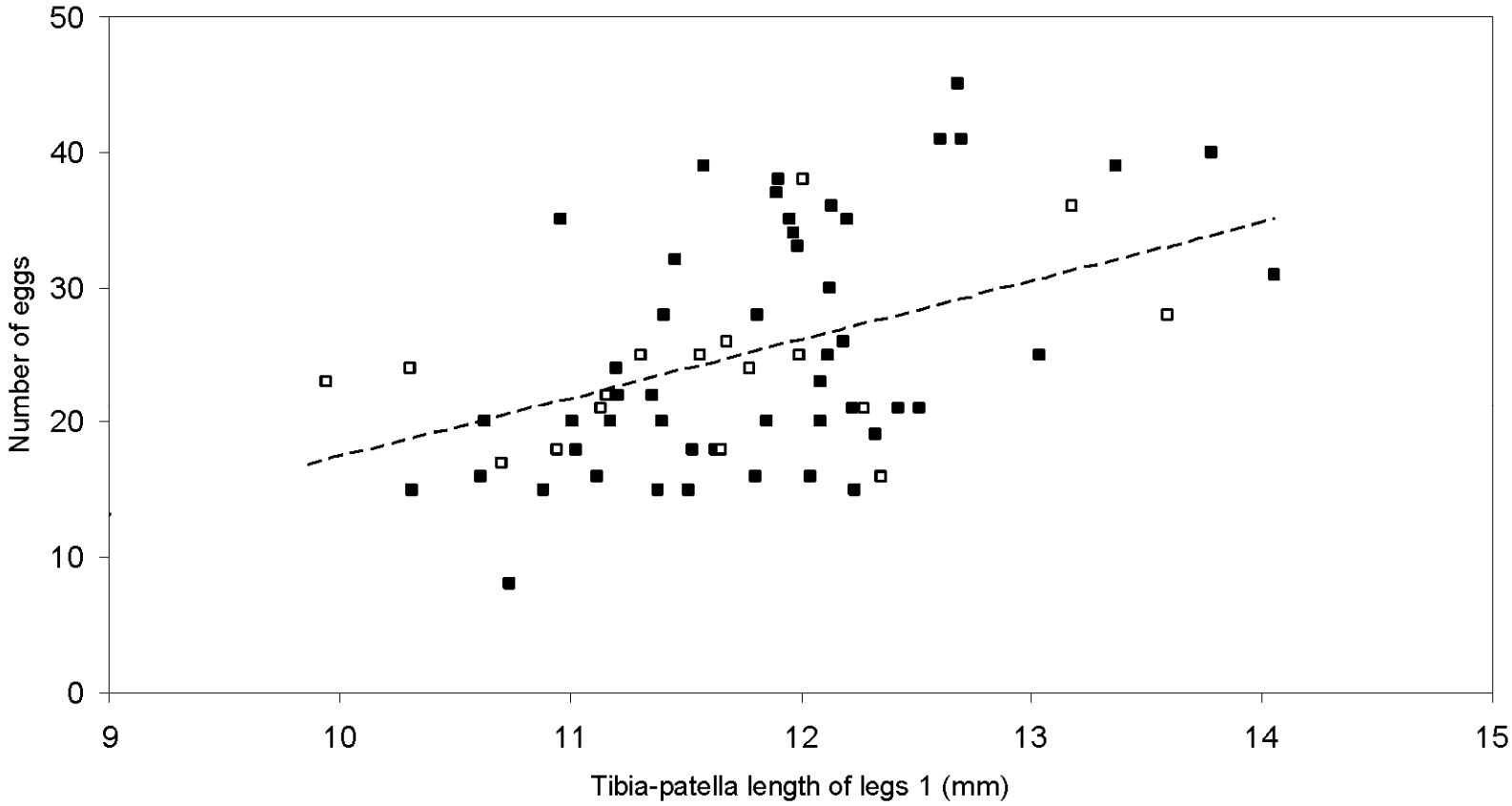
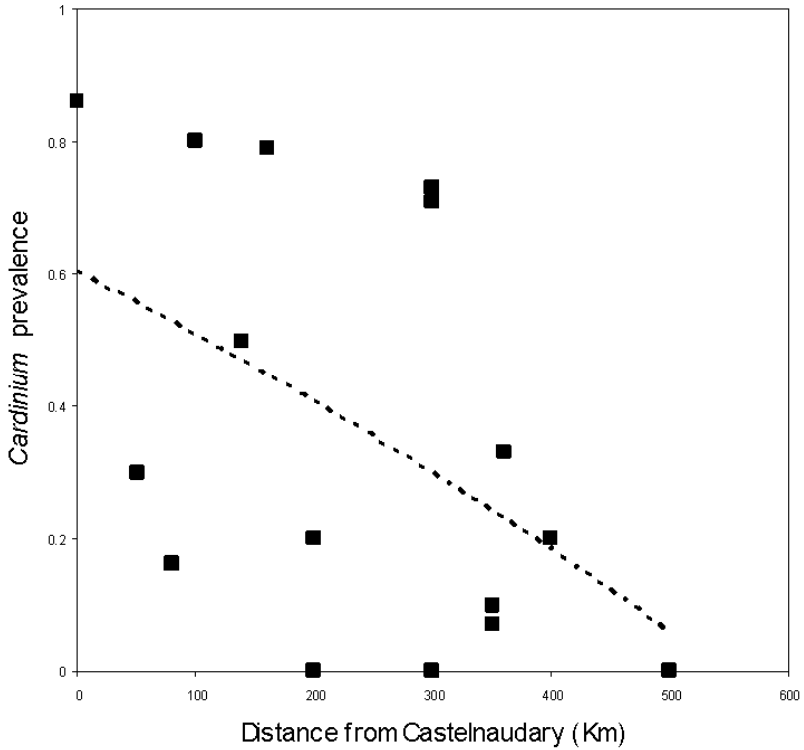

50
40
30
20
10
0
Number of eggs
9
10
11
12
13
14
15
Tibia-patella length of legs 1 (mm)

Figure S1


Cardinium prevalence
Distance from Castelnaudary (Km)
0
0.2
0.4
0.6
0.8
1
0
100
200
300
400
500
600

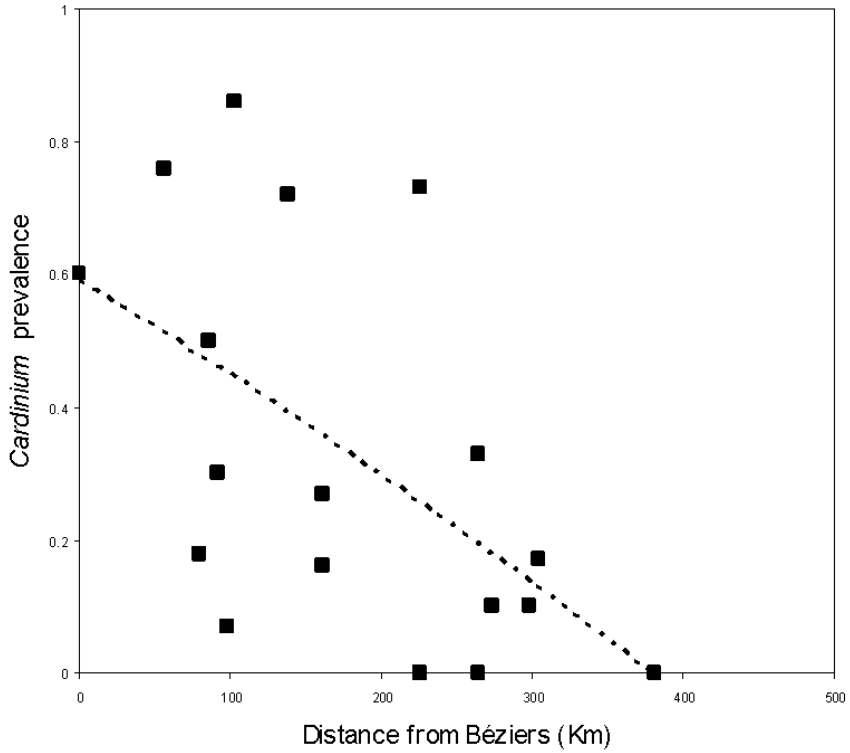

Cardinium prevalence
Distance from Béziers (Km)
0
0.2
0.4
0.6
0.8
1
0
100
200
300
400
500

Figure S2

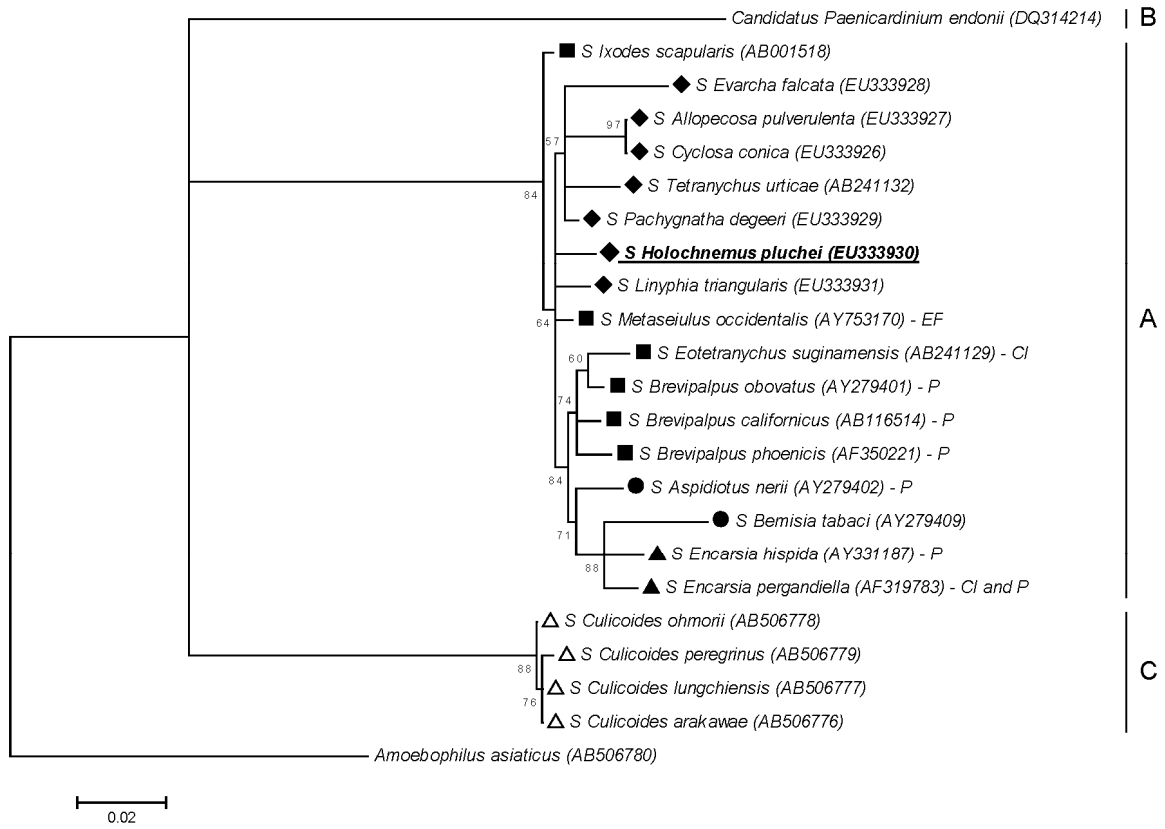

Candidatus Paenicardinium endonii (DQ314214)
S Ixodes scapularis (AB001518)
S Evarcha falcata (EU333928)
S Allopecosa pulverulenta (EU333927)
S Cyclosa conica (EU333926)
S Tetranychus urticae (AB241132)
S Pachygnatha degeeri (EU333929)
S Holochnemus pluchei (EU333930)
S Linyphia triangularis (EU333931)
S Metaseiulus occidentalis (AY753170) - EF
S Eotetranychus suginamensis (AB241129) - Cl
S Brevipalpus obovatus (AY279401) - P
S Brevipalpus californicus (AB116514) - P
S Brevipalpus phoenicis (AF350221) - P
S Aspidiotus nerii (AY279402) - P
S Bemisia tabaci (AY279409)
S Encarsia hispida (AY331187) - P
S Encarsia pergandiella (AF319783) - Cl and P
S Culicoides ohmorii (AB506778)
S Culicoides peregrinus (AB506779)
S Culicoides lungchiensis (AB506777)
S Culicoides arakawae (AB506776)
Amoebophilus asiaticus (AB506780)
97
57
84
64
60
74
84
71
88
88
76
B
A
C
0.02

Figure S3A

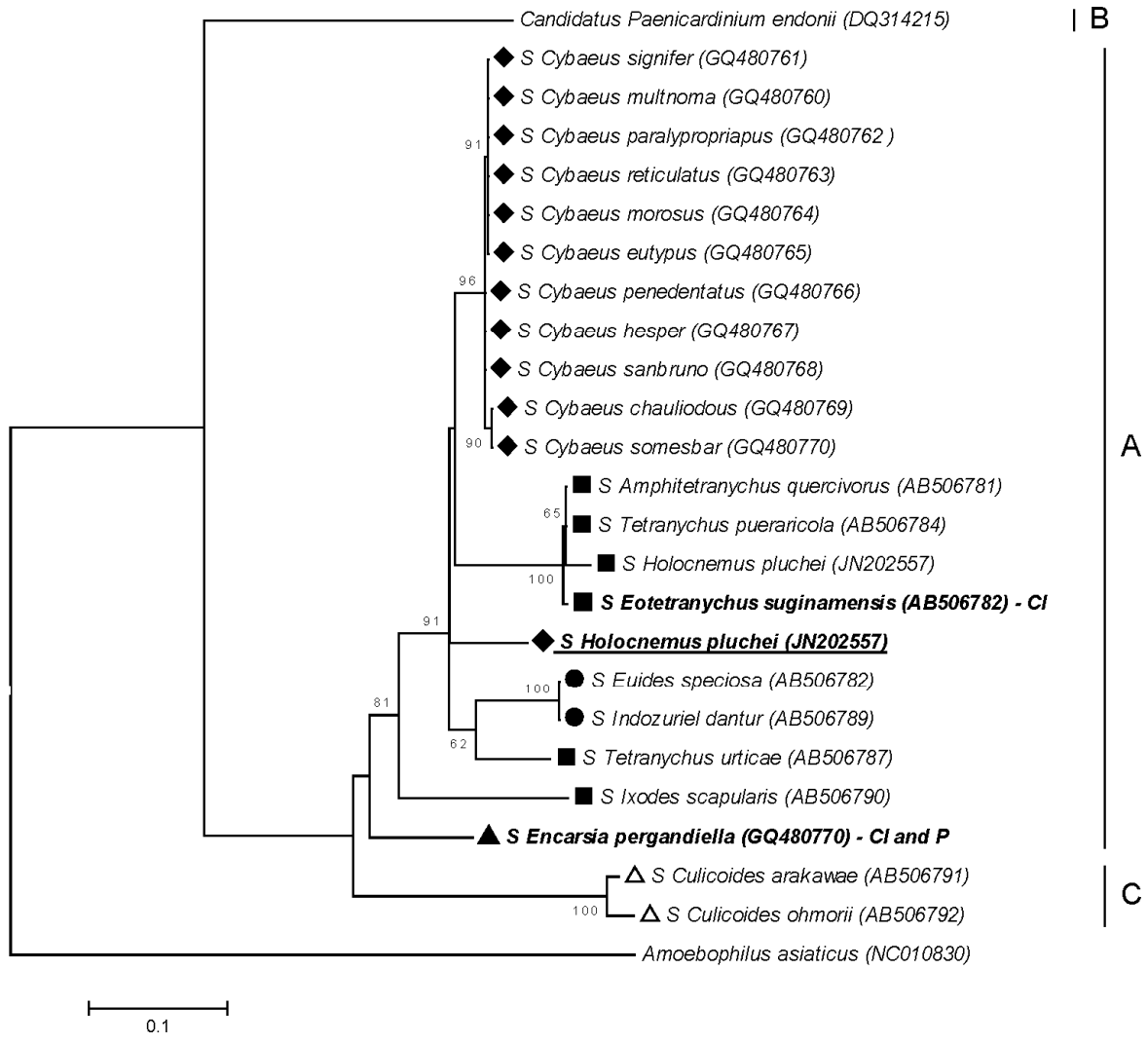

Candidatus Paenicardinium endonii (DQ314215)
S Cybaeus signifer (GQ480761)
S Cybaeus multnoma (GQ480760)
S Cybaeus paralypropriapus (GQ480762)
S Cybaeus reticulatus (GQ480763)
S Cybaeus morosus (GQ480764)
S Cybaeus eutypus (GQ480765)
S Cybaeus penedentatus (GQ480766)
S Cybaeus hesper (GQ480767)
S Cybaeus sanbruno (GQ480768)
S Cybaeus chauliodous (GQ480769)
S Cybaeus somesbar (GQ480770)
S Amphitetranychus quercivorus (AB506781)
S Tetranychus pueraricola (AB506784)
S Holocnemus pluchei (JN202557)
S Eotetranychus suginamensis (AB506782) - Cl
S Holocnemus pluchei (JN202557)
S Euides speciosa (AB506782)
S Indozuriel dantur (AB506789)
S Tetranychus urticae (AB506787)
S Ixodes scapularis (AB506790)
S Encarsia pergandiella (GQ480770) - Cl and P
S Culicoides arakawae (AB506791)
S Culicoides ohmorii (AB506792)
Amoebophilus asiaticus (NC010830)
91
96
90
65
100
91
100
81
62
100
B
A
C
0.1

Figure S3B

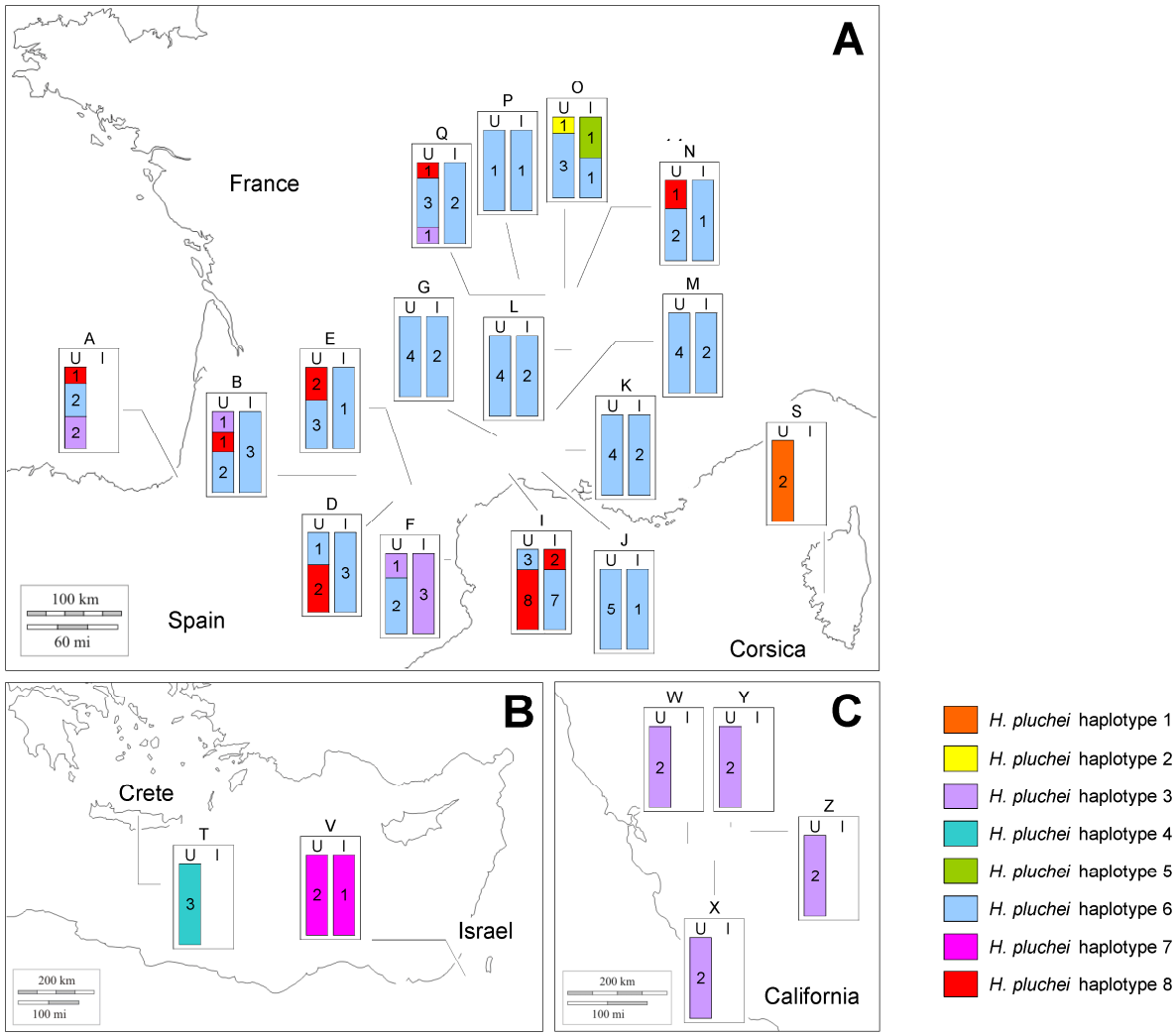

A
France
Spain
Corsica
100 km
60 mi
B
Crete
Israel
200 km
100 mi
C
California
200 km
100 mi
H. pluchei haplotype 1
H. pluchei haplotype 2
H. pluchei haplotype 3
H. pluchei haplotype 4
H. pluchei haplotype 5
H. pluchei haplotype 6
H. pluchei haplotype 7
H. pluchei haplotype 8

Figure S4