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Evolutionary changes in symbiont community structure in ticks

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1 **Community dynamics of maternally inherited bacteria in ticks**

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29 **Key words:** Symbiosis, Maternally inherited bacteria, Heritable symbiont communities,
30 Coevolution, Tick

31

32 **Running title:** Communities of maternally inherited bacteria

33 **Abstract**

34 Ecological specialization to restricted diet-niches is driven by obligate, and often maternally
35 inherited, symbionts in many arthropod lineages. These heritable symbionts typically form
36 evolutionary stable associations with arthropods that can last for millions of years. Ticks were
37 recently found to harbor such an obligate symbiont, *Coxiella*-LE, that synthesizes B vitamins
38 and cofactors not obtained in sufficient quantities from blood diet. In this study, the
39 examination of 81 tick species however shows that *Coxiella*-LE symbioses are characterized
40 by low evolutionary stability with frequent host shifts and extinction events. Further
41 examinations revealed the presence of nine other genera of maternally inherited bacteria in
42 ticks. Although these nine symbionts were primarily thought to be facultative, their
43 distribution pattern rather suggests that at least four may have independently replaced
44 *Coxiella*-LE during tick evolution and may be novel obligate symbionts. Phylogenetic
45 evidence next indicates that cocladogenesis is rare in these symbioses since most originate
46 following horizontal transfer of an existing symbiont from one tick species to another. A
47 result of these processes is that symbiotic communities commonly reach high levels of
48 diversity with up to six unrelated maternally inherited bacteria coexisting in some tick
49 species. Remarkably, the structure of symbiotic communities is not fixed and stable across the
50 tick phylogeny: the dynamic of symbiotic interactions drives important variations both among
51 and within tick species.

52

53 **Introduction**

54 Symbiosis with microorganisms is an important driver of evolutionary novelty in eukaryotes
55 (Moran *et al.* 2008; Wernegreen 2012). Arthropods frequently engage in associations with
56 bacterial endosymbionts that live exclusively within host cells and undergo maternal
57 (transovarial) transmission to offspring (Moran *et al.* 2008; Wernegreen 2012). In some cases,

58 these maternally inherited symbionts have evolved towards obligate mutualists and determine
59 ecologically important traits: obligate symbionts enable arthropod specialization on
60 unbalanced dietary source, such as plant sap or vertebrate blood, by providing biosynthetic
61 pathways absent from their hosts (Moran *et al.* 2008; Wernegreen 2012). However, in most
62 cases, they are facultative symbionts manipulating reproduction, protecting against natural
63 enemies or facilitating adaptation to changing environments (Moran *et al.* 2008; Engelstadter
64 & Hurst 2009; Oliver *et al.* 2010). As hosts can vary in the numbers and types of maternally
65 inherited symbionts they harbor, heritable and functionally important phenotypic variation can
66 exist within arthropod populations (Ferrari & Vavre 2011; Jaenike 2012).

67

68 Obligate symbionts are, by definition, present in most individuals of a given host species:
69 their mutualistic relationship is obligate for both two organisms as they can only live together.
70 Obligate symbionts typically form evolutionary stable associations that last for millions of
71 years (Moran *et al.* 2008) and that exhibit strict co-cladogenesis, resulting in congruent host-
72 symbiont phylogenies as observed in aphids, leafhoppers or tsetse flies (Chen *et al.* 1999;
73 Moran *et al.* 2005b; Takiya *et al.* 2006; Joussetin *et al.* 2009). Facultative symbionts, in
74 contrast, exhibit variable infection frequencies across temporal and spatial gradients (Jaenike
75 *et al.* 2010; Ferrari *et al.* 2012; Stefanini & Duron 2012; Russell *et al.* 2013; Smith *et al.*
76 2015a). They can also undergo occasional horizontal transfers (HT) across arthropod species,
77 resulting in limited phylogenetic congruence between hosts and symbionts (Russell *et al.*
78 2009; Duron *et al.* 2010; Russell *et al.* 2012; Joussetin *et al.* 2013). By combining maternal
79 inheritance with HT, unrelated maternally inherited bacteria can co-infect a single individual
80 host thereby forming an endosymbiotic community (Vautrin & Vavre 2009; Ferrari & Vavre
81 2011). Coexistence of symbionts within these communities is expected to involve complex
82 interactions, which can range from cooperation to competition and can, in turn, determine

83 aggregation and exclusion patterns, respectively (Moran *et al.* 2008; Vautrin & Vavre 2009;
84 Ferrari & Vavre 2011).

85

86 Among arthropods, ticks (Arachnida: Ixodidea) form a diversified group of *ca.* 900 species,
87 almost all depending on vertebrate blood as the sole food source (Guglielmone *et al.* 2010).
88 Ticks are well known as vectors of a wide diversity of infectious diseases, but they recently
89 were shown to harbor high numbers of endosymbionts: at least 10 distinct genera of
90 maternally inherited bacteria have been reported in ticks over the last decade (listed in Table
91 1). Among them, a *Coxiella*-like endosymbiont (*Coxiella*-LE hereafter) has been recently
92 identified as an obligate symbiont required for tick survival and reproduction (Zhong *et al.*
93 2007). An examination of *Coxiella*-LE intra-host localization revealed a pronounced tissue
94 tropism: this endosymbiont was typically found to infect ovaries (to ensure maternal
95 transmission) and the distal part of Malpighian tubules, suggesting a possible role in nutrition,
96 osmoregulation or excretion (Klyachko *et al.* 2007; Lalzar *et al.*, 2014; Machado-Ferreira *et*
97 *al.*, 2011). Its genome was further shown to encode pathways for synthesis of amino acids and
98 vitamins (*i.e.*, major B vitamins and cofactors) that fit closely with the expected nutritional
99 complements required for strict hematophagy (Gottlieb *et al.* 2015; Smith *et al.* 2015b). The
100 discovery of *Coxiella*-LE in numerous tick groups (Jasinskas *et al.* 2007; Clay *et al.* 2008;
101 Machado-Ferreira *et al.* 2011; Almeida *et al.* 2012; Lalzar *et al.* 2012; Duron *et al.* 2014a;
102 Duron *et al.* 2015a) corroborates the hypothesis of an obligate and mutualist symbiont.

103 However, around one third of examined tick species has been found devoid of *Coxiella*-LE or
104 harbor *Coxiella*-LE at much lower frequencies than expected for an obligate endosymbiont
105 upon which host survival and reproduction depends (Duron *et al.* 2014a; Duron *et al.* 2015a).
106 Most importantly, the species in which *Coxiella*-LE is missing are scattered among major tick
107 families and genera, a pattern suggesting the repeated loss of *Coxiella*-LE infections during

108 tick evolution (Duron *et al.* 2015a). Another surprising result was that closely related
109 *Coxiella*-LE infected unrelated tick species suggesting recurrent HT of this obligate symbiont
110 (Duron *et al.* 2015a). Interestingly, only a few case studies in insects have also shown that an
111 obligate endosymbiont may occasionally be lost but, in all these cases, the ancestral
112 endosymbiont has been replaced by another one recently acquired from a different host
113 species following HT (Fukatsu & Ishikawa 1992, 1996; Moran *et al.* 2005; Conord *et al.*
114 2008; Toju *et al.* 2013; Husnik & McCutcheon 2016).

115

116 Little is known about the incidence and distribution of the nine other maternally inherited
117 bacteria reported in ticks: they are usually thought to be facultative symbionts but their effects
118 on tick biology remain largely unknown (Table 1). Two genera are only found in ticks:
119 *Midichloria*, which inhabits the mitochondria of some tick species, and a *Francisella*-like
120 endosymbiont (*Francisella*-LE), which was reported in only a few tick species (Table 1).
121 Interestingly, the recent sequencing of a *Francisella*-LE strain suggests that this symbiont has
122 recently replaced a *Coxiella*-LE in at least one tick species (Gerhart *et al.* 2016). The seven
123 remaining endosymbiont genera are more or less frequently found in other arthropod groups,
124 including well-studied insects. Five endosymbionts (*Wolbachia*, *Cardinium*, *Arsenophonus*,
125 *Spiroplasma* and *Rickettsia*) are remarkably common and known to manipulate insect
126 reproduction through the induction of parthenogenesis, feminization, male-killing and
127 cytoplasmic incompatibility (Table 1). Several strains of *Wolbachia*, *Rickettsia* and
128 *Spiroplasma* are also defensive symbionts, protecting their insect-hosts against infections by
129 pathogens or against pathogen induced-mortality (Table 1). Two other endosymbionts,
130 *Rickettsiella* and *Lariskella*, are reported from only a couple of other arthropod taxa in
131 addition to ticks and their effects are unknown in most cases (Table 1).

132

133 The diversity of maternally inherited bacteria found in ticks provides an ideal system to study
134 the factors that shape endosymbiotic communities such as fidelity of maternal inheritance,
135 host specificity, frequency of HT and interactions among symbionts, including competitive
136 and cooperative interactions. The absence of the *Coxiella*-LE in distinct tick lineages raises
137 the pivotal question of whether this obligate symbiont has been repeatedly replaced by
138 alternative mutualist symbiont(s) and, if so, under which conditions. Does the distribution of
139 endosymbionts across tick taxa reflect random acquisitions through HT followed by vertical
140 inheritance or rather does it depend on exclusion or aggregation processes acting within
141 endosymbiotic communities? Here, we address this issue by analyzing variation in tick
142 endosymbiont communities at different geographic and phylogenetic scales using a
143 representative collection of tick specimens covering *ca.* 10% of tick species diversity. We first
144 examined the incidence and strain composition of 10 maternally inherited symbiotic bacteria
145 across tick families, genera, species and populations. Second, we estimated the relatedness
146 between endosymbiotic strains, retraced their respective evolutionary histories and contrasted
147 this pattern with tick phylogeny. Finally, we used this data set to infer the ecological and
148 evolutionary processes structuring tick endosymbiotic communities.

149

150 **Methods**

151 Tick collection

152 Specimens belonging to the two major families of ticks, Ixodidae (hard ticks) and Argasidae
153 (soft ticks), were collected from a variety of field sites around the world and from laboratory
154 colonies (Supplementary Table 1). One population per tick species was generally analyzed,
155 except for six focal species for which five distinct populations were collected. From 1 to 40
156 individuals per population were analyzed individually. Samples were either directly used for
157 molecular analyses or preserved in 70–90% ethanol until use.

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Molecular screening and typing

Tick DNA was individually extracted using the DNeasy Blood & Tissue Kit (QIAGEN) following manufacturer instructions. Each individual extract was then tested by PCR for infection for ten genera of maternally inherited bacteria: *Coxiella*, *Rickettsiella*, *Midichloria*, *Lariskella*, *Francisella*, *Arsenophonus*, *Cardinium*, *Wolbachia*, *Rickettsia* and *Spiroplasma*. Independent assays for each endosymbiont were performed by amplifying a fragment of either the 16S *rRNA* gene or another housekeeping gene using specific primers (see Supplementary Table 2 for procedures). DNA template quality of symbiont-negative specimens was systematically verified by PCR amplification of the eukaryotic 18S *rRNA* gene using universal primers (Supplementary Table 2); if no reaction was obtained, the tick extract was not retained in the study. Positive PCR products of one to ten randomly sampled individuals per infected species were purified and sequenced in both directions (EUROFINS) to ensure that the record represented a true positive and not a PCR artifact or related bacterium. Sequence chromatograms were manually cleaned with CHROMAS LITE (http://www.technelysium.com.au/chromas_lite.html) and alignments were performed using CLUSTALW (Thompson *et al.* 2002), implemented in the MEGA software (Kumar *et al.* 2004).

After initial screening, additional PCR amplifications were conducted to acquire multi-locus sequences for the *Coxiella* intrageneric phylogeny and to test for co-divergence with tick hosts (Supplementary Table 2). To this end, we used a subsample of tick specimens to obtain (1) additional *Coxiella* sequences (including the *rpoB*, *GroEL* and *dnaK* genes), and (2) tick mitochondrial DNA (mtDNA) sequences (including the 12S *rRNA*, the 16S *rRNA* and the *C01* genes). All PCR products were processed as described above.

184 Phylogenetic and statistical analyses

185 The GBLOCKS program (Castresana 2000) with default parameters was used to remove
186 poorly-aligned positions and to obtain non-ambiguous sequence alignments. Phylogenetic
187 analyses were based on sequence alignments done with single or concatenated sequences
188 from bacteria or tick mitochondria. Concatenated sequence alignments were checked for
189 putative recombinant regions using the GENECONV (Sawyer, 1999) and RDP (Martin, 2000)
190 methods available in the RDP3 computer analysis package (Martin, 2010). Closely-related
191 organisms obtained from GenBank were also included in the analyses. The evolutionary
192 models that best fit the sequence data were determined using the Akaike information criterion
193 with the program MEGA (Kumar *et al.* 2004). Sequence data and best-fitting evolutionary
194 models are detailed in Supplementary Table 3. Tree-based phylogenetic analyses were done
195 using maximum-likelihood (ML) analyses. ML heuristic searches using a starting tree
196 obtained by neighbor-joining was conducted in MEGA (Kumar *et al.* 2004). Clade robustness
197 was assessed by bootstrap analysis using 1,000 replicates. To test for associations between
198 *Coxiella*-LE and tick multi-locus data sets, we used the Procrustean Approach to
199 Cophylogeny (PACo) program (Balbuena *et al.* 2013) in R (<http://www.r-project.org>) using
200 the *ape* (Paradis *et al.* 2004) and *vegan* (Oksanen *et al.* 2013) packages. The significance of
201 cophylogenetic tests was established by 10,000 random permutations of the association
202 matrix.

203

204 We tested for differences in bacterial diversity among tick populations of a given species
205 using a Fisher's exact test (Raymond & Rousset 1995a) as implemented in the GENEPOP
206 program (Raymond & Rousset 1995b). For each population, occurrence and co-occurrence of
207 different symbionts infecting ticks at the individual level were visualized using the *Mondrian*
208 package (<https://cran.r-project.org/web/packages/Mondrian>) in R. We further investigated

209 potential differences between tick species in the composition of their endosymbiont
210 communities using correspondence analyses (CA). These computations were carried out using
211 the FactoMineR package (<http://factominer.free.fr>) in R. We first constructed a table in which
212 rows represent different populations and columns correspond to the prevalence of symbionts
213 (one column per symbiont). This table was further used to perform the CA analysis, which
214 allowed us to visualize among-population variation on a factorial map.

215

216 **Results**

217 Distribution of maternally inherited bacteria in ticks

218 We assayed for the presence of 10 maternally inherited bacteria in 861 individual ticks from
219 81 species belonging to the two major families of ticks, Argasidae (soft ticks: 3 genera, 26
220 species) and Ixodidae (hard ticks: 6 genera, 55 species) (Figure 1 and Supplementary Table
221 1). Of the 861 specimens, 706 (82%) were PCR-positive for at least one of the 10 bacteria.
222 The 155 remaining specimens (18%) were devoid of any of the targeted bacteria but had
223 satisfactory DNA template quality as shown by the positive PCR amplification of the 18S
224 gene fragment; these specimens were therefore considered as uninfected. Within tick
225 populations, species and genera, maternally inherited bacteria were detected at diverse
226 frequencies, ranging from 0% to 100%, as detailed below.

227

228 Of the 81 species examined, sampled individuals of two species (2.5%), the soft tick species
229 *Antricola guglielmonei* (n=4) and the hard tick *Ixodes apronophorus* (n=1), were found
230 uninfected (Figure 1 and Supplementary Table 1). The 79 other tick species (97.5%) were
231 found positive for at least one of the targeted bacteria in at least some of the specimens
232 (Figure 1 and Supplementary Table 1). The number of infected species did not vary between
233 tick families (Fisher's exact test, $p=0.54$) or genera ($p=0.15$). However, the number of

234 detected bacterial species per tick species positively covaried with the screening depth, *i.e.* the
235 number of examined specimens per tick species (Spearman's rank correlation, $N=81$, $r=0.34$,
236 $p=0.002$): tick species observed with higher bacterial diversity were those for which we
237 examined more specimens, such as the sheep tick *Ixodes ricinus* ($n=94$ individuals and 6
238 detected bacteria) and the African blue tick *Rhipicephalus decoloratus* ($n=91$ individuals and
239 6 detected bacteria).

240

241 All 10 maternally inherited bacteria previously reported in ticks (Table 1) were observed in
242 our samples. However, their respective incidences differed dramatically. *Coxiella*-LE and
243 *Rickettsia* infect more tick species than any other bacteria (Fisher's exact tests, all $p<10^{-3}$):
244 *Coxiella*-LE was detected in 49 tick species (60.5%), *Rickettsia* in 45 species (55.6%),
245 *Francisella*-LE in 17 species (21.0%), *Spiroplasma* in 13 species (16.4%), *Midichloria* in 12
246 species (14.8%), *Rickettsiella* in 10 species (12.3%), *Cardinium* in five species (6.2%),
247 *Lariskella* in three species (3.7%), *Arsenophonus* as well as *Wolbachia* in one species (1.2%).
248 A few PCR assays resulted in false positives (a band was present, but the sequence was
249 outside the targeted clade); they were excluded from further analyses.

250

251 Each of the 10 bacterial genera had a patchy distribution along the tick phylogeny. Some
252 showed a non-random distribution among tick species: their presence and prevalence may
253 vary considerably in the different tick groups (Figure 1 and Supplementary Table 1). Indeed,
254 *Coxiella*-LE was uniformly distributed between tick families (Fisher's exact test, $p=0.14$), but
255 not among tick genera ($p=2.10^{-6}$): it was common in *Rhipicephalus* (13 of 13 examined
256 species), *Argas* (3/3), *Ornithodoros* (17/22), *Amblyomma* (7/8) and *Haemaphysalis* (2/2), but
257 was rarer in *Dermacentor* (2/5), *Hyalomma* (1/5), *Ixodes* (5/22) and *Antricola* (0/1).
258 Conversely, there is no evidence to reject a uniform distribution of *Rickettsia* between tick

259 families ($p=0.32$) and genera ($p=0.11$): *Argas* (1/3), *Ornithodoros* (15/22), *Amblyomma* (5/8),
260 *Dermacentor* (4/5), *Haemaphysalis* (1/2), *Hyalomma* (1/5), *Ixodes* (11/22) and *Rhipicephalus*
261 (3/13).

262

263 Two of the eight other targeted bacterial genera, *Francisella*-LE and *Spiroplasma*, showed
264 variable distributions among tick families and genera (Figure 1 and Supplementary Table 1).

265 *Francisella*-LE was uniformly distributed between tick families ($p=0.56$) but not among
266 genera ($p=0.01$): common in *Hyalomma* (4/5), *Amblyomma* (4/8) and *Dermacentor* (2/5) and
267 rare or absent in the other genera. *Spiroplasma* was not uniformly distributed among tick
268 families ($p=0.007$) and genera ($p=0.02$): absent in soft ticks (0/26) but present in hard ticks
269 (13/55), it was only detected in *Ixodes* (8/22), *Dermacentor* (1/5) and *Rhipicephalus* (4/13).

270 The six remaining bacteria (*Midichloria*, *Rickettsiella*, *Cardinium*, *Lariskella*, *Arsenophonus*
271 and *Wolbachia*) did not show significant variation in incidence among tick families (all
272 $p>0.32$) or tick genera (all $p>0.18$).

273

274 When considering the whole bacterial community, as many as 33 different endosymbiotic
275 combinations were observed at the tick species level (Figure 1 and Supplementary Table 1).
276 Thirty-five of the 81 tick species (43.2%) were found infected by only one bacterium, but 44
277 (56.3%) harbored two or more bacteria: 23 species were infected by two bacteria (28.4%), 16
278 species by three bacteria (19.8%), two species by four bacteria (2.5%), one species by five
279 bacteria (1.2%) and two species by six bacteria (2.5%). At the tick species level, bacteria were
280 randomly associated in most cases (Fisher's exact tests, all $p>0.06$), suggesting that their
281 distribution across tick species was independent from the presence of other symbionts. Non-
282 random associations between bacteria were however observed. (1) Two bacteria co-occurred
283 in the same tick species more frequently than expected by chance in two cases (aggregation

284 pattern): *Midichloria* and *Rickettsia* ($p=0.03$), *Midichloria* and *Spiroplasma* ($p=0.02$). (2)
285 Conversely, two bacterial combinations co-occurred in the same tick species less frequently
286 than expected by chance (exclusion pattern): *Coxiella*-LE and *Rickettsiella* ($p=0.01$),
287 *Coxiella*-LE and *Francisella*-LE ($p=0.02$) (Figure 1 and Supplementary Table 1).
288
289 In the six focal tick species for which five geographic populations were sampled (Figure 2 and
290 Supplementary Table 1), each tick species hosted from two to six bacteria and each
291 population from one to five bacteria. The presence of two or more bacteria was often reflected
292 by coinfection at the individual level. Indeed, 25 of the 94 *I. ricinus* individuals (26.6%)
293 harbored more than one symbiont. In the soft tick *O. sonrai*, double or triple infections were
294 fixed or almost fixed in some populations, but absent in others. There was significant
295 variation in infection patterns among populations in four of the six tick species: the tree-hole
296 tick *I. arboricola* ($\chi^2_8=26.55$, $p=8.10^{-4}$), the polar seabird tick *I. uriae* ($\chi^2_{10}=30.79$, $p=6.10^{-4}$),
297 the African blue tick *R. decoloratus* ($\chi^2_{12}=34.65$, $p=5.10^{-4}$) and the soft tick *O. sonrai* ($\chi^2_4 \rightarrow \infty$,
298 $p < 10^{-10}$). These community level effects were due to variation in prevalence of one or two
299 bacteria per tick species: *Rickettsiella* (Fisher's exact test, $p=4.10^{-4}$) and *Spiroplasma* ($p=0.01$)
300 in *I. arboricola*, *Coxiella*-LE ($p=5.10^{-4}$) and *Rickettsiella* ($p=0.002$) in *I. uriae*, *Spiroplasma*
301 ($p=2.10^{-6}$) in *R. decoloratus*, *Midichloria* ($p < 10^{-10}$) and *Rickettsia* ($p < 10^{-10}$) in *O. sonrai*
302 (Figure 2 and Supplementary Table 1). In contrast, the composition of bacterial communities
303 were homogeneous among populations of the sheep tick *I. ricinus* ($\chi^2_{12}=14.04$, $p=0.30$) and
304 the cattle tick *R. microplus* ($\chi^2_2=0.75$, $p=0.69$).
305
306 The correspondence analysis (CA) showed again that infection patterns tended to differ more
307 among the six focal tick species than among populations of each tick species (Figures 3A and
308 B). In this analysis, the first two axes account for 78.4% of the total variability. The first axis

309 of the CA discriminates populations infected by *Coxiella*-LE (on the left) from those infected
310 by *Rickettsia* (on the right); the second axis discriminates populations infected by *Rickettsiella*
311 (on top) from those infected by either *Spiroplasma* or *Midichloria* (Figure 3A). Indeed,
312 *Coxiella*-LE was (almost) fixed in all *R. microplus*, *R. decoloratus* and *O. sonrai* populations,
313 but rare or absent otherwise. *Midichloria* was the most commonly detected bacterium in *I.*
314 *ricinus* and *Rickettsia* in *I. arboricola*; no bacterium was common in *I. uriae* (Figures 2 and
315 3B, Supplementary Table 1).

316

317 Evolutionary history of maternally inherited bacteria

318 DNA sequence analysis detected genetic diversity within each bacterial genus. A total of 165
319 genetically distinct strains were found: 50 strains of *Coxiella*-LE (30.3%), 47 of *Rickettsia*
320 (28.5%), 17 of *Francisella*-LE (10.3%), 16 of *Rickettsiella* (9.7%), 13 of *Spiroplasma* (7.9%),
321 12 of *Midichloria* (7.3%), five of *Cardinium* (3.0%), three of *Lariskella* (1.8%), one of
322 *Arsenophonus* (0.6%) and one of *Wolbachia* (0.6%).

323

324 The partitioning of *Coxiella*-LE diversity among tick species revealed a complex structure
325 with signatures of both co-divergence and HT events. A pattern suggestive of co-divergence
326 was detected for the *Coxiella*-LE harbored by *I. ricinus*, *I. hexagonus* and *I. uriae* since they
327 clustered together within the *Coxiella* phylogeny (Figure 4). A more compelling example is
328 found in the genus *Rhipicephalus* where the *Coxiella*-LE found in the 13 examined tick
329 species clustered within the same clade. In addition, there was perfect topological congruence
330 between the phylogenies of concatenated *Coxiella*-LE gene sequences (*rpoB*, *GroEL* and
331 *dnaK*; no recombination was detected in this data set using the GENECONV and RDP tests,
332 all $p > 0.10$) and concatenated *Rhipicephalus* mtDNA genes (12S *rRNA*, 16S *rRNA* and *COI*),
333 corroborating the co-divergence hypothesis (PACo analysis, $p = 5.10^{-6}$; Figure 5). Conversely,

334 absence of clustering in related tick species was also observed (Figure 4). For instance, the
335 *Coxiella*-LE of *Ornithodoros* soft ticks were scattered among different *Coxiella* branches.
336 Similarly, the *Coxiella*-LE of *Amblyomma* hard ticks belonged to a minimum of five distinct
337 phylogenetic clusters. These patterns are the signatures of repeated HT events, revealing the
338 ability of some *Coxiella*-LE to extensively move among tick species.

339

340 Examination of phylogenies of the nine other bacteria showed that they all undergo occasional
341 HT events. For example, the *Francisella*-LE and *Midichloria* strains of soft ticks are scattered
342 among those of hard ticks (Supplementary Figures 1-2). The other symbionts are not only
343 circulating among tick species but also among arthropod classes: the *Cardinium*,
344 *Arsenophonus*, *Lariskella*, *Wolbachia*, *Rickettsiella*, *Rickettsia* and *Spiroplasma* strains found
345 in ticks were frequently related to bacterial strains found in insects (Supplementary Figures 3-
346 9). However, in one case, the infection pattern remains difficult to interpret: the *Wolbachia*
347 strain detected in the sheep tick *I. ricinus* is 100% identical to that of the tick parasitoid
348 *Ixodiphagus hookeri* (Supplementary Figure 6). This suggests that it is likely the result of a
349 cross-contamination due the presence of parasitoid DNA in the tick DNA sample, a pattern
350 previously observed in *I. ricinus* (Plantard *et al.* 2012).

351

352 Worthy of note is that three endosymbionts are closely related, albeit genetically distinct, to
353 vertebrate pathogens. This includes *Coxiella*-LE, which are closely related to the Q fever
354 agent *C. burnetii* (Duron *et al.* 2015b), *Francisella*-LE, closely related to the causative agent
355 of tularaemia, *F. tularensis* (Sjodin *et al.* 2012), and *Rickettsia*, closely related to many (often
356 tick-borne) *Rickettsia* pathogens (Perlman *et al.* 2006; Weinert *et al.* 2009). There was thus a
357 potential risk of misidentification of endosymbionts in some tick species: the bacteria
358 observed may be tick-borne pathogens rather than maternally inherited endosymbionts.

359 However, in most cases, endosymbionts can be unambiguously distinguished from their
360 pathogenic relatives on the basis of their DNA sequences, as for *Coxiella*: all the strains we
361 found in ticks were genetically distinct from *C. burnetii* and can be unambiguously assigned
362 to *Coxiella*-LE (Figure 4; Duron *et al.* 2015b). For *Francisella*, the strains we found in ticks
363 formed a monophyletic clade related to, but distinct from the pathogenic species
364 (Supplementary Figure 1; see also Goethert & Telford 2005; Sjodin *et al.* 2012). For
365 *Rickettsia*, the situation is more complex because endosymbiotic forms and vertebrate
366 pathogens are scattered along the phylogeny (Perlman *et al.* 2006; Weinert *et al.* 2009): the
367 distinction of endosymbiotic forms and vertebrate pathogens on the basis of DNA sequences
368 alone may not be reliable in some cases. For instance, in several *Rhipicephalus* and
369 *Amblyomma* tick species, we observed *Rickettsia* strains closely related to, but genetically
370 distinct from, the causative agent of the African tick-bite fever, *Rickettsia africae*
371 (Supplementary Figure 8) and we cannot state whether these strains are pathogenic or not.
372 There are nevertheless other cases where the *Rickettsia* we detected are highly likely
373 endosymbionts: this is the case for those found in the blacklegged tick *I. scapularis* and the
374 American dog tick *Dermacentor variabilis* which clustered with *Rickettsia buchneri*
375 (Supplementary Figure 8), a non-pathogenic species known to be maternally inherited in ticks
376 (Kurtti *et al.* 2015). The high frequency of *Rickettsia* infections in tick species such as the
377 tree-hole tick *I. arboricola* also suggests the presence of *Rickettsia* endosymbiont (Figure 2
378 and Supplementary Table 1).

379

380 **Discussion**

381 That ecological specialization to restricted diet-niches is driven by evolutionary stable
382 symbiotic interactions is beyond doubt for many arthropod lineages (Moran *et al.* 2008;
383 Wernegreen 2012). The present study nevertheless shows that obligate symbioses are unstable

384 in ticks. We screened 81 tick species for *Coxiella*-LE, a formally-recognized obligate
385 symbiont of ticks, and for nine other maternally inherited symbionts whose incidence,
386 prevalence and diversity were largely unknown in tick populations. We detected maternally
387 inherited bacteria in almost all tick species (79 of 81) with many of them (44) hosting more
388 than one symbiont. In multi-infected tick species, symbionts are assembled in communities
389 which can reach high levels of complexity. Indeed, six distinct genera of symbionts co-exist
390 in populations of the sheep tick *I. ricinus* and in populations of the African blue tick *R.*
391 *decoloratus*. No symbiotic community structure was fixed and stable across the tick
392 phylogeny and communities varied both among tick species and among tick populations and
393 individuals within species. Notably, two transmission modes, both apparent in symbiont
394 phylogenies, act together: maternal inheritance, which ensures the persistence of infections
395 within a host clade, and occasional HT, which enables symbionts to disperse beyond their
396 primary host species. One of the most remarkable outcomes of these processes is the low
397 evolutionary stability of the symbiosis between ticks and *Coxiella*-LE.

398

399 As expected, *Coxiella*-LE was the most common maternally inherited symbiont associated
400 with ticks, recorded in 49 (60.5%) of the 81 sampled species. The wide distribution of
401 genetically differentiated *Coxiella*-LE strains across the tick phylogeny suggests that the
402 symbiosis is ancient and arose in early tick evolution. We did not, however, observe the co-
403 diversification pattern typically found for obligate mutualists. Instead, the spread of *Coxiella*-
404 LE infections was surprisingly complex, depending on two distinct evolutionary strategies.
405 Some *Coxiella*-LE symbionts seem to be highly specialized on their tick hosts, with ancient
406 acquisition followed by co-diversification. This is best exemplified by the co-divergence
407 observed between *Rhipicephalus* and *Coxiella*-LE lineages. The *Rhipicephalus* genus is
408 thought to have emerged in the Middle Miocene, *ca.* 14Mya (Murrell *et al.* 2001), which can

409 be assumed as an approximate minimal date for the original *Coxiella*-LE infection in this tick
410 genus. On the contrary, other *Coxiella*-LE symbionts are more generalist and seem to have
411 been acquired through recent HT events from unrelated tick species. Such a pattern is also
412 observed for other endosymbionts, like *Wolbachia* that experiments common host shifts in
413 insects and occasional transition to co-cladogenesis, as observed in filarial nematodes
414 (Werren *et al.*, 2008). Overall, the present data on *Coxiella*-LE supports the hypothesis that a
415 replacement of obligate symbiont takes place in ticks. Interestingly, we did not observe any
416 tick species infected by more than one *Coxiella*-LE strain, a result also corroborated by other
417 studies (Jasinskas *et al.* 2007; Clay *et al.* 2008; Machado-Ferreira *et al.* 2011; Almeida *et al.*
418 2012; Lalzar *et al.* 2012). The absence of co-infection pattern despite frequent HT events
419 further suggests that different *Coxiella*-LE strains cannot stably coexist within the same tick
420 species.

421
422 Exclusion processes are however not limited to *Coxiella*-LE, but also involve other symbiont
423 genera. In 30 of the 32 tick species not infected by *Coxiella*-LE, we detected the presence of
424 one to five other symbionts among which one is at fixation in most cases. Indeed, we found
425 significant exclusion patterns between *Coxiella*-LE and two other symbionts, *Francisella*-LE
426 and *Rickettsiella*. A hypothesis explaining these patterns is that these latter may be alternative
427 obligate symbionts which have replaced *Coxiella*-LE, as in some tick species of the
428 *Ornithodoros* and *Dermacentor* genera. Moreover, in some tick species of the *Ixodes* genus,
429 other alternative obligate symbionts may exist: *Rickettsia* and *Midichloria* strains were fixed,
430 or were close to fixation, in two tick species free of *Coxiella*-LE infection, *i.e.* the tree-hole
431 tick *I. arboricola* and in the sheep tick *I. ricinus*, respectively. A formal testing through
432 nutritional and physiological experiments is now required to validate this hypothesis but
433 available data on the bacterial genomes recently published provide pivotal highlights. Indeed,

434 genomic data suggest that these possible alternative obligate symbionts have evolved adaptive
435 mechanisms enabling tick survival: their genomes encode functions suggesting that they are
436 obligate tick mutualists in a very similar way to *Coxiella*-LE since they also have, at least
437 partially, the genetic capability for *de novo* B vitamin synthesis. For instance, recent
438 metabolic reconstructions of *Rickettsia* genomes revealed that all genes required for folate
439 (B9 vitamin) biosynthesis are present in the genome of the *Rickettsia* endosymbionts of the
440 black legged tick *I. scapularis* and of the Western black-legged tick *I. pacificus* (Hunter *et al.*
441 2015). Similarly, the genomes of *Francisella*-LE (isolated from the fowl tick *Argas persicus*
442 and from the Gulf Coast tick *Amblyomma maculatum*) and of *Midichloria* (from *I. ricinus*)
443 contain complete or nearly complete genetic pathways for biotin (B7 vitamin) and riboflavin
444 (B2) biosyntheses (Sassera *et al.* 2011; Sjodin *et al.* 2012; Gerhart *et al.* 2016).

445

446 The genetic capability for *de novo* B vitamin synthesis of some symbionts may explain why
447 they do not widely coexist with *Coxiella*-LE. In such cases where different symbionts provide
448 the same benefit for the host, maintenance of multiple infections is not expected (Vautrin &
449 Vavre 2009): as there is no additional benefit provided by the multiple infection, single
450 infection is expected to become fixed through regular imperfect transmission of the multiple
451 infection, a process that can be further accelerated if multiple infection is costly.

452 Alternatively, loss of multiple infections would be prevented if each symbiont becomes
453 indispensable through complementary retention of needed pathways: the two endosymbiont
454 then encode gene sets that form an interdependent metabolic patchwork, as observed in
455 several lineages of Hemiptera (Pérez-Brocal *et al.*, 2006; Bennett & Moran, 2013; Husnik &
456 McCutcheon 2016). Such process may possibly act in ticks and explain why a few tick
457 species remain co-infected by *Coxiella*-LE and *Francisella*-LE or *Rickettsia*. Another
458 interesting possibility is that some tick species may have lost their *Coxiella*-LE but have

459 acquired some functionally important symbiont genes (including those of B vitamin synthesis
460 pathways) via lateral gene transfer. In this context, ticks may retain the adaptive trait (B
461 vitamin provisioning) without the symbiont. Indeed, this pattern was reported from some
462 filarial nematodes to explain their ability of to live and reproduce without obligate symbiont
463 (McNulty *et al.* 2010). However, examination of the genome of a tick species lacking
464 *Coxiella*-LE, the black legged tick *I. scapularis*, did not show evidence of such lateral gene
465 transfer (Gulia-Nuss *et al.* 2016). Worthy of note is that this tick species is infected by a
466 *Rickettsia* endosymbiont which may synthesize B9 vitamin (Hunter *et al.* 2015), a pattern that
467 may explain, at least partially, the absence of *Coxiella*-LE.

468

469

470

471 An important consideration for future studies is the role of ecological connections between
472 tick species in facilitating HT. Feeding on a shared vertebrate may be an important
473 determinant: examination of tick internal organs revealed a high concentration of *Coxiella*-LE
474 within salivary glands in some species (Klyachko *et al.* 2007; Qiu *et al.* 2014). This tissue
475 tropism may allow the release of *Coxiella*-LE in the vertebrate during biting and then favor
476 HT between ticks feeding on a same vertebrate. In such cases, vertebrates may thus act as
477 ecological arenas for the global exchange of symbionts, serving as a possible intermediate
478 host for HT among tick species. Such HT events mediated by bacterial inoculums in
479 vertebrates may also favor the emergence of novel bacterial phenotypes. This is the most
480 parsimonious explanation for the origin of Q fever since *C. burnetii* likely evolved from a
481 *Coxiella*-LE ancestor that succeeded in infecting vertebrate cells (Duron *et al.* 2015a).
482 Phylogenetic data of *Francisella* and *Rickettsia* also suggest the occurrence of regular
483 transitions between pathogenic and endosymbiotic forms along their evolutionary history

484 (Perlman *et al.* 2006; Darby *et al.* 2007; Weinert *et al.* 2009; Sjodin *et al.* 2012). An obvious
485 question is then to what extent interactions within the symbiotic communities of ticks, and
486 more precisely the selection for HT-symbionts, may have facilitated the evolutionary
487 emergence of novel vertebrate pathogens.

488

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506

507 **References**

508 Almeida AP, Marcili A, Leite RC *et al.* (2012) *Coxiella* symbiont in the tick *Ornithodoros*
509 *rostratus* (Acari: Argasidae). *Ticks and Tick-borne Diseases*, **3**, 203-206.

510 Andreotti R, Perez de Leon AA, Dowd SE *et al.* (2011) Assessment of bacterial diversity in
511 the cattle tick *Rhipicephalus (Boophilus) microplus* through tag-encoded
512 pyrosequencing. *BMC Microbiology*, **11**, 6.

513 Anstead CA, Chilton NB (2014) Discovery of novel *Rickettsiella* spp. in ixodid ticks from
514 Western Canada. *Applied and Environmental Microbiology*, **80**, 1403-1410.

515 Balbuena JA, Miguez-Lozano R, Blasco-Costa I. (2013) PACo: a novel procrustes application
516 to cophylogenetic analysis. *PLoS One*, **8**, e61048.

517 Benson MJ, Gawronski JD, Eveleigh DE *et al.* (2004) Intracellular symbionts and other
518 bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet,
519 Cape Cod, Massachusetts. *Applied and Environmental Microbiology*, **70**, 616-620.

520 Bennett GM, Moran NA (2013) Small, smaller, smallest: the origins and evolution of ancient
521 dual symbioses in a Phloem-feeding insect. *Genome Biology and Evolution*, **5**, 1675-
522 1688. Bouchon D, Cordaux R, Grève P (2012) *Rickettsiella*, intracellular pathogens of
523 arthropods. In: *Manipulative Tenants* (eds. Zchori-Fein E & Bourtzis K), pp. 127-148.
524 CRC Press.

525 Brownlie JC, Johnson KN (2009) Symbiont-mediated protection in insect hosts. *Trends*
526 *Microbiology*, **17**, 348-354.

527 Burger TD, Shao R, Beati L *et al.* (2012) Phylogenetic analysis of ticks (Acari: Ixodida) using
528 mitochondrial genomes and nuclear rRNA genes indicates that the genus *Amblyomma*
529 is polyphyletic. *Molecular Phylogenetics and Evolution*, **65**, 45-55.

530 Burger TD, Shao RF, Labruna MB *et al.* (2014) Molecular phylogeny of soft ticks (Ixodida:
531 Argasidae) inferred from mitochondrial genome and nuclear rRNA sequences. *Ticks*
532 *and Tick-borne Diseases*, **5**, 195-207.

533 Cafiso A, Bazzocchi C, De Marco L, *et al.* (in press) Molecular screening for *Midichloria* in
534 hard and soft ticks reveals variable prevalence levels and bacterial loads in different
535 tick species. *Ticks and Tick-borne Diseases*.

536 Carpi G, Cagnacci F, Wittekindt, NE *et al.* (2011) Metagenomic profile of the bacterial
537 communities associated with *Ixodes ricinus* ticks. *PLoS One*, **6**, e25604.

538 Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in
539 phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540-552.

540 Chen X, Li S, Aksoy S (1999) Concordant evolution of a symbiont with its host insect
541 species: molecular phylogeny of genus *Glossina* and its bacteriome-associated
542 endosymbiont, *Wigglesworthia glossinidia*. *Journal of Molecular Evolution*, **48**, 49-
543 58.

544 Clay K, Klyachko O, Grindle N *et al.* (2008) Microbial communities and interactions in the
545 lone star tick, *Amblyomma americanum*. *Molecular Ecology*, **17**, 4371-4381.

546 Clayton KA, Gall CA, Mason, KL *et al.* (2015) The characterization and manipulation of the
547 bacterial microbiome of the Rocky Mountain wood tick, *Dermacentor andersoni*.
548 *Parasites & Vectors*, **8**, 632.

549 Conord C, Despres L, Vallier A *et al.* (2008) Long-term evolutionary stability of bacterial
550 endosymbiosis in Curculionoidea, additional evidence of symbiont replacement in the
551 Dryophthoridae family. *Molecular Biology and Evolution*, **25**, 859–868.

552 Cordaux, R., Paces-Fessy, M., Raimond, M., Michel-Salzat, A., Zimmer, M. & Bouchon, D.
553 (2007) Molecular characterization and evolution of arthropod-pathogenic *Rickettsiella*
554 bacteria. *Applied and Environmental Microbiology*, **73**, 5045-5047.

555 Darby AC, Cho NH, Fuxelius HH, *et al.* (2007) Intracellular pathogens go extreme: genome
556 evolution in the Rickettsiales. *Trends in Genetics*, **23**, 511-520.

557 Dergousoff SJ, Chilton NB (2010) Detection of a new *Arsenophonus*-type bacterium in
558 Canadian populations of the Rocky Mountain wood tick, *Dermacentor andersoni*.
559 *Experimental and Applied Acarology*, **52**, 85-91.

560 Dergousoff SJ, Chilton, NB (2011) Novel genotypes of *Anaplasma bovis*, "*Candidatus*
561 *Midichloria*" sp. and *Ignatzschineria* sp. in the Rocky Mountain wood tick,
562 *Dermacentor andersoni*. *Veterinary Microbiology*, **150**, 100-106.

563 Duron O, Bouchon D, Boutin S *et al.* (2008a) The diversity of reproductive parasites among
564 arthropods: *Wolbachia* do not walk alone. *BMC Biology*, **6**, 27.

565 Duron O, Cremaschi J, McCoy KD (2016) The high diversity and global distribution of the
566 intracellular bacterium *Rickettsiella* in the polar seabird tick *Ixodes uriae*. *Microbial*
567 *Ecology*, **71**, 761-770.

568 Duron O, Hurst GD, Hornett EA, *et al.* (2008b) High incidence of the maternally inherited
569 bacterium *Cardinium* in spiders. *Molecular Ecology*, **17**, 1427-1437.

570 Duron O, Jourdain E, McCoy KD (2014a) Diversity and global distribution of the *Coxiella*
571 intracellular bacterium in seabird ticks. *Ticks and Tick-borne Diseases*, **5**, 557-563.

572 Duron O, Noel V, McCoy KD *et al.* (2015a) The recent evolution of a maternally inherited
573 endosymbiont of ticks led to the emergence of the Q Fever pathogen, *Coxiella*
574 *burnetii*. *PLoS Pathogens*, **11**, e1004892.

575 Duron O, Schneppat UE, Berthomieu A *et al.* (2014b) Origin, acquisition and diversification
576 of heritable bacterial endosymbionts in louse flies and bat flies. *Molecular Ecology*,
577 **23**, 2105-2117.

578 Duron O, Sidi-Boumedine K, Rousset E *et al.* (2015b) The importance of ticks in Q fever
579 transmission: what has (and has not) been demonstrated? *Trends in Parasitology*, **31**,
580 536-552.

581 Duron O, Wilkes TE, Hurst GD (2010) Interspecific transmission of a male-killing bacterium
582 on an ecological timescale. *Ecology Letters*, **13**, 1139-1148.

583 Engelstadter J, Hurst GDD (2009) The ecology and evolution of microbes that manipulate
584 host reproduction. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 127-
585 149.

586 Epis S, Mandrioli M, Genchi M *et al.* (2014) Localization of the bacterial symbiont
587 *Candidatus* Midichloria mitochondrii within the hard tick *Ixodes ricinus* by whole-
588 mount FISH staining. *Ticks and Tick-borne Diseases*, **4**, 19-45.

589 Epis S, Sasser D, Beninati T *et al.* (2008) *Midichloria mitochondrii* is widespread in hard
590 ticks (Ixodidae) and resides in the mitochondria of phylogenetically diverse species.
591 *Parasitology*, **135**, 485-494.

592 Ferrari J, Vavre F (2011) Bacterial symbionts in insects or the story of communities affecting
593 communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
594 **366**, 1389-1400.

595 Ferrari J, West JA, Via S *et al.* (2012) Population genetic structure and secondary symbionts
596 in host-associated populations of the pea aphid complex. *Evolution*, **66**, 375-390.

597 Fukatsu T, Ishikawa H (1992) A novel eukaryotic extracellular symbiont in an aphid,
598 *Astegopteryx styraci* (Homoptera, Aphididae, Hormaphidinae). *Journal of Insect*
599 *Physiology*, **38**, 765-773.

600 Fukatsu T, Ishikawa H (1996) Phylogenetic position of yeast-like symbiont of *Hamiltonaphis*
601 *styraci* (Homoptera, Aphididae) based on 18S rDNA sequence. *Insect Biochemistry*
602 *and Molecular Biology*, **26**, 383-388.

603 Gerhart JG, Moses AS, Raghavan R (2016) A *Francisella*-like endosymbiont in the Gulf
604 Coast tick evolved from a mammalian pathogen. *Scientific Reports*, **6**, 33670.

605 Goethert HK, Telford SR (2005) A new *Francisella* (Beggiatiales: Francisellaceae) inquiline
606 within *Dermacentor variabilis* say (Acari: Ixodidae). *Journal of Medical Entomology*,
607 **42**, 502-505.

608 Gottlieb Y, Lalzar I, Klasson L (2015) Distinctive genome reduction rates revealed by
609 genomic analyses of two *Coxiella*-Like endosymbionts in ticks. *Genome Biology and*
610 *Evolution*, **7**, 1779-1796.

611 Guglielmono AA, Robbing RG, Apanaskevich DA *et al.* (2010) The Argasidae, Ixodidae and
612 Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa*,
613 **2528**, 1–28.

614 Gulia-Nuss M, Nuss AB, Meyer JM *et al.* (2016) Genomic insights into the *Ixodes scapularis*
615 tick vector of Lyme disease. *Nature Communications*, **7**,10507.

616 Hamilton PT, Perlman SJ (2013) Host defense via symbiosis in *Drosophila*. *PLoS Pathogens*,
617 **9**, e1003808.

618 Henning K, Greiner-Fischer S, Hotzel H *et al.* (2006) Isolation of Spiroplasma sp. from an
619 Ixodes tick. *International Journal of Medical Microbiology*, **296**, 157-161.

620 Hilgenboecker K, Hammerstein P, Schlattmann P *et al.* (2008) How many species are infected
621 with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiology Letters*,
622 **281**, 215-220.

623 Hunter DJ, Torkelson JL, Bodnar J *et al.* (2015) The *Rickettsia* endosymbiont of *Ixodes*
624 *pacificus* contains all the genes of de novo folate Biosynthesis. *Plos One*, **10**,
625 e0144552.

626 Husnik F, McCutcheon JP (2016) Repeated replacement of an intrabacterial symbiont in the
627 tripartite nested mealybug symbiosis. *Proc Natl Acad Sci USA*, **113**, E5416-24.

628 Kruh L, Weintraub PG, Mozes-Daube N *et al.* (2013) Novel *Rickettsiella* bacterium in

629 the leafhopper *Orosius albicinctus* (Hemiptera: Cicadellidae). *Applied and*
630 *Environmental Microbiology*, **79**, 4246-4252.

631 Jaenike J (2012) Population genetics of beneficial heritable symbionts. *Trends in Ecology and*
632 *Evolution*, **27**, 226-232.

633 Jaenike J, Unckless R, Cockburn SN *et al.* (2010) Adaptation via symbiosis: recent spread of
634 a *Drosophila* defensive symbiont. *Science*, **329**, 212-215.

635 Jasinskas A, Zhong J, Barbour AG (2007) Highly prevalent *Coxiella* sp. bacterium in the tick
636 vector *Amblyomma americanum*. *Applied and Environmental Microbiology*, **73**, 334-
637 336.

638 Jouselin E, Coeur d'Acier A, Vanlerberghe-Masutti F *et al.* (2013) Evolution and diversity of
639 *Arsenophonus* endosymbionts in aphids. *Molecular Ecology*, **22**, 260-270.

640 Jouselin E, Desdevises Y, Coeur d'acier A (2009) Fine-scale cospeciation between
641 *Brachycaudus* and *Buchnera aphidicola*: bacterial genome helps define species and
642 evolutionary relationships in aphids. *Proceedings of the Royal Society of London,*
643 *Series B*, **276**, 187-196.

644 Klyachko O, Stein BD, Grindle N *et al.* (2007) Localization and visualization of a *Coxiella*-
645 type symbiont within the lone star tick, *Amblyomma americanum*. *Applied and*
646 *Environmental Microbiology*, **73**, 6584-6594.

647 Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary
648 Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150-163.

649 Kurtti TJ, Felsheim RF, Burkhardt NY *et al.* (2015) *Rickettsia buchneri* sp. nov., a rickettsial
650 endosymbiont of the blacklegged tick *Ixodes scapularis*. *International Journal of*
651 *Systematic and Evolutionary Microbiology*, **65**, 965-970.

652 Kurtti TJ, Munderloh UG, Andreadis TG *et al.* (1996) Tick cell culture isolation of an
653 intracellular prokaryote from the tick *Ixodes scapularis*. *Journal of Invertebrate*
654 *Pathology*, **67**, 318-321.

655 Kurtti TJ, Palmer AT, Oliver JH (2002) *Rickettsiella*-like bacteria in *Ixodes woodi* (Acari:
656 Ixodidae). *Journal of Medical Entomology*, **39**, 534-540.

657 Lalzar I, Harrus S, Mumcuoglu KY *et al.* (2012) Composition and seasonal variation of
658 *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities.
659 *Applied and Environmental Microbiology*, **78**, 4110–4116.

660 Lalzar I, Friedmann Y, Gottlieb Y (2014) Tissue tropism and vertical transmission of *Coxiella*
661 in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. *Environmental*
662 *Microbiology*, **16**, 3657-3668.

663 Leclerque A, Hartelt K, Schuster C *et al.* (2011) Multilocus sequence typing (MLST) for the
664 infra-generic taxonomic classification of entomopathogenic *Rickettsiella* bacteria.
665 *FEMS Microbiology Letters*, **324**, 125-134.

666 Lo N, Beninati T, Sasser D *et al.* (2006) Widespread distribution and high prevalence of an
667 alpha-proteobacterial symbiont in the tick *Ixodes ricinus*. *Environmental*
668 *Microbiology*, **8**, 1280-1287.

669 Lukasik P, Dawid MA, Ferrari J *et al.* (2013a) The diversity and fitness effects of infection
670 with facultative endosymbionts in the grain aphid, *Sitobion avenae*. *Oecologia*, **173**,
671 985-996.

672 Lukasik P, van Asch M, Guo H *et al.* (2013b) Unrelated facultative endosymbionts protect
673 aphids against a fungal pathogen. *Ecology Letters*, **16**, 214-218.

674 Machado-Ferreira E, Dietrich G, Hojgaard A *et al.* (2011) *Coxiella* symbionts in the Cayenne
675 tick *Amblyomma cajennense*. *Microbial Ecology*, **62**, 134-142.

676 Martin D, Rybicki E (2000) RDP: detection of recombination amongst aligned sequences.
677 *Bioinformatics*. **16**, 562–563.

678 Martin D, Lemey P, Lott M, Moulton V, Posada D, Lefevvre P (2010) RDP3: a flexible and
679 fast computer program for analyzing recombination. *Bioinformatics*, **26**, 2462–2463.

680 Matsuura Y, Kikuchi Y, Meng XY *et al.* (2012) Novel clade of alphaproteobacterial
681 endosymbionts associated with stinkbugs and other arthropods. *Applied and*
682 *Environmental Microbiology*, **78**, 4149-4156.

683 McNulty SN, Foster JM, Mitreva M *et al.* (2010) Endosymbiont DNA in endobacteria-free
684 filarial nematodes indicates ancient horizontal genetic transfer. *PLoS One*, **5**, e11029.

685 Moran NA, Tran P, Gerardo NM (2005) Symbiosis and insect diversification, an ancient
686 symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Applied and*
687 *Environmental Microbiology*, **71**, 8802–8810.

688 Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and evolution of heritable
689 bacterial symbionts. *Annual Review of Genetics*, **42**, 165-190.

690 Moran NA, Tran P, Gerardo NM (2005b) Symbiosis and insect diversification: an ancient
691 symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Applied and*
692 *Environmental Microbiology*, **71**, 8802–8810.

693 Murrell A, Campbell NJ, Barker SC (2001) A total-evidence phylogeny of ticks provides
694 insights into the evolution of life cycles and biogeography. *Molecular Phylogenetics*
695 *and Evolution*, **21**, 244-258.

696 Najm NA, Silaghi C, Bell-Sakyi, L (2012) Detection of bacteria related to *Candidatus*
697 *Midichloria mitochondrii* in tick cell lines. *Parasitology Research*, **110**, 437-442.

698 Niebylski ML, Peacock MG, Fischer ER *et al.* (1997a) Characterization of an endosymbiont
699 infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*.
700 *Applied and Environmental Microbiology*, **63**, 3933-3940.

701 Niebylski ML, Schrumpf ME, Burgdorfer W *et al.* (1997b) *Rickettsia peacockii* sp. nov., a
702 new species infecting wood ticks, *Dermacentor andersoni*, in western Montana.
703 *International Journal of Systematic Bacteriology*, **47**, 446-452.

704 Nikoh N, Hosokawa T, Moriyama M *et al.* (2014) Evolutionary origin of insect-*Wolbachia*
705 nutritional mutualism. *Proceedings of the National Academy of Sciences of the United*
706 *States of America*, **111**, 10257-10262.

707 Novakova E, Hypsa V, Moran NA (2009) *Arsenophonus*, an emerging clade of intracellular
708 symbionts with a broad host distribution. *BMC Microbiology*, **9**, 143.

709 Oksanen JF, Blanchet G, Kindt R *et al.* (2013) Vegan: Community Ecology Package. R
710 *package version 2.0-9*.

711 Oliver KM, Degnan PH, Burke GR *et al.* (2010) Facultative symbionts in aphids and the
712 horizontal transfer of ecologically important traits. *Annual Review of Entomology*, **55**,
713 247-266.

714 Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R
715 language. *Bioinformatics*, **20**, 289-290.

716 Pérez-Brocá V, Gil R, Ramos S, Lamelas A, Postigo M *et al.* (2006) A small microbial
717 genome: the end of a long symbiotic relationship? *Science*, **314**, 312–13

718 Perlman SJ, Hunter MS, Zchori-Fein E (2006) The emerging diversity of *Rickettsia*.
719 *Proceedings of the Royal Society of London, Series B*, **273**, 2097-2106.

720 Plantard O, Bouju-Albert A, Malard MA, *et al.* (2012) Detection of *Wolbachia* in the tick
721 *Ixodes ricinus* is due to the presence of the hymenoptera endoparasitoid *Ixodiphagus*
722 *hookeri*. *PLoS One*, **7**, e30692.

723 Qiu Y, Nakao R, Ohnuma A *et al.* (2014) Microbial population analysis of the salivary glands
724 of ticks; a possible strategy for the surveillance of bacterial pathogens. *PLoS One*, **9**,
725 e103961.

726 Raymond M, Rousset F (1995a) An exact test for population differentiation. *Evolution*, **49**,
727 1283-1286.

728 Raymond M, Rousset F (1995b) Genepop (version 1.2), a population genetics software for
729 exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.

730 Russell JA, Funaro CF, Giraldo YM *et al.* (2012) A veritable menagerie of heritable bacteria
731 from ants, butterflies, and beyond: broad molecular surveys and a systematic review.
732 *PLoS One*, **7**, e51027.

733 Russell JA, Goldman-Huertas B, Moreau CS *et al.* (2009) Specialization and geographic
734 isolation among *Wolbachia* symbionts from ants and lycaenid butterflies. *Evolution*,
735 **63**, 624-640.

736 Russell JA, Weldon S, Smith AH, *et al.* (2013) Uncovering symbiont-driven genetic diversity
737 across North American pea aphids. *Molecular Ecology*, **22**, 2045-2059.

738 Sassera D, Lo N, Epis S, D'Auria G *et al.* (2011) Phylogenomic evidence for the presence of a
739 flagellum and *cbb(3)* oxidase in the free-living mitochondrial ancestor. *Molecular*
740 *Biology and Evolution*, **28**, 3285-3296.

741 Sawyer SA (1999) GENECONV: A computer package for the statistical detection of gene
742 conversion. <http://www.math.wustl.edu/~sawy>.

743 Scoles GA (2004) Phylogenetic analysis of the *Francisella*-like endosymbionts of
744 *Dermacentor* Ticks. *Journal of Medical Entomology*, **41**, 277-286.

745 Sjodin A, Svensson K, Ohrman C *et al.* (2012) Genome characterization of the genus
746 *Francisella* reveals insight into similar evolutionary paths in pathogens of mammals
747 and fish. *BMC Genomics*, **13**, 268.

748 Smith AH, Lukasik P, O'Connor MP *et al.* (2015a) Patterns, causes and consequences of
749 defensive microbiome dynamics across multiple scales. *Molecular Ecology*, **24**, 1135-
750 1149.

751 Smith TA, Driscoll T, Raghavan R (2015b) A *Coxiella*-like endosymbiont is a potential
752 vitamin source for the Lone Star Tick. *Genome Biology and Evolution*, **7**, 831-838.

753 Stefanini A, Duron O (2012) Exploring the effect of the *Cardinium* endosymbiont on spiders.
754 *Journal of Evolutionary Biology*, **25**, 1521-1530.

755 Subramanian G, Sekeyova Z, Raoult D *et al.* (2012) Multiple tick-associated bacteria in
756 *Ixodes ricinus* from Slovakia. *Ticks and Tick-borne Diseases*, **3**, 406-410.

757 Takiya DM, Tran PL, Dietrich CH *et al.* (2006) Co-cladogenesis spanning three phyla:
758 leafhoppers (Insecta: Hemiptera: Cicadellidae) and their dual bacterial symbionts.
759 *Molecular Ecology*, **15**, 4175-4191.

760 Thompson JD, Gibson TJ, Higgins DG (2002) Multiple sequence alignment using ClustalW
761 and ClustalX. *Curr. Protoc. Bioinformatics*, Chapter 2, Unit 2.3.

762 Toju H, Tanabe AS, Notsu Y, Sota T, Fukatsu T (2013) Diversification of endosymbiosis,
763 replacements, co-speciation and promiscuity of bacteriocyte symbionts in weevils.
764 *ISME Journal*, **7**, 1378–1390.

765 Tsuchida T, Koga R, Fujiwara A *et al.* (2014) Phenotypic effect of "*Candidatus* Rickettsiella
766 viridis," a facultative symbiont of the pea aphid (*Acyrtosiphon pisum*), and its
767 interaction with a coexisting symbiont. *Applied and Environmental Microbiology*, **80**,
768 525-533.

769 Tsuchida T, Koga R, Horikawa M *et al.* (2010) Symbiotic bacterium modifies aphid body
770 color. *Science*, **330**, 1102-1104.

771 Tully JG, Rose DL, Yunker CE *et al.* (1995) *Spiroplasma ixodetis* sp. nov., a new species
772 from *Ixodes pacificus* ticks collected in Oregon. *International Journal of Systematic*
773 *Bacteriology*, **45**, 23 -28.

774 Tully JG, Rose DL, Yunker CE *et al.* (1981) Helical mycoplasmas (spiroplasmas) from
775 *Ixodes ticks*. *Science*, **212**, 1043-1045.

776 Vautrin E, Vavre F (2009) Interactions between vertically transmitted symbionts: cooperation
777 or conflict? *Trends in Microbiology*, **17**, 95-99.

778 Venzal JM, Estrada-Pena A, Portillo A *et al.* (2008) Detection of Alpha and Gamma-
779 Proteobacteria in *Amblyomma triste* (Acari: Ixodidae) from Uruguay. *Experimental*
780 *and Applied Acarology*, **44**, 49-56.

781 Vilcins IM, Old JM, Deane E (2009) Molecular detection of *Rickettsia*, *Coxiella* and
782 *Rickettsiella* DNA in three native Australian tick species. *Experimental and Applied*
783 *Acarology*, **49**, 229-242.

784 Weinert LA, Tinsley MC, Temperley M *et al.* (2007) Are we underestimating the diversity
785 and incidence of insect bacterial symbionts? A case study in ladybird beetles. *Biology*
786 *Letters*, **3**, 678-681.

787 Weinert LA, Werren JH, Aebi A *et al.* (2009) Evolution and diversity of *Rickettsia* bacteria.
788 *BMC Biology*, **7**, 6.

789 Wernegreen JJ (2012) Endosymbiosis. *Current Biology*, **22**, R555-561.

790 Werren JH, Skinner SW, Huger AM (1986) Male-killing bacteria in a parasitic wasp. *Science*,
791 **231**, 990-992.

792 Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate
793 biology. *Nature Review Microbiology*, **6**, 741-751.

794 Williams-Newkirk AJ, Rowe LA, Mixson-Hayden TR *et al.* (2012) Presence, genetic
795 variability, and potential significance of "*Candidatus* Midichloria mitochondrii" in the
796 lone star tick *Amblyomma americanum*. *Experimental and Applied Acarology*, **58**,
797 291-300.

798 Zchori-Fein E, Perlman SJ (2004) Distribution of the bacterial symbiont *Cardinium* in
799 arthropods. *Molecular Ecology*, **13**, 2009-2016.

800 Zhong J, Jasinskas A, Barbour AG (2007) Antibiotic treatment of the tick vector *Amblyomma*
801 *americanum* reduced reproductive fitness. *PLoS One*, **2**, e405.

802 Zug R, Hammerstein, P (2012) Still a host of hosts for *Wolbachia*: analysis of recent data
803 suggests that 40% of terrestrial arthropod species are infected. *PLoS One*, **7**, e38544.

804

805 **Data accessibility statement:** Novel nucleotide sequences have been deposited in the GenBank
806 nucleotide database (accession numbers pending). All other data are presented in the
807 supplementary materials.

808

809 **Author contributions:** OD, FB, VN, CA and JC performed molecular and phylogenetic
810 analyses, OD, FB and JC performed statistical analyses, KDMcC, OP, JG, AAPdL, DJAH,
811 ARVO, YG, GB, AAG, AE-P, MNO, LZ, FV and CC performed field missions, tick
812 sampling, morphological identifications and preliminary analyses. OD wrote the first draft of
813 the manuscript, and all authors contributed substantially to revisions.

814 **Table 1.** List of the ten maternally inherited bacteria found in ticks and illustrative (non-exhaustive) references.

Maternally inherited bacteria	Distribution in arthropods	Major properties
GAMMA-PROTEOBACTERIA		
1 - <i>Coxiella</i> -LE	Very common in ticks, not found in other arthropods (Jasinskas <i>et al.</i> 2007; Clay <i>et al.</i> 2008; Machado-Ferreira <i>et al.</i> 2011; Almeida <i>et al.</i> 2012; Lalar <i>et al.</i> 2012; Duron <i>et al.</i> 2014a; Carpi <i>et al.</i> 2011; Duron <i>et al.</i> 2015a)	Obligate symbiont in most tick species (Zhong <i>et al.</i> 2007; Gottlieb <i>et al.</i> 2015; Smith <i>et al.</i> 2015b). Closely related to the agent of Q fever, <i>C. burnetii</i> (Duron <i>et al.</i> 2015a)
2 - <i>Rickettsiella</i>	Scattered distribution in arthropods (Tsuchida <i>et al.</i> 2010; Bouchon <i>et al.</i> 2012; Iasur-Kruh <i>et al.</i> 2013), common in ticks (Kurtti <i>et al.</i> 2002; Vilcins <i>et al.</i> 2009; Anstead & Chilton 2014; Duron <i>et al.</i> 2015a; Duron <i>et al.</i> 2016)	Unknown effect in ticks. Facultative mutualist in aphids (Tsuchida <i>et al.</i> 2010; Lukasik <i>et al.</i> 2013a; Tsuchida <i>et al.</i> 2014) and likely in other insects (Iasur-Kruh <i>et al.</i> 2013). Some strains are entomopathogenic (Cordaux <i>et al.</i> 2007; Leclerque <i>et al.</i> 2011)
3 - <i>Arsenophonus</i>	Common in arthropods (Duron <i>et al.</i> 2008a; Novakova <i>et al.</i> 2009), present in ticks (Clay <i>et al.</i> 2008; Dergousoff & Chilton 2010; Clayton <i>et al.</i> 2015)	Male-killer in parasitoid wasps (Werren <i>et al.</i> 1986; Duron <i>et al.</i> 2010), putative obligate symbionts in bat flies and louse flies (Duron <i>et al.</i> 2014b), facultative symbionts in other insects (Novakova <i>et al.</i> 2009; Jousselein <i>et al.</i> 2013)
4 - <i>Francisella</i> -LE	Rare in ticks, not found in other arthropods (Niebylski <i>et al.</i> 1997a; Scoles 2004; Goethert & Telford 2005; Clayton <i>et al.</i> 2015; Gerhart <i>et al.</i> 2016)	Unknown effect in most cases but alternative obligate symbiont in at least one tick species (Gerhart <i>et al.</i> 2016) ; closely related to the agent of tularaemia (<i>F. tularensis</i>) (Sjodin <i>et al.</i> 2012)
ALPHA-PROTEOBACTERIA		
5 - <i>Wolbachia</i>	Very common in arthropods (Duron <i>et al.</i> 2008a; Hilgenboecker <i>et al.</i> 2008; Zug & Hammerstein 2012), present in ticks (Andreotti <i>et al.</i> 2011; Carpi <i>et al.</i> 2011; Subramanian <i>et al.</i> 2012)	Unknown effect in ticks. Reproductive manipulation in many arthropods (Engelstadter & Hurst 2009), facultative mutualist (defensive symbiosis) in others as mosquitoes (Brownlie & Johnson 2009; Hamilton & Perlman 2013), obligate symbiont in bed bugs (Nikoh <i>et al.</i> 2014). At least in the case of the sheep tick, <i>Ixodes ricinus</i> , it has been demonstrated that the detection of <i>Wolbachia</i> was due to a contamination by a hymenopteran parasitoid (Plantard <i>et al.</i> 2012).
6 - <i>Rickettsia</i>	Common in arthropods (Perlman <i>et al.</i> 2006; Weinert <i>et al.</i> 2009), present in ticks (Niebylski <i>et al.</i> 1997b; Clayton <i>et al.</i> 2015; Kurtti <i>et al.</i> 2015)	Unknown effect in ticks. Reproductive manipulator in diverse insect species (Engelstadter & Hurst 2009) and defensive symbiont in other insects (Lukasik <i>et al.</i> 2013b); closely related to pathogenic strains, often tick-borne, infecting vertebrates (Perlman <i>et al.</i> 2006; Weinert <i>et al.</i> 2009; Kurtti <i>et al.</i> 2015)
7 - <i>Midichloria</i>	Present in ticks, not found in other arthropods (Lo <i>et al.</i> 2006; Epis <i>et al.</i> 2008; Venzal <i>et al.</i> 2008; Dergousoff & Chilton 2011; Najm <i>et al.</i> 2012; Subramanian <i>et al.</i> 2012; Williams-Newkirk <i>et al.</i> 2012; Qiu <i>et al.</i> 2014; Cafiso <i>et al.</i> in press)	Unknown effect; inhabit tick mitochondria (Epis <i>et al.</i> 2014)
8 - <i>Lariskella</i>	Rare and with a scattered distribution in arthropods (Matsuura <i>et al.</i> 2012; Toju <i>et al.</i> 2013), reported once in ticks (Qiu <i>et al.</i> 2014)	Unknown effect
MOLLICUTES		
9 - <i>Spiroplasma</i>	Common in arthropods (Weinert <i>et al.</i> 2007; Duron <i>et al.</i> 2008a), present in ticks (Tully <i>et al.</i> 1981; Tully <i>et al.</i> 1995; Henning <i>et al.</i> 2006)	Unknown effect in ticks. Male-killer in diverse insect species (Engelstadter & Hurst 2009)
BACTEROIDETES		
10 - <i>Cardinium</i>	Common in arthropods (Zchori-Fein & Perlman 2004; Duron <i>et al.</i> 2008a; Duron <i>et al.</i> 2008b), present in ticks (Kurtti <i>et al.</i> 1996; Benson <i>et al.</i> 2004)	Unknown effect in ticks. Reproductive manipulator in diverse insect species (Engelstadter & Hurst 2009)

815 **Figure legends**

816 **Figure 1.** Distribution of heritable bacterial symbionts in ticks. The left part of the figure shows
817 a simplified phylogeny of tick genera adapted from Burger *et al.* (2012; 2014) and lists the 81
818 tick species under investigation. n, number of individuals examined in each tick species. The
819 top part of the figure shows a simplified bacterial phylogeny of the ten bacterial symbionts
820 examined in this study. Boxes representing presence of infections are colored differently
821 depending on symbionts. The right part of the figure shows the number of symbiont genera
822 observed in each tick species. The triangles indicate the tick species for which five
823 populations were examined. The geographical origin of tick populations and infection
824 prevalence are detailed in Supplementary Table 1.

825

826 **Figure 2.** Variations of individual infection status between populations of six tick species. The
827 left part of the figure shows a simplified tick phylogeny based from Burger *et al.* (2012;
828 2014). Each graph shows the frequency of bacterial infection types in one tick population.
829 The geographical origin of the tick populations and the number of individuals tested are
830 indicated at the top of the graphs. Blue: *Coxiella*-LE (CO), green: *Rickettsiella* (RL), maroon,
831 *Francisella* (F), red: *Midichloria* (M), orange: *Rickettsia* (RS), black: *Wolbachia* (W), violet:
832 *Spiroplasma*, grey: *Cardinium* (CA). The 2 other bacteria, *i.e.*, *Lariskella* (L) and
833 *Arsenophonus* (A), were not detected in these tick populations.

834

835 **Figure 3.** Correspondence analysis (CA) performed on the symbiotic communities of 30
836 populations of six tick species (five populations per species). Each community is represented
837 by a dot. (A) Projection of infection status on a factorial map. The first axis of the CA
838 discriminates populations infected by *Coxiella*-LE (on the left) from those infected by
839 *Rickettsia* (on the right); the second axis discriminates populations infected by *Rickettsiella*
840 (on top) from those infected by either *Spiroplasma* or *Midichloria*. (B) Populations grouped

841 according to the six tick species (six groups are delimited by the 95% confidence ellipses
842 drawn around the barycenter of each species).

843

844 **Figure 4.** Cladogram depicting the majority-rule consensus (60%) of *Coxiella* phylogenetic
845 trees constructed using maximum-likelihood (ML) estimations based on *rpoB* sequences (491
846 unambiguously aligned bp) from *Coxiella*-LE strains of ticks, the agent of Q fever (*C.*
847 *burnetii*) and outgroups (*Legionella pneumophila* and *Rickettsiella grylli*). Two *rpoB* *Coxiella*
848 sequences per infected tick species are shown in most cases. White circles, *Coxiella*-LE of
849 soft ticks; black circles, *Coxiella*-LE of hard ticks; black triangles, *C. burnetii*. Branch
850 numbers indicate percentage bootstrap support (1,000 replicates).

851

852 **Figure 5.** Congruence between *Rhipicephalus* tick species and their *Coxiella*-LE symbionts.
853 (A) Tick mtDNA cladogram depicting the majority-rule consensus (50%) of phylogenetic
854 trees constructed using maximum-likelihood (ML) estimations based on concatenated 12S
855 *rRNA*, 16S *rRNA* and *COI* gene sequences (1,465 unambiguously aligned bp); (B) *Coxiella*-
856 LE cladogram depicting the majority-rule consensus (50%) of phylogenetic trees constructed
857 using ML estimations based on concatenated *rpoB*, *GroEL* and *dnaK* gene sequences (1,252
858 unambiguously aligned bp). Branch numbers indicate percentage bootstrap support for major
859 branches (1,000 replicates; only values >70% are shown).