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Abstract

Ecological specialization to restricted diet niches is driven by obligate, and often maternally inherited, symbionts in many arthropod lineages. These heritable symbionts typically form evolutionarily stable associations with arthropods that can last for millions of years. Ticks were recently found to harbour such an obligate symbiont, *Coxiella*-LE, that synthesizes B vitamins and cofactors not obtained in sufficient quantities from blood diet. In this study, the examination of 81 tick species shows that some *Coxiella*-LE symbioses are evolutionarily stable with an ancient acquisition followed by codiversification as observed in ticks belonging to the *Rhipicephalus* genus. However, many other *Coxiella*-LE symbioses are characterized by low evolutionary stability with frequent host shifts and extinction events. Further examination revealed the presence of nine other genera of maternally inherited bacteria in ticks. Although these nine symbionts were primarily thought to be facultative, their distribution among tick species rather suggests that at least four may have independently replaced *Coxiella*-LE and likely represent alternative obligate symbionts. Phylogenetic evidence otherwise indicates that cocladogenesis is globally rare in these symbioses as most originate via horizontal transfer of an existing symbiont between unrelated tick species. As a result, the structure of these symbiont communities is not fixed and stable across the tick phylogeny. Most importantly, the symbiont communities commonly reach high levels of diversity with up to six unrelated maternally inherited bacteria coexisting within host species. We further conjecture that interactions among coexisting symbionts are pivotal drivers of community structure both among and within tick species.

Keywords: co-evolution, heritable symbiont communities, maternally inherited bacteria, symbiosis, tick

Introduction

Symbiosis with microorganisms is an important driver of evolutionary novelty in eukaryotes (Moran *et al.* 2008; Wernegreen 2012). Arthropods frequently engage in associations with bacterial endosymbionts that live exclusively within host cells and undergo maternal (transovarial) transmission to offspring (Moran *et al.* 2008; Wernegreen 2012). In some cases, these maternally inherited symbionts have evolved towards obligate mutualists and determine ecologically important traits: obligate symbionts enable arthropod specialization on unbalanced dietary resources, such as plant sap or vertebrate blood, by providing biosynthetic pathways absent from their hosts (Moran *et al.* 2008; Wernegreen 2012). However, in most cases, they are facultative symbionts manipulating reproduction, protecting against natural enemies or facilitating adaptation to changing environments (Moran *et al.* 2008; Engelstadter & Hurst 2009; Oliver *et al.* 2010). As hosts can vary in the number and types of maternally inherited symbionts they harbour, heritable and functionally important phenotypic variation can exist within arthropod populations (Ferrari & Vavre 2011; Jaenike 2012).

Obligate symbionts are, by definition, present in most individuals of a given host species: their mutualistic relationship is obligate for the survival of both organisms. Obligate symbionts typically form evolutionary stable associations that last for millions of years (Moran *et al.* 2008) and that exhibit strict cocladogenesis, resulting in congruent host–symbiont phylogenies as observed in aphids, leafhoppers or tsetse flies (Chen *et al.* 1999; Moran *et al.* 2005b; Takiya *et al.* 2006; Jousselin *et al.* 2009). Facultative symbionts, in contrast, exhibit variable infection frequencies across temporal and spatial gradients (Jaenike *et al.* 2010; Ferrari *et al.* 2012; Stefanini & Duron 2012; Russell *et al.* 2013; Smith *et al.* 2015a). They can also undergo occasional horizontal transfers (HT) across arthropod species, resulting in limited phylogenetic congruence between hosts and symbionts (Russell *et al.* 2009, 2012; Duron *et al.* 2010; Jousselin *et al.* 2013). By combining maternal inheritance with HT, unrelated maternally inherited bacteria can coinfect a single individual host, thereby forming an endosymbiotic community (Vautrin & Vavre 2009; Ferrari & Vavre 2011). Coexistence of symbionts within these communities is expected to involve complex interactions, which can range from cooperation to competition and can, in turn, determine aggregation and exclusion patterns (Moran *et al.* 2008; Vautrin & Vavre 2009; Ferrari & Vavre 2011).

Among arthropods, ticks (Arachnida: Ixodidea) form a diversified group of *ca.* 900 species, almost all depending on vertebrate blood as their sole food source (Guglielmone *et al.* 2010). Ticks are well known as vectors of a wide diversity of infectious diseases, but they recently were shown to harbour high numbers of endosymbionts: at least 10 distinct genera of maternally inherited bacteria have been reported in ticks over the last decade (listed in Table 1). Among them, a *Coxiella*-like endosymbiont (*Coxiella*-LE hereafter) has been recently identified as an obligate symbiont required for tick survival and reproduction (Zhong *et al.* 2007). An examination of *Coxiella*-LE intrahost localization revealed a pronounced tissue tropism: this endosymbiont typically infects the ovaries (to ensure maternal transmission) and the distal part of Malpighian tubules, suggesting a possible role in nutrition, osmoregulation or excretion (Klyachko *et al.* 2007; Machado-Ferreira *et al.* 2011; Lalzar *et al.* 2014). Its genome was further shown to encode pathways for the synthesis of amino acids and vitamins (*i.e.* major B vitamins and cofactors) that fit closely with the expected nutritional complements required for strict haematophagy (Gottlieb *et al.* 2015; Smith *et al.* 2015b). The discovery of *Coxiella*-LE in numerous tick groups (Jasinskas *et al.* 2007; Clay *et al.* 2008; Machado-Ferreira *et al.* 2011; Almeida *et al.* 2012; Lalzar *et al.* 2012; Duron *et al.* 2014a, 2015a) corroborates the hypothesis of an obligate symbiont. However, around one-third of examined tick species have been found to lack *Coxiella*-LE or to harbour *Coxiella*-LE at much lower frequencies than expected for an obligate endosymbiont (Duron *et al.* 2014a, 2015a). Most importantly, the species missing *Coxiella*-LE are scattered among major tick families and genera, a pattern suggesting the repeated loss of *Coxiella*-LE infections during tick evolution (Duron *et al.* 2015a). Another surprising result was that closely related *Coxiella*-LE infect distantly related tick species suggesting recurrent HT of this obligate symbiont (Duron *et al.* 2015a). Interestingly, in the few cases where it has been shown that an obligate endosymbiont has been lost in an insect host, the ancestral endosymbiont has always been replaced by another, more recently acquired, species from a different host species via HT (Fukatsu & Ishikawa 1992, 1996; Moran *et al.* 2005; Conord *et al.* 2008; Toju *et al.* 2013; Husnik & McCutcheon 2016).

Little is known about the incidence and distribution of the nine other maternally inherited bacteria reported in ticks: they are usually thought to be facultative symbionts, but their effects on tick biology remain largely unstudied (Table 1). Two genera are only found in ticks: *Midichloria*, which inhabits the mitochondria of some tick species, and a *Francisella*-like endosymbiont (*Francisella*-LE), which has only been reported in a few tick species (Table 1). Interestingly, the recent sequencing of a *Francisella*-LE strain suggests that this symbiont has recently replaced a *Coxiella*-LE in at least one tick

Table 1 List of the ten maternally inherited bacteria found in ticks and illustrative (nonexhaustive) references

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

species (Gerhart *et al.* 2016). The seven remaining endosymbiont genera are more or less frequently found in other arthropod groups, including well-studied insects. Five endosymbionts (*Wolbachia*, *Cardinium*, *Arsenophonus*, *Spiroplasma* and *Rickettsia*) are common in some arthropod groups and known to manipulate insect reproduction through the induction of parthenogenesis, feminization, male killing and cytoplasmic incompatibility (Table 1). Several strains of *Wolbachia*, *Rickettsia* and *Spiroplasma* are also defensive symbionts, protecting their insect hosts against infections by pathogens or against pathogen-induced mortality (Table 1). Two other endosymbionts, *Rickettsiella* and *Lariskella*, are reported from only a couple of other arthropod taxa in addition to ticks, and their effects are unknown in most cases (Table 1).

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

Methods

Tick collection

Specimens belonging to the two major families of ticks, Ixodidae (hard ticks) and Argasidae (soft ticks), were collected from a variety of field sites around the world and from laboratory colonies (Table S1, Supporting information). One population per tick species was generally analysed, except for six focal species for which five distinct populations were collected. From 1 to 40 individuals per population were analysed individually. Samples were either directly used for molecular analyses or preserved in 70–90% ethanol until use.

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 by 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 Table S2, Supporting information for procedures). DNA template quality of symbiont-negative specimens was systematically verified by PCR amplification of the eukaryotic 18S *rRNA* gene using universal primers (Table S2, Supporting information); 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 artefact 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 multilocus sequences for the *Coxiella* intrageneric phylogeny and to test for codivergence with tick hosts (Table S2, Supporting information). To this end, we used a subsample of tick specimens to obtain (i) additional *Coxiella* sequences (including the *rpoB*, *GroEL* and *dnaK* genes), and (ii) tick mitochondrial DNA (mtDNA) sequences (including the 12S *rRNA*, the 16S *rRNA* and the *C01* genes). All PCR products were processed as described above.

Phylogenetic and statistical analyses

The GBLOCKS program (Castresana 2000) with default parameters was used to remove poorly aligned positions and to obtain nonambiguous sequence alignments. Phylogenetic analyses were based on sequence alignments performed with single or concatenated sequences from bacteria or tick mitochondria. Concatenated sequence alignments were checked for putative recombinant regions using the GENECONV and RDP algorithms available in RDP3 package (Martin *et al.* 2010). Closely

related organisms obtained from GenBank were also included in the analyses. The evolutionary models that best fit the sequence data were determined using the Akaike information criterion with the program MEGA (Kumar *et al.* 2004). Sequence data and best-fitting evolutionary models are detailed in Table S3 (Supporting information). Tree-based phylogenetic analyses were performed using maximum-likelihood (ML) analyses. ML heuristic searches using a starting tree obtained by neighbour joining was conducted in MEGA (Kumar *et al.* 2004). Clade robustness was assessed by bootstrap analysis using 1000 replicates. To test for associations between *Coxiella*-LE and tick multilocus data sets, we used the Procrustean Approach to Cophylogeny (PACo) program (Balbuena *et al.* 2013) in R (http://www.r-project.org) using the APE (Paradis *et al.* 2004) and VEGAN (Oksanen *et al.* 2013) packages. The significance of cophylogenetic tests was established by 10 000 random permutations of the association matrix.

We tested for differences in bacterial diversity among tick populations of a given species using a Fisher's exact test (Raymond & Rousset 1995a) as implemented in the GENEPOP program (Raymond & Rousset 1995b). For each population, occurrence and co-occurrence of different symbionts infecting ticks at the individual level were visualized using the MONDRIAN package (https://cran.r-project.org/web/packages/Mondrian) in R. We further investigated potential differences among tick species in the composition of their symbiont communities using correspondence analyses (CA). These computations were carried out using the FACTOMINER package (http://factominer.free.fr) in R. We constructed a table in which rows represent different populations and columns correspond to the prevalence of symbionts (one column per symbiont) to perform the CA analysis, which allowed visualizing the among-population variation on a factorial map.

Deviations from random associations among tick species of any two bacterial genera were tested using Fisher's exact test implemented in the GENEPOP program (Raymond & Rousset 1995b). This analysis was completed at the individual tick levels to test whether the aggregation or exclusion patterns among the B1 and B2 bacterial genera persist when taking into account other cocirculating bacteria. For each bacterial genus X detected in at least five individual ticks, we defined the categorical variable Bx with a value 0 or 1 to describe its presence or absence within each of the screened individual ticks. Using the glm function in R (https://stat.ethz.ch/R-manual/R-patched/library/stats/html/glm.html), we built a maximal model to explain the variations in B1 prevalence as a linear function integrating all the additive and interactive effects among the factors Bx≠1. Model simplification was classically achieved by removing the terms without signification effect ($P > 0.05$) on the variations in B1 prevalence. We finally investigated whether the factor B2 remained in the minimal adequate model fitting the variation in B1 prevalence.

Results

Distribution of maternally inherited bacteria in ticks

We assayed for the presence of 10 maternally inherited bacterial genera in 861 individual ticks from 81 species belonging to the two major tick families, Argasidae (soft ticks: three genera, 26 species) and Ixodidae (hard ticks: six genera, 55 species) (Fig. 1 and Table S1, Supporting information). Of the 861 specimens, 706 (82%) were PCR-positive for at least one of the 10 bacterial genera. The 155 remaining specimens (18%) were devoid of any of the targeted bacteria but had satisfactory DNA template quality as shown by the positive PCR amplification of the 18S gene fragment; these specimens were therefore considered as uninfected. Within tick populations, species and genera, maternally inherited bacteria were detected at diverse frequencies, ranging from 0% to 100%, as detailed below.

Of the 81 species examined, sampled individuals of two species (2.5%), the soft tick species *Antricola guglielmonei* ($n = 4$) and the hard tick *Ixodes apronophorus* ($n = 1$), were found uninfected (Fig. 1 and Table S1, Supporting information). The 79 other tick species (97.5%) were found positive for at least one of the targeted bacteria in at least some of the specimens (Fig. 1 and Table S1, Supporting information). The number of infected species did not vary between tick families (Fisher's exact test, $P = 0.54$) or genera ($P = 0.15$). However, the number of detected bacterial genera per tick species covaried positively with the screening effort, *that is* the number of examined specimens per tick species (Spearman's rank correlation, $N = 81$, $r = 0.34$, $P = 0.002$): tick species observed with higher bacterial diversity were those for which we examined more specimens, such as the sheep tick *Ixodes ricinus* ($n = 94$ individuals and 6 detected bacteria) and the African blue tick *Rhipicephalus decoloratus* ($n = 91$ individuals and 6 detected bacteria).

All 10 maternally inherited bacterial genera previously reported in ticks (Table 1) were observed in our samples. However, their respective incidences differed dramatically. *Coxiella*-LE and *Rickettsia* infect more tick species than any other bacteria (Fisher's exact tests, all $P < 10^{-3}$): *Coxiella*-LE was detected in 49 tick species (60.5%), *Rickettsia* in 45 species (55.6%), *Francisella*-LE in 17 species (21.0%), *Spiroplasma* in 13 species (16.4%), *Midichloria* in 12 species (14.8%), *Rickettsiella* in 10

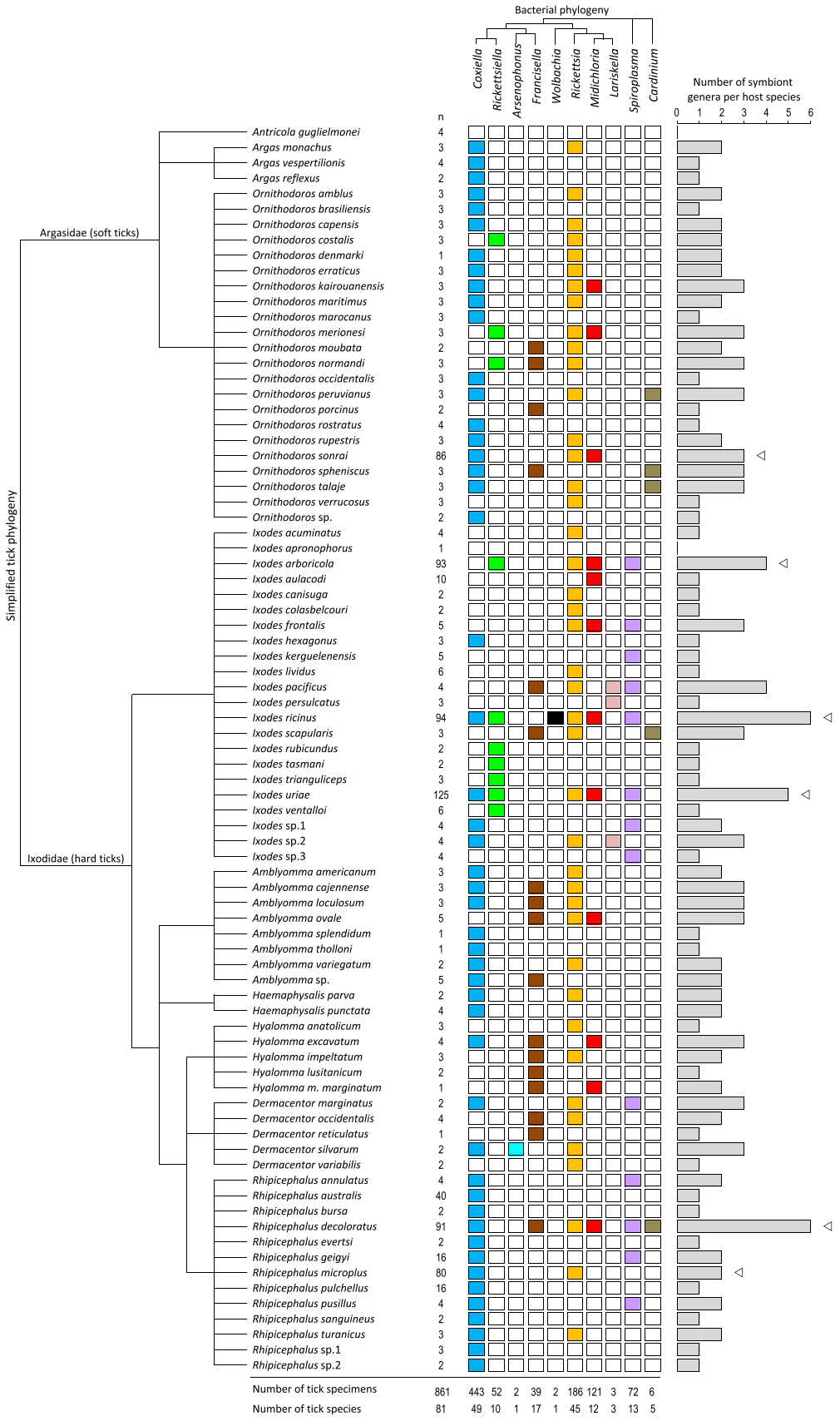
Bacterial phylogeny
Coxiella
Rickettsiella
Arsenophonus
Francisella
Wolbachia
Rickettsia
Midichloria
Lariskella
Spiroplasma
Cardinium
Number of symbiont genera per host species
0 1 2 3 4 5 6
n
Simplified tick phylogeny
Argasidae (soft ticks)
Ixodidae (hard ticks)
Antricola guglielmonei 4
Argas monachus 3
Argas vespertilionis 4
Argas reflexus 2
Ornithodoros amblus 3
Ornithodoros brasiliensis 3
Ornithodoros capensis 3
Ornithodoros costalis 3
Ornithodoros denmarki 1
Ornithodoros erraticus 3
Ornithodoros kairouanensis 3
Ornithodoros maritimus 3
Ornithodoros marocanus 3
Ornithodoros merionesi 3
Ornithodoros moubata 2
Ornithodoros normandi 3
Ornithodoros occidentalis 3
Ornithodoros peruvianus 3
Ornithodoros porcinus 2
Ornithodoros rostratus 4
Ornithodoros rupestris 3
Ornithodoros sonrai 86
Ornithodoros spheniscus 3
Ornithodoros talaje 3
Ornithodoros verrucosus 3
Ornithodoros sp. 2
Ixodes acuminatus 4
Ixodes apronophorus 1
Ixodes arboricola 93
Ixodes aulacodi 10
Ixodes canisuga 2
Ixodes colasbelcouri 2
Ixodes frontalis 5
Ixodes hexagonus 3
Ixodes kerguelenensis 5
Ixodes lividus 6
Ixodes pacificus 4
Ixodes persulcatus 3
Ixodes ricinus 94
Ixodes scapularis 3
Ixodes rubicundus 2
Ixodes tasmani 2
Ixodes trianguliceps 3
Ixodes uriae 125
Ixodes ventalloi 6
Ixodes sp.1 4
Ixodes sp.2 4
Ixodes sp.3 4
Amblyomma americanum 3
Amblyomma cajennense 3
Amblyomma loculosum 3
Amblyomma ovale 5
Amblyomma splendidum 1
Amblyomma tholloni 1
Amblyomma variegatum 2
Amblyomma sp. 5
Haemaphysalis parva 2
Haemaphysalis punctata 4
Hyalomma anatolicum 3
Hyalomma excavatum 4
Hyalomma impeltatum 3
Hyalomma lusitanicum 2
Hyalomma m. marginatum 1
Dermacentor marginatus 2
Dermacentor occidentalis 4
Dermacentor reticulatus 1
Dermacentor silvarum 2
Dermacentor variabilis 2
Rhipicephalus annulatus 4
Rhipicephalus australis 40
Rhipicephalus bursa 2
Rhipicephalus decoloratus 91
Rhipicephalus evertsi 2
Rhipicephalus geigyi 16
Rhipicephalus microplus 80
Rhipicephalus pulchellus 16
Rhipicephalus pusillus 4
Rhipicephalus sanguineus 2
Rhipicephalus turanicus 3
Rhipicephalus sp.1 3
Rhipicephalus sp.2 2
Number of tick specimens 861 443 52 2 39 2 186 121 3 72 6
Number of tick species 81 49 10 1 17 1 45 12 3 13 5

Fig. 1 Distribution of heritable bacterial symbionts in ticks. The left part of the figure shows a simplified phylogeny of tick genera adapted from Burger *et al.* (2012, 2014) and lists the 81 tick species under investigation. *n*, number of individuals examined in each tick species. The top part of the figure shows a simplified bacterial phylogeny of the ten bacterial symbionts examined in this study. Boxes representing presence of infections are coloured differently depending on symbionts. The right part of the figure shows the number of symbiont genera observed in each tick species. The triangles indicate the tick species for which five populations were examined. The geographic origin of tick populations and infection prevalence are detailed in Table S1 (Supporting information).

species (12.3%), *Cardinium* in five species (6.2%), *Lariskella* in three species (3.7%), *Arsenophonus* as well as *Wolbachia* in one species (1.2%). A few PCR assays resulted in false positives (a band was present, but the sequence was outside the targeted clade); these were excluded from further analyses.

Each of the 10 bacterial genera had a patchy distribution along the tick phylogeny. Some showed a nonrandom distribution among tick species: their presence and prevalence varied considerably in the different tick groups (Fig. 1 and Table S1, Supporting information). Indeed, *Coxiella*-LE was uniformly distributed between the two tick families (Fisher's exact test, $P = 0.14$), but not among tick genera ($P = 2.10^{-6}$): it was common in *Rhipicephalus* (13 of 13 examined species), *Argas* (3/3), *Ornithodoros* (17/22), *Amblyomma* (7/8) and *Haemaphysalis* (2/2), but was rarer in *Dermacentor* (2/5), *Hyalomma* (1/5), *Ixodes* (5/22) and *Antricola* (0/1). Conversely, there is no evidence to reject a uniform distribution of *Rickettsia* between tick families ($P = 0.32$) and genera ($P = 0.11$): *Argas* (1/3), *Ornithodoros* (15/22), *Amblyomma* (5/8), *Dermacentor* (4/5), *Haemaphysalis* (1/2), *Hyalomma* (1/5), *Ixodes* (11/22) and *Rhipicephalus* (3/13).

Two of the eight other targeted bacterial genera, *Francisella*-LE and *Spiroplasma*, showed variable distributions among tick families and genera (Fig. 1 and Table S1, Supporting information). *Francisella*-LE was uniformly distributed between tick families ($P = 0.56$) but not among genera ($P = 0.01$): common in *Hyalomma* (4/5), *Amblyomma* (4/8) and *Dermacentor* (2/5) and rare or absent in the other genera. *Spiroplasma* was not uniformly distributed among tick families ($P = 0.007$): absent in soft ticks (0 of 26 screened species) but present in hard ticks (13/55). Although *Spiroplasma* was uniformly distributed among hard tick genera ($P = 0.17$), it was only detected in *Ixodes* (8/22), *Dermacentor* (1/5) and *Rhipicephalus* (4/13). *Midichloria*, *Rickettsiella*, *Cardinium* and *Lariskella* did not show significant variation in incidence between the tick families (all $P > 0.32$) or among tick genera (all $P > 0.18$). No preferential distribution can be also detected for *Arsenophonus* and *Wolbachia* as each was present each in only one tick species.

When considering the whole bacterial community, as many as 33 different endosymbiont combinations were observed at the tick species level (Fig. 1 and Table S1, Supporting information). Thirty-five of the 81 tick species (43.2%) were infected by only one bacterium, but 44 (56.3%) harboured two or more bacterial genera: 23 species were infected by two genera (28.4%), 16 species by three (19.8%), two species by four (2.5%), one species by five (1.2%) and two species by six (2.5%). At the tick species level, bacteria were randomly associated in most cases (Fisher's exact tests, all $P > 0.06$), suggesting that their distribution across tick species was independent of the presence of other symbionts. Nonrandom associations between bacteria were however observed at the tick species levels. (i) Two pairs of bacteria co-occurred in the same tick species more frequently than expected by chance in two cases (aggregation pattern): *Midichloria* and *Rickettsia* ($P = 0.03$), *Midichloria* and *Spiroplasma* ($P = 0.02$). (ii) Conversely, two bacterial combinations co-occurred in the same tick species less frequently than expected by chance (exclusion pattern): *Coxiella*-LE and *Rickettsiella* ($P = 0.01$), *Coxiella*-LE and *Francisella*-LE ($P = 0.02$) (Fig. 1 and Table S1). Interestingly, despite the variations in bacterial community structure observed across the entire data set, these conclusions of exclusion patterns driven at host species levels were also supported by the analyses performed at individual tick levels (see detailed results in the Table S4, Supporting information). Indeed, *Coxiella* prevalence dropped from 0.68 among the mono-infected individual ticks to zero among the *Rickettsiella*-infected specimens and to 0.27–0.50 among the *Francisella*-infected ticks (Table S4, Supporting information). Complementarily, *Rickettsiella* prevalence dropped from 0.13 among the mono-infected individual ticks to zero among the individual ticks harbouring *Coxiella* while *Francisella* prevalence dropped from 0.062 among the mono-infected ticks to 0.029 among the ticks bearing *Coxiella* (Table S4, Supporting information). The analyses performed at individual tick levels also revealed that the aggregation between *Midichloria* and *Rickettsia* occurred in *Coxiella*-infected ticks (the prevalence of either *Midichloria* or *Rickettsia* increased in the ticks bearing both *Coxiella* and either *Rickettsia* or *Midichloria*) whereas the aggregation between *Midichloria* and *Spiroplasma* occurred in *Coxiella*-free ticks (Table S4, Supporting information).

In the six focal tick species for which five geographic populations were sampled (Fig. 2 and Table S1), each tick species hosted from two to six bacterial genera and each population from one to five bacteria. The presence

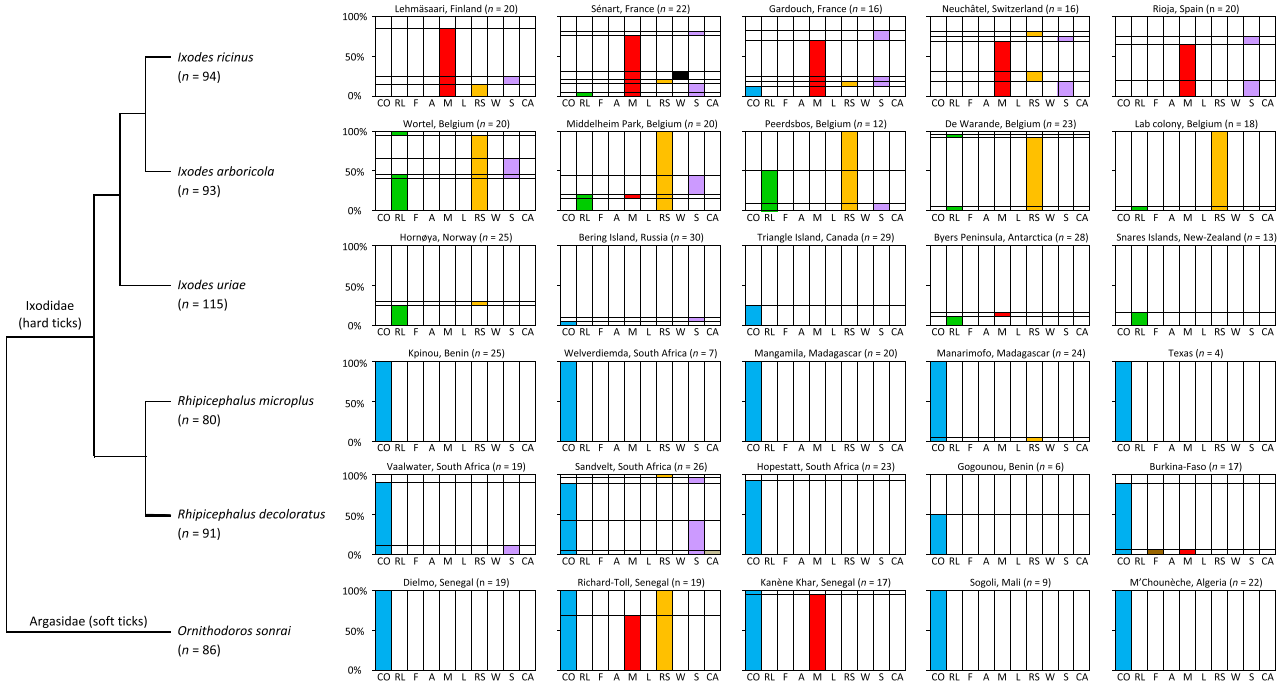

Fig. 2 Variations in individual infection status between populations of six tick species. The left part of the figure shows a simplified tick phylogeny based from Burger *et al.* (2012, 2014). Each graph shows the frequency of bacterial infection types in one tick population. The geographic origin of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

of two or more bacteria was often reflected by coinfection at the individual level. Indeed, 25 of the 94 *I. ricinus* individuals (26.6%) harboured more than one symbiont. In the soft tick *Ornithodoros sonrai*, double or triple infections were fixed or almost fixed in some populations, but absent in others. There was significant variation in infection patterns among populations in four of the six tick species: the tree-hole tick *Ixodes arboricola* ($\chi^2_8 = 26.55$, $P = 8.10^{-4}$), the polar seabird tick *Ixodes uriae* ($\chi^2_{10} = 30.79$, $P = 6.10^{-4}$), the African blue tick *R. decoloratus* ($\chi^2_{12} = 34.65$, $P = 5.10^{-4}$) and the soft tick *O. sonrai* ($\chi^2_4 \to \infty$, $P < 10^{-10}$). These community level effects were due to variation in prevalence of one or two bacteria per tick species: *Rickettsiella* (Fisher's exact test, $P = 4.10^{-4}$) and *Spiroplasma* ($P = 0.01$) in *I. arboricola*, *Coxiella*-LE ($P = 5.10^{-4}$) and *Rickettsiella* ($P = 0.002$) in *I. uriae*, *Spiroplasma* ($P = 2.10^{-6}$) in *R. decoloratus*, and *Midichloria* ($P < 10^{-10}$) and *Rickettsia* ($P < 10^{-10}$) in *O. sonrai* (Fig. 2 and Table S1, Supporting information). In contrast, the composition of bacterial communities was homogeneous among populations of the sheep tick *I. ricinus* ($\chi^2_{12} = 14.04$, $P = 0.30$) and the cattle tick *Rhipicephalus microplus* ($\chi^2_2 = 0.75$, $P = 0.69$).

The correspondence analysis (CA) showed again that infection patterns tended to differ more among the six focal tick species than among populations of each tick species (Fig. 3A and B). In this analysis, the first two axes account for 78.4% of the total variability. The first axis of the CA discriminates populations infected by *Coxiella*-LE (on the left) from those infected by *Rickettsia* (on the right); the second axis discriminates populations infected by *Rickettsiella* (on top) from those infected by either *Spiroplasma* or *Midichloria* (Fig. 3A). Indeed, *Coxiella*-LE was (almost) fixed in all *R. microplus*, *R. decoloratus* and *O. sonrai* populations, but was rare or absent otherwise. *Midichloria* was the most commonly detected bacterium in *I. ricinus* and *Rickettsia* in *I. arboricola*; no bacterium was common in *I. uriae* (Figs 2 and 3B, Table S1, Supporting information).

Evolutionary history of maternally inherited bacteria

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

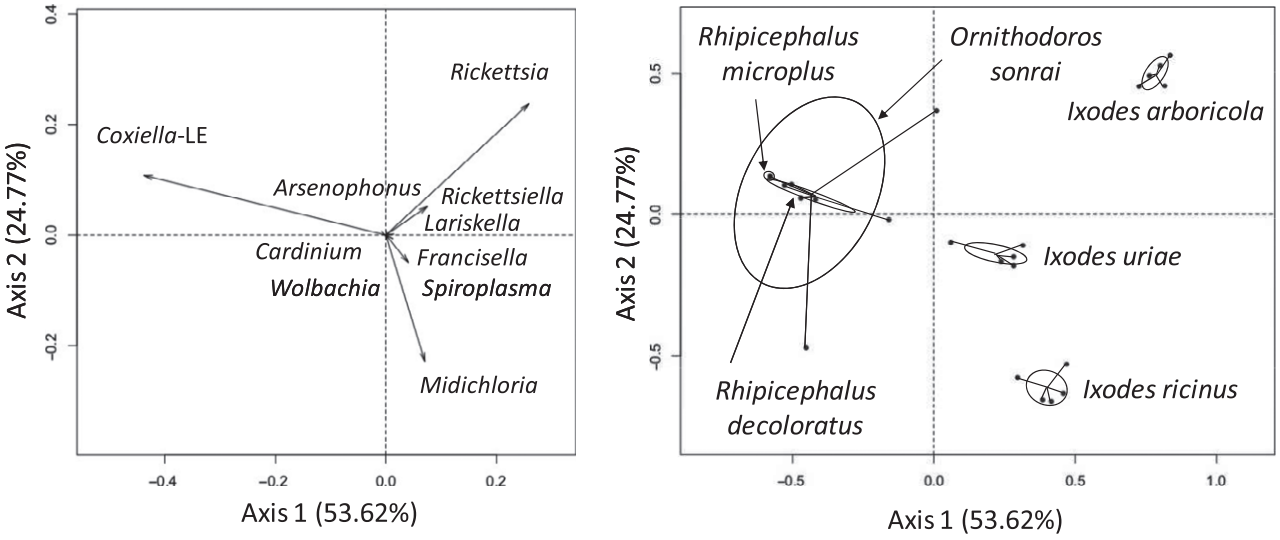

Fig. 3 Correspondence analysis (CA) performed on the symbiotic communities of 30 populations of six tick species (five populations per species). Each community is represented by a dot. (A) Projection of infection status on a factorial map. The first axis of the CA discriminates populations infected by *Coxiella*-LE (on the left) from those infected by *Rickettsia* (on the right); the second axis discriminates populations infected by *Rickettsiella* (on top) from those infected by either *Spiroplasma* or *Midichloria*. (B) Populations grouped according to the six tick species (six groups are delimited by the 95% confidence ellipses drawn around the barycenter of each species).

The partitioning of *Coxiella*-LE diversity among tick species revealed a complex structure with signatures of both codivergence and HT events. A pattern suggestive of codivergence was detected for the *Coxiella*-LE harboured by *I. ricinus*, *I. hexagonus* and *I. uriae* as they clustered together within the *Coxiella* phylogeny (Fig. 4). A more compelling example is found in the genus *Rhipicephalus* where the *Coxiella*-LE found in the 13 examined tick species clustered within the same clade. In addition, there was perfect topological congruence between the phylogenies of concatenated *Coxiella*-LE gene sequences (*rpoB*, *GroEL* and *dnaK*; no recombination was detected in this data set using the GENECONV and RDP tests, all $P > 0.10$) and concatenated *Rhipicephalus* mtDNA genes (12S *rRNA*, 16S *rRNA* and *CO1*), corroborating the codivergence hypothesis (PACo analysis, $P = 5.10^{-6}$; Fig. 5). Conversely, absence of clustering in related tick species was also observed (Fig. 4). For instance, the *Coxiella*-LE of *Ornithodoros* soft ticks were scattered among different *Coxiella* branches. Similarly, the *Coxiella*-LE of *Amblyomma* hard ticks belonged to a minimum of five distinct phylogenetic clusters. These patterns are the signatures of repeated HT events, revealing the ability of some *Coxiella*-LE to extensively move among tick species.

Examination of phylogenies of the nine other bacteria showed that they all undergo occasional HT events. For example, the *Francisella*-LE and *Midichloria* strains of soft ticks are scattered among those of hard ticks (Figs S1–S2, Supporting information). The other symbionts are not only circulating among tick species but also among arthropod classes: the *Cardinium*, *Arsenophonus*, *Lariskella*, *Wolbachia*, *Rickettsiella*, *Rickettsia* and *Spiroplasma* strains found in ticks were frequently related to bacterial strains found in insects (Figs S3–S9, Supporting information). However, the infection pattern was difficult to interpret in one case: the *Wolbachia* strain detected in the sheep tick *I. ricinus* is 100% identical to that of the tick parasitoid *Ixodiphagus hookeri* (Fig. S6, Supporting information). This suggests that it is likely the result of a cross-contamination due the presence of parasitoid DNA in the tick DNA sample, a pattern previously observed in *I. ricinus* (Plantard *et al*. 2012).

It is worthy of note that three endosymbionts are closely related, albeit genetically distinct, to vertebrate pathogens. This includes *Coxiella*-LE, which are closely related to the Q fever agent *Coxiella burnetii* (Duron *et al*. 2015b), *Francisella*-LE, closely related to the causative agent of tularaemia, *Francisella tularensis* (Sjodin *et al*. 2012), and *Rickettsia*, closely related to many (often tick-borne) *Rickettsia* pathogens (Perlman *et al*. 2006; Weinert *et al*. 2009). There was thus a potential risk of misidentification of endosymbionts in some tick species: the bacteria observed may be tick-borne pathogens rather than maternally inherited endosymbionts. However, in most cases, endosymbionts can be unambiguously distinguished from their pathogenic relatives on the basis of their DNA sequences, as for *Coxiella*: all the strains we found in ticks were genetically distinct from *C. burnetii* and can be unambiguously assigned to

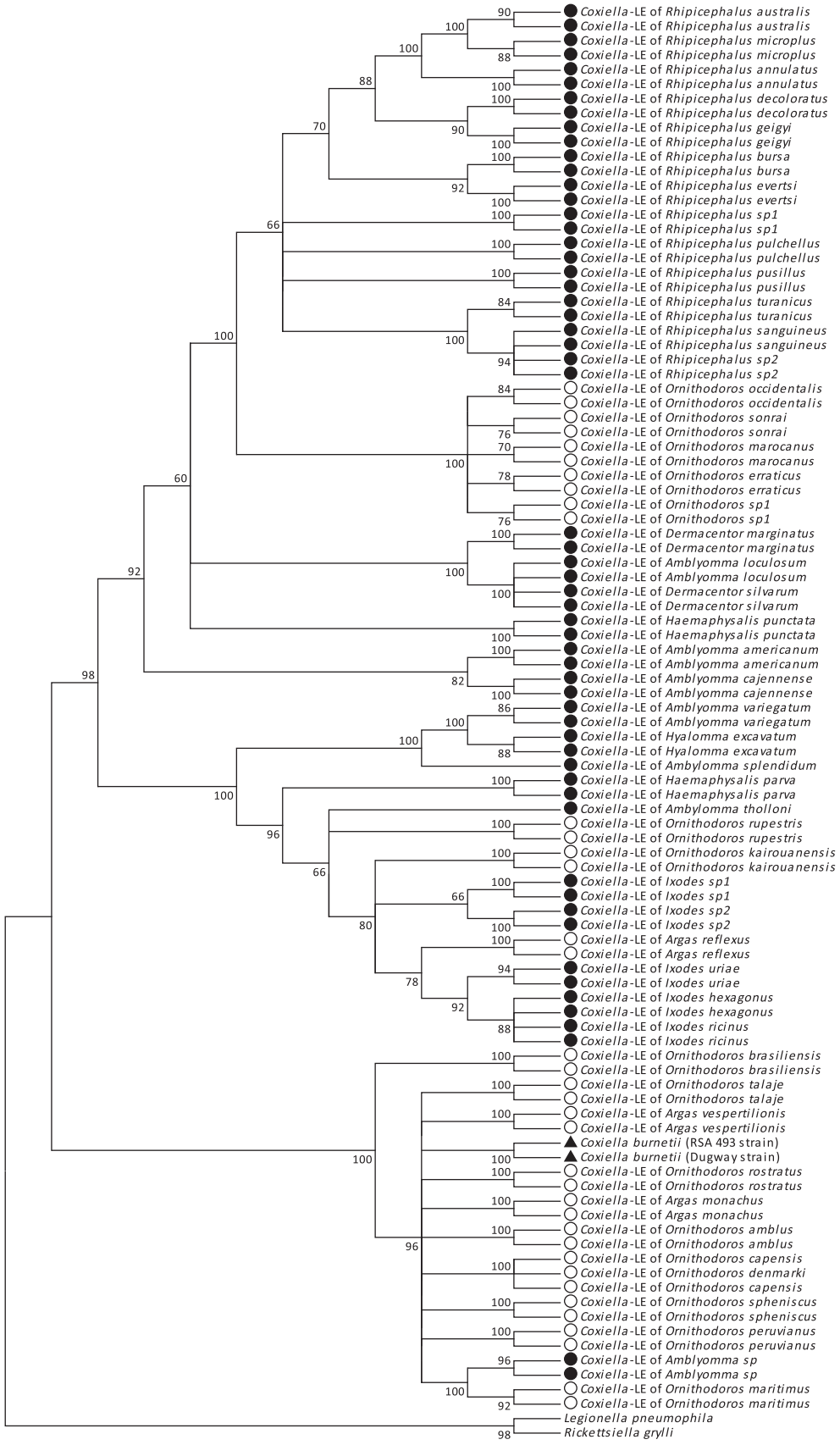
Coxiella-LE of Rhipicephalus australis
Coxiella-LE of Rhipicephalus australis
Coxiella-LE of Rhipicephalus microplus
Coxiella-LE of Rhipicephalus microplus
Coxiella-LE of Rhipicephalus annulatus
Coxiella-LE of Rhipicephalus annulatus
Coxiella-LE of Rhipicephalus decoloratus
Coxiella-LE of Rhipicephalus decoloratus
Coxiella-LE of Rhipicephalus geigyi
Coxiella-LE of Rhipicephalus geigyi
Coxiella-LE of Rhipicephalus bursa
Coxiella-LE of Rhipicephalus bursa
Coxiella-LE of Rhipicephalus evertsi
Coxiella-LE of Rhipicephalus evertsi
Coxiella-LE of Rhipicephalus sp1
Coxiella-LE of Rhipicephalus sp1
Coxiella-LE of Rhipicephalus pulchellus
Coxiella-LE of Rhipicephalus pulchellus
Coxiella-LE of Rhipicephalus pusillus
Coxiella-LE of Rhipicephalus pusillus
Coxiella-LE of Rhipicephalus turanicus
Coxiella-LE of Rhipicephalus turanicus
Coxiella-LE of Rhipicephalus sanguineus
Coxiella-LE of Rhipicephalus sanguineus
Coxiella-LE of Rhipicephalus sp2
Coxiella-LE of Rhipicephalus sp2
Coxiella-LE of Ornithodoros occidentalis
Coxiella-LE of Ornithodoros occidentalis
Coxiella-LE of Ornithodoros sonrai
Coxiella-LE of Ornithodoros sonrai
Coxiella-LE of Ornithodoros marocanus
Coxiella-LE of Ornithodoros marocanus
Coxiella-LE of Ornithodoros erraticus
Coxiella-LE of Ornithodoros erraticus
Coxiella-LE of Ornithodoros sp1
Coxiella-LE of Ornithodoros sp1
Coxiella-LE of Dermacentor marginatus
Coxiella-LE of Dermacentor marginatus
Coxiella-LE of Amblyomma loculosum
Coxiella-LE of Amblyomma loculosum
Coxiella-LE of Dermacentor silvarum
Coxiella-LE of Dermacentor silvarum
Coxiella-LE of Haemaphysalis punctata
Coxiella-LE of Haemaphysalis punctata
Coxiella-LE of Amblyomma americanum
Coxiella-LE of Amblyomma americanum
Coxiella-LE of Amblyomma cajennense
Coxiella-LE of Amblyomma cajennense
Coxiella-LE of Amblyomma variegatum
Coxiella-LE of Amblyomma variegatum
Coxiella-LE of Hyalomma excavatum
Coxiella-LE of Hyalomma excavatum
Coxiella-LE of Ambylomma splendidum
Coxiella-LE of Haemaphysalis parva
Coxiella-LE of Haemaphysalis parva
Coxiella-LE of Ambylomma tholloni
Coxiella-LE of Ornithodoros rupestris
Coxiella-LE of Ornithodoros rupestris
Coxiella-LE of Ornithodoros kairouanensis
Coxiella-LE of Ornithodoros kairouanensis
Coxiella-LE of Ixodes sp1
Coxiella-LE of Ixodes sp1
Coxiella-LE of Ixodes sp2
Coxiella-LE of Ixodes sp2
Coxiella-LE of Argas reflexus
Coxiella-LE of Argas reflexus
Coxiella-LE of Ixodes uriae
Coxiella-LE of Ixodes uriae
Coxiella-LE of Ixodes hexagonus
Coxiella-LE of Ixodes hexagonus
Coxiella-LE of Ixodes ricinus
Coxiella-LE of Ixodes ricinus
Coxiella-LE of Ornithodoros brasiliensis
Coxiella-LE of Ornithodoros brasiliensis
Coxiella-LE of Ornithodoros talaje
Coxiella-LE of Ornithodoros talaje
Coxiella-LE of Argas vespertilionis
Coxiella-LE of Argas vespertilionis
Coxiella burnetii (RSA 493 strain)
Coxiella burnetii (Dugway strain)
Coxiella-LE of Ornithodoros rostratus
Coxiella-LE of Ornithodoros rostratus
Coxiella-LE of Argas monachus
Coxiella-LE of Argas monachus
Coxiella-LE of Ornithodoros amblus
Coxiella-LE of Ornithodoros amblus
Coxiella-LE of Ornithodoros capensis
Coxiella-LE of Ornithodoros denmarki
Coxiella-LE of Ornithodoros capensis
Coxiella-LE of Ornithodoros spheniscus
Coxiella-LE of Ornithodoros spheniscus
Coxiella-LE of Ornithodoros peruvianus
Coxiella-LE of Ornithodoros peruvianus
Coxiella-LE of Amblyomma sp
Coxiella-LE of Amblyomma sp
Coxiella-LE of Ornithodoros maritimus
Coxiella-LE of Ornithodoros maritimus
Legionella pneumophila
Rickettsiella grylli

Fig. 4 Cladogram depicting the majority-rule consensus (60%) of *Coxiella* phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *rpoB* sequences (491 unambiguously aligned bp) from *Coxiella*-LE strains of ticks, the agent of Q fever (*Coxiella burnetii*) and out-groups (*Legionella pneumophila* and *Rickettsiella grylli*). Two *rpoB Coxiella* sequences per infected tick species are shown in most cases. White circles, *Coxiella*-LE of soft ticks; black circles, *Coxiella*-LE of hard ticks; black triangles, *C. burnetii*. Branch numbers indicate percentage bootstrap support (1000 replicates).

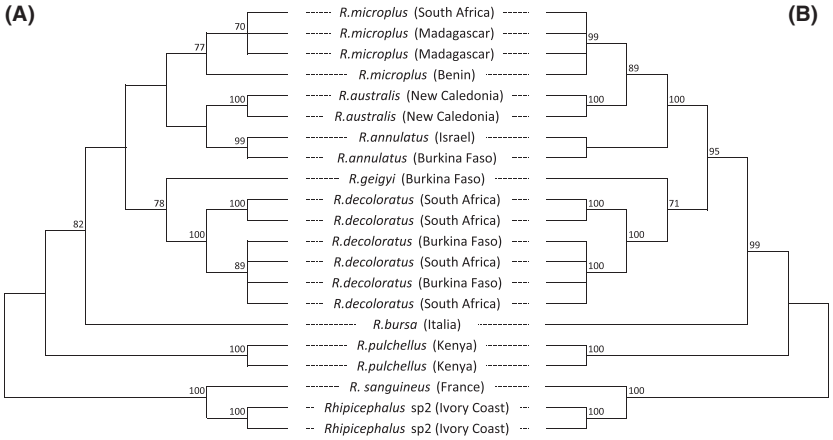

Fig. 5 Congruence between *Rhipicephalus* tick species and their *Coxiella*-LE symbionts. (A) Tick mtDNA cladogram depicting the majority-rule consensus (50%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on concatenated 12S *rRNA*, 16S *rRNA* and *C01* gene sequences (1465 unambiguously aligned bp); (B) *Coxiella*-LE cladogram depicting the majority-rule consensus (50%) of phylogenetic trees constructed using ML estimations based on concatenated *rpoB*, *GroEL* and *dnaK* gene sequences (1252 unambiguously aligned bp). Branch numbers indicate percentage bootstrap support for major branches (1000 replicates; only values >70% are shown).

Coxiella-LE (Fig. 4; Duron *et al*. 2015b). For *Francisella*, the strains we found in ticks formed a monophyletic clade related to, but distinct from the pathogenic species (Fig. S1, Supporting information; see also Goethert & Telford 2005; Sjodin *et al*. 2012). For *Rickettsia*, the situation is more complex because endosymbiotic forms and vertebrate pathogens are scattered along the phylogeny (Perlman *et al*. 2006; Weinert *et al*. 2009): the distinction of endosymbiotic forms and vertebrate pathogens on the basis of DNA sequences alone may not be reliable in some cases. For instance, in several *Rhipicephalus* and *Amblyomma* tick species, we observed *Rickettsia* strains closely related to, but genetically distinct from, the causative agent of African tick bite fever, *Rickettsia africae* (Fig. S8, Supporting information), and we cannot state whether these strains are pathogenic or not. There are nevertheless other cases where the *Rickettsia* we detected are likely endosymbionts: this is the case for those found in the black-legged tick *Ixodes scapularis* and the American dog tick *Dermacentor variabilis* which clustered with *Rickettsia buchneri* (Fig. S8, Supporting information), a nonpathogenic species known to be maternally inherited in ticks (Kurtti *et al*. 2015). The high frequency of *Rickettsia* infections in tick species such as the tree-hole tick *I. arboricola* also suggests the presence of *Rickettsia* endosymbionts (Fig. 2 and Table S1, Supporting information).

Discussion

That ecological specialization to restricted diet niches is driven by evolutionary stable symbiotic interactions is beyond doubt for many arthropod lineages (Moran *et al*. 2008; Wernegreen 2012). The present study nevertheless shows that obligate symbioses are relatively unstable in ticks. We screened 81 tick species for *Coxiella*-LE, a formally recognized obligate symbiont of ticks, and for nine other maternally inherited symbionts whose incidence, prevalence and diversity were largely unknown in tick populations. We detected maternally inherited bacteria in almost all tick species (79 of 81) with many of them (44) hosting more than one symbiont. In multi-infected tick species, symbionts are assembled in communities which can reach high levels of complexity. Indeed, six distinct genera of symbionts coexist in populations of the sheep tick *Ixodes ricinus* and in populations of the African blue tick *Rhipicephalus decoloratus*. However, no symbiotic community structure was fixed and stable across the tick phylogeny and communities varied both among tick species and among tick populations and individuals within species. Two transmission modes, both apparent in symbiont phylogenies, act together to produce these patterns: maternal inheritance (*i.e.* vertical transmission), which ensures the persistence of infections within a host clade,

and occasional HT, which enables symbionts to disperse beyond their primary host species. The success of these transmission patterns will then modify and may also be modified by interactions among the symbionts coexisting within infracommunities. One of the most remarkable outcomes of these processes is the low evolutionary stability of the symbiosis between ticks and *Coxiella*-LE.

As expected, *Coxiella*-LE was the most common maternally inherited symbiont associated with ticks, recorded in 49 (60.5%) of the 81 sampled species. The wide distribution of genetically differentiated *Coxiella*-LE strains across the tick phylogeny suggests that the symbiosis is ancient and arose in early tick evolution. We did not, however, observe the codiversification pattern typically found for obligate mutualists. Instead, the spread of *Coxiella*-LE infections was surprisingly complex, depending on two distinct evolutionary strategies. Some *Coxiella*-LE symbionts seem to be highly specialized on their tick hosts, with an ancient acquisition followed by codiversification. This is best exemplified by the codivergence observed between *Rhipicephalus* and *Coxiella*-LE lineages. The *Rhipicephalus* genus is thought to have emerged in the Middle Miocene, *ca*. 14Mya (Murrell *et al.* 2001), which can be assumed as an approximate minimal date for the original *Coxiella*-LE infection in this tick genus. On the contrary, other *Coxiella*-LE symbionts are more generalist and seem to have been acquired through recent HT events from unrelated host species. Such a pattern is also observed for other endosymbionts, like *Wolbachia* that experiences frequent host shifts in insects, with occasional transitions to cocladogenesis in other host groups (Werren *et al.* 2008). Overall, the present data on *Coxiella*-LE supports the hypothesis that a replacement of obligate symbionts frequently occurs in ticks. Interestingly, we did not observe any tick species infected by more than one *Coxiella*-LE strain, a result also corroborated by other studies (Jasinskas *et al.* 2007; Clay *et al.* 2008; Machado-Ferreira *et al.* 2011; Almeida *et al.* 2012; Lalzar *et al.* 2012). The absence of coinfection despite frequent HT events further suggests that different *Coxiella*-LE strains cannot stably coexist within the same tick species.

Exclusion processes are not limited to *Coxiella*-LE, but may also involve other symbiont genera. In 30 of the 32 tick species not infected by *Coxiella*-LE, we detected the presence of one to five other symbionts, among which one was typically close to fixation. Indeed, we found significant exclusion patterns between, on the one hand, *Coxiella*-LE and, on the other hand, *Francisella*-LE and *Rickettsiella*. This suggests that these latter bacterial genera may be alternative obligate symbionts which have replaced *Coxiella*-LE in some tick species (notably in *Ornithodoros* and *Dermacentor* species where they are often observed in all specimens). Moreover, in some *Ixodes* species, *Rickettsia* and *Midichloria* strains were fixed, or were close to fixation, in two tick species free of *Coxiella*-LE infection, *that is* the tree-hole tick *Ixodes arboricola* and the sheep tick *I. ricinus*, respectively. Although formal testing through nutritional and physiological experiments is now required to validate this hypothesis, recent data on the bacterial genomes suggest that these possible alternative symbionts have evolved adaptive mechanisms enabling tick survival. Indeed, their genomes encode functions suggesting that they are obligate tick mutualists in a very similar way to *Coxiella*-LE as they also have, at least partially, the genetic capability for *de novo* B vitamin synthesis. Recent metabolic reconstructions of *Rickettsia* genomes revealed that all genes required for folate (B9 vitamin) biosynthesis are present in the genome of the *Rickettsia* endosymbionts of both the black-legged tick *Ixodes scapularis* and the Western black-legged tick *Ixodes pacificus* (Hunter *et al.* 2015). Similarly, the genomes of *Francisella*-LE (isolated from the fowl tick *Argas persicus* and from the Gulf Coast tick *Amblyomma maculatum*) and of *Midichloria* (from *I. ricinus*) contain complete or nearly complete genetic pathways for biotin (B7 vitamin) and riboflavin (B2) biosynthesis (Sassera *et al.* 2011; Sjodin *et al.* 2012; Gerhart *et al.* 2016).

The genetic capability for *de novo* B vitamin synthesis may explain why some endosymbionts do not coexist with *Coxiella*-LE. In such cases, where different symbionts provide the same benefit for the host, the maintenance of multiple infections is not expected (Vautrin & Vavre 2009) as there is no additional benefit. Single infection is thus expected to become fixed through regular imperfect transmission of the different bacteria. This process is expected to be further accelerated if multiple infections are costly. Alternatively, loss of multiple infections would be prevented if each symbiont becomes mutually indispensable through the complementary retention of needed pathways. In several lineages of Hemiptera, for example, different endosymbionts encode different gene sets and thus form an interdependent metabolic patchwork (Pérez-Brocal *et al.* 2006; Bennett & Moran 2013; Husnik & McCutcheon 2016). Such a process may be possible in ticks and explain why some tick species remain coinfected by *Coxiella*-LE and *Francisella*-LE/*Rickettsia*. Another interesting possibility is that some tick species may have lost their *Coxiella*-LE but have acquired some functionally important symbiont genes (including those of B vitamin synthesis pathways) via lateral gene transfer. In this context, ticks may retain the adaptive trait (B vitamin provisioning) without the symbiont. Indeed, this pattern was reported from some filarial nematodes to explain their ability of to live and reproduce without

obligate symbiont (McNulty *et al.* 2010). However, examination of the genome of a tick species lacking *Coxiella*-LE, the black-legged tick *I. scapularis*, did not show evidence of this lateral gene transfer (Gulia-Nuss *et al.* 2016). However, this tick species is infected by a *Rickettsia* endosymbiont which may synthesize B9 vitamin (Hunter *et al.* 2015), providing thus a possible explanation for the absence of *Coxiella*-LE.

An important consideration for future studies will be to understand the ecological pathways that facilitate the HT of symbionts among tick species. Feeding on a shared vertebrate may be a particularly important determinant. Indeed, the examination of tick internal organs has revealed a high concentration of *Coxiella*-LE within the salivary glands of some species (Klyachko *et al.* 2007; Qiu *et al.* 2014). Vertebrates may thus act as ecological arenas for the global exchange of symbionts, serving as possible intermediate hosts for HT among tick species. Such HT events may also favour the emergence of novel bacterial phenotypes. Indeed, this is the most parsimonious explanation for the origin of Q fever as *C. burnetii* seems to have evolved from a *Coxiella*-LE ancestor that infected vertebrate cells (Duron *et al.* 2015a). Phylogenetic data from *Francisella* and *Rickettsia* also suggest the occurrence of regular transitions between pathogenic and endosymbiotic forms along their evolutionary history (Perlman *et al.* 2006; Darby *et al.* 2007; Weinert *et al.* 2009; Sjodin *et al.* 2012). An obvious question is then to determine to what extent interactions within the symbiotic communities of ticks may facilitate the evolutionary emergence of novel vertebrate pathogens.

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O.D., F.B., V.N., C.A. and J.C. performed molecular and phylogenetic analyses; O.D., C.C., F.B. and J.C. performed statistical analyses; K.D.M., O.P., J.G., A.A.P.dL., D.J.A.H., A.R.V.O., Y.G., G.B., A.A.G., A.E.-P., M.N.O., L.Z., F.V. and C.C. performed field missions, tick sampling, morphological identifications and preliminary analyses; O.D. wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Data accessibility

Novel nucleotide sequences have been deposited in the GenBank nucleotide database (Accession nos.: *Coxiella*, KY677980-KY677995, KY678138-KY678200; *Rickettsiella*, KY677996-KY678006; *Arsenophonus*, KY677978-KY677979; *Francisella*, KY678009-KY678036; *Wolbachia*, KY6780 07-KY678008; *Midichloria*, KY674359-KY674396; *Lariskella*, KY674397-KY674399; *Spiroplasma*, KY674400-KY674 420; *Cardinium*, KY660634-KY660639; *Rickettsia*, KY67803 7-KY678116; tick mtDNA, KY676804-KY676845, KY678 117-KY678137). All other data are presented in the supplementary materials.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 List of tick species and populations included in the analysis, with details on their origin, the sample size (*n*), and the prevalence of the ten maternally-inherited endosymbiotic bacteria (prevalence highlighted in grey when present).

Table S2 Genes and primers used in this study.

Table S3 List of best-fitting evolutionary models used for maximum-likelihood (ML) analyses.

Table S4 Testing at individual-tick levels the exclusion and aggregation patterns detected at tick-species levels.

Fig. S1 *Francisella* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *rpoB* sequences (306 unambiguously aligned bp) from *Francisella*-LE strains of ticks, the agent of tularemia (*F. tularensis*) and other *Francisella* species.

Fig. S2 *Midichloria* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *16S rRNA* gene sequences (386 unambiguously aligned bp) from *Midichloria* strains of ticks, the type species *M. mitochondrii* and outgroups (*Lariskella arthropodarum* and *Rickettsia* spp.).

Fig. S3 *Cardinium* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees (60% consensus tree) constructed using maximum-likelihood (ML) estimations based on *16S rRNA* gene sequences (351 unambiguously aligned bp) from *Cardinium* strains of ticks, *Cardinium* found in other arthropods (including insects and arachnids) and outgroups (*Amoebophilus* spp.).

Fig. S4 *Arsenophonus* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *yaeT* sequences (430 unambiguously aligned bp) from *Arsenophonus* strains of ticks, *Arsenophonus* found in insects and outgroups (*Proteus mirabilis* and *Providencia stuartii*).

Fig. S5 *Lariskella* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *16S rRNA* gene sequences (385 unambiguously aligned bp) from *Lariskella* strains of ticks, *Lariskella* of insects and outgroups (*Midichloria mitochondrii* and *Rickettsia* spp.).

Fig. S6 *Wolbachia* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *wsp* sequences (490 unambiguously aligned bp) from *Wolbachia* strains of ticks and of other arthropods (including insects and arachnids).

Fig. S7 *Rickettsiella* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *GroEL* sequences (578 unambiguously aligned bp) from *Rickettsiella* strains of ticks, formally described *Rickettsiella* species and outgroups (*Coxiella burnetii* and *Legionella* spp.).

Fig. S8 *Rickettsia* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *gltA* sequences (590 unambiguously aligned bp) of *Rickettsia* strains of ticks, *Rickettsia* of insects, formally described *Rickettsia* species and outgroups (*Midichloria mitochondrii* and *Ehrlichia canis*).

Fig. S9 *Spiroplasma* cladogram depicting the majority-rule consensus (60%) of phylogenetic trees constructed using maximum-likelihood (ML) estimations based on *16S rRNA* gene sequences (764 unambiguously aligned bp) from *Spiroplasma* strains of ticks, *Spiroplasma* of other arthropods (including insects and arachnids), and formally described *Spiroplasma* species.