



HAL
open science

Microbial community structure reveals instability of nutritional symbiosis during the evolutionary radiation of *Amblyomma* ticks

Florian Binetruy, Marie Buysse, Quentin Lejarre, Roxanne Barosi, Manon Villa, Nil Rahola, Christophe Paupy, Diego Ayala, Olivier Duron

► To cite this version:

Florian Binetruy, Marie Buysse, Quentin Lejarre, Roxanne Barosi, Manon Villa, et al.. Microbial community structure reveals instability of nutritional symbiosis during the evolutionary radiation of *Amblyomma* ticks. *Molecular Ecology*, 2020, 29 (5), pp.1016-1029. 10.1111/mec.15373 . hal-03001756

HAL Id: hal-03001756

<https://hal.science/hal-03001756>

Submitted on 18 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Microbial community structure reveals instability of nutritional symbiosis**
2 **during evolutionary radiation of *Amblyomma* ticks**

3 Florian Binetruy^{a*}, Marie Buysse^a, Quentin Lejarre^{a,b}, Roxanne Barosi^a, Manon Villa^a, Nil
4 Rahola^{a,b}, Christophe Paupy^a, Diego Ayala^{a,b} and Olivier Duron^a

5

6 ^a MIVEGEC (Maladies Infectieuses et Vecteurs : Ecologie, Génétique, Evolution et
7 Contrôle), Centre National de la Recherche Scientifique (CNRS) - Institut pour la Recherche
8 et le Développement (IRD) - Université de Montpellier (UM), Montpellier, France

9 ^b CIRMF (Centre International de Recherches Médicales de Franceville), Franceville, Gabon

10 * Correspondance: florian.binetruy@ird.fr

11 **Abstract**

12 Mutualistic interactions with microbes have facilitated the adaptation of major eukaryotic
13 lineages to restricted diet niches. Hence, ticks with their strictly blood-feeding lifestyle are
14 associated with intracellular bacterial symbionts through an essential B vitamin
15 supplementation. In this study, examination of the whole bacterial diversity in 25 tick species
16 of the *Amblyomma* genus showed that three intracellular bacteria, *Coxiella*-like
17 endosymbionts (LE), *Francisella*-LE and *Rickettsia*, are remarkably common. No other
18 bacterium is so uniformly present in *Amblyomma* ticks. Almost all *Amblyomma* species were
19 found to harbour a nutritive obligate symbiont, *Coxiella*-LE or *Francisella*-LE, able to
20 synthesize B vitamins. However, despite the co-evolved and obligate nature of these
21 mutualistic interactions, the structure of microbiomes does not mirror the *Amblyomma*
22 phylogeny with a clear exclusion pattern between *Coxiella*-LE and *Francisella*-LE across tick
23 species. *Coxiella*-LE, but not *Francisella*-LE, form evolutionarily stable associations with
24 ticks commonly leading to co-cladogenesis. We further evidenced symbiont replacements
25 during radiation of *Amblyomma*, with recent, and likely ongoing, invasions by *Francisella*-LE
26 and subsequent replacements of ancestral *Coxiella*-LE through transient co-infections.
27 Nutritional symbiosis in *Amblyomma* ticks is thus not a stable evolutionary state, but instead
28 arises from conflicting origins between unrelated but competing symbionts with similar
29 metabolic capabilities.

30 **Introduction**

31 Macro-organisms harbour complex microbial communities living inside and on their body
32 (Margulis, 1993; Theis et al., 2016). These microbial communities, known as the
33 microbiomes, can determine pivotal phenotypic traits of their hosts, driving a variety of
34 ecological and evolutionary processes including major nutritive, reproductive and immune
35 functions (Gould et al., 2018; Groussin et al., 2017; Hanning & Diaz-Sanchez, 2015; Ley et
36 al., 2008; Turner, James, & Poole, 2013). As hosts vary in the microbiomes they harbour, an
37 associated functionally important phenotypic variation exists within host populations (Falony
38 et al., 2016; Ferrari & Vavre, 2011; Jaenike, 2012; Oliver, Russell, Moran, & Hunter, 2003;
39 Scarborough, Ferrari, & Godfray, 2005). In arthropods, these microbiomes notably include
40 highly specialized intracellular bacteria depending almost exclusively on maternal
41 (transovarial) transmission to ensure their persistence in host populations (Moran,
42 McCutcheon, & Nakabachi, 2008; Wernegreen, 2012). Some of these maternally inherited
43 symbionts are essential for the life cycle of their arthropod hosts: They are obligate mutualists
44 able to synthesize biochemical products favouring the specialization of arthropods to novel
45 habitats or to particular feeding niches such as strict haematophagy or phloemophagy (Moran
46 et al., 2008; Wernegreen, 2012). Overall, these mutualistic interactions have facilitated the
47 radiation of major arthropod lineages, leading to remarkable host–symbiont phylogenetic
48 congruence with a strict co-cladogenesis pattern in many cases (Chen, Li, & Aksoy, 1999;
49 Duron et al., 2017; Jousset, Desdevises, & Coeur d’acier, 2009; Moran, Tran, & Gerardo,
50 2005; Takiya, Tran, Dietrich, & Moran, 2006).

51

52 Arthropods and beneficial maternally inherited symbionts can form evolutionary stable
53 associations lasting for millions of years, but that are not necessarily permanent (Bennett &
54 Moran, 2015; McCutcheon, Boyd, & Dale, 2019; Moran et al., 2008; Wernegreen, 2012).

55 Recent phylogenetic reconstructions suggest that beneficial symbiotic relationships can break
56 down: Recently acquired symbionts can replace ancestral beneficial symbionts and provide
57 similar benefits to the host (McCutcheon et al., 2019; Sudakaran, Kost, & Kaltenpoth, 2017).
58 An alternative scenario is that recently acquired symbionts may cooperate with ancestral
59 beneficial symbionts (Moran et al. 2008; Vautrin & Vavre, 2009). Vertical transmission
60 actually locks the different symbionts together as coinfection and creates then privileged
61 situations for symbiont–symbiont interactions, especially cooperation and dependence
62 between symbionts. Functions of ancestral beneficial symbionts may be complemented by
63 recently acquired cosymbionts and their coexistence can be ultimately stable over millions of
64 years (Meseguer et al 2017). New beneficial symbionts often originate from microbes
65 abundant in the host environment, potentially including entomopathogens, parasites vectored
66 by arthropods or other maternally inherited symbionts, primarily facultative (i.e. not essential)
67 for host survival (Koga & Moran, 2014; Matsuura et al., 2018; McCutcheon et al., 2019;
68 Sachs, Skophammer, & Regus, 2011). These facultative symbionts, however, determine
69 important traits in arthropods: protection against natural enemies, adaptation to changing
70 environments or reproductive traits (Engelstädter & Hurst, 2009; Ferrari & Vavre, 2011;
71 Moran et al., 2008; Oliver et al., 2003). Contrary to beneficial obligate symbionts, facultative
72 symbionts undergo occasional horizontal transfers (HT) across arthropod species, resulting in
73 limited phylogenetic congruence between hosts and symbionts (Duron, Wilkes, & Hurst,
74 2010; Jouselin, Cœur d’Acier, Vanlerberghe-Masutti, & Duron, 2013; Russell et al., 2009).
75 Overall, the diverse range of microbial lifestyle strategies creates a complex web of
76 interactions mediating the dynamics of beneficial symbioses in arthropods (McCutcheon et
77 al., 2019).
78

79 Co-existence of symbionts within microbial communities is expected to involve interactions
80 ranging from cooperation to competition and that can, in turn, determine aggregation and
81 exclusion patterns (Ferrari & Vavre, 2011; Moran et al., 2008; Vautrin & Vavre, 2009).
82 Exclusion patterns have been recently detected between maternally inherited symbionts of
83 ticks, suggesting that replacements of beneficial symbionts occur in this system (Duron et al.,
84 2017). Among arthropods, ticks (Arachnida: Ixodidae) are well known to engage in symbiotic
85 associations with at least 10 different genera of maternally inherited bacteria (Ahantrag,
86 Trinachartvanit, Baimai, & Grubhoffer, 2013; Duron et al., 2017). Ticks are specialized for an
87 exclusive diet of vertebrate blood, and have evolved intimate interactions with beneficial
88 symbionts that provide essential B vitamins and co-factors deficient in the blood diet (Bonnet,
89 Binetruy, Hernández-Jarguín, & Duron, 2017; Duron et al., 2017, 2018; Gerhart, Moses, &
90 Raghavan, 2016; Gottlieb, Lalzar, & Klasson, 2015; Guizzo et al., 2017; Hunter et al., 2015;
91 Olivieri et al., 2019; Smith, Driscoll, Gillespie, & Raghavan, 2015). Approximately two thirds
92 of tick species harbour *Coxiella*-like endosymbionts (*Coxiella*-LE hereafter), which are
93 required for tick survival and reproduction (Gottlieb et al., 2015; Guizzo et al., 2017; Smith et
94 al., 2015; Zhong, Jasinskas, & Barbour, 2007). *Coxiella*-LE genomes encode pathways for the
95 biosynthesis of major B vitamins and co-factors that fit closely with the expected nutritional
96 complements required for strict haematophagy (Gottlieb et al., 2015; Guizzo et al., 2017;
97 Smith et al., 2015). *Coxiella*-LE are abundant in two organs of ticks: ovaries, that is
98 consistent with vertical transmission into developing oocytes, and Malpighian tubules, where
99 B vitamins are possibly synthesized (Buisse, Plantard, McCoy, Duron, & Menard, 2019;
100 Wang et al., 2018)(Lalzar et al 2012). Owing to their maternal inheritance and beneficial
101 nature, *Coxiella*-LE are present in most individuals within infected host species [16, 41]. In
102 the *Rhipicephalus* tick genus, the acquisition of *Coxiella*-LE was followed by co-

103 diversification resulting in deeply congruent *Rhipicephalus*–*Coxiella*-LE phylogenies (Duron
104 et al., 2017).

105

106 In a few tick species, however, *Coxiella*-LE are present at much lower frequencies than
107 expected for obligate nutritional symbionts, suggesting that they are instead facultative
108 symbionts in these hosts (Duron et al., 2015). Phylogenetic evidence corroborates this
109 hypothesis, since closely related *Coxiella*-LE may infect distantly related tick species
110 suggesting recurrent HT of some *Coxiella*-LE (Duron et al., 2015). In other tick species, no
111 *Coxiella*-LE were detected at all, but alternative obligate beneficial symbionts have been
112 identified or hypothesized (Duron et al., 2017, 2018; Gerhart et al., 2016; Kurtti et al., 2015;
113 Olivieri et al., 2019). Indeed, *Francisella*-like endosymbionts (*Francisella*-LE) are commonly
114 found in tick species lacking *Coxiella*-LE. A recent analysis of endosymbiotic communities in
115 81 tick species also showed that there is a significant exclusion pattern between *Francisella*-
116 LE and *Coxiella*-LE (Duron et al., 2017). Like *Coxiella*-LE, *Francisella*-LE are essential for
117 tick nutrition: Ticks deprived of their *Francisella*-LE completely cease development but
118 resume normal growth upon supplementation with B vitamins (Duron et al., 2018). Genomes
119 of *Francisella*-LE contain roughly the same biosynthesis pathways of B vitamins and co-
120 factors as observed in *Coxiella*-LE genomes (Duron et al., 2018; Gerhart et al., 2016).
121 *Francisella*-LE also presents the same tropism than *Coxiella*-LE: *Francisella*-LE are
122 abundant in ovaries and Malpighian tubules of the ticks they infect (Duron et al 2018, current
123 biology). Although *Francisella*-LE and *Coxiella*-LE are distantly related, they have
124 converged towards an analogous nutritional mutualism with ticks [32, 33].

125

126 While *Coxiella*-LE symbioses are likely ancestral in ticks, replacements by *Francisella*-LE
127 having recently transitioned to an endosymbiotic lifestyle (Duron et al., 2018; Gerhart et al.,

128 2016) appear across the tick phylogeny (Duron et al., 2017). Yet, the factors favouring
129 *Francisella*-LE over *Coxiella*-LE in this evolutionary dynamic are not well understood. In
130 this study, we examined the evolutionary dynamic of tick microbiomes, with a focus on
131 beneficial nutritional symbioses, in an ecologically diverse genus of hard ticks, *Amblyomma*.
132 This genus is the third largest in the family Ixodidae, with its species primarily occupying the
133 tropical zones. The centre of species diversity is on the American continent, where half of all
134 the 130 *Amblyomma* species are found (Guglielmone, Estrada-Peña, Keirans, & Robbins,
135 2003). The *Amblyomma* genus includes major vectors of tick-borne disease agents, including
136 the lone star tick, *A. americanum*, which is the primary vector of *Ehrlichia* spp. (Childs &
137 Paddock, 2003). Only a few studies have examined the microbial diversity in *Amblyomma*
138 species, showing that they are infected either by *Coxiella*-LE or by *Francisella*-LE in addition
139 to other maternally inherited bacteria (Binetruy, Dupraz, Buysse, & Duron, 2019; Budachetri
140 et al., 2014; Clay et al., 2008; Duron et al., 2017; Gerhart et al., 2016). Here, we investigated
141 the variation in tick microbial communities at different geographic and phylogenetic scales
142 using a representative collection of specimens covering ca. 20% of *Amblyomma* species
143 diversity. First, we reconstructed the *Amblyomma* phylogeny through the sequencing of large
144 nuclear rDNA sequences (18S, ITS1, 5.8S, ITS2, and 28S rDNA). Second, we extensively
145 characterized bacterial communities in *Amblyomma* species through a DNA barcoding
146 approach targeting the 16S rDNA. Third, we traced the evolutionary histories of *Coxiella*-LE
147 and *Francisella*-LE using multilocus sequence typing (MLST) systems. While the *Coxiella*-
148 LE MLST already exists (Duron et al., 2015), in this study we developed a specific
149 *Francisella*-LE MLST. Finally, we compared *Amblyomma* phylogeny with microbiome
150 structure and further used co-phylogenetics, by comparing *Amblyomma*, *Coxiella*-LE and
151 *Francisella*-LE phylogenies, to reveal the global dynamics of symbiotic interactions.

152

153 **Materials and methods**

154 *Tick collection and processing*

155 We examined a total of 144 tick specimens belonging to 25 *Amblyomma* species (1–11
156 specimens per species) collected from field sites in America and Africa or from laboratory
157 colonies (Supplementary Table S1). Samples were preserved in 70% ethanol until use. To
158 eliminate external (i.e. cuticular) microbes, tick specimens were surface cleaned with bleach
159 prior to DNA extraction (Binetruy, Dupraz, et al., 2019). A few specimens (*A. loculosum*,
160 *n*=4; *A. sculptum*, *n*=1; *Amblyomma* sp., *n*=1, obtained from a previous study (Duron et al.,
161 2017)) were, however, not bleach-treated prior to DNA extraction (Supplementary Table S1).
162 All tick DNA was individually extracted using the DNeasy Blood & Tissue Kit (Qiagen,
163 Hilden, Germany).

164

165 *Molecular typing of ticks*

166 To reconstruct *Amblyomma* phylogeny, we sequenced almost complete rDNA sequences,
167 including the 5' end of 18S, the entire ITS1, 5.8S and ITS2 sequences, and the 3' end of 28S
168 rDNA (Supplementary Table S2) from 44 specimens (indicated in yellow in supplementary
169 Table S1). Depending on the tick species, the size of the rDNA amplicon obtained varies from
170 4270 bp to 5054 bp. PCR products were purified using the kit Cleanpcr (CleanNA,
171 Waddinxveen, The Netherlands), fragmented to a size of 300 bp and further used to construct
172 libraries with the kit Nextera XT (Illumina, San Diego, California, USA). These libraries were
173 then pooled together, indexed and pair-end sequenced on a Miseq (Illumina) sequencer using
174 a flow cell equipped with a V3, 600-cycle reagent cartridge.

175

176 We obtained >24 million reads after quality filtering and removing adaptors using the
177 Cutadapt tool on a Galaxy workbench (Goecks, Nekrutenko, Taylor, & The Galaxy Team,

178 2010; M. Martin, 2011). Paired-end reads were *de novo* assembled using metaSPAdes v3.11
179 with k-mer sizes of 21, 33, 55, and 73 bp (Nurk, Meleshko, Korobeynikov, & Pevzner, 2017).
180 The resulting metaSPAdes contigs were binned into tick nuclear DNA and non-tick nuclear
181 DNA groups through megablast in the GenBank nucleotide collection (Boratyn et al., 2013).
182 These contigs were scaffolded, when applicable, by aligning to the complete sequences of the
183 rRNA genes operon of *A. americanum* (GenBank AF291874), *A. hebraeum* (GenBank
184 KY457489) and the partial sequence of *A. marmoreum* (GenBank KY457492) using MEGA
185 (Kumar, Stecher, & Tamura, 2016).

186

187 *Bacterial metabarcoding*

188 A 251-bp portion of the V4 variable region of the bacterial 16S rRNA gene was amplified
189 individually for each DNA sample using a Multiplex PCR Kit (Qiagen) and universal primers
190 (16SV4F: 5'-GTGCCAGCMGCCGCGGTAA-3' and 16SV4R: 5'-
191 GGACTACHVGGGTWTCTAATCC-3') (Galan et al., 2016). Amplified bacterial 16S rDNA
192 products were purified and sequenced using an Illumina MiSeq platform (GenSeq,
193 Montpellier University) and 251-bp end sequence reads were obtained,. All bioinformatic
194 analyses were conducted using the pipeline Frogs
195 (<https://github.com/geraldinepascal/FROGS>) (Escudié et al., 2018) as previously described
196 (Binetruy et al., 2019). One step of post-process operational taxonomic unit (OTU) affiliation
197 was additionally performed through the Frogs pipeline on a Galaxy workbench (Escudié et
198 al., 2018; Goecks et al., 2010). This step consists in aggregating OTUs that share 97% of
199 identity within 99% of the amplicon length, and it reduced the probability of keeping
200 artefactual OTUs by resolving multi-hit ambiguities. To control the contamination during
201 these procedure negative controls were performed (three negative extraction controls were
202 included in all extraction series, two negative PCR controls were included in all PCR series).

203 Moreover. OTUs having a maximal abundance in negative controls were discarded and false-
204 positive OTUs were removed by filtering OTU representing less than 0.005% of the OTU
205 total abundance (Bokulich et al., 2013). Following this procedure, the microbiome was
206 determined individually for almost all *Amblyomma* specimens ($n=142/143$); however, the
207 amount of DNA for the single specimen of *A. sculptum* was not sufficient.

208 A phylogenetic tree using OTU sequences and beta-diversity matrices based on this tree was
209 assessed with FastTree and GUnifrac packages in R (J. Chen et al., 2012; Price, Dehal, &
210 Arkin, 2009), using the computational procedure described in (Binetruy, Dupraz, et al., 2019).
211 Multidimensional scaling (MDS) plots were then generated using the package ggplot2 in R
212 (Wickham, 2016). Permutational multivariate analysis of variance (PERMANOVA)
213 implemented in the vegan package in R or pairwise PERMANOVA (Arbizu, 2017/2018) was
214 further performed on the generalized UniFrac ($\alpha = 0.5$) dissimilarity matrix to evaluate the
215 potential impact of the presence of symbionts and co-infection on bacterial diversity. The *P*-
216 values of the pairwise PERMANOVA were corrected for multiple comparisons using Holm's
217 method (Holm, 1979). A Mantel test was used to examine the association between microbial
218 diversity (GUnifrac distance) and *Amblyomma* phylogeny using the ecodist package in R
219 ($n=9999$ permutations) (Goslee & Urban, 2007).

220

221 *Multilocus typing of Coxiella-LE and Francisella-LE*

222 *Coxiella*-LE were genotyped through nested or semi-nested PCR amplification and
223 sequencing of three housekeeping genes (16S rRNA, *rpoB* and *groEL*) previously developed
224 for the *Coxiella* MLST methodology (Duron et al., 2015) (Supplementary Table S2). No
225 *Francisella*-LE MLST was previously developed and we thus used the published genome of
226 *Francisella*-LE F-Om strain (isolated from the soft tick *Ornithodoros moubata* [32];
227 GenBank accession number: QAPC000000000) as reference to design specific PCR primers

228 for five genes (16S rRNA, *rpoB*, *groEL*, *ftsZ* and *gyrB*; Supplementary Table S2). Positive
229 PCR products were purified and sequenced in both directions by Eurofins (Ebersberg,
230 Germany). Sequence chromatograms were manually cleaned with Chromas Lite
231 (http://www.technelysium.com.au/chromas_lite.html) and aligned with CLUSTALW
232 implemented in MEGA 7 (Kumar et al., 2016; Thompson, Gibson, & Higgins, 2002).

233

234 *Phylogenetic analyses*

235 Phylogenetic relationships were assessed using *Amblyomma*, *Coxiella*-LE and *Francisella*-LE
236 sequences produced in this study and additional sequences available in GenBank (including
237 *Amblyomma*, *Coxiella*-LE and *Francisella*-LE relatives and outgroups). GBLOCKS
238 (Castresana, 2000) was used to remove poorly aligned positions and to obtain unambiguous
239 sequence alignments. All sequence alignments were also checked for putative recombinant
240 regions using the RDP3 analysis package (D. P. Martin et al., 2010). The best fitting
241 evolutionary models were determined using the Akaike information criterion and Bayesian
242 information criterion with MEGA 7 (Kumar et al., 2016). Phylogenetic analyses were based
243 on Bayesian inferences (BI) with MrBayes v3.2.7 (Ronquist et al., 2012). Two replicate
244 analyses were run for 1 million generations. For each replicate, we ran one cold chain and
245 three hot chains of the Markov chain Monte Carlo method, using a random starting tree and
246 sampling trees every 100 generations and discarding 25% as burn-in. The remaining trees
247 were used to calculate 50% majority-rule consensus trees.

248

249 The BI phylogenies of *Amblyomma*, *Coxiella*-LE and *Francisella*-LE were then used to
250 conduct co-phylogenetic analyses using the Procrustean Approach to Cophylogeny (PACo)
251 package in R (Balbuena, Míguez-Lozano, & Blasco-Costa, 2013; Hutchinson, Cagua,
252 Balbuena, Stouffer, & Poisot, 2017). The significance of the co-phylogenetic tests was

253 established by 100,000 random permutations of the two-association matrix. To test an effect
254 of *Rickettsia* co-infection on the evolution of *Francisella*-LE and *Coxiella*-LE, linear
255 regressions were computed in R using the matrix of genetic distances of these symbionts and
256 the presence/absence of *Rickettsia* as an explicative variable.

257

258 *Ethics statement*

259 The use of the genetic resources was declared to the French Ministry of the Environment
260 (reference TREL19028117S/156) and to Gabon government (entry authorization
261 #AE16008/PR/ANPN/SE/CS/AEPN and #research authorization
262 #AR0013/16/MESRS/CENAREST/CG/CST/CSAR).

263

264 **Results**

265 *Phylogeny of Amblyomma ticks*

266 We first reconstructed the phylogenetic relationships among the 25 *Amblyomma* species using
267 BI analyses based on large fragments of nuclear rDNA sequences obtained from one-to-two
268 specimens per species (Figure 1). The findings support a monophyletic origin of the five
269 African *Amblyomma* species (AF group): They cluster together in a robust clade nested
270 among the 20 New World *Amblyomma* species (NW group), suggesting an American origin
271 of these African species (Figure 1). Moreover, the phylogeny of *Amblyomma* also parallels
272 the tick host-range at least in the NW group: Closely related *Amblyomma* species often share
273 the same host species, such as *A. dissimile* and *A. rotundatum* feeding on poikilotherms
274 (reptiles and amphibians) or *A. latepunctatum*, *A. scalpturatum* and *A. naponense* feeding
275 mainly on tapirs and Suidae (Figure 1). However, in other cases, closely related *Amblyomma*
276 species use different host species, such as *A. ovale*, a generalist species feeding on a diversity

277 of domestic and wild animals, and *A. varium*, a specialized species feeding on arboreal
278 vertebrates.

279

280 *Microbial diversity and symbiont prevalence*

281 We further examined the whole bacterial diversity of 24 out of the 25 *Amblyomma* species
282 ($n=142$ specimens, one-to-ten specimens were examined per species) via high-throughput 16S
283 rDNA sequencing. The amount of DNA for the 25th *Amblyomma* species, *A. sculptum* ($n=1$),
284 was not sufficient to perform bacterial barcoding (this specimen was already PCR-typed for
285 *Coxiella*-LE and *Francisella*-LE, as further detailed). After filtration of false-positive OTUs
286 and contaminants (Binetruy, Dupraz, et al., 2019; Birer, Tysklind, Zinger, & Duplais, 2017),
287 4,364,360 reads distributed in 195 OTUs were obtained (Table S3). *Coxiella*-LE and
288 *Francisella*-LE were the most abundant bacterial genera representing 33.6% and 33.1% of the
289 total number of reads, respectively (Figure 2 and Supplementary Table S1). Other bacterial
290 genera were found: In most cases, each represented a negligible part of the 16S rDNA reads
291 when present. A remarkable exception to this pattern was the presence of abundant
292 intracellular bacteria belonging to the *Rickettsia* genus in 16 *Amblyomma* species (Figure 2
293 and Supplementary Table S1). Other exceptions included the *A. romitii* samples for which no
294 *Coxiella*-LE and *Francisella*-LE reads were detected: Most reads were assigned to a tick-
295 borne pathogen, *Ehrlichia* sp. In *A. loculosum* and *Amblyomma* sp. samples, *Coxiella*-LE
296 reads were detected but reads of other bacteria were more abundant. Since the *A. loculosum*
297 and *Amblyomma* sp. samples were not bleach-treated prior to DNA extraction (as done for the
298 other samples), the abundant presence of these bacteria may be due to the cuticular
299 bacteriome, as recently observed in another *Amblyomma* species (Binetruy, Dupraz, et al.,
300 2019).

301

302 The structure of microbial diversity was not globally impacted by the phylogenetic proximity
303 among *Amblyomma* species: The dendrogram of microbial diversity did not parallel the
304 *Amblyomma* phylogeny (Mantel two-tailed test, $R=0.16$, $P=0.19$) (Supplementary Figure S1).
305 However, the MDS plot suggested an effect of tick species on bacterial diversity
306 (Supplementary Figure S2), as confirmed by the PERMANOVA analysis ($R^2=0.51$,
307 $P=0.001$). Further testing showed a clear separation between *Coxiella*-LE-infected tick
308 species/specimens and *Francisella*-LE-infected ones (pairwise PERMANOVA analysis,
309 $R^2=0.31$, adjusted P for multiple comparisons= 0.0003 ; Figure 3A). *Rickettsia* also structures
310 the bacterial diversity but to a lesser extent than *Coxiella*-LE and *Francisella*-LE
311 (PERMANOVA, $R^2=0.11$, $P=0.001$; Figure 3B). The *Coxiella*-LE and *Francisella*-LE
312 clusters are actually structured into two sub-clusters each, fitting with the presence of
313 *Rickettsia* (Figure 3c) as corroborated by PERMANOVA analyses: (1) *Coxiella*-LE without
314 *Rickettsia* vs. *Coxiella*-LE with *Rickettsia* ($R^2=0.2$, adjusted P for multiple
315 comparisons= 0.0015), and (2) *Francisella*-LE without *Rickettsia* vs. *Francisella*-LE with
316 *Rickettsia* ($R^2=0.22$, adjusted P for multiple comparisons= 0.0015).
317
318 Of the 25 *Amblyomma* species (including *A. sculptum*) examined for the presence of *Coxiella*-
319 LE and *Francisella*-LE, 24 were infected by one or both of these symbionts: 11 *Amblyomma*
320 species harbour only *Coxiella*-LE, 13 species only *Francisella*-LE and three species both
321 (Figure 2, Supplementary Table S1). Only *A. romitii* was not infected by *Coxiella*-LE and
322 *Francisella*-LE but this may be explained by the presence of the tick-borne pathogen
323 *Ehrlichia* sp., which may mask the presence of other bacteria. In the 24 infected *Amblyomma*
324 species, *Coxiella*-LE and *Francisella*-LE were not randomly associated (Fisher's exact test,
325 $P=0.001$): These two symbionts co-occurred in the same tick species less frequently than
326 expected by chance (exclusion pattern), meaning that their distribution across tick species was

327 strongly dependent on each other. Conversely, neither *Coxiella*-LE nor *Francisella*-LE
328 showed a non-random association with *Rickettsia* (Fisher's exact tests, $P=0.99$ and 0.68 ,
329 respectively). However, *Coxiella*-LE and *Francisella*-LE had a patchy and quite uniform
330 distribution along the *Amblyomma* phylogeny: While some closely related *Amblyomma*
331 species were infected by the same symbiont genus (e.g. *A. dissimile* and *A. rotundatum* by
332 *Francisella*-LE, or *A. latepunctatum*, *A. sculpturatum* and *A. naponense* by *Coxiella*-LE),
333 others were not (e.g. *A. americanum*, infected by *Coxiella*-LE, and *A. oblongoguttatum* by
334 *Francisella*-LE) (Figure 2). This distribution pattern paralleled partly the tick host-range:
335 Indeed, *A. dissimile* and *A. rotundatum* that are related and specialized for poikilotherms were
336 both infected by *Francisella*-LE (Figure 1, 2).

337

338 In the tick species they infect, *Coxiella*-LE and *Francisella*-LE were present in most
339 specimens examined (Figure 2, Supplementary Table S1). However, a more contrasted pattern
340 was apparent in the three *Amblyomma* species that were co-infected by *Coxiella*-LE and
341 *Francisella*-LE: (i) in *A. geayi*, of the 10 examined specimens, seven were infected by
342 *Coxiella*-LE in one locality, but in other localities, two specimens were infected by
343 *Francisella*-LE and one was co-infected by *Coxiella*-LE and *Francisella*-LE; (ii) in *A.*
344 *latepunctatum*, of the four examined specimens, three specimens of the same locality were co-
345 infected by *Coxiella*-LE and *Francisella*-LE but one specimen from another locality was only
346 infected by *Coxiella*-LE; (iii) in *A. sculptum*, the single examined specimen was co-infected
347 by *Coxiella*-LE and *Francisella*-LE (Figure 2, Supplementary Table S1). In contrast to
348 *Coxiella*-LE and *Francisella*-LE, the prevalence of *Rickettsia* was heterogeneous, with
349 infection frequencies ranging from 14% to 100% depending on tick species (Figure 2,
350 Supplementary Table S1). In all cases, *Rickettsia* was found with either *Coxiella*-LE (seven

351 *Amblyomma* species) or *Francisella*-LE (seven species) or both (two species). Neither
352 *Rickettsia* nor *Coxiella*-LE nor *Francisella*-LE shows infection-biased sex ratio: within each
353 *Amblyomma* species, the prevalence of infection did not differ between males and females
354 (Fisher's exact test, all $P>0.1$).

355

356 *Evolutionary history of Coxiella-LE and Francisella-LE symbioses*

357 Sequencing of three *Coxiella* MLST genes (16S rRNA, *rpoB* and *groEL*) led to the
358 identification of 14 genetically different *Coxiella*-LE in a subset of 32 specimens representing
359 the 14 infected *Amblyomma* species (one to four specimens per species were examined). Each
360 *Amblyomma* species was infected by a genetically distinct *Coxiella*-LE and no variation of
361 *Coxiella*-LE was observed among specimens belonging to the same *Amblyomma* species. We
362 observed no sign of recombination in the *Coxiella*-LE data set (all $P>0.05$ for the
363 GENECONV and RDP recombination-detection tests) and we thus used the 16S rRNA, *rpoB*
364 and *groEL* concatenated sequences for BI analyses. Comparisons with other sequences
365 available on GenBank showed that the *Coxiella*-LE of *Amblyomma* are polyphyletic: They
366 were scattered into different well-supported clusters among *Coxiella*-LE of other tick species
367 (Supplementary Figure S3). Indeed, the *Coxiella*-LE of *A. variegatum*, *A. tholloni* and *A.*
368 *splendidum* form a monophyletic clade that is more closely related to the *Coxiella*-LE of
369 *Ixodes* tick species than to the *Coxiella*-LE of other *Amblyomma* species. Similarly, the
370 *Coxiella*-LE clade of *A. cajennense*, *A. sculptum* and *A. americanum* is more closely related to
371 the *Coxiella*-LE of *Dermacentor* tick species. This pattern is suggestive of recurrent HT
372 events of *Coxiella*-LE among tick species. However, the *Coxiella*-LE clusters of *Amblyomma*
373 species can be gathered into two main groups, one with all the *Coxiella*-LE of NW
374 *Amblyomma* species and the other with all the *Coxiella*-LE of AF *Amblyomma* species
375 (Supplementary Figure S3). This pattern suggests an effect of phylogeographic drivers in

376 structuring the evolution of *Coxiella*-LE. This is strongly supported by the co-phylogeny
377 analysis between *Coxiella*-LE and *Amblyomma* phylogenies: There is a significant topological
378 congruence between their phylogeny (PACo analysis, $P=0.0001$; Figure 4A). This shows that
379 co-cladogenesis with *Coxiella*-LE occurred during the radiation of *Amblyomma*.

380

381 Sequencing of five *Francisella* MLST genes (16S rRNA, *rpoB*, *groEL*, *ftsZ* and *gyrB*) led to
382 the identification of 15 genetically different *Francisella*-LE in a subset of 28 tick specimens
383 representing the 13 infected *Amblyomma* species (one to four specimens per species were
384 used). There was only one *Francisella*-LE in each *Amblyomma* species, except *A. pacae* and
385 *A. rotundatum* in which two and three genetically distinct *Francisella*-LE, respectively, were
386 present in specimens from different localities. Each *Amblyomma* species harbours genetically
387 distinct *Francisella*-LE, except for *A. geayi* and *A. latepunctatum* that harbour identical
388 *Francisella*-LE on the basis of their MSLT sequences (Supplementary Figure S4). Owing to
389 the lack of *Francisella*-LE *rpoB*, *groEL*, *ftsZ* and *gyrB* gene sequences available in GenBank
390 before this study (with the exception of two published *Francisella*-LE genomes), the BI
391 phylogenetic analysis between the *Francisella*-LE of *Amblyomma* and those of other tick
392 species (with sequences available in GenBank) was made using only their 16S rRNA
393 nucleotidic sequences (Supplementary Figure S5). No *Francisella*-LE subclade specific to
394 *Amblyomma* exists along the 16S rRNA phylogenetic tree: The *Francisella*-LE of
395 *Amblyomma* are instead scattered among *Francisella*-LE of other tick genera suggesting
396 recurrent HT events among unrelated tick species (Supplementary Figure S5). However, the
397 inner topology of the *Francisella*-LE clade based on 16S rRNA gene sequences remained too
398 poorly resolved in many cases (as shown by low support values of inner branches) to infer the
399 exact relatedness among all *Francisella*-LE. We thus further reconstructed the phylogenetic
400 relationships between *Francisella*-LE using BI analyses based on their 16S rRNA, *rpoB*,

401 *groEL*, *ftsZ* and *gyrB* nucleotidic sequences. We observed no sign of recombination in the
402 *Francisella*-LE data set (all $P > 0.05$ for the GENECONV and RDP recombination-detection
403 tests) and we thus used the 16S rRNA, *rpoB*, *groEL*, *ftsZ* and *gyrB* concatenated sequences
404 for analyses. Conversely to *Coxiella*-LE, there was no apparent co-cladogenesis or
405 phylogeographic pattern along the *Francisella*-LE phylogeny (Supplementary Figure S4).
406 Indeed, the *Francisella*-LE of *A. sculptum* (NW group) is closely related to the *Francisella*-
407 LE of an unrelated *Amblyomma* species, *A. paulopunctatum* (AF group). In addition, the
408 *Francisella*-LE of *A. sculptum* and *A. paulopunctatum* are more closely related to the
409 *Francisella*-LE of the soft tick *O. moubata* than to the *Francisella*-LE of other *Amblyomma*
410 species. The co-phylogeny analysis also showed no significant signal of congruence between
411 the *Francisella*-LE and *Amblyomma* phylogenies (PACo analysis, $P = 0.06$): Only HT events
412 seem to have impacted the distribution *Francisella*-LE across *Amblyomma* phylogeny (Figure
413 4B). Interestingly, while co-infection with *Rickettsia* along the *Coxiella*-LE phylogeny is
414 random (linear model, adjusted $R^2 = -0.01$, F-stat=0.60, $P = 0.56$; Supplementary Figure S6A),
415 it is not so along the *Francisella*-LE phylogeny (adjusted $R^2 = 0.15$, F-stat=6.90, $P = 0.002$;
416 Figure S6B): Co-infections with *Rickettsia* are more common with certain *Francisella*-LE
417 subclades than with others.

418

419 **Discussion**

420 Three intracellular bacterial genera, *Coxiella*-LE, *Francisella*-LE and *Rickettsia*, are
421 widespread across the 25 species of *Amblyomma* ticks we examined in this study. Only a few
422 other bacteria have been detected and none is so uniformly present in *Amblyomma*. However,
423 the structure of the microbiomes does not mirror the *Amblyomma* phylogeny and closely
424 related *Amblyomma* commonly harbour divergent microbiomes. As expected, almost all
425 *Amblyomma* species were found to harbour a nutritive obligate symbiont, *Coxiella*-LE or

426 *Francisella*-LE, both able to synthesize B vitamins but with a clear exclusion pattern between
427 them: *Coxiella*-LE was found as a single infection in 11 *Amblyomma* species, *Francisella*-LE
428 in 13 species and co-infection was seen in only three species. Despite the co-evolved and
429 obligate interactions of ticks with their mutualistic partners, we detected evidence of symbiont
430 replacements during radiation of *Amblyomma*, raising questions regarding the ecological and
431 evolutionary factors underlying replacements.

432

433 The comparison of symbiont and tick phylogenies revealed that the *Coxiella*-LE symbiosis is
434 ancient and arose in the early evolution of the *Amblyomma* genus. Hence, some *Coxiella*-LE
435 are specialized for their *Amblyomma* hosts, with an ancient acquisition followed by co-
436 diversification, meaning that the persistence of *Coxiella*-LE through vertical transmission is
437 stable over the duration of *Amblyomma* species diversification. A very similar co-
438 diversification pattern has also been reported for *Coxiella*-LE symbiosis in the *Rhipicephalus*
439 tick genus (Duron et al., 2017). However, the spread of *Coxiella*-LE was more complex in
440 *Amblyomma*: The infections found in some *Amblyomma* species are distantly related and do
441 not form an *Amblyomma*-specific clade. Rather, phylogenetics shows that *Coxiella*-LE of
442 *Amblyomma* are actually scattered among *Coxiella*-LE of other tick genera such as *Ixodes* and
443 *Dermacentor*. Only extensive HT of *Coxiella*-LE among tick genera may explain these
444 phylogenetic incongruences. Since facultative, but not obligate, symbionts can undergo HT
445 between host species (Bennett & Moran, 2015; McCutcheon et al., 2019; Nancy A. Moran et
446 al., 2008; Wernegreen, 2012), this suggests that some *Coxiella*-LE are facultative symbionts
447 of ticks. Interestingly, *Coxiella*-LE is a facultative symbiont in some *Ixodes* species, such as *I.*
448 *ricinus* and *I. uriae* (Duron et al., 2017; Duron, Jourdain, & McCoy, 2014; Duron et al.,
449 2015). The phylogenetic proximity of *Coxiella*-LE of *Ixodes* spp. with the *Coxiella*-LE of *A.*
450 *variegatum*, *A. splendidum* and *A. tholloni* thus suggests that a facultative *Coxiella*-LE of

451 *Ixodes* spp. had an early jump to the *Amblyomma* ancestor of these species before replacing
452 the ancestral obligate symbiont and evolving obligate nutritional symbioses with current
453 species.

454

455 The infection dynamics of *Francisella*-LE is different to that of *Coxiella*-LE. While the
456 *Francisella*-LE of ticks form a monophyletic clade within the *Francisella* genus (Duron et al.,
457 2017, 2018), we observed frequent HT events between unrelated tick species. No co-
458 cladogenesis signal with *Amblyomma* is apparent along the phylogenies, meaning that current
459 *Francisella*-LE arose only recently in this genus. The presence of unrelated *Francisella*-LE in
460 *Amblyomma* further indicates that several independent acquisitions of *Francisella*-LE have
461 occurred during the radiation of this tick genus. These acquisitions have likely come at the
462 expense of the *Coxiella*-LE with their ultimate replacement by *Francisella*-LE. The AF
463 *Amblyomma* group is illustrative of this process: Most species (*A. splendidum*, *A. variegatum*,
464 *A. tholloni* and *A. loculosum*) are infected by *Coxiella*-LE with a strong co-cladogenesis
465 pattern, but one species (*A. paulopunctatum*) is infected by *Francisella*-LE. This pattern
466 suggests that *Francisella*-LE has replaced the *Coxiella*-LE primarily present in the *A.*
467 *paulopunctatum* ancestor. Other examples include the monophyletic group formed by *A.*
468 *americanum*, *A. oblongoguttatum*, *A. cajennense* and *A. sculptum*: *Coxiella*-LE has co-
469 diverged with all species but one, since here *Francisella*-LE eliminated the *Coxiella*-LE
470 primarily present in the *A. oblongoguttatum* ancestor. In addition, *A. sculptum* is co-infected
471 at the individual level by an ancestral *Coxiella*-LE (i.e. showing a co-cladogenesis pattern
472 with *Coxiella*-LE of the *A. sculptum* relatives) and a recently acquired *Francisella*-LE. This
473 pattern suggests that HT of *Francisella*-LE within *Amblyomma* communities is recent, likely
474 ongoing, and that co-infections with ancestral *Coxiella*-LE are only transitory. The
475 preferential association of some *Francisella*-LE with *Rickettsia* further implies that these co-

476 infections may be important drivers since some *Rickettsia* are also able to synthesize folate
477 (B9 vitamin) (Hunter et al., 2015) and thus to participate in nutritional symbiosis along with
478 *Francisella*-LE. Under this hypothesis, *Francisella*-LE and *Rickettsia* are cooperating
479 together, and each may fulfil essential metabolic functions not ensured by the others. They
480 may also act together to replace ancestral *Coxiella*-LE.

481

482 Some biological traits of these symbioses are indicative of how and why *Francisella*-LE and
483 some *Coxiella*-LE are both eliminating ancestral *Coxiella*-LE in *Amblyomma* ticks.
484 *Francisella*-LE or *Coxiella*-LE were occasionally detected in the salivary glands of several
485 tick species (Budachetri et al., 2014; Buysse et al., 2019; Klyachko, Stein, Grindle, Clay, &
486 Fuqua, 2007) suggesting that ticks may inject part of their symbionts during feeding. Ticks,
487 unlike other arthropod vectors, often attach and aggregate on the host for several days to
488 obtain a meal, a process termed ‘co-feeding’. The spatiotemporal proximity of ticks during
489 co-feeding may favour the HT of *Francisella*-LE and *Coxiella*-LE between conspecifics but
490 also between different tick species, as commonly observed for tick-borne pathogens
491 (Voordouw, 2015; Wright, Sonenshine, Gaff, & Hynes, 2015). This process may lead to local
492 or systemic infections in vertebrates since a few cases of opportunistic *Coxiella*-LE infections
493 have been reported after tick feeding (Shivaprasad et al., 2008; Vapniarsky, Barr, & Murphy,
494 2012; Woc-Colburn et al., 2008, p.). This mode of transmission may be particularly
495 significant for *Francisella*-LE and *Coxiella*-LE by leading to co-infections with ancestral
496 *Coxiella*-LE in ticks. Interestingly, we found two *Amblyomma* species, *A. geayi* and *A.*
497 *latepunctatum*, that are co-infected by their respective ancestral *Coxiella*-LE but that also
498 share the same *Francisella*-LE, which is also closely related to the *Francisella*-LE of *A.*
499 *dissimile*. The genetic proximity of *Francisella*-LE in these three unrelated *Amblyomma*
500 species is suggestive of recent HT events through co-feeding: While these three *Amblyomma*

501 species feed on very different vertebrate hosts, hampering the possibility of co-feeding, the
502 immature stages of *A. dissimile* are commonly found in diverse mammals or birds (Binetruy,
503 Chevillon, de Thoisy, Garnier, & Duron, 2019; Guglielmone & Nava, 2010; Scott & Durden,
504 2015), suggesting that they may be ecological bridges driving HT of *Francisella*-LE across
505 tick species.

506

507 By replacing the ancestral *Coxiella*-LE, the novel symbiont colonizes a pre-adapted tick
508 physiological environment that requires the provision of B vitamin: Thus, it must be able to
509 synthesize these compounds as the ancestral *Coxiella*-LE did. The presence of several B
510 vitamin biosynthesis pathways is ancestral in the *Coxiella* and *Francisella* genera, and all
511 members of these genera, including pathogenic species that are also all intracellular, have
512 conserved these abilities through their radiation (Duron et al., 2018; Gerhart et al., 2016;
513 Meibom & Charbit, 2010; Rowe & Huntley, 2015; Smith et al., 2015; van Schaik, Chen,
514 Mertens, Weber, & Samuel, 2013): All *Francisella*-LE and *Coxiella*-LE are already pre-
515 adapted to nutritional symbioses with ticks. Beyond B vitamins, the replacement of ancestral
516 *Coxiella*-LE suggests that the new symbiont could supply an additional benefit that the
517 ancestral *Coxiella*-LE was unable to supply to ticks, thereby out-competing them. The
518 genomes of ancient beneficial endosymbionts have lost most of their gene contents from their
519 ancestor, being usually small in size and dense in gene content but also suffering Muller's
520 ratchet, with fixation of deleterious mutations through genetic drift (McCutcheon et al., 2019;
521 McCutcheon & Moran, 2012; N. A. Moran, 1996; Rispé & Moran, 2000). This evolution
522 towards massive genomic reduction is obvious for the *Coxiella*-LE of *A. americanum*, which
523 have a genome of only 0.66 Mb (Smith et al., 2015), but not for the *Francisella*-LE of *A.*
524 *maculatum*: Its genome is 1.56 Mb, and although half is pseudogenized, it may have a higher
525 biosynthetic capability (Gerhart et al., 2016). Similar variation is also reported between

526 *Coxiella*-LE with some genomes reaching ca. 1.5 Mb (Gottlieb et al., 2015; Ramaiah &
527 Dasch, 2018), suggesting that some *Coxiella*-LE have greater biosynthetic capabilities than
528 others. However, other processes may act on the *Coxiella*-LE replacement. Indeed, ancestral
529 *Coxiella*-LE may have evolved too reduced (degraded) genomes and become maladapted,
530 opening the road to replacement by a new symbiont. This degeneration–replacement model
531 has been proposed for other arthropods such as cicadas (Campbell et al., 2015; Łukasik et al.,
532 2018; Matsuura et al., 2018), but replacements are expected to be transient making them
533 difficult to observe (McCutcheon et al., 2019). In *Amblyomma*, the observations of three
534 species with co-infections by ancestral *Coxiella*-LE and recently acquired *Francisella*-LE
535 may correspond to this transient state before extinction of *Coxiella*-LE.

536

537 That ecological specialization to strict haematophagy is driven by nutritional symbiotic
538 interactions is beyond doubt for ticks. The present study, nevertheless, shows that nutritional
539 symbiosis in the *Amblyomma* genus is not stable state, being impacted by competition
540 between *Coxiella*-LE and *Francisella*-LE or between *Coxiella*-LE themselves. We potentially
541 underestimate the amplitude of this dynamics: our intraspecific sampling was low for some
542 tick species and this may have led to an underestimation of the *Francisella*-LE frequency.
543 Indeed, in the cases of low prevalence, most samples will be found not infected by
544 *Francisella*-LE while other members of the species, not sampled and tested, are in fact
545 infected. Precisely, this pattern is found in this study: we found variation *Francisella*-LE
546 infection pattern between sampling localities in three tick species in which just few few
547 specimens were infected, which clearly indicates this potential for false negatives arising from
548 insufficient sampling. Anyway, ticks now march on with their recently acquired *Francisella*-
549 LE, at the expense of *Coxiella*-LE, but the precise mechanisms providing the advantage to
550 *Francisella*-LE remain to be determined, including endosymbiosis intrinsic factors, such as

551 competition between symbionts with similar metabolic capabilities and the differential degree
552 of genome reduction.

553

554 **References**

555 Ahantarig, A., Trinachartvanit, W., Baimai, V., & Grubhoffer, L. (2013). Hard ticks and their bacterial
556 endosymbionts (or would be pathogens). *Folia Microbiologica*, 58(5), 419–428. doi:
557 10.1007/s12223-013-0222-1

558 Arbizu, P. M. (2018). *Pairwise multilevel comparison using adonis*. Contribute to
559 *pmartinezarbizu/pairwiseAdonis* development by creating an account on GitHub [R].
560 Retrieved from <https://github.com/pmartinezarbizu/pairwiseAdonis> (Original work published
561 2017)

562 Balbuena, J. A., Míguez-Lozano, R., & Blasco-Costa, I. (2013). PACo: A Novel Procrustes Application to
563 Cophylogenetic Analysis. *PLOS ONE*, 8(4), e61048. doi: 10.1371/journal.pone.0061048

564 Bennett, G. M., & Moran, N. A. (2015). Heritable symbiosis: The advantages and perils of an
565 evolutionary rabbit hole. *Proceedings of the National Academy of Sciences of the United*
566 *States of America*, 112(33), 10169–10176. doi: 10.1073/pnas.1421388112

567 Binetruy, F., Chevillon, C., de Thoisy, B., Garnier, S., & Duron, O. (2019). Survey of ticks in French
568 Guiana. *Ticks and Tick-Borne Diseases*, 10(1), 77–85. doi: 10.1016/j.ttbdis.2018.09.003

569 Binetruy, F., Dupraz, M., Buysse, M., & Duron, O. (2019). Surface sterilization methods impact
570 measures of internal microbial diversity in ticks. *Parasites & Vectors*, 12(1), 268. doi:
571 10.1186/s13071-019-3517-5

572 Birer, C., Tysklind, N., Zinger, L., & Duplais, C. (2017). Comparative analysis of DNA extraction
573 methods to study the body surface microbiota of insects: A case study with ant cuticular
574 bacteria. *Molecular Ecology Resources*, 17(6), e34–e45. doi: 10.1111/1755-0998.12688

575 Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., ... Caporaso, J. G.
576 (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon
577 sequencing. *Nature Methods*, 10(1), 57–59. doi: 10.1038/nmeth.2276

578 Bonnet, S. I., Binetruy, F., Hernández-Jarguín, A. M., & Duron, O. (2017). The Tick Microbiome: Why
579 Non-pathogenic Microorganisms Matter in Tick Biology and Pathogen Transmission. *Frontiers*
580 *in Cellular and Infection Microbiology*, 7. doi: 10.3389/fcimb.2017.00236

581 Boratyn, G. M., Camacho, C., Cooper, P. S., Coulouris, G., Fong, A., Ma, N., ... Zaretskaya, I. (2013).
582 BLAST: a more efficient report with usability improvements. *Nucleic Acids Research*, 41(Web
583 Server issue), W29–W33. doi: 10.1093/nar/gkt282

584 Budachetri, K., Browning, R. E., Adamson, S. W., Dowd, S. E., Chao, C.-C., Ching, W.-M., & Karim, S.
585 (2014). An insight into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae).
586 *Journal of Medical Entomology*, 51(1), 119–129. doi: 10.1603/me12223

587 Buysse, M., Plantard, O., McCoy, K. D., Duron, O., & Menard, C. (2019). Tissue localization of Coxiella-
588 like endosymbionts in three European tick species through fluorescence in situ hybridization.
589 *Ticks and Tick-Borne Diseases*, 10(4), 798–804. doi: 10.1016/j.ttbdis.2019.03.014

590 Campbell, M. A., Leuven, J. T. V., Meister, R. C., Carey, K. M., Simon, C., & McCutcheon, J. P. (2015).
591 Genome expansion via lineage splitting and genome reduction in the cicada endosymbiont
592 *Hodgkinia*. *Proceedings of the National Academy of Sciences*, 112(33), 10192–10199. doi:
593 10.1073/pnas.1421386112

594 Castresana, J. (2000). Selection of Conserved Blocks from Multiple Alignments for Their Use in
595 Phylogenetic Analysis. *Molecular Biology and Evolution*, 17(4), 540–552. doi:
596 10.1093/oxfordjournals.molbev.a026334

597 Chen, J., Bittinger, K., Charlson, E. S., Hoffmann, C., Lewis, J., Wu, G. D., ... Li, H. (2012). Associating
598 microbiome composition with environmental covariates using generalized UniFrac distances.
599 *Bioinformatics (Oxford, England)*, 28(16), 2106–2113. doi: 10.1093/bioinformatics/bts342

600 Chen, X., Li, S., & Aksoy, S. (1999). Concordant evolution of a symbiont with its host insect species:
601 molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont,
602 *Wigglesworthia glossinidia*. *Journal of Molecular Evolution*, *48*(1), 49–58.

603 Childs, J. E., & Paddock, C. D. (2003). The ascendancy of *Amblyomma americanum* as a vector of
604 pathogens affecting humans in the United States. *Annual Review of Entomology*, *48*, 307–
605 337. doi: 10.1146/annurev.ento.48.091801.112728

606 Clay, K., Klyachko, O., Grindle, N., Civitello, D., Oleske, D., & Fuqua, C. (2008). Microbial communities
607 and interactions in the lone star tick, *Amblyomma americanum*. *Molecular Ecology*, *17*(19),
608 4371–4381.

609 Duron, O., Binetruy, F., Noël, V., Cremaschi, J., McCoy, K. D., Arnathau, C., ... Chevillon, C. (2017).
610 Evolutionary changes in symbiont community structure in ticks. *Molecular Ecology*, *26*(11),
611 2905–2921. doi: 10.1111/mec.14094

612 Duron, O., Jourdain, E., & McCoy, K. D. (2014). Diversity and global distribution of the *Coxiella*
613 intracellular bacterium in seabird ticks. *Ticks and Tick-Borne Diseases*, *5*(5), 557–563. doi:
614 10.1016/j.ttbdis.2014.04.003

615 Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., ... Vial, L. (2018). Tick-Bacteria
616 Mutualism Depends on B Vitamin Synthesis Pathways. *Current Biology*, *28*(12), 1896-
617 1902.e5. doi: 10.1016/j.cub.2018.04.038

618 Duron, O., Noël, V., McCoy, K. D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., ... Chevillon, C. (2015).
619 The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence
620 of the Q Fever Pathogen, *Coxiella burnetii*. *PLoS Pathogens*, *11*(5), e1004892. doi:
621 10.1371/journal.ppat.1004892

622 Duron, O., Wilkes, T. E., & Hurst, G. D. D. (2010). Interspecific transmission of a male-killing bacterium
623 on an ecological timescale. *Ecology Letters*, *13*(9), 1139–1148. doi: 10.1111/j.1461-
624 0248.2010.01502.x

625 Engelstädter, J., & Hurst, G. D. D. (2009). The Ecology and Evolution of Microbes that Manipulate
626 Host Reproduction. *Annual Review of Ecology, Evolution, and Systematics*, 40(1), 127–149.
627 doi: 10.1146/annurev.ecolsys.110308.120206

628 Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., ... Pascal, G. (2018). FROGS:
629 Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics (Oxford, England)*, 34(8), 1287–
630 1294. doi: 10.1093/bioinformatics/btx791

631 Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., ... Raes, J. (2016). Population-
632 level analysis of gut microbiome variation. *Science (New York, N.Y.)*, 352(6285), 560–564. doi:
633 10.1126/science.aad3503

634 Ferrari, J., & Vavre, F. (2011). Bacterial symbionts in insects or the story of communities affecting
635 communities. *Philosophical Transactions of the Royal Society of London. Series B, Biological
636 Sciences*, 366(1569), 1389–1400. doi: 10.1098/rstb.2010.0226

637 Galan, M., Razzauti, M., Bard, E., Bernard, M., Brouat, C., Charbonnel, N., ... Cosson, J.-F. (2016). 16S
638 rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. *MSystems*,
639 1(4). doi: 10.1128/mSystems.00032-16

640 Gerhart, J. G., Moses, A. S., & Raghavan, R. (2016). A Francisella-like endosymbiont in the Gulf Coast
641 tick evolved from a mammalian pathogen. *Scientific Reports*, 6, 33670. doi:
642 10.1038/srep33670

643 Goecks, J., Nekrutenko, A., Taylor, J., & The Galaxy Team. (2010). Galaxy: a comprehensive approach
644 for supporting accessible, reproducible, and transparent computational research in the life
645 sciences. *Genome Biology*, 11(8), R86. doi: 10.1186/gb-2010-11-8-r86

646 Goslee, S. C., & Urban, D. L. (2007). The ecodist Package for Dissimilarity-based Analysis of Ecological
647 Data. *Journal of Statistical Software*, 22(1), 1–19. doi: 10.18637/jss.v022.i07

648 Gottlieb, Y., Ghanim, M., Gueguen, G., Kontsedalov, S., Vavre, F., Fleury, F., & Zchori-Fein, E. (2008).
649 Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. *FASEB*

650 *Journal: Official Publication of the Federation of American Societies for Experimental Biology,*
651 *22(7), 2591–2599. doi: 10.1096/fj.07-101162*

652 Gottlieb, Y., Lazar, I., & Klasson, L. (2015). Distinctive Genome Reduction Rates Revealed by Genomic
653 Analyses of Two Coxiella-Like Endosymbionts in Ticks. *Genome Biology and Evolution, 7(6),*
654 *1779–1796. doi: 10.1093/gbe/evv108*

655 Gould, A. L., Zhang, V., Lamberti, L., Jones, E. W., Obadia, B., Korasidis, N., ... Ludington, W. B. (2018).
656 Microbiome interactions shape host fitness. *Proceedings of the National Academy of*
657 *Sciences, 115(51), E11951–E11960. doi: 10.1073/pnas.1809349115*

658 Groussin, M., Mazel, F., Sanders, J. G., Smillie, C. S., Lavergne, S., Thuiller, W., & Alm, E. J. (2017).
659 Unraveling the processes shaping mammalian gut microbiomes over evolutionary time.
660 *Nature Communications, 8, 14319. doi: 10.1038/ncomms14319*

661 Guglielmone, A. A., Estrada-Peña, A., Keirans, J. E., & Robbins, R. G. (2003). *Ticks (Acari: Ixodida) of*
662 *the Neotropical Zoogeographic Region*. Universiteit Utrecht, J. Bovy-Verbeek (public relations
663 ICTTD).

664 Guglielmone, A. A., & Nava, S. (2010). Hosts of *Amblyomma dissimile* Koch, 1844 and *Amblyomma*
665 *rotundatum* Koch, 1844 (Acari: Ixodidae). *Zootaxa, 2541, 27–49.*

666 Guizzo, M. G., Parizi, L. F., Nunes, R. D., Schama, R., Albano, R. M., Tirloni, L., ... Oliveira, P. L. (2017). A
667 *Coxiella* mutualist symbiont is essential to the development of *Rhipicephalus microplus*.
668 *Scientific Reports, 7(1), 17554. doi: 10.1038/s41598-017-17309-x*

669 Hanning, I., & Diaz-Sanchez, S. (2015). The functionality of the gastrointestinal microbiome in non-
670 human animals. *Microbiome, 3. doi: 10.1186/s40168-015-0113-6*

671 Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of*
672 *Statistics, 6(2), 65–70. Retrieved from JSTOR.*

673 Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X.-Y., & Fukatsu, T. (2010). *Wolbachia* as a bacteriocyte-
674 associated nutritional mutualist. *Proceedings of the National Academy of Sciences of the*
675 *United States of America, 107(2), 769–774. doi: 10.1073/pnas.0911476107*

676 Hunter, D. J., Torkelson, J. L., Bodnar, J., Mortazavi, B., Laurent, T., Deason, J., ... Zhong, J. (2015). The
677 Rickettsia Endosymbiont of Ixodes pacificus Contains All the Genes of De Novo Folate
678 Biosynthesis. *PLoS ONE*, *10*(12). doi: 10.1371/journal.pone.0144552

679 Hutchinson, M. C., Cagua, E. F., Balbuena, J. A., Stouffer, D. B., & Poisot, T. (2017). paco:
680 implementing Procrustean Approach to Cophylogeny in R. *Methods in Ecology and Evolution*,
681 *8*(8), 932–940. doi: 10.1111/2041-210X.12736

682 Jaenike, J. (2012). Population genetics of beneficial heritable symbionts. *Trends in Ecology &*
683 *Evolution*, *27*(4), 226–232. doi: 10.1016/j.tree.2011.10.005

684 Jousselin, E., Cœur d’Acier, A., Vanlerberghe-Masutti, F., & Duron, O. (2013). Evolution and diversity
685 of Arsenophonus endosymbionts in aphids. *Molecular Ecology*, *22*(1), 260–270. doi:
686 10.1111/mec.12092

687 Jousselin, E., Desdevises, Y., & Coeur d’acier, A. (2009). Fine-scale cospeciation between
688 Brachycaudus and Buchnera aphidicola: bacterial genome helps define species and
689 evolutionary relationships in aphids. *Proceedings. Biological Sciences*, *276*(1654), 187–196.
690 doi: 10.1098/rspb.2008.0679

691 Klyachko, O., Stein, B. D., Grindle, N., Clay, K., & Fuqua, C. (2007). Localization and visualization of a
692 coxiella-type symbiont within the lone star tick, Amblyomma americanum. *Applied and*
693 *Environmental Microbiology*, *73*(20), 6584–6594. doi: 10.1128/AEM.00537-07

694 Koga, R., & Moran, N. A. (2014). Swapping symbionts in spittlebugs: evolutionary replacement of a
695 reduced genome symbiont. *The ISME Journal*, *8*(6), 1237–1246. doi: 10.1038/ismej.2013.235

696 Koga, R., Nikoh, N., Matsuura, Y., Meng, X.-Y., & Fukatsu, T. (2013). Mealybugs with distinct
697 endosymbiotic systems living on the same host plant. *FEMS Microbiology Ecology*, *83*(1), 93–
698 100. doi: 10.1111/j.1574-6941.2012.01450.x

699 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis
700 Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. doi:
701 10.1093/molbev/msw054

702 Kurtti, T. J., Felsheim, R. F., Burkhardt, N. Y., Oliver, J. D., Heu, C. C., & Munderloh, U. G. (2015).
703 *Rickettsia buchneri* sp. nov., a rickettsial endosymbiont of the blacklegged tick *Ixodes*
704 *scapularis*. *International Journal of Systematic and Evolutionary Microbiology*, 65(Pt 3), 965–
705 970. doi: 10.1099/ij.s.0.000047

706 Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P., Ramey, R. R., Bircher, J. S., ... Gordon, J. I. (2008).
707 Evolution of mammals and their gut microbes. *Science (New York, N.Y.)*, 320(5883), 1647–
708 1651. doi: 10.1126/science.1155725

709 Łukasik, P., Nazario, K., Leuven, J. T. V., Campbell, M. A., Meyer, M., Michalik, A., ... McCutcheon, J. P.
710 (2018). Multiple origins of interdependent endosymbiotic complexes in a genus of cicadas.
711 *Proceedings of the National Academy of Sciences*, 115(2), E226–E235. doi:
712 10.1073/pnas.1712321115

713 Margulis, L. (1993). Origins of species: acquired genomes and individuality. *Biosystems*, 31(2), 121–
714 125. doi: 10.1016/0303-2647(93)90039-F

715 Martin, D. P., Lemey, P., Lott, M., Moulton, V., Posada, D., & Lefevre, P. (2010). RDP3: a flexible and
716 fast computer program for analyzing recombination. *Bioinformatics (Oxford, England)*,
717 26(19), 2462–2463. doi: 10.1093/bioinformatics/btq467

718 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
719 *EMBnet.Journal*, 17(1), 10–12. doi: 10.14806/ej.17.1.200

720 Matsuura, Y., Kikuchi, Y., Hosokawa, T., Koga, R., Meng, X.-Y., Kamagata, Y., ... Fukatsu, T. (2012).
721 Evolution of symbiotic organs and endosymbionts in lygaeid stinkbugs. *The ISME Journal*,
722 6(2), 397–409. doi: 10.1038/ismej.2011.103

723 Matsuura, Y., Moriyama, M., Łukasik, P., Vanderpool, D., Tanahashi, M., Meng, X.-Y., ... Fukatsu, T.
724 (2018). Recurrent symbiont recruitment from fungal parasites in cicadas. *Proceedings of the*
725 *National Academy of Sciences*, 115(26), E5970–E5979. doi: 10.1073/pnas.1803245115

726 McCutcheon, J. P., Boyd, B. M., & Dale, C. (2019). The Life of an Insect Endosymbiont from the Cradle
727 to the Grave. *Current Biology: CB*, 29(11), R485–R495. doi: 10.1016/j.cub.2019.03.032

728 McCutcheon, J. P., & Moran, N. A. (2012). Extreme genome reduction in symbiotic bacteria. *Nature*
729 *Reviews Microbiology*, 10(1), 13–26. doi: 10.1038/nrmicro2670

730 Meibom, K. L., & Charbit, A. (2010). The unraveling panoply of Francisella tularensis virulence
731 attributes. *Current Opinion in Microbiology*, 13(1), 11–17. doi: 10.1016/j.mib.2009.11.007

732 Moran, N. A. (1996). Accelerated evolution and Muller's ratchet in endosymbiotic bacteria.
733 *Proceedings of the National Academy of Sciences of the United States of America*, 93(7),
734 2873–2878. doi: 10.1073/pnas.93.7.2873

735 Moran, Nancy A., McCutcheon, J. P., & Nakabachi, A. (2008). Genomics and Evolution of Heritable
736 Bacterial Symbionts. *Annual Review of Genetics*, 42(1), 165–190. doi:
737 10.1146/annurev.genet.41.110306.130119

738 Moran, Nancy A., Tran, P., & Gerardo, N. M. (2005). Symbiosis and insect diversification: an ancient
739 symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Applied and*
740 *Environmental Microbiology*, 71(12), 8802–8810. doi: 10.1128/AEM.71.12.8802-8810.2005

741 Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H. E., Moran, N. A., & Hattori, M. (2006).
742 The 160-kilobase genome of the bacterial endosymbiont Carsonella. *Science (New York, N.Y.)*,
743 314(5797), 267. doi: 10.1126/science.1134196

744 Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. A. (2017). metaSPAdes: a new versatile
745 metagenomic assembler. *Genome Research*, 27(5), 824–834. doi: 10.1101/gr.213959.116

746 Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in
747 aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences*
748 *of the United States of America*, 100(4), 1803–1807. doi: 10.1073/pnas.0335320100

749 Olivieri, E., Epis, S., Castelli, M., Varotto Boccazzi, I., Romeo, C., Desirò, A., ... Sasserà, D. (2019).
750 Tissue tropism and metabolic pathways of Midichloria mitochondrii suggest tissue-specific
751 functions in the symbiosis with Ixodes ricinus. *Ticks and Tick-Borne Diseases*. doi:
752 10.1016/j.ttbdis.2019.05.019

753 Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: computing large minimum evolution trees
754 with profiles instead of a distance matrix. *Molecular Biology and Evolution*, 26(7), 1641–
755 1650. doi: 10.1093/molbev/msp077

756 Ramaiah, A., & Dasch, G. A. (2018). Genome Sequence of Coxiella-Like Endosymbiont Strain CLE-
757 RmD, a Bacterial Agent in the Cattle Tick, Rhipicephalus microplus Deutsch Strain. *Genome*
758 *Announcements*, 6(13). doi: 10.1128/genomeA.00003-18

759 Rispe, C., & Moran, N. A. (2000). Accumulation of Deleterious Mutations in Endosymbionts: Muller's
760 Ratchet with Two Levels of Selection. *The American Naturalist*, 156(4), 425–441. doi:
761 10.1086/303396

762 Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... Huelsenbeck, J. P.
763 (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a
764 Large Model Space. *Systematic Biology*, 61(3), 539–542. doi: 10.1093/sysbio/sys029

765 Rowe, H. M., & Huntley, J. F. (2015). From the Outside-In: The Francisella tularensis Envelope and
766 Virulence. *Frontiers in Cellular and Infection Microbiology*, 5, 94. doi:
767 10.3389/fcimb.2015.00094

768 Russell, J. A., Goldman-Huertas, B., Moreau, C. S., Baldo, L., Stahlhut, J. K., Werren, J. H., & Pierce, N.
769 E. (2009). Specialization and geographic isolation among Wolbachia symbionts from ants and
770 lycaenid butterflies. *Evolution; International Journal of Organic Evolution*, 63(3), 624–640.
771 doi: 10.1111/j.1558-5646.2008.00579.x

772 Sachs, J. L., Skophammer, R. G., & Regus, J. U. (2011). Evolutionary transitions in bacterial symbiosis.
773 *Proceedings of the National Academy of Sciences*, 108(Supplement 2), 10800–10807. doi:
774 10.1073/pnas.1100304108

775 Scarborough, C. L., Ferrari, J., & Godfray, H. C. J. (2005). Aphid protected from pathogen by
776 endosymbiont. *Science (New York, N.Y.)*, 310(5755), 1781. doi: 10.1126/science.1120180

777 Scott, J. D., & Durden, L. A. (2015). *Amblyomma dissimile* Koch (Acari: Ixodidae) parasitizes bird
778 captured in Canada. *Systematic and Applied Acarology*, 20(8), 854–860. doi:
779 10.11158/saa.20.8.2

780 Shivaprasad, H. L., Cadenas, M. B., Diab, S. S., Nordhausen, R., Bradway, D., Crespo, R., &
781 Breitschwerdt, E. B. (2008). Coxiella-like infection in psittacines and a toucan. *Avian Diseases*,
782 52(3), 426–432. doi: 10.1637/8192-120707-Reg

783 Smith, T. A., Driscoll, T., Gillespie, J. J., & Raghavan, R. (2015). A Coxiella-Like Endosymbiont Is a
784 Potential Vitamin Source for the Lone Star Tick. *Genome Biology and Evolution*, 7(3), 831–
785 838. doi: 10.1093/gbe/evv016

786 Sudakaran, S., Kost, C., & Kaltenpoth, M. (2017). Symbiont Acquisition and Replacement as a Source
787 of Ecological Innovation. *Trends in Microbiology*, 25(5), 375–390. doi:
788 10.1016/j.tim.2017.02.014

789 Takiya, D. M., Tran, P. L., Dietrich, C. H., & Moran, N. A. (2006). Co-cladogenesis spanning three
790 phyla: leafhoppers (Insecta: Hemiptera: Cicadellidae) and their dual bacterial symbionts.
791 *Molecular Ecology*, 15(13), 4175–4191. doi: 10.1111/j.1365-294X.2006.03071.x

792 Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., ... Bordenstein, S.
793 R. (2016). Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts
794 and Their Microbiomes. *MSystems*, 1(2), e00028-16. doi: 10.1128/mSystems.00028-16

795 Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2002). Multiple sequence alignment using ClustalW
796 and ClustalX. *Current Protocols in Bioinformatics*, Chapter 2, Unit 2.3. doi:
797 10.1002/0471250953.bi0203s00

798 Turner, T. R., James, E. K., & Poole, P. S. (2013). The plant microbiome. *Genome Biology*, 14, 209. doi:
799 10.1186/gb-2013-14-6-209

800 van Schaik, E. J., Chen, C., Mertens, K., Weber, M. M., & Samuel, J. E. (2013). Molecular pathogenesis
801 of the obligate intracellular bacterium *Coxiella burnetii*. *Nature Reviews. Microbiology*, 11(8),
802 561–573. doi: 10.1038/nrmicro3049

803 Vapniarsky, N., Barr, B. C., & Murphy, B. (2012). Systemic Coxiella-like infection with myocarditis and
804 hepatitis in an eclectus parrot (*Eclectus roratus*). *Veterinary Pathology*, *49*(4), 717–722. doi:
805 10.1177/0300985811409251

806 Vautrin, E., & Vavre, F. (2009). Interactions between vertically transmitted symbionts: cooperation or
807 conflict? *Trends in Microbiology*, *17*(3), 95–99. doi: 10.1016/j.tim.2008.12.002

808 Voordouw, M. J. (2015). Co-feeding transmission in Lyme disease pathogens. *Parasitology*, *142*(2),
809 290–302. doi: 10.1017/S0031182014001486

810 Wang, M., Zhu, D., Dai, J., Zhong, Z., Zhang, Y., & Wang, J. (2018). Tissue Localization and Variation of
811 Major Symbionts in *Haemaphysalis longicornis*, *Rhipicephalus haemaphysaloides*, and
812 *Dermacentor silvarum* in China. *Applied and Environmental Microbiology*, *84*(10). doi:
813 10.1128/AEM.00029-18

814 Wernegreen, J. J. (2012). Endosymbiosis. *Current Biology: CB*, *22*(14), R555-561. doi:
815 10.1016/j.cub.2012.06.010

816 Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (2nd ed.). Retrieved from
817 <https://www.springer.com/gp/book/9783319242750>

818 Woc-Colburn, A. M., Garner, M. M., Bradway, D., West, G., D’Agostino, J., Trupkiewicz, J., ...
819 Nordhausen, R. W. (2008). Fatal coxiellosis in Swainson’s Blue Mountain Rainbow Lorikeets
820 (*Trichoglossus haematodus moluccanus*). *Veterinary Pathology*, *45*(2), 247–254. doi:
821 10.1354/vp.45-2-247

822 Wright, C. L., Sonenshine, D. E., Gaff, H. D., & Hynes, W. L. (2015). *Rickettsia parkeri* Transmission to
823 *Amblyomma americanum* by Cofeeding with *Amblyomma maculatum* (Acari: Ixodidae) and
824 Potential for Spillover. *Journal of Medical Entomology*, *52*(5), 1090–1095. doi:
825 10.1093/jme/tjv086

826 Zhong, J., Jasinskas, A., & Barbour, A. G. (2007). Antibiotic treatment of the tick vector *Amblyomma*
827 *americanum* reduced reproductive fitness. *PloS One*, *2*(5), e405. doi:
828 10.1371/journal.pone.0000405

829

830 **Acknowledgements**

831 Financial support was provided by the French government's Programmes Investissement
832 d'Avenir (Laboratoire d'Excellence CEBA, the MicroBIOMES Strategic Project 2016–2018
833 and the MiTick Annual Project 2016) and recurring funding from CNRS and IRD (OD, MB,
834 DA). FB benefits from a PhD fellowship financed by the CEBA Laboratoire d'Excellence and
835 Montpellier University. We wish to thank the platforms MGX (Montpellier Genomics and
836 Bioinformatics Facility) and GenSeq (Montpellier University), for the technical help and the
837 high-throughput sequencing. We are grateful to the members of Institut Pasteur de Guyane,
838 particularly Benoît de Thoisy and Agathe Chavy, for technical support. Special
839 acknowledgement to the “Agence Nationale de la Preservation de la Nature” (ANPN) and the
840 “Centre National de la Recherche Scientifique et Technologique of Gabon” (CENAREST)
841 that authorized and facilitated the access to the national parks in Gabon, and Marc F. Ngangué
842 (ANPN) that participated to the field collections in Gabon. We also thank Frédéric Delsuc,
843 Xavier Bailly, Laurence Mouton and Christine Chevillon for helpful discussions. The authors
844 acknowledge the IRD itrop HPC (South Green Platform) at IRD Montpellier for providing
845 HPC resources that have contributed to the research results reported in this paper. We also
846 acknowledge useful discussions with members of the Tiques et Maladies à Tiques (TMT)
847 working group from the Réseau Ecologique des Interactions Durables (REID).

848

849 **Conflict of interest**

850 The authors declare that they have no conflict of interest.

851 **Figure legends**

852 **Figure 1** Phylogenetic relationship of the 25 *Amblyomma* species examined in this study. The
853 phylogenetic tree was inferred using Bayesian inferences (BI) from a large fragment of rDNA
854 sequences (18S, ITS1, 5.8S, ITS2, and 28S rDNA; 3312 bp unambiguously aligned; best-fit
855 approximation for the evolutionary model: GTR+G+I), the numbers on nodes indicate the BI
856 probability. One to two specimens per *Amblyomma* species (45 specimens in total) were
857 analysed. The vertebrate species on which tick species mainly feed (host-range) is shown.
858 White circles, African group (AF); black circles, New World group (NW).

859

860 **Figure 2** Diversity of the microbiome and bacterial prevalence in *Amblyomma* species. The
861 left part of the Figure shows the *Amblyomma* phylogenetic tree adapted from Figure 1. One to
862 two specimens per *Amblyomma* species (45 specimens in total) were included in the
863 phylogenetic analysis. At the middle part, the heatmap shows the abundance and diversity of
864 the nine most abundant OTUs characterized in the 16S rRNA bacterial data set for each tick
865 species. The number on the right of the heatmap indicates the number of tick specimens per
866 species used to construct the heatmap (one to ten specimens per *Amblyomma* species (142
867 specimens in total) were included in the microbiome analysis). On the right of the figure, the
868 prevalence of the three most common symbionts is illustrated by the coloured squares
869 (*Coxiella*-LE, blue square; *Francisella*-LE, green square and *Rickettsia*, red square): full
870 square indicate a prevalence of 100%, while empty or partly colored squares indicate
871 prevalences from 0 to <100% (the numbers besides these squares indicate the prevalences).
872 The amount of DNA for the single specimen of *A. sculptum* was not sufficient to perform the
873 16S rRNA barcoding, therefore the heatmap for this species is not applicable (N/A). In this
874 latter species, the presence of *Coxiella*-LE and *Francisella*-LE was asserted through specific

875 PCR assays as detailed in the text. White circles, *Amblyomma* African group (AF); black
876 circles, *Amblyomma* New World group (NW).

877

878 **Figure 3** Structuration of the *Amblyomma* microbiome. **A** Multidimensional scaling plot
879 (MDS) of the microbial diversity of each *Amblyomma* species depending on the presence of
880 both *Coxiella*-LE (red dots) and *Francisella*-LE (green dots) or the absence of obligate
881 symbiont (blue dots). **B** MDS plot of the microbial diversity depending on the presence (red
882 dots) / absence (blue dots) of *Rickettsia*. **C** MDS plot of the microbial diversity depending on
883 the association / no association between the three symbionts (*Coxiella*-LE, *Francisella*-LE
884 and *Rickettsia*): C_R (red dots) correspond to *Amblyomma* specimens co-infected by
885 *Coxiella*-LE and *Rickettsia*; C (brownish dots) to specimens only infected by *Coxiella*-LE; F
886 (green dots) to specimens only infected by *Francisella*-LE; F_R to specimens co-infected by
887 *Francisella*-LE and *Rickettsia*; C_F_R (light blue dots) to specimens multi-infected by
888 *Coxiella*-LE, *Francisella*-LE and *Rickettsia*; dark blue dots to specimens not infected by these
889 symbionts. Each MDS plot is based on the generalized Unifrac ($\alpha = 0.5$) distance matrix and
890 all confidence ellipses are been drawn with the confidence level of 0.95.

891

892 **Figure 4** Evolutionary histories between *Amblyomma* and their nutritional symbionts. **A** Co-
893 phylogeny between the phylogenies of *Amblyomma* (on the left) and *Coxiella*-LE (on the
894 right): The tick phylogeny is based on 3312-bp nuclear rDNA sequences (18S, ITS1, 5.8S,
895 ITS2, and 28S rDNA; best-fit approximation for the evolutionary model: GTR+G+I); the
896 *Coxiella*-LE phylogeny was reconstructed using a concatenated gene sequence (16S rRNA,
897 *rpoB* and *groEL* concatenated sequences, 2045 unambiguously aligned base pairs; best-fit
898 approximation for the evolutionary model: GTR+G+I). **B** Cophylogeny between the
899 phylogenies of *Amblyomma* (on the left) and *Francisella*-LE (on the right): The tick

900 phylogeny is based on 3312-bp nuclear rDNA sequences (18S, ITS1, 5.8S, ITS2, and 28S
901 rDNA; best-fit approximation for the evolutionary model: GTR+G+I); the *Francisella*-LE
902 phylogeny was reconstructed using a concatenated gene sequence (16S rRNA, *rpoB*, *groEL*,
903 *ftsZ* and *gyrB* concatenated sequences, 3506 unambiguously aligned base pairs; best-fit
904 approximation for the evolutionary model: GTR+G+I). Each phylogeny was reconstructed
905 using BI and node numbers are the posterior probabilities (only values >70 are shown). White
906 circles, *Amblyomma* African group (AF); black circles, New World group (NW).