



HAL
open science

Two novel *Rickettsia* species of soft ticks in North Africa: ‘*Candidatus Rickettsia africaseptentrionalis*’ and ‘*Candidatus Rickettsia mauretunica*’

Marie Buysse, Olivier Duron

► **To cite this version:**

Marie Buysse, Olivier Duron. Two novel *Rickettsia* species of soft ticks in North Africa: ‘*Candidatus Rickettsia africaseptentrionalis*’ and ‘*Candidatus Rickettsia mauretunica*’. *Ticks and Tick-borne Diseases*, 2020, 11 (3), pp.101376. 10.1016/j.ttbdis.2020.101376 . hal-03001768

HAL Id: hal-03001768

<https://hal.science/hal-03001768>

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 **Two novel *Rickettsia* species of soft ticks in North Africa: ‘*Candidatus***
2 ***Rickettsia africaseptentrionalis*’ and ‘*Candidatus Rickettsia mauretaniae*’**

3

4 Marie Buysse¹ and Olivier Duron^{1*}

5

6 ¹ Laboratoire Maladies Infectieuses et Vecteurs : Ecologie, Génétique, Evolution et Contrôle

7 (MIVEGEC), Centre National de la Recherche Scientifique (CNRS) - Institut pour la

8 Recherche et le Développement (IRD) - Université de Montpellier (UM), 911 Avenue

9 Agropolis, F-34394 Montpellier, France

10 * Correspondence: olivier.duron@ird.fr

11 **Abstract**

12 *Rickettsia* are obligate intracellular bacteria often associated with hard ticks but more rarely
13 with soft ticks. In this study, we detected two putative novel *Rickettsia* species in three soft
14 species from North Africa: *Ornithodoros occidentalis* from Morocco, *Ornithodoros erraticus*
15 from Algeria and *Ornithodoros normandi* from Tunisia. We characterized these two novel
16 *Rickettsia* species on the basis of comparative DNA sequence analyses and phylogenetics of
17 four genes (*gltA*, 16S rRNA, *coxA* and *ompB*). These *Rickettsia*, provisionally named
18 ‘*Candidatus Rickettsia africaseptentrioalis*’ and ‘*Candidatus Rickettsia mauretaniae*’, differed
19 in nucleotide sequence from those of other *Rickettsia* species by 0.14–21.43% depending on
20 the gene examined. Phylogenetics further showed that the two novel *Rickettsia* species are
21 closely related to each other and represent sister taxa to *R. hoogstraalii*, *R. felis* and *R.*
22 *asemboensis* within the transitional *Rickettsia* group. While *Ornithodoros* host species of
23 ‘*Candidatus Rickettsia africaseptentrioalis*’ and ‘*Candidatus Rickettsia mauretaniae*’ are
24 among the most common soft ticks to bite humans, their pathogenicity remains to be
25 investigated.

26

27 **Key words**

28 Soft ticks; *Ornithodoros*; *Rickettsia*; North Africa

29 1. Introduction

30 Members of the genus *Rickettsia* are obligate intracellular bacteria infecting eukaryotes. There
31 are currently more than 30 recognized species but the advent of multilocus sequence typing
32 (MLST) and molecular phylogenetics has recently led to the description of several new
33 putative species (Labruna, 2009; Parola et al., 2005; Weinert, 2015; Weinert et al., 2009).
34 Nowadays, the best known *Rickettsia* species are the causative agents of severe diseases in
35 humans and other mammals, including Rocky Mountain spotted fever and epidemic typhus
36 (Parola et al., 2005; Perlman et al., 2006; Weinert, 2015). These *Rickettsia* species are all
37 associated with blood-feeding arthropods (ticks, mites, lice and fleas) which widely transmit
38 infections to vertebrates. However, not all *Rickettsia* are pathogens since many are
39 exclusively found in arthropods (e.g., ladybirds, spiders and book lice) in which they undergo
40 maternal (transovarial) transmission to offspring (Behar et al., 2010; Gottlieb et al., 2006;
41 Perlman et al., 2006; Weinert et al., 2009), as exemplified with *R. buchneri* in the blacklegged
42 tick *Ixodes scapularis* (Kurtti et al., 2015).

43
44 In recent years, *Rickettsia* spp. have been discovered in a diverse range of hosts, but hard ticks
45 (Ixodid) remain undoubtedly among the main arthropod hosts (Labruna, 2009; Perlman et al.,
46 2006; Weinert et al., 2015, 2009). By contrast, only a few *Rickettsia* have been reported from
47 few soft ticks (Argasid): this includes *R. bellii* and *R. hoogstraalii*, which are both regularly
48 identified in *Argas* spp. and *Ornithodoros* spp., but also the *R. wissemanii*, *R. nicoyana*, *R.*
49 *lusitaniae* and undetermined species, which are known only from a few *Ornithodoros* and
50 *Argas* species (Duh et al., 2010; Duron et al., 2018; Hornok et al., 2019; Milhano et al., 2014;
51 Moreira-Soto et al., 2017; Sánchez-Montes et al., 2016; Tahir et al., 2016; Yan et al., 2019).
52 In a recent study, Duron et al. (2017) examined a collection of 26 soft tick species and
53 detected infection by *Rickettsia* in one *Argas* and 15 *Ornithodoros* species. While some of
54 these *Rickettsia* infections were not assigned to known species, examination of *gltA*

55 phylogeny showed that most of them were closely related to *R. lusitaniae* and *R. bellii*
56 (Duron et al., 2017).

57

58 In this paper, we report on the discovery of two putative new species of *Rickettsia* in nymphs
59 of three soft tick species from North Africa: *O. occidentalis* from Morocco, *O. erraticus* from
60 Algeria and *O. normandi* from Tunisia. We used MLST gene sequences, including *gltA*, *coxA*,
61 *ompB* and 16S rRNA, and phylogenetics for the description of these infections. We further
62 examined their genetic proximity with known *Rickettsia* species and strains.

63

64 **2. Materials and Methods**

65 *2.1. Tick DNA collection*

66 Our preliminary examination of the *gltA* *Rickettsia* sequences obtained from soft ticks by
67 Duron et al. (2017) showed that six sequences (GenBank accession numbers: *O. erraticus*,
68 KY678045; *O. normandi*, KY678051; *O. occidentalis*, pending) cannot be assigned to known
69 *Rickettsia* species and strains, as we detailed below in the Results section. These six *gltA*
70 *Rickettsia* sequences were obtained from nymphs of *O. occidentalis* from Morocco (locality
71 Fez, 2010: *n*=3; locality Taza, 2010: *n*=1), *O. erraticus* from Algeria (locality Taher, 2010:
72 *n*=1) and *O. normandi* from Tunisia (locality Bizerte, 2010: *n*=1). The two most distant
73 localities (Fez and Bizerte) are approximately 1000 km apart. These six nymphs were
74 primarily collected through the examination of rodent burrows (Trape et al., 2013). They were
75 further individually washed in three sterile water baths, air dried and collected in sterile
76 microtubes. DNA was individually crushed by shaking with a bead beater (mixer mill
77 MM301, Qiagen, Hilden, Germany), and then DNA was isolated and purified using the
78 DNeasy Blood and Tissue Extraction Kit (Qiagen) following the manufacturer's instructions
79 as described in Trape et al., 2013.

80

81 2.2. *Molecular typing*

82 DNA templates of the six nymphs mentioned above were used for *Rickettsia* molecular
83 typing. Along with the *gltA* gene already sequenced, we amplified three other genes, 16S
84 rRNA, *coxA*, *ompB*, using semi-nested or nested PCR assays (Table 1). The use of semi-
85 nested or nested PCR was efficient at decreasing the probability of contamination from
86 unwanted amplification products (false positives). To prevent possible contamination,
87 different parts of this process were physically separated from one another, in entirely separate
88 rooms. All amplicons were also sequenced to control for false positive amplifications. Gene
89 features, primers and PCR conditions are detailed in Table 1.

90

91 Semi-nested and nested PCR amplifications were performed as follows: the first PCR run
92 with the external primers was performed in a 10 μ L volume containing ca. 20 ng of genomic
93 DNA, 3 mM of each dNTP (Thermo Scientific), 8 mM of MgCl₂ (Roche Diagnostics), 3 μ M
94 of each primer, 1 μ L of 10X PCR buffer (Roche Diagnostics), and 0.5 U of Taq DNA
95 polymerase (Roche Diagnostics). A 1 μ L aliquot of the PCR product from the first reaction
96 was then used as a template for the second round of amplification. The second PCR was
97 performed in a total volume of 25 μ L and contained 8 mM of each dNTP (Thermo Scientific),
98 10 mM of MgCl₂ (ThermoScientific), 7.5 μ M of each of the internal primers, 2.5 μ L of 10X
99 PCR buffer (Thermo Scientific), and 1.25 U of Taq DNA polymerase (Thermo Scientific). All
100 PCR amplifications were performed under the following conditions: initial denaturation at
101 93°C for 3 min, 35 cycles of denaturation (93°C, 30 s), annealing (T_m=52–56°C, depending
102 on primers, 30 s), extension (72°C, 1 min), and a final extension at 72°C for 5 min. Known
103 positive and negative individuals were used as controls in each PCR assay. All PCR products
104 were visualized with electrophoresis in a 1.5% agarose gel. Positive PCR products were
105 purified and sequenced in both directions (EUROFINS). The chromatograms were manually
106 inspected and cleaned with CHROMAS LITE

107 (http://www.technelysium.com.au/chromas_lite.html) and sequence alignments were done
108 using CLUSTALW (Thompson et al., 2003), both implemented in MEGA (Tamura et al.,
109 2007). Novel nucleotide sequences have been deposited in the GenBank nucleotide database
110 (Accession numbers: '*Candidatus Rickettsia africaseptentrioalis*', *gltA* [pending], 16S rRNA
111 [pending], *coxA* [pending] and *ompB* [pending]; '*Candidatus Rickettsia mauretaniae*', *gltA*
112 [pending], 16S rRNA [pending], *coxA* [pending] and *ompB* [pending]).

113

114 2.3. Molecular phylogenetics

115 The GBLOCKS program (Castresana, 2000) with default parameters was used to remove
116 poorly aligned positions and to obtain unambiguous sequence alignments. All sequence
117 alignments were also checked for putative recombinant regions using the RDP3 computer
118 analysis package (Martin et al., 2010). Given a set of aligned nucleotide sequences, RDP3 can
119 rapidly analyze these with a range of powerful non-parametric recombination detection
120 methods, including the GENECONV (Sawyer, 1999) and RDP (Martin and Rybicki, 2000).
121 Phylogenetic relationships were evaluated between *Rickettsia* strains using *gltA*, 16S rRNA,
122 *coxA* and *ompB* gene sequences. In addition to the sequences produced in this study,
123 additional *Rickettsia* sequences, representative of the diversity in the genus and available from
124 GenBank, were also used. The evolutionary models most closely fitting the sequence data
125 were determined using Akaike information criterion with the MEGA program (Tamura et al.,
126 2007). Phylogenetic analyses were based on maximum likelihood (ML) analyses. A ML
127 heuristic search, using a starting tree obtained by neighbor-joining, was conducted and clade
128 robustness was further assessed by bootstrap analysis using 1,000 replicates in MEGA
129 (Tamura et al., 2007).

130

131 3. Results

132 3.1. Multilocus typing of *Rickettsia*

133 The diversity of *Rickettsia* in *O. occidentalis*, *O. erraticus* and *O. normandi* was examined
134 using *gltA* (589 bp), 16S rRNA (729 bp), *coxA* (562 bp) and *ompB* (672 bp) gene sequences.
135 The *Rickettsia* 16S rRNA, *coxA* and *ompB* genes were amplified and sequenced here from all
136 tick DNA templates ($n=6$), while all the *gltA* gene sequences were already available in
137 GenBank from Duron et al. (2017). All sequences were easily readable without double peaks,
138 indicating a confident degree of primer specificity for *Rickettsia* PCR amplifications. All
139 these sequences belong unambiguously to the *Rickettsia* genus as described below.

140

141 On the basis of DNA sequences, we characterized one to three distinct alleles depending on
142 the *Rickettsia* gene (Table 2), including three alleles for *gltA* (98.3–99.66% pairwise
143 nucleotide identity), one for 16S rRNA, two for *coxA* (99.29% pairwise nucleotide identity)
144 and three for *ompB* (98.08–99.68% pairwise nucleotide identity). Overall, the allelic variation
145 at the five gene markers led to the identification of three different *Rickettsia* genotypes (A, B
146 and C hereafter): the A genotype was found in *O. erraticus* ($n=1$), the B genotype in *O.*
147 *normandi* ($n=1$), while the C genotype was shared by all *O. occidentalis* specimens ($n=4$)
148 (Table 2). None of these three genotypes showed 100% nucleotide identity at the four gene
149 markers with other *Rickettsia* species and strains available in GenBank, including other
150 *Rickettsia* spp. previously documented in soft ticks (i.e., *R. bellii*, *R. hoogstraalii*, *R.*
151 *wissemanii*, *R. nicoyana* and *R. lusitaniae*; Table 3). In terms of nucleotide identity, the
152 closest *Rickettsia* species of the A, B and C *Rickettsia* genotypes are *R. felis*, *R. asemboensis*,
153 *R. hoogstraalii*, *R. senegalensis* and *R. lusitaniae*, which all belong to the transitional
154 *Rickettsia* group (Table 3). If compared together, the A, B and C genotypes showed a
155 substantial nucleotide divergence at the four gene sequences examined but they had a better
156 pairwise nucleotide identity with each other than with any other *Rickettsia* species and strains
157 (Table 3).

158

159 3.2. Phylogenetics of *Rickettsia*

160 Maximum-likelihood (ML) analyses were further used to examine the evolutionary
161 relationships of the A, B and C *Rickettsia* genotypes of *O. occidentalis*, *O. erraticus* and *O.*
162 *normandi* with 38 other *Rickettsia* species, including other known *Rickettsia* from soft ticks.
163 We observed no sign of recombination in the data set: (i) the comparison between *Rickettsia*
164 *gltA*, 16S rRNA, *coxA* and *ompB* single gene phylogenies revealed the same phylogenetic
165 branching (Figure 1A–D) and (ii) the analysis of concatenated sequences did not detect
166 significant recombination events between the *Rickettsia* sequences used in the ML analyses
167 (all $P > 0.10$ for the GENECONV and RDP recombination-detection tests). The single *gltA*,
168 16S rRNA, *coxA* and *ompB* gene phylogenies and concatenated phylogeny consistently
169 showed that the A, B and C *Rickettsia* genotypes of *O. occidentalis*, *O. erraticus* and *O.*
170 *normandi* are markedly divergent to known *Rickettsia* species and strains (Figures 1A–D and
171 2). The A and B *Rickettsia* genotypes clustered together, suggesting that they belong to the
172 same species, while the C *Rickettsia* genotype belongs to a distinct, albeit close, species.
173 These two putative species are phylogenetically related to but divergent from some other
174 *Rickettsia* species belonging to the transitional phylogenetic group. It clustered within a
175 robust monophyletic clade including other *Rickettsia* species reported from soft ticks, *R.*
176 *hoogstraalii* and *R. lusitaniae*, and *Rickettsia* reported from fleas, *R. felis*, *R. senegalensis*
177 and *R. asemboensis* (Figures 1A–D and 2).

178

179 **4. Discussion**

180 While only a few *Rickettsia* species have been reported from soft ticks (Duh et al., 2010;
181 Duron et al., 2018; Hornok et al., 2019; Milhano et al., 2014; Moreira-Soto et al., 2017;
182 Sánchez-Montes et al., 2016; Tahir et al., 2016; Yan et al., 2019), in this study we identified
183 two novel *Rickettsia* species in soft tick *Ornithodoros* species from North Africa. The MLST
184 of four gene fragments fulfills the criteria usually used to designate new *Rickettsia* species

185 (e.g. Anstead and Chilton, 2013; Milhano et al., 2014; Moreira-Soto et al., 2017; Tahir et al.,
186 2016; Turebekov et al., 2019): their allelic profiles, showing a substantial magnitude of
187 difference, are unique relative to all recognized and putative species within the *Rickettsia*
188 genus. On account of its distinct genetic and phylogenetic traits described, we propose the
189 designation ‘*Candidatus Rickettsia africaseptentrioalis*’ (a’fri.ca sep.ten.tri.o’a.lis, referring to
190 North Africa where the organism was isolated) for the species found in *O. erraticus* (A
191 *Rickettsia* genotype) and *O. normandi* (B *Rickettsia* genotype), and ‘*Candidatus Rickettsia*
192 *mauretaniae*’ (ma.o.re.’ta.njae, referring to Mauretania [a region in the ancient Maghreb
193 during Antiquity] where the organism was isolated) for the species found in *O. occidentalis*
194 (C *Rickettsia* genotype). Interestingly, the MLST data set also indicated the presence of at
195 least two clearly distinct genotypes of ‘*Candidatus R. africaseptentrioalis*’, one present in *O.*
196 *erraticus* from Algeria and the other in *O. normandi* from Tunisia. These localities are more
197 than 350 km apart, suggesting that a greater diversity of ‘*Candidatus R. africaseptentrioalis*’
198 genotypes may circulate across North Africa.

199
200 The soft ticks *O. occidentalis*, *O. erraticus* and *O. normandi* all belong to the *O. erraticus*
201 species complex. While small mammals are the most common hosts of these species, they are
202 also among the most common soft ticks to bite humans (Trape et al., 2013). Two of these
203 ticks, *O. occidentalis* and *O. normandi*, are known to be only from North Africa, but *O.*
204 *erraticus* is more widespread and is reported from many Mediterranean regions, including the
205 Iberian Peninsula in Europe and the Middle East. These *Ornithodoros* species commonly
206 carry the relapsing fever agents *Borrelia crocidurae* and *Borrelia hispanica* and the African
207 swine fever viruses (Boinas et al., 2011; Trape et al., 2013), but they have never been found to
208 carry a pathogenic *Rickettsia*. In this context, whether or not ‘*Candidatus Rickettsia*
209 *africaseptentrioalis*’ and ‘*Candidatus Rickettsia mauretaniae*’ are potential human pathogens
210 is a question worthy of interest. These two novel species belong the transitional phylogenetic

211 group of *Rickettsia* and are closely related to *R. asemboensis*: this *Rickettsia* species was
212 described in 2013 in fleas and was latter reported in ticks (Troyo et al., 2016). Its pathogenesis
213 in vertebrate hosts is unknown (Jiang et al., 2013). Another close relative is *R. felis* which is
214 the causative agent of flea-borne spotted fever. This cosmopolitan pathogen, first described as
215 a human pathogen from the United States in 1991, is now considered a common cause of
216 fever in Africa (Brown and Macaluso, 2016). However, at least one *R. felis* strain, called LSU,
217 is a non-infectious maternally inherited symbiont inducing parthenogenesis in book lice
218 (Behar et al., 2010; Gillespie et al., 2014). This last example clearly shows that not all
219 *Rickettsia* of the transitional phylogenetic group are pathogens. Because the pathogenicity of
220 ‘*Candidatus Rickettsia africaseptentrioalis*’ and ‘*Candidatus Rickettsia mauretaniae*’ is still
221 unknown, considering these species as important pathogens might be premature. As pointed
222 out by Labruna and Walker (2014), the current view in rickettsiology has a strong
223 anthropocentric bias and tends to describe all novel *Rickettsia* species as pathogenic forms. In
224 many arthropods (e.g., ladybirds, spiders and book lice, but also ticks), *Rickettsia* are non-
225 pathogenic and undergo exclusive maternal transmission to offspring, which functions as both
226 mutualist and reproductive manipulator (Bonnet et al., 2017; Perlman et al., 2006; Weinert,
227 2015; Weinert et al., 2009). As a result, the putative pathogenicity of ‘*Candidatus Rickettsia*
228 *africaseptentrioalis*’ and ‘*Candidatus Rickettsia mauretaniae*’ should be the aim of further
229 studies before a definitive decision is reached on this effect.

230

231 **Competing interests**

232 The authors declare that they have no competing interests.

233

234 **Acknowledgments**

235 We are grateful to Céline Arnathau, Georges Diatta, Patrick Durand, François Renaud, Jean-
236 François Trape and Laurence Vial for help at different stages of this work. We also

237 acknowledge useful discussions with members of the French group *Tiques et Maladies à*
238 *Tiques* (TMT). Financial support was provided by recurrent funding from the *Centre National*
239 *de la Recherche Scientifique* (CNRS) and *Institut de Recherche pour le Développement*
240 (IRD).

241

242 **References**

- 243 Anstead, C.A., Chilton, N.B., 2013. A Novel *Rickettsia* Species Detected in Vole Ticks
244 (*Ixodes angustus*) from Western Canada. *Appl. Environ. Microbiol.* 79, 7583–7589.
245 <https://doi.org/10.1128/AEM.02286-13>
- 246 Behar, A., McCormick, L.J., Perlman, S.J., 2010. *Rickettsia felis* Infection in a Common
247 Household Insect Pest, *Liposcelis bostrychophila* (Psocoptera: Liposcelidae). *Appl.*
248 *Environ. Microbiol.* 76, 2280–2285. <https://doi.org/10.1128/AEM.00026-10>
- 249 Boinas, F.S., Wilson, A.J., Hutchings, G.H., Martins, C., Dixon, L.J., 2011. The persistence
250 of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF
251 endemic period in Portugal. *PLoS One* 6. <https://doi.org/10.1371/journal.pone.0020383>
- 252 Bonnet, S.I., Binetruy, F., Hernandez, A.M., Duron, O., 2017. The Tick Microbiome: Why
253 Non-pathogenic Microorganisms Matter in Tick Biology and Pathogen Transmission.
254 *Front. Cell. Infect. Microbiol.* 7. <https://doi.org/10.3389/fcimb.2017.00236>
- 255 Brown, L.D., Macaluso, K.R., 2016. *Rickettsia felis*, an Emerging Flea-Borne Rickettsiosis.
256 *Curr. Trop. Med. Reports* 3, 27–39. <https://doi.org/10.1007/s40475-016-0070-6>
- 257 Castresana, J., 2000. Selection of Conserved Blocks from Multiple Alignments for Their Use
258 in Phylogenetic Analysis. *Mol. Biol. Evol.* 17, 540–552.
259 <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- 260 Duh, D., Punda-Polic, V., Avsic-Zupanc, T., Bouyer, D., Walker, D.H., Popov, V.L.,
261 Jelovsek, M., Gracner, M., Trilar, T., Bradaric, N., Kurtti, T.J., Strus, J., 2010. *Rickettsia*
262 *hoogstraalii* sp. nov., isolated from hard- and soft-bodied ticks. *Int. J. Syst. Evol.*

263 Microbiol. 60, 977–984. <https://doi.org/10.1099/ijs.0.011049-0>

264 Duron, O., Binetruy, F., Noel, V., Cremaschi, J., McCoy, K., Arnathau, C., Plantard, O.,
265 Goolsby, J., Perez De Leon, A.A., Heylen, D.J.A., Raoul Van Oosten, A., Gottlieb, Y.,
266 Baneth, G., Guglielmono, A.A., Estrada-Pena, A., Opara, M.N., Zenner, L., Vavre, F.,
267 Chevillon, C., 2017. Evolutionary changes in symbiont community structure in ticks.
268 Mol. Ecol. 26, 2905–2921. <https://doi.org/10.1111/mec.14094>

269 Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., Loire, E., Ménard, C.,
270 Bouchez, O., Vavre, F., Vial, L., 2018. Tick-Bacteria Mutualism Depends on B Vitamin
271 Synthesis Pathways. Curr. Biol. 28, 1–7. <https://doi.org/10.1016/j.cub.2018.04.038>

272 Gillespie, J.J., Drisco, T.P., Verhoeve, V.I., Utsuki, T., Husseneder, C., Chouljenko, V.N.,
273 Azad, A.F., MacAluso, K.R., 2014. Genomic Diversification in Strains of *Rickettsia felis*
274 Isolated from Different Arthropods. Genome Biol. Evol. 7, 35–56.
275 <https://doi.org/10.1093/gbe/evu262>

276 Gottlieb, Y., Ghanim, M., Chiel, E., Gerling, D., Portnoy, V., Steinberg, S., Tzuri, G.,
277 Horowitz, R.A., Belausov, E., Mozes-daube, N., Kontsedalov, S., Gershon, M., Gal, S.,
278 Katzir, N., Zchori-fein, E., 2006. Identification and Localization of a *Rickettsia sp.* in
279 *Bemisia tabaci* (Homoptera: Aleyrodidae). Appl. Environ. Microbiol. 72, 3646–3652.
280 <https://doi.org/10.1128/AEM.72.5.3646>

281 Hornok, S., Szoke, K., Meli, M.L., Sandor, A.D., Gorfal, T., Estok, P., Wang, Y., Tu, V.T.,
282 Kovats, D., Boldogh, S.A., Corduneanu, A., Sulyok, K.M., Gyuranecz, M., Kontschan,
283 J., Takacs, N., Halajian, A., Epis, S., Hofmann-Lehmann, R., 2019. Molecular detection
284 of vector-borne bacteria in bat ticks (Acari: Ixodidae, Argasidae) from eight countries of
285 the Old and New Worlds. Parasit. Vectors 12, 1–7. [https://doi.org/10.1186/s13071-019-](https://doi.org/10.1186/s13071-019-3303-4)
286 3303-4

287 Jiang, J., Maina, A.N., Knobel, D.L., Cleaveland, S., Laudisoit, A., Wamburu, K., Ogola, E.,
288 Parola, P., Breiman, R.F., Njenga, M.K., Richards, A.L., 2013. Molecular detection of

289 *Rickettsia felis* and *Candidatus Rickettsia Asemboensis* in Fleas from Human Habitats,
290 Asembo, Kenya . Vector-Borne Zoonotic Dis. 13, 550–558.
291 <https://doi.org/10.1089/vbz.2012.1123>

292 Kurtti, T.J., Felsheim, R.F., Burkhardt, N.Y., Oliver, J.D., Heu, C.C., Munderloh, U.G., 2015.
293 *Rickettsia buchneri* sp. nov., a rickettsial endosymbiont of the blacklegged tick *Ixodes*
294 *scapularis*. Int. J. Syst. Evol. Microbiol. 65, 965–970.
295 <https://doi.org/10.1099/ijms.0.000047>

296 Labruna, M.B., 2009. Ecology of *Rickettsia* in South America, in: Hechemy, K.E., Brouqui,
297 P., Samuel, J.E., Raoult, D.A. (Eds.), Fifth International Conference on Rickettsiae and
298 Rickettsial Diseases. Blackwell Publishing, Marseille, France, pp. 156–165.

299 Labruna, M.B., Walker, D.H., 2014. *Rickettsia felis* and Changing Paradigms about
300 Pathogenic *Rickettsia*. Emerg. Infect. Dis. 20, 1768–1769.
301 <https://doi.org/10.3201/eid2010.131797>

302 Martin, D., Rybicki, E., 2000. RDP: detection of recombination amongst aligned sequences.
303 Bioinformatics 16, 562–563. <https://doi.org/10.1093/bioinformatics/16.6.562>

304 Martin, D.P., Lemey, P., Lott, M., Moulton, V., Posada, D., Lefevre, P., 2010. RDP3: A
305 flexible and fast computer program for analyzing recombination. Bioinformatics 26,
306 2462–2463. <https://doi.org/10.1093/bioinformatics/btq467>

307 Milhano, N., Palma, M., Marcili, A., Nuncio, M.S., de Carvalho, I.L., De Sousa, R., 2014.
308 *Rickettsia lusitaniae* sp. nov. isolated from the soft tick *Ornithodoros erraticus* (Acarina:
309 Argasidae). Comp. Immunol. Microbiol. Infect. Dis. 37, 189–193.
310 <https://doi.org/10.1016/j.cimid.2014.01.006>

311 Moreira-Soto, R.D., Moreira-Soto, A., Corrales-Aguilar, E., Calderón-Arguedas, Ó., Troyo,
312 A., 2017. ‘*Candidatus Rickettsia nicoyana*’: A novel *Rickettsia* species isolated from
313 *Ornithodoros knoxjonesi* in Costa Rica. Ticks Tick. Borne. Dis. 8, 532–536.
314 <https://doi.org/10.1016/j.ttbdis.2017.02.015>

315 Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-Borne Rickettsioses around the World:
316 Emerging Diseases Challenging Old Concepts. *Clin. Microbiol. Rev.* 18, 719–756.
317 <https://doi.org/10.1128/CMR.18.4.719-756.2005>

318 Perlman, S.J., Hunter, M.S., Zchori-Fein, E., 2006. The emerging diversity of *Rickettsia*.
319 *Proc. R. Soc. B* 273, 2097–2106. <https://doi.org/10.1098/rspb.2006.3541>

320 Sánchez-Montes, S., Guzmán-Cornejo, C., Martínez-Nájera, Y., Becker, I., Venzal, J.M.,
321 Labruna, M.B., 2016. *Rickettsia lusitaniae* associated with *Ornithodoros yumatensis*
322 (Acari: Argasidae) from two caves in Yucatan, Mexico. *Ticks Tick. Borne. Dis.* 7, 1097–
323 1101. <https://doi.org/10.1016/j.ttbdis.2016.09.003>

324 Sawyer, S.A., 1999. GENECONV: A computer package for the statistical detection of gene
325 conversion. Distrib. by author, Dep. Math. Washingt. Univ. St. Louis. Available at [http://](http://www.math.wustl.edu/~sawy)
326 www.math.wustl.edu/~sawy.

327 Tahir, D., Socolovschi, C., Marié, J. Lou, Ganay, G., Berenger, J.M., Bompar, J.M., Blanchet,
328 D., Cheuret, M., Mediannikov, O., Raoult, D., Davoust, B., Parola, P., 2016. New
329 *Rickettsia* species in soft ticks *Ornithodoros hasei* collected from bats in French Guiana.
330 *Ticks Tick. Borne. Dis.* 7, 1089–1096. <https://doi.org/10.1016/j.ttbdis.2016.09.004>

331 Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics
332 Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.* 24, 1596–1599.
333 <https://doi.org/10.1093/molbev/msm092>

334 Thompson, J.D., Gibson, T.J., Higgins, D.G., 2003. Multiple Sequence Alignment Using
335 ClustalW and ClustalX. *Curr. Protoc. Bioinforma.* 2.3.1-2.3.22.
336 <https://doi.org/10.1002/0471250953.bi0203s00>

337 Trape, J.F., Diatta, G., Arnathau, C., Bitam, I., Sarih, M., Belghyti, D., Bouattour, A.,
338 Elguero, E., Vial, L., Mané, Y., Baldé, C., Pugnolle, F., Chauvancy, G., Mahé, G.,
339 Granjon, L., Duplantier, J.M., Durand, P., Renaud, F., 2013. The Epidemiology and
340 Geographic Distribution of Relapsing Fever Borreliosis in West and North Africa, with a

341 Review of the *Ornithodoros erraticus* Complex (Acari: Ixodida). PLoS One 8.
342 <https://doi.org/10.1371/journal.pone.0078473>

343 Troyo, A., Moreira-Soto, R.D., Calderon-Arguedas, Ó., Mata-Somarribas, C., Ortiz-Tello, J.,
344 Barbieri, A.R.M., Avendaño, A., Vargas-Castro, L.E., Labruna, M.B., Hun, L., Taylor,
345 L., 2016. Detection of rickettsiae in fleas and ticks from areas of Costa Rica with history
346 of spotted fever group rickettsioses. Ticks Tick. Borne. Dis. 7, 1128–1134.
347 <https://doi.org/10.1016/j.ttbdis.2016.08.009>

348 Turebekov, N., Abdiyeva, K., Yegemberdiyeva, R., Dmitrovsky, A., Yeraliyeva, L.,
349 Shapiyeva, Z., Amirbekov, A., Oradova, A., Kachiyeva, Z., Ziyadina, L., Hoelscher, M.,
350 Froeschl, G., Dobler, G., Zinner, J., Frey, S., Essbauer, S., 2019. Prevalence of *Rickettsia*
351 species in ticks including identification of unknown species in two regions in
352 Kazakhstan. Parasites and Vectors 12, 1–16. <https://doi.org/10.1186/s13071-019-3440-9>

353 Weinert, L.A., 2015. The diversity and phylogeny of *Rickettsia*, in: Morand, S., Krasnov,
354 B.R., Littlewood, D.T.J. (Eds.), Parasite Diversity and Diversification, Evolutionary
355 Ecology Meets Phylogenetics. University Printing House Cambridge, pp. 150-.

356 Weinert, L.A., Araujo-Jnr, E. V., Ahmed, M.Z., Welch, J.J., 2015. The incidence of bacterial
357 endosymbionts in terrestrial arthropods. Proc. R. Soc. B 282.
358 <https://doi.org/10.1098/rspb.2015.0249>

359 Weinert, L.A., Werren, J.H., Aebi, A., Stone, G.N., Jiggins, F.M., 2009. Evolution and
360 diversity of *Rickettsia* bacteria. BMC Biol. 7, 1–15. [https://doi.org/10.1186/1741-7007-](https://doi.org/10.1186/1741-7007-7-6)
361 [7-6](https://doi.org/10.1186/1741-7007-7-6)

362 Yan, P., Qiu, Z., Zhang, T., Li, Y., Wang, W., Li, M., Yu, Z., Liu, J., 2019. Microbial
363 diversity in the tick *Argas japonicus* (Acari: Argasidae) with a focus on *Rickettsia*
364 pathogens. Med. Vet. Entomol. 327–335. <https://doi.org/10.1111/mve.12373>

365

366 **Table 1.** Genes and primers used for *Rickettsia* sequencing.

Gene	Hypothetical product	Primers (5'-3')	T _m	Fragment size	Reference	
16S rRNA	Small ribosomal unit	R16SF1	CGTGGGAATCTGCCCATCAG	55°C	Semi-nested PCR assay: 1st round PCR: R16SF1/R16SR1: 854bp 2nd round PCR: R16SF2/R16SR1: 772bp	This study
		R16SF2	CGCTGATGGATGAGCCCGCGTC			
		R16SR1	GGTGGTYGCGGATCGCAGAG			
<i>gltA</i>	Citrate synthase	RgltAF1	CCTATGGCTATTATGCTTGCGGC	56°C	Semi-nested PCR assay: 1st round PCR: RgltAF1/RgltAR1: 664bp 2nd round PCR: RgltAF2/RgltAR1: 637bp	Duron et al. 2017
		RgltAF2	GGTTCTCTTTCKGCATTTTATCC			
		RgltAR1	CTTGAAGCTATCGCTCTTAAAGATG			
<i>ompB</i>	Outer membrane protein	RompBF1	GGCTGGACCTGAAGCTGGAGC	52°C	Nested PCR assay: 1st round PCR: RompBF1/RompBR2: 834bp 2nd round PCR: RompBF2:RompBR1: 715bp	This study
		RompBF2	GTTGCTGCAGGTGACGAAGCTG			
		RompBR1	GTCCATCTAACTGAGACTGAG			
		RompBR2	GCATCAGGTCTTATGCTTGAC			
<i>coxA</i>	Cytochrome C oxydase subunit I	RcoxAF2	CCYGATATGGCATTTCACGCC	55°C	Semi-nested PCR assay: 1st round PCR: RcoxAF2/RcoxAR2: 886bp 2nd round PCR: RcoxAF2/RcoxAR1: 607bp	This study
		RcoxAR1	ATCGTATGGGCTCACCATATGT			
		RcoxAR2	AAGCACCGAGCGACATCGTA			

367

368 **Table 2.** Sequence profiles of the four genes (16S rRNA, *gltA*, *coxA* and *ompB*) in the three *Rickettsia* genotypes (A–B: ‘*Candidatus Rickettsia*
 369 *africaseptentrioalis*’; C: ‘*Candidatus Rickettsia mauretaniae*’) identified in this study. Letters a–c represent the different alleles at each gene locus. *n*,
 370 number of specimens by each *Rickettsia* genotype.

<i>Rickettsia</i> host species (<i>n</i>)	<i>Rickettsia</i> genes				<i>Rickettsia</i> haplotypes
	16S rRNA	<i>gltA</i>	<i>ompB</i>	<i>coxA</i>	
<i>Ornithodoros erraticus</i> (<i>n</i> =1)	a	a	a	a	A
<i>Ornithodoros normandi</i> (<i>n</i> =1)	a	b	b	a	B
<i>Ornithodoros occidentalis</i> (<i>n</i> =4)	a	c	c	c	C

371

372 **Table 3.** Sequence similarity of the 16S rRNA, *gltA*, *coxA* and *ompB* gene sequences of the three *Rickettsia* genotypes (A–B: ‘*Candidatus Rickettsia*
373 *africaseptentrionalis*’; C: ‘*Candidatus Rickettsia mauretaniae*’) detected in *O. erraticus*, *O. normandi* and *Ornithodoros occidentalis* to those of closely
374 related *Rickettsia* species or *Rickettsia* species of other soft tick species available in GenBank.

Gene	<i>Rickettsia</i> (Genbank accession no.)	% Sequence similarity		
		<i>Rickettsia</i> of <i>O. erraticus</i>	<i>Rickettsia</i> of <i>O. normandi</i>	<i>Rickettsia</i> of <i>O. occidentalis</i>
16s rRNA	<i>R. lusitaniae</i>	–	–	–
	<i>R. asemboensis</i> (JWSW000000000)	99.73 (727 of 729bp)	99.73 (727 of 729bp)	99.73 (727 of 729bp)
	<i>R. felis</i> (CP000053)	99.86 (728 of 729bp)	99.86 (728 of 729bp)	99.86 (728 of 729bp)
	<i>R. hoogstraalii</i> (CCXM000000000)	99.86 (728 of 729bp)	99.86 (728 of 729bp)	99.86 (728 of 729bp)
	<i>R. wissemanii</i> (LT558851)	99.45 (725 of 729bp)	99.45 (725 of 729bp)	99.45 (725 of 729bp)
	<i>R. nicoyana</i> (KX228147)	99.59 (726 of 729bp)	99.59 (726 of 729bp)	99.59 (726 of 729bp)
	<i>R. bellii</i> (CP000849)	99.04 (722 of 729bp)	99.04 (722 of 729bp)	99.04 (722 of 729bp)
<i>gltA</i>	<i>R. lusitaniae</i> (JQ771933)	97.96 (577 of 589bp)	97.62 (575 of 589bp)	97.28 (573 of 589bp)
	<i>R. asemboensis</i> (JWSW000000000)	97.28 (573 of 589bp)	96.94 (571 of 589bp)	96.60 (569 of 589bp)
	<i>R. felis</i> (CP000053)	96.43 (568 of 589bp)	96.10 (566 of 589bp)	95.76 (564 of 589bp)
	<i>R. hoogstraalii</i> (CCXM000000000)	96.77 (570 of 589bp)	96.43 (568 of 589bp)	96.10 (566 of 589bp)
	<i>R. wissemanii</i> (LT558852)	92.36 (544 of 589bp)	92.02 (542 of 589bp)	92.02 (542 of 589bp)
	<i>R. nicoyana</i> (KX228143)	92.36 (544 of 589bp)	92.02 (542 of 589bp)	92.02 (542 of 589bp)
	<i>R. bellii</i> (CP000849)	85.91 (506 of 589bp)	85.74 (505 of 589bp)	85.57 (504 of 589bp)
<i>ompB</i>	<i>R. lusitaniae</i>	–	–	–
	<i>R. asemboensis</i> (JWSW000000000)	98.07 (659 of 672bp)	97.92 (658 of 672bp)	97.32 (654 of 672pb)
	<i>R. felis</i> (CP000053)	98.07 (659 of 672bp)	97.92 (658 of 672bp)	97.17 (653 of 672pb)
	<i>R. hoogstraalii</i> (CCXM000000000)	98.51 (662 of 672bp)	98.36 (661 of 672bp)	97.62 (656 of 672pb)
	<i>R. wissemanii</i> (LT558854)	97.44 (304 of 312bp)	97.12 (303 of 312bp)	97.12 (303 of 312pb)
	<i>R. nicoyana</i>	–	–	–

	<i>R. bellii</i> (CP000849)	78.87 (530 of 672bp)	78.72 (529 of 672bp)	78.57 (528 of 672pb)
<i>coxA</i>	<i>R. lusitaniae</i>	–	–	–
	<i>R. asemboensis</i> (JWSW00000000)	96.62 (543 of 562pb)	96.62 (543 of 562pb)	95.91 (539 of 562pb)
	<i>R. felis</i> (CP000053)	95.73 (538 of 562pb)	95.73 (538 of 562pb)	95.02 (534 of 562pb)
	<i>R. hoogstraalii</i> (CCXM00000000)	95.91 (539 of 562pb)	95.91 (539 of 562pb)	95.19 (535 of 562pb)
	<i>R. wissemanii</i>	–	–	–
	<i>R. nicoyana</i>	–	–	–
	<i>R. bellii</i> (CP000849)	86.83 (488 of 562pb)	86.83 (488 of 562pb)	86.83 (488 of 562pb)

376 **Figure legends**

377 **Fig. 1.** Phylogeny of *Rickettsia* constructed using maximum-likelihood (ML) estimations
378 based on (A) *gltA* gene sequences (589 unambiguously aligned nucleotide sites; best-fit
379 approximation for the evolutionary model: GTR + G+I); (B) 16S rRNA sequences (729
380 unambiguously aligned nucleotide sites; best-fit approximation for the evolutionary model:
381 HKY+G+I); (C) *coxA* gene sequences (562 unambiguously aligned nucleotide sites; best-fit
382 approximation for the evolutionary model: GTR + G+I); (D) *ompB* gene sequences (672
383 unambiguously aligned nucleotide sites; best-fit approximation for the evolutionary model:
384 GTR + G). Circles indicate *Rickettsia* species found in soft ticks (black circles: sequences
385 from *Rickettsia* characterized in this study from *Ornithodoros occidentalis*, *O. erraticus* and
386 *O. normandi*; white circles: sequences from *Rickettsia* characterized from other tick species
387 and available in GenBank). Sequences from representative *Rickettsia* groups, species and
388 strains available in GenBank were also added to the analysis. The grey boxes delineate the
389 different *Rickettsia* groups (their names are indicated in upper case). Bacterial name, host
390 species and GenBank accession numbers are shown on the tree. Branch numbers indicate
391 percentage bootstrap support for major branches (1000 replicates; only bootstrap values >70%
392 are shown). The scale bar is in units of substitution/site.

393

394 **Fig. 2.** Phylogeny of *Rickettsia* constructed using maximum-likelihood (ML) estimations
395 based on concatenated *gltA*, 16S rRNA, *coxA* and *ompB* sequences (2552 unambiguously
396 aligned nucleotide sites; best-fit approximation for the evolutionary model: GTR + G+I).
397 Circles indicate *Rickettsia* species found in soft ticks (black circles: sequences from *Rickettsia*
398 characterized in this study from *Ornithodoros occidentalis*, *O. erraticus* and *O. normandi*;
399 white circles: sequences from *Rickettsia* characterized from other tick species and available in
400 GenBank). Sequences from representative *Rickettsia* groups, species and strains available in
401 GenBank were also added to the analysis. The grey boxes delineate the different *Rickettsia*

402 groups (their names are indicated in upper case). Bacterial name, host species and GenBank
403 accession numbers are shown on the tree. Branch numbers indicate percentage bootstrap
404 support for major branches (1000 replicates; only bootstrap values >70% are shown). The
405 scale bar is in units of substitution/site.