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Two novel *Rickettsia* species of soft ticks in North Africa: '*Candidatus* Rickettsia africaseptentrioalis' and '*Candidatus* Rickettsia mauretaniae'

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Abstract

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

Key words

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

1. Introduction

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

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

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

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

2. Materials and Methods

2.1. Tick DNA collection

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

2.2. Molecular typing

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

Semi-nested and nested PCR amplifications were performed as follows: the first PCR run with the external primers was performed in a 10 µL volume containing ca. 20 ng of genomic DNA, 3 mM of each dNTP (Thermo Scientific), 8 mM of $MgCl_2$ (Roche Diagnostics), 3 µM of each primer, 1 µL of 10X PCR buffer (Roche Diagnostics), and 0.5 U of Taq DNA polymerase (Roche Diagnostics). A 1µL aliquot of the PCR product from the first reaction was then used as a template for the second round of amplification. The second PCR was performed in a total volume of 25 µL and contained 8 mM of each dNTP (Thermo Scientific), 10 mM of $MgCl_2$ (ThermoScientific), 7.5 µM of each of the internal primers, 2.5 µL of 10X PCR buffer (Thermo Scientific), and 1.25 U of Taq DNA polymerase (Thermo Scientific). All PCR amplifications were performed under the following conditions: initial denaturation at 93°C for 3 min, 35 cycles of denaturation (93°C, 30 s), annealing (Tm=52–56°C, depending on primers, 30 s), extension (72°C, 1 min), and a final extension at 72°C for 5 min. Known positive and negative individuals were used as controls in each PCR assay. All PCR products were visualized with electrophoresis in a 1.5% agarose gel. Positive PCR products were purified and sequenced in both directions (EUROFINS). The chromatograms were manually inspected and cleaned with CHROMAS LITE

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

2.3. Molecular phylogenetics

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

3. Results

3.1. Multilocus typing of Rickettsia

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

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

3.2. Phylogenetics of Rickettsia

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

4. Discussion

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

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

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

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

Competing interests

The authors declare that they have no competing interests.

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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:	This study
		R16SF2	CGCTGATGGATGAGCCCGCGTC		1st round PCR: R16SF1/R16SR1: 854bp	
		R16SR1	GGTGGTYGCGGATCGCAGAG		2nd round PCR: R16SF2/R16SR1: 772bp	
gltA	Citrate synthase	RgltAF1	CCTATGGCTATTATGCTTGCGGC	56°C	Semi-nested PCR assay:	Duron et *al*. 2017
		RgltAF2	GGTTCTCTTTCKGCATTTTATCC		1st round PCR: RgltAF1/RgltAR1: 664bp	
		RgltAR1	CTTGAAGCTATCGCTCTTAAAGATG		2nd round PCR: RgltAF2/RgltAR1: 637bp	
ompB	Outer membrane protein	RompBF1	GGCTGGACCTGAAGCTGGAGC	52°C	Nested PCR assay:	This study
		RompBF2	GTTGCTGCAGGTGACGAAGCTG		1st round PCR: RompBF1/RompBR2: 834bp	
		RompBR1	GTCCATCTAACTGAGACTGAG		2nd round PCR: RompBF2:RompBR1: 715bp	
		RompBR2	GCATCAGGTCTTATGCTTGCAC			
coxA	Cytochrome C oxydase subunit I	RcoxAF2	CCYGATATGGCATTTCCACGCC	55°C	Semi-nested PCR assay:	This study
		RcoxAR1	ATCGTATGGGCTCACCATATGT		1st round PCR: RcoxAF2/RcoxAR2: 886bp	
		RcoxAR2	AAGCACCGAGCGACATCGTA		2nd round PCR: RcoxAF2/RcoxAR1: 607bp	

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

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

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 africaseptentrioalis'; 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	*Rickettsia* (Genbank accession no.)	% Sequence similarity		
		Rickettsia of *O. erraticus*	*Rickettsia* of *O. normandi*	*Rickettsia* of *O. occidentalis*
16s rRNA	*R. lusitaniae*	–	–	–
	R. asemboensis (JWSW00000000)	99.73 (727 of 729bp)	99.73 (727 of 729bp)	99.73 (727 of 729bp)
	*R. felis (*CP000053)	99.86 (728 of 729bp)	99.86 (728 of 729bp)	99.86 (728 of 729bp)
	R. hoogstraalii (CCXM00000000)	99.86 (728 of 729bp)	99.86 (728 of 729bp)	99.86 (728 of 729bp)
	R. wissemanii (LT558851)	99.45 (725 of 729bp)	99.45 (725 of 729bp)	99.45 (725 of 729bp)
	R. nicoyana (KX228147)	99.59 (726 of 729bp)	99.59 (726 of 729bp)	99.59 (726 of 729bp)
	R. bellii (CP000849)	99.04 (722 of 729bp)	99.04 (722 of 729bp)	99.04 (722 of 729bp)
gltA	*R. lusitaniae* (JQ771933)	97.96 (577 of 589bp)	97.62 (575 of 589bp)	97.28 (573 of 589bp)
	R. asemboensis (JWSW00000000)	97.28 (573 of 589bp)	96.94 (571 of 589bp)	96.60 (569 of 589bp)
	*R. felis (*CP000053)	96.43 (568 of 589bp)	96.10 (566 of 589bp)	95.76 (564 of 589bp)
	R. hoogstraalii (CCXM00000000)	96.77 (570 of 589bp)	96.43 (568 of 589bp)	96.10 (566 of 589bp)
	R. wissemanii (LT558852)	92.36 (544 of 589bp)	92.02 (542 of 589bp)	92.02 (542 of 589bp)
	R. nicoyana (KX228143)	92.36 (544 of 589bp)	92.02 (542 of 589bp)	92.02 (542 of 589bp)
	R. bellii (CP000849)	85.91 (506 of 589bp)	85.74 (505 of 589bp)	85.57 (504 of 589bp)
ompB	*R. lusitaniae*	–	–	–
	R. asemboensis (JWSW00000000)	98.07 (659 of 672bp)	97.92 (658 of 672bp)	97.32 (654 of 672pb)
	*R. felis (*CP000053)	98.07 (659 of 672bp)	97.92 (658 of 672bp)	97.17 (653 of 672pb)
	R. hoogstraalii (CCXM00000000)	98.51 (662 of 672bp)	98.36 (661 of 672bp)	97.62 (656 of 672pb)
	R. wissemanii (LT558854)	97.44 (304 of 312bp)	97.12 (303 of 312bp)	97.12 (303 of 312pb)
	R. nicoyana	–	–	–

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

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Figure legends

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

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

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