Molecular detection of novel *Anaplasmataceae* closely related to *Anaplasma platys* and *Ehrlichia canis* in the dromedary camel (*Camelus dromedarius*)

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Abstract

Serological surveys have confirmed *Anaplasma marginale* and *Anaplasma phagocytophilum* infections in dromedary camels, but molecular surveys and genetic characterisation of camel-associated *Anaplasma* species are lacking. In this study, we detected tick-borne *Anaplasmataceae* in 30 of 100 (30%) healthy dromedary camels screened using a combined 16S rRNA - *groEL* PCR-sequencing approach. Nucleotide sequencing confirmed *Anaplasmataceae* genome presence in 28 of the 33 16S rRNA PCR-positive samples, with two additional positive samples, for which 16S rRNA sequence data were ambiguous, being identified by g*roEL* gene characterisation. Phylogenetic analyses of a 1,289 nt segment of the 16S rRNA gene confirmed the presence of a unique *Ehrlichia* lineage and a discrete *Anaplasma* lineage, comprising three variants, occurring at an overall prevalence of 4% and 26%, respectively. Genetic characterisation of an aligned 559 nt *groEL* gene region revealed the camel-associated *Anaplasma* and *Ehrlichia* lineages to be novel and most closely related to *Anaplasma platys* and *Ehrlichia canis*. Based on the confirmed monophyly, minimum pairwise genetic distances between each novel lineage and its closest sister taxon, and the inability to isolate the bacteria, we propose that *Candidatus* status be assigned to each. This first genetic characterisation of *Anaplasmataceae* from naturally infected, asymptomatic dromedary camels in Saudi Arabia confirms the presence of two novel lineages that are phylogenetically linked to two pathogenic canid species of increasing zoonotic concern.

Keywords: *Anaplasma*, *Ehrlichia*, tick-borne *Anaplasmataceae*, alphaproteobacteria, 16S rRNA, *groEL*, *Camelus dromedarius*, phylogeny, Saudi Arabia

Introduction

Anaplasma and *Ehrlichia* are obligate, intra-cellular, gram-negative, tick-borne bacteria that infect a wide range of animals, including humans. These bacteria typically cycle asymptomatically between enzootic ticks and domestic or wild vertebrate hosts, but can cause severe wasting and anaemia, when transmitted to hosts outside of this natural cycle (Nicholson et al. 2010). The mammalian reservoir host(s) play an important role in maintenance and propagation of the bacteria, which although transtadially transmitted, cannot be maintained in ticks because transovarial transmission appears to be inefficient (Parola et al. 2005).

Ehrlichioses and anaplasmoses have long been recognised in domesticated animals, but are considered emerging zoonotic infections due to steadily increasing numbers of medical cases since their initial, and relatively recent detection in humans in 1986 and 1994, respectively (Doudier et al. 2010). This increased incidence is related to technological advances in diagnosis, intensified surveillance, changing environmental factors that impact reservoir host and vector distributions and densities, and increased tick-human contact opportunities (Doudier et al. 2010; Nicholson et al. 2010). Bacterial species associated with clinical disease in humans include *Ehrlichia chaffeensis*, *Anaplasma phagocytophylum* and *E. ewingii.* However, growing evidence indicates that three additional species, namely *E. canis*, *E. ruminantium* and *A. platys* may also be pathogenic in humans (Doudier et al. 2010, Nicholson et al. 2010, Maggi et al. 2013).

Although the bacterial species within these two *Anaplasmataceae* genera are ubiquitous (Rar & Golovljova 2011), competent vectors and vertebrate species involved in maintenance, may differ regionally. Molecular surveys have demonstrated the existence of geographically distinct bacterial variants (Kawahara et al. 2006; Hsieh et al. 2010; Dergousoff & Chilton 2011) and have identified locality-specific tick and vertebrate reservoir host species (Kawahara et al. 2006; Harrison et al. 2013). In order to control disease and limit spill-over to susceptible hosts in a particular area, it is important to determine the mammalian reservoirs of infection, the enzootic tick species involved in the natural cycle, and to assess regional bacterial species prevalence and diversity. Here we investigated the *Anaplasmataceae* status of dromedary camels (*Camelus dromedarius*) which, on the basis of numerous serological surveys, are known to be infected with *A. marginale* in a number of countries (reviewed by Menteberre et al. 2013) and with *A. phagocytophilum* in Tunisia (Ben Said et al. 2013). This was achieved using a molecular approach which recovered an overall *Anaplasmataceae* prevalence of 30% and identified novel *Anaplasma* and *Ehrlichia* lineages in dromedary camels, closely related to *A. platys* and *E. canis*, occurring at a prevalence of 26% and 4%, respectively.

Materials and Methods

Eighty male and 20 female dromedary camels were sampled post-mortem at an abbatoir in Unizah, Saudi Arabia. All females were 6-15 years old, whereas all, except four males were 1-2 years old. Animals were inspected prior to slaughter by a single observer (ANA) and samples of spleen were collected aseptically, from each animal, post-mortem, and transported on ice to the laboratory. The spleen samples were divided into equal parts, with one being stored at -80°C and the other in absolute ethanol. Prior to DNA extraction with the Roche High Pure PCR Template Preparation Kit, each 0.2 – 0.4 g ethanol stored sample was serially rehydrated using double-distilled water. *Anaplasma* and *Ehrlichia* genome presence was initially assessed with tick-borne *Anaplasmataceae*-specific primers, EHR16SD and EHR16SR that target a 345 bp region of the 16S rRNA gene (Parola et al., 2000; Primer set 'A' in Table 1). DNA extracts of all first-round PCR positives were subjected to two additional rounds of amplification in order to generate overlapping 16S rRNA gene fragments, using primer sets B and C / D (Table 1). Genomic amplification reactions contained 3 µl of template DNA and were performed in a final reaction volume of 50 µl using Biotools *Taq* polymerase (Biotools, Spain) reaction conditions and a previously described touchdown PCR thermal cycling approach (Harrison et al. 2013). A contiguous 16S rRNA sequence > 1400 nucleotides (nt) in length was generated for each of the variants identified through sequencing and phylogenetic analysis of the diagnostic 345 bp 16S rRNA amplicons. The species informative heat-shock operon or *groEL* gene was also targeted for amplification with diverse combinations of published (Chae et al. 2000; Ybañez et al. 2012) and newly designed primers (Table 1). All amplicons of the expected size were purified directly from the tube using the Roche High Pure PCR product purification kit and each strand was cycle sequenced with BigDye v3.1 (Applied Biosystems) and run on an ABI 3130 automated sequencer (Applied Biosystems). The resulting sequence chromatograms were viewed and edited in Mega5 (Tamura et al. 2011) and used in nucleotide blast searches against the Genbank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Each gene dataset was complemented with homologous reference sequences for eleven of the *Anaplasma* and *Ehrlichia* species listed in the 'prokaryotic names with standing in nomenclature' site (http://www.bacterio.net/-allnamesdl.html), newly described *Ehrlichia mineirensis* (Cabezas-Cruz et al. 2012; 2013) and three *Candidatus* lineages, namely "*Ca*. Ehrlichia shimanensis", "*Ca.* Ehrlichia ovata" and "*Ca.* Anaplasma odocoilei". *Wolbachia*, a non-tick-borne, endosymbiotic *Anaplasmataceae* genus, was included for outgroup purposes. The final 16S rRNA dataset comprising of 27 taxa was aligned in the ClustalW programme embedded in Mega5 (Tamura et al. 2011) and optimised further by eye. As unambiguous alignment of the hypervariable 5' end of the gene was not possible, this indel-rich region was removed, reducing the initial 1,422 nt dataset to 1,289 nt, prior to phylogenetic analysis. For the *groEL* dataset, which comprised 31 taxa, an initial amino acid level alignment was used to guide indel insertions at nucleotide level, resulting in a final aligned dataset 559 nt in length. The HKY+G+I and the GTR+G+I models of sequence evolution were identified as the bestfit models under the Akaike Information Criterion, for 16S rRNA and *groEL*, respectively. Each model was used for minimum evolution (ME) and maximum likelihood (ML) analyses in Mega5 (Tamura et al. 2011) and guided selection of priors for Bayesian inference (BI) in MrBayes (Huelsenbeck & Ronquist 2001). Nodal support values from ME and BI were transferred onto the ML tree nodes (Fig 1a & b), consistently recovered across all methods of analysis.

Results

Screening of dromedary camel spleen DNA extracts with the type-specific primers (Parola et al. 2000) resulted in amplification of the expected 345 bp band in 33 of the 100 samples, all of which were purified and sequenced in order to confirm bacterial genus identity. Unambiguous 16S rRNA gene sequences > 250 nt in length were obtained for 28 of the 33 positive samples and used in nucleotide Blast searches which confirmed the presence of *Ehrlichia* in four of the 28 samples, and *Anaplasma* in the remaining 24 samples. Subsequent amplification and phylogenetic analysis of a near full-length 16S rRNA gene region 1,289 nt in length, of a subset of samples, revealed the presence of three *Anaplasma* variants and a single *Ehrlichia* variant, for each of the camel-associated *Anaplasmataceae* lineages (Fig. 1a). *GroEL* analyses incorporated data generated for 15 samples representative of each of the three *Anaplasma* 16S rRNA variants and two PCR-positive samples for which 16S data were ambiguous. In contrast to the 16S rRNA gene region, *groEL* recovered a single camel *Anaplasma* variant and two *Ehrlichia* variants (Fig. 1b). The combined 16S rRNA and *groEL* results revealed that 23 of the 80 males (29%) and seven of the 20 females (35%) were infected with *Anaplasmataceae*, with three (13%) of the 23 positive males carrying *Ehrlichia* and the remaining 20 (87%) *Anaplasma*. Just one of the seven PCR-positive females was infected with *Ehrlichia*. BlastN searches with 16S rRNA gene sequences submitted to Genbank under accession numbers KF843823-KF843828, revealed the camel *Anaplasma* and *Ehrlichia* strains to be unique and to differ at ≥6 (of 1405) and at ≥13 (of 1407) nucleotide sites, respectively from their closest published matches (JN558826 and GU810149) in the Genbank database. Similarly, the *groEL* gene sequences of camel-associated *Anaplamataceae*, submitted under accession numbers KJ814955–KJ814961, were found to be genetically distinct and to differ by ≥ 55 nt and ≥ 18 nt from their closest *Anaplasma* and *Ehrlichia* Genbank matches, respectively, across the ~550 nt region characterised in this study. All methods of phylogenetic analysis, performed for both gene regions confirmed the taxonomic relatedness of the camel lineages to *A. platys* and to the *E. canis*-*E. mineirensis* clade, with high levels of support (Fig. 1). Uncorrected *groEL* pairwise distances (p-distances) revealed that each of the camel *Anaplasmataceae* lineages had the highest nucleotide sequence identity to *A. platys* and *E. canis*, and that each of these pairwise distances exceeded those between formally recognised congeneric species (supplementary Table 1S). It therefore appears that each of the camel-associated lineages potentially corresponds to a novel species. Based on this we propose that the *Anaplasma* camel-associated lineage be named "*Candidatus* Anaplasma camelii" and that "*Candidatus* Ehrlichia regneryi" be assigned to denote the new *Ehrlichia* lineage, in honour of Russell L. Regnery for his extensive contributions on diverse arthropod-borne alpha-proteobacterial disease agents.

Discussion

Anaplasma marginale has been recorded in *Camelus dromedarius* from various Middle Eastern, African, European and Asian countries at prevalence estimates that range from 0.4 – 10.7%(reviewed by Mentaberre et al. 2013), inclusive of Saudi Arabia at 1.86% (Al Shaikh et al. 2007). In these studies *A. marginale* was identified on the basis of serology alone, and molecular methods to detect and characterise the bacterial agent in seropositive camels from the Canary Islands proved unsuccessful (Mentaberre et al. 2013). In a parallel serological survey, Ben Said and co-workers (2014) found that 29% of the healthy dromedary camels evaluated in Tunisia were positive for *A. phagocytophilum*. In our study, which is the first to genetically characterise tick-borne *Anaplasmataceae* in dromedary camels, we observed a similarly high overall prevalence of 30%. However, instead of *A. marginale* or *A. phagocytophilum*, a potentially novel species that is sister to *A. platys* (100% bootstrap support) was instead identified in 26% of the animals sampled. In addition 4% of the camels were shown to harbour a lineage that is most closely related to *E. canis* (100% bootstrap support, Fig. 1). It is notable that the two most closely related *Anaplasmataceae* species are pathogenic in dogs, causing canine cyclic thrombocytopenia (CCT) and canine monocytic ehrlichiosis (CME), respectively.

As with the Tunisian study (Ben Said et al. 2014), the camels sampled in this Saudi Arabian study appeared healthy and had no apparent clinical manifestations. It is not clear whether these results are attributable to spill-over, as would seem to be the case for goats and sheep, which based on recent reports have an *A. platys* prevalence of 0.8% (Liu et al. 2012; Djiba et al. 2013), or whether dromedary camels may be reservoirs of infection. However, the high prevalence of the strain sister to *A. platys*, namely "*Candidatus* Anaplasma camelii" (26%), in these asymptomatic large (pseudo)ruminants, suggests a broader role in maintenance and propagation. The results further signal the need for additional investigations to establish the vector(s), as well as the veterinary and medical significance of these apparently novel species. This would require evaluation of the predominant camel-associated tick species, *Hyalomma dromedarii*, and its congenerics (van Straten and Jongejan 1993; Elghali and Hassan 2009). In addition, as dogs occur in high numbers and in close proximity to camels in Saudi Arabia (A.N. Alagaili pers. obs.), and are companion animals, canid molecular surveys are warranted. This is of importance as, although rare, cases of human infection with *E. canis* and *A. platys*, the two species shown to be most closely related to the camel strains, have been reported and are of increasing zoonotic concern (Doudier et al. 2010; Maggi et al. 2013).

Acknowledgements

We wish to express our sincere thanks to Lara Nicholson and Eduard Roos, University of Pretoria, for technical assistance. This research was supported by the Deanship of Scientific Research at King Saud University through the research group project number RGP_020 (ANA, OBM, NCB), and by individual (ADSB), chair (NCB) and facilities (No: UID78566) grants awarded by the National Research Foundation of South Africa.

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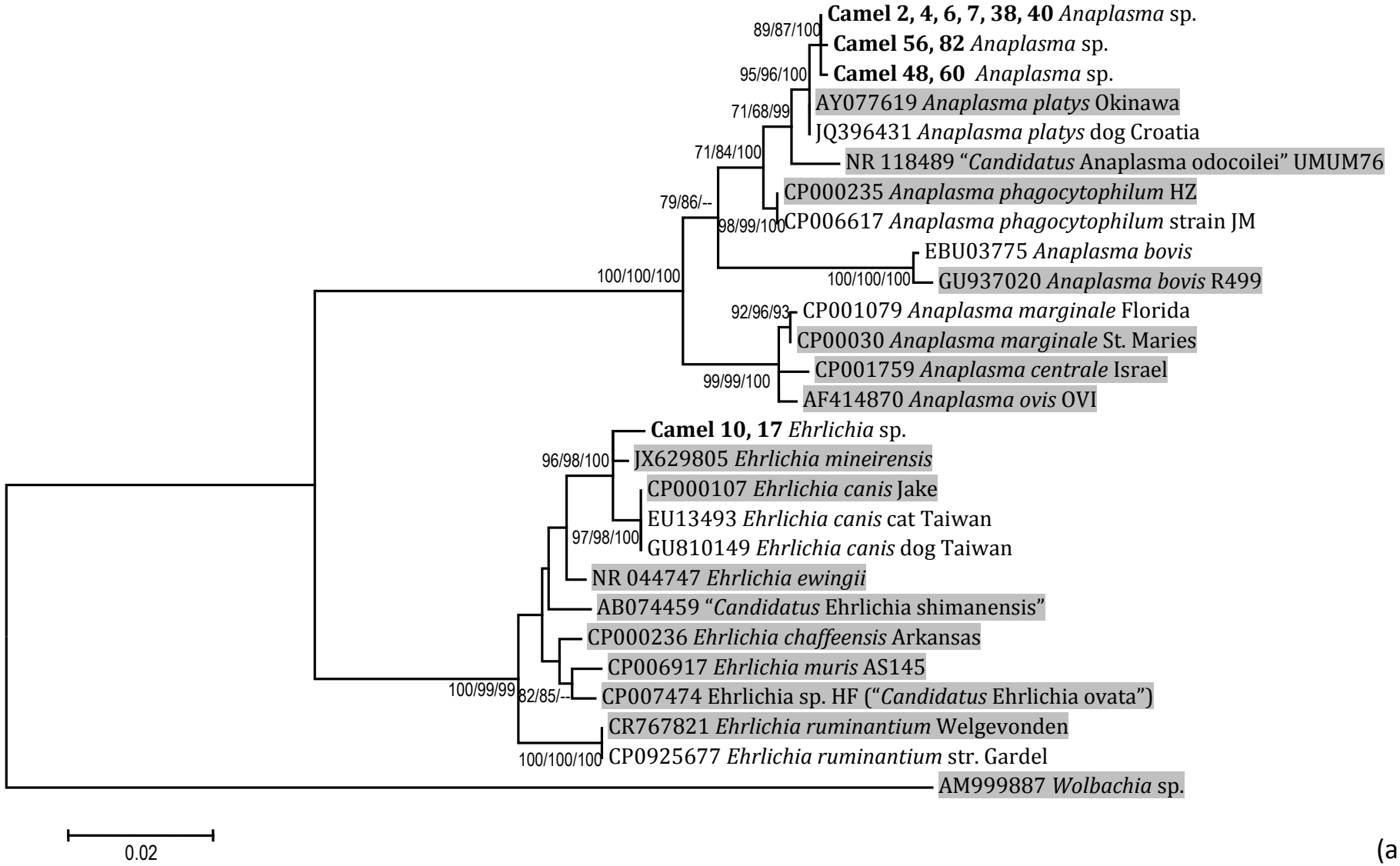
TABLE 1 Summary of primers used in this study and their relative performance with respect to amplification of the tick-borne camel *Anaplasmataceae* lineages characterised in this study

Reaction Code	Primer set Primer name: sequence (5' – 3')	Primer reference	Tm (°C)	Amplicon size	Gene target	Touchdown PCR Ta x2, x3, x35
A	EHR16SD: GGTACCYACAGAAGAAGTCC EHR16SR: TAGCACTCATCGTTTACAGC	Parola et al. 2000 Parola et al. 2000	58 57	~345 bp	16S rRNA	58, 57, 56
[1]B	pA (27F): AGAGTTTGATCCTGGCTCAG EHR16SR: TAGCACTCATCGTTTACAGC	Edwards et al. 1989 Parola et al. 2000	57 57	~790 bp	16S rRNA	58, 57, 56
C	EHR16SD: GGTACCYACAGAAGAAGTCC pH (1492R): GGCTACCTTGTTACGACTT	Parola et al. 2000 Reysenbach et al. 1992	58 55	~1030 bp	16S rRNA	58, 57, 56
[2]D	EHR16SD: GGTACCYACAGAAGAAGTCC pH (1522R): AAGGAGGTGATCCAGCCGCA	Parola et al. 2000 Edwards et al. 1989	58 61	~1060 bp	16S rRNA	58, 57, 56
[3]E1 [3]E2	ELF1: GAGTTCGACGGTAAGAAGTTCA AnaGro712R: CCGCGATCAAACTGCATACC	Chae et al. 2000 Ybañez et al. 2012	57 59	~709 bp	*groEL*	57, 56, 55 55, 54, 53
[4]F1 F2	AnaPlatF2: GCGTAGTCCGATTCTCCAGT AnaGro712R: CCGCGATCAAACTGCATACC	This study Ybañez et al. 2012	59 59	~650 bp	*groEL*	57, 56, 55 58, 57, 56
[5]G	EhrlCanF3: GACATGGCAAATGTAGTTGTAAC AnaGro712R: CCGCGATCAAACTGCATACC	This study Ybañez et al. 2012	57 59	~595 bp	*groEL*	53, 52, 51

Tm: Melting temperature; Ta: Annealing temperature; [1]Amplifies / co-amplifies *Caulobacter* genome; [2]Targets a host and 16S gene resulting in double bands in some samples; [3]Failed to amplify either camel lineage; [4]Preferentially amplifies the camel *Anaplasma* lineages and co-amplified a host gene in some dromedary camel samples; [5]Primer set which amplified all camel *Ehrlichia groEL* gene variants. The EhrlCanF3 primer which was designed to match available *Ehrlichia canis* and closely related sequences.


Camel 2, 4, 6, 7, 38, 40 *Anaplasma* sp.
89/87/100
Camel 56, 82 *Anaplasma* sp.
95/96/100
Camel 48, 60 *Anaplasma* sp.
AY077619 *Anaplasma platys* Okinawa
71/68/99
JQ396431 *Anaplasma platys* dog Croatia
71/84/100
NR 118489 "*Candidatus* Anaplasma odocoilei" UMUM76
CP000235 *Anaplasma phagocytophilum* HZ
79/86/--
98/99/100
CP006617 *Anaplasma phagocytophilum* strain JM
EBU03775 *Anaplasma bovis*
100/100/100
100/100/100
GU937020 *Anaplasma bovis* R499
92/96/93
CP001079 *Anaplasma marginale* Florida
CP00030 *Anaplasma marginale* St. Maries
CP001759 *Anaplasma centrale* Israel
99/99/100
AF414870 *Anaplasma ovis* OVI
Camel 10, 17 *Ehrlichia* sp.
96/98/100
JX629805 *Ehrlichia mineirensis*
CP000107 *Ehrlichia canis* Jake
EU13493 *Ehrlichia canis* cat Taiwan
97/98/100
GU810149 *Ehrlichia canis* dog Taiwan
NR 044747 *Ehrlichia ewingii*
AB074459 "*Candidatus* Ehrlichia shimanensis"
CP000236 *Ehrlichia chaffeensis* Arkansas
CP006917 *Ehrlichia muris* AS145
100/99/99
82/85/--
CP007474 Ehrlichia sp. HF ("*Candidatus* Ehrlichia ovata")
CR767821 *Ehrlichia ruminantium* Welgevonden
100/100/100
CP0925677 *Ehrlichia ruminantium* str. Gardel
AM999887 *Wolbachia* sp.
0.02

(a)

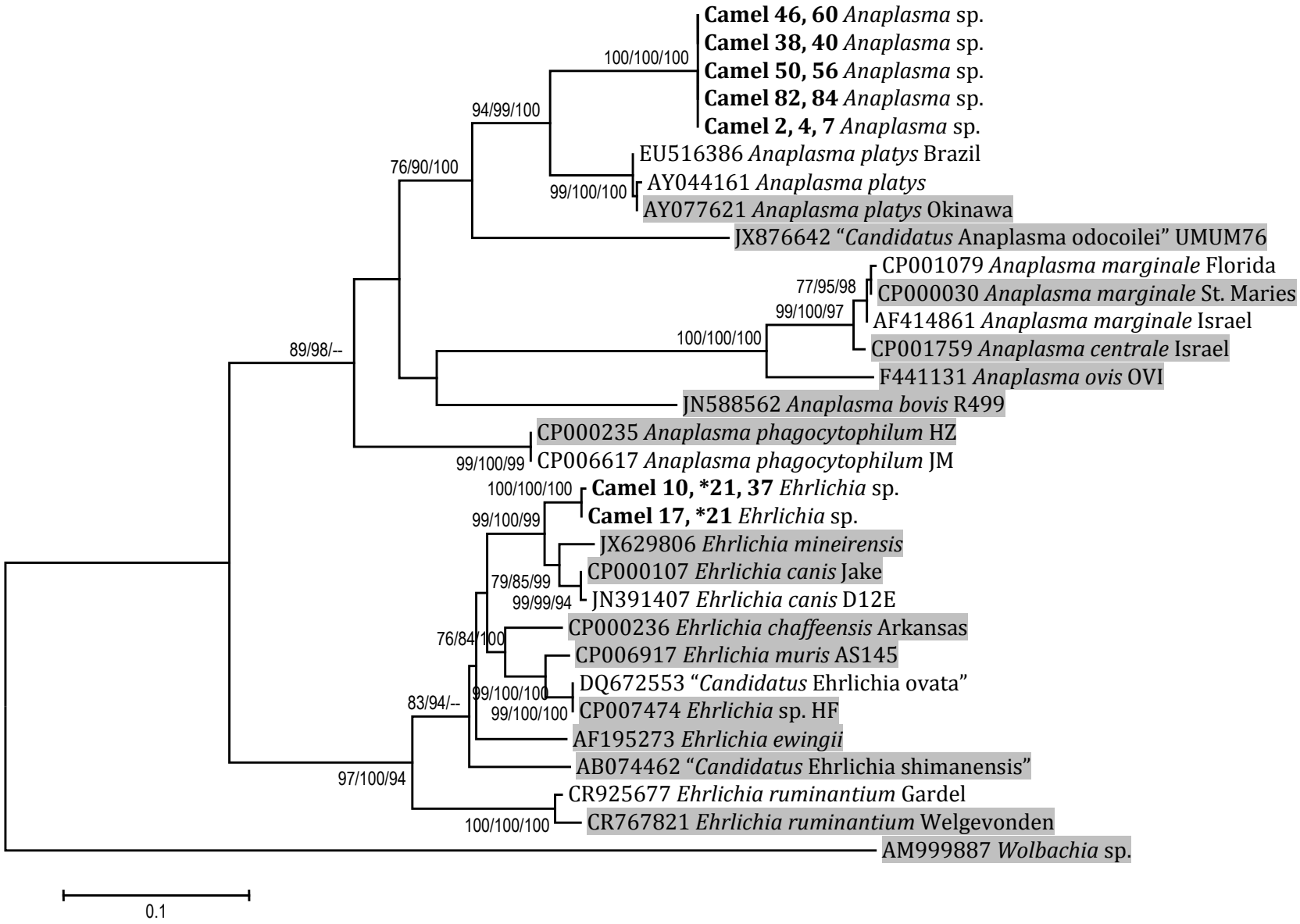

Camel 46, 60 *Anaplasma* sp.
Camel 38, 40 *Anaplasma* sp.
Camel 50, 56 *Anaplasma* sp.
Camel 82, 84 *Anaplasma* sp.
Camel 2, 4, 7 *Anaplasma* sp.
EU516386 *Anaplasma platys* Brazil
AY044161 *Anaplasma platys*
AY077621 *Anaplasma platys* Okinawa
JX876642 "*Candidatus* Anaplasma odocoilei" UMUM76
CP001079 *Anaplasma marginale* Florida
CP000030 *Anaplasma marginale* St. Maries
AF414861 *Anaplasma marginale* Israel
CP001759 *Anaplasma centrale* Israel
F441131 *Anaplasma ovis* OVI
JN588562 *Anaplasma bovis* R499
CP000235 *Anaplasma phagocytophilum* HZ
CP006617 *Anaplasma phagocytophilum* JM
Camel 10, *21, 37 *Ehrlichia* sp.
Camel 17, *21 *Ehrlichia* sp.
JX629806 *Ehrlichia mineirensis*
CP000107 *Ehrlichia canis* Jake
JN391407 *Ehrlichia canis* D12E
CP000236 *Ehrlichia chaffeensis* Arkansas
CP006917 *Ehrlichia muris* AS145
DQ672553 "*Candidatus* Ehrlichia ovata"
CP007474 *Ehrlichia* sp. HF
AF195273 *Ehrlichia ewingii*
AB074462 "*Candidatus* Ehrlichia shimanensis"
CR925677 *Ehrlichia ruminantium* Gardel
CR767821 *Ehrlichia ruminantium* Welgevonden
AM999887 *Wolbachia* sp.
100/100/100
94/99/100
76/90/100
99/100/100
77/95/98
99/100/97
100/100/100
89/98/--
99/100/99
100/100/100
99/100/99
79/85/99
99/99/94
76/84/100
99/100/100
99/100/100
83/94/--
97/100/94
100/100/100
0.1
(b)

Figure 1. Maximum Likelihood gene tree depicting genetic relatedness of *Anaplasma* and *Ehrlichia* variants detected in dromedary camels, *Camelus dromedarius*, from Saudi Arabia (indicated in bold), based on (a) an aligned 1289 nucleotide (nt) segment of the 16S rRNA gene and (b) an aligned 559 nt region of the *groEL* gene. Reference *Anaplasma* and *Ehrlichia* strains common to both trees and used for the pairwise-distance comparisons (in supplementary Table 1S) are denoted by grey shading. Bootstrap support values > 65 % from minimum evolution (ME; 1,000 bootstrap replicates) and maximum likelihood (ML; 1,000 replicates), and posterior probability support values > 90 % from Bayesian inference (BI; two independent runs of 5 million generations, sampled every 1000 generations, and 20% burn-in) are indicated ME/ML/BI next to those nodes recovered with high levels of support across all methods of analysis.
* Denotes a sample co-infected with the two "*Candidatus* Ehrlichia regneryi" *groEL* gene variants.

Supplementary Table 1S Uncorrected (p-distance) matrix summarising *groEL* gene pairwise nucleotide distances in the top-right and 16S rRNA gene nucleotide distances in the bottom left of the table. Shaded blocks indicate the minimum pairwise distances between formally recognised *Anaplasma* and *Ehrlichia* species.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	**[9]**	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]
[1] Camel Anaplasma sp.	--	**0.100**	0.165	0.224	0.231	0.235	0.211	0.178	0.245	0.251	0.242	0.240	0.235	0.258	0.231	0.240	0.256
[2] AY077621 *Anaplasma platys*	**0.002**	--	0.167	0.216	0.218	0.225	0.211	0.178	0.229	0.238	0.238	0.222	0.222	0.244	0.238	0.227	0.238
[3] JX876642 *Ca.* Anaplasma odocoilei	0.010	0.009	--	0.233	0.235	0.225	0.205	0.175	0.240	0.251	0.258	0.245	0.256	0.251	0.251	0.242	0.269
[4] CP000030 *Anaplasma marginale*	0.027	0.027	0.030	--	0.015	0.093	0.222	0.213	0.267	0.267	0.269	0.262	0.258	0.262	0.282	0.256	0.273
[5] CP001759 *Anaplasma centrale*	0.031	0.030	0.032	0.005	--	0.085	0.220	0.216	0.260	0.260	0.265	0.256	0.253	0.258	0.278	0.253	0.273
[6] F441131 *Anaplasma ovis*	0.029	0.027	0.028	0.004	0.006	--	0.209	0.218	0.260	0.258	0.271	0.260	0.262	0.269	0.276	0.260	0.256
[7] JN588562 *Anaplasma bovis*	0.030	0.031	0.031	0.038	0.038	0.040	--	0.200	0.244	0.236	0.244	0.255	0.247	0.249	0.240	0.245	0.251
[8] CP000235 *A. phagocytophilum*	0.009	0.008	0.012	0.024	0.027	0.025	0.032	--	0.216	0.220	0.218	0.211	0.218	0.216	0.216	0.215	0.229
[9] Camel Ehrlichia sp.	0.080	0.080	0.082	0.072	0.076	0.074	0.089	0.074	--	**0.033**	0.045	0.082	0.076	0.080	0.091	0.095	0.136
[10] CP000107 *Ehrlichia canis*	0.078	0.078	0.080	0.069	0.073	0.071	0.085	0.072	**0.007**	--	0.027	0.080	0.078	0.078	0.091	0.095	0.135
[11] JX629806 *Ehrlichia mineirensis*	0.078	0.078	0.081	0.072	0.076	0.074	0.089	0.073	0.006	0.005	--	0.085	0.080	0.080	0.093	0.100	0.140
[12] CP007474 *Ca.* Ehrlichia ovata	0.073	0.073	0.076	0.067	0.069	0.069	0.082	0.067	0.016	0.017	0.015	--	0.025	0.056	0.078	0.085	0.131
[13] CP006917 *Ehrlichia muris*	0.074	0.074	0.078	0.067	0.071	0.070	0.084	0.070	0.017	0.018	0.016	0.007	--	0.056	0.075	0.096	0.133
[14] CP000236 *Ehrlichia chaffeensis*	0.073	0.073	0.076	0.067	0.071	0.069	0.083	0.067	0.012	0.013	0.010	0.008	0.009	--	0.076	0.085	0.122
[15] AF195273 *Ehrlichia ewingii*	0.074	0.074	0.078	0.068	0.074	0.071	0.086	0.069	0.012	0.013	0.010	0.013	0.013	0.009	--	0.085	0.120
[16] AB074462 *Ca.* Ehrlichia shimanensis	0.076	0.076	0.079	0.070	0.074	0.072	0.086	0.071	0.014	0.016	0.014	0.013	0.014	0.012	0.010	--	0.136
[17] CR767821 *Ehrlichia ruminantium*	0.075	0.074	0.076	0.071	0.076	0.072	0.087	0.070	0.022	0.023	0.022	0.021	0.022	0.018	0.015	0.017	--