# Molecular assessment of *Bartonella* in *Gerbillus nanus* from Saudi Arabia reveals high levels of prevalence, diversity and co-infection

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ABSTRACT

Bartonellae bacteria are associated with several re-emerging human diseases. These vector-borne pathogens have a global distribution, yet data on *Bartonella* prevalence and diversity in the Arabian Peninsula are limited. In this study we assessed the *Bartonella* infection status of the Baluchistan gerbil (*Gerbillus nanus*), a species associated with pastoral communities throughout the Middle East region, using a multi-gene PCR screening approach. The results demonstrated that 94 (68.1%) of the 138 gerbils trapped on a monthly basis, over a period of one year, were PCR-positive. Sequencing of the *gltA* gene region confirmed the presence of four discrete *Bartonella* lineages (I-IV) and high levels of co-infection (33.0%). Each of the four lineages, varied in overall abundance (7.5% - 47.9%) and had discernible seasonal peaks. *Bartonella* status was significantly correlated with ectoparasite presence, but not with sex, nor with season. Statistical analyses further revealed that co-infected individuals had a significantly higher relative body condition. Multi-locus sequence analysis (MLSA) performed with a concatenated dataset of three genetic loci (*gltA*, *nuoG*, and *rpoB*), 1452 nucleotides (nt) in length confirmed that lineage IV, which occurred in 24 PCR-positive animals (25.5%), is most closely related to zoonotic *B. elizabethae*. The remaining three lineages (I-III) formed a monophyletic clade which, on the basis of *gltA* was shown to contain bartonellae from diverse Gerbillinae species from the Middle East, suggestive of a gerbil-associated species complex in this region. Lineage I was identical to a *Candidatus* B. sanaae strain identified previously in Bushy-tailed jirds (*Sekeetamys calurus*) from Egypt, wherease MLSA indicate that lineages II and III are novel. The high levels of infection and co-infection, together with the presence of multiple *Bartonella* lineages indicate that *Gerbillus nanus* is likely a natural reservoir of *Bartonella* in the Arabian Peninsula.

**Keywords:** MLSA; *Candidatus* B. sanae; *Bartonella elizabethae*; Gerbillinae; Middle East; seasonal variation

HIGHLIGHTS

- 68% of *Gerbillus nanus* from Saudi Arabia infected with *Bartonella*
- Four *Bartonella* lineages recovered; one closely related to zoonotic *B. elizabethae*
- Lineages vary seasonally and in overall abundance, ranging from 7% to 48%
- High level of co-infected individuals (33%)

INTRODUCTION

Species of *Bartonella*, a bacterial genus of intracellular parasites, infect erythrocytes and endothelial cells of a wide range of animal taxa (Dehio, 2001; Harms and Dehio, 2012). The genus currently comprises more than 30 recognised species, of which at least 17 are associated with human disease (Breitschwerdt, 2014; Kaiser et al., 2011). *Bartonella* is considered a re-emerging human pathogen capable of causing latent bacteraemia (Mogollon-Pasapera et al., 2009). Acute infections can result in clinical conditions such as endocarditis, myocarditis and neuroretinitis (Breitschwerdt, 2014; Gray et al., 2004), whereas chronic infections manifest as a range of non-specific symptoms, which are often misdiagnosed (Breitschwerdt et al., 2010; Harms and Dehio, 2012; Maggi et al., 2012, 2011).

Bartonellae are predominantly transmitted by haematophagous arthropod vectors such as fleas, lice, ticks and mites (Billeter et al., 2008; Chomel et al., 1996; Kabeya et al., 2010) and rodents are important natural reservoirs (Kosoy et al. 2017). More than 20 *Bartonella* species, including several zoonotic species, have been recorded in rodents, many of which are reservoir hosts, and display no overt signs of infection (Breitschwerdt, 2014; Buffet et al., 2013; Jiyipong et al., 2014; Saisongkorh et al., 2009). Depending on the *Bartonella* species and vector, infections in vertebrate reservoirs can range from being highly host-restricted, to infecting a diverse range of species (Bastos, 2007; Inoue et al., 2011; Kosoy et al., 2000).

Bartonellae are widely distributed throughout the world, including the Middle Eastern region (Çelebi et al., 2015; Cohen et al., 2015; Malania et al., 2016; Marciano et al., 2016; Morick et al., 2011, 2010, 2009). However, data for the Arabian Peninsula are limited to two diagnostic reports of human *B. henselae* infections (Eiger-Moscovich et al., 2016; Yousif et al., 1996). Further, the *Bartonella* status of wildlife reservoirs, and rodents in particular, remains unknown despite the commensal nature of many rodent species and the generally high *Bartonella* infection rates recorded elsewhere.

Saudi Arabia, a large country within the Arabian Peninsula, is home to 22 rodent species, half of which belong to the murid subfamily Gerbillinae (Harrison and Bates, 1991). The distributions of these species often extend beyond the borders of Saudi Arabia to other areas of the Middle East. In particular, *Gerbillus nanus*, the Baluchistan gerbil, occurs widely throughout the arid regions of the Middle East, extending from the

Mediterranean to the western parts of India. This species occurs in natural environments and is often associated with pastoral societies and villages in rural areas (Azizi et al., 2011; Shanas et al., 2006). Although it is a species from which *Bartonella* has previously been isolated (Morick *et al.*, 2011), data are geographically limited, and seasonal and host factors remain largely unknown. To address this, we determined the prevalence and diversity of *Bartonella* in *G. nanus* from Saudi Arabia, in individuals sampled monthly over a one-year period, by performing multi-gene PCR screening of all samples, followed by multi-locus sequence analysis (MLSA) of all variants to ensure accurate infection estimates and phylogenetic placement of lineages, respectively.

## 2. MATERIALS & METHODS

### *2.1 Study site and sample collection*

Samples were collected from Prince Saud Al-Faisal Wildlife Research Centre (21°15.1640 N, 40°42.9840 E) in Saudi Arabia. A total of 138 Baluchistan gerbils (77 males and 61 females) were trapped monthly over the course of a full calendar year, from December 2011 to November 2012 as part of a prior study (Sarli et al., 2015; Fig. 1), using approved trapping, euthanization and dissection protocols (University of Pretoria Ethical Clearance Certificate EC060-17). Fleas, mites and lice were collected from 115 individuals (Suppl. Table 1S) for 11 of the 12 months (from January to November) by thorough combing of the animal's pelt on all sides with a toothbrush, over a table covered with white paper. This was supplemented with thorough visual inspection and removal of any remaining ectoparasites with tweezers.

### *2.2 Molecular detection*

Genomic DNA was extracted from heart tissue using the Roche High Pure DNA kit (Roche Diagnostics, UK) according to the manufacturer's specifications for mammalian tissues. Samples were initially screened using *Bartonella*-specific primer sets, previously shown to be valuable for studies on southern African rodent species (Bastos, 2007; Brettschneider et al. 2012). Primers Bart-EF and Bart-ER target a ~500 base pair (bp) region of the citrate synthase gene (*gltA*) (Bastos, 2007; Brettschneider et al., 2012), and primers NuoGF and NuoGR amplify a 366 bp region of the gamma subunit of NADH dehydrogenase (*nuoG*) gene (Colborn et al., 2010). To ensure consistency, PCR thermal cycling was performed on the same ABI 2720 (Applied Biosystems, MA, USA) machine. The risk of cross-contamination was minimised by preparing PCR reactions in a DNA-free fume hood following decontamination with UV radiation, by performing pre- and post-PCR procedures in separate laboratories, by limiting the number of samples handled each day to <15, and by including negative control reactions (to which no DNA was added), as well as a positive control (*Bartonella*-positive *Micaelamys namaquensis*, EZ11-4, Brettschneider et al., 2012) with every subset of samples screened.

PCR products were resolved by 1.5% agarose gel electrophoresis and amplicon size was estimated against a 1 kb molecular weight marker (Fermentas). The presence of *Bartonella* was confirmed by the successful amplification of both gene regions or through two independent amplifications of the same gene region, in combination with bi-directional nucleotide sequencing of each product. Briefly, PCR products were purified directly from the tube using a Roche PCR Product Purification Kit (Roche Diagnostics, UK) and cycle sequenced using the BigDye v.3.1 terminator cycle-sequencing kit (PerkinElmer, USA). Sequencing reactions were run on an Applied Biosystems 3500xl (Life Technologies, Carlsbad, US) at the DNA Sanger Sequencing facility of the University of Pretoria. The resulting sequence chromatograms were viewed and edited using the Chromas programme embedded in MEGA version 7.02 (Kumar et al., 2016).

All *gltA* and *nuoG* PCR-positive samples were sequenced in order to identify the sequence variants present. Four additional gene regions were amplified and sequenced for a representative subset of samples to improve the robustness of phylogenetic analyses and to place sequence variants with greater confidence (La Scola et al. (2003). These additional gene regions, summarised in Table 1, included a 722 bp *gltA* fragment amplified with the BhCS1137n and Bart-EF primer combination (Bastos, 2007; Norman et al., 1995), a 509 bp *rpoB* fragment amplified with the 1600F and 2030R primers (Drancourt and Raoult, 1999), a 306 bp *ribC* fragment targeted with ZRib1F and ZRib1R primers (Zeaiter et al., 2003) and a 753 bp *ftsZ* fragment amplified with ftsZ-F and ftsZ-R primers (Colborn et al., 2010). Due to high levels of co-infection, some sequences contained multiple peaks in chromatograms corresponding to polymorphic sites between the *Bartonella* lineages. The latter two primer sets were excluded from the analyses as it was not possible to obtain representative data for all lineages initially identified through sequencing of the smaller, ~500 bp, *gltA* fragment.

*2.3 Genetic characterization*

Sequence data were aligned using the ClustalW algorithm in MEGA 7.02 (Kumar et al., 2016) and discrete sequence variants were identified based on the initial *gltA* gene tree inferred using the neighbour-joining algorithm and uncorrected p-distances in MEGA 7.02. Sequences containing ambiguities, identified based on the presence of multiple polymorphic sites on chromatograms, were visually compared to unambiguous sequence chromatograms representative of each sequence variant, in an attempt to identify the constituent sequence variants present in each of the co-infections.

A BLAST nucleotide search (www.ncbi.nlm.nih.gov/blast) was conducted for each lineage using the 327 nucleotide *gltA* fragment advocated for species delineation (La Scola et al., 2003) to ensure retrieval and incorporation of the most closely related *Bartonella* sequences currently available in Genbank (www.ncbi.nlm.nih.gov). A reference sequence database was compiled from whole genome sequences sourced

from the NCBI sequence database of representative species within the *Bartonella* genus (Suppl. Table 2S).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted using individual gene (*gltA*, *nuoG* and *rpoB*) and concatenated datasets in PhyML 3.1 (Guindon and Gascuel, 2003) and MrBayes v3.2.6 (Ronquist et al., 2012), respectively. These analyses were guided by the best-fit model of sequence evolution selected under the corrected Akaike Information Criterion (AICc) in jModeltest 2.1.10 (Darriba et al., 2015; Suppl. Table 3S). Nucleotide sequences corresponding to each gene region and variant characterised in this study were submitted to Genbank under the accession numbers provided in Table 1.

*2.4 Statistical analyses*

Generalized linear models (GLM) with a binomial family (link = logit) were used to correlate probability of *Bartonella* infection to (a) sex (male and female), (b) relative body condition , (c) season (wet and dry) and the (d) presence of ectoparasites (present or not). Body condition was calculated using weight and body length (measured from the back of the skull to the base of the tail) and converted to a continuous relative body condition index as described by Peig and Green (2009). A similar analysis was conducted to determine whether the prevalence of different lineages varied significantly between seasons. An ordered logistic regression was run to evaluate whether co-infections amongst infected individuals correlated to any of these factors. In addition, an analysis of variance (ANOVA) was used to investigate the correlation of relative body condition with three infection categories, *viz*. (i) uninfected, (ii) infected by a single strain/lineage and (iii) co-infected with two or more strains. Results were regarded as statistically significant for p-values of 0.05 or less. Two individuals for which complete body measurements were not available were excluded from all statistical analyses, as was the month of December for analyses pertaining to the ectoparasite component. All statistical analyses were conducted within R (R Core Team, 2017).

## 3. RESULTS

*3.1* Bartonella *infection rates and ectoparasite presence*

*Bartonella* infections were detected by PCR in combination with nucleotide sequencing in 94 (68.1%) of the 138 *G. nanus* individuals evaluated in this study. Of these, a positive *Bartonella* status was assigned to 89 on the basis of amplification and sequencing of both the *gltA* and *nuoG* gene regions, and to five individuals based on repeat amplification and sequencing of the *nuoG* gene.

Ectoparasites were present on 92 (78.6%) of the 117 animals sampled from January to November. Of these 92 infested animals, fleas were present on 91 (98.9%),

mites were present on 21 (22.8%), and lice were present on five (5.4%).Both mites and lice shared hosts with fleas in 20 and 3 instances, respectively., but mites and lice never shared a host (Suppl. Table 1S).

*3.2 Genetic characterization of bartonellae*

Analysis of the *gltA* and *nuoG* gene sequences revealed that all unambiguous sequences could be assigned to one of five discrete sequence variants, which will henceforth be referred to as sequence variants 1 to 5. These variants could be assigned to four lineages, with lineage I consisting of sequence variants 1 and 2, and each of the remaining variants corresponding to a specific lineage as follows: variant 3 to lineage II, variant 4 to lineage III and variant 5 to lineage IV. ML and BI analyses of a concatenated sequence dataset, 1452 nucleotides (nt) in length and comprising of 677 nt *gltA*, 328 nt *nuoG* and 447 nt *rpoB* data, produced well-supported phylogenies of similar topology (Fig. 2). Two distinct clades, denoted A and B (Figs 2 & 3), each contained three lineages (I to III) and one lineage (IV), respectively. Within clade B, there was strong support for the sister relationship between lineage IV and *B. elizabethae*, however it was not clear whether, on the basis of genetic distances, lineage IV is sufficiently distinct to be considered a novel species (Suppl. Table 4S). In contrast, the MLSA phylogeny confirmed that each of the three lineages within Clade A was distinct from all formally recognized *Bartonella* species for which homologous data were available (Figs. 2 & 3; Suppl. Table 4S). However, when considering available, homologous data for individuals genes, it was found that sequence variants 1 and 2 were identical across a 528 nt *gltA* gene sequence span to sequences that Alsarraf et al. (2017) detected in *Dipodillus dasyurus* and *Sekeetamys calurus* from Egypt (Suppl. Fig. 1S) and assigned to "*Candidatus* Bartonella sanaae". Additional database searches using the shorter *gltA* fragment, which is best-represented in the Genbank, identified additional closely related taxa and revealed that, with the exception of lesser and greater Egyptian jerboas (family Dipodidae) exported from Egypt, all clade A lineages occur in rodents belonging to the murid family Gerbillinae (Fig. 3).

A large proportion of sequences, 31 of 94 (33.0%) contained multiple polymorphic sites indicative of co-infection with multiple *Bartonella* sequence variants. This was determined through visual inspection of polymorphic sites and comparison to the corresponding sites in chromatograms of each of the five sequence variants recovered, which allowed for the identification of the individual variants in 22 of the 31 co-infected sequences (71.0%; Table 1).

The five lineages also varied in abundance (summarised in Table 1), with lineage I sequence variants 1 and 2 being rare, and neither being detected as co-infections. In contrast, lineage II, which was most abundant, was detected in 45 (47.9%) *Bartonella*-positive animals and occurred as a co-infection with lineage III in seven individuals and with lineage IV in nine individuals. Lineage IV was present in 24 of the *Bartonella* infected rodents (25.5%), predominantly as part of co-infections with either lineage III

(in six individuals) or with lineage II. Different lineages also reached peak prevalence during different seasons (Fig 4). Lineage III prevalence was significantly higher in the wet *versus* the dry season ($\chi^2 = 6.6771$; df = 92; $p < 0.05$), whereas lineages II and IV both reached peak prevalence during the dry season. However, the seasonal difference in prevalence was not significant for either of these lineages ($\chi^2 = 1.0183$; df = 92; p = 0.31 and $\chi^2 = 1.8828$; df = 92; p = 0.17, respectively). The rarity of lineage I precluded analysis or comment on its variation across seasons.

*3.3 Factors associated with Bartonella infection*

Overall *Bartonella* prevalence varied with season (73.3% for the dry season compared to 62.3% for the wet season) and was higher in females (72.1%) than in males (65.3%; Suppl. Table 5S), however, these differences were not significant ($\chi^2 = 0.4652$; df = 114; p = 0.4952 and $\chi^2 = 1.3070$; df = 114; p = 0.2529, respectively). *Bartonella* infection rate was significantly higher when ectoparasites were present ($\chi^2 = 5.5288$; df = 114; $p < 0.05$) and a higher relative body condition were also significantly correlated to *Bartonella* infection($\chi^2 = 4.5995$; df = 114; $p < 0.05$). However, the presence of ectoparasites was not significantly correlated with body condition ($\chi^2 = 0.4253$; df = 114; p = 0.5143).

Further investigation of the relationship of infection status with body condition using multinomial logistic regressions revealed that co-infected individuals' relative body condition was significantly higher than that of uninfected individuals (LO = 0.164, z = 2.69; $p < 0.01$). The difference in relative body condition between co-infected individuals and those infected with a single lineage approached significance (LO = 0.081, z = 1.88, p = 0.0598), but the difference in relative body condition between uninfected individuals and those infected with a single lineage was not significant (LO = 0.083, z = 1.49, p = 0.1374; Suppl. Fig. 2S). Although the number of co-infected females (19) was higher than males (12), this difference was not significant (LO = 0.882, z = 1.60, p = 0.1098).

## 4. DISCUSSION

We report, for the first time, *Bartonella* infection in a natural rodent host population, *Gerbillus nanus*, from Saudi Arabia. These small mammals appear to be natural reservoirs of diverse bartonellae based on the relatively high prevalence (68%), compared to other rodent species (Gutiérrez et al., 2015), and have previously been shown to be capable of maintaining *Bartonella* infections for a period of up to 101 days (Morick et al., 2011). *Gerbillus nanus* is also known to harbour ectoparasites capable of transmitting *Bartonella*. In particular, *Xenopsylla conformis mycerini*, which was recorded on *G. nanus* as well as several other rodent species from our study site (Harrison et al., 2015), is a congener of *Xenopsylla ramesis* for which Morick *et al.* (2011) previously illustrated vector competence.

*Bartonella* prevalence is known to vary substantially amongst species, even those inhabiting similar environments, (Bai et al., 2002; Çelebi et al., 2015). It is therefore notable that reported *Bartonella* infection rates in other Gerbillinae species from the Middle Eastern region are similarly high, ranging from 40.7% to 86.0% (Cohen et al., 2015; Malania et al., 2016; Marciano et al., 2016). This consistently high infection rate across a range of Gerbillinae species and across multiple localities points to common predisposing host characteristics (Cohen et al., 2015), in addition to confirming their importance as natural reservoirs of *Bartonella*.

Our molecular analyses identified the presence of five unique sequence variants (1-5), belonging to four distinct monophyletic lineages (I-IV), that clustered within two clades. Clade A, consisting of the lineages I to III (Fig. 2), appears to be associated with the Gerbillinae subfamily as all closely related sequences were detected in rodents of this subfamily (Suppl. Fig. 1S). The only exception to this was *Bartonella* isolated from lesser and greater Egyptian jerboas (family Dipodidae), which were exported to Japan as pets (Inoue et al., 2009), and may thus not represent naturally acquired infections. The distinctiveness of this clade, first reported by Inoue et al. (2009) in exotic small mammals imported to Japan, was confirmed by Malania et al. (2016) using distance measures across multiple gene regions. Alsarraf et al. (2017) have subsequently proposed two new *Candidatus* species within this clade, namely "*Candidatus* B. sanaae" and "*Candidatus* B. fadhilae" (Suppl. Fig. 1S).

Clade A (Fig. 1 & 2) comprising lineages I-III appears, on the basis of data from wild-caught animals, to represent a primarily Gerbillinae-associated species complex within the broader *Bartonella* phylogeny. Lineages of this complex have been reported throughout the Middle East and into the Caucasus region (Inoue et al., 2009; Malania et al., 2016; Marciano et al., 2016; Morick et al., 2011, 2010) (Fig. 2), indicating a widespread distribution. Similarly, clade B, which incorporates lineage IV (Fig. 2), and is sister to *Bartonella elizabethae*, contains *Bartonella* strains previously detected in the same range of gerbil, jird and jerboa host species, as is observed for clade A. Although the zoonotic potential of members of these species complexes is not known, it is clear that focussed studies within rural communities where rodents are abundant and transmission opportunities likely to be high, will likely to be of regional relevance, given the similarities in small mammal communities and their associated *Bartonella* species.

*Bartonella* co-infections are a well-documented phenomenon (Abbot et al., 2007; Chan and Kosoy, 2010), with most studies typically recording values below 20% (Birtles et al., 2001; Brettschneider et al., 2012; Gundi et al., 2010; Inoue et al., 2009; Kosoy et al., 2004b; Telfer et al., 2007b). Notwithstanding a recent study reporting co-infection rates of 89% based on pyrosequencing rather than Sanger sequencing, suggesting that low co-infection rates may be linked to technical limitations (Gutiérrez et al., 2014), the level of co-infections detected in *G. nanus* from Saudi Arabia (33%) in our study was >10% higher than levels previously reported using similar methods.

The prevalence of individual lineages varied considerably. This has been hypothesised to be related to differences in host-susceptibility between particular lineages (Telfer et al., 2007b). This could also be associated with interactions between lineages within the host, as illustrated Gutiérrez et al. (2014), and may be a key component in maintaining *Bartonella* diversity. The differences in lineage representation in co-infections and the significant correlation of higher relative body condition in co-infected *G. nanus* is suggestive of competitive interactions within the host, and is another aspect requiring further investigation. In common with the results of previous studies (Gutiérrez et al., 2014; Telfer et al., 2007a, 2007b), we observed temporal changes in prevalence for different lineages, with peaks in prevalence occurring in different seasons. Telfer et al. (2007b) proposed that this may be due to associations with different vectors and their seasonal fluctuations, changes in host behaviour between seasons or differences in the duration of infection by different lineages. The presence of multiple lineages displaying seasonal variation in prevalence could explain why no significant difference in prevalence was observed between seasons, despite this being identified as a driver of *Bartonella* prevalence from previous work (Kosoy et al., 2004a).

In conclusion, this study has confirmed the presence of diverse bartonellae in *G. nanus* in the Arabian Peninsula, and expands the geographical extent of two clades that appear to have strong *Bartonella* -host associations throughout the Middle East region. This together with the high levels of infection pointing to the natural reservoir host potential of this murid rodent species calls for further investigation of *Bartonella* prevalence, diversity and zoonotic potential in the region.

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**TABLE 1** Number of *Gerbillus nanus* infected with single and multiple *Bartonella* lineages, with the relevant Genbank accession numbers provided for each of the five sequence variants characterised in this study.

| | | | **Coinfections** | **GenBank accession numbers** | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sequence variant** | **Lineage** | **No. of infections (%)** | **No. (%)** | ***gltA*** (677 nt) | ***nuoG*** (328 nt) | ***rpoB*** (447 nt) | ***ribC*** (265 nt) | ***ftsZ*** (734 nt) |
| **1** | **I** | 4 (4.3) | 0 (0) | MG887833* | MG887838 | MG887850 | - | MG887845 |
| **2** | **I** | 3 (3.2) | 0 (0) | MG887832 | MG887837 | MG887849 | - | MG887844 |
| **3** | **II** | 45 (47.9) | 16 (35.6) | MG887834 | MG887839 | MG887851 | MG887842 | MG887846 |
| **4** | **III** | 30 (31.9) | 13 (43.3) | MG887831 | MG887836 | MG887848 | MG887841 | MG887843 |
| **5** | **IV** | 24 (25.5) | 15 (62.5) | MG887830 | MG887835 | MG887847 | MG887840 | - |

* Identical to KX137114, *Bartonella sanaae*, over an homologous 528 nt region (Figure 1S)

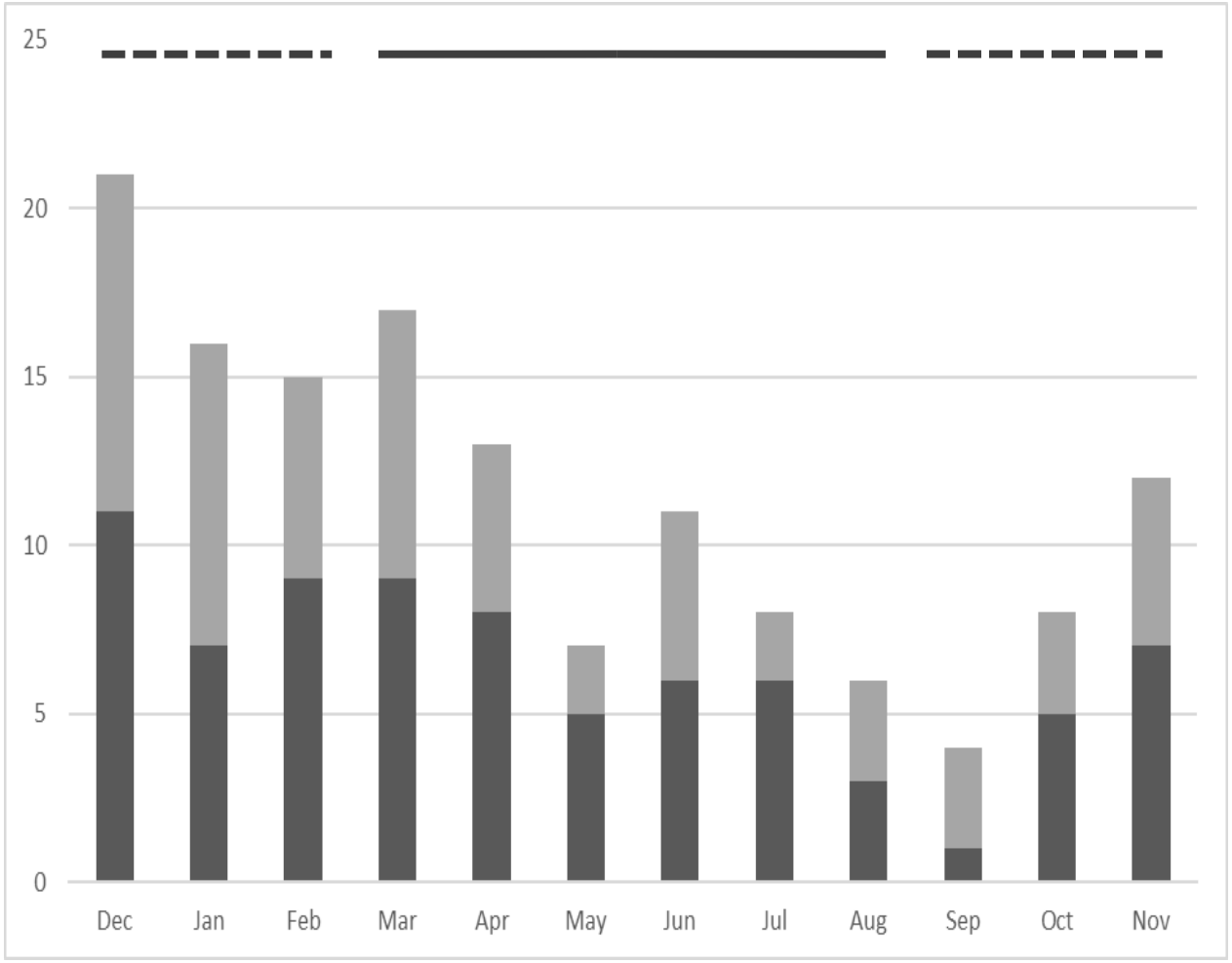


**Figure 1** The number of Baluchistan gerbils trapped per month (number of males and females indicated by dark grey and light grey columns, respectively). The dry season is indicated by dashed bars and the wet season demarcated by a solid bar

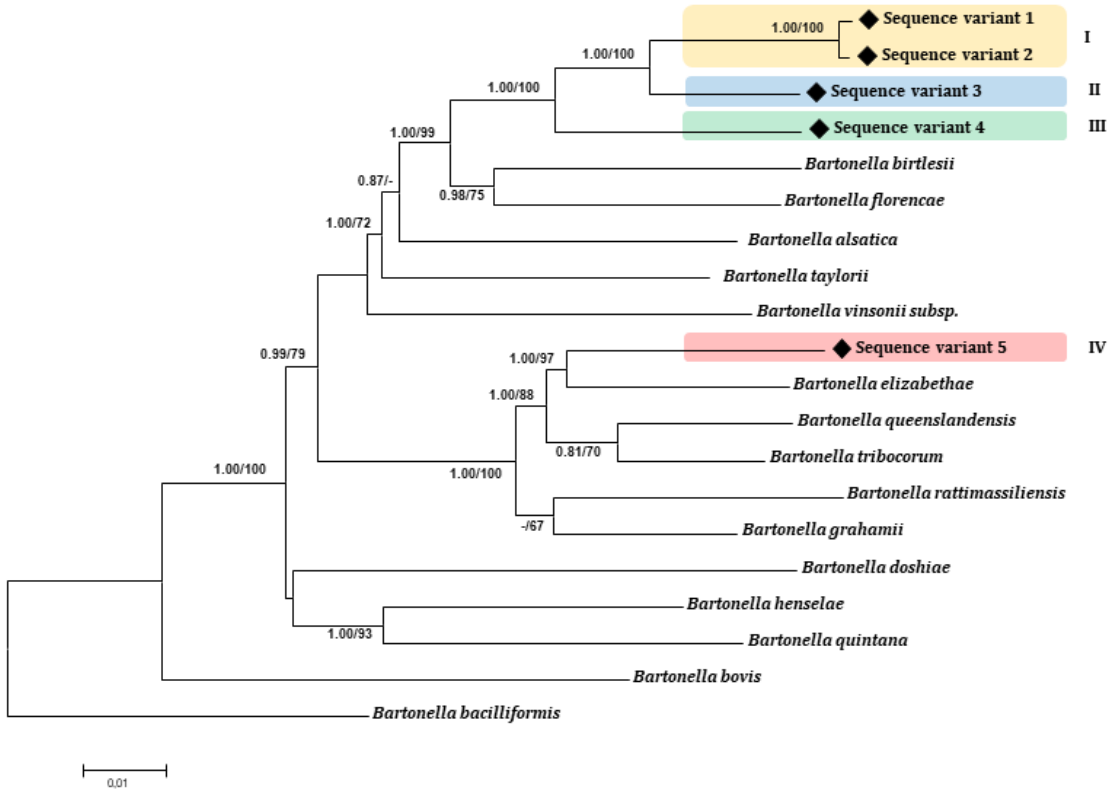


**Figure 2** Genetic relatedness of the four *Bartonella* lineages present in *Gerbillus nanus* from Saudi Arabia and other formally recognised *Bartonella* species (Table 2S), based on a concatenated dataset, 1452 nucleotides (nt) in length (677 nt *gltA*, 328 nt *nuoG* and 447 nt *rpoB*). Nodal support values from Bayesian inference (BI) and maximum likelihood, ≥ 0.80 and ≥ 65, respectively, are indicated BI/ML on the relevant nodes of the p-distance neighbour-joining tree which recovered a topology consistent with that produced by the BI and ML analyses. ♦ Indicates sequence variants characterised in this study.

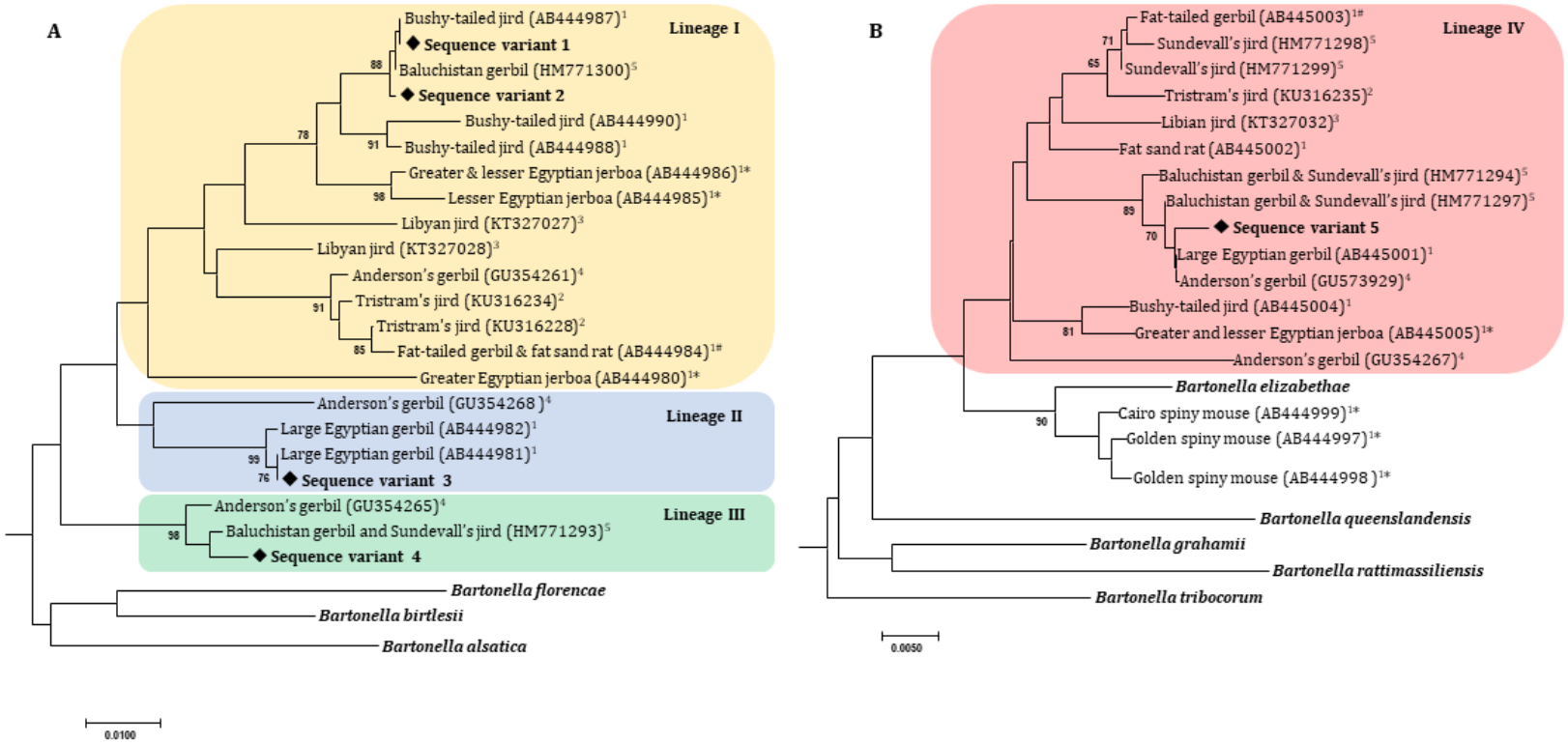


**Figure 3** Tree based on the 338 nt *gltA* gene fragment that is most frequently used for detection and characterisation *of Bartonella* strains, illustrating [A] Clade A (lineages I-III) and [B] Clade B (lineage IV) relatedness to closely related sequences identified through BlastN searches against the Genbank database (www.ncbi.nlm.nih.gov/blast) as well as select *Bartonella* species (Table 2S). The tree was constructed in MEGA 7.02 using the neighbour-joining algorithm and uncorrected (p) distances. Nodal support values are based on 2,500 non-parametric bootstrap replications and only those with values ≥ 65 are shown. are linked to the relevant reference sequences as follows: (1) Inoue et al., 2009; (2) Marciano et al., 2016; (3) Malania et al., 2016; (4) Morick et al., 2010; (5) Morick et al., 2011. ♦ indicates sequence variants characterised in this study; * denotes hosts that do not belong to the murid sub-family Gerbillinae; # indicates fat-tailed gerbils imported from breeders located in the Netherlands.

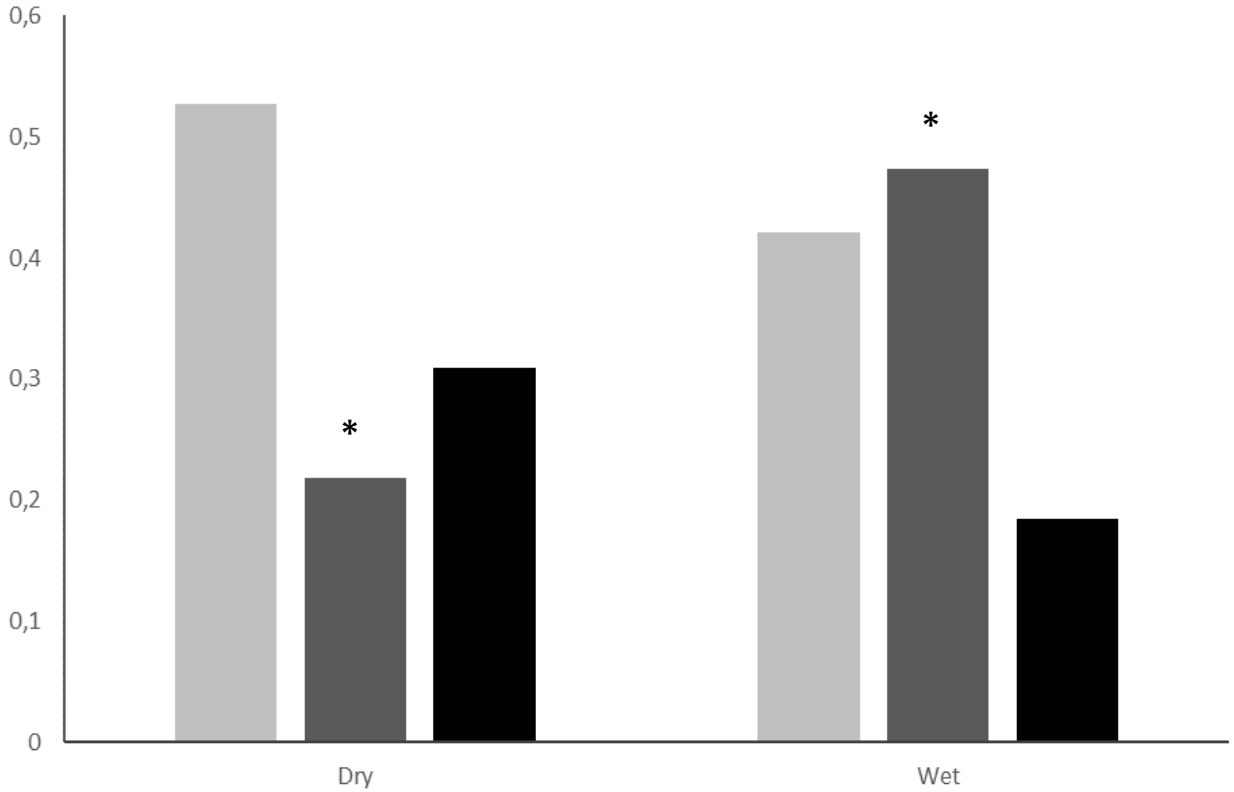


**Figure 4** Variation in lineage prevalence by season (dry *vs* wet). Lineage II (light grey), lineage III (dark grey) and lineage IV (black). Significant differences between the dry and wet season are indicated by an asterisk.

**TABLE 1S** *Gerbillus nanus* ectoparasite abundance by host gender and ectoparasite type

| | | Mites | | Fleas | | Lice | |
|---|---|---|---|---|---|---|---|
| | n | No. | Abundance | No. | Abundance | No. | Abundance |
| **Male** | 51 | 11 | 32 | 50 | 269 | 3 | 3 |
| **Female** | 40 | 9 | 17 | 40 | 210 | 2 | 3 |
| **Total** | 91 | 20 | 49 | 90 | 479 | 5 | 6 |

**TABLE 2S** GenBank accession numbers for reference sequences used in phylogenetic analyses

| Species | Strain | *gltA* | *nuoG* | *rpoB* |
|---|---|---|---|---|
| ***B. alsatica*** | IBS 382 | NZ_JH725020 | NZ_JH725020 | NZ_JH725020 |
| ***B. bacilliformis*** | KC583 | NC_008783 | NC_008783 | NC_008783 |
| ***B. birtlesii*** | IBS 325 | NZ_CM001557 | NZ_CM001557 | NZ_CM001557 |
| ***B. bovis*** | 91-4 | NZ_CM001844 | NZ_CM001844 | NZ_CM001844 |
| ***B. doshiae*** | R18 | NZ_JH725094 | NZ_JH725094 | NZ_JH725094 |
| ***B. elizabethae*** | F9251 | NZ_JH725033 | NZ_JH725033 | NZ_JH725033 |
| ***B. florencae*** | R4 | NZ_HE997451 | NZ_HE997451 | NZ_HE997451 |
| ***B. grahamii*** | as4aup | NC_012846 | NC_012846 | NC_012846 |
| ***B. henselae*** | Houston-1 | NC_005956 | NC_005956 | NC_005956 |
| ***B. queenslandensis*** | AUST/NH15 | NZ_HE998003 | NZ_HE997985 | NZ_HE998003 |
| ***B. quintana*** | Toulouse | NC_005955 | NC_005955 | NC_005955 |
| ***B. rattimassiliensis*** | 15908 | NZ_CALY02000044 | NZ_CALY02000044 | NZ_CALY02000039 |
| ***B. taylorii*** | 8TBB | NZ_JH725052 | NZ_JH725052 | NZ_JH725052 |
| ***B. tribocorum*** | IBS 506 | NC_010161 | NC_010161 | NC_010161 |
| ***B. vinsonii subsp. berkhoffii*** | Winnie | NC_020301 | NC_020301 | NC_020301 |

**TABLE 3S** The best-fit model of sequence evolution selected under the corrected Akaike Information Criterion (AICc) in jModeltest 2.1.10 for each gene region as well as the concatenated dataset comprising *gltA*, *nuoG* and *rpoB*

| | Model of seqence evolution |
|---|---|
| ***gltA*** | GTR + G |
| ***nuoG*** | HKY + I |
| ***rpoB*** | HKY + G |
| ***ribC*** | GTR + I |
| ***ftsZ*** | HKY |
| ***gltA & nuoG & rpoB*** | HKY + G |

**TABLE 4S***:* Nucleotide pairwise distances for *gltA* (327 nt; bottom left) and *rpoB* (509 nt; top right)

| | Seq. variant 1 | Seq. variant 2 | Seq. variant 3 | Seq. variant 4 | Seq. variant 5 | *B.alsatica* | *B.bacilliformi* | *B.birtlesii* | *B.bovis* | *B.doshiae* | *B.elizabethae* | *B.florencae* | *B.grahamii* | *B.henselae* | *B.queenslandensis* | *B.quintana* | *B.rattimassiliensis* | *B.taylorii* | *B.tribocorum* | *B.vinsonii subsp. berkhoffii* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Seq. variant 1** | \ | 0.000 | 0.047 | 0.072 | 0.136 | 0.094 | 0.136 | 0.076 | 0.145 | 0.134 | 0.110 | 0.078 | 0.116 | 0.123 | 0.121 | 0.116 | 0.128 | 0.087 | 0.121 | 0.092 |
| **Seq. variant 2** | 0.004 | \ | 0.047 | 0.072 | 0.136 | 0.094 | 0.136 | 0.076 | 0.145 | 0.134 | 0.110 | 0.078 | 0.116 | 0.123 | 0.121 | 0.116 | 0.128 | 0.087 | 0.121 | 0.092 |
| **Seq. variant 3** | 0.041 | 0.040 | \ | 0.074 | 0.130 | 0.092 | 0.139 | 0.078 | 0.136 | 0.123 | 0.107 | 0.081 | 0.112 | 0.121 | 0.121 | 0.119 | 0.116 | 0.089 | 0.116 | 0.105 |
| **Seq. variant 4** | 0.063 | 0.060 | 0.059 | \ | 0.139 | 0.089 | 0.125 | 0.087 | 0.134 | 0.128 | 0.116 | 0.074 | 0.110 | 0.112 | 0.128 | 0.119 | 0.125 | 0.096 | 0.121 | 0.107 |
| **Seq. variant 5** | 0.117 | 0.117 | 0.095 | 0.107 | \ | 0.128 | 0.168 | 0.141 | 0.145 | 0.139 | 0.065 | 0.125 | 0.083 | 0.145 | 0.072 | 0.132 | 0.089 | 0.132 | 0.065 | 0.132 |
| ***B.alsatica*** | 0.091 | 0.089 | 0.082 | 0.081 | 0.101 | \ | 0.125 | 0.092 | 0.116 | 0.116 | 0.105 | 0.081 | 0.103 | 0.098 | 0.103 | 0.101 | 0.112 | 0.074 | 0.101 | 0.067 |
| ***B.bacilliformi*** | 0.152 | 0.152 | 0.135 | 0.151 | 0.136 | 0.138 | \ | 0.148 | 0.105 | 0.132 | 0.145 | 0.119 | 0.152 | 0.130 | 0.159 | 0.139 | 0.143 | 0.130 | 0.150 | 0.136 |
| ***B.birtlesii*** | 0.098 | 0.097 | 0.083 | 0.075 | 0.098 | 0.079 | 0.142 | \ | 0.141 | 0.132 | 0.116 | 0.072 | 0.121 | 0.107 | 0.123 | 0.110 | 0.134 | 0.087 | 0.123 | 0.096 |
| ***B.bovis*** | 0.152 | 0.151 | 0.139 | 0.139 | 0.130 | 0.133 | 0.127 | 0.124 | \ | 0.094 | 0.132 | 0.121 | 0.128 | 0.112 | 0.139 | 0.123 | 0.125 | 0.132 | 0.134 | 0.125 |
| ***B.doshiae*** | 0.151 | 0.152 | 0.138 | 0.141 | 0.142 | 0.136 | 0.157 | 0.124 | 0.151 | \ | 0.128 | 0.110 | 0.112 | 0.101 | 0.121 | 0.114 | 0.116 | 0.116 | 0.130 | 0.110 |
| ***B.elizabethae*** | 0.126 | 0.127 | 0.107 | 0.116 | 0.047 | 0.114 | 0.146 | 0.108 | 0.132 | 0.138 | \ | 0.103 | 0.067 | 0.123 | 0.049 | 0.116 | 0.085 | 0.110 | 0.047 | 0.110 |
| ***B.florencae*** | 0.092 | 0.091 | 0.083 | 0.083 | 0.119 | 0.086 | 0.141 | 0.069 | 0.139 | 0.135 | 0.123 | \ | 0.101 | 0.103 | 0.105 | 0.103 | 0.110 | 0.094 | 0.105 | 0.094 |
| ***B.grahamii*** | 0.107 | 0.107 | 0.089 | 0.097 | 0.047 | 0.101 | 0.138 | 0.100 | 0.127 | 0.135 | 0.056 | 0.117 | \ | 0.116 | 0.069 | 0.116 | 0.072 | 0.107 | 0.067 | 0.107 |
| ***B.henselae*** | 0.119 | 0.119 | 0.105 | 0.117 | 0.122 | 0.102 | 0.135 | 0.104 | 0.119 | 0.117 | 0.120 | 0.111 | 0.111 | \ | 0.110 | 0.081 | 0.112 | 0.096 | 0.112 | 0.103 |
| ***B.queenslandensis*** | 0.127 | 0.127 | 0.111 | 0.122 | 0.050 | 0.111 | 0.142 | 0.110 | 0.132 | 0.149 | 0.054 | 0.129 | 0.044 | 0.122 | \ | 0.110 | 0.085 | 0.105 | 0.038 | 0.112 |
| ***B.quintana*** | 0.139 | 0.139 | 0.120 | 0.136 | 0.132 | 0.124 | 0.148 | 0.126 | 0.119 | 0.141 | 0.139 | 0.129 | 0.129 | 0.082 | 0.139 | \ | 0.123 | 0.094 | 0.105 | 0.094 |
| ***B.rattimassiliensis*** | 0.130 | 0.130 | 0.113 | 0.113 | 0.063 | 0.119 | 0.148 | 0.114 | 0.141 | 0.151 | 0.073 | 0.126 | 0.044 | 0.129 | 0.070 | 0.133 | \ | 0.121 | 0.085 | 0.119 |
| ***B.taylorii*** | 0.101 | 0.100 | 0.098 | 0.089 | 0.114 | 0.088 | 0.138 | 0.089 | 0.130 | 0.129 | 0.123 | 0.085 | 0.108 | 0.111 | 0.126 | 0.119 | 0.127 | \ | 0.107 | 0.081 |
| ***B.tribocorum*** | 0.119 | 0.119 | 0.108 | 0.111 | 0.053 | 0.098 | 0.142 | 0.098 | 0.130 | 0.141 | 0.060 | 0.123 | 0.042 | 0.114 | 0.051 | 0.136 | 0.056 | 0.116 | \ | 0.112 |
| ***B.vinsonii subsp. berkhoffii*** | 0.111 | 0.110 | 0.101 | 0.097 | 0.108 | 0.097 | 0.146 | 0.102 | 0.139 | 0.138 | 0.114 | 0.110 | 0.107 | 0.111 | 0.120 | 0.122 | 0.111 | 0.089 | 0.110 | \ |

**TABLE 5S** *Bartonella* infections by season and host gender

| | | Season | | Sex | |
|---|---|---|---|---|---|
| | **n** | **Dry** | **Wet** | **Male** | **Female** |
| - | 44 | 21 | 23 | 27 | 17 |
| + | 94 | 55 | 39 | 50 | 44 |
| **Total** | 138 | 76 | 62 | 77 | 61 |

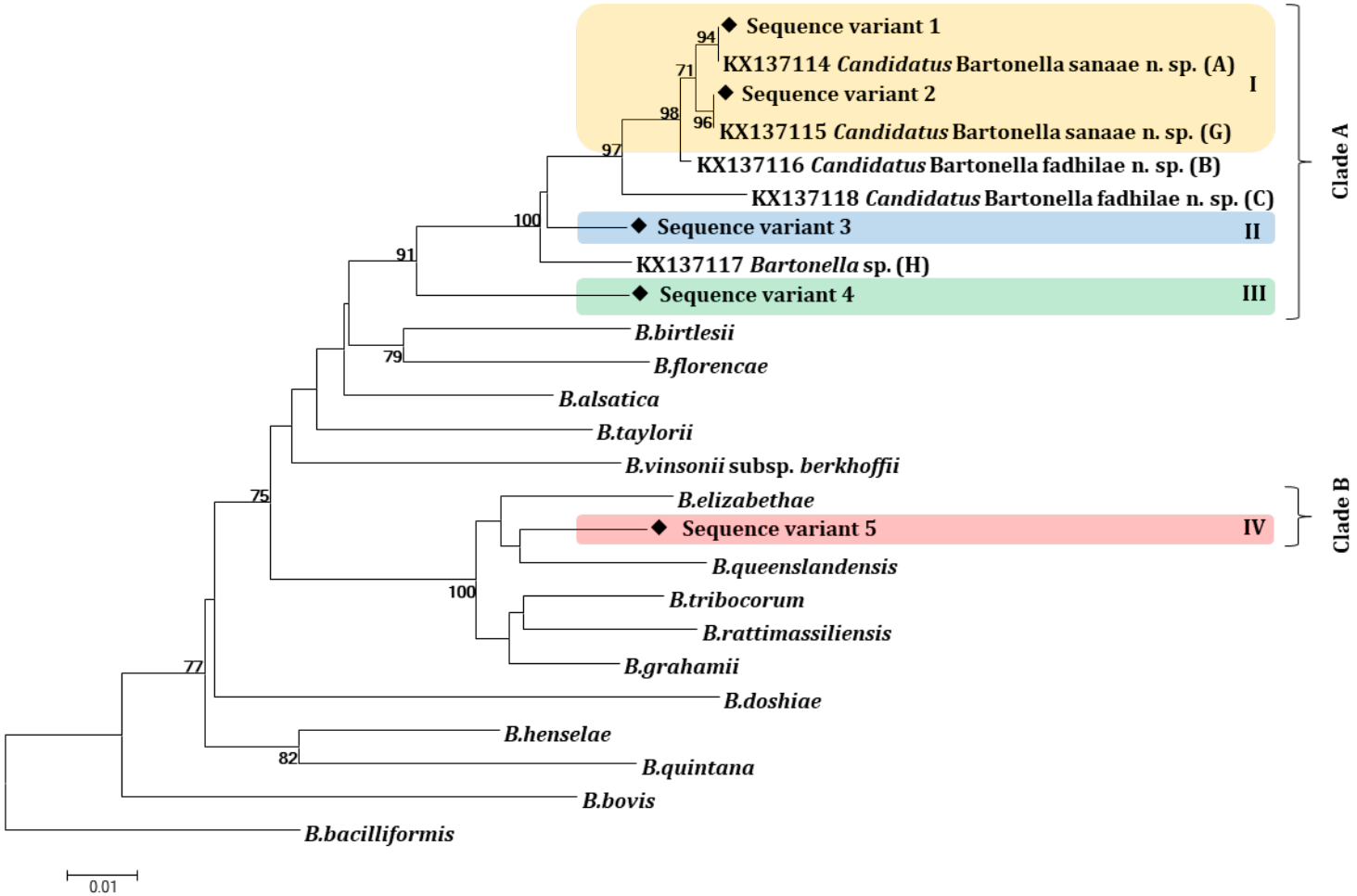


**Figure 1S** Phylogenetic relationship of *Bartonella* strains characterised in the current study, the closely related *Candidatus* species proposed by Alsarraf et al. (2017) and reference sequences (as deatailed in Table 2S). The phylogeny was inferred based on a 528 nt *gltA* fragment in MEGA 7.*02* using the neighbour-joining algorithm. Nodal support values are based on 2500 non-parametric bootstrap replications and values $\geq 65$ are shown.

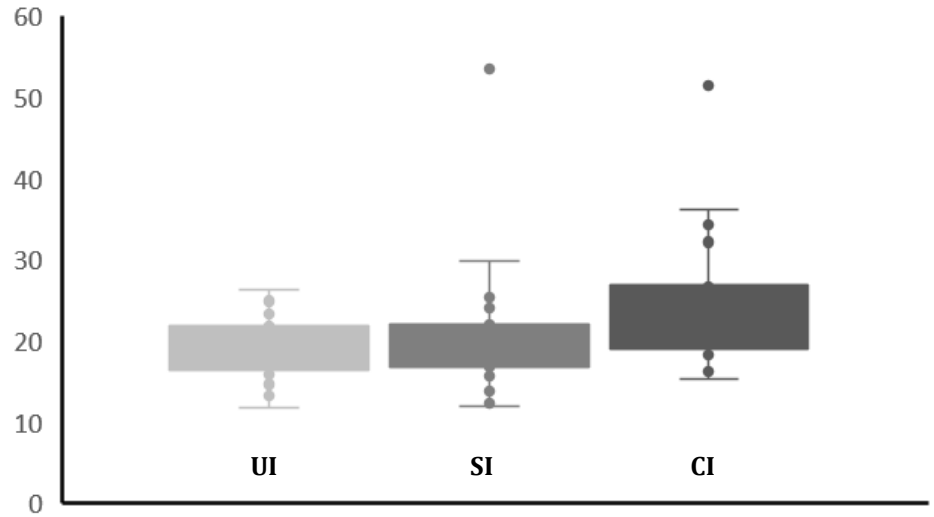


**Figure 2S** Relative body condition of uninfected individuals (UI), individuals infected by a single lineage (SI) and individuals infected by multiple lineages (CI). The difference between UI and SI were not significant, the difference between SI and CI approached significance (p=0.0598) and the difference between UI and CI was significant.