Bartonellae of synanthropic four-striped mice (*Rhabdomys pumilio*) from the Western Cape Province, South Africa

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Abstract

Bartonella is a species-rich bacterial genus that infects a wide variety of wild and domestic animals, including rodents. Despite high levels of murid rodent diversity in Africa, associated *Bartonella* prevalence and diversity remains under-studied, particularly within the southern African sub-region. To address this, we sampled endemic four-striped mice, *Rhabdomys pumilio*, from three rural and two urban localities in the Western Cape Province, South Africa. PCR screening and multi-locus sequence analysis (MLSA) inclusive of five genome regions (*glt*A, *nuo*G, *rib*C, *rpo*B, and ITS), was respectively used to evaluate *Bartonella* status and diversity in these synanthropic rodent populations. An overall infection rate of 15% was recovered, ranging from 0% for an urban locality to 36.4% for a rural locality, consistent with the higher flea abundance recorded at the latter sites. Nucleotide sequencing and phylogenetic analyses confirmed the presence of three distinct *Bartonella* lineages (I - III), with lineages II and III grouping with bartonellae previously detected in *R. pumilio* from nature reserves in the Free State Province of South Africa, and lineage I being novel and sister to *Bartonella* strains identified previously in *Micaelamys namaquensis.* Our results indicate significant landscape effects on infection rates, highlight differential PCR assay performance and identify three host-associated *Bartonella* lineages in *Rhabdomys* from South Africa.

Keywords: Phylogeny; multi-locus sequence analysis (MLSA); *Candidatus* Bartonella species; urban; rural

Introduction

Bartonellae are fastidious, Gram-negative α2-proteobacteria (Houpikian and Raoult 2001) that infect a wide range of mammalian hosts including rodents, insectivores, carnivores, lagomorphs, primates, ungulates and humans (Chomel and Kasten 2010). These bacteria typically infect erythrocytes of vertebrate reservoir hosts and can induce pathology in multiple organs (Young, et al. 2014). Transmission among vertebrate hosts is facilitated by blood-sucking arthropod vectors such as fleas, ticks, mites and sand flies but fleas, in particular, are considered major vectors among rodent hosts (Billeter, et al. 2008, Tsai, et al. 2011). At least 34 species and subspecies of *Bartonella* are currently recognized with the majority of these (> 80%) recorded in rodents (Jiyipong, et al. 2014). The ability of rodents to invade anthropogenically-transformed areas where they can attain pest status, and their role as reservoir hosts for a wide range of infectious disease agents, including *Bartonella*, underscore the importance of assessing the bacterial infection status of rodents across different habitat types, and particularly within commensal settings where contact opportunities are high (Bastos, et al. 2005, Berglund, et al. 2010).

Members of the genus *Rhabdomys* are relatively small (40-60 g), widely distributed rodents, occurring throughout southern and East Africa (Skinner and Chimimba 2005). Initially considered a monotypic genus comprising between 14 and 20 *Rhabdomys pumilio* subspecies, the genus has undergone a number of revisions, with molecular data now suggesting the presence of at least two southern African species, *R. pumilio* and *R. dilectus*, and two *R. dilectus* subspecies (Castiglia, et al. 2012, Du Toit, et al. 2012, Ganem, et al. 2012, Rambau, et al. 2003). Areas of sympatry in South Africa have been inferred from environmental niche modelling (Meynard et al., 2012) and confirmed through molecular characterisation (Le Grange, et al. 2015).

Rhabdomys pumilio is a broad-niche, peri-domestic rodent that can adapt to and benefit from anthropogenic habitat change (Froeschke, et al. 2013, Van der Mescht, et al. 2013). In line with the generalist nature of this rodent, it is host to a diverse array of parasitic species. In the Western and Northern Cape Provinces of South Africa more than 30 ectoparasite species, including ixodid ticks, mesostigmatid and trombiculid mites, fleas and anoplurid lice have been recorded from *R. pumilio* (Froeschke, et al. 2013, Matthee, et al. 2007, Matthee, et al. 2010, Matthee and Krasnov 2009, Matthee and Ueckermann 2008), with some of the recorded fleas (e.g. *Chiastopsylla rossi* and *Dinopsyllus ellobius*) being known vectors of zoonotic disease agents such as *Yersinia pestis* (Ingram 1927). More recently, Van Der Mescht and Matthee (2017) reported the presence of at least 14 flea species from *R. pumilio*, viz. *Chiastopsylla capensis*, *Chiastopsylla carus*, *Chiastopsylla mulleri simplex, Chiastopsylla pitchfordi, Chiastopsylla quadrisetis*, *Chiastopsylla rossi, Ctenophthalmus calceatus, Dinopsyllus ellobius, Dinopsyllus tenax, Hysophthalmus temporis, Listropsylla agrippinae, Listropsylla fouriei, Xenopsylla brasiliensis* and *Xenopsylla eridos*.

A prior study of bartonellae in terrestrial small mammals from nature reserves in the Free State Province of South Africa revealed high levels of infection and diversity in the 10 mammalian host species evaluated (Pretorius, et al. 2004). Although just nine of the 86 animals were *R. pumilio* specimens, characterization of the citrate synthase (*glt*A) gene revealed that the three *Bartonella*-

positive *Rhabdomys* specimens harbored two novel lineages. The uniqueness of these lineages and relatively high *Rhabdomys* infection rate (44%) prompted the current expanded assessment of *Bartonella* prevalence and diversity in *R. pumilio* sampled from rural and urban settlements in the Western Cape Province of South Africa. Using multi-locus screening in combination with phylogenetic analyses we were able to obtain refined estimates of infection, across these modified habitat types and enhanced taxonomic placement of the *Bartonella* lineages present in *Rhabdomys*.

Materials and Methods

Rodent sampling

A total of 80 *R. pumilio* individuals were sampled from five localities in the Western Cape Province as part of a previous study (Froeschke and Matthee 2014). Two of the five localities were urban settings (Franschhoek and Somerset West) and three occurred within rural landscapes (Franschhoek, Wellington and Gordon's Bay). Line transects, consisting of Sherman-like live traps were placed approximately 10 m apart and baited with a mixture of peanut butter and oats. *Rhabdomys pumilio* individuals were euthanized with 2–4 ml Sodium Pentobarbitone (200 mg/kg), depending on individual weight, as approved by the Ethics Committee of Stellenbosch University (ref no. 2006B01007 and SUACUM11- 00004(p)) and under collection permits issued by Cape Nature (ref no. 317/2003, 360/2003, AAA004-00221-0035). Body measurements and weight were recorded for each animal, and the ectoparasites were removed by pelage brushing and stored in 70% ethanol. Fleas were mounted using standard methods and identified to species level (Segerman 1995) by a single investigator (LvdM).

Genetic characterization

DNA extracted from frozen *Rhabdomys* heart tissue with the High Pure PCR template preparation kit (Roche) was screened for *Bartonella* genome presence as previously described using primer sets targeting the citrate synthase (*glt*A), NADH dehydrogenase gamma subunit (*nuo*G), riboflavin synthase (*Rib*C), RNA polymerase subunit B (*rpo*B) and the 16S-23S ribosomal RNA intergenic spacer (ITS) gene regions (Table 1S). As fleas were mounted for identification purposes, they were not evaluated for *Bartonella* genome presence.

Samples were considered positive based on at least two independent amplifications of one or more target gene regions and on nucleotide sequence verification of *Bartonella* presence. The latter was achieved by purifying amplicons of the correct size with the High Pure PCR product purification kit (Roche), and performing Sanger cycle sequencing with Big Dye Terminator cycle sequencing ready reaction kit (Perkin-Elmer), with each of the external PCR primers, in separate reactions. Reactions were purified, denatured and submitted to the core Sanger sequencing facility at the University of Pretoria.

Phylogenetic analyses

Individual *Bartonella* gene datasets complemented with homologous, reference sequence data from the GenBank database (www.ncbi.nlm.nih.gov) were generated for the five bacterial genes characterized in this study. Sequences were aligned using the ClustalX program embedded in Mega 6 (Tamura, et al. 2013), and individual p-distance neighbor-joining trees were initially inferred in order to delineate *Bartonella* species boundaries on the basis of genetic distances (La Scola et al., 2003), and high nodal support values (Pretorius et al., 2004). Maximum likelihood (ML) and Bayesian inference (BI) analyses were subsequently performed using PhyML (Guindon and Gascuel 2003) and MrBayes (Huelsenbeck and Ronquist 2001), respectively, for the individual gene and concatenated datasets.

Statistical analyses

A stepwise Akaike Information Criterion (AIC) analysis was conducted to determine whether host sex, landscape type (urban *versus* rural) and load of each flea species or total host flea load significantly explained the variation in *Bartonella* status providing the best fit predictive model. A binomial logistic regression was conducted on the best fit model to determine whether any of the predictors had a significant effect on the log probability of *Bartonella* status. A Fisher's exact test, to account for limited flea species data, and a Pearson's Chi-squared test (with Yates' continuity correction) were conducted to test whether *Bartonella* status is dependent on the species or sex of fleas, respectively. All analyses and assumption testing were conducted in the Rstudio interface of R v3.4.3 (R Core Team 2017).

Results

Bartonella amplification ranged from 6.5% to 13.8% (Table 1), across the different gene regions. Based on two or more independent, sequence-confirmed *Bartonella* genome amplifications, an overall *Bartonella* infection rate of 15% was recovered (Table 1). Whereas the *glt*A and *rib*C primer sets each detected 10 positive individuals, the *nuo*G, *rpo*B and *ITS* assays identified eleven, eight and five *Bartonella*-positive animals, respectively.

Sequence data generated in this study were submitted to GenBank under accession numbers indicated in Supplementary Table 2S. These data, when complemented with reference strain sequences in the Genbank database, were used to infer individual gene phylogenies (Supplementary Fig. 1 & 2) to confirm the consistency of phylogenetic placement across the different gene regions. In addition, samples containing discernible levels of co-infections across the different gene regions characterized (Supplementary Table 3S) were excluded prior to concatenation. As most samples were co-infected, this ultimately restricted enhanced phylogenetic placement of lineages within the broader genus phylogeny to just two samples, FHC6 and GB17, respresentative of lineages I and III, respectively. *Glt*A and *rib*C gene phylogenies each recovered three discrete lineages (denoted I-III in the *glt*A gene tree; Fig. 1A), of which two (II and III)

grouped with bartonellae previously identified in *R. pumilio* from the Free State Province of South Africa (Pretorius et al. 2004). In contrast, lineage I was novel and sister to bartonellae strains previously identified in *Micaelamys namaquensis* from Gauteng Province, South Africa (Brettschneider et al., 2010). A number of close matches (≥ 97% identity), were identified for the *glt*A gene, through nucleotide Blast searches against the Genbank database (wwww.ncbi.nlm.nih.gov). As *glt*A is the marker of choice and best-represented in the Genbank database, these sequences were included in the single gene *glt*A gene phylogeny (Fig. 1A). Whilst some of these close matches included rodent bartonellae from Kenya (Halliday, et al. 2015), all were distinct from the *Bartonella* strains in *Rhabdomys* from South Africa, indicating that lineages II and III have a strong *Rhabdomys* host-association. Further, the pairwise genetic distances between the three lineages, and that between lineage I and closely-related *Bartonella* strains identified previously in *Micaelamys*, matched or exceeded distances between formally recognized species such as *B. grahamii* (NC012846) and *B. rattimassiliensis* (AY515124). The consistent recovery of three discrete monophyletic lineages for each of the gene regions characterized, in combination with published genetic distance guidelines (La Scola, et al. 2003) suggests that the three lineages detected in *Rhabdomys* each represent discrete species. However, as PCR assay performance varied markedly (Table 1 and supplementary Table 3S), it was not possible to generate data for all lineages across all gene regions. Our taxonomic recommendations based on the five-gene concatenated phylogeny (Fig. 1B) are therefore limited to lineages I and III. Phylogenetic placement of these two lineages within the broader *Bartonella* phylogeny was achieved using a concatenated *glt*A-*rib*C-*rpo*B-*nuo*G-*ITS* dataset (Fig. 1B), 2560 nt in length. The higher levels of support for the internal nodes obtained with this dataset confirmed the sister-taxon relationship for lineage I and *B. elizabethae* (72% bootstrap support), which were nested within a larger clade containing *B. tribocorum*, *B. queenslandensis* and *Rhabdomys*-associated lineage III (96% and 90% support from ML and BI analyses, respectively; Fig. 1B).

Three flea species, *C. rossi*, *Dinopsyllus tenax,* and *Listropsylla agrippinae,* were recorded from *R. pumilio* at the five localities (Table 2). Although abundance varied between localities, two species (*C. rossi* and *L. agrippinae*) predominated at all sites. Tick larvae and nymphs (*Rhipicephalus*, *Haemaphysalis* and *Ixodes*) as well as mesostigmatid mites (*Laelaps giganteus*) were also present.

Under the binomial logistic regression; landscape type (urban *versus* rural) had a significant effect on the log probability of *Bartonella* occurrence ($\chi^2 = 8.66$, df= 1, $p = 0.003245$) with rodents from the rural landscape being significantly more likely to have a higher infection rate compared to those sampled from the urban landscape ($p = 0.02552$). However, the best fit predictive model did not contain load of each flea species or total host flea load, thus our flea data had no significant role in explaining the variation in *Bartonella* prevalence. Additionally, neither the Fisher's exact test, nor the Pearson's Chi-squared test (with Yates continuity) were significant, indicating that *Bartonella* status was independent of flea species composition and sex, respectively.

Discussion

The overall *Bartonella* infection rate in *R. pumilio* samples collected in the present study was 15%, ranging from 0% (for Somerset West) to 36.4% (for Wellington) by locality, and from 6.5% (for *ITS*) to 13.8% (for *nuo*G) in the individual gene assessments (Table 1). As frozen heart samples, rather than spleen were used in this study, this likely represents an underestimation of the infection rate. The marked variation in *Bartonella* genome detection capabilities of the assays precluded characterization of all gene regions for the three lineages detected in *Rhabdomys* from the western Cape and highlights the importance of using multiple assays in parallel to ensure accurate estimates of *Bartonella* prevalence. Despite variable assay performance the individual gene phylogenies *glt*A (Fig. 1A) as well as the *rib*C, *rpo*B, and ITS (Supplementary Fig. 1S) consistently recovered two of the three *Bartonella* lineages (I-III) in *R. pumilio*. However, the *nuo*G marker (Colborn et al. 2010) identified two additional lineages (Supplementary Fig. 2S). These results are at odds with the more widely used gene markers that have proven valuable for delineating rodent-associated *Bartonella* species (La Scola, et al. 2003,Pretorius, et al. 2004), suggesting that whilst the *nuoG* marker is valuable for detecting *Bartonella* genome presence in clinical specimens, its phylogenetic utility is less clear.

Using the prescribed p-distance cut-offs for the *glt*A and *rpo*B gene regions (< 96% and < 95%, respectively; La Scola et al., 2003), it was confirmed that six positive specimens harbored a strain that is sister to the zoonotic *B. elizabethae* species complex, and which is denoted lineage I in this study. The remaining *R. pumilio* individuals harboured one of two lineages (denoted II and III in this study) previously identified in *R. pumilio* individuals (Pretorius, et al. 2004) sampled from a natural setting more than 800 km north of our study site. Of interst is that the *glt*A p-distance phylogeny placed one sample from lineage III (GB9) outside the La Scola et al. (2003) species delineation criteria, whereas p-distances for other gene regions placed the same sample well within the proposed cut-off values (La Scola et al., 2003). Hence, in accordance with Pretorius et al. (2004) we suggest that the cut-off values for individual gene regions should not be applied too strictly and should also be guided by nodal support values confirming monophyly of the novel lineage.

On the basis of the five-gene MLSA (Fig. 1B) it appears that two of the three lineages (I and III) present in *Rhabdomys* are novel with lineage III corresponding to the strain previously detected in *Rhabdomys* (Fig. 1A; Pretorius et al. 2004)The close association between lineage I strains and *B. elizabethae* warrants further investigation as strains of the latter *Bartonella* species complex are zoonotic and ubiquitous. It is noteworthy that whereas lineage I predominated at the three rural sampling sites evaluated in this study (Table 1), it was not recorded in *Rhabdomys* previously sampled from natural landscapes (Pretorius et al. 2004).

In the present study, the *Bartonella* infection rates in *R. pumilio* varied between habitat types with rural landscapes generally having higher levels of infection than urban landscapes. Despite the relatively small sample size and similar densities of *R. pumilio* hosts in both landscape types (Froeschke and Matthee, 2014), statistically significant differences were observed between urban and rural localities. The highest infection rate was recorded at the rural locality of

Wellington (36.4%), whereas at the urban locality of Somerset West there was an absence of *Bartonella*. The former infection rate is comparable to the 44% infection rate in *R. pumilio* sampled from natural landscapes in the Free State Province and reported by Pretorius et al. (2004). This, together with the observation that *Bartonella* infection rates were higher at the Franschhoek rural locality (25%) compared to the Franschhoek urban locality (7.7%) suggests that land usage type and the associated degree of anthropogenic modification appears to be a strong driver of *Bartonella* prevalence.

Ectoparasites, especially fleas, are known vectors of *Bartonella* species among rodents (Billeter, et al. 2008,Tsai, et al. 2011) and it is therefore anticipated that higher infestation rates would be associated with higher *Bartonella* infection rates. However, our statistical analyses suggest that this is not the case for the Western Cape population of *R. pumilio* evaluated in this study.

Conclusions

From the limited number of studies of *Bartonella* in murid rodents sampled from natural settings in South Africa (Brettschneider et al. 2012, Pretorius et al. 2004), it is clear that the high levels of regional murid rodent biodiversity (Skinner and Chimimba 2005) reflect similarly high levels of *Bartonella* species diversity and infection rates. Whilst studies that determine overall infection and diversity for multiple rodent host species at a single sampling site are valuable, focused single-host studies provide a means for unravelling *Bartonella* transmission dynamics. In an assessment of *Micaelamys namaquensis*, an indigenous murid rodent that can attain pest status, seasonal variation in *Bartonella* infection was detected by Brettschneider et al. (2012), whereas in the current study a landscape effect was discernible, despite the small sample size. Both studies underscore the value of expanded, single-host species investigations in reaching a better understanding of factors influencing rodent *Bartonella* ecology in southern Africa.

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Author Disclosure Statement.

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Figure 1: Maximum likelihood (ML) trees inferred using: (A) a 452 nt citrate synthase gene (*gltA*) region and (B) a concatenated 2560 nt dataset comprising *gltA* (452 nt), *ribC* (283 nt), *rpoB* (655 nt), *nuoG* (359 nt), *ITS* (811 nt). For dataset (A), the *Bartonella* lineages identified in *Rhabdomys pumilio* from rural and urban sampling sites in Western Cape Province, South Africa in this study are indicated in bold and the closest matches identified through BlastN searches against the Genbank database (inclusive of *Rhabdomys* bartonellae from the Pretorius et al. (2004) study, are denoted with a *). Reference sequences that are common to both phylogenies (A and B) are shaded grey in Fig. 1A. Bootstrap values ≥ 70 (from 1000 ML bootstrap replications) and posterior probabilities ≥ 90 from Bayesian inference (BI) are indicated ML/BI on the relevant nodes.

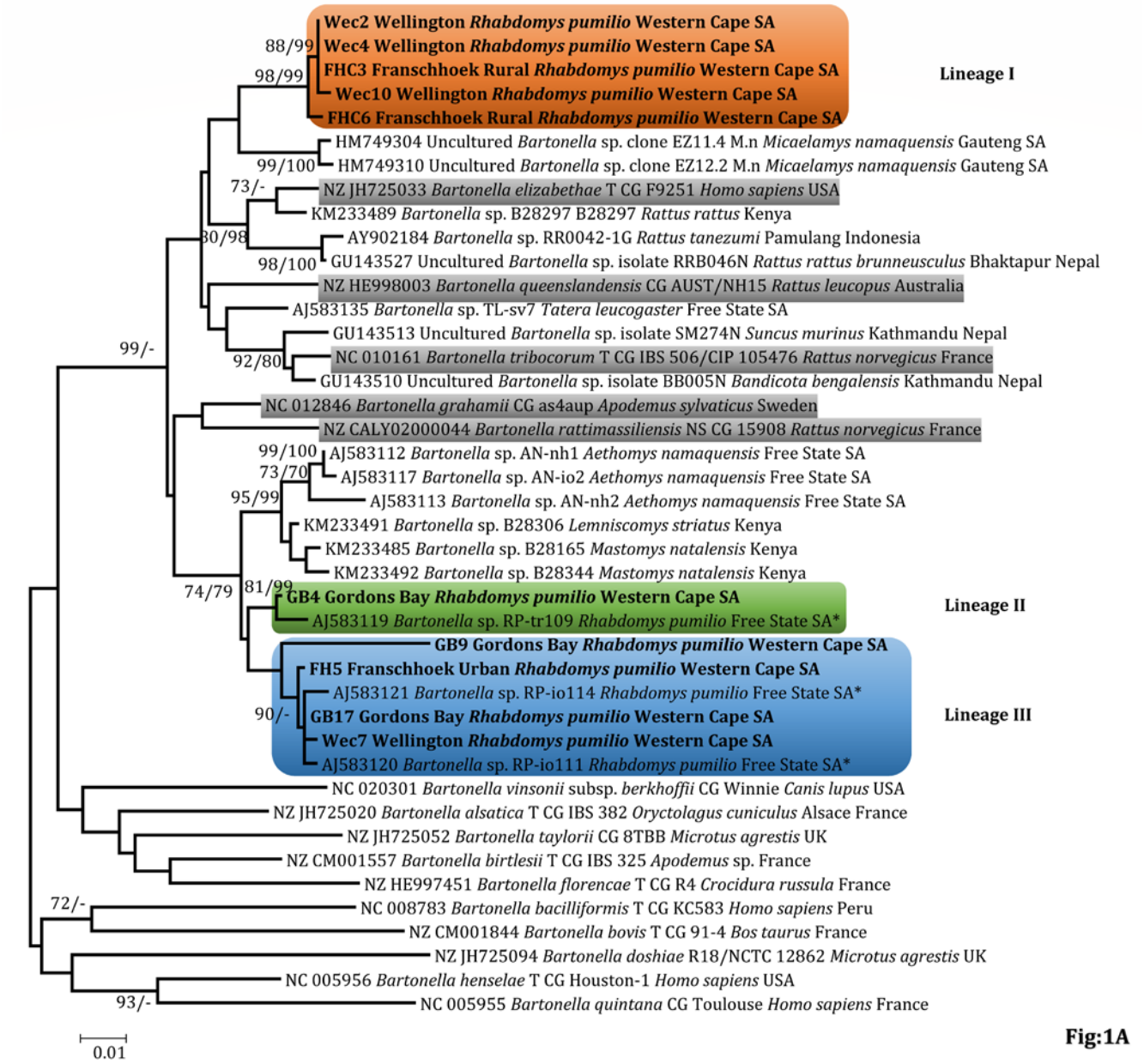
Wec2 Wellington Rhabdomys pumilio Western Cape SA
Wec4 Wellington Rhabdomys pumilio Western Cape SA
FHC3 Franschhoek Rural Rhabdomys pumilio Western Cape SA
Wec10 Wellington Rhabdomys pumilio Western Cape SA
FHC6 Franschhoek Rural Rhabdomys pumilio Western Cape SA
Lineage I
HM749304 Uncultured Bartonella sp. clone EZ11.4 M.n Micaelamys namaquensis Gauteng SA
HM749310 Uncultured Bartonella sp. clone EZ12.2 M.n Micaelamys namaquensis Gauteng SA
NZ JH725033 Bartonella elizabethae T CG F9251 Homo sapiens USA
KM233489 Bartonella sp. B28297 B28297 Rattus rattus Kenya
AY902184 Bartonella sp. RR0042-1G Rattus tanezumi Pamulang Indonesia
GU143527 Uncultured Bartonella sp. isolate RRB046N Rattus rattus brunneusculus Bhaktapur Nepal
NZ HE998003 Bartonella queenslandensis CG AUST/NH15 Rattus leucopus Australia
AJ583135 Bartonella sp. TL-sv7 Tatera leucogaster Free State SA
GU143513 Uncultured Bartonella sp. isolate SM274N Suncus murinus Kathmandu Nepal
NC 010161 Bartonella tribocorum T CG IBS 506/CIP 105476 Rattus norvegicus France
GU143510 Uncultured Bartonella sp. isolate BB005N Bandicota bengalensis Kathmandu Nepal
NC 012846 Bartonella grahamii CG as4aup Apodemus sylvaticus Sweden
NZ CALY02000044 Bartonella rattimassiliensis NS CG 15908 Rattus norvegicus France
AJ583112 Bartonella sp. AN-nh1 Aethomys namaquensis Free State SA
AJ583117 Bartonella sp. AN-io2 Aethomys namaquensis Free State SA
AJ583113 Bartonella sp. AN-nh2 Aethomys namaquensis Free State SA
KM233491 Bartonella sp. B28306 Lemniscomys striatus Kenya
KM233485 Bartonella sp. B28165 Mastomys natalensis Kenya
KM233492 Bartonella sp. B28344 Mastomys natalensis Kenya
GB4 Gordons Bay Rhabdomys pumilio Western Cape SA
AJ583119 Bartonella sp. RP-tr109 Rhabdomys pumilio Free State SA*
Lineage II
GB9 Gordons Bay Rhabdomys pumilio Western Cape SA
FH5 Franschhoek Urban Rhabdomys pumilio Western Cape SA
AJ583121 Bartonella sp. RP-io114 Rhabdomys pumilio Free State SA*
GB17 Gordons Bay Rhabdomys pumilio Western Cape SA
Wec7 Wellington Rhabdomys pumilio Western Cape SA
AJ583120 Bartonella sp. RP-io111 Rhabdomys pumilio Free State SA*
Lineage III
NC 020301 Bartonella vinsonii subsp. berkhoffii CG Winnie Canis lupus USA
NZ JH725020 Bartonella alsatica T CG IBS 382 Oryctolagus cuniculus Alsace France
NZ JH725052 Bartonella taylorii CG 8TBB Microtus agrestis UK
NZ CM001557 Bartonella birtlesii T CG IBS 325 Apodemus sp. France
NZ HE997451 Bartonella florencae T CG R4 Crocidura russula France
NC 008783 Bartonella bacilliformis T CG KC583 Homo sapiens Peru
NZ CM001844 Bartonella bovis T CG 91-4 Bos taurus France
NZ JH725094 Bartonella doshiae R18/NCTC 12862 Microtus agrestis UK
NC 005956 Bartonella henselae T CG Houston-1 Homo sapiens USA
NC 005955 Bartonella quintana CG Toulouse Homo sapiens France
88/99
98/99
99/100
73/-
30/98
98/100
99/-
92/80
99/100
73/70
95/99
74/79
81/99
90/-
72/-
93/-
0.01

Fig:1A

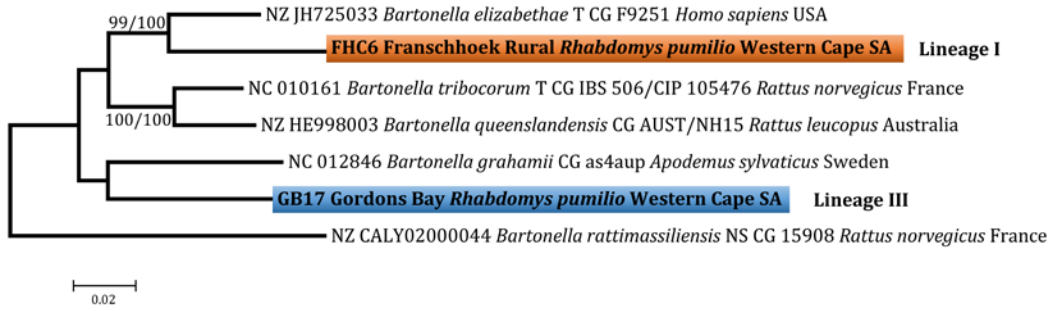
NZ JH725033 Bartonella elizabethae T CG F9251 Homo sapiens USA
FHC6 Franschhoek Rural Rhabdomys pumilio Western Cape SA
Lineage I
NC 010161 Bartonella tribocorum T CG IBS 506/CIP 105476 Rattus norvegicus France
NZ HE998003 Bartonella queenslandensis CG AUST/NH15 Rattus leucopus Australia
NC 012846 Bartonella grahamii CG as4aup Apodemus sylvaticus Sweden
GB17 Gordons Bay Rhabdomys pumilio Western Cape SA
Lineage III
NZ CALY02000044 Bartonella rattimassiliensis NS CG 15908 Rattus norvegicus France
99/100
100/100
0.02

Fig. 1B

TABLE 1 Locality, geographical coordinates, sample size and *Bartonella* PCR assay detection rates in *Rhabdomys pumilio* sampled from rural and urban sites in the Western Cape Province, South Africa.

Habitat type	Locality	Geographical locality	Size [km^2]	Sample size	No of positives	Prevalence by locality (%)	*GltA*	*NuoG*	*RibC*	*RpoB*	*ITS*	Lineage/s Present
Rural	Wellington	33° 31' 44.40" S 19° 02' 27.49" E	0.46	11	4	36.4	4	3	4	3	2	I & III
	Gordons Bay	34° 08 '48.55" S 18° 53' 16.29" E	0.13	30	4	13.3	3	5	4	2	2	I, II & III
	Franschhoek	33° 51' 11.48" S 18° 58' 20.60" E	0.38	8	2	25	2	2	2	2	1	I & III
Urban	Franschhoek	33° 54' 34.77" S 19° 07' 33.16" E	0.03	13	1	7.7	1	1	0	1	0	III
	Somerset West	34° 03' 37.36" S 18° 49' 42.49" E	0.10	18	0	0	0	0	0	0	0	_
Total %				**80**	**11**	**13.6**	**10 12.5%**	**11 12.5%**	**10 12.5%**	**8 10%**	**5 6.5%**	

TABLE 2 Locality, geographical coordinates, sample size and *Bartonella* PCR assay detection rates in *Rhabdomys pumilio* sampled from rural and urban sites in the Western Cape Province, South Africa.

Habitat type	Locality	Geographical locality	Size [km²]	Sample size	No of positives	Prevalence by locality (%)	*GltA*	*NuoG*	*RibC*	*RpoB*	*ITS*	Lineage/s Present
Rural	Wellington	33° 31' 44.40" S 19° 02' 27.49" E	0.46	11	4	36.4	4	3	4	3	2	I & III
	Gordons Bay	34° 08 '48.55" S 18° 53' 16.29" E	0.13	30	4	13.3	3	5	4	2	2	I, II & III
	Franschhoek	33° 51' 11.48" S 18° 58' 20.60" E	0.38	8	2	25	2	2	2	2	1	I & III
Urban	Franschhoek	33° 54' 34.77" S 19° 07' 33.16" E	0.03	13	1	7.7	1	1	0	1	0	III
	Somerset West	34° 03' 37.36" S 18° 49' 42.49" E	0.10	18	0	0	0	0	0	0	0	_
Total %				**80**	**11**	**13.6**	**10 12.5%**	**11 12.5%**	**10 12.5%**	**8 10%**	**5 6.5%**	

TABLE 3 Summary of GenBank accession numbers assigned to each of the *Bartonella* sequence variants identified in *Rhabdomys pumilio* from the Western Cape Province, South Africa.

Sample codes	*glt*A Accession Number	*rpoB* Accession Number	*NuoG* Accession Number	*rib*C Accession Number	*ITS* Accession Number
FH5	MG840442	MG840446	MG840448	_	_
FHC3	MG840443	MG840447	KY906293	KY906285	_
FHC6	KY800497	KY906280	KY906288	KY906284	MG836980
GB4	KY800493	_	KY906289	KY906287	_
GB7	_	_	KY906290	_	_
GB9	KY800494	KY906282	KY906291	KY906286	KY887021
GB17	MG840444	KY906283	KY906291	KY906285	MG836981
GB24	_	_	KY906291	KY906285	_
Wec2	MG840443	_	KY906292	KY906284	MG836982
Wec4	MG840443	KY906280	_	KY906284	KY887022
Wec7	KY800496	KY906283	KY906291	KY906285	_
Wec10	MG840445	KY906281	KY906294	KY906284	_