Bartonella diversity and zoonotic potential in indigenous Tete Veld rats (*Aethomys ineptus*) from South Africa

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Abstract

Bartonellosis is a vector-borne disease that is often misdiagnosed due to a broad range of clinical symptoms, compounded by a lack of awareness regarding the prevalence, diversity and public health impacts of regional strains. Despite recent PCR-based confirmation of *Bartonella* in 9.7% of non-malarial, acute febrile patients in South Africa, data regarding reservoirs of infection are limited. As the majority of *Bartonella* species described to date are associated with rodent species globally, including zoonotic species such as *B. elizabethae*, and as rodent biodiversity is high in southern Africa, we evaluated *Bartonella* in the Tete Veld rat (*Aethomys ineptus*), a highly adaptable murid rodent that thrives in both natural and commensal settings. These rodents are infested with a broad range of ectoparasite species, and often occur in sympatry with *Micaelamys namaquensis,* an indigenous rodent previously shown to host *B. elizabethae*. DNA extracts from heart samples of 75 *A. ineptus* trapped over an eight-month period, from the Roodeplaat Nature Reserve (RNR), were evaluated using a multi-locus sequence analysis (MLSA) approach. Nucleotide sequencing and phylogenetic analyses of individual (*gltA, ribC, rpoB* and *nuoG*) and concatenated gene datasets confirmed the presence of three discrete *Bartonella* lineages (I-III). Lineages I and II, are genetically distinct from all currently recognised *Bartonella* species but cluster with strains present in other indigenous rodents from South and East Africa, whereas lineage III contained *B. elizabethae*, a zoonotic species associated with *Rattus* species globally. Records confirming *R. tanezumi* presence in this nature reserve, which is situated in close proximity to Pretoria, the administrative capital of South Africa, suggests the likelihood of spill-over from invasive to indigenous species. These results together with the high levels of infection (86.7%) and co-infection (33.8%), indicate that *A. ineptus* is a natural reservoir for multiple *Bartonella* species in South Africa, including one with zoonotic potential.

Keywords: *Bartonella elizabethae*, co-infection, *Rattus*, *Micaelamys*, multi-locus sequence analysis (MLSA), phylogeny

Rodents and their ectoparasites play an important role in the maintenance and transmission of a broad range of infectious diseases, including vector-borne pathogens, such as *Rickettsia, Anaplasma* and *Bartonella* (Bowman and Nuttall, 2008; Labuda and Nuttall, 2004). Globally, the public health burden of rodent-associated, vector-borne diseases is increasingly recognised (Billeter et al., 2008; Boulouis et al., 2005), yet has received limited attention in southern Africa. Recent confirmation of acute bartonellosis (9.7% PCR-positivity) in patients presenting with non-malarial, acute febrile illness in South Africa (Simpson et al., 2018), highlights the importance of reservoir host identification, particularly at the interfaces between humans and wildlife.

Globally, studies have shown that rodents host a wide spectrum of *Bartonella* species including zoonotic species such as *B. elizabethae* and *B. tribocorum* (Gundi et al., 2009; Inoue et al., 2010; Kosoy et al., 2004a) and that infection rates are generally high, averaging ~50% (Gutierrez et al., 2015; Kosoy et al., 2004b). Previous studies investigating *Bartonella* prevalence in small mammals from South Africa recovered infection rates of 15% (Hatyoka et al. 2019), 37% (Pretorius et al., 2004), and 44% (Brettschneider et al. 2012). Together these studies confirmed the presence of strains closely related to zoonotic *Bartonella elizabethae*, in three indigenous rodent genera, *viz. Saccostomys*, *Gerbilliscus* and *Micaelamys*, sampled from natural settings in South Africa.

Aethomys ineptus and *M. namaquensis* are highly adaptable species that occur within natural and disturbed/modified habitats, such as rural villages and croplands (Russo et al., 2006). Both species host a broad range of ectoparasites, some of which have been implicated as vectors of zoonotic pathogens such as *Yersinia pestis* (Braack et al., 1996; Fagir et al., 2014).

Given the high *Bartonella* infection rate previously recorded for *M. namaquensis* (Brettschneider et al., 2012) and current lack of data for *A. ineptus,* we set out to assess *Bartonella* diversity for this species, which occurs in sympatry with *M. namaquensis.* The Roodeplaat Nature Reserve (www.roodeplaat-reserve.co.za) was selected due to its close proximity to Pretoria, the administrative capital of South Africa, the known co-occurrence of *M. namaquensis* and the availability of samples collected for prior reproductive physiology study (Muteka et al., 2006). Although seven small mammal species have been recorded from this nature reserve (Table 1S), sample availability was restricted to *Aethomys*. A multi-locus sequence analysis (MLSA) approach, was used to evaluate *Bartonella* genome presence in DNA extracts from heart samples of 75 *A. ineptus* (40 females, 35 males) sampled as previously described (Muteka et al. 2006). Briefly, the citrate synthase (*gltA*), NADH dehydrogenase gamma subunit (*nuoG*), riboflavin synthase (*ribC*), and RNA polymerase subunit B *(rpoB*) gene regions were amplified with genus-specific primers, as previously described (Hatyoka et al. 2018). Amplicons of the expected size were purified and submitted to the core Sanger sequencing facility of the University of Pretoria. Sequence chromatograms were viewed and edited using Chromas, aligned using ClustalX in MEGA 7 (Kumar et al., 2016). All co-infections, discerned by the presence of multiple peaks, were excluded from further downstream sequence analyses. Sequences generated in this study and submitted to

GenBank under accession numbers MH177938–65 and MH507273-77 (Table 2S), were used in individual BLAST nucleotide searches (www.ncbi.nlm.nih.gov/blast) to identify closely-related sequences. Individual gene datasets complemented with reference sequences were used to infer p-distance neighbour-joining trees in order to delineate *Bartonella* species boundaries on the basis of genetic distance (La Scola et al., 2003). Subsequent Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed in PhyML (Guindon and Gascuel, 2003) and in MrBayes (Huelsenbeck and Ronquist, 2001), respectively, using appropriate models of sequence evolution and priors. Nodal support values ≥70 from ML bootstrap resampling and BI posterior probabilities ≥95 in combination with genetic distance (Tables 3S) guided clade delineation (Pretorius et al. 2004).

The MLSA approach employed in this study identified 65 (86.7%) *Bartonella*-positive animals (Table 1), of which 22 (33.8%) had ambiguities at 10% (or fewer) nucleotide sites, consistent with co-infection with two or more *Bartonella* strains. The *gltA* p-distance gene tree recovered 13 sequence variants that could be assigned to three discrete lineages (I-III), with high levels of support in individual (Fig. 1A) and concatenated (*gltA*, *rpoB, ribC,* and *nuoG*) gene phylogenies (Fig. 1B). Lineage I, which was present in 15 animals and is genetically distinct from all currently recognised *Bartonella* species, has previously been detected in *Saccostomys, Rhabdomys* and *Micaelamys* rodents from South Africa (Pretorius et al., 2004) and in *Mastomys* and *Lemniscomys* from Kenya (Halliday et al., 2015). Lineage II, previously reported in *Micaelamys namaquensis* from the Gauteng and Free State provinces of South Africa (Brettschneider et al., 2012; Pretorius et al., 2004), was the dominant lineage, occurring in at least 26 *A. ineptus* (Table 1-2). Lineage III, which was limited to two individuals, clustered with *B. elizabethae*, a species typically associated with invasive rats globally. The lineage III *gltA* sequence variant in *Aethomys* was identical to strains previously detected in *Micaelamys namaquensis* (Brettschneider et al. 2012) and in *Rattus tanezumi* (Mostert 2009) from South Africa.

Generalized linear models (GLM) with a binomial family (link=logit) conducted in the Rstudio interface of Rv3.4.3 (R Core Team, 2017) were used to determine whether (i) *Bartonella* infection is correlated to rodent sex and sampling season and (ii) lineage prevalence varies between males and females, and between seasons. Although *Bartonella* infection peaked during the winter months (93.3%) and females had a slightly higher infection rate compared to males (Tables 1-2), the statistical analyses revealed that neither season, nor sex was significantly correlated with *Bartonella* infection status ($\chi2$=0.01848; df=74; p=0.8919 and $\chi2$=2.70019; df=74; p=0.2592, respectively). Similarly, there were no significant differences in lineage I and II infections either by sex ($\chi2$=1.0877; df=40; p=0.2970) or by season ($\chi2$=0.2317; df=40; p=0.8906).

This first assessment of *Bartonella* in a natural population of *Aethomys ineptus*, revealed an overall infection rate of 86.7%. This is more than two-fold higher than the combined ~40% prevalence previously reported for other rodent species sampled from nature reserves in South Africa (Brettschneider et al., 2012; Pretorius et al., 2004). In particular, *Bartonella*

Table 1: *Bartonella* prevalence and diversity in *Aethomys ineptus* sampled from Roodeplaat Nature Reserve (South Africa)

	No. of individuals	*Bartonella*-positive individuals	Percentage (%)	Number of unambiguous (co-infected) sequences	†Lineage I	†Lineage II	†Lineage III
Males	35	30	85.7	20(7)	10	13	0
Females	40	35	87.5	23 (15)	5	13	2
Total	75	65	86.7	43 (22)	15	26	2

† Based on 43 unambiguous sequences generated in this study

Table 2: Seasonal variation in *Bartonella* lineages and co-infection rates in *Aethomys ineptus* sampled from Roodeplaat Nature Reserve (South Africa)

	Male	Female	Lineage I	Lineage II	Lineage III	Number of co-infected individuals (%)	†Prevalence per season (%)
Winter	16/18	4/4	7	10	0	11 (39.3%)	28/30 (93.3)
Spring	10/12	12/12	5	11	2	11 (37.9%)	29/36 (80.6)
Summer	4/5	19/24	3	5	0	0 (0%)	8/9 (88.9)
Total	30/35	35/40	15	26	2	22 (33.8%)	65/75 (87.6)

†Based on PCR screening with two gene regions (*gltA* and *nuoG*), followed by sequence confirmation of *Bartonella* genome presence

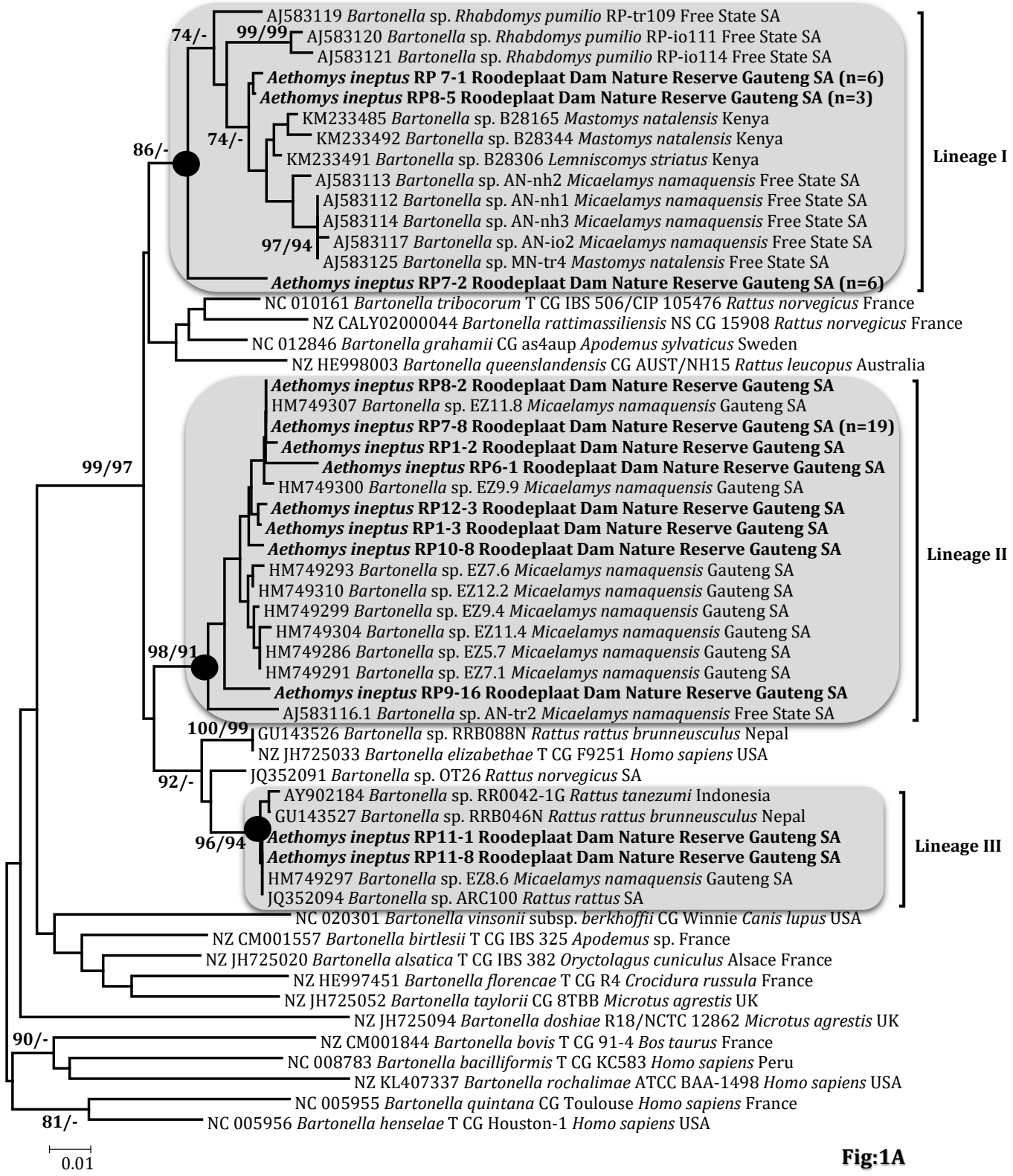
AJ583119 Bartonella sp. Rhabdomys pumilio RP-tr109 Free State SA
74/-
99/99
AJ583120 Bartonella sp. Rhabdomys pumilio RP-io111 Free State SA
AJ583121 Bartonella sp. Rhabdomys pumilio RP-io114 Free State SA
Aethomys ineptus RP 7-1 Roodeplaat Dam Nature Reserve Gauteng SA (n=6)
Aethomys ineptus RP8-5 Roodeplaat Dam Nature Reserve Gauteng SA (n=3)
KM233485 Bartonella sp. B28165 Mastomys natalensis Kenya
74/-
KM233492 Bartonella sp. B28344 Mastomys natalensis Kenya
86/-
KM233491 Bartonella sp. B28306 Lemniscomys striatus Kenya
AJ583113 Bartonella sp. AN-nh2 Micaelamys namaquensis Free State SA
AJ583112 Bartonella sp. AN-nh1 Micaelamys namaquensis Free State SA
AJ583114 Bartonella sp. AN-nh3 Micaelamys namaquensis Free State SA
97/94
AJ583117 Bartonella sp. AN-io2 Micaelamys namaquensis Free State SA
AJ583125 Bartonella sp. MN-tr4 Mastomys natalensis Free State SA
Aethomys ineptus RP7-2 Roodeplaat Dam Nature Reserve Gauteng SA (n=6)
Lineage I
NC 010161 Bartonella tribocorum T CG IBS 506/CIP 105476 Rattus norvegicus France
NZ CALY02000044 Bartonella rattimassiliensis NS CG 15908 Rattus norvegicus France
NC 012846 Bartonella grahamii CG as4aup Apodemus sylvaticus Sweden
NZ HE998003 Bartonella queenslandensis CG AUST/NH15 Rattus leucopus Australia
Aethomys ineptus RP8-2 Roodeplaat Dam Nature Reserve Gauteng SA
HM749307 Bartonella sp. EZ11.8 Micaelamys namaquensis Gauteng SA
Aethomys ineptus RP7-8 Roodeplaat Dam Nature Reserve Gauteng SA (n=19)
Aethomys ineptus RP1-2 Roodeplaat Dam Nature Reserve Gauteng SA
99/97
Aethomys ineptus RP6-1 Roodeplaat Dam Nature Reserve Gauteng SA
HM749300 Bartonella sp. EZ9.9 Micaelamys namaquensis Gauteng SA
Aethomys ineptus RP12-3 Roodeplaat Dam Nature Reserve Gauteng SA
Aethomys ineptus RP1-3 Roodeplaat Dam Nature Reserve Gauteng SA
Aethomys ineptus RP10-8 Roodeplaat Dam Nature Reserve Gauteng SA
HM749293 Bartonella sp. EZ7.6 Micaelamys namaquensis Gauteng SA
HM749310 Bartonella sp. EZ12.2 Micaelamys namaquensis Gauteng SA
HM749299 Bartonella sp. EZ9.4 Micaelamys namaquensis Gauteng SA
HM749304 Bartonella sp. EZ11.4 Micaelamys namaquensis Gauteng SA
98/91
HM749286 Bartonella sp. EZ5.7 Micaelamys namaquensis Gauteng SA
HM749291 Bartonella sp. EZ7.1 Micaelamys namaquensis Gauteng SA
Aethomys ineptus RP9-16 Roodeplaat Dam Nature Reserve Gauteng SA
AJ583116.1 Bartonella sp. AN-tr2 Micaelamys namaquensis Free State SA
Lineage II
100/99
GU143526 Bartonella sp. RRB088N Rattus rattus brunneusculus Nepal
NZ JH725033 Bartonella elizabethae T CG F9251 Homo sapiens USA
92/-
JQ352091 Bartonella sp. OT26 Rattus norvegicus SA
AY902184 Bartonella sp. RR0042-1G Rattus tanezumi Indonesia
GU143527 Bartonella sp. RRB046N Rattus rattus brunneusculus Nepal
96/94
Aethomys ineptus RP11-1 Roodeplaat Dam Nature Reserve Gauteng SA
Aethomys ineptus RP11-8 Roodeplaat Dam Nature Reserve Gauteng SA
HM749297 Bartonella sp. EZ8.6 Micaelamys namaquensis Gauteng SA
JQ352094 Bartonella sp. ARC100 Rattus rattus SA
Lineage III
NC 020301 Bartonella vinsonii subsp. berkhoffii CG Winnie Canis lupus USA
NZ CM001557 Bartonella birtlesii T CG IBS 325 Apodemus sp. France
NZ JH725020 Bartonella alsatica T CG IBS 382 Oryctolagus cuniculus Alsace France
NZ HE997451 Bartonella florencae T CG R4 Crocidura russula France
NZ JH725052 Bartonella taylorii CG 8TBB Microtus agrestis UK
NZ JH725094 Bartonella doshiae R18/NCTC 12862 Microtus agrestis UK
90/-
NZ CM001844 Bartonella bovis T CG 91-4 Bos taurus France
NC 008783 Bartonella bacilliformis T CG KC583 Homo sapiens Peru
NZ KL407337 Bartonella rochalimae ATCC BAA-1498 Homo sapiens USA
NC 005955 Bartonella quintana CG Toulouse Homo sapiens France
81/-
NC 005956 Bartonella henselae T CG Houston-1 Homo sapiens USA
0.01

Fig:1A

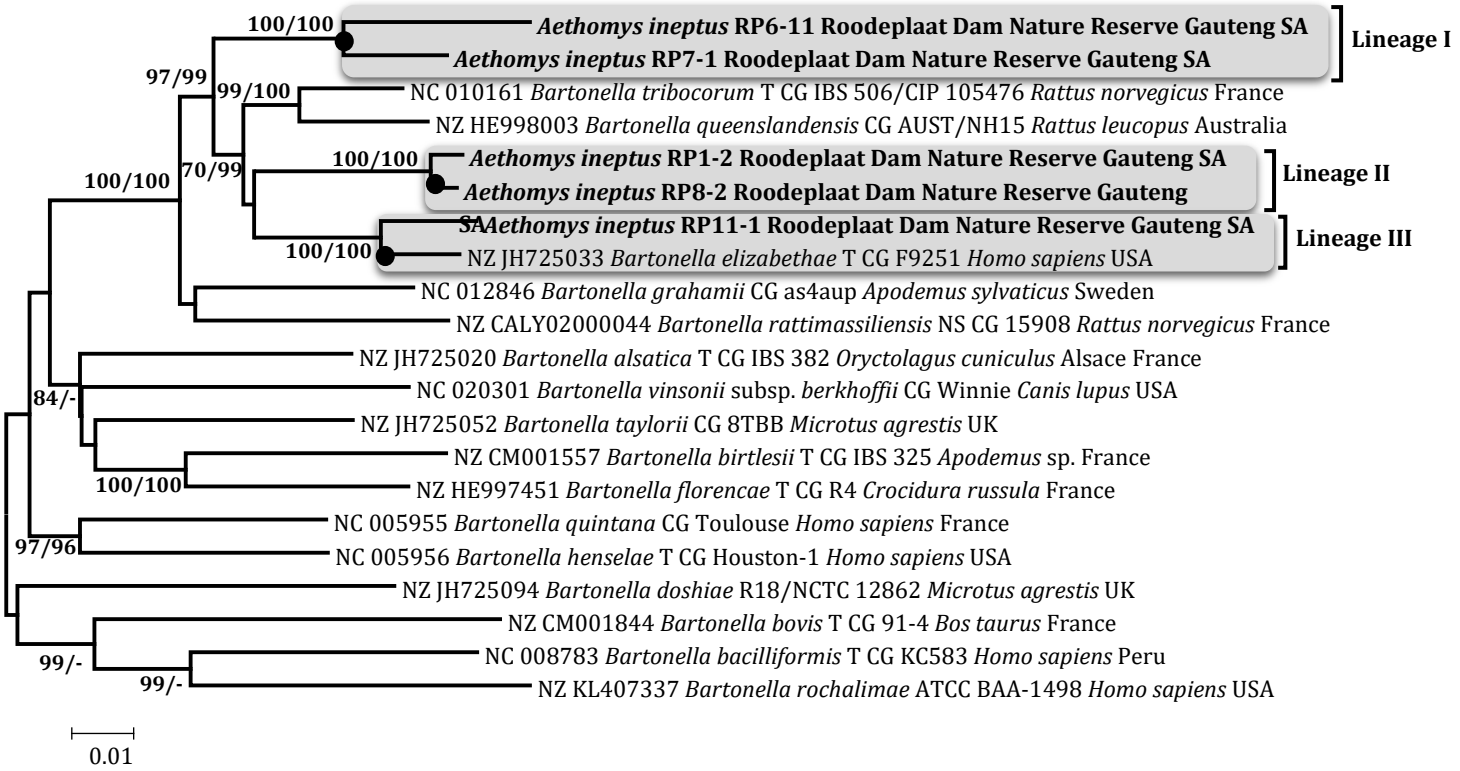

Fig:1B

Figure 1: Maximum Likelihood (ML) tree inferred using (A) a 51-taxon partial *gltA* gene dataset (395 nt in length) and (B) a 21-taxon concatenated dataset (1672 nt in length) comprising partial *gltA* (395 nt), *rpoB* (753 nt), *ribC (*245 nt), and *nuoG* (264 nt) gene sequences. All *Bartonella* strains detected in *Aethomys ineptus* Tete Veld rats, Gauteng Province (South Africa) from this study are indicated in bold. Those sourced from public databases are prefixed with the corresponding accession number. The *gltA* gene phylogeny (A) contains the closest matches identified through BlastN searches against the GenBank database. Nodal support indices ≥70 and ≥90 are indicated ML/Bayesian Inference (BI) on the relevant nodes. The country code "SA" corresponds to samples from South Africa characterised in this and in previous studies.

prevalence in *Micaelamys namaquensis*, a species which, like *A. ineptus,* is highly adaptable and with which it often occurs in sympatry, was comparatively low, ranging from 44.0% (n=100) to 57.9% (n=19) at the Gauteng and Free State province sampling sites, respectively (Brettschneider et al., 2012; Pretorius et al., 2004). Similarly, the 33.8% co-infection level in *A. ineptus* is nearly two-fold higher than the 18.2% recorded for *M. namaquensis* (Brettschneider et al., 2012). Although this co-infection rate is higher than the 22% maximum generally reported in studies employing similar experimental procedures to ours (Birtles et al., 2001; Brettschneider et al., 2012; Gundi et al., 2010; Inoue et al., 2009; Kosoy et al., 2004c; Telfer et al., 2007), it is on par with that reported for Baluchistan gerbils (*Gerbillus nanus*) from Saudi Arabia (Kleynhans et al., 2018). In common with *A. ineptus*, the latter species also displayed high levels of infection and lineage diversity.

Sequence analyses identified the presence of 13 *gltA* variants that clustered within three distinct lineages (I–III), of which two (I and II) are unrelated to all formally recognised species. Lineage I has a broad host and geographical range (Fig. 1A), being present in *Saccostomys, Rhabdomys* and *Micaelamys* from South Africa (Pretorius et al., 2004) and in *Mastomys* and *Lemniscomys* from East Africa (Halliday et al., 2015). Similarly, the host species range of Lineage II, the dominant lineage in *A. ineptus* (Tables 1-2), is broader than previously anticipated from reports indicating that it is limited to *Micaelamys namaquensis* (Pretorius et al. 2004; Brettschneider et al. 2012). Lineage III which is closely related to zoonotic *B. elizabethae* strains detected in *Rattus* species globally, is identical to a strain identified in invasive *Rattus* sampled from rural households (Mostert, 2009) and in *M. namaquensis* from a nature reserve (Brettschneider et al., 2012) in South Africa. The presence of this *Rattus*-associated zoonotic *Bartonella* species in *A. ineptus* and prior genetically-confirmed records of invasive *Rattus tanezumi* at Roodeplaat Nature Reserve (Bastos et al., 2011), are strongly suggestive of spill-over between invasive rats and indigenous rodents. This finding underscores concerns raised by Neves et al. (2018) regarding the potential for spill-over of exotic, zoonotic *Bartonella* from invasive to indigenous rodent populations and establishment of novel epidemiological cycles. Continuous community-level monitoring of natural rodent populations, in combination with rodent pest-control programmes targeting invasive species, are important mitigation strategies, particularly in newly-invaded, peri-urban areas that are frequented for outdoor activities.

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Conflict of Interest

The authors declare no conflict of interest.

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