

Table S1: Barcode primers used for PCR amplification of the 16S rRNA gene.

Name	Code	*Multiplex identifier (MID) sequence	†Primer
MID1-27F	F1	CGT ATC GCC TCC CTC GCG CCA TCAG ACG AGT GCGT	27F
MID2-27F	F2	CGT ATC GCC TCC CTC GCG CCA TCAG ACG CTC GACA	27F
MID3-27F	F3	CGT ATC GCC TCC CTC GCG CCA TCAG AGA CGC ACTC	27F
MID4-27F	F4	CGT ATC GCC TCC CTC GCG CCA TCAG AGC ACT GTAG	27F
MID5-27F	F5	CGT ATC GCC TCC CTC GCG CCA TCAG TCA GAC ACGA	27F
MID1-1435R	R1	CTA TGC GCC TTG CCA GCC CGC TCAG ACG AGT GCGT	1435R
MID2-1435R	R2	CTA TGC GCC TTG CCA GCC CGC TCAG ACG CTC GACA	1435R
MID3-1435R	R3	CTA TGC GCC TTG CCA GCC CGC TCAG AGA CGC ACTC	1435R
MID4-1435R	R4	CTA TGC GCC TTG CCA GCC CGC TCAG AGC ACT GTAG	1435R
MID5-1435R	R5	CTA TGC GCC TTG CCA GCC CGC TCAG ATC AGA CACG	1435R

*red font= Adaptor sequence, blue font= Key sequence, green font= MID sequence.

†27F = 5'-AGA GTT TGA TCM TGG CTC AGA ACG; 1435R= 5'-CGA TTA CTA GCG ATT CCR RCT TCA

Table S2: Information for rodent samples.

Sample number	Origin of rodent	Habitat	Rodent species ^a	Sex	Barcode primer ID ^b
R19	Gottenburg	urban/peri-urban	<i>M. natalensis</i>	male	F2R5
R20	Gottenburg	urban/peri-urban	<i>M. coucha</i>	male	F3R4
R29	Gottenburg	urban/peri-urban	<i>M. natalensis</i>	male	F3R1
R30	Gottenburg	urban/peri-urban	<i>M. natalensis</i>	male	F3R2
R31	Gottenburg	urban/peri-urban	<i>M. natalensis</i>	female	F3R3
R171	Gottenburg	urban/peri-urban	<i>M. natalensis</i>	female	F1R2
R172	Gottenburg	urban/peri-urban	<i>M. natalensis</i>	female	F1R3
R74	Hlalakahle	urban/peri-urban	<i>M. natalensis</i>	male	F4R5
R75	Hlalakahle	urban/peri-urban	<i>M. natalensis</i>	male	F5R1
R78	Hlalakahle	urban/peri-urban	<i>M. natalensis</i>	female	F5R2
R95	Hlalakahle	urban/peri-urban	<i>M. natalensis</i>	female	F4R4
R98	Hlalakahle	urban/peri-urban	<i>M. natalensis</i>	male	F1R5
R99	Hlalakahle	urban/peri-urban	<i>M. natalensis</i>	female	F5R3
R2	Tlhavekisa	communal rangeland	<i>M. coucha</i>	female	F1R1
R5	Tlhavekisa	communal rangeland	<i>M. coucha</i>	male	F2R2
R6	Tlhavekisa	communal rangeland	<i>M. coucha</i>	male	F1R4
R11	Tlhavekisa	communal rangeland	<i>M. coucha</i>	female	F2R3
R12	Tlhavekisa	communal rangeland	<i>M. coucha</i>	female	F2R4
R84	Tlhavekisa	communal rangeland	<i>M. natalensis</i>	female	F2R1
R21	Manyeleti	protected area	<i>M. natalensis</i>	female	F3R5
R53	Manyeleti	protected area	<i>Steatomys sp.</i>	female	F4R2
R61	Manyeleti	protected area	<i>M. coucha</i>	male	F4R3
R159	Manyeleti	protected area	<i>M. coucha</i>	male	F5R4
R177	Manyeleti	protected area	<i>M. coucha</i>	female	F5R5
R179	Manyeleti	protected area	<i>M. coucha</i>	male	F4R1

^a Species identification was confirmed by molecular typing of the cytochrome c oxidase gene and the cytochrome *b* gene.

^b Sample-specific primer combination used to amplify each sample to construct the library for circular consensus sequencing (CCS); See Table 1 for detailed primer information.

TABLE S3. Sequence read archive (SRA) accession numbers of bacterial sequences obtained from rodents.

Accession number	Sample number	Rodent species
SRX5967121	R172	<i>Mastomys natalensis</i>
SRX5967122	R21	<i>Mastomys natalensis</i>
SRX5967123	R29	<i>Mastomys natalensis</i>
SRX5967124	R30	<i>Mastomys natalensis</i>
SRX5967125	R31	<i>Mastomys natalensis</i>
SRX5967126	R171	<i>Mastomys natalensis</i>
SRX5967127	R98	<i>Mastomys natalensis</i>
SRX5967128	R99	<i>Mastomys natalensis</i>
SRX5967129	R19	<i>Mastomys natalensis</i>
SRX5967130	R20	<i>Mastomys coucha</i>
SRX5967131	R179	<i>Mastomys coucha</i>
SRX5967132	R61	<i>Mastomys coucha</i>
SRX5967133	R53	<i>Steatomys</i>
SRX5967134	R177	<i>Mastomys coucha</i>
SRX5967135	R159	<i>Mastomys coucha</i>
SRX5967136	R74	<i>Mastomys natalensis</i>
SRX5967137	R75	<i>Mastomys natalensis</i>
SRX5967138	R12	<i>Mastomys coucha</i>
SRX5967139	R84	<i>Mastomys natalensis</i>
SRX5967140	R6	<i>Mastomys coucha</i>
SRX5967141	R11	<i>Mastomys coucha</i>
SRX5967142	R2	<i>Mastomys coucha</i>
SRX5967143	R5	<i>Mastomys coucha</i>
SRX5967144	R78	<i>Mastomys natalensis</i>
SRX5967145	R95	<i>Mastomys natalensis</i>

TABLE S4. Genbank accession numbers of sequences used for the phylogenetic analysis of the 16S rRNA gene of *Bartonella* spp.

Accession	Organism	Strain/Country	Host
NR104902	<i>Bartonella vinsonii</i> subsp. <i>arupensis</i>	OK 94-513/Sweden	Culture
DQ228135	<i>Bartonella vinsonii</i> subsp. <i>berkhofii</i>	Q64SHD/China	Dog
AJ250247	<i>Bartonella quintana</i>	Italy	<i>Pediculus humanus corporis</i>
NR074335	<i>Bartonella henselae</i>	Houston-1/USA	Cat
NR178638	<i>Bartonella mastomydis</i>	008/Senegal	<i>Mastomys erythroleucus</i>
EU111756	<i>Bartonella queenslandensis</i>	AUST/NH8/Australia	<i>Melomys</i> sp.
NR074354	<i>Bartonella tribocorum</i>	IBS 506/France	<i>Rattus</i> sp.
NR115255	<i>Bartonella rattimassiliensis</i>	15908/France	<i>Rattus norvegicus</i>
CP001562	<i>Bartonella grahamii</i>	as4aup/Sweden	<i>Apodemus flavicollis</i>
NR116175	<i>Bartonella rattaaustraliani</i>	AUST/NH4/Australia	<i>Rattus tunneyi</i>
NR025889	<i>Bartonella elizabethae</i>	F9251/USA	Human
NR115254	<i>Bartonella phoceensis</i>	16120/France	<i>Rattus norvegicus</i>
AB602532	<i>Bartonella pachyuromydis</i>	FN18-1/Netherlands	<i>Pachyuromys duprasi</i>
AY993936	<i>Bartonella</i> sp.	RF255YX /China	<i>Rattus tanezumi flavipectus</i>
NR_074243	<i>Ochrobactrum anthropi</i>	ATCC 49188/USA	Culture

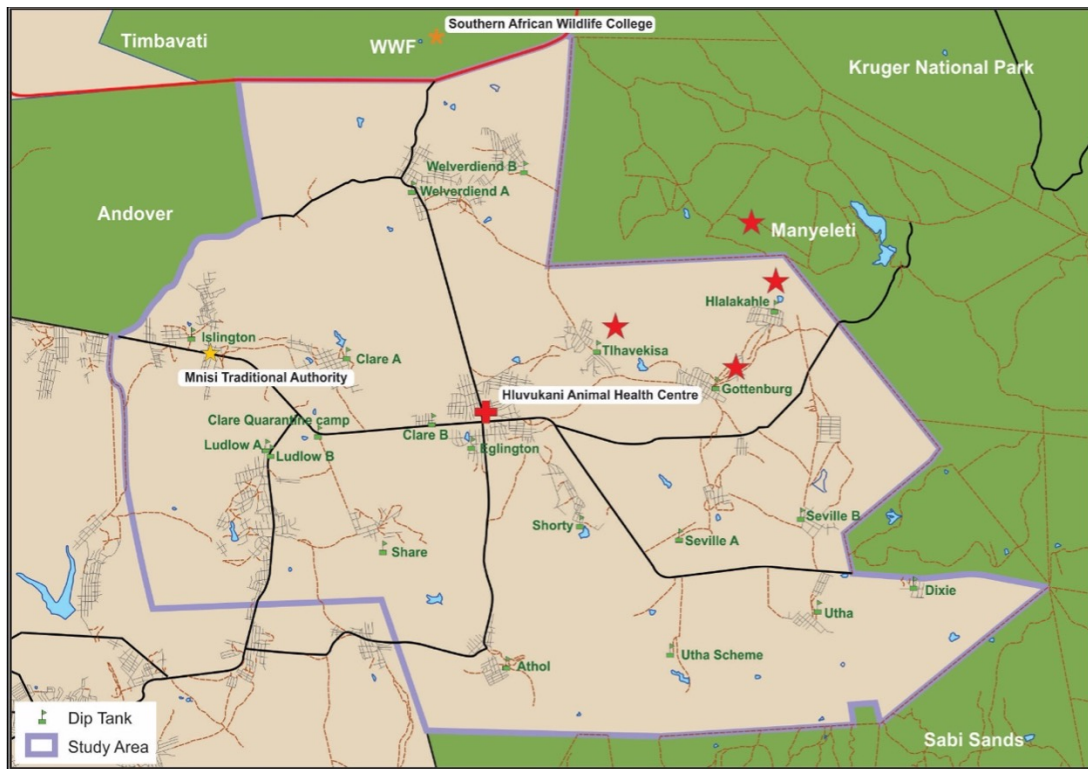


Figure S1. Map of the study area in Bushbuckridge-East Community, Mpumalanga Province, South Africa. Red stars show sites of rodent capture. Gottenburg and Hlalakahle are urban or peri-urban areas, while Tihavekisa is in the communal rangelands. Manyeleti is a protected wildlife reserve. Dark green represents protected areas where wildlife roam freely.

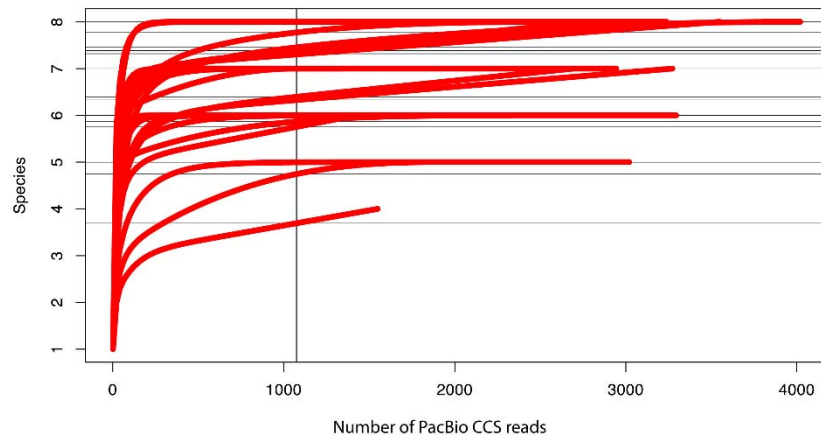


Figure S2. Rarefaction curves of bacterial species (OTUs) identified in rodent blood samples. The number of bacterial species detected in rodent blood samples was plotted as a function of read depth. The vertical line in the plot indicates the value where rarefaction criterion was satisfied.

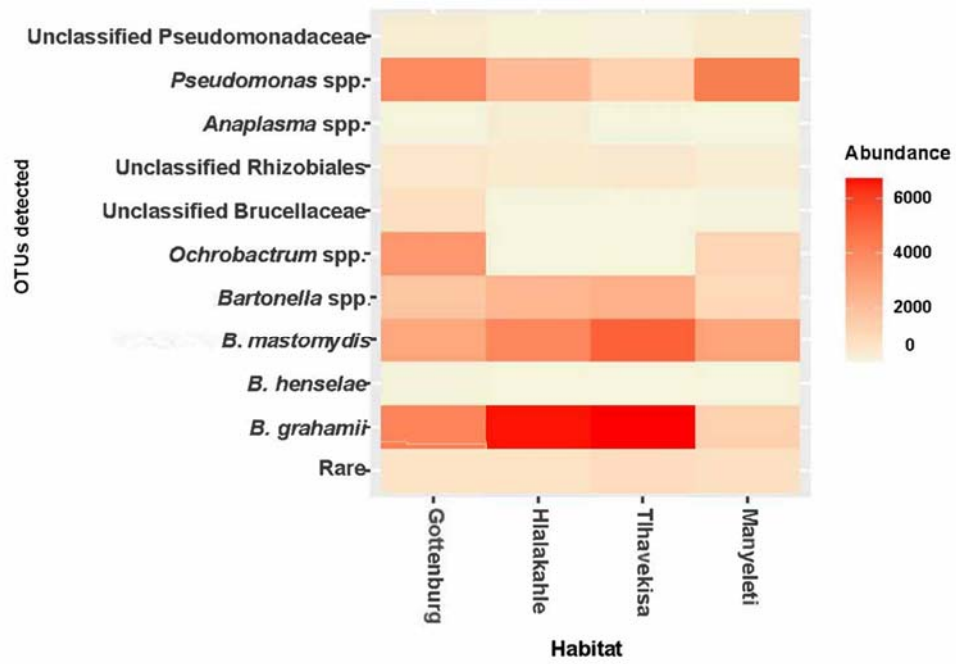


FIGURE S3. Heatmap showing the abundance of operational taxonomic units (OTUs) detected in rodent samples from the four habitats. Habitat names are shown on the X axis and bacterial sequences detected in rodent blood are depicted on the Y axis.

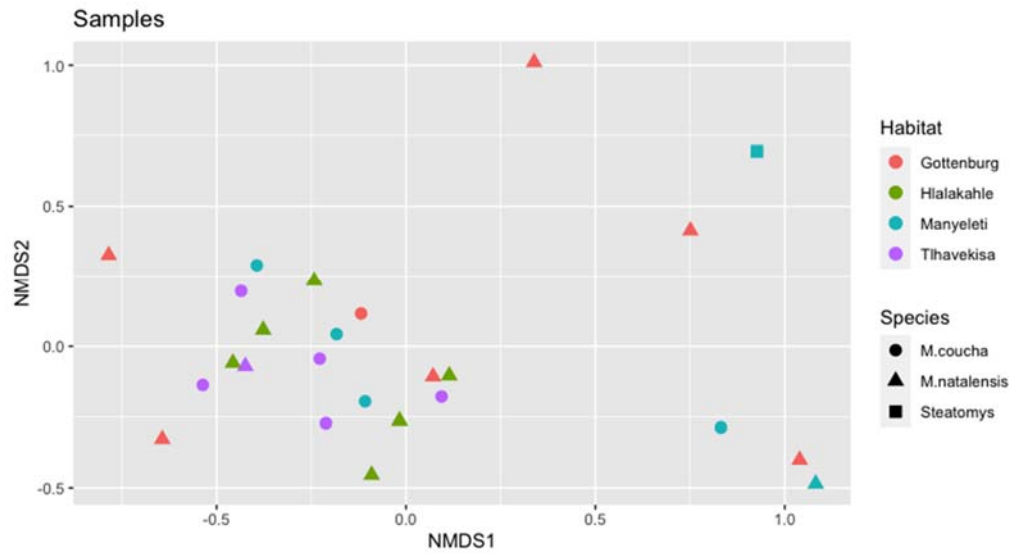


FIGURE S4. NMDS plot of generalized ordination distances between rodent samples. Red shapes indicate rodents captured from Gottenburg, green shapes – rodents from Hlalakahle, blue shapes – rodents from Manyeleti and purple shapes – rodents from Tihavekisa. *Mastomys coucha* are represented by the circles, *M. natalensis* by the triangles and *Steatomys* sp. by the square.

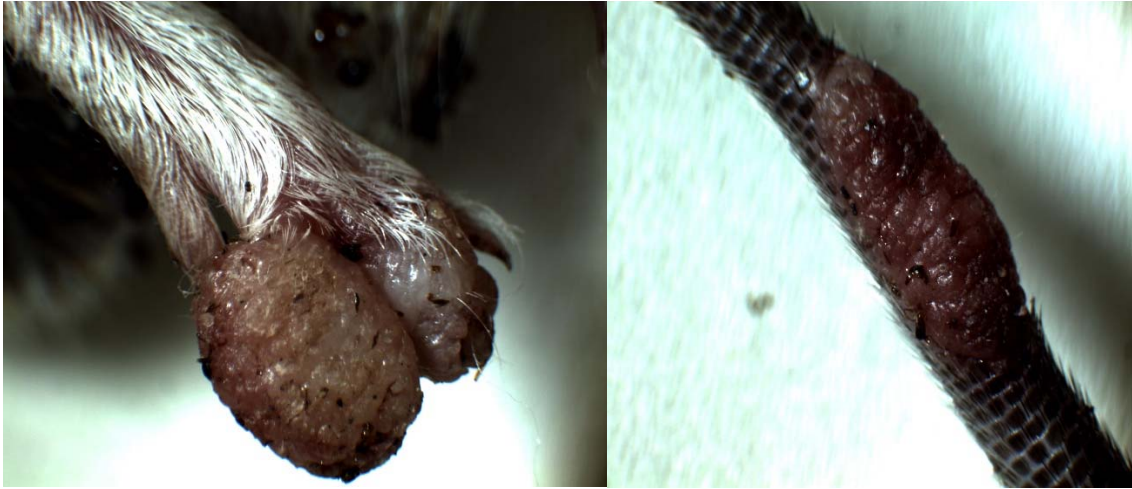


FIGURE S5. Ulcerative pododermatitis lesions in foot and tail of some rodents in this study. Previous research indicates such lesions are associated with *Pseudomonas* spp. infection.

R scripts for microbiome analysis

R studio was used to import sample data, and the `vegan` package was used to calculate the mean species diversity of bacteria in all rodents from the different habitat areas using rarefaction curves. `FactoMineR` package was used to conduct Principal Component Analysis (PCA) to quantify the compositional similarity or dissimilarity of the bacterial population in the blood of the rodents across the habitat areas. Statistical test of PerMANOVA (Permutational ANOVA) was conducted using the `adonis` function in the `vegan` package. Nonmetric multidimensional scaling (NMDS) ordination to compare samples based on their rarefied bacterial populations was done using the `phyloseq` package.

Rarefaction curves

```
>setwd("C:/Users/User/Desktop")
>rodent <- read.table("OtuRodent.txt", header=T, row.names=1, sep="\t")
>library(vegan)
>raremax <- min(rowSums(rodent))
>col <- c("purple")
>lty <- c("solid")
>lwd <- c("4")
>pars <- expand.grid(col = col, lty = lty, lwd = lwd, stringsAsFactors = FALSE)
>head(pars)
>out <- with(pars, rarecurve(rodent, step = 20, sample = raremax, col = col, lty = lty, lwd =lwd, label = FALSE))
```

Principal component analysis (FactoMineR)

```
>rodent <- read.table("rodent.txt", header=T, row.names=1, sep="\t")
>library(FactoMineR)
>library (factoextra)
>res <- PCA(rodent[,1:11])
>summary(res)
>res <- PCA(rodent, quali.sup=12)
>plot(res, cex=0.8, habillage="Habitat")
>fviz_pca_ind(res, cex=1.2, label="none", habillage="Habitat")
```

Description of the dimensions

```
>dimdesc(res)
```

PerMANOVA (Vegan)

```
>rm <- read.table("Rodentmeta.txt", header=T, row.names=1, sep="\t")
>library(vegan)
```

```

> R.dist=vegdist(rm, distance="bray")
>adonis(R.dist ~ Gottenburg*Manyeleti, data =rm, permutations = 1000)
>adonis(R.dist ~ Manyeleti*Tlhavekisa, data=rm, permutations = 1000)
>adonis(R.dist ~ Hlalakahle*Gottenburg, data=rm, permutations = 1000)
>adonis(R.dist ~ Gottenburg*Hlalakahle, data=rm, permutations = 1000)
>adonis(R.dist ~ Manyeleti*Hlalakahle, data=rm, permutations = 1000)
>adonis(R.dist ~ Gottenburg*Tlhavekisa, data=rm, permutations = 1000)
>adonis(R.dist ~ Hlalakahle*Tlhavekisa, data=rm, permutations = 1000)

```

Ordination Script (Phyloseq)

```

>library("phyloseq")
>library("ggplot2")
>library("plyr")
>library("vegan")
>library(microbiome)
>otu_mat <- read.table("Rodent1.txt", header=T, row.names=1, sep="\t")
>tax_mat <- read.table("TaxRodent.txt", header=T, row.names=1, sep="\t")
>samples_df<- read.table("SampleRodent.txt", header=T, row.names=1, sep="\t", check.names =
TRUE, stringsAsFactors = TRUE)
>row.names(otu_mat) <- otu_mat$otu
>row.names(tax_mat) <- tax_mat$otu
>row.names(samples_df) <- samples_df$sample
>otu_mat <- as.matrix(otu_mat)
>tax_mat <- as.matrix(tax_mat)
>OTU = otu_table(otu_mat, taxa_are_rows = TRUE)
>TAX = tax_table(tax_mat)
>rownames(samples_df)=colnames(otu_mat)
>samples=as.data.frame(samples_df)
>mysamples=sample_data(as.data.frame(samples_df))
>set2 <- phyloseq(OTU, TAX, mysamples)
>set2
>sample_names(set2)
>rank_names(set2)

```

```
>sample_variables(set2)
>plot_bar(set2, fill = "Genus")
>plot_bar(set2, fill = "Species")
>plot_heatmap(set2, method = "NMDS", distance = "bray")
>plot_heatmap(set2, method = "MDS", distance = "(A+B-2*J)/(A+B-J)",
  taxa.label = "Genus", taxa.order = "Genus",
  trans=NULL, low="beige", high="red", na.value="beige")
>plot_heatmap(set2, method = "MDS", distance = "(A+B-2*J)/(A+B-J)",
  taxa.label = "Species", taxa.order = "Species",
  trans=NULL, low="beige", high="red", na.value="beige")
>plot_heatmap(set2, method = "NMDS", distance = "bray", taxa.label = "Species", taxa.order =
"Species",
  low="beige", high="red", na.value="beige")
>dist_methods <- unlist(distanceMethodList)
>print(dist_methods)
>plot_richness(set2, measures=c("Chao1", "Shannon"))
>plot_richness(set2, measures=c("Chao1", "Shannon"), x="Species", color="Habitat")
>set2.ord <- ordinate(set2, "NMDS", "bray")
>plot_ordination(set2, set2.ord, type="taxa", color="Species", shape="Genus",
  title="OTUs")
>plot_ordination(set2, set2.ord, type="samples", color="Habitat",
  shape="Species", title="Samples") + geom_point(size=3)
```

